
THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

The incidence of goitre in newborn lambs from ewes fed fodder radish, rape or Italian ryegrass with or without iodine supplementation

H.G. JUDSON[1]* and G.K. BARRELL[2]

[1]Agricom, P.O. Box 3761, Christchurch 8140, New Zealand
[2]Faculty of Agriculture and Life Sciences, P.O. Box 84, Lincoln University, Lincoln 7647, New Zealand
*Corresponding author: gjudson@agricom.co.nz

ABSTRACT

Brassicas are an important forage species on many New Zealand sheep farms. However, glucosinolates present in some brassicas can cause iodine deficiency in lambs when pregnant ewes are fed such crops during gestation. To investigate the effect of differences in glucosinolate profile of fodder radish and a brassica on ewe thyroid function and incidence of goitre in lambs born to ewes grazing these crops during pregnancy, a 3 x 2 factorial study utilising three diets (Italian ryegrass, fodder radish, rape) and two rates of iodine (I) supplementation (with, without) was conducted. Thyroid hormone (T_3, T_4) response to a thyroid releasing factor (TRF) challenge in ewes and incidence of goitre in newborn lambs was determined. A mild goitre, based on thyroid weight:lamb weight ratio (g/kg) of >0.4, was recorded only in newborn lambs from ewes fed rape and the incidence was higher (75% *vs* 11%) in those without I supplementation. Pregnant ewes grazing radish or rape had the lowest T_3 and T_4 responses to TRF, except in the case of T_4 where there had been I supplementation. Differences in glucosinolate content and composition may explain the differing results for sheep fed radish or rape.

Keywords: brassicas; ewe; pregnancy; thyroid; goitrogens.

INTRODUCTION

Brassicas are an important source of forage on many farms. They are generally used to support high winter stocking rates or to provide quality feed for finishing young stock during summer. Radishes (*Raphanus spp.)* offer many of the features of brassicas such as swedes, turnips, kales and rapes with the added advantages of improved growth under hot conditions and tolerance to both turnip and cauliflower mosaic viruses.

Severe thyroid deficiency can occur in newborn lambs when pregnant ewes are fed brassica crops for long periods prior to lambing (Sinclair & Andrews, 1958). Members of the brassica species and the closely related radish genus produce glucosinolates which hydrolyse during digestion to produce thiocyanate or thiouracil ions that either block iodine (I) uptake by the thyroid glands or otherwise reduce the synthesis of the thyroid hormones, T_3 and T_4. As with I deficiency this can cause goitre, an enlargement of the thyroid glands, and the occurrence of weak newborn lambs that are vulnerable to early post-natal death. Iodine supplementation has been shown to reduce such perinatal mortality. It increased lambing percentage by 14 to 21% in a study of pasture-fed ewes (Sargison *et al.*, 1998) and reduced the incidence of goitre in lambs from ewes grazing swedes or swedes, turnips and kale (Grace *et al.*, 2001). Forage rape and fodder radishes differ in both total glucosinolate content and the relative amount of individual glucosinolates (H.G. Judson, Unpublished data). In general, fodder radishes appear to contain higher concentrations of total glucosinolates than forage rapes but they have progoitrin concentrations below the level of detection compared with approximately 2 µmol/g in rape.

The aim of this experiment was to determine if these differences in glucosinolate profile between rape and fodder radish conferred differences in ewe thyroid function and the incidence of goitre in lambs born to ewes grazing these crops during pregnancy. Thyroid function was examined by use of a thyroid releasing factor (TRF) challenge. TRF is the hypothalamic signal that stimulates secretion of thyroid stimulating hormone (TSH) from the anterior pituitary gland. Response of the thyroid glands to this challenge cascade is gauged by measuring the increase in plasma concentration of T_3 and T_4 following intravenous administration of TRF.

MATERIALS AND METHODS

Procedure

A 3 x 2 factorial experiment was conducted utilising pregnant ewes grazing three forages and two rates of I supplementation. The three forages were grazed for 63 days during gestation from 13 June to 15 August, comprised Italian ryegrass (*cv* Ceres Crusader), fodder radish (*cv* Graza) and rape (*cv* Rangi). The two rates of I were zero and 390 mg (1.5 ml Flexidine, Bomac Laboratories Limited, Manakau City, Auckland, New Zealand) administered as a single intramuscular injection to the neck.

In mid-February, 126 mixed-age Romney ewes were allocated to three feeding treatment groups such that mean group live weights of approximately 48 kg were similar. Half the ewes in each group were injected with the I supplement. The ewes were joined with rams on 20 March and managed as a single mob through mating until pregnancy was confirmed by ultrasonic scanning then they were separated into the winter feeding treatment groups (n = 34 per group) to begin the separate feeding regimes in mid June. At the estimated start of lambing on 15 August, the ewes were grouped into a single mob and transferred to perennial ryegrass (*Lolium perenne*) pasture. During lambing, the flock was checked twice daily and the lambs caught, individually identified and weighed. For each ewe, lambing date, litter size, lamb birth weight, gender and whether lambs were born alive, still born or suffered perinatal death was recorded. Thyroid glands were dissected from 16 to 18 lambs of each feeding treatment (8 or 9 each from I supplemented and non supplemented group of ewes) that were either euthanased (captive bolt), collected as still born or were casualties of perinatal loss. The ratio of the combined weight of both thyroid glands (g) to the weight of the lamb (kg) was calculated for each of these lambs. This measurement is considered to be useful for the diagnosis of goitre when this ratio exceeds 0.4 (Clark *et al*., 1998). Throughout the forage grazing period the ewes were offered a daily allowance of approximately 1.5 kg of DM. The herbages were analysed for glucosinolate content prior to the feeding regimes being imposed on 1 May and once again during the trial.

Prior to joining (20 February) and again prior to lambing (14 August) seven ewes from each treatment were challenged with a thyroid releasing factor (TRF, 200 µg of thyrotropin releasing factor, Calbiochem-Novabiochem Corporation, La Jolla, California, USA) delivered as a single intravenous bolus. Plasma T_4 and T_3 concentration was determined from blood samples taken at zero and four hours after TRF administration. The zero hour value was subtracted from the four hour concentration to give the thyroid hormone response to the TRF challenge.

The study was carried out at Ceres Research Centre, Prebbleton, in Canterbury, New Zealand. All animal procedures were approved by the Lincoln University Animal Ethics Committee.

TABLE 1: Concentration (µmol/g) (mean of two samples) of specific glucosinolates and total glucosinolate in samples taken from Italian ryegrass, fodder radish and rape feed treatments. ND = Not detectable.

Glucosinolate	Winter forage crop		
	Ryegrass	Radish	Rape
Progoitrin	ND	ND	1.75
4-hydroxyglucobrassicin	ND	ND	0.5
Glucoraphanin	ND	0.35	ND
Glucobrassicin	ND	1.9	1.0
Total glucosinolates	ND	3.0	7.45

TABLE 2: Mean ± standard error of the mean of plasma total T_3 concentration (nmol/L) in pregnant ewes grazing Italian ryegrass, fodder radish or rape during winter (June-August) without or with iodine supplementation. Values are for blood samples collected before and after an intravenous injection of 200 µg TRF.

Winter forage crop	Iodine supplementation	Prior to feeding treatment		After feeding treatment	
		pre TRF	post TRF	pre TRF	post TRF
Ryegrass	Without	1.1 ± 0.2	2.4 ± 0.1	1.4 ± 0.2	2.4 ± 0.2
	With	1.2 ± 0.1	2.6 ± 0.2	1.5 ± 0.1	2.2 ± 0.2
Radish	Without	1.2 ± 0.1	2.9 ± 0.1	1.1 ± 0.1	1.7 ± 0.1
	With	1.3 ± 0.1	2.4 ± 0.1	1.6 ± 0.1	2.0 ± 0.1
Rape	Without	1.1 ± 0.1	2.5 ± 0.2	1.7 ± 0.1	2.0 ± 0.2
	With	1.0 ± 0.1	2.2 ± 0.2	1.6 ± 0.1	1.8 ± 0.2

TABLE 3: Mean ± standard error of the mean of plasma total T_4 concentration (nmol/L) in pregnant ewes grazing Italian ryegrass, fodder radish or rape during winter (June-August) without or with iodine supplementation. Values are for blood samples collected before and after an intravenous injection of 200 µg TRF.

Winter forage crop	Iodine supplementation	Prior to feeding treatment		After feeding treatment	
		pre TRF	post TRF	pre TRF	post TRF
Ryegrass	Without	37 ± 9	82 ± 11	43 ± 8	86 ± 9
	With	44 ± 5	88 ± 8	52 ± 6	87 ± 7
Radish	Without	34 ± 4	72 ±7	34 ± 7	59 ± 7
	With	38 ± 4	76 ± 6	50 ± 6	83 ± 6
Rape	Without	38 ± 5	79 ± 7	65 ± 5	90 ± 6
	With	40 ± 6	77 ± 9	94 ± 13	131 ± 18

Laboratory analyses

Samples were freeze-dried and ground through a 1 mm screen. Glucosinolates were extracted from a 3.75 g sample using the methods of van Etten *et al.* (1976). The concentration of various glucosinolates were determined by high performance liquid chromatography (HPLC) (Hewlett Packard 1100 series) using HPLC Phenomen columns (5 µ ODS, 250 x 4.6 mm), column heater and automated pre-derivation of the sample. Initial isolation and identification of individual glucosinolates was made with the aid of a photo-diode array (PDA) (200 – 350 nm and specified at 235 nm) verified at Waikato University using mass spectrometry (VG Platform II; Fisons instruments). Total glucosinolates are presented as the combined total of individual glucosinolates.

Plasma total T_4 and T_3 concentrations were measured in duplicate 25 µL and 100 µL aliquots, respectively, using commercial solid-phase ^{125}I radioimmunoassay kits (Count-A-Coat Total T_4 and Total T_3, Diagnostic Products Corporation, Los Angeles, California, USA) which had previously been validated for ovine plasma in this and other laboratories (Moenter *et al.*, 1991; Saleh *et al.*, 1998). The intra-assay coefficient of variation was 5.3% and 6.7%, and the sensitivity was 3.2 and 0.11 nmol/L for the T_4 and T_3 assays, respectively.

Effects with feeding treatments were compared using a paired Student's *t*-test.

RESULTS

Concentrations of some specific and total glucosinolates from each of the three feed sources are given in Table 1.

Forage crops differed in glucosinolate profile. For example, rape contained more than twice the concentration of total glucosinolates compared with fodder radish. There were no detectable concentrations of either progoitrin or 4-hydroxyglucobrassicin in radish, yet in rape, these two compounds made up 30% of the total glucosinolate content. In contrast, glucoraphanin was present in radish but undetectable in rape. Glucosinolates were not detected in ryegrass.

After grazing the different winter forages there was, in general, an elevation of plasma T_3 and T_4 concentrations in all ewes (Tables 2 and 3). Unsupplemented ewes grazing rape had higher ($P < 0.01$) plasma concentrations of T_3 and T_4 than unsupplemented ewes on the other diets (Tables 2 and 3).

The increase in plasma concentration of T_3 (Figure 1) in pregnant ewes as a result of the TRF challenge at the end of the feeding treatments was reduced ($P < 0.05$) in comparison with the pre-treatment results, with ewes grazing rape and radish

FIGURE 1: The increase in plasma total T_3 concentration in response to a TRF challenge in ewes prior to the grazing treatment (white) and after grazing Italian ryegrass, fodder radish or rape (black) with no I supplementation or with (+) I supplementation. Vertical bars represent the standard error of the mean.

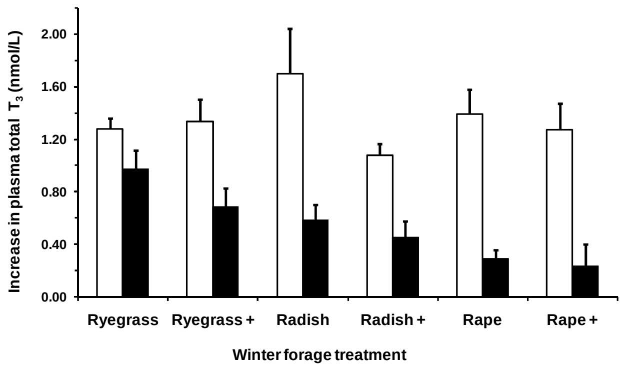


FIGURE 2: The change in plasma total T_4 concentration in response to a TRF challenge in ewes prior to the grazing treatment (white) and after grazing Italian ryegrass, fodder radish or rape (black) with no I supplementation or with (+) I supplementation. Vertical bars represent the standard error of the mean.

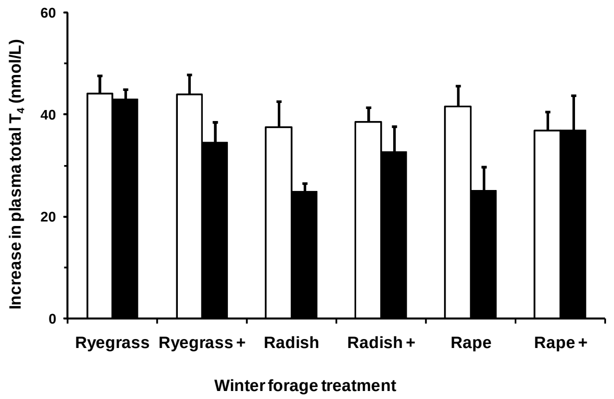


FIGURE 3: Mean thyroid weight:lamb weight ratio (g/kg) of lambs born to ewes grazing Italian ryegrass, fodder radish or rape with no I supplementation or with (+) I supplementation. Ratios above 0.40 (horizontal line) in individuals constituted a diagnosis of goitre. Vertical bars represent the standard error of the mean.

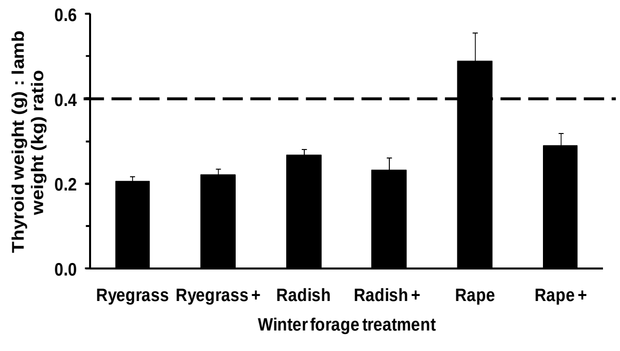
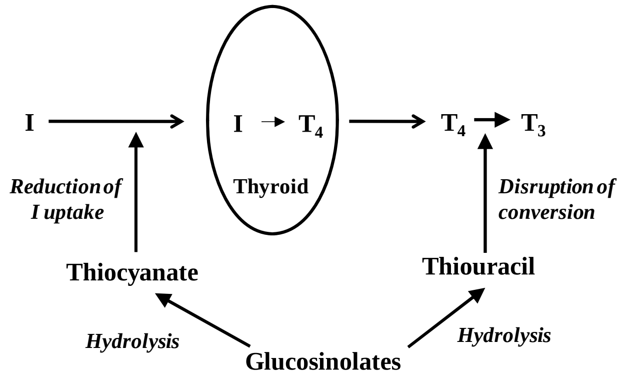


FIGURE 4: Mechanism through which glucosinolates affect both I uptake by the thyroid glands and the conversion of T_4 to T_3.



without supplementary I showing the greatest reductions (P <0.05). The mean increases in plasma concentration of T_4 (Figure 2) in pregnant ewes as a result of the TRF challenge at the end of the feeding treatments were not significantly different (P >0.05) apart from those grazing fodder radish and rape that were not supplemented with I. In the latter cases, there was a reduction (P <0.05) in the T_4 response to TRF (Figure 2).

Lambs born to ewes fed rape during gestation and not supplemented with I had a significantly higher (P <0.01) mean thyroid weight:lamb weight ratio (0.49 g/kg) than lambs born to ewes in all other treatments (0.24 g/kg) (Figure 3). Goitre, diagnosed as a mean thyroid weight:lamb weight ratio above 0.4 g/kg, only occurred in lambs from the ewes fed rape and not in other treatments. The proportion diagnosed with goitre on rape was higher in lambs from unsupplemented ewes (75%) compared with those from supplemented ewes (11%).

DISCUSSION

The incidence of goitre, as recorded from the thyroid weight to lamb weight ratio, of newborn lambs in this study was confined to those whose mothers had grazed rape during pregnancy. It did not occur in lambs from ewes that grazed Italian ryegrass nor, notably, where the ewes had grazed fodder radish, in spite of the presence of glucosinolates in this forage. Supplementation of ewes grazing rape with I lowered the incidence of goitre in their lambs as shown also by Grace *et al.* (2001) in the case of ewes grazing brassicas. Feeding rape or fodder radish to ewes during gestation attenuated the response of their thyroid glands, as determined by diminished increase in plasma T_3 and T_4 concentrations to a TRF challenge. This effect was overcome by I supplementation in the case of T_4.

In this study, the incidence of goitre in newborn lambs was associated with high concentrations of plasma T_3 and T_4 in their mothers that were grazing rape. It is possible that these high maternal plasma thyroid hormone concentrations have some direct deleterious effect on thyroid gland development in the fetuses against which supplemental I appears to provide some degree of protection. Alternatively, goitrogenic compounds derived from ingestion of rape by the ewes may interfere directly with fetal thyroid development or are additional to their maternal effects.

Absence of goitre in newborn lambs from ewes grazing fodder radish supports the view that the glucosinolate profile of forages is an important factor in their goitrogenic effects. The presence of progoitrin and 4-hydroxyglucobrassicin in rape and their absence in fodder radish and Italian ryegrass may account for the differences observed here. These two compounds generate thiouracil which is known to disrupt the conversion of T_4 to T_3 (see Figure 4). This could account for the raised plasma concentration of T_4 recorded in the ewes that were grazing rape. However, glucosinolates that are common to radish and rape, such as glucobrassicin, may generate thiocyanate following ingestion of these crops and this can block I uptake (see Figure 4). Disruption of thyroid function in ewes by this mode of action appears to be less able to cause goitre in their lambs and, as indicated by the present results, is readily overcome by the provision of supplemental I.

Other brassicas have negative effects on thyroid functionality in sheep. For instance, lambs fed kale for a period of 24 weeks also showed suppressed thyroid function as determined by plasma T_4 concentration (Barry *et al.*, 1983). However, measurement of basal plasma concentrations of T_3 and T_4 does not always provide a reliable indication of thyroid gland activity (Clark *et al.*, 1998; Grace *et al.*, 2001) and this is evident in the present results. Nevertheless, there are clear differences in the thyroid hormone response in terms of increased plasma concentration following an intravenous TRF challenge, between ewes grazing Italian ryegrass and those on the glucosinolate-containing forages used here. Diminished thyroid hormone responsiveness to a TRF challenge in sheep may be a sensitive indicator of impaired thyroid function arising from the presence of interfering compounds in grazed forages, but the current results show that this is not necessarily indicative of their potential goitrogenicity to developing fetuses.

Where goitrogenic forages, such as brassicas, make up a large proportion of the gestational diet of ewes, I supplementation can largely protect against goitre in their newborn lambs and thus, in some environments, may increase ewe productivity by improving lamb survival. However, it has been shown here that there are similar forages, such as fodder radish, which by virtue of differing glucosinolate profiles may not present the same risk of goitre incidence in lambs.

ACKNOWLEDGEMENTS

We thank Martin Wellby for the T_4 and T_3 analyses and Lincoln University Feed Laboratory for glucosinolate analysis.

REFERENCES

Barry, T.N.; Duncan, S.J.; Sadler, W.A.; Millar, K.R.; Sheppard, A.D. 1983: Iodine metabolism and thyroid hormone relationships in growing sheep fed on kale (*Brassica oleracea*) and ryegrass (*Lolium perenne*) – clover (*Trifolium repens*) fresh-forage diets. *British Journal of Nutrition* **49**: 241-253.

Clark, R.G.; Sargison, N.D.; West, D.M.; Littlejohn R.P. 1998: Recent information on iodine deficiency in New Zealand sheep flocks. *New Zealand Veterinary Journal* **46**: 216-222.

Grace, N.D.; Knowles, S.O.; Sinclair, G.R. 2001: Effect of premating iodine supplementation of ewes fed pasture or a brassica crop prelambing on the incidence of goitre in newborn lambs. *Proceedings of the New Zealand Society of Animal Production* **61**: 164-167.

Moenter, S.M.; Woodfill, C.J.I.; Karsch, F.J. 1991: Role of the thyroid gland in seasonal reproduction: thyroidectomy blocks seasonal suppression of reproductive neuroendocrine activity in ewes. *Endocrinology* **128**: 1337-1334.

Saleh, D.M.; Barrell, G.K.; Bailey, C.I.; Frampton, C.M.A. 1998: Effects of exogenous triidothyronine (T_3) and a goitrogen, methylthiouracil (MTU), on thyroid gland function in sheep. *Small Ruminant Research* **30**: 49-56.

Sargison, N.D.; West, D.M.; Clark R.G. 1998: The effect of iodine deficiency on ewe fertility and perinatal lamb mortality. *New Zealand Veterinary Journal* **46**: 72-75.

Sinclair, D.P.; Andrews, E.D. 1958: Prevention of goitre in newborn lambs from kale-fed ewes. *New Zealand Veterinary Journal* **6**: 87-95.

van Etten, C.H; Daxenbichler, M.E; Williams, P.H; Kwolek, W.F. 1976: Glucosinolates and derived products in cruciferous vegetables. Analysis of the edible parts from 22 varieties of cabbage. *Journal of Agriculture, Food and Chemistry* **24**: 452-455.