QUALITATIVE EVALUATION OF D.H.P. (DIHIDROXIPIRIDINE) IN THE URINE OF BUFFALOES FED WITH LEUCAENA

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ABSTRACT
The aim of this study was to evaluate the DHP elimination in the urine of buffaloes fed with three levels of leucaena (0,10 and 20g of leucaena's DM/kg $LW^{0.75}$), plus corn silage at 2.5% of LW in D.M. The main purpose was to estimate the levels of mimosine toxicity in those resistant animals. Data showed that DHP started to be eliminated at the first urination in both levels of leucaena. This occurred one hour after ingestion. DHP was present up to the third urination (5 hours after ingestion) in the highest level.

KEYWORDS
buffalo, leucaena toxicity, mimosine, dihidroxipiridine.

INTRODUCTION
One of the best solutions to supply the feed scarceness during the dry season in the tropics is through different forms of utilization of leucaena (*Leucaena leucocephala*) (Alcântara, 1993). However, its consumption has presented some toxicity problems which can be evaluated through different methodologies (Acamovic & D'Mello, 1981). The ingested mimosine is primarily degradated at rumen level with the resultant DHP absorbed in the intestines. Therefore, the DHP's amount detected in the urine is the reflex of that quantity found in the blood.

Thus the urine-DHP can be considered a good indicator for the toxicity evaluation by mimosine. The simplest way to measure this kind of toxicity is through the mimosine's DHP excretion into the urine. Meanwhile, linearity was not found between urine-DHP recovery and the one present in the diet. (Jones & Megarrity, 1983). This statement was supported by Franzolin & Velloso (1987) who found a relationship between ingested mimosine and excreted DHP in the urine equal to 4.09 this relationship being four times superior to that reported by Jones & Megarrity (1983). There is no doubt that urine's DHP detection and quantification is a good auxiliar on predicting toxical problems. Among the available techiniques, the one used by Megarrity (1987) is of great interest because of its simplicity. It is based upon the reaction between DHP, HCL 0.1N and $FeCl_3$. Method precision is limited because some poliphenolic compounds and some other pigments may react with the salts giving uncertain results. This study was made to estabilish the best moment to evaluate the DHP in the urine of Murrah buffalo fed with fresh leucaena in order to identify toxicity symptoms more accurately.

METHODS
Six young, male, Murrah buffalo between 12 and 14 months of age were used to test three treatments: control (corn silage corresponding to 2.5% of L.W.); corn silage plus 10g of leucaena D.M./kg $L.W.^{0.75}$ and corn silage plus 20g of leucaena D.M./kg $L.W.^{0.75}$. Adaptation to the diet and for the rumen flora was made feeding the animals with corn silage 10 days before the tests. After adaptation the animals were taken to metabolic cages were the collections took place. The meals were distributed in the morning period and total volume of three following micturitions after consumption was collected. Basal ration (corn silage) was fed to the animals only after leucaena consumption.

The study was arranged in a complete randomized design with two replications with split plots being the plots and combinations of three periods of time and three treatments and the sub-plots the periods between the micturitions.

Detection of DHP was made through the reaction of 0.25 ml of urine and 5 ml of the solution (1g of $FeCl_3$ plus 3 ml of HCl in one liter of distilated water).

From this reaction three different colours may occur:
purple - 3.4 DHP presence
blue - 2.3 DHP presence
yellow - DHP absence

RESULTS AND DISCUSSION
The buffalo accepted poorly the fresh leucaena; it was necessary to mix it with corn silage. As the liveweight did change from one period of collection to the other, the amount of fresh leucaena was corrected according to the metabolic weight and to the percentage of dry matter in the plants. On average, the amounts of leucaena varies from 2.3kg (level one) to 3.8kg (level two).

The micturition volumes from 8:00 am to 5:00 pm varied from 15 up to 1,100 ml in each urination. Total excreted urine in a nine hour period varied from 15 up to 1,100 ml in each urination. Total excreted urine in a nine hour period varied from 240 to 1,710 ml the average equal to 901 ml the average. It is presumed that a great part of the micturitions did take place during the night as water was left "ad libitum" for the animals.

The reactions between urine samples and the solution indicated that corn silage consuming animals gave yellow or strawish color, meaning DHP absence. One and two levels gave purple and blue colors, pointing out the presence of DHP in the urine according to Megarrity (1978). Table 1 summarizes the colorimetric behaviour of the urine samples according to the three different diets within the three periods of time that the experiment ran. DHP excretion took place at the first micturition just after the leucaena consumption. It occurred one hour and 10 minutes after ingestion. Urine's color at reaction was more intense the higher the level of ingested fresh leucaena (see Table 1). The quantities of excreted DHP were higher in the two first micturitions that happened one hour and twenty minutes after consumption.

After the second urination DHP excretion was almost nothing.

CONCLUSIONS
DHP appeared in the urine of buffalo just after the ingestion of fresh leucaena (1h 20, after consumption) poisting out the very rapid metabolization of the mimosine.

Colorimetric method to detect the presence of DHP in the urine of ruminants showed efficacy and accuracy to detect mimosine latent toxicity in buffalo.

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Table 1
Partial and total micturition volumes, time of collecting and colorimetric reaction of buffaloes 'urine according to 3 different diets

		micturitions/reaction color			
Animal	Dieta	1st	2nd	3rd	Total (ml)
266	silage*	733-ml 8:35h yellow 93-ml	676-ml 10:30h dark yellow 68-ml	160-ml 13:55h light yellow 200-ml	1569
283	silage*	8:50h yellow	10:40h yellow	13:50h light yellow	361
270	10g leucena D.M./kg $L.W.^{0.75}$**	438-ml 8:35h light purple	300-ml 10:30h purple	145-ml 12:40h light purple	883
277	10g leucena D.M./kg $L.W.^{0.75}$**	450-ml 9:05h purple	508-ml 10:40h dark purple	380-ml 16:10h purple	1338
279	20g leucena D.M./kg $L.W.^{0.75}$**	166-ml 9:05h purple	137-ml 10:35h dark purple	135-ml 10:42h purple	438
288	20g leucena D.M./kg $L.W.^{0.75}$**	410-ml 9:55h purple	221-ml 13:10 dark purple	418-ml 13:30 dark purple	1049

* 2.5% of the L.W. in D.M. basis
** plus corn silage to lead the quantily up to 2.5% of L.W. in D.M. basis