Iodine deficiency in dairy cattle

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ABSTRACT

Clinical signs of iodine deficiency (*e.g*. non-visible oestrus, low first service conception rate, stillbirths), plus low (<45 nmol/L) serum thyroxine (T4) concentrations were observed in dairy cows from 12 farms in the Manawatu.

Goitre, as determined by diffuse thyroid hyperplasia, plus significantly ($p<0.05$) increased thyroid size, weight and thyroid:bodyweight ratio, was found in 16% (6 of 38) of stillborn calves from these farms. Goitre was present in calves from 42% (5 of 12) of the herds. One of these farms was chosen to investigate temporal changes in concentrations of the thyroid hormones, thyroxine (T4) and tri-iodothyronine (T3), as an indicator of iodine status, and to attempt to determine the optimum time for sampling. Blood samples were collected from a cohort of animals from the herd (20 representative animals from 400 cows) 8 times over a 12 month period, for measurement of total serum iodine (TSI), T3 and T4 concentrations in both individual cows and pooled (10 cows) samples. T4 was maximal in June (58.6 nmol/L) and lowest (39.2 nmol/L) in October ($P<0.05$). Between November and Augusts mean TSI varied (NS) from 31 ± 9 to 47 ± 9 μg/L, but was significantly ($P<0.01$) higher in October (91 ± 16 μg/L). T4 concentrations were ($P<0.05$) related ($r = 0.35$) to TSI between November and August (7/8 blood samplings), but were widely divergent ($r = 0.08$) in October. Concentrations of T3 were unrelated to TSI ($r = 0.07$). In pooled samples, concentrations of TSI, but not T3 or T4, were closely related to the mean concentrations in individual animals.

An iodine-response study was also carried out on the same herd. Half the herd (200 of 400) were given (6 mL i/m) iodised oil (Flexidine, Bomac) before mating and before calving. The pregnancy rate in anoestrous cows treated with iodine was 13.9% higher than in untreated cows.

It was concluded that T4 (individual animals) or TSI (pools of 10 cows) give equivalent information about the iodine status of a herd, except in the post calving period. However, the reference range needs to be validated by further response trials.

Keywords: Iodine deficiency; thyroxine, cattle; stillborn calves; goitre.

INTRODUCTION

Iodine is an essential micronutrient that has a critical function in metabolism and general health, but whose significance is often overlooked in livestock production systems. The primary function of iodine is in the synthesis of the thyroid hormones (Grodsky, 1979) thyroxine (tetra-iodothyronine: T4) and tri-iodothyronine (T3). Thyroxine is virtually inactive, but is converted in the tissues to the active hormone T3, which controls energy metabolism and protein synthesis in cells (Underwood & Suttle, 1999).

The majority of evidence indicates that the physiological state and metabolic status of the cow are the most important factors to affect the synthesis and concentrations of thyroid hormones. There is much evidence to show that T4 and/or T3 concentrations are lowest near parturition (Mixner *et al.,* 1962; Kesler *et al*., 1981; Refsal *et al*., 1984; Pezzi *et al.,* 2003), probably as a consequence of the metabolic changes and initiation of lactation which occurs at that time.

Thyroid hormone concentrations in cattle are affected by many environmental factors; including temperature (Magdub *et al*., 1982), botanical composition of pasture (Grace & Waghorn, 2004), selenium status (Arthur *et al.,* 1991; Wichtel, 1998) and the presence of goitrogens in the diet (Kearney, 2004). Diet can alter thyroid hormone concentrations via effects mediated by growth hormone releasing factor, somatotrophin (Kahl *et al.*, 1995); fat or starch contents (Blum *et al*., 2000). Likewise, liver damage and concurrent disease can also affect thyroid hormone concentrations (Kahl *et al.,* 2000).

Measuring thyroxine concentrations is widely used as a proxy for the iodine status of cattle. However, because the production of thyroxine is under homeostatic regulation, and because of the recycling and storage of iodine within the thyroid gland, it has been suggested that this measure may not be satisfactory (Grace *et al*., 1996). It is also difficult to interpret T4 concentrations, since the reference range that is used in New Zealand is based on the range found in apparently normal

animals, rather than on iodine-response studies. Wichtel (1995) considered that this situation is not ideal for the interpretation of field samples.

Iodine deficiency was first recognised as a problem of livestock in New Zealand by Gilruth in 1901 (Sinclair & Andrews, 1954), as a cause of goitre in calves and lambs. Likewise the intake of the human population of New Zealand is generally deficient (Skeaff *et al.*, 2003). Iodine deficiency is currently recognised as being highly prevalent in cattle populations, and affecting many aspects of production, including reproduction (oestrous cycles, conception rate and stillbirths), peri-natal calf mortality and ill-thrift in growing animals (Underwood & Suttle, 1999; McDowell, 2003). Animals displaying such signs of impaired production and reproductive performance were observed by the authors between 2002 and 2004 in the Manawatu region of New Zealand; measurement of low serum T4 concentrations (<45 nmol/L) or amelioration of symptoms after administration of iodine was taken as a preliminary indication that iodine deficiency might be present.

The present studies were undertaken in order to:

i) investigate evidence for iodine deficiency affecting cows in the Manawatu region of New Zealand,
ii) evaluate the value of T4 measurements as an indicator of iodine deficiency and,
iii) determine whether iodine supplementation could improve aspects of animals' productivity.

MATERIALS AND METHODS

Study 1: Iodine deficiency in stillborn calves

During the calving periods of 2003 and 2004, 44 stillborn calves were collected from 12 herds in the Manawatu district. These were from herds suspected of being iodine deficient from a previous history of low serum thyroxine concentrations, neonates with goitre, stillbirths, or non-cycling cows that could not be explained with reference to overall plane of nutrition. Following weighing and examination of each stillborn, the thyroid was removed, weighed and its principal dimensions measured. Samples of thyroid tissue were collected for histological determination of the presence of goitre. Blood and tissue were collected for measurements of thyroid hormone concentration and iodine content. Blood samples were also collected from at least 10 cows in each herd for serum thyroxine analysis.

Study 2: Temporal changes

The study was carried out on a 400-cow, spring-calving dairy farm in the Manawatu region. This herd was selected for the study as:

i) low T4 concentrations of <45 nmol/L had been found in 90% of individuals from a random sample of cows in the herd and,
ii) goitre had been diagnosed in stillborn calves.

A group of 20 representative cows were selected (matched for age, date of calving, days in milk and production of milk solids). Blood samples were collected from these cows 8 times (Nov, Dec, Jan, Feb, May, June, Aug and Oct) for T3, T4 and total serum iodine (TSI) concentrations. Copper and selenium concentrations were monitored to ensure normal status. Condition scores were assessed and cows were weighed on each occasion. Pasture samples were collected for iodine determination in May, June and October.

Study 3: Effect of iodine supplementation on reproduction

An iodine-response study was also carried out on the same herd used in Study 2. Half the herd (200 of 400) were given (6 mL i/m) iodised oil (Flexidine, Bomac) on 2 occasions; 19th October 04 (*i.e.* pre-mating) and 28th July 05 (*i.e.* pre-calving).

The following data were recorded:

i) calving to first service interval and conception rate to first service,
ii) the proportion of animals that remained anoestrous by 15th November (only cows that had calved at least 45 days were included; all received CIDR treatment) and the pregnancy rate achieved by 15th March in these animals,
iii) the pregnancy rate of all cows at 15th March and,
iv) the proportion of stillbirths and peri-natal calf deaths at the subsequent calving.

Blood samples were collected from 15 representative animals from each group prior to the October treatment and in November and January for T4 measurement.

Analytical methods

Iodine concentrations in serum and pasture were determined by extracting the iodine using an alkaline ashing procedure followed by iodine determination using inductively coupled plasma mass spectroscopy (Fecher *et al*., 1998). Serum T3 and T4 concentrations were determined in duplicate using a coated tube radioimmunoassay kit, that has been validated for use in cattle (Millar & Albyt, 1985) according to the manufacturer's instructions (Coat-A-Count® Total T3 and Coat-A-Count® Total T4; Diagnostic Products Corporation, Los Angeles, California, USA).

Analysis of data

Data from Study 1 were interpreted as indicated in Table 1. These data (thyroid histopathology, thyroid weight, thyroid:body weight ratio, thyroid iodine content, foetal thyroxine concentration, mean herd thyroxine concentration and the presence/absence of goitre in the herds) were subjected to two-tailed independent *t* test or Mann-Whitney test to compare the differences between the measured parameters among the groups. Mean serum thyroxine concentrations between herds, calves with thyroid hyperplasia *versus* normal thyroid histology were subjected to Kruskal-Wallis one way analysis of variance. Differences were considered significant when $P<0.05$.

Concentrations of T4 (Studies 2 and 3) and T3 and TSI (Study 2) were subjected to repeated measures analysis of variance (GLM procedure) followed by pair-wise comparisons among periods using the Bonferroni test. Relationships between body weight and condition; T4 and TSI concentrations; T4 concentration and body weight were investigated using regression analysis.

The results for the pooled samples were compared to the mean for the group at each period, using a one-sample t-test. Differences were considered significant when $P<0.05$. Regression analysis was carried out to investigate the relationship between: body weight and condition; T4 and TSI concentrations; T4 concentration and body weight.

Ethical Approval

All experimental procedures involving the cows were approved by the Massey University Animal Ethics Committee (Protocol No. 04/170).

RESULTS

Study 1: Iodine deficiency in stillborn calves

The results of this study are summarised in Table 1. Goitre, as defined by histological lesions of diffuse thyroid hyperplasia, was confirmed in six neonates (15.8%), from 5 herds (41.7%). The range in weight of the thyroid gland was 7 g to 287 g, so the thyroids of all calves in the study (n = 42) exceeded 6.5 g (considered the upper limit for fresh weight for the thyroid in full-term normal calves). Two thirds of the neonates in the study had thyroid weights in excess of 14 g (considered indicative of goitre). A large proportion (80.5%) of calves had a thyroid:body weight ratio in excess of 0.033 (considered the upper limit of normality).

Thyroid gland weight, dimensions (length, width and isthmus) and thyroid:body weight ratio, were significantly higher in calves with thyroid hyperplasia than in the calves with normal histology ($P<0.001$), whereas differences in carcass weight and serum T4 concentrations between these groups were not significant ($P>0.05$).

The calves with a thyroid iodine content of >1000 mg/kg DM (9/16) had normal thyroid histology, while those calves with an iodine content of <1000 mg/kg (7/16), all had thyroid hyperplasia.

A large proportion of stillborn calves (82.4%) had serum T4 concentrations below the limit (80 nmol/L) indicated for neonates. Calves without thyroid hyperplasia had significantly higher ($P<0.001$) T4 concentrations than the dams from herds with no goitre cases.

In the adults, overall mean T4 concentration for all herds (42.8 ± 1.0 nmol/L) was below the threshold of 45 nmol/L. When herds with calves confirmed with thyroid hyperplasia were compared to those without, no significant differences ($P>0.05$) in T4 concentrations were found.

Study 2: Temporal changes

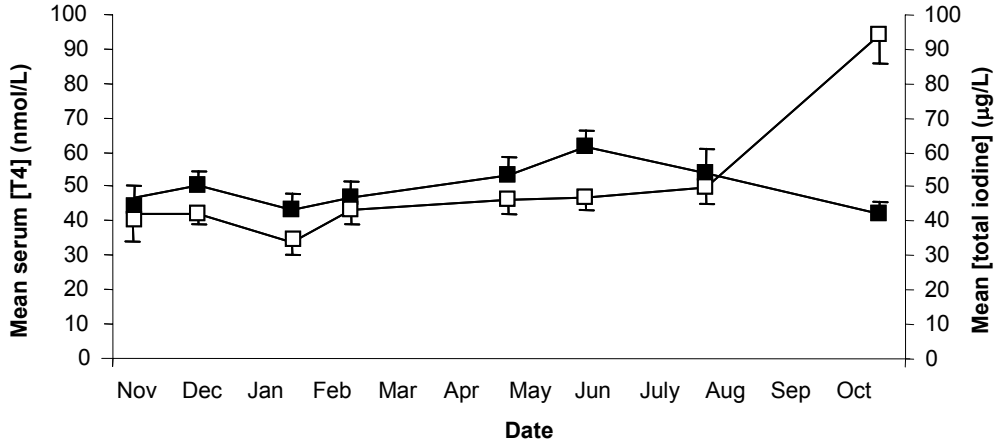
Changes in mean concentrations of T4, T3 and TSI differed significantly (all $P<0.001$) with time. Concentrations of T4 (Figure 1) were 43.9 ± 7.1 nmol/L (mean ± SD) at the start of the study, were maximal between May and August (50.4 to 58.6 nmol/L) and were lowest in the final month of the trial (October: 39.2 ± 7.1 nmol/l). Overall mean concentrations over the 12 month period were 46.8 ± 10.9 nmol/L.

Table 1: Indicators of iodine deficiency and results.

Parameter	Indicator	Reference	Results	
Thyroid histology	Diffuse hyperplasia	Radostits *et al.*, 2000	15.8%	(6 of 38)
Thyroid weight	> 6.5 g	Radostits *et al.*, 2000	100.0%	(42 of 42)
Thyroid weight	> 14 g	Andrews, 2003	66.6%	(28 of 42)
Thyroid:body weight ratio	> 0.033	Synge, 1982	80.5%	(33 of 41)
Thyroid iodine content	< 1000 mg/kg	VLA, 2004	44.0%	(7 of 16)
Foetal serum thyroxine	< 80 nmol/L	Andrews, 2003	82.4%	(28 of 34)
Herd mean thyroxine	< 45 nmol/L	Ruakura, 1985	50.0%	(6 of 12)

VLA = Veterinary Laboratory Agency, Weybridge, United Kingdom
Ruakura = Ruakura Animal Health Laboratory, Hamilton, New Zealand

Figure 1: Mean (± SEM) concentrations of thyroxine (■) and total serum iodine (□) in 20 dairy cows over a 12-month period.



Concentrations of T3 at the start of the study were 1.70 ± 0.22 nmol/L; a value which remained relatively constant between November and February (ranging between 1.67 ± 0.24 and 1.77 ± 0.34 nmol/L). Concentrations were significantly ($P<0.05$) lower in May (1.56 ± 0.26 nmol/L) than previously and declined thereafter, reaching a nadir of 1.35 ± 0.16 nmol/L ($P<0.01$) in October. The ratio of T4 to T3 concentrations (T4:T3 ratio) varied with significantly over time ($P<0.05$), following a similar pattern to that of T4 concentrations.

Between November and February, mean TSI concentrations were relatively constant (range of values (mean ± SD): 31 ± 9 to 40 ± 9 µg/L) with lowest values occurring in January. Thereafter, concentrations slowly increased over the winter (May: 43 ± 7 µg/L, August: 47 ± 9 µg/L; $P<0.05$), but were markedly higher in October (91 ± 16 µg/L; $P<0.01$) (Figure 1).

Concentrations of TS1 were significantly ($P<0.05$) correlated ($r=0.35$) with those of T4 during the period between November and August. However, TSI and T4 were unrelated to each other during October, such that when data for the entire year were included in the analysis, the correlation ($r=0.08$) was not significant. Conversely, T3 concentrations were unrelated to those of TSI, either through the entire study, or when data for October were excluded from analysis ($r=0.07$).

Body weight and condition score were maximal between November and February (approximately 500 kg body weight and BCS 4.2 and 4.3). A steady increase occurred over the subsequent six months, with maximum values occurring in August (589 kg body weight and 5.3 condition score). A substantial loss in condition and body weight then occurred over the next two months, resulting in a return to values in October that were similar to those at the start of the study (498 kg body weight and 4.4 condition score).

Comparison of trends in body weight with those of T4 revealed a similar pattern for both of these variables. Changes in T4 preceded the changes in body weight by approximately one month. Regression analysis, using data that were adjusted for 30 days, showed that a linear relationship existed [r^2 (adj) = 81.8%].

Iodine concentrations in pasture samples were 0.25, 0.20 and 1.25 mg/kg dry matter in May, June and October, respectively.

Study 3: Effect of iodine supplementation on reproduction

Total serum iodine concentrations were significantly ($P<0.001$) raised in treated cows one month (November) after iodine administration (Table 2). However, plasma T4 concentrations were not significantly ($p=0.4$) increased above pre-treatment values after iodine administration when blood samples were collected one month (November) or 3 months (January) later (Table 2).

There were no significant differences in the conception rates to first insemination, the total number of cows that were pregnant by the end of the mating period or the peri-natal calf losses between treated and control cows (Table 3).

Table 2: Total serum iodine (TSI) and thyroxine (T4) concentrations before and after iodine administration.

		Pre-treatment	+1 month	+3 months
TSI	Control	84.0 ± 4.7	89.6 ± 12.0	33.2 ± 3.0
	Treated	95.9 ± 4.8	176.3 ± 14.3	66.8 ± 3.1
T4	Control	44.1 ± 2.6	42.3 ± 1.9	38.4 ± 1.7
	Treated	40.0 ± 1.9	40.0 ± 1.4	39.4 ± 1.8

Table 3: Reproductive outcomes after iodine treatment prior to the planned start of mating (October) and the start of calving (July).

	NRR to 1st AI (%)	Total cows pregnant (%)	Total anoestrous cows* pregnant (%)	Peri-natal calf losses (next calving) (%)
Control	45.0	83.5	70.0	7.3
Treated	48.8	85.7	83.9	5.9

*presented for anoestrous treatment within 26 days of the planned start of mating

When only those cows that had been presented for anoestrus treatment by 26 days after the planned start of mating were considered, more treated than control cows subsequently became pregnant. This result approached, but did not reach, significance (p=0.10).

DISCUSSION

The occurrence of goitre in new-born calves is considered the cardinal manifestation of iodine deficiency in cattle (Radostits *et al.,* 2000). A key finding in Study 1 was that goitre was common in stillborn calves. Histological lesions of diffuse thyroid hyperplasia, excess thyroid weights and excess thyroid to body weight ratios were seen in many of the calves, along with widespread low concentrations of foetal serum thyroxine.

In Study 2 (longitudinal study of T3, T4 and TSI concentrations) there was little variation in concentrations of any of the measures of iodine status through much of the year. Concentrations of T4 generally remained in the range 44-58 nmol/L between November and June and, although there was a nadir of T4 concentrations in January, this was not reflected in changes in T3 concentrations. It was, however, reflected in TSI concentrations, which also displayed a nadir in January. Conversely, in the spring (August and October), when T4 concentrations declined to <40 nmol/L, T3 concentrations declined in parallel. Importantly, however, TSI concentrations, which had exhibited little fluctuation over the preceding period, increased markedly during the spring, such that they were unrelated to concentrations of either T3 or T4 at that time. In terms of the dynamics of thyroid hormones in the animal, such results are to be expected. Iodine is sequestered in the thyroid and released, as needed, for T4 synthesis. Hence, there is no reason to suppose a short-term temporal relationship between TSI and T4. Circulating concentrations of T4 are controlled by upstream endocrine regulators and are maintained by the presence of binding proteins. All of these mechanisms are designed to ensure that the supply of T3 to tissues is maintained within narrow homeostatic bounds. However, it is not impossible that the decline in T3 concentrations during the spring could be interpreted as these homeostatic mechanisms coming under duress at the time when animals' metabolic load is maximal.

Lack of a relationship between TSI and T4 was also evident in Study 3. Despite a two-fold increase in TSI concentrations one month after iodine administration, there was no change in T4 concentrations. This suggests that, despite the herd having T4 concentrations that were below the value suggested as the lower limit of the normal range, T4 concentrations (and, by inference, thyroid activity) were not affected by the supplementation of iodine. In other words, as T4 concentrations of <45 nmol/L were not responsive to iodine supplementation, it implies that either:

(a) concentrations of <45 nmol/L do not represent a clinical deficiency, or;

(b) the animals are unable to utilise the additional iodine.

If the latter was the case, it might be explained in terms of sequestration of iodine within the thyroid rather than its use for synthesis of hormones; the present study provides no information on this subject.

However, it seems more likely that T4 concentrations did not change in response to additional iodine as the cows had enough of the element for their immediate metabolic needs. Support for this conjecture is also derived from the minimal response of reproductive performance to supplementation of iodine at the time of the planned start of mating.

On the other hand, it was found that there were no significant differences in mean T4 concentration between herds where neonates had thyroid hyperplasia and those that did not, which could support the notion that measuring T4 concentrations is of little value in the diagnosis of iodine deficiency. Such an argument has been made by previous authors (*e.g.* McCoy *et al.,* 1997; Rogers, 1999, Randhawa & Randhawa, 2001). However, recent data from the Veterinary Laboratories Agency in the United Kingdom (Livesey & Payne, 2006, unpublished data), suggests that the problem may, in fact, be related to the threshold at which concentrations of T4 are regarded as 'normal' or 'abnormal'. In their data, the lower quartile of samples submitted to their

laboratory is 26-43 nmol/L, from which they suggest that values >43 nmol/L are 'unlikely to be associated with iodine deficiency', and even where values are below this figure, herds may or may not experience clinical deficiency.

Such an interpretation would be compatible with the results of the present study. All of the herds had mean T4 concentrations of <45 nmol/L, which was variably associated with stillbirths and, possibly, iodine-responsive limitations to production. Thus, whilst in the intervention study, T4 concentrations of ~45 nmol/L were unaffected by iodine supplementation and the stillbirth rate was only marginally affected, the presence of goitre in this, and other farms with T4 concentrations of <45 nmol/L, is indicative that iodine deficiency is present. It may be that, as suggested by Livesey & Payne, 2006 (unpublished data) the expression of clinical iodine deficiency is dependant upon other factors such as the nutritional status of the cows, the presence of T4-binding proteins in plasma, *etc*. For the foetus, its higher demands for iodine *versus* those of its dam, together with the fact that it has to obtain its iodine via maternal T4 (*i.e*. not directly as inorganic iodine), makes it more susceptible to marginal iodine status, especially when other factors push cows in marginal iodine status to display clinical iodine deficiency. Hence, this explains the presence of neonatal goitre, despite the lack responsiveness of adults to additional iodine.

CONCLUSIONS

Either T4 or TSI could be used outside the post-calving period as a measure of iodine status in cattle herds. Pooled samples are adequate for use as a measure of TSI concentration, but are not suitable for T4.

When clinical signs indicative of iodine deficiency are present in a herd, necropsy of stillborn calves should be carried out to confirm the diagnosis. Along with thyroid histology, thyroid weight/dimensions, carcass weight, thyroid iodine content and serum thyroxine concentration (*e.g*. from foetal heart blood) are all useful measures.

Iodine supplementation is probably not justified in cattle unless T4 concentrations are <45 nmol/L. In addition, during the post-calving period, protein and energy intake should also be assessed. Supplementation could be beneficial in the late mating period to avoid the low concentrations of iodine that occur in January (both animal and pasture) and also during late pregnancy (after drying off) to ensure adequate concentrations in the developing foetus.

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