

## STUDIES ON THE PREVALENCE AND DIAGNOSIS OF SUBCLINICAL IODINE DEFICIENCY IN BUFFALOES (*Bubalus bubalis*)

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### ABSTRACT

A baseline survey for iodine status was conducted by measuring plasma inorganic iodine (PII) levels in 73 buffaloes from 67 dairy units located in the districts of Ludhiana, Jalandhar, Ferozepur and Hoshiarpur of Punjab. Concentrations of triiodothyronine ( $T_3$ ), thyroxine ( $T_4$ ), total cholesterol and free fatty acids (FFA) were compared between iodine deficient and normal buffaloes. Circulating  $T_4$  and  $T_3$  levels were determined before and after injection of ethiodised oil in animals with low PII levels. The prevalence rate of low iodine status was recorded to be 38.4 percent and varied between 26.3 to 50.0 percent among three age groups and 29.0 to 60.0 percent in different districts. Basal thyroxine ( $T_4$ ) and triiodothyronine ( $T_3$ ),  $T_4:T_3$  ratio, total cholesterol and free fatty acids were not significantly affected by low iodine status. However, the activity of circulating  $T_4$ ,  $T_4:T_3$  ratio increased and that of  $T_3$  decreased following iodine supplementation, thus suggesting that subclinical deficiency is prevalent. It may also be concluded from this study that elevation in  $T_4$  and  $T_4:T_3$  ratio in response to iodine supplementation is a more sensitive diagnostic test of iodine deficiency than a single basal  $T_4$  and  $T_3$  assay.

**Keywords:** iodine deficiency, buffaloes, plasma inorganic iodine, thyroxine, triiodothyronine, iodized oil, iodine injection

### INTRODUCTION

Iodine exerts its physiological role as a component of thyroid hormones which exert global effects on body metabolism by virtue of control of cellular energy exchange, basal metabolic rate, tissue growth, reproduction and lactation. Recent figures from the surveys by the Government of India and state governments show that 82 percent of the districts surveyed (197/239) are endemic for iodine deficiency in the human population and no single state in the country is free from iodine deficiency disorders in people (Pandav and Anand, 1997). However, iodine deficiency disorders in animals have yet to receive the same attention as that of human beings. It is believed that due to ecology of iodine, the geographic pattern of dietary iodine availability to farm animals is similar to that of the human population. Based on this assumption, about 100 million animals (cattle, buffaloes, sheep and goats) have been estimated in iodine deficient zones of India (Bedi, 1997). This has also been reflected to some extent by few observations (Rajkumar,

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1970; Raina and Pachauri, 1984) in small ruminants of these areas. In spite of this, no survey on iodine status has been done to determine iodine status of livestock, probably because clear signs are not commonly seen and marginal deficiency may be prevalent. However, reports are there that productivity is inevitably reduced in all animals even with marginal deficiency that does not cause clinical signs (Knights *et al.*, 1979; Salakhutdinov, 1985; Sedina, 1987). Thus, it seemed necessary to determine iodine status and its effect on livestock health. Therefore, the objective of the present study was to assess iodine status of dairy buffaloes in Punjab state and to investigate the role of iodine injection in the control and prevention of iodine deficiency.

## MATERIALS AND METHODS

### Animals

Seventy-three buffaloes were selected randomly from 371 buffaloes in 67 dairy units located in four districts (Ludhiana, Jalandhar, Ferozepur, Hoshiarpur) of Punjab. They were classed by age (Table 1) as heifers (1 to 3 years), young buffaloes (3 to 6 years) and old buffaloes (>6 years). No iodophors or iodine-containing compounds for teat dipping or udder sanitation were used at any of these dairy units.

### Plasma collection

Blood was collected by venipuncture in to acid washed heparinised vials during the months of July to September 1997. Plasma was separated by centrifugation and stored at -10°C for analysis within a month.

### Biochemical assay

*Plasma inorganic iodine (PII)*: Since PII is very sensitive to iodine intake in ruminants (Rogers,

1992), it was employed to assess iodine status. The concentration was measured by a chromatographic and colorimetric technique (Aumont and Tressol, 1987). On the basis of their PII values, the animals were classified as normal (>105 µg/L), marginal (51 to 104 µg/L) and low (<51 µg/L) (Rogers, 1992).

*Cholesterol*: Total plasma cholesterol was measured colorimetrically (Zak, 1957).

*Free Fatty acids (FFA)*: The concentration of FFA was determined by the method of Lowry and Tinsley (1976).

*Thyroxine (T<sub>4</sub>)*: Activity was measured by radio-immunoassay using kits from Bhaba Atomic Centre (Radio-pharmaceuticals Operation Board of Radiation and Isotope Technology, BARC's Vashi Complex, Navi Mumbai, India). Duplicate tubes of T<sub>4</sub> assay for blank, zero standard and four standard concentrations (3.22, 6.44, 12.80 and 25.70 nmol/L) and single tubes for two controls were prepared according to instructions provided with the kit. Single tubes were prepared for the samples by adding 100 µL each of diluted plasma (1:10 with assay buffer), radio-labelled T<sub>4</sub>, T<sub>4</sub> antiserum and assay buffer. Polyethylene glycol solution (1 ml) was added after incubation (at 37°C for 30 minutes) to all tubes except the blank and were centrifuged at 2000 g for 20 minutes. Radioactivity of the precipitate was measured as counts per minute using a gamma counter (model LB2105; Berthold, Germany).

*Triiodothyronine (T<sub>3</sub>)*: Activity was measured by a radio-immunoassay (kit from Bhaba Atomic Research Centre). Duplicate tubes of T<sub>3</sub> assay for blank, zero standard, five standard concentrations (0.23, 0.46, 0.92, 1.84, 3.68 nmol/L) and two controls were prepared according to instructions. Single tubes were prepared for samples by adding 50 µL of plasma sample and

100 µL each of radio-labelled T<sub>3</sub>, T<sub>3</sub> antiserum and 300 µL of assay buffer and incubated at 37°C for 45 minutes after mixing. The rest of the procedure was similar to the T<sub>4</sub> assay. All the samples were run in a single assay. The intra- assay variation was 5.90% for T<sub>3</sub> and 5.01% for T<sub>4</sub>.

#### **Experimental Design**

A baseline survey was conducted to determine the PII concentration of all the animals. Concentration of plasma thyroxine (T<sub>4</sub>), triiodothyronine (T<sub>3</sub>), cholesterol and FFA were measured to assess their usefulness in the diagnosis of iodine deficiency. Plasma samples from 15 buffaloes with PII levels below 105 µg/L were compared with 15 animals with PII levels above 105 µg/L.

Since the herdsmen had not observed perinatal weakness, stillbirths or neonatal goitre in any of the herds, the low PII values in some animals suggested that a non-clinical deficiency might be prevalent (Rogers, 1992). Therefore, the effect of parenteral iodine supplementation on thyroid activity was examined in five iodine deficient cows. A single subcutaneous injection of 1 mL of 78% ethiodised oil was given to each of the cows. Levels of T<sub>4</sub> and T<sub>3</sub> were assayed before and 70 days after injection.

### **RESULTS AND DISCUSSION**

Measurement of PII in this study showed that iodine intake was low in buffaloes of Ludhiana, Jalandhar, Ferozepur and Hoshiarpur districts of Punjab. The mean ( $\pm$ SEM) PII value of the sampled buffaloes from four districts of Punjab was  $222.6 \pm 24.4$  µg/L (range 10.5 - 780 µg/L). Marginally low ( $83.9 \pm 3.61$  µg/L) and low ( $34.1 \pm 3.19$  µg/L) PII levels were recorded equally in 19.2 percent of the

buffaloes (Table 1). In relation to age, prevalence rate was 27.8, 50.0 and 36.3 percent in heifers, young buffaloes (3- 6 years) and mature buffaloes (> 6 years), respectively (Table 1). Low PII levels were observed in 29, 47, 33.3 and 60 percent buffaloes of Ludhiana, Jalandhar, Ferozepur and Hoshiarpur districts. The district wise distribution of 24 dairy herds that had low PII levels was 8 of 36 in Ludhiana, 7 of 14 in Jalandhar, 4 of 11 in Ferozepur and 5 of 6 in Hoshiarpur.

However, Chhabra (2006) recorded lower mean PII levels of 43.4 and 49.1 mg/ml during summer and winter seasons, respectively, in buffaloes from Amritsar, Ludhiana, and Patiala districts. The prevalence rate was also higher (84.5%) compared to the present study. The difference might be due to soil type and area of study. Similar baseline surveys in sub-mountainous regions (Singh *et al.*, 2006) and districts of Ludhiana, Hoshiarpur, Jalandhar and Ferozepur (Randhawa and Randhawa, 2001) in cows demonstrated low iodine intake in 48.8% and 38.4% of crossbred cattle.

Ramakrishna and Prasad (1991) found that 41.5 percent of goat thyroids from Barielly abattoirs had goitrous lesions. Occurrence of visible goitre varied between 0.5-5.5 percent at a goat farm at Laxmipur (Rajkumar, 1970) and 0.16-5.66 percent in Terai region at Uttar Pradesh. Indirect evidence supporting our results is also provided by the low soil iodine content of the region and the occurrence of endemic goitre (assessed by thyroid palpation) in 11.6 and 46.7 percent school children from two different areas of Ludhiana district (Jain, 1990).

Perinatal weakness and stillbirths were observed only in only one of the sampled dairy herds. However, non-specific clinical signs viz. anoestrus, suboestrus and prolonged postpartum anoestrus were observed in one of eight iodine deficient herds in Ludhiana, two of seven in Jalandhar, two

of four in Ferozepur and two of five in Hoshiarpur. Our findings on few characteristic clinical signs in iodine deficient buffaloes are supported by those of McCoy *et al.* (1997) who also demonstrated that dietary iodine deficiency (4-5 months) sufficient to produce clinical and pathological changes in thyroids of pregnant cattle may still allow normal births.

#### **Plasma thyroid hormones, cholesterol and free fatty acids in iodine deficient buffaloes**

The mean PII level of iodine deficient cows was  $59.3 \pm 5.40$   $\mu\text{g/L}$ , which was well below the value considered normal ( $105$   $\mu\text{g/L}$ ) by Rogers (1992) and was also lower to  $297.0$   $\mu\text{g/L}$  in normal group. Concentrations of mean  $T_3$ ,  $T_4$ , total cholesterol. A fall in plasma FFA levels showed that fat mobilisation was lower in buffaloes with low PII levels (Table 2). Non-observance of low  $T_3$  and  $T_4$  activities in cows having low PII values in this study concurred with the suggestion of Underwood (1981) that serum  $T_3$  and  $T_4$  are poorly related to thyroidal activity in domestic animals as had also been confirmed by Rogers (1992) in cows. In contrast, Baysu and Dundar (1984) measured lower iodothyronines concentrations in cattle having low milk production and fertility problems in areas where goitre was endemic in humans than those from non-goitrous areas. Pichaicharnarong *et al.* (1982), however, did not monitor clinical sign or iodine status of Swamp buffaloes, but recorded low  $T_3$  ( $2.4 \pm 0.07$  vs.  $2.20 \pm 0.08$  nmol/L) and  $T_4$  activities ( $98.71 \pm 3.94$  vs.  $57.40 \pm 3.47$  nmol/L) in human goitre endemic areas in comparison to non-goitrous areas in Thailand.

Diagnosis of severe iodine deficiency may be easily done from the clinical signs of goitre, perinatal weakness, hairlessness and stillbirths in neonates. However, less severe iodine deficiency

is more difficult to diagnose (McDowell, 1992). Various indicators that have been used to assess iodine deficiency in cattle including thyroid function tests ( $T_3$  and  $T_4$ ), PBI, MI and total serum iodine (Allcroft *et al.*, 1954; Puls, 1981), all have limitations.

Andrewartha *et al.* (1980) also concluded from their studies on iodine deficient sheep that single thyroxine assay provided little information on iodine status. Rogers (1992) examined several tests on cattle blood. He found that total iodine; protein bound iodine and thyroid hormones were difficult or impossible to interpret, and advocated the use of MI or PII. Aumont and Tressol (1987) had also shown that direct determination of PII was useful in diagnosis of dietary iodine imbalance.

#### **Effect of iodine supplementation on thyroid activity of iodine deficient buffaloes**

Since specific clinical signs of iodine deficiency were not apparent and the activities of thyroid hormone were not lower in the buffaloes in spite of low PII values, it appeared that deficiency might be non-clinical. However, after subcutaneous injection of ethiodised oil, there was a large increase ( $P < 0.01$ ) in mean PII concentration and elevation of  $T_4$  ( $P < 0.005$ ) for more than 70 days post injection (Table 3). Simultaneously, plasma  $T_3$  concentration declined ( $P < 0.05$ ) and  $T_4:T_3$  ratio increased ( $P < 0.005$ ). These alterations suggested that thyroid hormonogenesis improved following iodine supplementation.

Walton and Humphrey (1979) have also recorded similar increase in plasma thyroxine level in clinically iodine deficient sheep (premature births, stillbirths and neonatal mortality) treated with iodised oil. Low  $T_4$  levels,  $T_4:T_3$  ratio and a remarkable production of  $T_3$  had also been observed by Morinaga *et al.* (1990) in iodine-deficient

Table 1. Prevalence of iodine deficiency in buffaloes in Punjab.

<b>PII class (µg/L)</b>	<b>Heifers (n=18)</b>	<b>Young buffaloes (n=22)</b>	<b>Old buffaloes (n=33)</b>	<b>Total (n=73)</b>	<b>Percent of total buffaloes</b>
Normal (105)	13	11	21	45	61.6
Marginal (51-105)	3	4	7	14	19.2
Low (>51)	2	7	5	14	19.2
% deficient (<105)	27.8	50.0	36.3		38.4

Table 2. Effect of plasma iodine concentration on biochemical constituents involved in thyroid dysfunction in buffaloes.

<b>Class of buffaloes</b>	<b>PII (µg/L)</b>	<b>T<sub>3</sub> (nmol/L)</b>	<b>T<sub>4</sub> (nmol/L)</b>	<b>Cholesterol (nmol/L)</b>	<b>FFA (mg/L)</b>
Normal	297.0 <sup>a</sup>	1.73 <sup>a</sup>	37.9 <sup>a</sup>	1.88 <sup>a</sup>	52.9 <sup>a</sup>
Deficient	59.3 <sup>b</sup>	1.55 <sup>a</sup>	39.6 <sup>a</sup>	1.85 <sup>a</sup>	42.7 <sup>b</sup>

Means with different superscripts in a column differ significantly at P < 0.05.

Table 3. Effect of iodised oil administration on PII and thyroidal activity of iodine deficient buffaloes (n=5).

<b>Variable</b>	<b>Day after injection</b>	
	<b>0</b>	<b>70</b>
PII(µg/L)	66.6 ± 18.0	285.2 ± 20.5**
T <sub>4</sub> (nmol/L)	39.1 ± 1.28	63.3 ± 6.45***
T <sub>3</sub> (nmol/L)	1.18 ± 0.15	0.72 ± 0.11*
T <sub>4</sub> : T <sub>3</sub>	35.2 ± 4.82	92.1 ± 7.94***

\*P<0.05

\*\*P<0.01

\*\*\*P<0.005

goitre-affected calves. Therefore, improved thyroid hormonogenesis in response to treatment in our buffaloes showing low PII values suggest that sub-clinical deficiency may be prevalent, which, however, needs to be further confirmed by monitoring the effect on production.

The data in Table 3 further suggested that increase in PII and plasma  $T_4$  concentration,  $T_4:T_3$  ratio persisted for more than 70 days after iodine injection. It may, therefore, be inferred that 780 mg of I as ethiodised oil is a useful long-term iodine supplement for treatment and prevention of subclinical iodine deficiency in buffaloes. High milk iodine concentrations after iodised oil (400 mg of I) have been observed in ewes of goitre-affected flocks for more than two consecutive pregnancies (Statham and Koen, 1982; Azuolas and Caple, 1984). The results also revealed that thyroid provocation by iodine supplementation was more sensitive diagnostic test of low iodine intake than single basal  $T_4$  and  $T_3$  assay.

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