Iodine Supplementation of *Leucaena leucocephala* Diet for Goats. II. Effects on Blood Metabolites and Thyroid Hormones

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ABSTRACT : Twelve adult male goats, comprising of six castrated and six intacts, (2.5-3 years; 24.4±0.62 kg) were randomly but evenly divided into two groups (I_0 and I_{100}) and fed conventional concentrate mixture along with *Leucaena leucocephala* leaf meal (100 g/head approx.), the latter to supply 50 per cent of the crude protein (CP) requirements. The I_{100} group was provided with supplemental iodine as potassium iodide solution at 0.1 mg/day/animal. Wheat straw was provided *ad libitum* as sole source of roughage during the experimental period of 105 d. Blood samples were collected at the begining (0 d) and thereafter at 30, 60 and 90 d of experimental feeding. The study revealed that the serum glucose level was significantly higher ($p<0.01$) in I_{100} group as compared to I_0. Haemoglobin, packed cell volume and serum concentrations of total protein, albumin, globulin, calcium, inorganic phosphorus and alkaline phosphatase did not show significant differences as a result of iodine supplementation. Though the serum levels of triiodothyronine (T_3) were comparable between the two groups, that of thyroxine (T_4) increased significantly ($p<0.001$) in the I_{100} group. The T_3:T_4 ratio was also similar between both the groups. The study indicated that the adverse effect of *Leucaena* feeding on thyroid gland could possibly be alleviated by provision of extra iodine. However, this needs further confirmation using long duration studies. ***(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 6 : 791-796)***

Key Words : *Leucaena*, Iodine, Blood Metabolites, Thyroid Hormones, Goats

INTRODUCTION

Most of the tropical and sub-tropical countries suffer from acute seasonal shortage of animal feeds, especially of high protein forages. In recent years, *Leucaena leucocephala* has been propagated extensively in India, Malaysia, Philippines, Sri Lanka and other tropical countries to bridge the gap between protein demands and its supply (Ranjhan, 1998). *Leucaena* is a draught-resistant ever green leguminous forage rich in protein, minerals and β carotene (Akbar and Gupta, 1985). However, feeding of ruminants with *Leucaena* either as a sole diet or as a supplement to basal ration produces adverse effects depending upon the proportion of *Leucaena* in the diet, mimosine intake, duration of feeding and diet composition (Jones and Hegarty, 1984). The major toxic effects of *Leucaena* feeding is associated with enlarged thyroid gland (Kailas, 1991; Senani et al., 1994), reduced levels of thyroid hormones (Senani, 1992; Gupta and Atreja, 1998) and altered blood metabolites (Kailas, 1991); all attributed to the presence of free plant amino acid mimosine {β-[N--β hydroxy-4(1H)-pyridone] α amino propionic acid} and its rumen degradable product 3, 4 DHP {3 hydroxy 4(1H) pyridone} (Tangendjaja et al., 1983). A number of studies have indicated that mimosine degradation products in rumen act as potent goitrogens (Hegarty et al., 1979) thereby reducing the thyroxine (T_4) secretion rate (Gupta and Atreja, 1998). Iodine supplementation has been successfully tried to alleviate the toxic effects of goitrogenic feeds like cabbage, canola and soybean meal etc. More recently, studies with *Leucaena* have indicated an improvement in utilization and retention of nutrients by goats when supplemented with iodine (Pattanaik et al., 2000). Hence, the present study was taken to assess the impact of iodine supplementation to a *Leucaena* diet on the blood biochemical constitutents and circulating thyroid hormones in goats.

MATERIALS AND METHODS

The study was carried out in Animal Nutrition Division of Indian Veterinary Research Institute, Izatnagar between November, 1998 and February, 1999 representing peak winter season.

Animals and experimental design

Twelve healthy adult male goats (2.5-3 years; 24.4 ±0.62 kg body weight), comprising six each of castrated and intact animals, were randomly allocated equally into two groups: control (I_0) and experimental (I_{100}), so that each group consisted of three each of castrated and intact animals. The goats were housed in a well ventilated cement floored barn having individual feeding facilities. All the animals were given an anthelmintic (albendazole at 5 mg/kg body weight) before the onset of the experiment and kept under

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strict hygienic and uniform managemental conditions throughout the experimental period.

Feeds and feeding

All the goats were fed on a conventional concentrate mixture (crushed maize 50%, deoiled groundnut cake 25%, wheat bran 22%, calcium carbonate 1%, dicalcium phosphate 1% and common salt 1%) mixed with *Leucaena leucocephala* leaf meal (LLM), the latter to meet 50 per cent of their crude protein (CP) requirements (NRC, 1981). Weighed quantities of concentrate mixture (125 g) and LLM (100 g) were offered daily at 09:00 h and wheat straw was offered *ad libitum* as basal roughage at 14:00 h after ensuring complete consumption of the concentrates. The I_{100} group animals were given supplemental iodine (as potassium iodide solution) mixed with concentrate at the time of feeding at the rate of 0.1 mg/day/head. The animals were offered clean drinking water twice in a day.

Feed analysis

Proximate composition of concentrate supplement, LLM and wheat straw were determined as per standard procedure (AOAC, 1995). The mimosine content of LLM was estimated colorimetrically (Megarrity, 1978). Iodine content of feeds were assayed as described by Bedi (1999).

Blood collection and analysis

Blood from individual animals was collected by jugular venipuncture with and without anticoagulant (heparin) at 0, 30, 60, 90 days on the experimental diet. The uncoagulated blood was used for haematological assessment. viz., hemoglobin (Hb) and packed cell volume (PCV). The non heparinised blood was allowed to clot and the separated serum samples were decanted into autoclaved plastic vials and stored at -20℃ till analysis. Hb and PCV were estimated using standard procedures as described by Benjamin (1985) and Hawk (1965), respectively. Serum samples were analysed for glucose, total protein, albumin, globulin, cholesterol, urea, alkaline phosphatase, calcium and inorganic phosphorus as described by Pattanaik et al. (1999). Thyroid hormone status was assessed by estimating serum levels of triiodothyronine (T_3) and thyroxine (T_4) by radioimmuno assay (RIA) method (Bhandarkar and Pillai, 1982) using RIA kits (Bhabha Atomic Research Centre, Mumbai, India).

Statistical analysis

All data were subjected to two-way analysis of variance test (Snedecor and Cochran, 1989). Statistical significances between means were determined by the method of the new multiple range test of Duncan (1955) where the F value was significant at 5 per cent level.

RESULTS AND DISCUSSION

Chemical composition

The chemical composition of different feeds is given in table 1. The CP content of LLM was in the same range as reported by various workers (Haque et al., 1996; Mahanta et al., 1999). The mimosine content of LLM (1.45% on DMB) was in the range of 1.02 to 3.10 per cent reported by D'Mello and Fraser (1981). Iodine content of *Leucaena* was higher than the prior report of Jones et al. (1978) but apparently similar to the range of 0.46 to 0.81 mg/kg DM reported by Bedi (1999). The net iodine content of the control and supplemented diets are 0.228 and 0.407 mg/kg DM, respectively.

Blood metabolites

The values of mean Hb, PCV were within the normal range for goats (Keneko, 1989) without any significant ($p<0.05$) variation among treatment groups (table 2). Srivastava and Sharma (1998b) have also observed no impact of *Leucaena* feeding on hemoglobin content of goat blood. However, serum glucose concentration was significantly higher ($p<0.01$) in I_{100} than I_0 group. Moreover, the serum glucose concentration showed an increase with time being significantly higher ($p<0.01$) in the I_{100} group during days 30 and 60. This finding is supported by eariler observations showing that higher serum glucose concentrations are associated with iodine supplementation by Bedi et al. (2000) and Vsyakikh (1992). The lower ($p<0.01$) glucose concentration in the non-supplemented group was also registered in

Table 1. Chemical composition (percent DM basis) of dietary ingredients

Attriutes	Concentrate mixture*	*Leucaena leucocephala* leaf meal	Wheat straw
Organic matter	91.64	81.36	94.02
Crude protein	19.68	25.14	3.66
Ether extract	2.82	2.01	0.71
Total carbohydrate	69.14	54.21	89.59
Crude fiber	8.56	11.84	39.57
Nitrogen free exract	60.58	42.37	50.02
Total ash	8.36	18.64	5.98
Calcium	1.84	2.91	0.49
Phosphorus	1.10	0.63	0.36
Mimosine	-	1.45	-
Iodine (mg/kg DM)	0.13	0.86	0.12

* Contained maize 50%, deoiled groundnut cake 25%, wheat bran 22% and 1% each of dicalcium phosphate, calcium carbonate and common salt, on air dry basis.

Table 2. Influence of iodine supplementation on some haematological and serum metabolites in goats fed *leucaena* leaf meal

Treatment	Days post-feeding				Mean
	0	30	60	90	
Haemoglobin (g/dl)					
Control	9.75 ± 0.21	10.08 ± 0.15	9.33 ± 0.21	10.75 ± 0.28	9.98 ± 0.15
+Iodine	9.83 ± 0.21	10.08 ± 0.33	9.83 ± 0.25	10.75 ± 0.31	10.13 ± 0.15
Packed cell volume (%)					
Control	ND[+]	31.83 ± 0.60	34.67 ± 0.80	31.83 ± 0.91	32.78 ± 0.53
+Iodine	ND	34.83 ± 1.08	33.83 ± 0.87	34.67 ± 1.43	33.44 ± 0.63
Glucose (mg/dl)					
Control	47.51 ± 1.57	47.42 ± 1.39^{n}*	50.83 ± 1.18^{n}*	54.24 ± 1.37	50.00 ± 0.87^{n}**
+Iodine	47.83 ± 2.04	53.32 ± 1.30^{m}	55.41 ± 1.66^{m}	57.49 ± 3.19	53.38 ± 1.23^{m}
Cholesterol (mg/dl)					
Control	135.69 ± 12.20	113.65 ± 5.48	118.42 ± 3.38	141.65 ± 5.66	127.35 ± 4.24
+Iodine	135.30 ± 6.04	132.24 ± 5.24	124.52 ± 3.95	146.74 ± 4.25	134.57 ± 2.89
Serum Ca (mg/dl)					
Control	11.10 ± 0.70	10.90 ± 0.31	10.70 ± 0.22	10.40 ± 0.34	10.78 ± 0.21
+Iodine	11.47 ± 0.45	11.37 ± 0.32	10.97 ± 0.28	11.33 ± 0.49	11.28 ± 0.19
Inorganic P (mg/dl)					
Control	4.47 ± 0.31	6.37 ± 0.48	7.11 ± 0.41	5.73 ± 0.27	5.92 ± 0.27
+Iodine	4.92 ± 0.43	7.34 ± 0.53	7.40 ± 0.46	6.24 ± 0.30	6.47 ± 0.29
Alkaline phosphatase (IU)					
Control	337.5 ± 27.49	389.3 ± 24.14	385.0 ± 49.95	401.2 ± 56.59	378.0 ± 20.20
+Iodine	380.2 ± 34.67	343.4 ± 34.13	394.2 ± 36.18	383.4 ± 45.95	375.3 ± 20.36

^{m,n} Means bearing different superscripts in a column differ significantly * ($p<0.05$); ** ($p<0.01$).
[+] ND: Not determined.

Leucaena fed calves (Kailas, 1991). This hypoglycaemic effect of *Leucaena* might have been overcome by higher secretion rate of T_4 (table 3) arising from iodine supplementation. The concentration of cholesterol in serum was within the reported physiological range for caprine (Kaneko, 1989) and not influenced by iodine supplementation. Hypothyroidism has been correlated with hypercholestremia (Kaneko, 1989). However, in the present study no increase in serum cholesterol level was evident in the non-supplemented group possibly due to absence of pronounced hypothyroid condition as indicated by the normal T_3 and T_4 levels in the serum (table 3).

The concentration of calcium, inorganic phosphorus in the serum and alkaline phosphatase activity (table 2) did not vary significantly due to iodine supplementation. Previous studies have shown a positive influence of iodine on the calcium and phosphorus retention by calves (Koval'skii et al., 1972) and goats (Pattanaik et al., 2000). Moreover Schone et al. (1987) have also observed greater inorganic phosphorus in serum of pigs because of iodine supplementation of mustard cake based diet. But the impact of iodine was not evident in the present investigation. This could be attributed to the homeostatic mechanism involved in maintenance of serum calcium and inorganic phosphorus level through bone reserves and possibly, the experimental duration of 90 d was not long enough to disturb the mechanism.

Serum concentrations of total protein, albumin, globulin and their ratio did not differ significantly between the two groups (table 3) and were within the normal physiological range reported for goats (Kaneko, 1989). However, the albumin concentration was significantly ($p<0.01$) higher in the iodine supplemented group at 90 d post feeding. Likewise, serum urea levels did not differ significantly between treatment groups similar to the findings of Srivastava and Sharma (1998a, b) in goats fed graded levels of *Leucaena*. However, this was contrary to the observation of Wizol et al. (1999), who have reported an increase in serum urea level in milch cows given supplemental iodine.

Thyroid hormones

The results of serum concentration of total triiodothyronine (T_3) and thyroxine (T_4) are presented in table 3. There was no significant difference in serum T_3 levels between iodine supplemented and non-supplemented group. On the other hand, iodine supplementation brought a significant ($p<0.01$) increase in the level of T_4 in the serum (47.68 ± 1.83 vs 40.85 ± 2.24 ng/ml). The iodine supplemented group had significantly ($p<0.05$) higher serum T_4 concentrations

Table 3. Influence of iodine supplementation on blood serum proteins and thyroid hormones of goats fed *leucaena* leaf meal

Treatment	Days post-feeding				Mean
	0	30	60	90	
Total Protein (g/dl)					
Control	7.71 ± 0.48	8.90 ± 0.33	9.50 ± 0.47	10.21 ± 0.21	9.08 ± 0.26
+Iodine	8.07 ± 0.27	9.00 ± 0.34	9.71 ± 0.33	10.20 ± 0.32	9.25 ± 0.22
Albumin (g/dl)					
Control	4.18 ± 0.23	4.32 ± 0.13	4.76 ± 0.19	4.66 ± 0.35^{m}*	4.56 ± 0.15
+Iodine	4.22 ± 0.04	4.49 ± 0.24	4.38 ± 0.13	5.56 ± 0.11^{n}	4.66 ± 0.13
Globulin (g/dl)					
Control	3.53 ± 0.44	4.58 ± 0.34	4.74 ± 0.54	5.55 ± 0.36	4.60 ± 0.25
+Iodine	3.85 ± 0.26	4.52 ± 0.25	5.33 ± 0.42	4.65 ± 0.35	4.59 ± 0.19
Albumin:globulin ratio					
Control	1.28 ± 0.17	0.98 ± 0.09	1.12 ± 0.20	0.87 ± 0.11	1.06 ± 0.08
+Iodine	1.12 ± 0.08	1.01 ± 0.08	0.85 ± 0.08	1.24 ± 0.011	1.06 ± 0.05
Urea (mg/dl)					
Control	25.47 ± 3.43	37.95 ± 2.47	29.85 ± 2.05	33.33 ± 3.46	31.65 ± 1.66
+Iodine	33.66 ± 3.85	33.13 ± 3.37	27.77 ± 4.31	32.24 ± 3.50	31.71 ± 1.82
Triiodothyronine (T_3; ng/ ml)					
Control	0.51 ± 0.06	1.14 ± 0.23	1.37 ± 0.11	1.33 ± 0.14	1.09 ± 0.10
+Iodine	0.87 ± 0.12	0.95 ± 0.09	1.27 ± 0.13	1.06 ± 0.08	1.04 ± 0.06
Thyroxine (T4; ng/ml)					
Control	31.63 ± 3.32^{n}*	44.51 ± 1.44^{n}*	54.54 ± 2.06	32.73 ± 2.09	40.85 ± 2.24^{n}**
+Iodine	46.32 ± 3.52^{m}	51.52 ± 2.30^{m}	54.34 ± 2.41	38.55 ± 3.05	47.68 ± 1.83^{m}
T_3:T_4 ratio					
Control	64.62 ± 6.78	46.39 ± 8.07	40.91 ± 3.30	25.71 ± 2.59	44.41 ± 3.93
+Iodine	55.98 ± 3.79	56.27 ± 4.20	45.60 ± 5.93	36.91 ± 2.90	48.67 ± 2.63

^{m,n} Means bearing different superscripts in a column differ significantly * ($p<0.05$); ** ($p<0.01$).

at days 0 and 30. While no plausible explanation could be given for the higher T_4 value at 0 day, it could possibly related to the prior nutrition of the experimental goats. Of the two thyroid hormones, thyroxine represents quantitatively the main form and triiodothyronine, the most active form; and most, but not all, of the physiological effects attributable to thyroid hormones are attributable to T_3 (Bernal, 1980). T_4 is converted to T_3 in kidney (Chiraseveenuprapund et al., 1978) and liver (Balsam et al., 1981) which is inhibited only in extreme cases of iodine deficiency and/or goitrogen feeding because of the depletion of T_4 reserve. Barry et al. (1983) have, therefore, suggested that serum T_3 concentration is better related to animal productivity than T_4. Considering the near similar serum T_3 concentration in the control and supplemental groups found in the present study, it appears that the dietary level of *Leucaena* and/or experimental duration were not sufficient to induce severe iodine deficiency. However, there exists reports that there is preferential production of the less-iodinated thyroid hormone i.e., T_3 at the expense of the more-iodinated T_4 during the early stages of iodine deficiency (Stanbury and Pinchera, 1994). The present findings of similar T_3 levels accompanying a lower T_4 in non-supplemented animals could be attributable to such a compensatory mechanism. The higher level of T_4 in the supplemented group, therefore, appears to indicate the possible beneficial effects of provision of extra iodine to *Leucaena* diet. Similar to the present findings, Barry et al. (1983) have also observed elevated serum T_4 concentrations in lambs fed on kale (*Brassica oleracea*) with supplementary iodine with no significant increase in the serum T_3 level. The serum T_3 concentration showed an increasing trend from 0 to 90 d post feeding implying that the quantum of mimosine/DHP available at tissue level might have been insufficient to inhibit iodination of tyrosine and acted as a stimulus instead, as have been suggested by Haque et al. (1996). They observed an increase in serum T_3 levels of goats, fed *Leucaena* at a level of 50% CP of total ration, upto 135 d post-feeding before declining. Senani (1992) has also observed a similar pattern of seum T_3 level upon long term feeding of *Leucaena* in goats which, infact, could be attributed to the compensatory mechanism by way of increase in the number of cells in the thyroid gland (Bahri et al., 1982).

Goitrogens are of equal or greater importance than low levels of iodine in feeds as contributing factor in iodine deficiency (Underwood, 1981). Since DHP is a potent goitrogen, there may be an increase in the iodine requirement. In the present study, the elevated serum T_4 level in the supplemented group, though not conclusive, could have resulted from a higher availability of iodine at the follicular level of the thyroid gland. However, this need further confirmation using long duration studies in goats.

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