Iodine metabolism and thyroid hormone relationships in growing sheep fed on kale (*Brassica oleracea*) and ryegrass (*Lolium perenne*)–clover (*Trifolium repens*) fresh-forage diets

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1. Kale (*Brassica oleracea*) and ryegrass (*Lolium perenne*)–clover (*Trifolium repens*) pasture, grown under similar soil conditions, were grazed in the vegetative state by growing lambs of 23·6 kg initial live weight for 24 weeks. The kale and pasture contained respectively 20 and 270 μg iodine/kg dry matter (DM). The kale also contained 8 μmol total glucosinolates/g DM and 11·5 g S-methyl-L-cysteine sulphoxide (SMCO)/kg DM, both of which were nondetectable in the pasture diet.

2. Intramuscular injections of I (475 mg) were given during weeks 1 and 12 to half the forty-eight lambs grazing each forage. Wool growth, live-weight gain and cytochrome oxidase (*EC* 1.9.3.1) activity of biopsied hind-limb muscle were measured at 6-week intervals. Jugular blood samples were removed every 6 weeks for the determination of haematological factors and serum thyroid hormone concentrations. All animals were slaughtered at the end of the experiment and thyroid weight, thyroid I content, and the weight and cytochrome oxidase activity of heart muscle determined.

3. Serum concentrations of thyroxine (T_4) increased from 20 to 48 nmol/l during the 24 weeks that control lambs grazed ryegrass–clover pasture. I supplementation increased the concentration and total amount of I in the thyroid gland and increased serum T_4 concentration, but did not affect any other values measured in the lambs grazing the pasture herbage. Serum concentrations of triiodothyronine (T_3) were stable at 2 nmol/l for both groups.

4. Control lambs grazing kale for 24 weeks showed marked thyroid enlargement and depletion of thyroid I. By week 6, serum T_4 and T_3 concentrations had declined to 2–5 nmol/l and 1 nmol/l respectively and were stable at these values for the remainder of the experiment. I supplementation eliminated the thyroid depletion of this element, caused serum T_4 concentration to rise and stabilize at 90 nmol/l by week 18, and T_3 concentration to stabilize at 2 nmol/l by week 6. From week 6 onwards, wool growth was increased 13% by I supplementation, whereas empty body growth was unaffected.

5. Lambs grazing kale developed haemolytic anaemia, due to rumen fermentation of SMCO. I supplementation enabled the lambs to resist the anaemia better by increasing erythrocyte reduced gluthathione (GSH) content. Relative to pasture-fed animals, lambs grazing kale and supplemented with I showed increased heart muscle weight and cytochrome oxidase activity. This represented a compensatory mechanism for the reduced blood oxygen-carrying capacity caused by the anaemia. I-deficient (i.e. control) lambs grazing kale showed reduced cytochrome oxidase activity in both heart and hind-limb muscle.

6. The findings are in accord with T_3 having a greater biological potency than T_4 for regulating rates of body and wool growth. Increases in heart weight, heart cytochrome oxidase content and erythrocyte GSH content of kale-fed lambs were, however, associated with elevation in serum T_4 and not T_3 concentration.

7. I requirements of growing sheep and cattle consuming the pasture diets are discussed. Because of its better relationship to production traits, it is considered that requirements should be based on the ability to maintain T_3 rather than T_4 concentrations. On this basis, requirements could be met by diets containing 180–270 μg I/kg DM.

Kale (*Brassica oleracea*) contains glucosinolates which are hydrolysed during autolysis of the plant, such as during chewing by animals, to produce nitriles and inorganic thiocyanate ions (Tapper & Reay, 1973; Forss & Barry, 1983). The thiocyanate ion is known to have

goitrogenic properties which are reversible by iodine therapy. However, in an 8-week study with lambs grazing kale, Sinclair & Andrews (1959) found that although I administration prevented thyroid enlargement it did not increase animal growth rates. The present study was designed to measure responses to I supplementation in both body growth and wool growth in growing lambs fed on kale for a much longer period (24 weeks). It was also desired to obtain more detailed knowledge of I metabolism, particularly rates of depletion, through measuring changes in total thyroid I content and in serum concentrations of thyroxine (T_4) and triiodothyronine (T_3).

Brassica diets are unique in containing the free amino acid S-methyl-L-cysteine sulphoxide (SMCO), which is metabolized by rumen bacteria to produce dimethyl disulphide, which in turn causes haemolytic anaemia (Smith, 1974) and depressed appetite (Barry *et al.* 1982). Both effects are particularly severe in ruminants fed on kale diets, and a further objective was to evaluate whether elevating serum T_4 and T_3 concentrations in lambs grazing kale would reduce the severity of the anaemia.

The content of many trace elements in forages is influenced by soil conditions under which the plants are grown as well as by the type of cultivar. To differentiate between the two effects, a second group of lambs grazed ryegrass (*Lolium perenne*)–clover (*Trifolium repens*) pasture grown under similar soil conditions to the kale.

The relative metabolic effects of T_4 and T_3 have received attention, with opinion currently favouring T_3 as the active form of the thyroid hormone (Ingbar & Braverman, 1975). These deductions have been made with non-ruminants, and the opportunity was taken here to test this hypothesis with the growing sheep. The study was particularly appropriate for this purpose as a range of serum T_4 and T_3 concentrations was expected, and depletion of thyroid hormones was probable in the group grazing kale without I supplementation. The results obtained were consistent with T_3 having a greater biological potency than T_4 for regulating rates of body and wool growth, and animal performance was not depressed until serum T_3 concentrations declined, with wool growth being most affected. I supplementation was beneficial in lambs grazing kale for periods longer than 6 weeks, for both increasing wool growth and enabling them partially to counteract haemolytic anaemia by several mechanisms.

EXPERIMENTAL

Experimental design

A $2 \times 2 \times 2$ factorial experiment was conducted utilizing two diets (kale and ryegrass–clover pasture), two rates of I supplementation (with and without) and two rates of copper supplementation (with and without). Ninety-six lambs were divided into groups of twelve on the basis of initial live weight and allocated to the eight groups. Twenty-four animals therefore, comprised each I-treatment group. There were no Cu × I interactions ($P > 0{\cdot}05$) for animals fed on either diet. Consequently, the responses to I supplementation only are presented in this paper.

Animals grazed the two forages for 24 weeks, with the experiment commencing in midsummer (February, 1980) and concluding in late winter (August, 1980). All animals were slaughtered at the end of the experiment. An initial slaughter (IS) group of twelve lambs was slaughtered at the start of the experiment, to give a measure of initial I in the thyroid gland and to derive equations from which the body composition of the ninety-six test lambs could be predicted.

Forages

Areas of kale (0·6 ha; variety Maris Kestrel) were sown on six occasions approximately 10 d apart during spring 1979 (November and December). Seed rate was 3·9 kg/ha and fertilizer

application was 60 kg N/ha and 35 kg P/ha, applied as a mixture of diammonium phosphate and urea. The permanent pasture areas (2 × 4 ha) were grown on the same soil type (Wingatui silt loam) at the same time as the kale and were adjoining the area of kale, 4 ha being on each side. Annual fertilizer application was 18 kg P/ha and 22 kg S/ha applied as single superphosphate.

Animals

Romney wether lambs aged 5 months and with a mean initial live weight of 23·6 kg (SD 2·2) were used as experimental animals.

All animals were drenched at the commencement of the experiment and thereafter at 6-weekly intervals with alternating brands of anthelmintic (Thibenzole®; Merck, Sharp and Dohme Ltd (Hoddesden, Hertfordshire), and Valbazen®; Smith, Kline and French Ltd (Welwyn, Hertfordshire)). Selenium (2·5 mg as sodium selenate) was given orally to all animals 3 weeks before the start of the experiment and during week 12. I was administered by intramuscular injection of 1 ml iodized poppy seed oil (Lipiodol®; May and Baker Ltd (Manchester)) containing 475 mg I at the start of the experiment and at the end of week 12.

The forty-eight animals allocated to each forage were grazed in one group. Break fences were adjusted such that the kale-fed group was moved to a new area every 4 weeks and the pasture group moved every week. Forage of relatively constant yield and composition was provided throughout the 24 weeks by moving the kale group on to progressively later sowings, and by always offering the pasture group recently grown herbage of approximately 2500 kg (DM)/ha. Forage DM yield was measured to soil level before each area was grazed, and the break fences adjusted such that the amount of forage on offer was always 3 kg DM/lamb per d for kale-fed lambs and 4·5 kg DM/lamb per d for pasture-fed lambs. These allowance levels were maintained for the entire length of the experiment, and they ensured that animal growth was not restricted by either the availability of kale (Barry, McDonald *et al.* 1981) or pasture (Jagusch *et al.* 1981).

Midside wool patches of 122·5 × 122·5 mm were clipped to skin level on the left side of all animals at the commencement of the experiment, and thereafter at 6-weekly intervals. The sheep were also weighed after a 24 h fast on these occasions.

Slaughter procedures

All lambs were fasted for 24 h and shorn before slaughter. After slaughter the weights of gut contents, carcass, liver, kidneys and thyroid glands were recorded. For the IS group, regression relationships were derived of empty body-weight (EBW) and carcass weight (CW) on fasted live weight. These relationships were then used to predict the EBW and CW of the ninety-six test lambs at the start of the experiment, and rates of EBW gain and CW gain calculated using standard comparative-slaughter procedures (Barry, 1981).

Sampling procedures

Representative samples of forage eaten were obtained by placing three cages measuring 2·0 × 1·5 m on each feed break the day the animals were introduced. At the termination of grazing the cages were removed and forage harvested corresponding to what the animals had eaten. Samples were collected in this manner and freeze-dried at 4-week intervals for kale and 2-week intervals for pasture.

Blood was withdrawn by venipuncture from the jugular vein of sixteen animals per treatment group at the start of the experiment and during weeks 6, 12, 18 and 24. Whole blood was used immediately for haematology and reduced glutathione (GSH) determinations, whilst serum was stored at −20° until required for thyroid hormone assays. Samples

of hind-limb muscle were removed by biopsy at the previously-mentioned times from four animals per treatment group and retained for determination of cytochrome oxidase (*EC* 1.9.3.1) activity.

Laboratory methods

I in plant and thyroid tissue was determined by the method of Moxon & Dixon (1980); recovery of ^{125}I added as NaI was 0·87 and all results were corrected by this factor. National Bureau of Standards sample NBS1571, certified to contain 17 μg I/kg DM, was analysed in the same batch as plant and thyroid tissue and 17 μg I/kg DM recorded.

Serum T_4 and T_3 were measured by the radioimmunoassay procedures of Sadler & Brownlie (1975). The thyroxine binding:globulin binding value (TBGbr) was measured utilizing the uptake of [^{125}I]triiodothyronine on to dextran-coated charcoal, and expressed relative to the result obtained in the same batch analysis for pooled normal human sera. The method works on the same principle as that described by Clark (1963), referred to as the T_3 resin-uptake test. Over 99·9% of thyroid hormones in serum are bound to the globulin, prealbumin and albumin fractions of serum proteins (Sutherland & Simpson-Morgan 1975). The TBGbr test measures the extent of saturation of these binding sites with thyroid hormones; increases in TBGbr represent increases in saturation.

Plant analysis, haematology procedures, and the determination of erythrocyte GSH concentration were carried out as described by Barry, McDonald *et al.* (1981) and Barry, Reid *et al.* (1981).

Statistical methods

The experiment was analysed as a $2 \times 2 \times 2$ factorial, with the main effects being diet, I supplementation and Cu supplementation. The error term comprised pooled-between-animal residual variability, and this was used for all statistical comparisons.

For erythrocyte GSH concentration, an analysis carried out on values from the initial sampling showed significant differences between treatment groups ($P < 0.05$). Consequently, all GSH values were analysed as differences from the initial value.

RESULTS

Diet composition

Chemical composition of forage similar to that consumed by the lambs is shown in Table 1. Kale contained higher concentrations of sulphur than pasture, and contained appreciable concentrations of SMCO and glucosinolates which were absent from the pasture diet. I concentration was much lower in kale than in pasture. None of the plant constituents in Table 1 showed any trends with time throughout the experiment.

Thyroid weight and I content

After 24 weeks of grazing on the respective diets, thyroid weight was significantly increased in the group fed on kale that did not receive I supplementation ($P < 0.001$) and this was prevented by I administration (Table 2). In the absence of I supplementation, thyroid-gland I concentration was less for lambs grazing kale than pasture ($P < 0.05$) and the same trend occurred for total thyroid-I content, although this did not attain significance ($P > 0.05$). I supplementation increased both these criteria to similar values in lambs fed on both diets ($P < 0.001$). In the absence of I supplementation, thyroid-I concentration increased in lambs grazing pasture for 24 weeks relative to the IS group, whereas it markedly decreased in lambs grazing kale.

Serum thyroid hormone concentrations

In samples taken during week 18, I supplementation produced a small increase in T_4 concentration ($P < 0.05$) in the pasture-fed lambs but did not affect T_3 concentration or

Table 1. *Chemical composition (g/kg dry matter (DM)) of the forage consumed*

(Mean values and standard deviations for six kale samples taken at 4-weekly intervals and twelve pasture samples taken at 2-weekly intervals)

Diet...	Kale (*Brassica oleracea*)		Pasture*	
	Mean	SD	Mean	SD
Organic matter digestibility	0·86	0·012	0·74	0·026
Total nitrogen	25·1	3·12	33·2	3·51
Total sulphur	7·2	0·71	3·1	0·20
SMCO	11·5	4·72	0·7	0·10
Iodine (μg/kg DM)	20	8·9	270	106
Glucosinolates (μmol/g DM)	8·2	1·31	0	

SMCO; S-methyl-L-cysteine sulphoxide.
* Ryegrass (*Lolium perenne*)–clover (*Trifolium repens*).

Table 2. *Thyroid weight (g), iodine concentration (μg/g) and total amount of I in the thyroid gland (mg) for lambs slaughtered at the beginning and end of the experiment*

(Mean values with their standard errors for twelve initial animals and twenty-four animals per treatment group at final slaughter)

	Initial slaughter group (week 0)		Final slaughter group (week 24)				
			Kale (*Brassica oleracea*)		Pasture*		
	Mean	SE	No I	+I	No I	+I	SEM
Thyroid wt	4·3	0·73	28·5	7·3	7·2	6·5	1·95
I concentration	50·3	6·15	12·3	500·8	100·2	559·2	28·6
Total thyroid I	0·21	0·42	0·26	3·55	0·95	3·75	0·335

* Ryegrass (*Lolium perenne*)–clover (*Trifolium repens*).

TBGbr (Table 3). In the absence of I supplementation, and relative to pasture-fed lambs, those fed on kale showed a large reduction in serum T_4 concentration ($P < 0·001$) and proportionately smaller reductions in T_3 concentration ($P < 0·01$) and TBGbr ($P < 0·05$). I supplementation in kale-fed lambs markedly increased serum concentrations of T_4, T_3 and also TBGbr ($P < 0·001$), these values all being significantly higher than those recorded for pasture-fed lambs given I ($P < 0·001$).

The time sequence by which the previously-mentioned differences developed was then studied by selecting four samples from each of the four groups taken during weeks 0, 6, 12, 18, 24. Samples were used from the same animals at each of these five times. The eighty samples so selected were then analysed as a single batch. Results obtained (Fig. 1) showed that serum T_4 concentration progressively increased from 20 to 48 nmol/l over 24 weeks in the group fed on pasture that did not receive I supplementation. The effects of I supplementation in increasing T_4 concentration was evident after lambs had been fed on the pasture diet for 6 weeks, and there was also some evidence that I caused an increase in TBGbr that was not significant ($P > 0·05$). T_3 concentration in the serum of pasture-fed lambs was relatively stable over the 24 weeks, showing little effect of either time or I administration.

Table 3. *Concentrations of total thyroxine (T_4) and triiodothyronine (T_3) in jugular blood serum taken during week 18 (nmol/l), and thyroxine-binding: globulin binding value (TBGbr) (relative units)*

(Mean values with their standard errors for sixteen lambs per treatment group)

Diet...	Kale (*Brassica oleracea*)		Pasture*		
	No I	+I	No I	+I	SEM
Total T_4 (nmol/l)	4·0	91·1	39·5	46·7	2·24
Total T_3 (nmol/l)	1·6	2·8	2·3	2·0	0·15
TBGbr (relative units)	0·84	1·15	0·93	0·95	0·026

* Ryegrass (*Lolium perenne*)–clover (*Trifolium repens*).

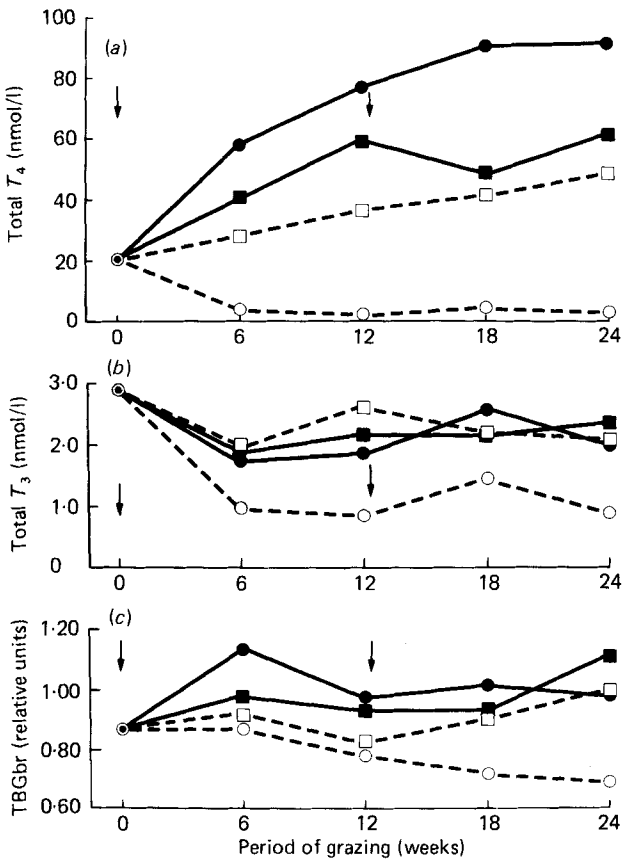

Fig. 1. Mean serum concentrations for four animals per treatment group of (*a*) thyroxine (T_4); (*b*) triiodothyronine (T_3); and (*c*) thyroxine binding:globulin binding value (TBGbr), often referred to as T_3 resin-uptake test. Standard errors of the means did not exceed (*a*) 4·71, (*b*) 0·17 (during the 6–18 week grazing period) and (*c*) 0·059. (○, ●), Lambs fed on the kale (*Brassica oleracea*) diet; (□, ■), lambs fed on the ryegrass (*Lolium perenne*)–clover (*Trifolium repens*) pasture diet. (○, □), Control animals; (●, ■) iodine-supplemented animals. ↓, 475 mg I administered by intramuscular injection.

Serum T_4 concentration in kale-fed animals that did not receive supplementary I declined to almost zero by week 6, TBGbr progressively declined from week 6 onwards, and T_3 declined to low levels by week 6 that were maintained for the remainder of the experiment (Fig. 1). The effect of I supplementation in increasing serum T_4 concentration gradually developed during the period of kale feeding, with maximum stable values only being attained by week 18. In contrast, effects of I in preventing the decreases in serum T_3 concentration and TBGbr in lambs fed on this diet were evident by week 6, and remained for the duration of kale feeding.

Biochemical properties of erythrocytes

The lambs grazing kale developed haemolytic anaemia, as expected. This is shown by the immediate increase in the proportion of erythrocytes containing Heinz bodies, and the rapid fall in packed cell volume (PCV) after introducing the lambs to this diet, followed by a reduction in erythrocyte GSH content (Fig. 2). In kale-fed lambs, erythrocyte GSH concentration was higher in the I-supplemented lambs than in control animals from week 6 onwards ($P < 0{\cdot}01$); PCV was also higher in week 18 ($P < 0{\cdot}05$) and tended to be higher in week 24, although this difference was not significant ($P < 0{\cdot}10$). Methaemoglobin comprised 10 mg/g haemoglobin in pasture-fed animals and 20 mg/g haemoglobin in kale-fed animals. I supplementation reduced methaemoglobin content by 5 mg/g haemoglobin in kale-fed lambs during weeks 18 and 24 ($P < 0{\cdot}05$).

Body growth

Averaged over the 24 weeks, live-weight gain was 110 g/d for pasture-fed lambs and was unaffected by I supplementation. Control kale-fed lambs grew at 108 g/d, which was increased to 118 g/d by I supplementation ($P < 0{\cdot}05$; SEM 3·2). There was little evidence of any changes in live-weight gain with time in any of the four treatment groups. The trend for I supplementation to increase EBW and CW gain of kale-fed lambs was too small to be significant ($P > 0{\cdot}05$). CW gain as a proportion of EBW gain was greater for kale-fed (0·660) than for pasture-fed (0·599) lambs ($P < 0{\cdot}05$; SEM 0·0067) and was reduced by 0·020 by I supplementation ($P < 0{\cdot}05$), with the effect being apparent in lambs fed on both diets.

Wool growth

During weeks 7–24, wool growth (Fig. 3) was greater for lambs fed on the pasture than those fed on the kale diet in the groups that did not receive I supplementation ($P < 0{\cdot}001$). I supplementation did not affect wool growth in lambs grazing pasture, but during weeks 7–24 it increased wool growth of lambs grazing kale by an average of 13% ($P < 0{\cdot}05$). Wool growth of kale-fed lambs given supplementary I was, however, still lower than that of pasture-fed lambs ($P < 0{\cdot}01$).

Weight of heart muscle and cytochrome oxidase activity of heart and hind-limb muscle

I supplementation increased the weight of heart muscle ($P < 0{\cdot}01$) and total heart cytochrome oxidase activity ($P < 0{\cdot}001$) in lambs fed on the kale diet (Table 4). I had no effect on these values in pasture-fed lambs, which were similar to those of the kale control group.

Mean initial cytochrome oxidase activity of hind-limb muscle was 5·7 units/mg wet muscle. During the experiment this showed some increase in pasture-fed lambs to reach a mean of 7·6 units/mg wet muscle by week 24 (Table 4) and was unaffected by I supplementation. Kale-fed lambs given supplementary I maintained similar values, but in the absence of I treatment a non-significant reduction in cytochrome oxidase activity occurred by week 24 ($P < 0{\cdot}10$).

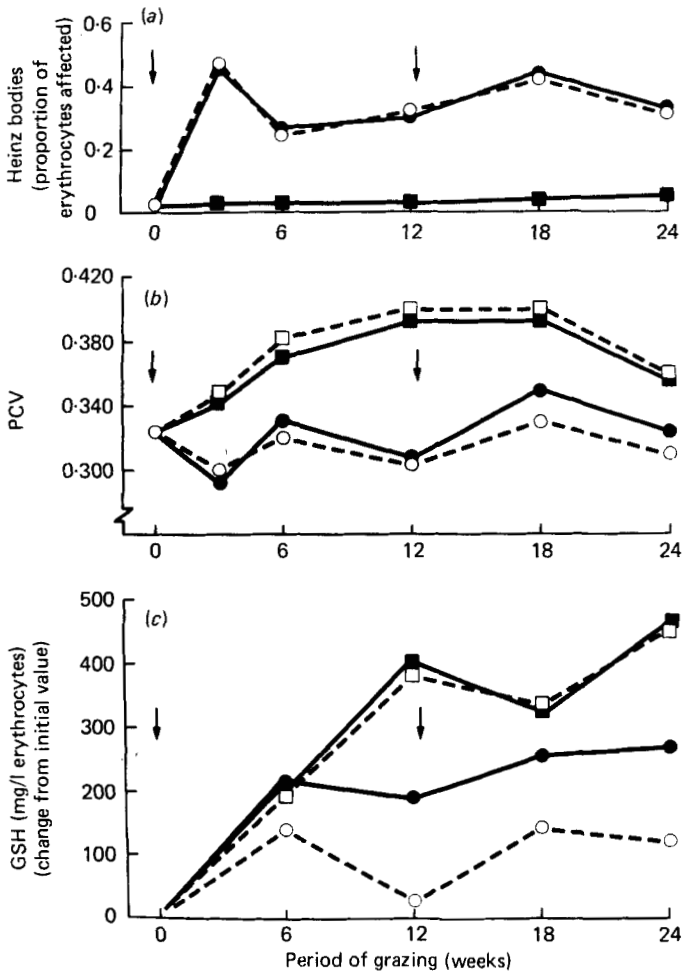

Fig. 2 Mean blood concentrations for sixteen animals per treatment group of (*a*) erythrocytes containing Heinz bodies; (*b*) packed cell volume of erythrocytes (PCV); and (*c*) reduced glutathione (GSH). Standard errors of the means did not exceed (*a*) 0·026, (*b*) 0·0079 and (*c*) 25·8. (○, ●), Lambs fed on the kale (*Brassica oleracea*) diet; (□, ■), lambs fed on the ryegrass (*Lolium perenne*)–clover (*Trifolium repens*) pasture diet. (○, □), Control animals; (●, ■), iodine-supplemented animals. ↓, 475 mg I administered by intramuscular injection.

Liver and kidney weights

After adjustment of values by analysis of co-variance to equal EBW, liver weight was less ($P < 0{\cdot}001$; SEM 7·8) for lambs fed on kale (685 g) than for those grazing pasture (771 g). Similarly, total kidney weight was less ($P < 0{\cdot}001$; SEM 2·1) for kale-fed (134 g) than for pasture-fed lambs (148 g).

Interrelationships involving thyroid hormones

In an attempt to assess the relative importance of T_4 and T_3, values collected at or near week 18 from lambs fed on the kale diet and not given supplementary I were divided into those lambs where serum T_4 concentration was not distinguishable from zero (0–2 nmol/l)

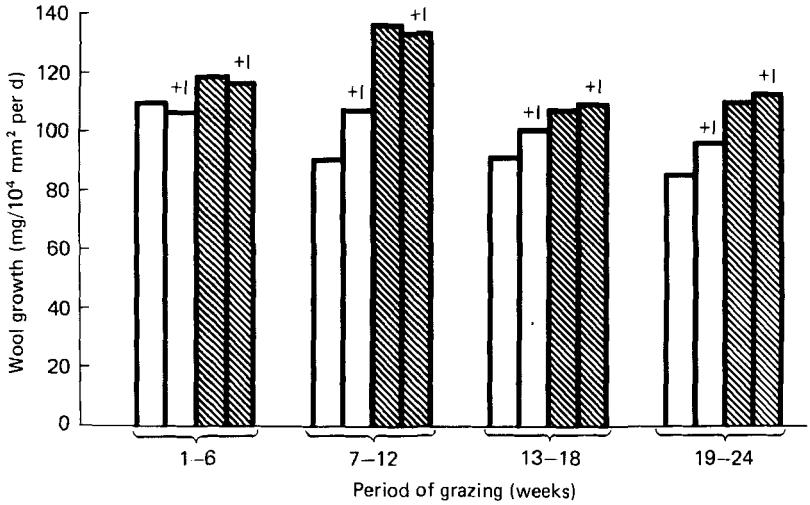

Fig. 3. Mean wool growth rates from midside areas for twenty-four animals per treatment group. (□), Lambs fed on the kale (*Brassica oleracea*) diet; (▨), lambs fed on the ryegrass (*Lolium perenne*)–clover (*Trifolium repens*) pasture diet. +I represents animals given iodine supplementation.

Table 4. *Heart muscle weight (g) and cytochrome oxidase* (EC *1.9.3.1*) *activity (cytochrome c oxidized/min) in heart and hind-limb muscle, determined at the end of week 24*

(Mean values with their standard errors for twenty-four determinations for heart weights and twelve for cytochrome oxidase per treatment group)

Diet...	Kale (*Brassica oleracea*)		Pasture*		
	No I	+I	No I	+I	SEM
Heart muscle wt	148	162	149	152	2·9
Cytochrome oxidase activity					
Heart (nmol/mg wet muscle)	47	54	40	40	2·6
Heart (mmol/heart)	6·6	8·9	6·2	6·2	0·40
Hind-limb (nmol/mg wet muscle)	4·9	7·8	8·3	7·0	1·03

* Ryegrass (*Lolium perenne*)–clover (*Trifolium repens*).

and those where measurable levels of T_4 were detected (3–7 nmol/l). Reductions in T_3 concentration accompanied the decrease in T_4 concentration, but none of the other values showed any difference between the two groups (Table 5).

DISCUSSION

I metabolism in sheep fed on kale

Diets are normally classified as I deficient if they contain 20 μg I/kg DM or less (Riesco *et al.* 1977; Potter *et al.* 1980). The kale diet used here can, therefore, be classified as both I-deficient and goitrogenic. After 24 weeks of grazing, severe I deficiency was evident, as shown by the increase in thyroid weights and reduction in thyroid-I concentration in animals that did not receive I supplementation. From the changes in thyroid-hormone concentrations in serum it is evident that almost all the depletion was complete after grazing the crop for

Table 5. *Blood composition (week 18), wool growth and live-weight gain (weeks 12–24) in the group of kale* (Brassica oleracea)*-fed lambs not given iodine supplementation, arranged in order of increasing serum T_4 concentration (week 18)*

(Mean values for animals within the stated T_4 ranges, with their standard errors)

	Serum T_4 concentration indistinguishable from zero (0–2 nmol/l)		Serum T_4 concentration (3–7 nmol/l)	
	Mean	SEM	Mean	SEM
No. of animals	9		6	
Serum T_3 (nmol/l)	1·3	0·09	1·8	0·15
Serum TBGbr (relative units)	0·85	0·050	0·81	0·020
Erythrocyte GSH (mg/l cells)	667	34	700	65
Wool growth (mg/10^4 mm^2 per d)	86	7·7	96	5·2
Live-wt gain (g/d)	101	10·4	106	12·0

T_3, triiodothyronine; T_4, thyroxine; TBGbr, thyroxine binding:globulin binding value; GSH, reduced glutathione.

6 weeks, from this time onwards T_4 concentrations being near zero and T_3 concentrations stable at 1 nmol/l; only the protein-binding system became progressively less saturated with thyroid hormones as grazing time exceeded 6 weeks.

Ferguson *et al.* (1965) found wool growth to be greatly reduced by thyroidectomy and to be increased by T_4 administration to both thyroidectomized and normal sheep. The responses in wool growth to I supplementation in lambs grazing kale in the present investigation confirm a role for thyroid hormones in regulating wool growth. I supplementation produced practically no increase in body growth of kale-fed lambs, as also found by Sinclair & Andrews (1959) in an 8-week study. Potter *et al.* (1980) gave an I-deficient (11 μg/kg DM) but goitrogen-free diet to sheep for 20 weeks, and also found that I supplementation increased wool growth but not body growth.

I deficiency reduced the ability of the lambs to counteract haemolytic anaemia produced from rumen fermentation of SMCO by two mechanisms, both of which were partially overcome by I supplementation. The first mechanism concerns biochemical properties of erythrocytes whilst the second concerns muscle cytochrome oxidase.

Dimethyl disulphide produced from rumen fermentation of SMCO combines with erythrocyte GSH, thus lowering the reducing environment and leading to the concomitant formation of Heinz bodies and methaemoglobin from haemoglobin (Smith, 1974). High erythrocyte GSH concentrations are, therefore, essential in counteracting kale anaemia, and in the present investigation these were reduced by I deficiency. Erythrocyte GSH concentrations can be altered either through utilization by gluthathione peroxidase (*EC* 1 . 11 . 1 . 9)/superoxide dismutase (*EC* 1 . 15 . 1 . 1) or by regeneration from glucose-6-P by glucose -6-P dehydrogenase (*EC* 1 . 1 . 1 . 49)/glutathione reductase (*EC* 1 . 6 . 4 . 2) (Barry, Reid *et al.* 1981). The two former enzymes were unaffected by I supplementation in kale-fed lambs, and it therefore seems that either the availability of glucose-6-P or the activity of the latter enzyme system must to some extent be dependent on thyroid hormones. The increase in erythrocyte GSH brought about by I supplementation was partially effective in maintaining PCV but had no effect in reducing Heinz-body formation.

Blood O_2-carrying capacity is reduced in kale anaemia, due to the reduction in PCV and to Heinz-body formation. In the presence of adequate I it seems that the animal counteracts

this by increasing both the weight of heart muscle and its cytochrome oxidase activity. Cytochrome oxidase functions in the respiratory chain, linking O_2 uptake to the production of $NADH_2$ from the tricarboxylic acid cycle, and increases in the enzyme are therefore likely to represent increased O_2 uptake. In turn this reflects an increase in the amount of work done by heart muscle, and that the animal probably counteracts the reduced O_2-carrying capacity of the blood by increased cardiac output. Relative to I-supplemented animals, control lambs grazing kale showed a reduction in cytochrome oxidase activity in both heart and hind-limb muscle by week 24. This is probably indicative of reductions in O_2 consumption and suggests that cytochrome oxidase may also be dependent on thyroid hormones to some extent. Thyroid hormones are known to be involved in oxidative phosphorylation, linking energy capture and heat production (Webster, 1975).

I metabolism in sheep fed on ryegrass–clover pasture

Supplementation with I only increased thyroid-I concentration and serum T_4 concentration in lambs grazing pasture, with no effect on any of the other factors measured. This shows that pasture I levels were inadequate for maximum T_4 production but adequate for T_3 production and to support normal rates of body and wool growth. Barry, Reid *et al.* (1981) reached the same conclusion for growing cattle grazing similar pasture.

Thyroid hormone metabolism

T_3 binds to nuclear receptors with a greater affinity than T_4 and in mammals T_3 is 3–5 times as potent as T_4 in thermogenesis (Chopra & Solomon, 1980). In the nucleus, T_3 stimulates the synthesis of messenger RNA and protein synthesis and phosphorylation (De Groot & Rue, 1980). This, and other evidence, has led to the view that most, but not all, of the physiological effects attributable to thyroid hormones are attributable to T_3 (Bernal, 1980). Data from the present experiment support the concept that T_3 is more active than T_4 in regulating wool and body growth in growing sheep. First, increasing serum T_4 but not T_3 concentration by I supplementation in lambs grazing pasture had no effect upon wool or body growth. Second, body and wool growth were still able to proceed at reasonable rates when serum T_4 concentration was indistinguishable from zero (Table 5). Third, depressions in wool growth were only recorded where serum T_3 concentration was depressed (i.e. in control lambs grazing kale).

T_4 is converted to T_3 in the kidney (Chiraseveenuprapund *et al.* 1978) and the liver (Balsam *et al.* 1981). It seems that animal productivity is not depressed unless dietary I concentration is so low, or dietary goitrogen concentration is so high, or both, such that the T_4 reserve becomes depleted and T_3 synthesis is impaired, thus explaining why serum T_3 concentration is better related to animal productivity. If this role for T_4 is accepted, correlations between production traits and plasma T_4 concentration in nutritional experiments, as calculated by Hart *et al.* (1978, 1979), could be misleading. A more meaningful result would be obtained if the production traits were correlated with plasma T_3 concentration. Techniques have recently become available for the measurement of free T_3 in the serum of domestic animals (Irvine, 1980), and it is probable that these would be of even better diagnostic value than total T_3 concentration.

Barry, Reid *et al.* (1981) found serum T_4 concentrations in lambs fed on kale and given supplementary I to increase to much higher values than those found for similar animals fed on ryegrass–clover pasture, and the same trend was repeated in the present investigation. T_4 stimulates erythroetin-induced erythropoesis in human and mouse bone marrow cells with a potency slightly greater than T_3 (Chopra & Solomon 1980), and the high T_4 concentrations in sheep with haemolytic anaemia may serve to stimulate synthesis of replacement erythrocytes. Other specific functions of T_4 can be assessed by comparing data

from the two I-supplemented groups, which had similar serum T_3 concentrations. Increased heart weight, heart cytochrome oxidase content and increased erythrocyte GSH content were all associated with the elevated serum T_4 concentration of kale-fed lambs. Injections of T_4 stimulate heart hypertrophy in rats (Goulding *et al.* 1976), and in muscle T_4 stimulates synthesis of hexokinase (Smith & Williams-Ashman, 1951). Hexokinase activity may limit glucose metabolism in erythrocytes, including GSH regeneration. Thus it is possible, but not proven, that the protection afforded against haemolytic anaemia by I supplementation in kale-fed lambs may be mediated more by T_4 than by T_3.

Dietary requirements for I

Based on measurements of T_4 secretion rate (TSR), the Agricultural Research Council (1980) estimated I requirements of all classes of ruminants to be safely met, in the absence of goitrogens, by diets containing 500 μg I/kg DM. In some instances the Agricultural Research Council (1980) suggests requirements may be met at dietary concentrations as low as 150 μg/ I/kg DM, although they considered this should be verified by further experimentation. Such verification can be provided by the control (i.e. non-I-supplemented) animals grazing pasture, both for lambs (present study) and for growing cattle (Barry, Reid *et al.* 1981). Because of its good relationship to production traits in both studies, it is considered that I requirements should be based on the ability to maintain serum T_3 concentrations rather than on TSR. On this basis a dietary concentration of 270 μg/kg DM was adequate for growing lambs (present study) and 180 μg/kg DM was adequate for growing cattle (Barry, Reid *et al.* 1981). Neither was adequate for optimum T_4 production or to prevent mild thyroid enlargement (cattle experiment only), but as serum T_3 concentration was not increased by I supplementation in either experiment it is apparent that T_3 synthesis from T_4 was not impaired at these dietary I concentrations.

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