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Influence of short-term *Leucaena leucocephala* feeding on milk yield and its composition, thyroid hormones, enzyme activity, and secretion of mimosine and its metabolites in milk of cattle

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SUMMARY

Four Karan/Friesian crossbred (Holstein × Tharparkar) dairy cows in late lactation (>200 days) were fed a basic diet of green maize and concentrates with an increasing proportion of *Leucaena leucocephala* (leucaena) leaf meal (LLM). The proportion was gradually increased from 0·25 of the dry matter intake (DMI) in the first week to 0·50 in the second, 0·75 in the third and *ad libitum* in the fourth week. Mimosine, 3,4-dihydroxy pyridine (3,4-DHP) and 2,3-dihydroxy pyridone (2,3-DHP) levels were determined in milk, serum, urine and faecal samples. On average DMI of leucaena was 0·023–0·025, 0·025–0·027, 0·027–0·028 and 0·022–0·025 of live weight (LW) during the first, second, third and fourth week, respectively. Mimosine, 3,4-DHP and 2,3-DHP appeared in the blood serum during leucaena feeding and continued appearing up to sixth week of experimental feeding even after the withdrawal of leucaena from the 34th day onwards. Similarly, excretion of mimosine, 3,4-DHP and 2,3-DHP were observed even after withdrawal of leucaena from the diet. The feeding of LLM resulted in a reduced level of T_3 (Triiodothyronine) and T_4 (Thyroxine) within a week of LLM feeding. The level of T_3 and T_4 improved to normal after withdrawal of LLM from the diet. The serum aspartate transferase (AST) and serum alanine transferase (ALT) activities were within the normal range. Leucaena feeding improved milk yield and composition only up to 3 weeks of feeding. The concentration of mimosine, 3,4-DHP and 2,3-DHP in milk was 0·33, 0·05 and 0·02 that of blood, respectively. The secretion of mimosine, 3,4-DHP and 2,3-DHP in the milk might be a concern for health of the offspring of leucaena-fed animals as well as human beings consuming such milk.

INTRODUCTION

Leucaena leucocephala (leucaena) is cheap (0·34 the price of green maize) and a good source of protein; therefore it is an economical feedstuff to use for milk production, also in addition, it is possible to produce 10–22 tonnes of edible dry matter (DM)/ha from leucaena (Hutton & Beattie 1976). Inclusion of leucaena in the diet of dairy animals, either as a pasture grass (Rai *et al*. 1994) or as seed (Talpada *et al*. 1994) in the concentrate mixture, improved (Flores *et al*. 1979) or sustained (Garcia *et al*. 1994, 1995; Garg & Kumar 1994; Richards *et al*. 1994) milk production with improved or similar composition (Flores *et al*. 1979; Garcia *et al*. 1994, 1995; Garg & Kumar 1994; Rai *et al*. 1994; Talpada *et al*. 1994).

The potential attributes of leucaena are severely limited because of the presence of toxic mimosine, β-(N-3-hydroxyl-4 (1H)-pyridone) α-amino propionic acid, a non-protein amino acid, and its immediate degradation product 3-hydroxy, 4 (1H)-pyridone 3,4-dihydroxy pyridine (3,4-DHP), which have depilatory effect and cause enlarged thyroid and several

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other toxic effects in ruminants as well as non-ruminants (Jones 1979; D'Mello 1992; Ram *et al.* 1994). No detrimental effects of mimosine on animal health or performance are observed when leucaena constitutes only a small proportion of the diet (Hamilton 1971). Gupta & Atreja (1998) did not observe any adverse effect on milk yield in goats fed gradually increasing levels of dietary leucaena DM (up to 750 g/kg). When leucaena was the sole component of the diet, a reduction in milk yield was reported that was attributed to either toxic effects or low nutrient intake; furthermore, these workers observed that neither mimosine nor DHP were secreted in goats' milk, thus rendering it safe for consumption. There is a paucity of information on secretion of mimosine or its metabolites, i.e. 3,4-DHP or 2,3-dihydroxy pyridone (2,3-DHP), through cows' milk; therefore the present study reports an attempt to elucidate the effect of feeding leucaena on the secretion of mimosine and dihydroxypyridone in milk, milk yield and composition, thyroid hormones and enzyme activities in lactating cattle.

MATERIALS AND METHODS

The experiment was conducted at the National Dairy Research Institute, Karnal, India. *L. leucocephala*, Hawaiian type K-8, was harvested from the institute farm, dried in the shade and the resultant leaf meal with twigs (LLM) used for feeding.

Experimental animals

Four Karan/Friesian crossbred (Holstein × Tharparkar) dairy cows in late lactation (>200 days), with mean LW of $400 \pm 15{\cdot}4$ kg, were fed a basic diet of green maize and concentrates with an increasing proportion of LLM which was gradually increased from 0·25 of the DM intake (DMI) in the first week to 0·50 in the second, 0·75 in the third and *ad libitum* in the fourth week. Concentrate mixture was offered at the time of milking. LLM was offered at 08.00 h and green maize was provided at 14.00 h. Fresh clean water was offered *ad libitum* twice daily. Daily records of DMI of LLM, concentrate mixture and green maize were maintained. LW were recorded weekly.

Collection of samples

Milk samples were collected for individual cows twice a week at the morning, noon and evening milkings. For each milking, the milk samples were collected separately and a suitable proportionate aliquot mixed. From each sample, 100 ml was kept for fat, solids not fat (SNF), total solids and ash estimation. From this, a small amount (15 ml) was centrifuged at 3000 rpm for 10 min to separate the fat and stored at −20 °C. In the morning, before offering meals, blood samples were drawn by jugular vein puncture and collected in vacutainer tubes. The serum was separated and stored at −20 °C for analysis. Twice a week, grab sampling of faeces was undertaken. The wet faeces were mixed with 0·1 N HCl and the volume was made so as to have a final concentration of 1 g faecal DM per 25 ml of 0·1 N HCl. Twice a week, urine samples were collected both in the morning and evening. To 75 ml of mixed urine sample, 5 ml 6 N HCl was added and stored until further analysis.

Chemical analysis

One gram DM of LLM or fresh faeces was extracted with 25 ml of 0·1 N HCl for 24 h and filtered through 0·22 μm filter paper (Lowry *et al.* 1983). Mimosine, 3,4-DHP and 2,3-DHP were estimated in Waters high performance liquid chromatography (HPLC) using 0·2% (w/v %) O-phosphoric acid as an eluent on a C_{18} microbondapack column (Tangendjaja & Wills 1980). Urine samples were analysed following the method of Tangendjaja & Wills (1980). The mimosine and DHP in serum were estimated following the method of Tangendjaja & Wills (1983). Milk samples were analysed in two different ways. Firstly, the whey serum samples were analysed and secondly, milk was defatted by centrifuging at 3000 rpm for 10 min. A sample of 3 ml defatted milk with equal volume of 10 N HCl was hydrolysed at 110 °C for 4 h under N_2 gas environment and pH was adjusted at 3·0 by adding sodium hydroxide (NaOH) solution. This was filtered through ordinary filter paper and finally through 0·22 μm mdi filter paper (Micro Devises Pvt. Ltd, India); 20 μl of the resulting filtrate was injected into HPLC to determine the mimosine and DHP. Milk fat was determined with the help of a Milco Tester (Rajasthan Electronics and Instruments Ltd, Jaipur, India). Corrected lactometer readings were taken into consideration to calculate SNF content following the standard procedure (ISI 1962). Microkjeldahl (AOAC 1984) was employed for total nitrogen determination and was multiplied by 6·38 to obtain the milk protein value. For total solids, 10 g of milk sample was taken in pre-weighed porcelain crucibles and kept at 80 °C in a hot air oven overnight. On the following day, the sample with crucible was weighed and total solids were estimated by difference. Dry samples, with crucible, were ashed at 550 °C in the muffle furnace for 3 h and total ash was estimated by difference. Lactose was calculated by subtracting fat, protein and ash from total solids.

The T_3 and T_4 in serum samples were determined by radioimmunoassay using RIAK4 and RIAK5 kits, respectively, supplied by the Board of Radiation and Isotope Technology (BRIT), Mumbai, India. AST

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Table 1. *Average daily DM and mimosine intake in lactating cows fed leucaena leaf meal (LLM)*

Period (days)	DM intake (kg) through (Mean ± S.E.) Maize fodder	LLM	Concentrate	Total	DMI (kg/100 kg body weight)	DM proportion through LLM	Mimosine intake (g/kg body weight)
1 to 3	6±0·5	2±0·0	2·3±0·0	10±0·6	2·51	0·18	0·08
4 to 7	5±0·2	2±0·0	2·4±0·0	9±0·4	2·27	0·20	0·08
8 to 10	4±0·2	4±0·0	2·4±0·0	10±0·2	2·47	0·37	0·15
11 to 14	5±0·1	4±0·0	2·4±0·0	12±0·3	2·67	0·34	0·15
15 to 17	3±0·3	5±0·0	2·4±0·0	11±0·4	2·76	0·48	0·23
18 to 21	3±0·2	6±0·3	2·4±0·0	11±0·4	2·74	0·51	0·24
22 to 24	3±0·6	4±0·7	1·6±0·3	9±0·5	2·18	0·48	0·18
25 to 28	5±0·5	3±0·1	2·4±0·0	10±0·4	2·48	0·25	0·11
29 to 33	6±0·2	2±0·1	2·4±0·0	11±0·4	2·65	0·23	0·10
34 to 36	8±0·9	Nil	2·4±0·0	11±1·2	2·65	Nil	Nil
37 to 39	8±0·2	Nil	2±0·0	10±1·1	2·60	Nil	Nil
40 to 43	8±0·3	Nil	2±0·0	11±1·0	2·63	Nil	Nil

As error values are on occasion very small their values were given as 0·0.

and ALT activities were determined with the help of SPAN Diagnostic Reagent Kits.

RESULTS

DM and mimosine intake

The LLM offered contained crude protein (CP) 196·0, ether extract (EE) 36·5, crude fibre (CF) 103·8, nitrogen free extract (NFE) 572·5, total ash 91·2 and mimosine 16·0 g/kg on a DM basis. The actual voluntary DMI through LLM feeding and mimosine intake is given in Table 1. During the last 10 days of LLM feeding both maize fodder and LLM were offered *ad libitum*. The initial consumption of mimosine (0·077 g/kg live body weight) increased to a maximum of 0·239 g/kg body weight during days 18–24. Subsequently, mimosine intake declined to 0·104 g/kg body weight when LLM was fed *ad libitum*. The concentrate mixture eaten was similar throughout except for days 22–24, when available concentrate was halved to induce an increase in voluntary LLM intake.

No external clinical symptoms were observed. Even average LW in the second (407±14·5 kg) and third (407±15·0 kg) week of the experiment were significantly higher than those in other weeks of the experiment (Table 2).

Table 2. *Weekly body weights of lactating cows (kg)*

Week	Animal number KF 5009	KF 5061	KF 5124	KF 5195	Average ± S.E.
0	380	430	430	360	400±15·4
1	382	428	431	365	402±14·3
2	390	435	435	368	407±14·5
3	390	436	435	366	407±15·0
4	385	432	428	364	402±14·4
5	378	427	428	364	399±14·3
6	381	428	428	366	401±13·4

Table 3. *Serum profile of mimosine and DHP in lactating cows (μg/ml)*

Week after leucaena feeding	Mimosine	3,4-DHP	2,3-DHP
1	25±5·7	318±35·4	70±19·1
2	28±3·8	992±142·2	491±18·5
3	47±8·2	12982±74	619±122·4
4	71±2·3	427 ±88·1	2716±355
5	28±7·0	155±27·1	1306±229
6	4±0. 7	9·44±0·81	14±12·2
7	ND	ND	ND

ND = not detectable.

Fate of mimosine and DHP

The mimosine, 3,4-DHP and 2,3-DHP concentrations differed significantly in the blood serum during different weeks of post-leucaena feeding (Table 3). Mimosine, 3,4-DHP and 2,3-DHP found during LLM feeding continued appearing in the serum up to the sixth week of experimental feeding, even after the withdrawal of leucaena from the 34th day onwards. Faecal concentrations of mimosine and 3,4-DHP fell sharply (Table 4) after 10 days on the diet while there

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Table 4. *Faecal excretion of mimosine and DHP in lactating cows (μg/g DM)*

Day post leucaena feeding	Mimosine	3,4-DHP	2,3-DHP
3	222±48·1	598±135·8	41±15·6
7	213±19·3	758±68·8	31±13·0
10	84±12·0	41±7·6	47±8·1
14	91±5·2	43±2·7	25±4·0
17	73±4·0	34±2·7	28±5·3
21	113±10·0	41±6·8	19±9·4
24	66±9·2	25±4·5	25±7·7
28	68±7·0	32±2·7	44±16·8
31	78±14·2	14±2·1	24±4·0
36	31±7·0	10±2·2	ND
39	12·5±0	6±0·5	ND
43	4±1·1	4±0·7	ND

ND = not detectable.

Table 5. *Urinary excretion of mimosine and DHP in lactating cows (μg/ml)*

Day post leucaena feeding	Mimosine	3,4-DHP	2,3-DHP
3	20±3·6	287±15·3	98±17·2
7	16±1·1	275±31·0	90±8·7
10	32±10·4	653±114·0	227±44·0
14	29±4·4	757±55·9	132±3·7
17	45±6·6	979±44·0	528±50·8
21	45±2·1	450±23·8	2126±366
24	43±2·2	312±29·9	2045±192
28	29±4·5	230±40·0	1582±178
31	25±1·9	247±14·3	1727±205
36	6·6±0·47	26±2·8	240±17·3
39	3·8±1·37	12±2·5	118±15·3
43	ND	2·2±0·63	7·4±0·91

ND = not detectable.

was no discernable trend in the changes of 2,3-DHP over time. After the withdrawal of LLM on the 34th day, both mimosine and 3,4-DHP continued to be excreted through faeces up to the 43rd day at appreciably lower concentrations than earlier. However, 2,3-DHP could not be detected in faeces when mimosine was not present in the feed.

The mimosine, 3,4-DHP and 2,3-DHP amounts excreted through urine when animals were on the leucaena-based diet are presented in Table 5. Even after withdrawal of leucaena from the diet on the 34th day of experimental feeding, mimosine was detected in the urine on the 39th day, but not on the 43rd day. Both 3,4-DHP and 2,3-DHP were detectable even up to the 43rd day, 8 days after withdrawal of mimosine.

Table 6. *Mimosine and DHP secretion through milk (μg/ml) in LLM-fed lactating cows*

Day after leucaena feeding	Mimosine	3,4-DHP	2,3-DHP
3	8·1±1·03	14±1·0	25±3·9
7	7·3±0·59	16±1·4	24±0·6
10	6·3±0·18	13±0·3	25±1·3
14	7·9±0·97	17±4·3	24±7·8
17	7·7±0·49	13±1·9	18±4·2
21	8·7±0·37	16±1·8	25±2·8
24	10±1·5	10±1·7	25±1·6
28	7·7±0·31	10±1·1	20±1·9
31	7·2±0·29	14±2·0	25±2·7
36	5·9±0·20	13±0·9	26±0·76
39	6·3±0·12	13±0·8	27±1·7
43	6·3±0·32	12±0·7	15±1·3
Average ± S.E.	7·4±0·33	14±0·6	23±1·0

Table 7. *Serum thyroid hormone profile (ng/ml) in leucaena-fed lactating cows*

Week post leucaena feeding	Tri-iodothyronine (T_3)	Thyroxine (T_4)
0	2·0±0·11	67±2·6
1	1·9±0·07	36±2·6
2	1·5±0·09	41±6·2
3	0·9±0·20	24±2·0
4	0·7±0·08	24±1·9
5	0·6±0·09	34±3·3
6	1·4±0·09	37±4·2
7	1·6±0·08	61±3·4

The defatted milk samples revealed no detectable amounts of mimosine, 3,4-DHP or 2,3-DHP. In the second procedure defatted milk samples were hydrolysed. The results showed that mimosine, 3,4-DHP and 2,3-DHP were secreted in the milk of dairy cows fed a leucaena-based diet even after 10 days of withdrawal of leucaena from their diet (Table 6). Serum enzyme and endocrinal analysis showed a drastic decline of T3 and T4 (Table 7) and a sharp rise of AST and ALT (Table 8) with leucaena feeding.

Influence of leucaena on milk yield and composition

Increase in milk yield was evident from the 5th day of leucaena feeding. The gradual increase was

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Table 8. *Serum enzymatic activities* (*units/ml*) *in leucaena-fed lactating cows*

Week post leucaena feeding	AST	ALT
0	20±0·5	17±0·4
1	29±2·9	26±1·4
2	29±0·8	28±2·5
3	34±4·9	28±2·5
4	37±4·2	31±2·1
5	37±2·5	39±2·4
6	45±5·0	31±2·2
7	52±4·7	34±1·4

Table 9. *Average milk and fat yield of leucaena-fed cows* (*kg*)

Day post leucaena feeding	Total milk	Fat corrected milk	Total fat
0	6±0·4	6±0·0	0·24±0·010
3	6±0·1	6±0·1	0·25±0·001
7	7±0·1	7±0·1	0·29±0·001
10	7±0·2	7±0·2	0·30±0·010
14	8±0·2	8±0·2	0·32±0·010
17	8±0·0	8±0·0	0·31±0·002
21	7±0·1	7±0·1	0·29±0·010
24	7±0·2	7±0·2	0·27±0·010
28	6·5±0·0	6·4±0·0	0·25±0·001
31	6·5±0·1	6·4±0·1	0·26±0·001
36	6·7±0·1	6·6±0·1	0·26±0·001
39	6·7±0·1	6·8±0·1	0·27±0·003
43	6·4±0·1	6·5±0·1	0·26±0·010

maintained until the 22nd day of feeding. The total milk yield as well as fat corrected milk (FCM) markedly differed on different days of milking after leucaena feeding (Table 9). After 24 days, the total milk yield tended to decline, hence immediately thereafter restriction of LLM feeding was lifted and *ad libitum* maize fodder was offered. The milk composition data are presented in Table 10. Higher total fat production was recorded on the 7th, 10th, 14th, 17th and 21st days of milking. The SNF and total solids percentage showed a rising trend from the 17th day onwards and increased protein content was noticeable from the 21st day onwards. The total ash content on all days was similar.

DISCUSSION

DMT on leucaena feeding

The voluntary DMI during 22–24th day of feeding the leucaena-based diet was less than the required level (Kearl 1982). Therefore, it was evident that as the animals were forced to increase their voluntary intake of leucaena they tended to decrease their total DMI, which might be a response towards toxic materials present in leucaena. However, in the present study none of the experimental animals showed any visible toxicity symptoms due to leucaena feeding. The retardation in voluntary DMI has been related to an increased proportion of leucaena in the diets of cattle (Blunt & Jones 1977; Ram *et al.* 1994), depending on the extent of biodegradation of mimosine and/or its metabolites (Hegarty *et al.* 1964; Ram *et al.* 1994; Feng & Atreja 1998; Gupta & Atreja 1998). Improvements in DMI (Table 1) were evident following withdrawal of leucaena from the animals' diets, thereby revealing that short-term negative effects observed due to leucaena feeding could be reversed by changing to a non leucaena-based diet without any adverse effect. During the last 10 days of LLM feeding (24th–33rd day of the experiment), animals were provided both LLM and maize fodder *ad libitum*. The voluntary intake of leucaena was found to be 0·23–0·25 of total DMI (Table 1). It seems that there is a mechanism within animals that allows them to adjust their intake. This hypothesis is supported by the findings of Jordan *et al.* (1995) who observed that, after 2 h of grazing on leucaena pastures, animals moved to non-leucaena pastures.

Mimosine and DHP in blood serum, faeces and urine

In ruminants, mimosine is degraded in the rumen (Hegarty *et al.* 1964; Ram *et al.* 1994; Samanta *et al.* 1994; Feng & Atreja 1998; Gupta & Atreja 1998), 3,4-DHP (Hegarty *et al.* 1979; Jones & Hegarty 1984; Gupta & Atreja 1998) and 2,3-DHP (Jones & Hegarty 1984; D'Mello, 1992) to various metabolites. Mimosine and its metabolites may be absorbed through the rumen wall, pass to the lower gut to be absorbed there, or excreted in the faeces. Unabsorbed mimosine may be metabolized in the caecum while some absorbed is also metabolized in the liver and it and metabolites may subsequently be excreted in urine or milk. As the experimental feeding of leucaena progressed, very high concentrations of 2,3-DHP were observed in serum of all cows (Table 3). Unabsorbed mimosine, 3,4-DHP and 2,3-DHP were excreted through faeces but, after day 7, the excretion of 2,3-DHP began to decline, suggesting improved absorption of these metabolites as LLM feeding continued. However, from a rough estimation of faecal output, total loss of mimosine and metabolites by the faecal route would be 0·01 or less of the total loss. Most of the toxin and its metabolites were eliminated in urine (Table 5).

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Table 10. *Average chemical milk composition (g/kg) of leucaena-fed lactating cows*

Day after leucaena feeding	Fat	SNF	Protein	Lactose	Total ash	Total solids
0	40±0·9	81±2·0	33±0	41±0·2	7·6±0·01	121±2·9
3	40±0·9	82±2·4	33±0	41±0·2	0·8±0·01	122±3·0
7	42±0·8	83±0·4	33±0	43±0·4	7·9±0·05	125±5·0
10	42±1·0	83±0·3	33±0	43±0·4	7·9±0·01	126±5·0
14	43±0·9	85±0·3	33±0	45±0·4	7·8±0·01	128±5·0
17	41±0·8	90±0·3	33±0	45±0·4	7·9±0·02	131±4·0
21	41±0·8	91±0·4	34±0	49±0·4	7·9±0·02	131±5·0
24	40±0·2	92±0·4	35±0	50±0·4	7·8±0·02	132±6·0
28	40±0·9	96±0·3	35±0	53±0·3	7·9±0·01	136±5·0
31	40±1·7	92±0·3	35±0	55±0·3	7·9±0·01	137±5·0
36	39±1·9	92±0·3	35±0	50±0·3	7·5±0·01	132±5·0
39	40±1·2	97±0·3	34±0	55±0·3	7·9±0·01	137±4·0
43	40±1·2	94±0·4	35±0	52±0·4	7·5±0·00	134±2·0

Mimosine and DHP in milk

Critically observing the data (Table 6) for mimosine and DHP secretion, it was found that, on average, 7±0·3 μg mimosine/ml, 14±0·6 μg 3,4-DHP/ml and 23±1·0 μg 2,3-DHP/ml were secreted through milk. Although the dietary intake of mimosine was variable, there was little difference in secretion of mimosine and its metabolic products in milk. This observation suggested that the mammary gland served as a regulatory organ for these toxins. All or most of the major milk constituents are known to be synthesized in the mammary glands from various precursors which are absorbed selectively from the blood. The mammary gland also exerts a selective filtering action on certain nutritional components. However, it seems to be freely permeable to the mimosine and its metabolites which are transferred directly from blood to the milk (McDonald *et al.* 1995). The selective absorption of mimosine and DHP also occurred in dairy cows in the present study, whereas this was not true in case of lactating goats. Although 0·01 or less of metabolites appeared in milk, the presence of such metabolites at all is of concern since it is not known to what extent these toxins might influence the health of consumers. The various toxins are known to influence different species of animals differently (Cheeke & Shull 1985; Cheeke 1997). Such milk should only be used for human consumption with caution.

Influence of mimosine and DHP on thyroid hormones

The marked reduction in T_3 and T_4 (Table 7) levels occurred from the first week onwards, which, on withdrawal of leucaena feeding, became normal on the seventh week of experimental feeding. The above effects of hypothyroidism, i.e. reduction in serum T_3 and/or T_4 levels, were because of leucaena feeding wherein mimosine is converted to 3,4-DHP and to 2,3-DHP. The latter two are known to have goitrogenic effects (Hegarty *et al.* 1979; Jones & Jones 1982; Jones & Hegarty 1984; Gupta *et al.* 1988; Senani *et al.* 1996; Haque *et al.* 1996).

Influence of mimosine and DHP on AST and ALT activities

AST and ALT concentrations give an indication of liver function in animals. In the present study, AST and ALT (Table 8) activity was within a normal physiological range, indicating no damage to the liver as increases of their activities have been reported to be associated with hepatic necrosis and other disease conditions related to histopathological changes (Benjamin 1985). Similar observations have also been recorded by other workers (Girdhar *et al.* 1991; Gupta & Atreja 1998).

Influence of leucaena on milk yield and its composition

The results for average milk and fat yield of leucaena-fed cows (Table 9) showed that beneficial effects in increasing milk yield and FCM were evident from the 7th–21st days of leucaena feeding (Flores *et al.* 1979; Rai *et al.* 1994). An increase was also observed in terms of SNF, protein, total solids (Rai *et al.* 1994) and fats which may be due to higher digestible protein and TDN values of leucaena (Singh & Mudgal 1967; Upadhayaya *et al.* 1974; Sobale *et al.* 1978; Deshmukh *et al.* 1983; Rodriguez & Borges 1989). The increase in fat might be due to increased acetate proportion in the leucaena-based diet (Gupta *et al.*

1986; Pathak *et al*. 1988; Samanta *et al*. 1994; Senani & Joshi 1995; Gupta & Atreja 1998) by enhancing supply of fat precursors in the mammary gland (McDonald *et al*. 1995). However, all these beneficial effects may be nullified if the recorded levels of mimosine, 3,4-DHP and 2,3-DHP have an untoward influence on the offspring of the animals or human health.

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