THE ROLE OF SELENIUM IN GRAZING RUMINANTS

A thesis presented in partial fulfilment
of the requirement for the
Degree of Doctor of Philosophy
at Massey University

Jeffrey Jay Wichtel

1995

SUMMARY

The objective of this research was to further the understanding of the production-limiting effects of selenium deficiency in grazing ruminants. The potential roles for selenium in antioxidant function, thyroid hormone metabolism, somatotropic function and appetite were investigated.

Initially, a series of field trials involving three levels of selenium treatment of dairy cattle was performed. Improvements in growth rate and milk production were recorded, however reproductive performance was not affected. The results of these trials support the use of the currently recommended selenium reference ranges for prediction of growth and milk production responses to supplementation of cattle.

A study was performed to evaluate the vitamin E status and polyunsaturated fatty acid intake of grazing cattle. Spring-calving dairy heifers managed under the conditions typical of New Zealand dairy farms did not appear to be at risk of periparturient disease resulting from high polyunsaturated fatty acid intake. These findings suggest that abnormal peroxide metabolism is unlikely to be an important mechanism in the impaired performance of selenium deficient adult cattle in New Zealand. Detailed examination of uterine involution and the events leading up to the first postpartum ovulation confirmed that supplementation with selenium, α-tocopherol, or both, does not affect postpartum reproductive function in first parity dairy cows fed pasture.

The effects of selenium supplementation on thyroid hormone metabolism, somatotropic function and growth were investigated. The results suggest that the adverse effects of selenium deficiency on calves may be mediated by altered thyroid hormone metabolism, but not through modulation of peripheral concentrations of growth hormone. Using Angora goat kids as a model, interactions between selenium and iodine supplementation were examined. The results suggest that selenium supplementation improves liveweight gain but that interactions between selenium and iodine status are of little importance in kids fed a diet moderately deficient in both selenium and iodine.

The effect of selenium supplementation on the voluntary feed intake of Angora goats was investigated. The results of this study suggested that selenium supplementation of kids does not affect the short-term intake of a selenium-deficient diet. It is concluded that increased appetite is unlikely to be the primary mechanism involved in the growth response to selenium supplementation in Angora goats.

Current concepts concerning the role of selenium deficiency in grazing ruminants are discussed and recommendations are made for future research.

ACKNOWLEDGMENTS

I thank my supervisor, Professor Norm Williamson, and the Department of Veterinary Clinical Sciences for providing me with the opportunity to combine the duties of research, teaching and clinical service more harmoniously than I ever envisaged. I am indebted to he and my other supervisor, Keith Thompson, for their support and scholarly advice throughout my doctoral studies.

Many friends and colleagues contributed to the research endeavour described in this thesis with Louise Craigie and Douglas Freeman deserving special mention. I am very grateful for their time, thought, friendship and undying sense of humour under conditions of duress. Thanks are also due to the following: Hugo Varela-Alvarez for statistical assistance; Alyson Illingworth, Robin Whitson, Laura Jacobson, Helen Hodge, Heidi Ward, Angus Fordham Yvette Cottam and Vanessa Tilson who provided technical assistance; and Stuart McCutcheon, John Bass, Margaret Scott and Gary Bentley who provided advice and assistance with assays.

Chris Russell and farmer managers Mark Power and Andrew Jull provided access to experimental animals of high quality for many of these projects, often at considerable inconvenience for their staff.

Funding of these studies was provided to a large degree by the New Zealand Lottery Grants Board and various Massey University and Departmental research funds. Additional funding was provided by Mallinckrodt Veterinary New Zealand Limited and Rhône Merieux.

Finally, my deepest appreciation goes to my wife, Maureen, and my daughters, Jocelyn and Nicola. Their love, encouragement and support made completion of this thesis possible.

To my parents, Patricia and Stuart, who helped their children discover the joy of learning and taught them that no goal is beyond their reach; and to my colleagues in agriculture and veterinary science who have dedicated their lives to perfecting the art and science of pastoral animal agriculture

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CHAPTER 1

Introduction

Introduction

Selenium (Se) deficiency has been recognised for over 30 years as a factor which limits production in grazing livestock. Diseases associated with selenium deficiency in New Zealand include nutritional myodegeneration (white muscle disease) of lambs, calves and goats, infertility of ewes and ill-thrift of sheep and cattle. Although white muscle disease is the most recognisable manifestation of deficiency, sub-clinical deficiencies are more common. Such deficiencies result in decreased growth rate, milk production and lambing percentage (Grace, 1994). The wide distribution of soils that are deficient in selenium (Watkinson, 1983) means that Se deficiency and its amelioration has considerable economic importance to New Zealand. Also of importance is lost revenue due to inappropriate supplementation leading to unnecessary expense and, on occasion, losses due to toxicity. There is a need to avoid excessive treatment to minimise the risk of selenium concentrations in ruminant liver and kidney tissue exceeding the acceptable concentration for human consumption.

The development of reference ranges for the prevention of ill-thrift and myodegeneration in young stock has been the primary aim of selenium research in New Zealand to date. This has left many other aspects of selenium and its role in grazing ruminants to be elucidated. For example, there are few data on which to base reference ranges for adult dairy cattle. This lack of information was an important impetus for the studies presented in this thesis.

Despite the accumulation of evidence linking selenium deficiency to disease, little is known concerning the biological role of selenium in mammals. The traditional model suggests that Se, as a component of the enzyme glutathione peroxidase, acts with vitamin E and other antioxidants as a peroxidation antagonist in cells throughout the body. This model seems reasonable for many of the disorders involving perturbation of cell membranes by free radicals (myopathies and liver necrosis, for example) but is less attractive in explaining how a lack of selenium causes the ill-thrift, infertility and lowered production commonly noted in ruminants grazing pastures which are deficient in selenium. Growth rate responses to selenium supplementation commonly occur in grazing calves in

the absence of signs of myodegeneration (McMurray *et al.*, 1983), suggesting the existence of a mechanism which does not involve abnormal tissue peroxidation. The need for a better understanding of the biological role of selenium in grazing ruminants led to the experimental work presented in Chapters 3 to 10. In these studies, the potential roles for selenium in antioxidant function, thyroid hormone metabolism, somatotropic function and appetite were investigated.

Because this work was performed as a sequence of studies, all of which are either in the process of publication or have already been published, this thesis is presented in the form of a series of discrete papers. References have been combined into a single bibliography.

CHAPTER 2

Review of the Literature

Selenium as an Essential Micronutrient

Selenium was established as an essential micronutrient in 1957 (Schwarz and Foltz, 1957) and later identified as an integral component of the cytosolic enzyme glutathione peroxidase (Rotruck *et al.*,1973) which is found in a wide range of tissues and animals. Closely following the discovery that selenium could protect against liver necrosis in rats fed a vitamin E-deficient diet, relationships between selenium deficiency and many diseases were established. These included hepatosis dietetica in pigs (Eggert *et al.*,1957), exudative diathesis in chickens (Patterson *et al.*, 1957) and nutritional myodegeneration in lambs and calves (Muth *et al.*1958; McLean *et al.*, 1959). All of these diseases responded to both vitamin E and selenium, suggesting to researchers that a relationship existed between these two nutrients. Since then many clinical disorders and syndromes have been described as responsive to either selenium or vitamin E or both, and those specific to ruminants are discussed below.

The many manifestations of selenium deficiency would seem to rule out a common mechanism for the selenium-responsive diseases. However, considerable evidence exists that a key mechanism is the peroxidation of the lipid of cellular membranes. The lipid peroxidation model suggests that selenium, vitamin E and other antioxidants act as peroxidation antagonists while other factors, such as dietary polyunsaturated fatty acids, act as protagonists. The resulting damage depends on the tissue's inherent ability to control these factors (McMurray and Rice, 1982). The modulation of immune function by selenium (Spallholz *et al.*, 1990) and its role in the metabolism of thyroid hormones (Berry *et al.*,1991) are additional or alternative mechanisms through which selenium might influence livestock health and production.

Selenium Availability and Metabolism

The availability and metabolism of selenium in domestic animals has been reviewed by Underwood (1977) and more recently by Grace (1994). Selenium intake in grazing ruminants is largely determined by the selenium content of soils on which they are grazing. About 30% of farmed land in New Zealand is considered to be selenium-deficient, with

soil selenium concentrations of less than 0.5 mg/kg air dried weight (Watkinson, 1983). The most deficient soils consist of rhyolitic pumice in the central volcanic plateau of the North Island. Peat soils in the Waikato River valley are also deficient. North Island coastal sands and stony soils in several locations are considered to be selenium-responsive, while most of the South Island is at least marginally deficient (Watkinson, 1983).

Plants vary in their uptake of selenium but selenium is not a requirement for plant growth. Using a factorial approach, Grace (1994) calculated the selenium requirement for sheep to be between 24 and 34 μg/kg of dry matter intake and for cattle between 35 and 44 μg/kg of dry matter intake. The selenium content of New Zealand pastures ranges from 5 to 70 μg/kg of dry matter, but is likely to be lower during periods of rapid pasture growth (Grace, 1994).

Selenium from ingested pasture is absorbed primarily in the duodenum, becomes associated with plasma proteins and is transported to the liver where it may become incorporated in selenoproteins (Symonds *et al.*, 1981). The principal route of excretion in ruminants is the faeces, however selenium is also expelled from the body via the lungs (Underwood, 1977). Selenium readily crosses the placenta and can enter the mammary gland. Thus changes in maternal selenium status tend to be reflected in the foetus and neonate (Abdelrahman and Kincaid, 1995).

The Biological Functions of Selenium

Glutathione peroxidase

Glutathione peroxidase and tissue peroxidation

The biological function of selenium in mammals has been studied mainly in the context of its antioxidant role, protecting tissues from oxidative degeneration. Selenium-dependant glutathione peroxidase (Se-GSHPx) is a selenoenzyme that reduces cytosolic peroxides (Tappel, 1984). Recently it has been demonstrated that there is more than one form of selenium-dependant glutathione peroxidase in tissues (Ursini *et al.*, 1985). The novel form, termed phospholipid hydroperoxide glutathione peroxidase (PLGSHPx), is associated with lipid membranes where it is capable of metabolising membrane phospholipid

hydroperoxides. Thus, the selenium-containing peroxidases appear to provide a system to protect cell membranes and cytosol from oxidative damage.

Each molecule of the enzyme glutathione peroxidase contains 4 atoms of selenium (Flohe *et al.*, 1973). Specifically, this enzyme catalyses the reduction of hydrogen peroxide, and other organic peroxides, following the general reactions depicted in Figure 2.1.

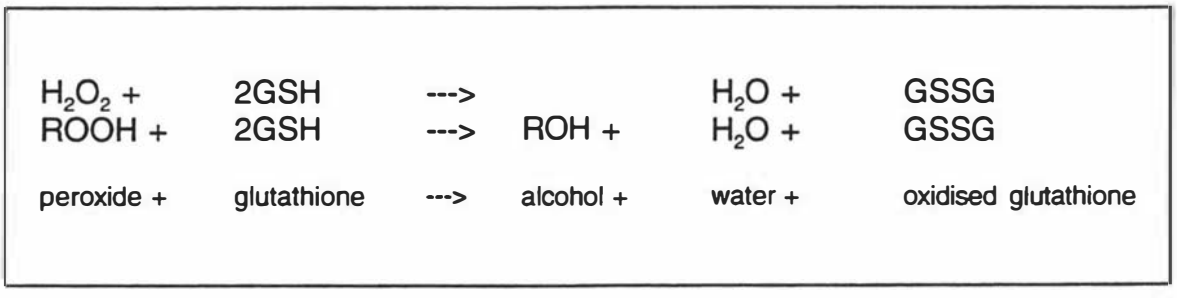

Figure 2.1. The general reactions involved in the reduction of hydrogen peroxide and organic peroxides by glutathione peroxidase.

In vitro the enzyme is relatively non-specific, reducing lipid and prostaglandin hydroperoxides as well as hydrogen peroxide (Flohe, 1983). It appears that glutathione peroxidase acts in conjunction with vitamin E and other antioxidants to prevent peroxidation of cellular microsomes (Scott *et al.*, 1974). Vitamin E has potent antioxidant properties, and selenium and vitamin E can exert a sparing effect on each other, at least in some situations (Scott *et al.*, 1974). Evidence for the antioxidant role of vitamin E is reviewed by Rice and Kennedy (Rice and Kennedy, 1988). In certain tissues, such as liver, there is a non-selenium-dependant glutathione peroxidase present in addition to the selenium-dependant enzyme (Scholz *et al.*,1981). Vitamin C, copper, zinc and β-carotene also appear to be involved in antioxidant systems but specific interactions are unclear. This large arsenal of antioxidants may mean deficiency in any one will not necessarily result in tissue damage.

Glutathione peroxidase and the arachidonic cascade

Evidence for the involvement of glutathione peroxidase in the arachidonic acid cascade has accumulated in recent years (Spallholz *et al.*,1990). Eicosanoids (products of arachidonic metabolism) are important mediators of immune and reproductive function (Spallholz *et al.*,1990; Thatcher, 1988). Anti-inflammatory effects attributed to selenium supplementation are likely to be mediated in part by glutathione peroxidase, as a result of its reduction of hydroperoxide and enhancement of eicosanoid synthesis (Spallholz *et al.*,1990).

Glutathione peroxidase could have roles in the production of $PGF_{2\alpha}$ from arachidonic cascade intermediaries such as PGH_2 and PGG_2 (Hong *et al.*,1989). The apparent relationship between selenium-dependant glutathione peroxidase and prostaglandin synthesis suggests several mechanisms by which selenium deficiency could alter reproductive function. It could be hypothesised that prostaglandin production in selenium-deficient animals is altered either in quantity produced by reproductive tissues, or by a change in the ratio of prostaglandins from different series (E series or F series) produced by these tissues. Many conditions that have been related to selenium deficiency (e.g. retained placenta (Julien *et al.*,1976), poor uterine involution (Harrison *et al.*,1986)) have also been related to alterations in $PGF_{2\alpha}$ production in dairy cattle (Thatcher, 1988; Wichtel, 1991), lending circumstantial evidence in support of this hypothesis.

Glutathione peroxidase, phagocytosis and the immune response

Decreased glutathione peroxidase activity in phagocytic cells has been reported in selenium-deficient heifers (Boyne and Arthur, 1979) and selenium deficiency has been associated with lower than normal bactericidal capacity of bovine neutrophils for *Candida albicans*, *Staphylococcus aureus* and *E.coli* (Boyne and Arthur, 1979; Gyang *et al.*, 1984)). Later studies have confirmed that leucocytes from cattle deficient in selenium have depressed microbicidal activity (Arthur and Boyne, 1985; Hogan et al., 1990) and that lymphocyte proliferation is inhibited (Yu-Zhang *et al.*,1992). Several studies, reviewed by Spallholz *et al* (Spallholz *et al.*,1990), have shown that selenium deficiency in animals leads to reduced glutathione peroxidase activity in phagocytic cells, release of increased amounts of H_2O_2 by macrophages and, in some instances, reduced cytotoxicity. In addition to its effects on inflammation, studies have suggested that humoral and cell-mediated

immunity may also be influenced by selenium supplementation (Spallholz *et al.*,1990). The mechanisms whereby selenium affects the immune response have not been firmly established, and the clinical significance of these findings is unclear at present.

Thyroid hormone 5′-deiodination

The discovery that type I iodothyronine 5′-deiodinase is a selenocysteine-containing enzyme (Berry *et al.*,1991) has led to renewed interest in the role of selenium in mammals. The deiodinases (types I and II) are enzymes responsible for the deiodination of L-thyroxine (T_4), converting it to its active form, 3,5,3′-triiodo-L-thyronine (T_3) (Leonard and Visser, 1986). Type I is the major deiodinase in liver, kidney and skeletal muscle, while type II is the major deiodinase in brain, pituitary and brown adipose tissue. It appears that only the type I enzyme contains selenium.

Changes in selenium status are reflected in plasma thyroid hormone concentrations. Peripheral concentrations of total thyroxine were higher and concentrations of total triiodothyronine lower in calves fed a synthetic selenium-deficient diet when compared to calves given the same diet supplemented with selenium (Arthur *et al.*, 1991a). Thyroid hormones are important in the control of growth hormone synthesis in the pituitary (Koenig *et al.*,1987), suggesting a role for selenium in growth that is independent of its function as a component of the enzyme glutathione peroxidase.

There is evidence that combined selenium and iodine deficiencies have important metabolic consequences (Goyens *et al.*,1987; Arthur, 1991). Deficiency of either of these nutrients has been shown to decrease thyroid iodine, thyroxine and triiodothyronine concentrations in rats, suggesting that selenium deficiency could exacerbate the effects of low iodine intake (Beckett *et al.*, 1993). Impaired thyroid hormone metabolism in the pituitary and depletion of thyroid iodine in selenium deficiency may be the biochemical basis for an interaction between selenium and iodine. Iodine deficiency of New Zealand livestock is recognised (Andrews and Sinclair, 1962) and is most likely to occur on soils of the low rainfall inland regions of Otago, Canterbury and Marlborough, and the pumice soils of the North Island. These are regions also known to be selenium-deficient.

In humans, a selenium/iodine interaction has also been suggested (Corvilain *et al.*, 1993). Selenium deficiency has been postulated to influence the outcome of iodine deficiency in areas of endemic goitre through its role in thyroid hormone metabolism, or through impaired peroxide metabolism in the thyroid gland.

Other roles for selenium

Several selenoproteins distinct from glutathione peroxidase and 5′-deiodinase have been identified. These include a protein isolated from the sperm of rats and cattle, and selenoprotein P isolated from rat liver and plasma. Read *et al.* (1990) studied selenoprotein P, the major selenoprotein of rats. It comprises 60% of the plasma selenoprotein in this species. When dietary selenium was limiting, selenoprotein P content was maintained better than liver and plasma glutathione peroxidase activity. This suggests selenoprotein P takes precedence over glutathione peroxidase in utilising selenium within the liver.

Ewan (1976) provided evidence that in selenium-deficient rats impaired growth could be partly explained by a direct depression of voluntary feed intake. Selenium deficiency may alter appetite via a mechanism which does not involve selenium-dependant glutathione peroxidase and type I 5′-deiodinase. In ruminants, however, feed intake after supplementation with selenium has not been investigated.

The Diagnosis of Selenium Deficiency

Intake of selenium is reflected in levels of selenium in various animal tissues, the highest concentrations occurring in liver and kidney. The enzyme glutathione peroxidase is present in several tissues including red blood cells, spleen, cardiac muscle, brain, thymus, adipose tissue and striated muscle (Scholz *et al.*, 1981). Tissue glutathione peroxidase activity is closely related to selenium intake and is a useful and convenient indicator of selenium intake in the grazing ruminant when selenium intake is relatively stable (Thompson *et al.*, 1981). Selenium-dependant glutathione peroxidase contained in red blood cells is responsible for most of the glutathione peroxidase activity in whole blood. Because of the lifespan of red blood cells, changes in their selenium content, and that of whole blood, lags

behind serum and plasma selenium concentrations by several weeks following altered dietary intake (Thompson *et al.*, 1980). Thus whole blood selenium concentration and glutathione peroxidase activity reflect selenium intake during the period 2 to 4 months preceding the sampling whereas serum or plasma selenium concentrations reflect recent selenium intake. There is no single test which can be considered superior in all situations because the method of choice depends on the objective of the testing procedure (Table 2.1). For example, where non-continuous supplementation methods are employed (e.g. a single injection of sodium selenate) measurements of duration of effect based on whole blood selenium or glutathione peroxidase will tend to over-estimate the duration of effect. Serum and plasma selenium should be used in this situation because selenium retained in red blood cells is not likely to be useful in preventing selenium-responsive conditions.

As a measure of selenium status in clinical research, glutathione peroxidase activity is less useful than selenium concentration in blood constituents. This is because the enzyme activity is difficult to compare between studies as the assay conditions vary between laboratories.

The samples used for measurement of selenium status are generally those that can be conveniently obtained from the animal. These include serum, plasma, erythrocytes, whole blood or liver selenium and serum, plasma, erythrocytes, whole blood or liver glutathione peroxidase. In cattle, serum and plasma concentrations of selenium and whole blood glutathione peroxidase activity are commonly used in the diagnosis of selenium deficiency (Thompson *et al.*, 1981) whereas in sheep, liver concentrations of selenium in culled animals are used most frequently. Although there is a close relationship between soil, plant and tissue concentrations of selenium, factors such as soil characteristics and pasture species will influence the uptake of selenium by plants. Some of these factors are reviewed by Grace (1994). The reference ranges for predicting responses to selenium supplementation are based on animal tissues and therefore soil and plant concentrations are less useful in the diagnosis of deficiency.

Table 2.1 **Recommended sampling procedures to be used in the diagnosis, monitoring and treatment of selenium deficiency in cattle[a].**

Reason for investigation	Time to sample	Age of animals to be sampled	Sample type	Sample number	Interpret-ation
Poor performance	At the time of the problem	The affected animals	EDTA blood	Three	Means only
Farm deficiency	Late spring or early summer	Unsupplemented lactating cows, rapidly growing calves	EDTA blood	Three	Means only
Assess reserves	Late winter or early spring	Unsupplemented lactating cows, rapidly growing calves	EDTA blood	Three	Means only
		If supplemented:	Serum/ liver	Ten	Individual values
Supplement-ation effectiveness	At the half-way point between planned treatments	Animals being supplemented	Serum/ liver	Ten	Individual values compared with previous samples

[a] From Clark and Ellison (1993).

Response trials and reference ranges

Several clinical and sub-clinical conditions of ruminants have been shown to be selenium-responsive. For extensive reviews refer to Underwood (1977), Hurley and Doane, (1989) and Ellison (1992). The selenium-responsive conditions reported include the following:

- Ill-thrift in lambs and calves
- Lowered milk production in cows
- White muscle disease in lambs, calves and goat kids
- Lowered fertility and embryonic death in sheep and perhaps cattle
- Metritis, poor uterine involution and cystic ovaries in cows
- Retained foetal membranes in cows
- Sub-clinical mastitis and impaired immune function in cattle
- Prematurity, perinatal death and abortion in cattle

Of these, only ill-thrift, lowered fertility, lowered milk production and white muscle disease have been reported in New Zealand literature (Ellison, 1992). Fertility responses have been documented most conclusively in ewes (Andrews, 1968) whereas there is little evidence of improved herd fertility following supplementation of New Zealand cattle (Tasker *et al.*, 1987). Notably absent from New Zealand literature are reports of selenium-responsive retained placenta, metritis and mastitis.

Table 2.2. Selenium reference ranges related to growth of young cattle[a].

	Whole blood (nmol/L)	Serum (nmol/L)	Glutathione peroxidase (kIU/L)	Liver (nmol/kg)
Responsive	<130	<52	<0.5	<380
Marginal	130-250	52-100	0.5-2.0	380-650
Adequate	>250	>100	>2.0	>650

[a] From Ellison (1992).

The recommended blood selenium reference ranges for New Zealand livestock (Ellison and Feyter, 1988) have been based on a limited number of published studies, reviewed by Ellison (Ellison, 1992). The most complete data is from growth trials in calves (Table 2.2) and lambs (Table 2.3). Values that fall in the marginal and deficient ranges indicate that the animals in question are at risk of disease, but do not necessarily guarantee a clinical or economic response to supplementation.

It has been suggested (Macky, 1991) that responses may occur when selenium is supplemented to achieve blood concentrations much higher than those currently recommended for cattle. This assertion is supported by the fact that recommended minimum blood selenium levels for cattle in New Zealand fall well below those accepted overseas (Julien *et al.*,1976; Smith *et al.*, 1988). However this assertion has not been tested in clinical response trials. The scarcity of trials involving supplementation of adult cattle is surprising considering the widespread use of selenium supplements in dairy cows.

Table 2.3. Selenium reference ranges related to growth of lambs[a].

	Whole blood (nmol/L)	Liver (nmol/kg)
Responsive	<127	<250
Marginal	128-250	250-450
Adequate	>250	>450

[a]. From Ellison (1992).

Protocols for Selenium Supplementation

A comprehensive review of supplementation options available to New Zealand farmers has been recently published (Tasker, 1992). Although many methods of supplementation are efficacious, they can differ widely in their cost and convenience. Dose rates and effective duration for selected selenium supplements for adult cattle are presented in Table 2.4.

Table 2.4. Dose rates and duration of effect for selected selenium supplements for adult cattle[a].

Product	Dose rate	Dose rate	Effective duration
Drench (sodium selenate)	10-30 mg	0.05 mg/kg	2-3 weeks
Daily drench (sodium selenate)	1-8 mg/d		
Deposel® injection (barium selenate)	1 ml/50 kg	1 mg/kg	>12 months
Injection (sodium selenate)	1-5 ml	0.1 mg/kg	6-8 weeks
Permasel® (selenium/iron pellet)	2 pellets	6 g/animal	12 months
Selpor® (pour-on)	10-15 ml	1.5 ml/50 kg	>8 months
Topdressing	1kg prills/ha	10 g/ha	12 months

[a] Adapted from Tasker (1992).

Aspects Requiring Further Research

This review raises several questions relating to the role of selenium in grazing ruminants that require further investigation. For example:

1. Are the selenium reference ranges used for New Zealand cattle reliable, given that they are considerably lower than those quoted for more intensively managed cattle overseas? This is addressed in Chapter 3.

2. What are the vitamin E and polyunsaturated fatty acid intakes of grazing adult dairy cattle? Are the selenium-responsive disorders noted in adult dairy cattle likely to be mediated by abnormal peroxide metabolism? These questions are examined in Chapter 4.

3. Is reproductive function in grazing dairy cattle influenced by selenium or vitamin E supplementation? This is addressed in Chapter 5.

4. Could alterations in thyroid or somatotropic function provide an alternative mechanism for selenium-responsive subclinical disease in grazing ruminants? This is the subject of the study presented in Chapter 6.

5. Are there interactions between selenium and iodine supplementation in ruminants fed diets deficient in both of these nutrients? The possibility of such interactions was examined using Angora goats as a model and the results are presented in Chapter 7.

6. Could the effects of selenium deficiency in ruminants be mediated by a direct depression of appetite, as has been suggested in other species? This was the subject of the study presented in Chapter 9 .

CHAPTER 3

The effect of intra-ruminal selenium pellets on growth rate, lactation and reproductive efficiency in dairy cattle[1]

[1] Published as: Wichtel JJ, Craigie AL, Varela-Alvarez H, Williamson NB. New Zealand Veterinary Journal 42, 205-10, 1994.

Abstract

In each of two dairy herds (A and B), rising yearling heifers (Trial 1) and adult cows (Trial 2) were assigned to three treatment groups. Untreated animals were compared to animals treated with either two or four intra-ruminal pellets containing 3 g of elemental selenium. The administration of pellets at the recommended dose (two pellets per animal) was effective in elevating whole blood glutathione peroxidase activity and selenium concentration to over 10 times those of control animals.

In Trial 1, a 15% response in liveweight gain ($P<0.001$) occurred in yearling heifers in the herd with the lowest pre-treatment selenium status. In Trial 2, cows receiving two pellets produced a greater milk volume ($P=0.06$) and more milk solids ($P=0.02$) than untreated controls; an increase in volume of 5.4% and 8%, and in milk solids of 6.5% and 6.4%, were noted in herds A and B respectively. There was a trend towards decreasing somatic cell counts in milk from the treated cows when compared to controls, the four-pellet group in Herd A and the two-pellet group in Herd B being significantly different from their respective control group. No between-group differences were noted in calving-first service or calving-conception intervals, nor in the proportion of animals pregnant to first or all services. The administration of selenium at twice the recommended dose rate yielded no additional response above that noted after the administration of the recommended dose.

The results of this study support the use of currently recommended Ministry of Agriculture and Fisheries selenium reference ranges in cattle for the prediction of a response to supplementation.

Introduction

The recommended blood selenium levels for cattle in New Zealand (Ellison and Feyter, 1988) have been based on a limited number of published studies, recently reviewed by Ellison (1992). The scarcity of trials involving supplementation of adult cattle is surprising considering the widespread use of selenium supplements in dairy cows. To date, research performed in New Zealand herds has implicated selenium deficiency as a cause of ill thrift (Fraser and Wright, 1984), white muscle disease (Andrews *et al.*, 1968) and reduced milk production (Hupkens van der Elst and Watkinson, 1980; Fraser *et al.*, 1987). Notably absent from this list are retained placenta and mastitis, diseases recognised by overseas researchers as selenium-responsive (Julien *et al.*,1976; Smith *et al.*, 1984; Weiss *et al.*, 1990; Ndiweni *et al.*, 1991; Braun *et al.*, 1991). More recently, New Zealand researchers have failed to demonstrate lactational or reproductive responses to selenium in herds considered marginally deficient (Clark *et al.*, 1992; Whelan *et al.*, 1992).

It has been suggested (Macky, 1991) that responses may occur when selenium is supplemented to achieve blood concentrations much higher than those currently recommended. This assertion is supported by the fact that recommended minimum blood selenium levels for cattle in New Zealand fall well below those accepted overseas (Julien *et al.*, 1976; Smith *et al.*, 1988). However, because of the limited number of trials performed, specific recommendations for adult cattle have been difficult to formulate.

The aims of this study were to examine the effect of intra-ruminal selenium-containing pellets (Permasel®: Mallinckrodt Veterinary, Upper Hutt) on the glutathione peroxidase activity and selenium concentration of whole blood, and to examine the effect of two different levels of selenium supplementation on growth rate, reproduction and lactation in grazing dairy cattle.

Materials and Methods

The selection of the two commercial spring-calving dairy herds used in this study was based upon a previous history of marginal to deficient selenium status. The mean whole blood glutathione peroxidase activity of control animals measured bimonthly over the study period in Herd A ranged from 0.3 to 1.4 kIU/L (mean 0.88 kIU/L) and in Herd B from 0.6 to 2.7 kIU/L (mean 1.46 kIU/L). An adequate selenium status is currently considered to be reflected by a glutathione peroxidase activity greater than 2.0 kIU/L, a marginal status by an activity of 0.5-2.0 kIU/L and a low status by an activity less than 0.5 kIU/L (Ellison and Feyter, 1988).

The herds consisted of Friesian cows and heifers grazing predominantly ryegrass and clover pasture, or supplemented with pasture hay harvested from the farm. Both herds were farmed on heavy clay soils known to be marginally deficient in selenium, with the pasture selenium content varying between 0.02 and 0.03 μg/kg. The copper content of liver and serum samples previously collected from cattle in Herd A indicated copper deficiency (Ellison and Feyter, 1988). All cattle in Herd A received 676 mg of calcium copper edetate (Coprin®: Mallinckrodt Veterinary, Upper Hutt) subcutaneously three times annually. The copper status of Herd B was adequate (Ellison and Feyter, 1988), so no copper supplementation was performed. The calves in Herd A were treated with 4.5 mg/kg liveweight of oxfendazole (Systamex®: Mallinckrodt Veterinary, Upper Hutt) every 4 to 6 weeks, while the calves in Herd B were treated with 8 mg/kg liveweight of levamisole (Nilverm®: Mallinckrodt Veterinary, Upper Hutt) every 4 weeks.

The 30 g intra-ruminal pellets used in this study contain 10% elemental selenium in a finely divided matrix of iron. It is claimed that selenium is released at a minimum rate of 3 mg per day (Marchant, 1991). The recommended dose for heifers and cows is two pellets repeated annually.

Trial 1

During July and August 1991, 70 rising 1-year-old heifer calves in Herd A and 92 rising 1-year-old heifer calves in Herd B were randomly assigned to one of the following three

treatment groups: control group, no treatment; low-dose group, two pellets orally; and high-dose group, four pellets orally.

The liveweight of the heifers was monitored approximately every two months using electronic scales (AG500®: Tru Test, Hamilton). The height of the heifers at their withers was measured using a height stick (Altitude Stick®: Adrian J. Paul, Duncan, Oklahoma) at start, midpoint and end of the study. Rectal palpation of the reproductive tract was performed 16 weeks after the start of mating for the diagnosis of pregnancy and estimation of conception dates. Data collection continued for 11 months and heifers were included in the analysis only if they remained in the herd until the end of the study.

Trial 2

During July 1991, 294 non-lactating cows in Herd A and 142 non-lactating cows in Herd B were assigned to one of three treatment groups using stratified random sampling with age as a blocking factor. Treatments were as for Trial 1: control group, no treatment; low-dose group, two pellets orally; and high-dose group, four pellets orally.

Milk production records and individual somatic cell counts were obtained from herd test records (Livestock Improvement Corporation, Hamilton) and the data used were derived from the monthly herd tests conducted during December and April. The planned start of calving was August 1. Data collection occurred up to the final herd test but cows were included in the analysis if they remained in the herd until the December herd test. Somatic cell count data were geometric means of all cell counts performed during the current lactation. There were no fewer than three herd tests for any cow. Pregnancy was diagnosed in each cow by rectal palpation at between 5 and 15 weeks after mating. Reproductive and health data were entered in a computer program for dairy herd data recording and analysis (DairyMAN® Version 5.1: Massey University, Palmerston North).

Blood analyses

In both trials, blood was collected into evacuated tubes containing EDTA anticoagulant after puncture of the tail vein. A random sample of five animals per group was chosen on each bleeding day for sampling pre-treatment, and about every 2 months thereafter.

Assay of glutathione peroxidase activity (Paglia and Valentine, 1967; Board and Peter, 1976) and selenium concentration (Watkinson, 1979; Watkinson and Brown, 1979) of whole blood was performed by the Ministry of Agriculture and Fisheries Animal Health Laboratories in Palmerston North and Hamilton respectively. Glutathione peroxidase activity is presented in katal units per litre (IU/L). One katal unit is that amount of enzyme activity which brings about oxidation of NADPH at the rate of 1 mole per second.

Statistical analysis

Data was analysed using analysis of variance and covariance, utilising the general linear model procedure of SAS® (SAS User's Guide, 1988a). Herd and age were considered random effects and treatment group a fixed effect. The effect of treatment was nested within herd. Interaction between treatment group and age was tested but was not significant for any dependant variable. Means reported are least square mean ± standard error of the mean. In Trial 1, means were adjusted by using initial live weight and initial withers height as covariates. In Trial 2, date of calving, previous production index and previous mean individual somatic cell count were used as covariates. Pairwise comparison of the least square mean of each treatment group to its control was performed using Student's *t*-test. Association between treatment group and categorical variables was tested by applying analysis of variance to ranks of each variable, which is equivalent to the Kruskal-Wallis k-sample test (SAS User's Guide, 1988b). Least square means of raw somatic cell count data are reported, but cell counts were analysed using both raw and $\log_{10}$-transformed data.

Results

For 2 months following the administration of pellets to treated cattle, glutathione peroxidase activity and selenium concentrations increased steadily and remained high to the end of the monitoring period, 11 months after administration (Table 3.1). The means presented were calculated for the period 2-11 months after administration.

Table 3.1. Comparison of glutathione peroxidase activity and selenium concentration of whole blood in cattle from two herds receiving 0, 2 or 4 intra-ruminal selenium pellets. Values are means for the period 2-11 months after-administration[a]

	Herd A			Herd B		
Trial	Control	Low	High	Control	Low	High
Trial A: Heifers						
GSHPx[b]	0.7 ± 0.1	9.8 ± 0.9	18.3 ± 1.2	1.3 ± 0.1	10.6 ± 1.4	16.4 ± 2.2
Selenium[c]	135 ± 9	1005 ± 77	1870 ± 96	181 ± 11	944 ± 123	1405 ± 178
Trial B: Cows						
GSHPx[b]	0.7 ± 0.1	15.0 ± 1.5	29.0 ± 1.6	1.5 ± 0.2	22.2 ± 1.2	23.6 ± 1.1
Selenium[c]	128 ± 9	1180 ± 119	2550 ± 165	273 ± 29	2293 ± 175	3107 ± 152

[a] In every case, values for treated animals are significantly different from untreated controls ($P<0.0001$).
[b] Glutathione peroxidase activity in whole blood (kIU/L).
[c] Selenium concentration in whole blood (nmol/L).

Selenium pellets administered at the recommended dose and high dose raised mean blood glutathione peroxidase activity and selenium concentration to levels above those currently considered adequate (Ellison and Feyter, 1988). These trials were not designed to test for pellet regurgitation, but regurgitation was neither observed nor suspected from the assays for selenium and glutathione peroxidase in the treated animals.

Trial 1

A 15% response in liveweight gain was noted in heifers in Herd A treated with either two or four pellets ($P<0.001$), whereas no growth response was noted in the treated heifers in Herd B (Table 3.2). A trend towards increasing gain in withers height in the treated animals was noted in Herd A ($P=0.2$) but not in Herd B. Pregnancy rate and start of mating-conception interval were not affected by treatment.

Trial 2

An analysis of milk production figures (Table 3.3) over the entire lactation showed that cows receiving two pellets produced a greater milk volume ($P=0.06$) and more milk solids ($P=0.02$) than untreated controls. An increase in volume of 5.4% and 8%, and in milk solids of 6.5% and 6.4%, were noted in herds A and B respectively. Interactions between treatment group and age were not significant. Milk production for the high-selenium group (four pellets) was not significantly different from values for the control group. Lactation-to-date figures for December, summarising the first 5 months of lactation, were also analysed (not presented). These results reflected the trend to increased milk solids production in the two-pellet group ($P<0.2$).

The incidence of clinical mastitis (3% overall) did not differ between groups, but there was a trend towards decreasing somatic cell counts in the treated cows compared to the controls (Table 3.3). The four pellet group in Herd A, and the two pellet group in Herd B were significantly different from their respective control group ($P<0.05$).

No between-group differences were noted in the reproductive parameters measured in the adult cows (Table 3.4). The incidence of retained placenta was less than 3% in both herds.

Table 3.2. Comparison of average daily weight gain, average daily height gain, and interval from planned start of mating to conception for Friesian replacement heifers in two herds receiving 0, 2 or 4 selenium pellets[a]

	Herd A			Herd B		
	Control	Low	High	Control	Low	High
n	23	24	23	32	27	32
Weight gain[b]	0.76 ± 0.03[y]	0.87 ± 0.01[z]	0.88 ± 0.02[z]	0.63 ± 0.01	0.63 ± 0.02	0.65 ± 0.01
Height gain[c]	0.22 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.19 ± 0.01
Pregnant(%)	95.7	87.5	95.2	97.1	86.2	96.9
Interval to conception[d]	27.7 ± 3.1	29.0 ± 2.7	23.5 ± 3.5	27.0 ± 3.0	24.3 ± 3.2	28.2 ± 2.6

[a] Values within each row not sharing the same superscript are significantly different ($P<0.001$).
[b] Average daily live weight gain (kg/day).
[c] Average daily height gain at withers (mm/day).
[d] Interval from herd start of mating date to estimated conception date (days).

Discussion

We found intra-ruminal selenium pellets to be effective in increasing blood selenium concentration and glutathione peroxidase activity in cattle. This is in agreement with the results of several overseas trials (Judson and McFarlane, 1984; McClure *et al.*, 1986; Hidiroglou *et al.*, 1985; Wilson *et al.*, 1991).

There was a 15% response in growth rate in Herd A, which had a mean control glutathione peroxidase activity of 0.88 kIU/L but there was no response in Herd B, which had a slightly higher mean control activity of 1.46 kIU/L. The results of Trial 1 fail to support the contention that the currently recommended reference ranges (Ellison and Feyter, 1988) are too low for calves. This is in agreement with Fraser and Wright (Fraser and Wright, 1990), who collated data from 22 New Zealand selenium response trials. Their results suggest that growth rate responses are unpredictable, but can be expected to occur at blood selenium concentrations of below 250 nmol/L and a glutathione peroxidase activity below 2.0 kIU/L.

Cows receiving two intra-ruminal selenium pellets in both herds produced more milk than untreated cows. Comparable milk production responses to selenium supplementation have been infrequently reported in New Zealand. Hupkens Van der Elst and Watkinson (1980) studied seven herds on Waikato peat land. Only one herd demonstrated a significant milk fat response (7.4%) to selenium supplementation. This herd had an average blood selenium concentration of 86 nmol/L in control animals, while herds that did not experience a response ranged from 110 to 200 nmol/L.

Table 3.3. Comparison of average daily milk production for the entire lactation for cows in two herds receiving 0, 2 or 4 selenium pellets[a]

	Herd A groups			Herd B groups		
	Control	Low	High	Control	Low	High
n	98	91	94	50	40	45
Litres /day	13.0 ± 0.3	13.7 ± 0.4[s]	13.2 ± 0.3	16.1 ± 0.4	17.4 ± 0.5[t]	16.7 ± 0.6
Fat /day	0.62 ± 0.02	0.66 ± 0.02[u]	0.63 ± 0.02	0.80 ± 0.01	0.85 ± 0.02[v]	0.82 ± 0.02
Protein /day	0.46 ± 0.01	0.49 ± 0.01[w]	0.47 ± 0.01	0.61 ± 0.01	0.65 ± 0.02[x]	0.62 ± 0.02
SCC[b]	235 ± 39	199 ± 28	139 ± 21[y]	235 ± 49	112 ± 17[z]	191 ± 38

[a] Within each herd, values with superscripts differ from the value for the untreated controls, with the following p-values: [s]P=0.081 [t]P=0.017 [u]P=0.008 [v]P=0.047 [w]P=0.024 [x]P=0.013 [y]P=0.020 [z]P=0.045.

[b] Somatic cell counts (x1000 cells/ml milk).

Table 3.4. Comparison of intervals from calving to first service and to conception, and summary statistics for conception for cows in two herds receiving 0, 2 or 4 selenium pellets[a]

	Herd A groups			Herd B groups		
	Control	Low	High	Control	Low	High
n	95	89	92	54	42	48
Calving-first service (days)	78.7 ± 2.3	73.6 ± 2.2	76.0 ± 2.2	73.6 ± 1.9	73.6 ± 2.9	76.0 ± 2.5
Calving-conception (days)	89.7 ± 2.6	91.3 ± 3.0	93.9 ± 2.9	92.1 ± 3.7	93.7 ± 4.6	90.4 ± 2.8
Pregnant to first service (%)	57.9	57.3	52.7	44.4	54.8	39.6
Services/conception	1.39 ± 0.08	1.38 ± 0.08	1.46 ± 0.08	1.63 ± 0.10	1.54 ± 0.11	1.63 ± 0.11
Pregnancy rate (%)	82.2	83.3	82.2	88.8	93.0	87.5

[a] Within herds, no significant differences occurred in any parameter measured.

Results from a selenium response trial (Fraser *et al.*, 1987) in twelve herds on alluvial pumice and peat soils of the Rangitaiki Plains demonstrated a milk fat response to supplementation averaging 3.7%. All herds in this trial had mean blood selenium concentrations less than 150 nmol/L prior to thestart of the trial. Recently, the results of two further trials were presented in herds considered marginal for selenium. In a herd of 120 cows, untreated cows were compared to cows supplemented with oral sodium selenite, a slow release bolus or sodium selenite by injection (Clark *et al.*, 1992). No response was noted in milk production or reproductive parameters in this trial. Mean glutathione peroxidase activity of control animals was about 3 kIU/L at the start of the trial in July, decreasing during spring. In another herd of 279 cows, untreated animals were compared to animals treated with 250 or 500 mg of barium selenate injected subcutaneously (Whelan *et al.*, 1992). Mean blood selenium concentrations ranged from 129 to 472 nmol/L over two seasons in the control cows. Despite significant differences in blood selenium concentration between the groups, no differences in production were noted. In both of the above trials, the within-season variation in selenium status was considerable and control blood selenium concentrations were in the range considered to be responsive for only a portion of the trial.

The results of our study and those of others presented above support the contention that milk production responses to selenium may occur in herds where mean glutathione peroxidase activity and blood selenium concentration are below 2.0 kIU/L and 250 nmol/L, respectively for the greater part of the season.

The reason for the small magnitude of the milk production response to treatment in the four-pellet (high-dose) group in our study is not apparent. The risk of toxicity is claimed to be low when this form of supplementation is used, except when administered in conjunction with intra-ruminal magnesium pellets (Marchant, 1991; Wakelin, 1993). Up to eight pellets have been administered to cattle, resulting in no ill effects (Judson and McFarlane, 1984; Wilson *et al.*, 1991). It would appear that the relationship between the number of pellets administered and blood selenium concentration is not linear when this form of selenium supplementation is used (Marchant, 1991). Elemental selenium contained in the pellets is converted to iron selenide via an electrolytic reaction between iron and

selenium in the presence of water. Iron selenide has poor availability to the animal, and absorption of the selenium from pellets is limited by the rate of its conversion to soluble organic molecules (selenomethionine and selenocysteine) by rumen microbes.

It is possible that iron released from the pellets could affect the response to supplemental selenium, through an unrecognised direct effect, or through interference with copper metabolism (Coup and Campbell, 1964). It has been reported that these pellets can provide elevated blood selenium concentrations for up to 3 years (Hidiroglou *et al.*, 1985). Based on complete dissolution over 3 years, four pellets would provide an additional 100 mg iron per day. Most New Zealand pastures contain in excess of 100 mg iron per kg of dry matter (Towers and Grace, 1983) and fully fed cows would have daily intakes of between 12 and 16 kg of dry matter. Based on these figures, the iron contribution from four pellets would be equivalent to no more than an 8% increase in daily iron intake. Nevertheless, further study of the effects of pellet accumulation in the rumen over several years may be warranted.

To our knowledge, this is the first New Zealand study to suggest a relationship between selenium status and udder health. These results support findings from overseas researchers (Smith *et al.*, 1984; Weiss *et al.*, 1990; Ndiweni *et al.*, 1991; Braun *et al.*, 1991), who found that low selenium status was associated with high somatic cell counts. The role of selenium in the immune response is supported by a growing body of evidence. Neutrophils from selenium-deficient cattle phagocytise bacteria but have impaired killing capacity when compared to neutrophils from selenium-adequate cattle (Boyne and Arthur, 1979; Gyang *et al.*, 1984). Glutathione peroxidase is thought to reduce oxidative cell damage to tissues and granulocytes following the release of free radicals produced by the respiratory burst during phagocytosis (Boyne and Arthur, 1981). It is also postulated that glutathione peroxidase modulates the production of immune mediators through the arachidonic cascade (Spallholz *et al.*, 1990).

It is interesting to note that in Herd A somatic cell counts were lowest in the four-pellet group but in Herd B they were lowest in the two-pellet group. Researchers studying immune responses *in vitro* have suggested that an optimal immune response might require

a higher level of selenium than that required to prevent other signs of deficiency (Boyne and Arthur, 1981; Hogan *et al.*, 1990; Bendich *et al.*, 1986). However, the relevance of these findings to disease resistance *in vivo* remains unclear (Suttle and Jones, 1989). Despite the trends in somatic cell counts in our study, the incidence of clinical mastitis was uniformly low in all treatment groups. The interaction between udder health and trace element status in New Zealand dairy cattle must be studied further before recommendations can be made.

Selenium supplementation trials performed overseas suggest selenium deficiency is related to retained placenta (Julien *et al.*, 1976), poor uterine involution (Harrison *et al.*, 1986), metritis and cystic ovaries (Harrison *et al.*, 1984). Similar studies in New Zealand herds have not been performed. Preliminary results from a response trial in three herds on marginal selenium soil types in New Zealand indicated that submission rates and conception rates were improved in two herds where mean blood selenium concentrations in control cows were 115 and 130 nmol/L, but not in a third herd where mean selenium concentration was 200 nmol/L (Tasker *et al.*, 1987). Selenium supplementation in this trial was 500 mg of barium selenate injected subcutaneously. This is the only data available from a controlled study where blood selenium concentrations have been related to reproductive efficiency in New Zealand dairy herds. We could not repeat these findings in our experimental herds, despite achieving responses in growth rate and milk production. From the information available, it appears that under New Zealand conditions blood selenium concentrations conducive to normal reproductive efficiency are no higher than those currently recommended for normal growth and milk production.

When current New Zealand reference ranges (Ellison and Feyter, 1988) are compared to overseas values, a discrepancy is evident, with our reference ranges being far lower than those commonly quoted in overseas literature. Researchers in the United Kingdom (Julien *et al.*, 1976) found plasma selenium concentrations of 1000 nmol/L (equivalent to blood selenium concentration of 1000-2000 nmol/L) adequate for prevention of retained placenta. Smith *et al.* (1988), studying mastitis, suggested that blood selenium concentrations of 2500 nmol/L were necessary to prevent impairment of immunity in North American herds. These compare to our recommended adequate range of greater than 250 nmol/L.

The reason for the apparent lower requirements of New Zealand livestock for selenium is not known. The biological function of selenium in mammals has been studied mainly in the context of its antioxidant role, protecting tissues from oxidative degeneration. Selenium-dependent glutathione peroxidase is a selenoenzyme that reduces cytosolic peroxides (O'Brien and Little, 1969). Vitamin E also has potent antioxidant properties, and it appears that it can reduce the requirements for selenium in some situations.

The biochemical interactions between selenium and vitamin E have been most extensively studied through their role in the pathogenesis of white muscle disease in calves (Walsh, 1991). Deficiencies of vitamin E and selenium, in conjunction with high levels of dietary polyunsaturated fatty acids, result in abnormal lipid peroxidation, leading to cell membrane damage. Polyunsaturated fatty acids tend to be in higher concentration in the grain components of diets fed to overseas cattle when compared to the forage components (Hakkarainen and Pehrson, 1987). Furthermore, the vitamin E content of feed often decreases during the northern winter after prolonged storage. Conversely, under New Zealand grazing conditions, dietary levels of vitamin E are unlikely to be as low, due to the feeding of green pasture for much of the year (Rammel and Cunliffe, 1983). The potentially higher ratio of oxidants to antioxidants in the base diet of overseas cattle may explain why their selenium requirements are apparently higher.

The assertion (McSporran, 1992) that New Zealand cattle could be deficient in antioxidants at certain times of the year has some theoretical basis. Polyunsaturated fatty acid levels in young growing grasses reach high concentrations in spring (McMurray and McEldowney, 1977; Larick and Turner, 1989) and in some situations are protected from hydrogenation in the rumen. These give rise to high plasma levels of linoleic and linolenic acids in cattle (Arthur, 1988; McMurray *et al.*, 1983). Linolenic acid has been incriminated as an important factor in nutritional muscular dystrophy in calves (Rice and Kennedy, 1988). Cattle may become depleted of vitamin E when fed predominantly stored feeds prior to calving, and the selenium content of pasture is often low at this time of year (Thompson *et al.*, 1981). Thus, spring-calving New Zealand cattle may be at risk of disease involving abnormal lipid peroxidation for a short period around calving time.

The mechanism for selenium-responsive sub-clinical disease in New Zealand cattle may be different from the tissue peroxidation model proposed for white muscle disease. A mechanism involving lipid peroxidation and cell damage is intuitively appealing in the case of white muscle disease. It is less attractive in explaining the sub-clinical syndromes of reduced weight gain and milk production seen in New Zealand. White muscle disease is almost unknown in young cattle in New Zealand and no studies have been published that link retained placenta to selenium deficiency, suggesting that the mechanisms involved in selenium responsive disease are dependent on diet and management.

The effects of selenium deficiency on thyroid and growth hormone function have been documented in rats and cattle (Arthur *et al.*, 1988; Arthur, 1991). This provides researchers with an alternative mechanism for the action of selenium in mammals which is unrelated to its antioxidant functions. Theoretically, the high levels of vitamin E typical of the diet of New Zealand cattle would not be protective, and this may offer a plausible explanation for the range of sub-clinical syndromes we observe in selenium-deficient cattle on pasture.

In conclusion, the results of this and other published New Zealand studies support the use of the currently recommended selenium reference ranges in cattle for prediction of response to supplementation. In herds where selenium status falls below the adequate range for the majority of the season, growth and milk production responses to supplementation can be expected. The effects of selenium supplementation on udder health and reproductive efficiency require further investigation.

CHAPTER 4

Alpha-tocopherol, selenium and polyunsaturated fatty acid concentrations in the serum and feed of spring-calving dairy heifers[2]

[2] Accepted as: Wichtel JJ, Freeman DA, Craigie AL, Varela-Alvarez H, Williamson NB. New Zealand Veterinary Journal, 1995.

Abstract

The objectives of this study were to provide baseline data for α-tocopherol, selenium and polyunsaturated fatty acid concentrations in the serum and feed of New Zealand dairy cattle, and to assess the likelihood that abnormal peroxide metabolism has a role in the impaired lactational and reproductive performance noted in selenium-deficient cattle. Twenty-four Friesian heifers were randomly allocated one of four winter diets consisting of hay with or without selenium supplementation, or pasture and silage with or without selenium supplementation. A winter diet consisting exclusively of hay (α-tocopherol concentration 19 mg/kg of dry matter) resulted in a pre-calving serum α-tocopherol concentration of 1.2 mg/L compared to 4.5 mg/L for pastured heifers ($P<0.01$). The pre-calving α-tocopherol concentration for the heifers fed hay fell into the range considered deficient (less than 2.0 mg/L), whereas heifers fed pasture and silage remained in the range considered adequate throughout the study period.

Serum fatty acid concentration, and the proportion of fatty acids that were polyunsaturated, were lowest in the hay-fed heifers before calving (1.0 mg/ml, 37.1% respectively), and remained unchanged following re-introduction to pasture after calving in late July and August. Serum fatty acid concentration did not increase following re-introduction of the heifers to pasture because of the unexpectedly low fatty acid concentration (4.8 g/kg of dry matter) of the mature winter pasture. In October, however, the proportion of fatty acids in serum that were polyunsaturated increased (50%), as did serum α-tocopherol concentrations (greater than 13 mg/L).

Mean serum selenium concentrations in the unsupplemented heifers ranged from 139 to 204 nmol/L, being lowest in October ($P<0.01$). Supplementation with intraruminal selenium pellets (two pellets delivering about 3 mg Se/day) increased serum selenium concentration and glutathione peroxidase activity ($P<0.01$) whereas the type of winter diet had no effect ($P>0.05$).

These results suggest that dairy cattle wintered on hay can become vitamin E-depleted, whereas the feeding of a pasture/silage-based diet should provide adequate vitamin E. The

pasture offered following calving during July and August provided a low dietary polyunsaturated fatty acid challenge, suggesting that abnormal peroxide metabolism is unlikely to be an important mechanism in the impaired performance of selenium-deficient adult cattle which calve at this time of year.

Introduction

Selenium deficiency in adult cattle has been implicated as a cause of decreased milk production (Hupkens van der Elst and Watkinson, 1980; Fraser *et al.*, 1987; Wichtel *et al.*, 1994), increased somatic cell counts (Wichtel *et al.*, 1994) and possibly diminished reproductive efficiency (Tasker *et al.*, 1987) in New Zealand dairy herds, yet the mechanism by which it impairs performance has not been established.

The antioxidant role of selenium as a component of the enzyme glutathione peroxidase is well documented. A deficiency of selenium and vitamin E has been found to be responsible for abnormal lipid peroxidation and damage to subcellular membranes (Rice and Kennedy, 1988; Walsh, 1991). Selenium and vitamin E appear to have a sparing effect on each other, while dietary polyunsaturated fatty acids can provide an oxidant challenge which increases the requirement for these nutrients. This model forms the biochemical basis for several selenium-responsive diseases, including nutritional degenerative myopathy in calves (Rice and Kennedy, 1988; McMurray *et al.*, 1983). Researchers in the United Kingdom found that when calves were fed a winter diet low in vitamin E and selenium, and were turned out on to lush spring pasture containing high concentrations of polyunsaturated fatty acids, alterations in the lipid structure of subcellular membranes led to destruction of membrane integrity and myopathy within 1 week of turn-out (McMurray *et al.*, 1983). It was hypothesised by these researchers that polyunsaturated fatty acids in spring pasture might in some way be protected from hydrogenation, giving rise to these high plasma concentrations. However, myopathy is not a common feature of selenium deficiency in adult cattle (Maas *et al.*, 1990) and it is not known whether abnormal peroxide metabolism plays a role in the impaired lactational and reproductive performance (Hupkens van der Elst and Watkinson, 1980; Fraser *et al.*, 1987; Wichtel *et al.*, 1994; Tasker *et al.*, 1987) noted in selenium-deficient adult cattle.

Several observations have led to questions regarding the mechanism of selenium-responsive disease in New Zealand cattle. Despite selenium intakes which are frequently much lower than those recommended overseas (National Research Council, 1988), reported cases of selenium-responsive myopathy are rare while those of retained placenta do not exist

(Ellison and Fraser, 1990). It has been argued that a deficiency of vitamin E is unlikely to occur in New Zealand cattle due to their pasture-based diet (Rammel and Cunliffe, 1983). However, the concentrations of vitamin E and polyunsaturated fatty acids in the serum and feed of grazing cattle managed under different conditions have not been documented. In the absence of an established mechanism for the responses to selenium supplementation, the role played by peroxide metabolism must still be considered. If a peroxidative challenge was to occur in adult New Zealand cattle and result in sub-clinical disease, it would be most likely to occur in the periparturient period when serum vitamin E concentrations tend to be lowest (McGillivray *et al.*, 1959; Weiss *et al.*, 1992) and abrupt changes in diet occur, analogous to the management of calves developing white muscle disease in the United Kingdom.

The objective of this study is to provide baseline data for vitamin E, selenium and polyunsaturated fatty acids in the serum and feed of adult cattle consuming one of two winter diets: pasture supplemented with silage (a diet likely to be adequate in vitamin E) or pasture hay (the diet most likely to be fed under New Zealand conditions that could result in vitamin E depletion). This study tests the hypotheses that a winter diet of hay will deplete heifers of circulating vitamin E when compared to a pasture/silage-based diet, and that feeding pasture immediately following calving to the heifers wintered on hay will lead to high concentrations of circulating polyunsaturated fatty acids. The latter situation would place these animals at risk of disease involving abnormal peroxide metabolism. Selenium supplementation is included in this design to test for interactions affecting serum concentrations of vitamin E and polyunsaturated fatty acids.

Materials and Methods

Experimental design

Twenty-four rising 2 year old pregnant Friesian heifers were randomly allocated to one of four experimental groups in a 2 by 2 factorial design, balanced for age and live weight. The heifers were wintered on hay, hay with selenium supplementation, pasture and silage (pasture/silage) or pasture/silage with selenium supplementation.

Prior to the first day of experimental treatment (2 June, 1992), all of the animals were kept

together as part of a herd of 100 rising 2-year-old heifers, grazing ryegrass and white clover pasture. The mean live weight of the experimental heifers on 2 June was 463 kg.

Dietary treatment

The 12 heifers wintered on hay were placed on a stony river bank sheltered by pine trees. These heifers were fed exclusively on pasture hay from round bales averaging 200 kg each. Hay was fed every 2 days and intakes estimated by observing wastage. Following 30 days on this diet, live weight was measured using electronic scales (AG500, Tru Test, Hamilton) and condition score was estimated to ensure adequate feed was being offered to meet requirements for maintenance and pregnancy (70-80 MJ metabolisable energy (ME)/heifer/day). There was continuous access to water from a trough. The heifers were maintained on the hay diet until the day of calving, a period varying from 48 to 59 days. In choosing hay for use in this experiment, three stacks of medium-quality pasture hay harvested from selenium-deficient areas were sampled. The three hay samples had a mean α-tocopherol content of 27 mg/kg dry matter, with a range 16-42 mg/kg. The hay with the lowest α-tocopherol content was chosen. When sampled again during the experiment, this hay contained 19 mg α-tocopherol/kg dry matter. The crude protein content of the chosen hay was 12% and the metabolisable energy content was estimated to be 8.7 MJ ME/kg dry matter, with an estimated digestibility of 60%.

The 12 heifers wintered on pasture/silage were fed ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture where allowances were intended to meet requirements for maintenance and pregnancy. The metabolisable energy content of the pasture was measured in June, August and October and ranged from 11.4 to 12.5 MJ ME/kg dry matter, with a digestibility of 76-83% and crude protein content of 19-23% dry matter. During periods of high rainfall, pastured animals were placed on a sawdust loafing pad, and were fed silage fed from troughs beside the pad. The silage was comprised of equal quantities of late spring pasture and well-eared maize. The metabolisable energy content of the silage was estimated as 9.2 MJ ME/kg dry matter, with a digestibility of about 59% and crude protein content of 9%, suggesting a moderate to poor quality silage. Silage constituted 40% of the animals' intake over the winter period for the pasture/silage group.

The α-tocopherol and fatty acid content of the hay, pasture and silage is presented in Table 4.1

Calving occurred between 20 July and 26 August, with the median date being 31 July. The average body condition scores of the heifers at calving were 4.8 and 4.7 (using a scale with a range 1-8) for the hay and pasture groups, respectively. One heifer in the hay-fed group was found not to be pregnant and was excluded from the study. Following calving, heifers joined the mixed-age milking cow herd which was grazed exclusively on ryegrass and white clover pasture.

Selenium supplementation

The unsupplemented animals in this study had not received previous selenium supplementation and were considered marginally selenium deficient. The mean serum selenium concentration and whole blood glutathione peroxidase activity for the unsupplemented animals were 139-204 nmol/L and 0.9-2.1 kIU/L, respectively, during the experimental period. Currently, adequate selenium status is considered to be reflected by a serum selenium concentration greater than 150 nmol/L (Anon., 1992) and whole blood glutathione peroxidase activity greater than 2.0 kIU/L (Ellison and Feyter, 1988).

The selenium-supplemented animals had received two or four intra-ruminal selenium pellets (Permasel®, Mallinckrodt Veterinary, Upper Hutt) administered orally 12 months previously, followed by a further two pellets on the first day of the experiment. The 30 g intra-ruminal pellets contained 10% elemental selenium in a finely divided matrix of iron. The pellets release about 3 mg of selenium per day for at least 1 year (Marchant, 1991).

Sampling and assays

Blood samples were obtained via jugular venepuncture and collected into plain and EDTA anticoagulant evacuated glass tubes. Pre-treatment blood samples were obtained on June 2, pre-calving samples on July 22, post-calving samples at 5 to 9 days after individual calving dates, and pre-mating samples on October 20. One heifer was not sampled pre-calving because she calved on July 20. Anticoagulant tubes were kept at 4 °C for up to 48 hours until the assay of whole blood glutathione peroxidase activity (Paglia and

Valentine, 1967; Board and Peter, 1976) was performed. Plain tubes were kept at room temperature for 6 hours, then centrifuged and the serum decanted. Serum was kept for up to 36 hours at 4 °C until the assays of selenium (Watkinson, 1966), α-tocopherol (Rammel and Hoogenboom, 1985) and fatty acids (Anon., 1980; Kates, 1972) were performed. Peroxidisable polyunsaturated fatty acids were defined as all detectable polyunsaturated fatty acids, with the exception of linoleic acid, which has been shown to be relatively resistant to peroxidation (Kornbrust and Mavis, 1980).

Pasture samples were obtained in June, August and October. On each occasion, the samples submitted for analysis were a pooled sample of at least three paddocks typical of that offered to the cattle. The acid detergent fibre content of the sample was estimated using the method of Clancey and Wilson (1966). The nitrogen content of the forage samples was determined by Kjeldahl digestion and analysed colorimetrically using the indophenol blue method (Bradstreet, 1965). Estimates of metabolisable energy and digestibility were based on the nitrogen and acid detergent fibre content of the sample using calculations in routine use by the New Zealand Agriculture Research Institute (J. Clark, personal communication).

Statistical analysis

The data were analysed using repeated measures analysis of variance, testing for the effects of the diet and selenium with repeated measures on time. Because time by treatment interactions were present, treatment effects within each time were tested separately using analysis of variance and covariance, with day of calving as a covariable. Treatments were considered fixed effects. The effects of time were tested separately for each time of year, using the initial sampling time (June) as the control level against which all others were compared.

Means reported are least square means ± standard error of the mean. Analysis was performed using the general linear model procedure of SAS (SAS User's Guide, 1988a). Results were considered to be significant at $P<0.05$. Probability values up to $P=0.20$ are included in tabular presentations.

Results

Interaction between winter diet and selenium supplementation was not significant for any of the outcomes measured; accordingly, the tabulated results are presented in terms of the main effects for the diet and selenium treatments.

Alpha-tocopherol and polyunsaturated fatty acids in feed

The winter-saved pasture sampled during August had a concentration of α-tocopherol of less than 25 mg/kg dry matter, as did the hay and silage (Table 4.1). The recommended minimum α-tocopherol content of diets for heifers is 25 mg/kg dry matter (National Research Council, 1988).

The greatest dietary fatty acid challenges to the study heifers occurred in October (Table 4.1), when the pasture α-tocopherol concentration was also high (40 mg/kg dry matter). A close relationship between concentration of α-tocopherol and peroxidisable polyunsaturated fatty acids in the forage samples is evident (Table 4.1) resulting in the feed α-tocopherol to peroxidisable polyunsaturated fatty acid ratio varying only between 7.1 and 17.9 mg/g. This observation suggests that forages with increased peroxidisable polyunsaturated fatty acid concentrations will also have increased vitamin E content.

Alpha-tocopherol and polyunsaturated fatty acids in serum

Serum α-tocopherol was affected by diet and time of year (Table 4.2). A winter diet consisting exclusively of hay resulted in a pre-calving (July) serum α-tocopherol concentration of 1.2 mg/L compared to 4.5 mg/L for pastured heifers ($P<0.01$). Although there is no reference range for serum α-tocopherol in New Zealand cattle, the pre-calving α-tocopherol concentration for the heifers fed hay fell into the range (less than 2.0 mg/L) considered deficient by overseas researchers (Hidiroglou *et al.*, 1992). Heifers fed pasture and silage remained in the range considered adequate throughout the study period. Serum α-tocopherol was not affected by selenium supplementation.

The serum α-tocopherol to peroxidisable polyunsaturated fatty acid ratio was also lowest in the group fed hay before calving (6.0 mg/g), primarily due to the low serum α-

tocopherol concentration at this time (Table 4.2). Like serum α-tocopherol, serum fatty acid concentration and the proportion of fatty acids that were polyunsaturated, were lowest in the hay-fed heifers before calving (1.0 mg/ml, 37.1% respectively), remaining unchanged after re-introduction to pasture following calving in late July and August (Table 4.3). The lack of increase in concentration of serum fatty acids following the re-introduction to pasture was explained by the low fatty acid concentration of the mature winter pasture fed after calving (4.8 g/kg). In October, however, the proportion of fatty acids in serum that were polyunsaturated increased to 50%, at which time serum α-tocopherol concentrations, were also very high (greater than 13 mg/L).

Selenium

During June, July and August, selenium supplementation had little effect on the fatty acid composition of serum (Table 4.3). In October, selenium supplementation resulted in an unexpected 8% reduction in the proportion of fatty acids that were polyunsaturated, reflecting both lower linoleic and α-linolenic acid content ($P<0.01$).

Table 4.1. Selenium, α-tocopherol and polyunsaturated fatty acid composition, on a dry matter basis, for hay, silage and pasture fed to experimental animals[a].

Nutrient	Feed				
	Hay	Silage	Pasture: June	Pasture: August	Pasture: October
α-tocopherol (mg/kg)	19	15	58	22	40
Selenium (μg/kg)	< 10	< 10	20	20	< 10
Total fatty acids (g/kg)	6.6	1.9	6.7	4.8	8.2
Linoleic (% w/w fatty acids)	16.4	18.4	9.0	11.5	10.9
α-linolenic (% w/w fatty acids)	34.6	43.5	73.4	52.7	65.6
α-tocopherol : PPUFA[b] (mg/g)	7.9	17.9	11.8	7.1	7.3

[a] On each occasion, pasture samples were pooled samples of at least three paddocks typical of that offered to cattle.

[b] The ratio of α-tocopherol to peroxidisable polyunsaturated fatty acids (mg/g) in the feed.

Table 4.2. **Means for the main effects of winter diet and selenium supplementation on serum α-tocopherol concentration and the ratio of α-tocopherol to peroxidisable polyunsaturated fatty acids in serum of spring-calving Friesian heifers at four times of the year**

Item	Treatment[a]				Treatment effects[b]			
	Hay diet	Pasture diet	No selenium	Selenium	Diet	Selenium	Time	Time x diet
June								
α-tocopherol[c]	4.1±0.2	3.9±0.2	4.3±0.2	3.7±0.2	NS	0.09		
α-tocopherol : PPUFA[d]	11.3±1.2	10.9±1.1	11.7±1.1	10.4±1.2	NS	NS		
July								
α-tocopherol[c]	1.2±0.3	4.5±0.2	3.0±0.2	2.6±0.2	<0.01	NS	<0.01	<0.01
α-tocopherol : PPUFA[d]	6.0±0.7	13.7±0.6	10.3±0.6	9.4±0.7	<0.01	NS	0.20	<0.01
August								
α-tocopherol[c]	2.6±0.2	3.5±0.2	3.2±0.2	2.9±0.2	0.01	NS	<0.01	0.02
α-tocopherol : PPUFA[d]	10.7±0.7	13.7±0.7	12.2±0.7	12.3±0.7	<0.01	NS	NS	0.08
October								
α-tocopherol[c]	9.3±0.5	8.9±0.5	9.3±0.5	8.0±0.5	NS	0.10	<0.01	NS
α-tocopherol : PPUFA[d]	14.0±1.1	14.3±1.1	13.4±1.0	14.8±1.0	NS	NS	0.01	NS

[a] Dietary treatments were hay; hay with selenium supplementation (two intra-ruminal pellets); pasture and silage; pasture and silage with selenium supplementation (n=6).

[b] Probability level: NS = not significant. Diet x selenium and selenium x time effects = NS ($P>0.10$).

[c] Serum α-tocopherol concentration (mg/L).

[d] The ratio of α-tocopherol to peroxidisable polyunsaturated fatty acids (mg/g) in serum.

Table 4.3. **Means for the main effects of winter diet and selenium supplementation on serum fatty acid concentration and fatty acid composition in spring-calving Friesian heifers at four times of the year**

Item	Treatment[a]				Treatment effects[b]			
	Hay diet	Pasture diet	No selenium	Selenium	Diet	Selenium	Time	Time x diet
June								
Total fatty acids[c]	2.1±0.1	2.0±0.1	2.1±0.1	2.0±0.1	NS	NS		
Linoleic[d]	19.3±0.4	19.2±0.4	18.8±0.4	19.7±0.4	NS	0.15		
α-linolenic[d]	12.8±0.4	12.7±0.4	12.9±0.4	12.6±0.4	NS	NS		
Arachidonic[d]	1.7±0.2	1.1±0.2	1.7±0.2	1.2±0.2	0.11	0.12		
Total PUFA[e]	38.1±0.9	38.5±0.9	38.6±0.9	37.9±0.9	NS	NS		
Total PPUFA[f]	18.8±0.6	19.3±0.6	18.9±0.6	19.1±0.6	NS	NS		
July								
Total fatty acids[c]	1.0±0.05	1.4±0.05	1.1±0.05	1.2±0.4	<0.01	NS	<0.01	0.04
Linoleic[d]	17.5±0.05	21.1±0.05	20.1±0.5	18.5±0.7	<0.01	0.05	NS	<0.01
α-linolenic[d]	9.9±0.3	15.7±0.3	12.8±0.3	12.7±0.3	<0.01	NS	NS	<0.01
Arachidonic[d]	2.4±0.1	1.8±0.1	2.3±0.1	1.9±0.1	0.02	0.08	<0.01	NS
Total PUFA[e]	37.1±0.8	45.0±0.7	41.8±0.6	40.3±0.8	<0.01	0.18	<0.01	<0.01
Total PPUFA[f]	19.5±0.4	24.0±0.3	21.7±0.4	21.8±0.3	<0.01	NS	<0.01	<0.01

(cont. over)

Table 4.3. (continued).

Item	Treatment[a]				Treatment effects[b]			
	Hay diet	Pasture diet	No selenium	Selenium	Diet	Selenium	Time	Time x diet
August								
Total fatty acids[c]	1.1±0.08	1.3±0.07	1.3±0.07	1.1±0.08	NS	0.18	<0.01	NS
Linoleic[d]	16.2±0.4	15.6±0.4	16.3±0.4	15.6±0.4	NS	NS	<0.01	NS
α-linolenic[d]	14.4±0.5	13.5±0.4	14.4±0.4	13.5±0.5	0.17	0.17	<0.01	NS
Arachidonic[d]	1.6±0.1	1.6±0.1	1.5±0.1	1.7±0.1	NS	0.12	NS	NS
Total PUFA[e]	37.7±0.7	36.0±0.7	37.3±0.7	36.4±0.7	0.12	NS	0.09	NS
Total PPUFA[f]	21.5±0.5	20.5±0.5	20.9±0.5	19.0±0.6	0.16	NS	<0.01	0.17
October								
Total fatty acids[c]	2.1±0.2	2.1±0.1	2.2±0.1	1.9±0.2	NS	0.14	NS	NS
Linoleic[d]	20.9±0.04	21.1±0.4	22.1±0.4	19.9±0.4	NS	<0.01	<0.01	NS
α-linolenic[d]	22.3±0.6	23.3±0.6	23.9±0.6	21.7±0.6	NS	<0.01	<0.01	NS
Arachidonic[d]	1.5±0.1	1.4±0.1	1.5±0.1	1.4±0.1	NS	NS	NS	NS
Total PUFA[e]	51.0±0.9	52.3±0.9	53.7±0.9	49.6±0.9	NS	<0.01	<0.01	NS
Total PPUFA[f]	30.1±0.6	31.2±0.5	31.7±0.5	29.6±0.6	0.15	0.02	< 0.01	NS

[a] Dietary treatments were hay; hay with selenium supplementation (two intra-ruminal pellets); pasture; pasture with selenium supplementation (n=6).
[b] Probability level: NS = not significant. Diet x selenium and selenium x time effects = NS (P>0.10).
[c] Total serum fatty acids (mg/ml).
[d] Proportion of total fatty acids (%, w/w).
[e] Total polyunsaturated fatty acids as a proportion of all fatty acids (%, w/w).
[f] Total peroxidisable polyunsaturated fatty acids as a proportion of all fatty acids (%, w/w).

Table 4.4. **Means for the main effects of winter diet and selenium supplementation on serum selenium concentration and whole blood glutathione peroxidase activity of spring-calving Friesian heifers at four times of the year.**

Item	Treatment[a]				Treatment effects[b]			
	Hay diet	Pasture diet	No selenium	Selenium	Diet	Selenium	Time	Time x selenium
June								
Selenium[c]	595 ± 18	602 ± 18	200 ± 16	997 ± 17	NS	< 0.01		
Glutathione peroxidase[d]	11.1 ± 0.9	12.3 ± 0.9	1.9 ± 0.8	21.4 ± 0.8	NS	< 0.01		
July								
Selenium[c]	549 ± 80	518 ± 84	186 ± 72	881 ± 82	NS	< 0.01	0.18	NS
Glutathione peroxidase[d]	13.2 ± 0.6	12.1 ± 0.6	2.1 ± 0.6	23.3 ± 0.6	NS	< 0.01	0.16	0.06
August								
Selenium[c]	547 ± 119	725 ± 114	204 ± 105	1068 ± 109	NS	< 0.01	NS	NS
Glutathione peroxidase[d]	11.5 ± 1.1	9.6 ± 1.0	1.8 ± 1.0	19.2 ± 1.0	NS	< 0.01	0.17	NS
October								
Selenium[c]	384 ± 43	425 ± 41	139 ± 38	670 ± 40	NS	< 0.01	< 0.01	< 0.01
Glutathione peroxidase[d]	12.4 ± 0.4	13.0 ± 0.3	0.9 ± 0.4	24.6 ± 0.4	NS	< 0.01	0.10	< 0.01

[a] Dietary treatments were hay; hay with selenium supplementation (2 Permasel intra-ruminal pellets); pasture; pasture with selenium supplementation (n=6).
[b] Probability level: NS = not significant. Diet x selenium and diet x time effects = NS ($P>0.10$).
[c] Serum selenium concentration (nmol/L).
[d] Whole blood glutathione peroxidase (kIU/L).

The mean serum selenium concentration in the unsupplemented heifers ranged from 139 to 204 nmol/L, and whole blood glutathione peroxidase activity ranged from 0.9-2.1 kIU/L, both measures being lowest in October ($P<0.01$, Table 4.4). Supplementation with intraruminal selenium pellets increased serum selenium concentration to about five-fold, and glutathione peroxidase activity ($P<0.01$) about ten-fold that of unsupplemented animals. The type of winter diet had no effect on selenium status ($P>0.05$).

Discussion

It appears that under certain management conditions, New Zealand dairy cattle can become depleted of α-tocopherol to a degree that has been associated with disease in cattle overseas. Heifers fed hay for 48-59 days prior to calving had an α-tocopherol concentration of 1.2 ± 0.3 mg/L, which is considered to reflect a deficient state (Hidiroglou *et al.*, 1992). There is a paucity of data on the vitamin E status of New Zealand dairy cattle. The only comparable values appear in a report published 36 years ago (McGillivray *et al.* 1959). In the present study, serum α-tocopherol concentrations measured during the winter and early spring periods were considerably lower than those reported in the earlier study.

Serum α-tocopherol concentrations were lowest around the time of calving, which agrees with the findings of other researchers (McGillivray *et al.*, 1959; Weiss *et al.*, 1992). This may in part be due to the low lipoprotein concentration of serum at this time (Weiss *et al.*, 1992), but serum lipoprotein concentration was not measured in our study.

The low concentration of α-tocopherol found in hay in this study has been noted by others (Lynch, 1983; Hakkarainen and Pehrson, 1987; Kivimae and Carpena, 1973) and is due to the destruction of α-tocopherol during maturation, harvesting and storage. However, the α-tocopherol content of hay samples tested in this study ranged from 16 to 42 mg/kg, suggesting that some hay will provide adequate vitamin E intake.

The dietary requirement for α-tocopherol cannot be determined without consideration of polyunsaturated fatty acid intake. Polyunsaturated fatty acids, particularly α-linolenic acid, provide the primary oxidant challenge for which α-tocopherol and selenium are protective (Rice and Kennedy, 1988). Thus, the ratio of α-tocopherol to peroxidisable polyunsaturated fatty acids in serum and tissue is likely to be a useful measure of α-tocopherol status. The ratio of vitamin E to peroxidisable polyunsaturated fatty acids has been shown to correlate well with *in vitro* peroxidisability of tissue microsomes and tissue homogenates (Kornbrust and Mavis, 1980). This ratio was lowest for pre-calving heifers on a hay diet (Table 4.2), reflecting the lower α-tocopherol content and perhaps the lower availability of α-tocopherol in hay when compared to pasture. No reference data is available for the ratio of α-tocopherol to peroxidisable polyunsaturated fatty acids in bovine tissues. However, it has been suggested that disease associated with α-tocopherol deficiency is unlikely to occur if the ratio of vitamin E to polyunsaturated fatty acids in feedstuffs exceeds 0.6 mg/g (Harris and Embree, 1963). This data was derived from studies involving monogastric animals, so caution must be exercised in extrapolating this information for ruminants. The ratios of α-tocopherol to peroxidisable polyunsaturated fatty acids in hay and pasture in August were 7.9 and 7.1 mg/g respectively (Table 4.1), equivalent to vitamin E to polyunsaturated fatty acid ratios of greater than 5.0 mg/g. This suggests that, despite the low absolute concentration of α-tocopherol, there was sufficient antioxidant present to protect the cattle from disease associated with the unsaturated fatty acid content of their diet.

In designing this study, we considered it possible that prolonged feeding of hay could alter ruminal ability to hydrogenate polyunsaturated fatty acids, at least temporarily during the period of adaptation to a grass diet following calving. Although serum linolenic acid (as a percentage of total fatty acids) increased from 9.9 % to 14.4% in the hay-fed heifers following their re-introduction to pasture (Table 4.3), the increase was not as dramatic as the 20-fold increase recorded in myopathic calves following introduction to spring pasture (McMurray *et al.*, 1983). The differences in these data may reflect the increased hydrogenation of linolenic acid in the adult rumen when compared to that of young calves and the maturity of the pasture fed to the heifers following calving. Surprisingly, the pasture fed to recently calved cows in August had a total fatty acid content of 4.8 g/kg,

which was lower than that of hay (Table 4.1), reflecting the maturity of this winter-saved grass. Cattle calving in July and August were therefore not exposed to a large dietary polyunsaturated fatty acid challenge. October pasture had a fatty acid content of 8.2 g/kg, suggesting that cattle re-introduced to pasture later in spring are more likely to experience a large increase in fatty acid intake. The higher chain (C-16 and C-18) fatty acid content of pasture has been shown to increase with use of nitrogen fertiliser (Barta, 1975) which is commonly applied in spring and autumn.

It is apparent from our data that forages with increased peroxidisable polyunsaturated fatty acid concentrations tend also to have increased protective α-tocopherol concentrations, an observation noted by other researchers (Hakkarainen and Pehrson, 1987).

In October, selenium supplementation resulted in an 8% reduction in the proportion of fatty acids that were polyunsaturated, reflecting both lower linoleic and α-linolenic acid content (Table 4.3). This effect was unexpected and is difficult to explain. Selenium supplementation may have enhanced the hydrogenation of unsaturated fatty acids in the rumen, or altered their absorbtion or metabolism. There was a trend towards decreased total fatty acid and α-tocopherol concentrations in the selenium-supplemented groups in October ($P<0.15$), which could indicate altered absorbtion of lipids.

Taken together, these findings would suggest that New Zealand cattle fed hay or pasture prior to calving in August are not at risk of periparturient disease resulting from high polyunsaturated fatty acid intake. This is primarily due to the low fatty acid content of mature winter pasture typically fed to these cattle at calving. Cattle re-introduced to immature pasture during late spring or autumn may be at greater risk of disease.

Roles for selenium and vitamin E in disease resistance have been suggested. It has been shown that these nutrients can affect disease resistance through modulation of eicosanoid biosynthesis (Watkinson and Brown, 1979) and alteration in immune cell function (Nockels, 1988; Spallholz *et al.*, 1990; Yu-Zhang *et al.*, 1992). Few studies have investigated the role of selenium or vitamin E in disease resistance under New Zealand

conditions, although pre-partum selenium supplementation was associated with decreased somatic cell counts in one trial (Wichtel *et al.*, 1994). Outside New Zealand, selenium or vitamin E deficiencies in cattle have been associated with high somatic cell counts in milk (Smith *et al.*, 1984; Weiss *et al.*, 1990; Batra *et al.*, 1992), reduced resistance to clinical mastitis (Smith *et al.*, 1984; Weiss *et al.*, 1990) and an impaired immune response (Nockels, 1988; Yu-Zhang *et al.*, 1992). The relationships between disease resistance and intake of selenium and vitamin E require further study before specific recommendations can be made.

In conclusion, spring-calving dairy heifers managed under the conditions of this study did not appear to be at risk of periparturient disease resulting from high polyunsaturated fatty acid intake. These data suggest that abnormal peroxide metabolism is unlikely to be an important mechanism in the impaired performance of selenium deficient adult cattle in New Zealand. However, serum α-tocopherol concentrations measured during the winter and the early spring periods were lower than those previously published for New Zealand dairy cattle.

CHAPTER 5

Effect of selenium and α-tocopherol supplementation on postpartum reproductive function of dairy heifers at pasture[3]

[3] Submitted as: Wichtel JJ, Craigie AL, Thompson KG and Williamson NB. Theriogenolgy, 1994.

Abstract

The objective of this study was to investigate the effects of selenium and α-tocopherol supplementation on uterine involution and ovarian function in dairy heifers fed a prepartum diet containing low concentrations of selenium and α-tocopherol. Twenty-four rising 2-year-old pregnant Friesian heifers were randomly allocated to one of four experimental groups in a 2 × 2 design balanced for age and body-weight. Prepartum treatments consisted of supplementation with either 2 intraruminal selenium pellets or 3600 mg of α-tocopherol *per os* 4 times per week, or both. Control animals received no supplementation. For 8 weeks before calving, the heifers were fed exclusively on pasture hay which contained less than 10 μg/kg of selenium and 19 mg/kg of α-tocopherol. After calving, the heifers grazed perennial ryegrass and white clover pasture. Concentrations of selenium and α-tocopherol in serum for the prepartum heifers of the control group were 125 nmol/L and 1.3 mg/L, respectively, indicating deficiencies of these nutrients. Treatment with selenium and α-tocopherol increased prepartum serum concentrations of selenium and α-tocopherol to 925 nmol/L and 5 mg/L, respectively ($P<0.001$). However, treatment with selenium, α-tocopherol, or both, failed to enhance uterine involution, hasten resumption of postpartum ovarian activity or reduce the incidence of clinical postpartum abnormalities. These findings suggest that postpartum reproductive dysfunction is not an important feature of moderate selenium or vitamin E deficiency of cattle at pasture.

Introduction

Deficiency of selenium has been linked to several reproductive disorders of cattle including retained placenta (Trinder *et al.*,1969), poor uterine involution (Harrison *et al.*,1986), metritis (Harrison *et al.*, 1984), cystic ovaries (Harrison *et al.*, 1984) and impaired fertilisation of ova (Segerson *et al.*,1977). For intensively grazed dairy cattle however, reproductive responses to selenium supplementation are rare, even in herds where lactational and growth responses to selenium have been recorded (Wichtel *et al.*, 1994).

The mechanism by which selenium deficiency impairs reproductive function in cattle has not been established. Selenium could influence uterine involution and postpartum ovarian activity through its postulated effects on immune function (Spallholz *et al.*, 1990), uterine contractility (Segerson *et al.*, 1980), thyroid hormone metabolism (Wichtel *et al.*,1995), or synthesis of prostaglandins (Reddanna *et al.*, 1989). Indices of reproductive efficiency such as conception rates and mean calving to conception intervals may be of insufficient sensitivity to detect the subtle effects of selenium deficiency on reproductive function, especially in herds with seasonal calving patterns. Detailed examination of uterine involution and the events leading up to the first postpartum ovulation may aid the identification of such effects.

Alternatively, impaired reproductive performance may not be an important feature of selenium deficiency of grazing cattle due to the concentration of vitamin E in pasture. Although it is often assumed that intensively grazed stock have adequate intakes of vitamin E (Rammel and Cunliffe, 1983), concentrations of vitamin E in serum, tissues and feed have not been adequately described for cattle fed diets based exclusively on pasture. Fresh pasture and pasture silage usually contain adequate amounts of vitamin E but pasture hay can contain very low concentrations (Hidiroglou amd Batra, 1994).

The purpose of this study was to investigate the effects of selenium and α-tocopherol supplementation on uterine involution and ovarian function in dairy heifers fed pasture.

Materials and Methods

Animals and treatments

The experimental units were rising 2-year-old pregnant Friesian heifers (n=24). Selenium (2 intraruminal selenium pellets; Permasel®, Mallinckrodt Veterinary, Upper Hutt) and α-tocopherol (3600 mg of dl-α-tocopherol *per os* in a gelatin capsule 4 times per week) were used in a 2 × 2 factorial arrangement stratified by age and body weight. Heifers receiving no supplementary α-tocopherol were treated with an empty gelatin capsule. The 30-g intraruminal pellets contained 10% elemental selenium in a finely divided matrix of iron and provided about 3 mg selenium per day for at least 1 year (Marchant, 1991). Supplementation with selenium and α-tocopherol began on the first day of the experiment (June 2), coincident with the beginning of the hay diet. Supplementation with α-tocopherol ceased on the day of calving, when the heifers were moved to fresh pasture. One heifer was later found to be not pregnant and was excluded from the study.

The heifers were fed exclusively on pasture hay which contained 95% perennial rye grass (*Lolium perenne*) and 5% white clover (*Trifolium repens*) and was known to contain low concentrations of selenium and vitamin E (Table 5.1). There was continuous access to water from a trough. Heifers were maintained on the pasture hay diet until the day of calving, a period varying from 48 to 59 d for individual heifers. Following calving, heifers joined a mixed-age milking cow herd grazing pasture which contained 85% perennial rye grass and 15% white clover (Table 5.1).

Performance monitoring

Body weight was recorded before treatment and monthly thereafter. A placenta was considered retained if it was not released within 24 hours of calving. Beginning 1 week after calving, each heifer was examined *per rectum* twice weekly. B-mode ultrasound with a 5 MHz linear array probe was utilized to measure the diameter of the involuting cervix and of the base of each uterine horn. Involution was considered to be completed when the following criteria were met: diameters of neither the cervix nor uterine horns exceeded 3.5 cm, the difference in diameter between the previously gravid and non-gravid horns was no greater than 5 mm, and there was no change in the diameter of the cervix or previously

gravid horn at two successive examinations.

Table 5.1. Composition on a dry matter basis for hay and pasture.

	Feed	
	Hay	**Pasture[a]**
Metabolisable Energy (MJ ME/kg)	8.7	11.7
Crude Protein (%)	11.7	22.5
Digestibility (%)	59.6	78.1
Se (μg/kg)	< 10	20
α-tocopherol (mg/kg)	19	22
Total fatty acids (g/kg)	6.6	4.8
PPUFA[b] (% w/w fatty acids)	36	54
α-tocopherol : PPUFA[c] (mg/g)	7.9	7.1

[a] On each occasion, pasture samples were pooled samples of at least 3 paddocks typical of that offered to cattle.
[b] Peroxidisable polyunsaturated fatty acids
[c] Ratio of α-tocopherol to peroxidisable polyunsaturated fatty acids (μg/mg).

A vaginal speculum was used to visually assess cervical discharge at 1 and 2 weeks postpartum. An abnormal discharge was defined as an excessive amount (about 100 ml or more) of vaginal discharge pooled in the anterior vagina, with a foul odour. A diagnosis of metritis was made when, in addition to an abnormal discharge, there was poor involution of the cervix and uterus, thickening of the uterine wall and excessive fluid in the lumen of the uterus as assessed by palpation and ultrasound.

The heifers were observed twice daily throughout the postpartum period for signs of oestrus. Tail paint was used to assist in oestrus detection. The interval from calving to the first postpartum rise in progesterone was determined from the concentration of progesterone in after-milk measured twice-weekly for 60 d postpartum. The first postpartum rise in progesterone was defined as a concentration of progesterone in whole milk greater than 10 ng/ml which persisted for 2 consecutive sampling periods. If a heifer

did not begin cycling during the 60 days following calving (2 heifers), an interval of 60 days to first progesterone rise was arbitrarily assigned to that heifer for the purposes of analysis.

Sample collection and methods of analysis

Blood samples were obtained via jugular venipuncture and collected into plain and EDTA-anticoagulant containing evacuated glass tubes. Pretreatment blood samples were obtained on June 2, prepartum samples on July 22 and postpartum samples at 5 to 9 days following individual calving dates.

Anticoagulant tubes were kept at 4°C for up to 48 hours until assay of whole blood glutathione peroxidase activity (Thompson *et al.*, 1981). Plain tubes were kept at room temperature in subdued lighting for 6 hours then centrifuged and the serum decanted. Serum was kept for up to 24 hours at 4°C before assays were performed. Concentration of selenium was determined using the fluorometric method of Watkinson (Watkinson, 1979). Concentration of α-tocopherol was measured using high performance liquid chromatography (Rammel and Hoogenboom, 1985).

Detergent-induced haemolysis, a biological test which has proven useful in assessing antioxidant adequacy in sheep (Stevenson and Jones, 1989) and cattle (Stevenson *et al.*, 1991), was measured using the spectrophotometric method described by Hamada and Matsumoto (Hamada and Matsumoto, 1980). Detergent solutions of polyoxyethylenesorbitan monolaurate at dilutions of 0.1%, 0.5%, 1.0% and 3.0% in 0.9% saline were used. For brevity, only results from assays using the 1.0% dilution are presented as dilution rate had no effect on outcome.

Milk samples (20 ml) were collected into sterile containers containing one potassium dichromate tablet. Samples were tightly capped and were stored at 4°C. The concentration of progesterone in milk was measured by direct radioimmunoassay, without extraction as described previously (Dobson *et al.*, 1975). Assay sensitivity was 0.2 nmol/L and the intra- and interassay coefficients of variation were 19.5% and 32.1%, respectively. Fatty acid composition was determined using gas chromatography (Kates, 1972). Peroxidisable

polyunsaturated fatty acids were defined as all detectable polyunsaturated fatty acids, with the exception of linoleic acid, which has been shown to be relatively resistant to peroxidation (Kornbrust and Mavis, 1980).

Statistical analysis

Analysis was performed using analysis of variance and covariance, utilizing the general linear model procedure of SAS (SAS User's Guide, 1988a). The full model included selenium treatment, α-tocopherol treatment, interaction between selenium and α-tocopherol, with pretreatment value or day of calving as covariants where appropriate. The model followed the general equation:

$$Y_{ijk} = \mu + S_i + T_j + ST_{ij} + \beta_1 X_{1ij} + e_{ijk}$$

where

Y_{ijk} =	dependant variable,
μ =	overall mean,
S_i =	effect of the selenium treatment group i (i = 1..2),
T_j =	effect of the α-tocopherol treatment group j (j = 1..2),
ST_{ij} =	effect of the interaction between selenium and α-tocopherol,
β_1 =	coefficient of regression of Y on the continuous variable X_1, and
e_{ijk} =	residual error.

Treatments were considered to be fixed effects. Associations between treatment and categorical variables were tested by applying analysis of variance to ranks of each variable, which is a nonparametric test equivalent to the Kruskal-Wallis k-sample test (SAS User's Guide, 1988b). Means reported are least squares means (LSM) ± SEM. Results were considered to be significant at $P<0.05$.

Results

Supplementation with selenium increased serum selenium concentration and blood glutathione peroxidase activity (Figure 5.1) but did not improve postpartum reproductive performance (Tables 5.2 and 5.3). Mean prepartum serum selenium concentration in the heifers receiving no supplementary selenium was 125 nmol/L, indicating a deficiency of selenium. Treatment increased prepartum serum concentrations of selenium to 925 nmol/L ($P<0.001$). The activity of glutathione peroxidase was less than 2 kIU/L of whole blood for heifers receiving no supplementary Se; activity of > 2 kIU/L is considered to reflect adequate selenium status in intensively grazed cattle (Ellison and Feyter, 1988). The glutathione peroxidase activity of heifers receiving supplementary selenium was increased to 24 kIU/L.

Supplementation with α-tocopherol (Figure 5.2) resulted in marked increases in the prepartum serum concentration of α-tocopherol, and the ratio of α-tocopherol to peroxidisable polyunsaturated fatty acids (PPUFA) . Serum α-tocopherol concentrations of 2.0 mg/L are considered to reflect adequate vitamin E status (Hidiroglou *et al.*, 1992). Heifers not receiving supplementary α-tocopherol had a serum α-tocopherol concentration of 1.3 mg/L and were therefore deficient in vitamin E before calving. Supplementary α-tocopherol increased the prepartum serum α-tocopherol concentration to 5 mg/L ($P<0.001$). Supplementation did not result in enhanced involution or improvement for any of the reproductive performance parameters measured (Tables 5.2 and 5.3). By 1 week postpartum, the serum concentration of α-tocopherol and ratio of α-tocopherol to peroxidisable polyunsaturated fatty acids were not significantly affected by prepartum supplementation with α-tocopherol (Figure 5.2).

The incidence of abnormal discharge was surprisingly high (53%) in this study (Table 5.2), and was affected by a selenium by α-tocopherol interaction ($P<0.05$). Despite the high incidence of abnormal discharge, the incidence of metritis was low (4%), and unaffected by supplementation with either selenium or α-tocopherol. A considerable degree of variation occurred in the reproductive interval data (Table 5.3), and no statistical significance occurred for the differences between treatments.

Table 5.2. **Clinical data describing postpartum reproductive function of heifers which were not supplemented or were supplemented with α-tocopherol, selenium (Se) or both α-tocopherol and selenium.**

	n	Retained Placenta	Abnormal Discharge	Metritis
			no	
Control	6	0	2	0
Se	6	1	4	0
α-tocopherol	5	2	5	0
Se + α-tocopherol	6	1	1	1
Effect			P	
Se		NS	NS	NS
α-tocopherol		NS	NS	NS
Se × α-tocopherol		NS	0.03	NS

Table 5.3. **Least squares means (LSM) and SEM for interval data describing the postpartum reproductive function of heifers which were not supplemented or were supplemented with α-tocopherol, selenium or both α-tocopherol and selenium.**

Treatment and Effect	n	Calving to Involution		Calving to Progesterone Rise		Calving to First Heat	
		d					
		LSM	SEM	LSM	SEM	LSM	SEM
Control	6	26.7	1.9	26.6	5.0	73.5	5.6
Se	6	29.8	2.1	18.3	5.4	86.6	6.1
α-tocopherol	5	30.1	2.0	17.7	5.1	82.8	6.5
Se + α-tocopherol	6	30.8	1.9	26.3	4.9	90.3	5.5
Effect		P					
Se		NS		NS		0.09	
α-tocopherol		NS		NS		NS	
Se × α-tocopherol		NS		NS		NS	

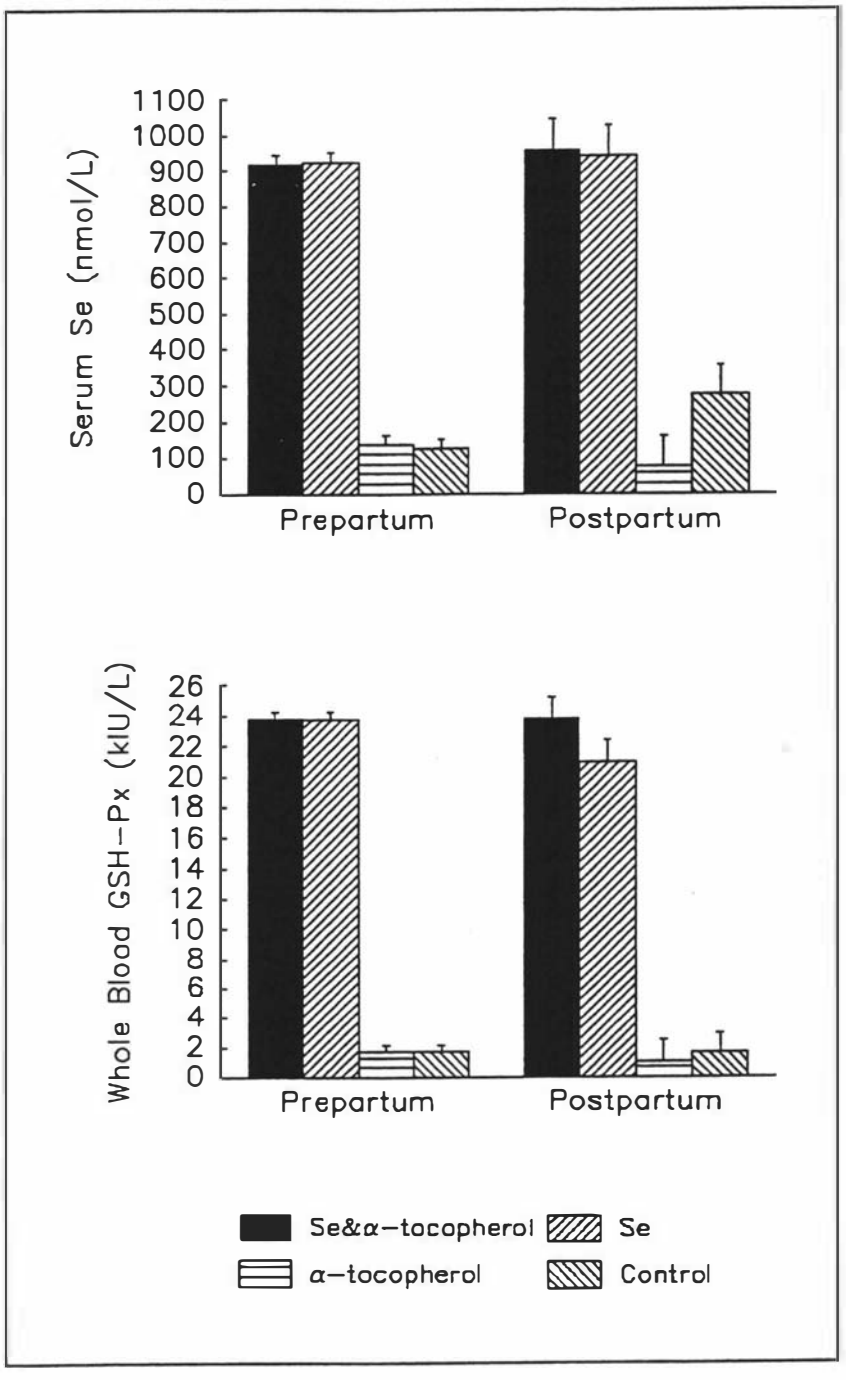

Figure 5.1. **The effect of treating heifers with selenium (Se), α-tocopherol or both selenium and α-tocopherol on the least squares means and SEM for serum selenium and blood glutathione peroxidase (GSH-Px).**

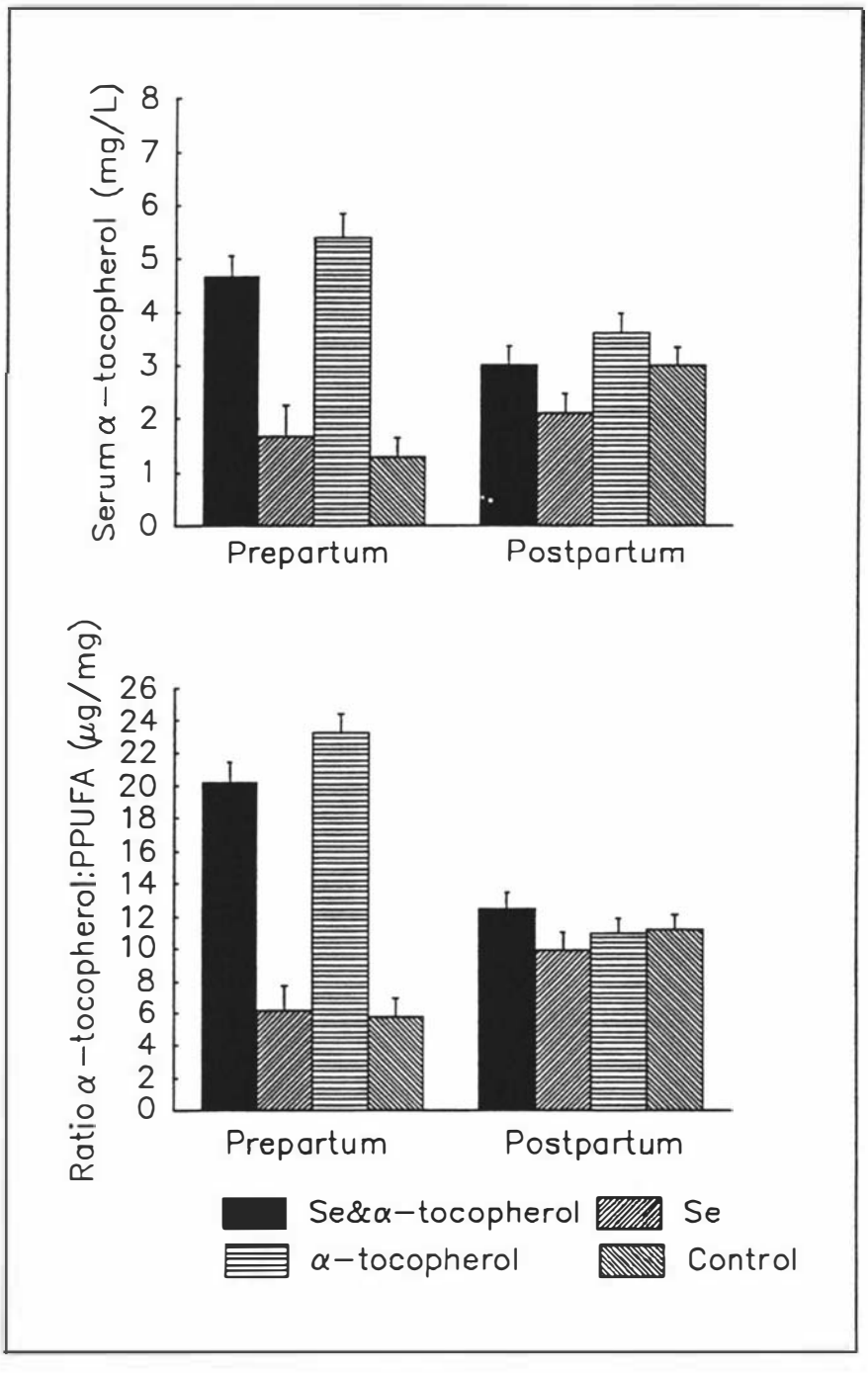

Figure 5.2. **The effect of treating heifers with selenium (Se), α-tocopherol or both selenium and α-tocopherol on the least squares means and SEM for serum α-tocopherol and the ratio of α-tocopherol to peroxidisable polyunsaturated fatty acids (PPUFA).**

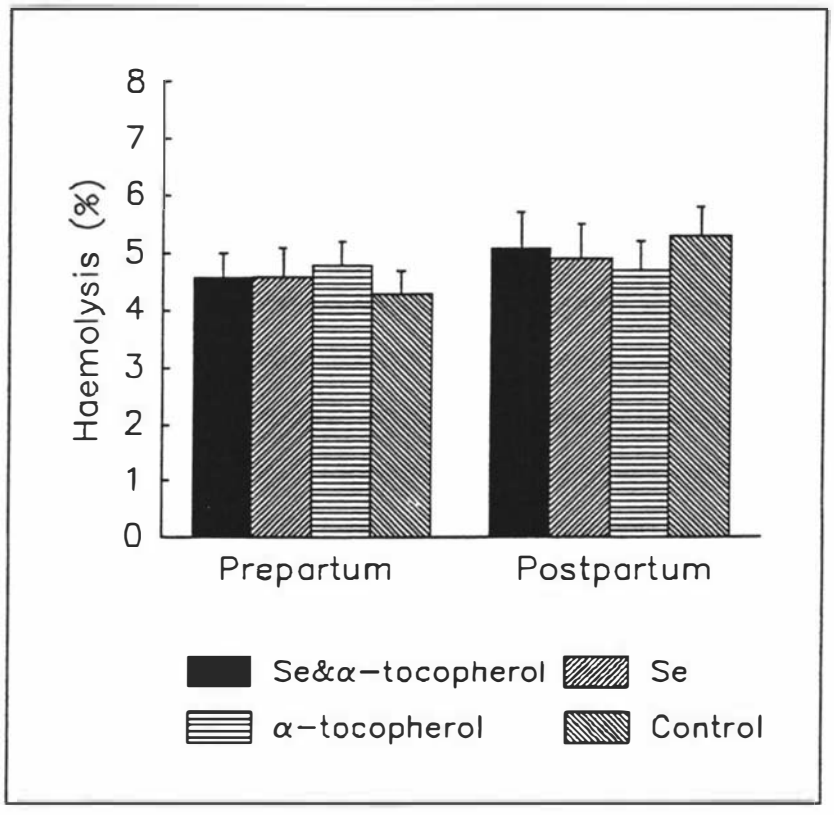

Figure 5.3. **The effect of treating heifers with selenium (Se), α-tocopherol or both selenium and α-tocopherol on the least squares means and SEM for detergent-induced haemolysis, expressed as a percentage of complete haemolysis.**

The rate of detergent-induced haemolysis was less than 7% for all experimental groups (Figure 5.3), and was not affected by supplementation with selenium or α-tocopherol. Average body weight at the start of the experiment was 462 kg and supplementation with either selenium or α-tocopherol did not affect body weight gain before or body weight loss after calving.

Discussion

Treatment of prepartum dairy heifers with selenium, α-tocopherol or both, failed to enhance uterine involution or to hasten resumption of postpartum ovarian activity. This is at variance with the reports of other workers (Harrison *et al.*, 1986; Harrison *et al.*, 1984) who found that treatment with selenium reduced the incidence of metritis and cystic ovaries and hastened uterine involution in cows that were affected by metritis. These and other studies, reviewed by Hurley and Doane (Hurley and Doane, 1989), have found that the incidence of retained placenta was reduced by treatment with selenium, or both selenium and vitamin E. In the current study, the incidence of retained placenta was unaffected by supplementation, however, the incidence of retained placenta (17%) was low compared to the incidence for other herds for which a response to supplementation was recorded (Harrison *et al.*, 1984; Hurley and Doane, 1989). These findings are consistent with the claim that, for intensively grazed dairy cattle, reproductive responses to selenium supplementation are rare (Wichtel *et al.*, 1994). It supports the contention that reproductive dysfunction is not an important feature of moderate selenium deficiency in cattle at pasture.

It has been argued that a deficiency of vitamin E is unlikely to occur in intensively grazed cattle due to the high concentration of vitamin E in their diet (Rammel and Cunliffe, 1983). In the current study, the serum concentration of α-tocopherol for prepartum heifers receiving no supplementary α-tocopherol could be considered to reflect a deficiency of vitamin E (Hidiroglou *et al.*, 1992). This is in agreement with Hidiroglou et al (Hidiroglou and Batra, 1994), who found a lower concentration of α-tocopherol in the plasma of cows fed hay compared to that of cows fed silage. Despite this, reproductive outcomes were not affected by supplementation with α-tocopherol and depletion of α-tocopherol did not appear to make cows more susceptible to the effects of low intakes of selenium. This calls into question the accepted normal ranges (Ellison and Feyter, 1988; Hidiroglou *et al.*, 1992) for serum selenium and α-tocopherol, which may overestimate the requirements of these nutrients for reproduction in intensively grazed herds fed pasture and pasture hay.

The dietary requirement for α-tocopherol cannot be determined without consideration of the intake of polyunsaturated fatty acids. Polyunsaturated fatty acids, particularly α-linolenic acid, provide the primary oxidant challenge for which α-tocopherol and selenium are protective (Rice and Kennedy, 1988). The ratio of serum α-tocopherol to peroxidisable polyunsaturated fatty acids (Kornbrust and Mavis, 1980) was measured as an estimation of the oxidant challenge for peripaturient heifers. This ratio was lowest for prepartum heifers receiving no supplementary α-tocopherol, largely reflecting the α-tocopherol content of the pasture hay. No reference data is available for this ratio in bovine serum or feed. However, it has been suggested that disease associated with vitamin E deficiency is unlikely to occur if the ratio of vitamin E to polyunsaturated fatty acids in feedstuffs exceeds 0.6 mg/g (Harris and Embree, 1963). The ratios of α-tocopherol to peroxidisable polyunsaturated fatty acids in hay and pasture were 7.9 and 7.1, respectively (Table 5.1) suggesting that, despite the low absolute concentration of α-tocopherol, there was sufficient antioxidant activity to protect the cattle from disease associated with the unsaturated fatty acid content of their diet.

The failure of supplementation to influence the rate of erythrocyte haemolysis suggests that the selenium and α-tocopherol status of the heifers was adequate for maintaining cell membrane stability (Stevenson and Jones, 1989), despite serum concentrations of these antioxidants being in the deficient range for prepartum heifers receiving no supplementation. This is a further indication that the selenium and α-tocopherol requirements of adult cattle on pasture may be lower than the currently accepted reference ranges. Other researchers have presented conflicting results. In one study (Schingoethe *et al.*, 1978), spontaneous haemolysis for cows at pasture was not different from that of cows fed a diet of stored feeds with low concentrations of vitamin E, but in that report selenium intake was adequate for both groups. In another study (Schingoethe *et al.*, 1979), a tendency for decreased spontaneous haemolysis was observed in lactating cows fed stored feeds supplemented with 300 mg D-α-tocopherol acetate.

In conclusion, examination of uterine involution and the events leading up to the first postpartum ovulation suggest that supplementation with selenium, α-tocopherol, or both, does not affect postpartum reproductive function in dairy heifers fed pasture.

CHAPTER 6

Effect of selenium and iodine supplementation on growth rate, thyroid and somatotropic function in dairy calves at pasture[4]

[4] Published as: Wichtel JJ, Craigie AL, Freeman DA, Varela-Alvarez H, Williamson NB. Journal of Dairy Science, 1995 (in press).

Abstract

The effects of selenium and iodine supplementation on growth rate and on thyroid and somatotropic function were examined for heifer calves from two herds at pasture. Supplementation of calves with intraruminal selenium pellets increased the basal plasma concentration of 3,5,3′-triiodothyronine and reduced the basal plasma concentration of thyroxine for both herds. For one herd, supplementation with selenium increased the triiodothyronine response to thyrotropin-releasing hormone challenge, increased body weight gain and tended to increase the plasma concentration of insulin-like growth factor-I. The plasma concentration of growth hormone was unaffected by selenium supplementation. Supplementation with iodine increased the response of thyroid hormones to thyrotropin-releasing hormone but did not increase body weight gain. Interaction between selenium and iodine treatment within the herds was not apparent for any outcome variable. These data suggest that the effects of selenium deficiency in grazing calves may be mediated by alterations in thyroid hormone metabolism, but apparently not through modulation of peripheral growth hormone concentration.

Introduction

Nutritional degenerative myopathy (white muscle disease) and poor growth rate are common manifestations of selenium deficiency of calves (Underwood, 1977). Although the antioxidant role of selenium and vitamin E in the prevention of myopathy is well understood (McMurray *et al.*,1983), the mechanism by which selenium deprivation causes growth retardation has not been established. Growth rate responses to selenium supplementation of calves on pasture commonly occur in the absence of signs of muscular degeneration (Wichtel *et al.*,1994), which suggests the existence of a mechanism that does not involve abnormal peroxide metabolism and is independent of the enzyme glutathione peroxidase.

Type I iodothyronine 5′-deiodinase is an selenium-dependent enzyme (Berry *et al.*,1991). The deiodinase enzymes are responsible for the deiodination of L-thyroxine, converting it to its more active form, 3,5,3′-triiodo-L-thyronine. Type I is the major deiodinase in liver, kidney, and skeletal muscle, and type II is the major deiodinase in brain, pituitary, and brown adipose tissue (Leonard and Visser, 1986).

The peripheral concentration of triiodothyronine was reduced and concentration of thyroxine was increased in calves fed a synthetic diet deficient in selenium when compared with concentrations for calves given the same diet supplemented with selenium (Arthur *et al.*, 1991a). In rats fed a diet deficient in selenium, similar changes in thyroid hormone concentration were noted (Arthur *et al.*, 1990). Although peripheral concentration of growth hormone was unchanged, pituitary concentration was decreased in the deficient rats when compared to rats supplemented with selenium. Plasma thyrotropin concentration was increased in the deficient rats, despite elevated plasma total and free thyroxine concentrations. The authors of this study concluded that these changes were consistent with impaired production of triiodothyronine by type II 5′-deiodinase in the pituitary (Arthur *et al.*, 1990) and suggested a role for selenium in growth that may be mediated by changes in thyroid hormone metabolism. Precisely how selenium affects somatotropic function is unclear; if peripheral concentration of growth hormone is unaffected, perhaps selenium deficiency affects mediators of growth hormone action such as insulin-like growth factor-I.

There is evidence (Arthur, 1991) that combined selenium and iodine deficiencies have important metabolic consequences. Deficiency of either of these nutrients decreases thyroid iodine, thyroxine and triiodothyronine concentrations in rats, suggesting that selenium deficiency could exacerbate the effects of low iodine intake. Although iodine deficiency of livestock is recognized (Barry *et al.*, 1981), few studies have investigated the effect of iodine supplementation on the growth of grazing cattle.

Our objective, therefore, was to test the hypothesis that supplementation with selenium, iodine, or both alters the growth rate and the plasma concentrations of thyroxine, triiodothyronine, growth hormone, and insulin-like growth factor-I in grazing dairy calves.

Materials and Methods

Calves

The experimental units were five-month-old Friesian heifer calves from two herds, herds 1 (n = 90) and 2 (n = 65). The calves grazed predominantly ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) pasture on their farms of origin. The pasture selenium concentration measured between 0.02 and 0.03 mg/kg of dry matter for both herds. The selenium requirement for calves grazing pasture has been estimated to be 0.03 mg/kg of dry matter (Ellison and Feyter, 1988). The pasture iodine concentration, measured at the start of the experiment, was 0.27 and 0.44 mg/kg of dry matter for herds 1 and 2, respectively. The iodine requirement for calves has been estimated at 0.25 mg/kg of dry matter (National Research Council, 1988).

Analysis of copper concentrations in liver and serum samples previously collected from cattle in herd 2 indicated copper deficiency (Ellison and Feyter, 1988). All calves in this herd received a subcutaneous injection of 676 mg of calcium-copper EDTA (Coprin®; Mallinckrodt Veterinary, Upper Hutt, New Zealand) prior to the start of the trial. The copper status of herd 1 was adequate based on current recommendations (Ellison and Feyter, 1988). Anthelmintic treatment of the calves consisted of 8 mg/kg of body weight of levamisole (Nilverm®; Mallinckrodt Veterinary) or 4.5 mg/kg of body weight of

oxfendazole (Systamex®; Mallinckrodt Veterinary) every 4 weeks for herds 1 and 2, respectively.

Experimental design

Calves were randomly assigned within herd to one of four groups according to a 2 × 2 factorial design, balanced for age and body weight. Treatments were 1) unsupplemented 2) supplemented with selenium 3) supplemented with iodine and 4) supplemented with both selenium and iodine.

Calves that were supplemented with selenium received two intraruminal selenium pellets (Permasel®; Mallinckrodt Veterinary) administered orally. These 30-g intraruminal pellets contain 10% elemental selenium in a finely divided matrix of iron, releasing selenium at a rate of about 3 mg/day for at least 1 year (Marchant, 1991). Calves that were supplemented with iodine received an intramuscular injection of 4 ml of iodinized poppyseed oil (Lipiodol®; Rhône Merieux, Wellington, New Zealand). This depot injection provided a total of 1.6 g of iodine, or 4.4 mg/day per calf if it is assumed that iodine is released from the injection site at a constant rate for 1 year (Grace, 1994).

Blood sampling and body weight gain

Prior to supplementation, blood samples were obtained from 8 calves per group in each of the two herds. The same calves were subsequently sampled at 6 and 9 weeks following commencement of treatment. On each occasion, blood was drawn from the jugular vein into plain and heparinized tubes and held at 4°C. Heparinized blood was centrifuged within four hours of collection and plasma was frozen at -20°C until assays for basal triiodothyronine, thyroxine, growth hormone and insulin-like growth factor-I were performed. Clotted blood was submitted directly to the Ministry of Agriculture and Fisheries Animal Health Laboratory (Hamilton, New Zealand) for analysis of serum selenium. Heifer body weight was monitored at the start of the trial and at subsequent sampling dates using electronic scales (AG500; Tru Test, Hamilton, New Zealand).

Thyrotropin-releasing hormone challenge

Nine weeks after commencement of treatment, a subgroup of 4 calves from each treatment group in each herd were chosen using a formal random sampling procedure and assigned to a thyrotropin-releasing hormone challenge study.

The calves were placed in a building and tethered in individual stalls on grates with rubber matting. Calves were allowed 8 days to adapt to a pelleted lucerne hay diet (selenium concentration 0.02 μg/kg of dry matter; iodine concentration 0.08 mg/kg of dry matter), fed once daily at 1600 h and ad libitum access to water. The amount of feed offered was 1.2 times maintenance requirements, calculated using the metabolisable energy requirements for calves (Holmes and Wilson, 1984) and body weight measured on entry to the building. Adaptation to the diet was assessed by weighing uneaten feed. There was no feed refusal after 3 days on the pelleted diet.

Intravenous canulas, consisting of polyethylene tubing (1 mm i.d.; Dural Plastics and Engineering, Dural, Australia), were placed in the jugular vein of each calf on day 9 and patency was maintained using 100 IU/ml of heparin in sterile, pyrogen-free saline. Feed was withheld from the afternoon of day 9. On day 10, 10-ml blood samples were obtained according to the following schedule: -20, -10, -5, 5, 10 20, 40, 60, 120, 240, 480, 720, and 960 min relative to thyrotropin-releasing hormone administration at time zero. Synthetic thyrotropin-releasing hormone (thyrotropin-releasing hormone®; Roche, Auckland, New Zealand) was diluted in saline to a concentration of 10 μg/ml and administered intravenously at a dose of 0.33 μg/kg of body weight (McCutcheon and Bauman, 1986).

Once collected into citrate anticoagulant tubes, blood samples were immediately placed on ice. The samples were centrifuged within 1 h; plasma was separated and then frozen at -20°C for the thyroid hormone, growth hormone, and insulin-like growth factor-I assays.

Assay of hormones and selenium

Total concentrations of triiodothyronine and thyroxine were determined using solid-phase radioimmunoassay kits (Coat-a-Count®; Diagnostic Products, Los Angeles, CA). Sensitivity

of the triiodothyronine assay was 0.05 nmol/L, and the half-displacement concentration was 1.94 nmol/L. The intra- and interassay coefficients of variation were 4.8 and 7.0%, respectively. Sensitivity of the thyroxine assay was 1.29 nmol/L, and the half-displacement concentration was 78.9 nmol/L. The intra- and interassay coefficients of variation were 5.1 and 7.7% respectively.

The concentration of growth hormone was determined by a double-antibody radioimmunoassay (Flux *et al.*, 1984) using pituitary-derived bovine growth hormone for iodination with ^{125}I (USDA-bGH-I1; 3.2 IU/mg) and for reference standards (USDA-bGH-B1; 1.9 IU/mg). Sensitivity of this assay was 0.79 ng/ml, and the half-displacement concentration was 15.5 ng/ml. Intra- and interassay coefficients of variation were 9.1 and 15.8%, respectively.

The concentration of insulin-like growth factor-I was determined for the three baseline plasma samples collected immediately before the thyrotropin-releasing hormone challenge. The method used a double-antibody radioimmunoassay after acid-ethanol extraction as described elsewhere (Hodgkinson *et al.*, 1991). The sensitivity of this assay was 1.06 ng/ml, and the half-displacement concentration was 3.73 ng/ml. Intra- and interassay coefficients of variation were 9.3 and 8.5%, respectively.

Assay of serum selenium concentration was performed by the Ministry of Agriculture and Fisheries Animal Health Laboratory in Hamilton, New Zealand using a fluorometric method developed by Watkinson (Watkinson, 1979).

Statistical analysis

Analysis was performed using analysis of variance and covariance, utilizing the general linear model procedure of SAS® (SAS, 1988a). Herd, selenium treatment, and iodine treatment were the main effects. selenium and iodine treatment were nested within herd. The initial models tested included terms for interaction between selenium and iodine treatments within herd, but interactions were not significant ($P>0.1$) for any of the independent variables measured. The final reduced models followed the general equation:

$$Y_{ijk} = \mu + H_i + S(H)_{j(i)} + I(H)_{k(i)} + \beta_1 X_{1ijk} + e_{l(ijk)}$$

where

Y_{ijk} = dependant variable,

μ = overall mean,

H_i = effect of the herd i (i = 1..2),

$S(H)_{j(i)}$ = effect of the selenium treatment group j within herd i (j = 1..2),

$I(H)_{k(i)}$ = effect of the I treatment group k within herd i (k = 1..2),

β_1 = coefficient of regression of Y on the continuous covariable (X_1), and

$e_{l(ijk)}$ = residual error.

Means for daily body weight gain were adjusted by using initial body weight as a covariant. Means for the basal plasma concentrations of selenium and hormones were adjusted by using the pretreatment values as covariants. The tabulated hormonal responses to thyrotropin-releasing hormone for the main effects were calculated as the peak hormone concentration and the area under the entire response curve, above the baseline concentration. Means reported are least squares means and standard error of the means. Plots of the hormonal response to thyrotropin-releasing hormone for each factor combination (Figure 6.1) were calculated without subtracting the baseline concentration, instead using the baseline value as a covariant.

Results

No interaction between selenium and iodine supplementation was apparent for any of the outcomes measured; therefore, the tabulated results are presented in terms of the main effects only.

Serum selenium concentration and body weight gain

Serum selenium concentration of the calves that were not supplemented with selenium remained in the deficient range throughout the study (Table 6.1). A minimum serum selenium concentration of 150 nmol/L (12 ng/ml) is recommended for cattle grazing pasture (Ellison and Feyter, 1988). Serum selenium concentration of the calves supplemented with selenium increased to a mean of over 800 nmol/L (63 ng/ml). The mean daily body weight gain of calves in herd 2 that were supplemented with selenium was 20% higher than that of calves receiving no selenium (P=0.01, Table 6.1). Supplementation with selenium had no effect on the growth rate of calves in herd 1. Supplementation with iodine had no effect on serum concentration of selenium or growth rate of calves.

Basal hormone concentrations

Basal hormone concentrations in plasma obtained 6 weeks after treatment are presented in Table 6.2. Concentrations of thyroid hormones were higher and concentration of growth hormone lower for calves in herd 1 when compared to those for calves in herd 2 (P<0.05).

Supplementation with selenium increased triiodothyronine concentration and decreased plasma thyroxine concentration for calves in both herds. Plasma growth hormone concentration was unaffected by selenium treatment. Supplementation with iodine had no effect on basal hormone concentrations except for a tendency to increase thyroxine concentration for calves in herd 2 (P=0.07).

Response to thyrotropin-releasing hormone challenge

Supplementation with selenium increased the baseline concentration of triiodothyronine, peak triiodothyronine concentration, and the area under the triiodothyronine response curve

in herd 2 ($P<0.05$; Table 6.3; Figure 6.1) but not in herd 1. Peak concentration of thyroxine and the area under the thyroxine response curve were not affected by selenium supplementation of either herd (Table 6.4; Figure 6.1) but supplementation decreased baseline thyroxine concentration for calves in herd 1 ($P<0.05$).

Supplementation with iodine increased peak concentrations of triiodothyronine and thyroxine and areas under the curves for triiodothyronine and thyroxine for both herds. This effect was additive to that of selenium supplementation (Figure 6.1), with no statistical interactions. No clear trend was apparent for baseline concentrations of triiodothyronine and thyroxine (Tables 6.3 and 6.4).

Baseline plasma growth hormone concentration and the growth hormone response to thyrotropin-releasing hormone challenge were unaffected by supplementation (Table 6.5 and Figure 6.1); however, concentration of insulin-like growth factor-I tended to increase for calves of herd 2 that were supplemented with selenium ($P=0.06$; Table 6.5).

Table 6.1. **Mean serum selenium concentration and daily body weight gain in heifer calves supplemented with selenium or iodine[a].**

Treatment and effect	Herd 1					Herd 2				
	Calves (no.)	Serum selenium (nmol/L)[b]		Gain (kg/d)		Calves (no.)	Serum selenium (nmol/L)		Gain (kg/d)	
		LSM	SE	LSM	SE		LSM	SE	LSM	SE
Selenium-	40	121.4	25.7	0.79	0.02	27	126.8	25.3	0.56	0.02
Selenium+	39	825.6	26.6	0.81	0.02	27	857.9	25.1	0.67	0.02
Iodine-	41	485.1	26.5	0.80	0.02	26	483.0	25.0	0.62	0.02
Iodine+	38	461.9	25.7	0.80	0.02	26	501.7	24.9	0.63	0.02
Effect[c]					**P**					
Selenium		<0.01		NS			<0.01		<0.01	
Iodine		NS		NS			NS		NS	
Herd							NS		<0.01	

[a] Treatments were selenium, 2 intraruminal selenium pellets; iodine, 4 ml 40% (*wt/wt*) iodinized poppyseed oil injected intramuscularly; + = supplemented, and - = not supplemented.

[b] Mean serum selenium of 16 calves per group 6 weeks after commencement of supplementation; ng/ml = nmol/L ÷ 12.67.

[c] NS = $P>0.10$; selenium x iodine interaction within herds = NS.

Table 6.2. **The effect of selenium or iodine supplementation on basal plasma concentrations of triiodothyronine (T_3), thyroxine (T_4) and growth hormone (GH) in heifer calves[a].**

Treatment and Effect	Herd 1							Herd 2					
	T3 (nmol/L)		T4 (nmol/L)		GH (ng/ml)			T3 (nmol/L)		T4 (nmol/L)		GH (ng/ml)	
	LSM	SE	LSM	SE	LSM	SE		LSM	SE	LSM	SE	LSM	SE
Se-	2.06	0.11	89.2	3.0	7.72	1.29		1.81	0.11	65.7	3.1	14.13	1.28
Se+	2.48	0.11	77.9	3.3	8.00	1.30		2.13	0.11	56.1	3.0	15.36	1.28
Iodine-	2.27	0.12	80.6	3.3	7.56	1.29		1.88	0.10	57.2	3.1	13.63	1.28
Iodine+	2.27	0.10	86.5	3.1	8.29	1.29		2.06	0.11	64.6	3.0	15.91	1.28
Effect[b]							**P**						
Selenium	<0.01		<0.01		NS			0.02		0.02		NS	
Iodine	NS		NS		NS			NS		0.07		NS	
Herd								0.04		<0.01		0.02	

[a] Treatments were selenium, 2 intraruminal selenium pellets; iodine, 4 ml 40% (*wt/wt*) iodinized poppyseed oil injected intramuscularly; 16 calves per group; + = supplemented, and - = not supplemented.

[b] NS = $P>0.10$; selenium x iodine interaction within herds = NS.

Table 6.3. **The effect of selenium or iodine supplementation on the plasma triiodothyronine (T_3) response to intravenous administration of thyrotropin-releasing hormone in heifer calves[a].**

Treatment and Effect	Herd 1				Herd 2		
	T3 Baseline (nmol/L)	T3 Peak[b] (nmol/L)	T3 AUC[c] (nmol•min/L)		T3 Baseline (nmol/L)	T3 Peak (nmol/L)	T3 AUC (nmol•min/L)
Selenium-	1.81	1.89	826		1.49	1.52	755
Selenium+	1.94	2.01	950		1.89	1.85	946
Iodine-	2.02	1.65	674		1.63	1.31	638
Iodine+	1.72	2.24	1102		1.75	2.05	1067
SE	0.11	0.10	57		0.11	0.10	57
Effect[d]				**P**			
Selenium	NS	NS	NS		0.02	0.03	0.03
Iodine	0.06	<0.01	<0.01		NS	<0.01	<0.01
Herd					NS	0.02	NS

[a] Treatments were selenium, 2 intraruminal selenium pellets; iodine, 4 ml 40% (*wt/wt*) iodinized poppyseed oil injected intramuscularly; 8 calves per group; + = supplemented, and - = not supplemented.

[b] Peak T3 concentration above the baseline concentration.

[c] Area under the T3 curve above the baseline concentration.

[d] NS = $P>0.10$; selenium x iodine interaction within herds = NS.

Table 6.4. **The effect of selenium or iodine supplementation on the plasma thyroxine (T_4) response to intravenous administration of thyrotropin-releasing hormone in heifer calves[a].**

Treatment and Effect	Herd 1				Herd 2		
	T4 Baseline (nmol/L)	T4 Peak[b] (nmol/L)	T4 AUC[c] (nmol•min/L)		T4 Baseline (nmol/L)	T4 Peak (nmol/L)	T4 AUC (nmol•min/L)
Selenium-	108.4	46.8	30463		80.6	54.9	35743
Selenium+	85.3	50.4	28125		74.0	47.8	30487
Iodine-	98.0	37.8	16990		68.4	39.7	23181
Iodine+	95.7	59.4	41597		86.2	62.9	43048
SE	6.8	5.5	4358		6.8	5.5	4358
Effect[d]				**P**			
Selenium	0.02	NS	NS		NS	NS	NS
Iodine	NS	0.01	<0.01		0.07	<0.01	<0.01
Herd					<0.01	NS	NS

[a] Treatments were selenium, 2 intraruminal selenium pellets; iodine, 4 ml 40% (*wt/wt*) iodinized poppyseed oil injected intramuscularly; 8 calves per group; + = supplemented, and - = not supplemented.

[b] Peak T4 concentration above the baseline concentration.

[c] Area under the T4 curve above the baseline concentration.

[d] NS = $P>0.10$; selenium x iodine interaction within herds = NS.

Table 6.5. **The effect of supplementing heifer calves with selenium or iodine on the plasma growth hormone (GH) response to intravenous administration of thyrotropin-releasing hormone and on plasma insulin-like growth factor-I (IGF-I) concentration[a].**

Treatment and Effect	Herd 1					Herd 2			
	GH Baseline (nmol/L)	GH Peak[b] (nmol/L)	GH AUC[c] (nmol•min/L)	IGF-I[d] (ng/ml)		GH Baseline (nmol/L)	GH Peak (nmol/L)	GH AUC (nmol•min/L)	IGF-I (ng/ml)
Selenium-	7.44	45.1	986	244.9		7.30	38.3	886	179.4
Selenium+	6.03	60.6	1058	243.4		8.23	42.8	848	210.0
Iodine-	7.80	46.5	688	257.2		7.60	38.0	544	183.7
Iodine+	5.66	59.1	1356	231.1		7.93	43.2	1191	205.7
SE	1.84	8.6	283	10.9		1.84	8.6	283	10.9
Effect[e]					**P**				
Selenium	NS	NS	NS	NS		NS	NS	NS	0.06
Iodine	NS	NS	NS	NS		NS	NS	NS	NS
Herd						NS	NS	NS	<0.01

[a] Treatments were selenium, 2 intraruminal selenium pellets; iodine, 4 ml 40% (*wt/wt*) iodinized poppyseed oil injected intramuscularly; 8 calves per group; + = supplemented, and - = not supplemented.

[b] Peak GH concentration above the baseline concentration.

[c] Area under the GH curve above the baseline concentration.

[d] Mean baseline concentration.

[e] NS = $P<0.10$; selenium x iodine interaction within herds = NS.

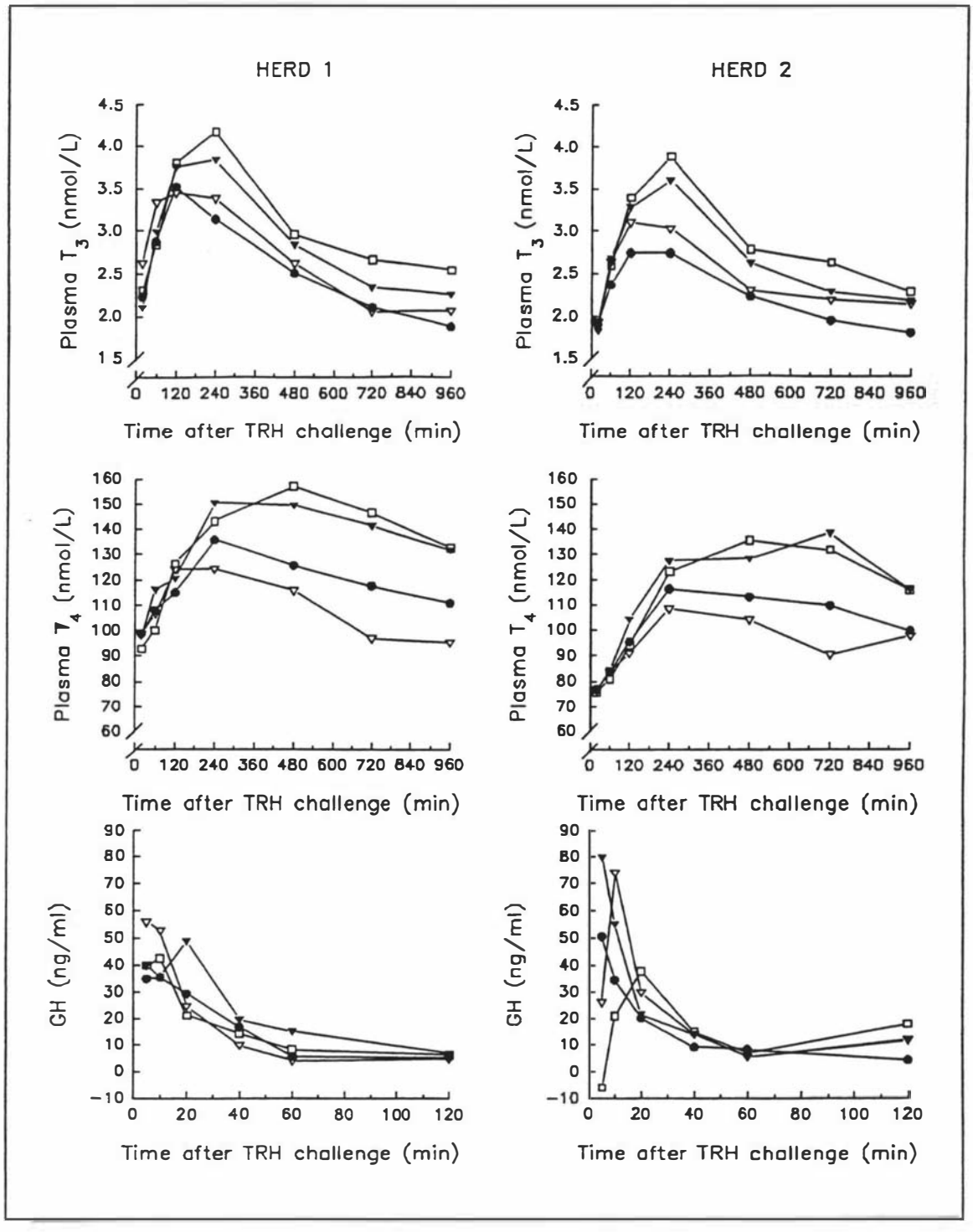

Figure 6.1. **Comparison of the effects of treatment of calves (n=4) with two selenium pellets (▽), 4 ml of 40% iodine in oil injected intramuscularly (▾), both selenium and iodine (□), or no treatment (•) on plasma concentrations of triiodothyronine (T_3), thyroxine (T_4), and growth hormone (GH) following challenge with thyrotropin-releasing hormone (TRH). Curves are adjusted for baseline values. Pooled SEM of the T_3 response (the area under the hormone curve) at 240 minutes was 0.18 and 0.13 nmol/L, of the T_4 response at 720 minutes was 6.5 and 9.7 nmol/L, of the GH response at 20 minutes was 17.8 and 18.3 ng/ml, for herds 1 and 2, respectively.**

Discussion

Supplementation of calves with intraruminal selenium pellets increased the basal plasma concentration of triiodothyronine and reduced the basal plasma concentration of thyroxine. This is in agreement with Arthur *et al.* (1991a) who reported similar changes in thyroid hormone concentration for supplemented calves fed a synthetic diet deficient in selenium. In the present study, supplementation with selenium increased the triiodothyronine response to thyrotropin-releasing hormone challenge, increased body weight gain and tended to increase the plasma concentration of insulin-like growth factor-I for one herd; however, the concentration of growth hormone was unaffected by selenium supplementation. Arthur *et al.* (1991a) reported no change in body weight gain in response to selenium supplementation, despite selenium intakes of less than 0.015 mg/kg of dry matter for the calves in their control group.

The response of triiodothyronine and thyroxine to thyrotropin-releasing hormone was increased in calves of both herds that were supplemented with iodine, however basal concentrations were largely unchanged and there was no growth rate response to iodine. This is in agreement with Barry et al (Barry *et al.*, 1981) who reported that supplementation with iodinized poppyseed oil resulted in no increase in the body weight gain or serum concentrations of triiodothyronine and thyroxine for cattle grazing pasture with an iodine concentration of 0.18 mg/kg of dry matter. The minimum iodine requirement for growth of calves fed pasture may be less than the 0.25 mg/kg of dry matter currently recommended by the National Research Council (National Research Council, 1988).

Plasma growth hormone concentrations (basal or responsive to thyrotropin-releasing hormone) in the present study were not significantly affected by selenium supplementation. This agrees with the findings of Arthur *et al.* (1990) in rats and suggests that the effects of selenium deficiency in grazing calves may not be mediated by alterations in peripheral growth hormone concentration. Deficiency of selenium could alter somatotropic function through effects on the endocrine or paracrine production of insulin-like growth factor-I, secretion of insulin-like growth factor-II, the number of somatotropic receptors or the

peripheral concentration of insulin-like growth factor-binding proteins. The concentration of insulin-like growth factor-I tended to be increased in the calves of herd 2 that had been supplemented with selenium.

A body weight gain response was noted only for calves in herd 2, despite the similar serum selenium concentrations of the two herds (Table 6.1). Overall, body weight gain (Table 6.1) and thyroid hormone concentrations (Table 6.2) were lower for calves in herd 2 when compared to those for calves in herd 1, but supplementation with iodine did not affect growth or the hormonal response to selenium supplementation for either herd. It is difficult to draw conclusions from comparisons between herds because of the possibility for confounding herd factors.

A lack of iodine may exacerbate the adverse effects of selenium deficiency on thyroid hormone metabolism (Arthur *et al.*, 1990), which may partially explain the inconsistent growth rate response to selenium supplementation. An interaction between selenium and iodine may have important implications for livestock grazing pasture grown on soils with low concentrations of both selenium and iodine. No such interaction was noted under the conditions of the present study; however, the intake of iodine for calves may have been in excess of minimum requirements during the period of this study. Further controlled experimentation is required to determine if an interaction between selenium and iodine occurs with lower iodine intakes.

Conclusions

These results suggest that the adverse effects of selenium deficiency on grazing cattle may be mediated by altered thyroid hormone metabolism, but not through modulation of peripheral concentrations of growth hormone. Interactions between selenium intake and the other determinants of thyroid metabolism, most notably iodine intake, require further study and may lead to formulation of more precise recommendations for selenium supplementation of grazing animals.

CHAPTER 7

Effects of selenium and iodine supplementation on the growth rate, mohair production and thyroid status of Angora goat kids[5]

[5] Submitted as: Wichtel JJ, Thompson KG, Craigie, AL and Williamson NB. New Zealand Journal of Agricultural Research, 1995.

Abstract

The objective of this study was to examine the effect of supplementation with selenium, iodine or both on growth rate, mohair production and plasma concentrations of thyroid hormones in Angora goat kids. Kids (n=54) were treated with 0 or 1 intraruminal selenium pellets, or treated intramuscularly with iodised oil at doses of 0, 200 or 400 mg of iodine in a 2 by 3 factorial arrangement. Supplementary selenium enhanced liveweight gain by 26% during the final month of the study, however mohair growth was not affected by either treatment. The plasma concentration of total thyroxine was increased by iodine treatment but decreased by selenium treatment. The plasma concentration of total triiodothyronine was reduced by iodine treatment but unaffected by selenium. Thus, selenium supplementation improved the liveweight gain of Angora goats but iodine supplementation had no effect on performance. Both selenium and iodine treatment altered thyroid hormone concentrations, but selenium by iodine interactions were not significant.

Introduction

The impaired performance of ruminants grazing forages deficient in selenium (Andrews *et al.*, 1968) may in part be due to alterations in thyroid hormone metabolism induced by selenium deficiency. The activity of the seleno-enzyme type I triiodothyronine 5′-deiodinase is sensitive to changes in selenium intake (Beckett *et al.*, 1993). Supplementary selenium causes plasma concentrations of triiodothyronine to be increased and thyroxine to be decreased in calves (Chapter 6) and ewes (Donald *et al.*, 1993) grazing pasture deficient in selenium.

Deficiency of selenium can exacerbate the hypothyroidism associated with deficiency of iodine in rats (Beckett *et al.*, 1993). Interactions between intakes of selenium and iodine that affect animal performance would have important implications for livestock grazing forages with low concentrations of both of these micronutrients. However, such interactions were not observed in lambs born to ewes which were treated with thiocyanate to induce hypothyroidism (Donald *et al.*, 1993). It is not known whether interactions between selenium and iodine supplementation occur in ruminants fed forages deficient in both selenium and iodine.

The objective of this study was to investigate the effect of supplementation with either selenium or iodine, or both, on growth rate, mohair production and plasma concentrations of thyroid hormones in Angora goat kids fed lucerne pellets deficient in both selenium and iodine.

Materials and Methods

Castrated male Angora goat kids (n=54), between 3 and 4 months old and weighing on average 12.5 ± 0.3 kg, were shorn and housed in a shed located on a research dairy farm at Massey University, Palmerston North. The experimental period was 95 days, beginning on 2 December, 1994. Pelleted lucerne (*Medicago sativa*) grown on the pumice soils of the central North Island was fed twice daily from troughs at twice the metabolisable energy requirement for maintenance (National Research Council, 1981; Table 7.1). Salt and copper (as copper sulphate) at rates of 5 g/kg and 4 mg/kg of dry matter, respectively, and vitamins A D and E, at rates of 16 iu/kg, 800 iu/kg and 7 iu/kg of dry matter, respectively, were added to the pellets daily.

Table 7.1 Composition on a dry matter basis of lucerne pellets.

Component	Concentration
Metabolisable Energy (MJ ME/kg)	9.5
Crude Protein (%)	19.2
Digestibility (%)	60.0
Selenium[a] (mg/kg)	0.02
Copper (mg/kg)	4.0
Iodine[a] (mg/kg)	0.09

[a] Requirements (dry matter basis, based on data for sheep) for selenium, copper and iodine are 0.03 mg/kg, 5 mg/kg and 0.3 mg/kg, respectively (Grace, 1994).

Treatment with intraruminal selenium pellets at doses of 0 or 1 pellet per kid and treatment with iodised oil at doses of 0, 200 or 400 mg of iodine per kid intramuscularly were administered in a 2 by 3 factorial arrangement in 3 randomised complete blocks, each block consisting of 18 goats assigned to 1 of 3 pens. The intraruminal pellets (Permasel Sheep®: Mallinckrodt Veterinary, Upper Hutt) contain 0.5 g of elemental selenium in a finely divided matrix of iron, and release selenium at a rate of about 0.5 mg/day for at least 1 year (S. Murray, personal communication). The depot injection of iodised poppy

seed oil (Lipiodol®: Rhône Merieux, Wellington) provides a total of 200 or 400 mg of iodine, equivalent to 0.27 or 0.55 mg/day per kid, respectively, if it is assumed that iodine is released from the injection site at a constant rate for 2 years (Grace, 1994).

Blood samples were obtained on the day of treatment and 95 days later. Blood was drawn from the jugular vein into plain and EDTA tubes and held at 4°C for up to 3 hours. After centrifugation, plasma and serum were decanted and frozen at -80°C until assays for total triiodothyronine, total thyroxine and selenium were performed. Body weight was monitored at the start of the trial and each month thereafter using electronic scales. The amount of mohair produced was determined by weighing the clean fleece on the last day of the experiment.

Total concentrations of triiodothyronine and thyroxine were determined using solid-phase radioimmunoassay kits (Coat-a-Count®: Diagnostic Products, Los Angeles, CA). The sensitivity of the triiodothyronine assay was 0.05 nmol/L and the half-displacement concentration was 1.6 nmol/L. The intra- and interassay coefficients of variation were 4.7 and 7.0%, respectively. Sensitivity of the thyroxine assay was 1.8 nmol/L, and the half-displacement concentration was 56.4 nmol/L. The intra- and interassay coefficients of variation were 4.8 and 7.7% respectively. Assay of serum selenium concentration was performed using the fluorometric method developed by Watkinson (1979).

The protein content of the lucerne was determined by Kjeldahl digestion and analysed colorimetrically using the indophenol blue method (Bradstreet, 1965). Metabolisable energy and digestibility were estimated using calculations based on nitrogen and acid detergent fibre content (Clancey and Wilson, 1966) of the sample.

Statistical analysis was performed using analysis of variance and covariance, utilizing the general linear model procedure of SAS (SAS User's Guide, 1988a). Pretreatment values were used as covariants for all dependant variables except fleece weight, for which initial body weight was used. The models followed the general equation:

$$Y_{ijk} = \mu + P_i + S_j + I_k + SI_{jk} + \beta_1 X_{1ijk} + e_{ijk}$$

where

Y_{ijk} =	dependant variable,
μ =	overall mean,
P_i =	effect of the pen i (i = 1..3),
S_j =	effect of the selenium treatment group j (j = 1..2),
I_k =	effect of the iodine treatment group k (k = 1..3),
SI_{jk} =	effect of the interaction between selenium and iodine,
β_1 =	coefficient of regression of Y on the continuous variable X_1, and
e_{ijk} =	residual error.

Treatments were considered fixed effects. Means reported are least squares means ± standard error (SE) of the means. Results were considered to be significant at $P<0.05$.

Results

Interaction between selenium and iodine supplementation was not significant for any of the outcomes measured; accordingly, the tabulated results are presented in terms of the main effects only. However, interactions may not have been detectable because of the limited number of observations. Therefore, the profiles of the least squares means for selected treatment combinations are presented in Figure 7.1.

Supplementation with selenium or iodine had no effect on fleece weight (Table 7.2). The daily liveweight gain (Table 7.2) of the goats that received supplementary selenium was 26% higher than that of the goats receiving no selenium ($P<0.001$) during the final month of the study. When measured over the entire 95-day study period, however, the liveweight gain was not significantly higher for the goats that received supplementary selenium ($P=0.43$). Serum selenium concentrations of the goats at the end of the study were 959

nmol/L and 139 nmol/L, respectively, for the goats that did or did not receive supplementary selenium ($P<0.001$; Table 7.3). The pretreatment serum selenium concentration (data not shown) was 232 ± 27.5 nmol/L for the goats that did not receive supplementary selenium (within-subject effect of time $P=0.2$).

The effect of iodine on liveweight gain and its interaction with selenium were not significant. However, the profile for the least squares means for liveweight gain during the third month (Figure 7.1) suggests that iodine treatment impaired growth, particularly in the goats treated with selenium. Liveweight gain was 92.9 g/day for goats receiving selenium and no supplementary iodine but 72.2 g/day for goats receiving selenium and 400 g of supplementary iodine ($P=0.08$).

Supplementation with selenium decreased plasma total thyroxine concentration by 18%, but the small increase in plasma total triiodothyronine concentration was not significant (Table 7.3). Supplementation with iodine increased plasma thyroxine concentration but decreased triiodothyronine concentration. The lack of significance of the selenium by iodine interaction for plasma thyroxine concentration suggests that the effects of selenium and iodine on plasma thyroxine are additive and this is supported by the appearance of the profile plot for thyroxine (Figure 7.1).

Table 7.2. **Least squares means for main effects on growth rate (overall and during the last month) and fleece weight for Angora goat kids treated with intraruminal selenium pellets at doses of 0 or 1 pellet per kid, or treated with depot iodine at doses of 0, 200 or 400 mg per kid.**

Treatment and effect	Growth rate (g/day)		Fleece weight (kg)
	Last month	Overall	
Selenium -	66.6	83.0	0.97
Selenium +	83.6	87.9	1.01
SE	4.8	4.3	0.03
Iodine 0 mg	83.2	88.6	0.98
Iodine 200 mg	73.2	83.5	0.98
Iodine 400 mg	69.0	84.3	1.00
SE	5.8	5.3	0.04
Effect		**P** [a]	
Selenium	*	NS	NS
Iodine	NS	NS	NS

[a] *= $P < 0.05$; NS= not significant.

Table 7.3. **Least squares means for main effects on concentrations of selenium in serum and of triiodothyronine (T_3) and thyroxine (T_4) in plasma for Angora goat kids treated with intraruminal selenium pellets at doses of 0 or 1 pellet per kid, or treated with depot iodine at doses of 0, 200 or 400 mg of iodine per kid.**

Treatment and effect	Selenium	T_3	T_4
		nmol/L	
Selenium -	139	1.77	91.1
Selenium +	959	1.85	74.4
SE	30	0.08	2.7
Iodine 0 mg	603	2.21	65.8
Iodine 200 mg	519	1.63	89.0
Iodine 400 mg	526	1.59	93.6
SE	36	0.10	3.3
Effect		**P** [a]	
Selenium	***	NS	***
Iodine	NS	***	***

[a] ***= $P < 0.001$; NS= not significant.

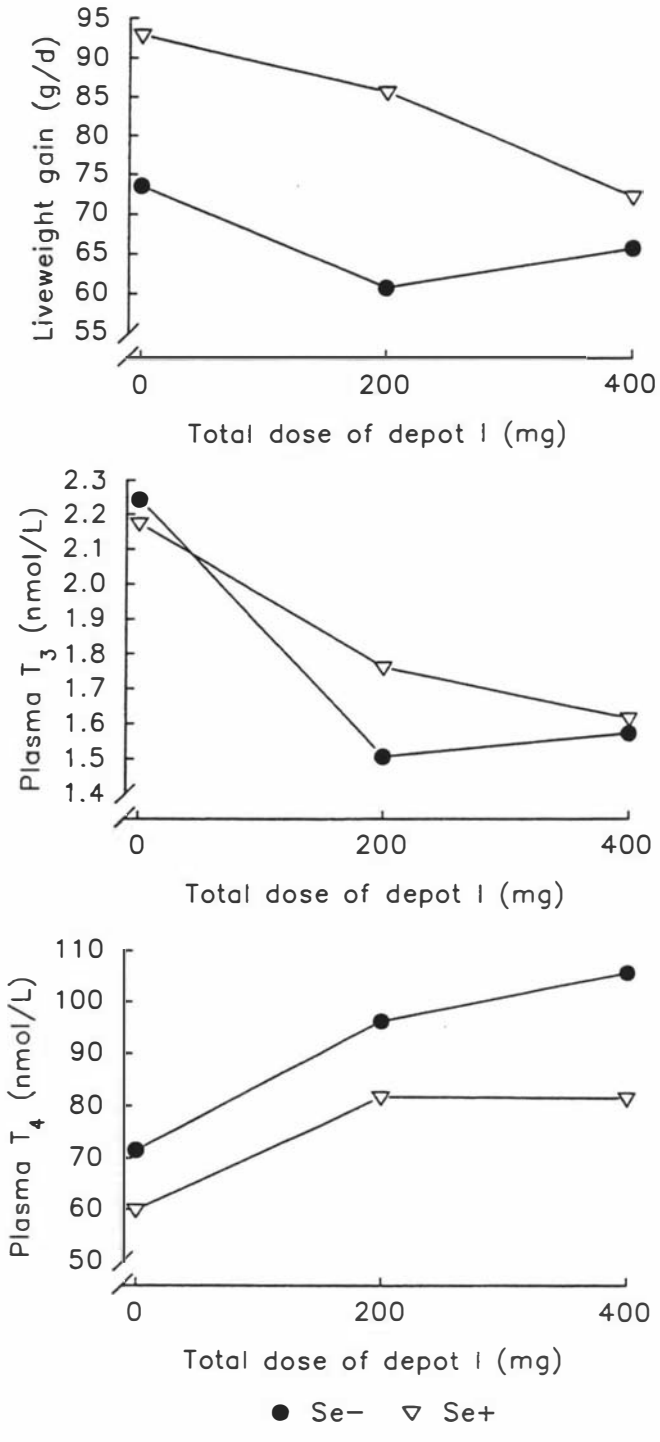

Figure 7.1 **Profiles of the least squares means for growth rate during the last month, and concentrations of triiodothyronine (T_3) and thyroxine (T_4) in plasma of Angora goat kids treated with intraruminal selenium pellets at doses of 0 or 1 pellet per kid, or treated with depot iodine at doses of 0, 200 or 400 mg per kid in a 2 by 3 factorial arrangement. Pooled SE for growth rate, T_3 and T_4 were 8.2 g/day , 0.15 nmol/L and 4.7 nmol/L, respectively.**

Discussion

To our knowledge, a growth response to selenium has not been documented in kids prior to this study. However, supplementary selenium increased liveweight gain in calves (Chapter 6) and lambs (Grace *et al.*, 1974). The tendency for reduced gain in kids supplemented with iodine in the present study might be due to the increased plasma thyroxine concentration induced by iodine treatment; in lambs (Coop and Clark, 1958) treatment with thyroxine decreased liveweight gain but increased fleece production.

The trace element requirements for mohair production have not been defined, but it appears from this study that liveweight gain may be more sensitive than mohair growth as an indicator of subclinical selenium deficiency in Angora kids.

Interpretation of serum selenium concentrations for goats is difficult because a reference range has not been formulated for this species. However, a minimum concentration of 150 nmol/L is recommended for calves grazing pasture (Ellison and Feyter, 1988), suggesting that the goats receiving no supplementary selenium were deficient by the end of the study. This was further supported by the growth response to supplementation observed during the final month of the study. We can conclude that the requirement for selenium for the growth of Angora kids is greater than 0.02 mg/kg of dry matter, and that a serum selenium concentration of less than 140 nmol/L reflects inadequate selenium intake.

Supplementation with selenium decreased plasma total thyroxine concentration, presumably reflecting increased peripheral metabolism of thyroxine by 5′-deiodinase (Beckett *et al.*, 1993). The additive nature of the effects of treatment with selenium and iodine on plasma thyroxine concurs with findings for calves (Chapter 6). The lack of significant increase in plasma total triiodothyronine concentration in response to selenium supplementation is at variance with other reports where supplementary selenium increased plasma triiodothyronine in calves (Chapter 6) and ewes (Donald *et al.*, 1993). If production responses to selenium are mediated via the thyroid axis, changes in the tissue concentration of triiodothyronine, the most active of the thyroid hormones, would be expected. It would appear, however, that circulating triiodothyronine is not as sensitive as thyroxine to a

deficiency of selenium, possibly because of changes in the secretion and catabolism of triiodothyronine that occur in response to selenium deficiency (Chanoine *et al.*, 1992).

The effect of supplementary iodine in reducing plasma triiodothyronine was unexpected and is difficult to explain. In one study (Donald *et al.*, 1993) plasma triiodothyronine concentration was reduced in lambs born to ewes treated with iodine before lambing. The profile for the effect of supplementary iodine on plasma triiodothyronine is similar to the profile for the effect of iodine treatment on liveweight gain (Figure 7.1), suggesting that iodine supplementation was associated with lower rates of gain and reduced plasma concentrations of triiodothyronine. The mechanisms involved in thyroid homeostasis are complex and it appears that our understanding of the metabolic consequences of iodine supplementation is incomplete.

This study has shown that selenium supplementation alters thyroid hormone metabolism and improves liveweight gain of Angora goat kids fed lucerne deficient in selenium.

Iodine supplementation did not improve performance and no interaction between iodine and selenium was observed.

CHAPTER 8

Serum glutathione peroxidase activity reflects short-term changes in selenium intake in goats[6]

[6] Submitted as: Wichtel JJ, Thompson KG and Williamson NB. New Zealand Veterinary Journal, 1995.

Abstract

The objective of this study was to determine if serum glutathione peroxidase activity reflects short-term changes in selenium status of goats. Angora goat kids (n=14) were fed pelleted lucerne containing 20 μg/kg of selenium, and treated orally with either selenium (0.1 mg/kg of liveweight weekly, as sodium selenate) or deionised water. Serum activity of glutathione peroxidase was increased in response to supplementation and differed from that of controls within 24 hours of supplementation. The change in serum glutathione peroxidase activity during the 21 days after supplementation closely followed changes in serum selenium concentration. The results of this study suggest that serum glutathione peroxidase activity reflects short-term improvement in the selenium status of Angora goat kids following oral supplementation with sodium selenate.

Introduction

Assays of erythrocyte glutathione peroxidase activity are commonly used in the diagnosis of selenium deficiency but are not sensitive to acute changes in selenium intake (Thompson *et al.*, 1981). Because of this, serum and liver concentrations of selenium are currently considered the most useful indicators of short-term changes in selenium status following supplementation (Clark and Ellison, 1993). Evidence regarding the diagnostic value of serum and plasma glutathione peroxidase activity is conflicting (Thompson *et al.*, 1981; Hussein and Jones, 1982; Maas *et al.*, 1993; Thompson *et al.*, 1980). Thompson *et al.* (1980; 1981) found that serum glutathione peroxidase activity was a sensitive indicator of selenium status in calves, but suggested that the low activity of glutathione peroxidase in serum may make routine estimation difficult.

Compared to assays of selenium, assays of glutathione peroxidase activity are simpler to perform, are less susceptible to problems of sample contamination and can be more readily adapted for automated analysis. Surprisingly, little research has been performed on the suitability of serum or plasma glutathione peroxidase activity for routine diagnostic purposes in livestock. This paper presents data which suggest that serum glutathione peroxidase activity reflects short-term changes in selenium status following oral supplementation with sodium selenate in Angora goat kids.

Materials and Methods

Animals and experimental design

Castrated male Angora goat kids (n=14), between 6 and 7 months old and weighing 21.7 kg (SE=1.0) were matched for liveweight and within each pair were assigned randomly to one of the following two treatment groups: treatment at weekly intervals with a drench containing sodium selenate dissolved in deionised water, providing selenium at a rate of 0.1 mg/kg of liveweight, or treatment with an equivalent volume of deionised water only. From weaning at 2 months of age until the end of the 21-d experimental period, the goats were fed pelleted lucerne (*Medicago sativa*) with a selenium concentration of 20 µg/kg of dry matter. All kids were treated 3 months previously with 400 mg of iodine as iodised

poppy seed oil (Lipiodol®: Rhône Merieux, Wellington). The kids each received 2 ml orally of a solution containing 28 mg/ml copper (as copper sulphate) and 50,000 iu/ml, 2500 iu/ml, 20 iu/ml of vitamins A, D and E, respectively, before the start and on day 11 of the experiment.

Collection and analysis of samples

Blood samples were obtained immediately before treatment, and on days 1, 3, 7, 14 and 21 after the start of treatment. Blood was drawn from the jugular vein into serum-separator tubes (SST Vacutainer®: Becton-Dickinson, Rutherford, N.J., USA). After centrifugation, serum was decanted and frozen at -80 °C until assays for glutathione peroxidase activity and selenium were performed. Glutathione peroxidase activity was measured using the method published by Thompson *et al.* (1980) modified for a Hitachi 911 clinical chemistry analyser (Boehringer Mannheim, Auckland) substituting undiluted serum for whole blood lysate and using t-butyl hydroperoxide as substrate. Results are presented in katal units (IU) of glutathione peroxidase activity. A katal unit is that amount of enzyme bringing about the oxidation of NADPH at the rate of 1 mole per second. Assay of serum selenium concentration was performed using the fluorometric method developed by Watkinson (1979).

Statistical analysis was performed by analysis of variance, utilising the general linear model procedure of SAS (SAS User's Guide, 1988a). The effect of treatment was tested separately for each time period. Means reported are least squares means ± standard error (SE). Results were considered to be significant at $P<0.05$.

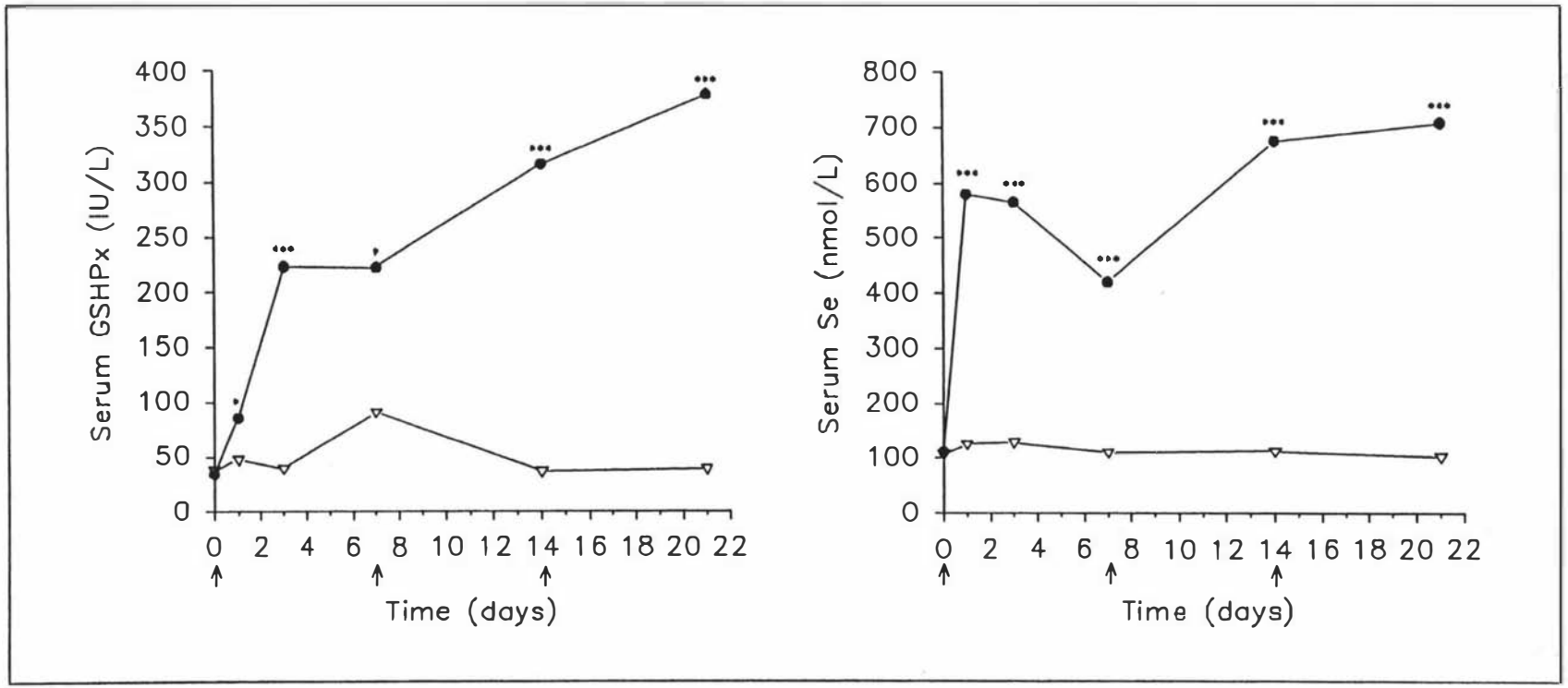

Figure 8.1. **The effect of oral treatment with sodium selenate (●) or deionised water (▽) on the activity of glutathione peroxidase (GSHPx) and concentration of selenium (Se) in serum for Angora goat kids. The arrows indicate the days on which treatment occurred. Pooled SE on day 14 for serum glutathione peroxidase and serum selenium were 10 and 20, respectively. Probability: * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.**

Results

Serum activity of glutathione peroxidase was increased ($P<0.05$, Figure 8.1) relative to that for controls within 24 hours of supplementation and remained so for the duration of the experiment. Changes in serum glutathione peroxidase activity closely followed changes in serum selenium concentration during the 21 days after the start of supplementation.

No significant modification of the standard assay procedure (Thompson *et al.*, 1980) was required when using undiluted serum instead of whole blood lysate. All observations made in this study fell above the detection limit of the assay.

Discussion

Assay of serum glutathione peroxidase appears to be as sensitive as serum selenium concentration to short-term improvement in selenium status in Angora goat kids. This observation agrees with previous findings in calves (Thompson *et al.*, 1980) but contrasts with the findings of Hussein and Jones (1982) who concluded that, in goats, glutathione peroxidase activity in plasma or serum was of no value as an indicator of selenium status. This disagreement may in part be due to differences in the selenium status of the experimental animals as the selenium intake for the goats in the latter study was not presented.

Thompson *et al.* (1980) found that plasma glutathione peroxidase activity in calves closely reflected both increasing and declining serum and liver selenium concentrations after a single intramuscular dose of sodium selenate. The present study did not measure glutathione peroxidase activity beyond 21 days after the start of supplementation. However, in comparing results from the present study to those of Thompson *et al.* (1980), it would appear that the baseline and peak serum selenium concentrations after a single treatment were similar for goats and calves, but the activity of glutathione peroxidase in serum was about 5 times greater in the goats. This suggests that, for a given selenium intake, serum

from goats has a greater glutathione peroxidase activity than serum from calves. Using serum glutathione peroxidase activity as a diagnostic test in species with relatively low serum activity may require modification of the standard assay procedure.

It has been suggested that glutathione peroxidase in serum is less stable than that in erythrocytes (Thompson *et al.*, 1981). Our data suggests that freezing serum at -80°C for up to 2 months does not compromise the diagnostic value of the samples. Unpublished data suggests that multiple freezing and thawing procedures may reduce the activity of the enzyme in caprine serum, and that there is a gradual reduction in activity when serum is stored at -20°C. Contamination of serum samples with red blood cells and haemolysis (*in vivo* or *in vitro*) are likely sources of error that require quantification in future studies.

Glutathione peroxidase is a functional marker of selenium status which, when compared to selenium, may better reflect the antioxidant status of the tissues in which it is assayed. However, neither the erythrocyte nor serum are known to be the principal site of dysfunction in selenium deficiency but are simply the most convenient samples to obtain from the live animal. Compared to erythrocyte glutathione peroxidase, activity of glutathione peroxidase in serum may more closely reflect the susceptibility of peripheral tissues to peroxidative damage.

This study has shown that the serum activity of glutathione peroxidase reflects short-term improvement in the selenium status of kids. Further research is required to determine if serum glutathione peroxidase activity could be used routinely as a sensitive and inexpensive test for monitoring the effect of selenium supplementation in goats and other livestock.

CHAPTER 9

Alteration of voluntary feed intake after selenium supplementation in Angora goat kids[7]

7 Submitted as: Wichtel JJ, Thompson KG, Craigie AL and Williamson NB. New Zealand Journal of Agricultural Research, 1995.

Abstract

The objective of this study was to examine the effect of selenium supplementation on voluntary feed intake in selenium-deficient Angora goat kids and its temporal relationship to alterations in the concentrations of thyroid hormones in plasma and the activity of glutathione peroxidase in serum. Angora goat kids (n=14) were fed pelleted lucerne containing 20 μg/kg of selenium and treated orally with either selenium (0.1 mg/kg of liveweight weekly, as sodium selenate) or deionised water. Supplementation with selenium had no effect on dry matter intake measured over 21 days. Serum activity of glutathione peroxidase was increased by day 1 of treatment (P<0.001), plasma concentration of thyroxine was decreased by day 21 of treatment (P<0.01) and plasma concentration of triiodothyronine was unaffected by treatment. It is concluded that increased appetite is unlikely to be the primary mechanism involved in the response to selenium supplementation in Angora goats.

Introduction

Impaired liveweight gain is commonly associated with selenium deficiency in growing ruminants (Underwood, 1977) but the mechanism is not known. Ewan (1976) provided evidence that in selenium-deficient rats impaired growth could be partly explained by a direct depression of voluntary feed intake. Selenium deficiency may alter appetite via a mechanism which does not involve selenium-dependant glutathione peroxidase and type I 5′-deiodinase. In ruminants, however, feed intake after supplementation with selenium has not been investigated.

The objective of this study was to examine the effect of selenium supplementation on voluntary feed intake in selenium-deficient Angora goat kids and its temporal relationship to alterations in the concentrations of thyroid hormones in plasma and the activity of glutathione peroxidase in serum.

Materials and Methods

Castrated Angora goat kids (n=14), between 6 and 7 months old and weighing 21.7 kg (SE=1.0), were shorn and housed in individual pens on sawdust. Kids were matched for liveweight and within each pair were assigned randomly to one of two treatment groups: treatment at weekly intervals with a drench containing sodium selenate dissolved in deionised water, providing selenium at a rate of 0.1 mg/kg of liveweight, or treatment with an equivalent volume of deionised water only. The goats had been fed pelleted lucerne (*Medicago sativa*) containing low concentrations of selenium since weaning at 2 months of age. Composition of the pellets is presented in Chapter 7. During the experimental period, the pellets were fed twice daily from buckets. Each kid received an identical ration which was 1.5 times the largest previously recorded intake. Feed refusals were weighed twice daily to calculate intake. One week was allowed for adaptation to the pens, and baseline daily intakes were recorded for 4 days prior to the first treatment. The experimental period was 21 days, beginning on 22 March. All kids had been treated 3 months previously with 400 mg of iodine as iodised poppy seed oil (Lipiodol®: Rhône

Merieux, Wellington). The kids each received 2 ml orally of a solution containing 28 mg/ml copper (as copper sulphate) and 50,000 iu/ml, 2500 iu/ml, 20 iu/ml of vitamins A, D and E, respectively, before the start and on day 11 of the experiment.

Blood samples were obtained immediately before treatment, and on days 1, 3, 7, 14 and 21 post-treatment. Blood was drawn from the jugular vein into EDTA-anticoagulant tubes and into plain serum-separator tubes (SST Vacutainer®: Becton-Dickinson, Rutherford, N.J., USA). After centrifugation, plasma and serum were decanted and frozen at -80 °C until assays for total triiodothyronine, total thyroxine and glutathione peroxidase were performed. Liveweight was recorded at the start, midpoint and end of the trial.

Details of the radioimmunoassay for total concentrations of triiodothyronine and thyroxine are presented in Chapter 7, as were the methods for feed analysis. Glutathione peroxidase activity was measured using the method published by Thompson *et al.*, (1980) modified for an Hitachi 911 clinical chemistry analyser (Boehringer Mannheim, Auckland) and using t-butyl hydroperoxide as substrate. One unit of glutathione peroxidase activity is that amount of enzyme bringing about the oxidation of NADPH at the rate of 1 mole per second.

Statistical analysis was performed using analysis of variance and covariance, utilizing the general linear model procedure of SAS (SAS User's Guide, 1988a). The effect of treatment was tested separately for each time period, with pre-treatment values used as covariants. Treatment was considered a fixed effect. For the purposes of statistical analysis, mean daily dry matter intake was calculated for the periods 0-3 days, 4-7 days, 8-14 days and 15-21 days post-treatment, adjusting for initial metabolic weight and using baseline intake as a covariant. Means reported are least squares means ± standard error (SE) of the means. Results were considered to be significant at $P<0.05$.

Results

Supplementation with selenium had no effect on the daily feed intake measured over 21 days (Figure 9.1) and the liveweight gain and gain-to-feed ratio did not differ between groups (Table 9.1). Serum selenium concentration measured prior to treatment (Table 9.1) indicated that these kids were deficient based on previous growth responses to selenium supplementation (see Chapter 7).

Serum activity of glutathione peroxidase was increased ($P<0.001$, Figure 9.1) and plasma concentration of thyroxine decreased ($P<0.01$, Figure 9.2) in response to supplementation. Plasma concentration of triiodothyronine was unaffected by treatment (Figure 9.2). The activity of glutathione peroxidase in serum increased rapidly in response to treatment, differing from that of controls by 24 hours after supplementation. Thyroxine tended to be lower ($P<0.10$) for the treated kids at 14 days and was significantly lower at 21 days after the start of treatment ($P<0.05$).

Table 9.1. The effect of oral treatment with sodium selenate on the least squares means for serum selenium, liveweight gain and feed-to-gain ratio for Angora goat kids fed lucerne pellets.

Variable	Control	Selenium	SE	Effect[a]
Serum selenium (nmol/L)				
Day 0	108	111	7	NS
Day 21	103	709	43	0.0001
Liveweight gain (g/day)	65.5	53.7	9.8	NS
Feed:Gain (g/kg)	64.7	55.3	8.5	NS

[a] Probability; NS = not significant.

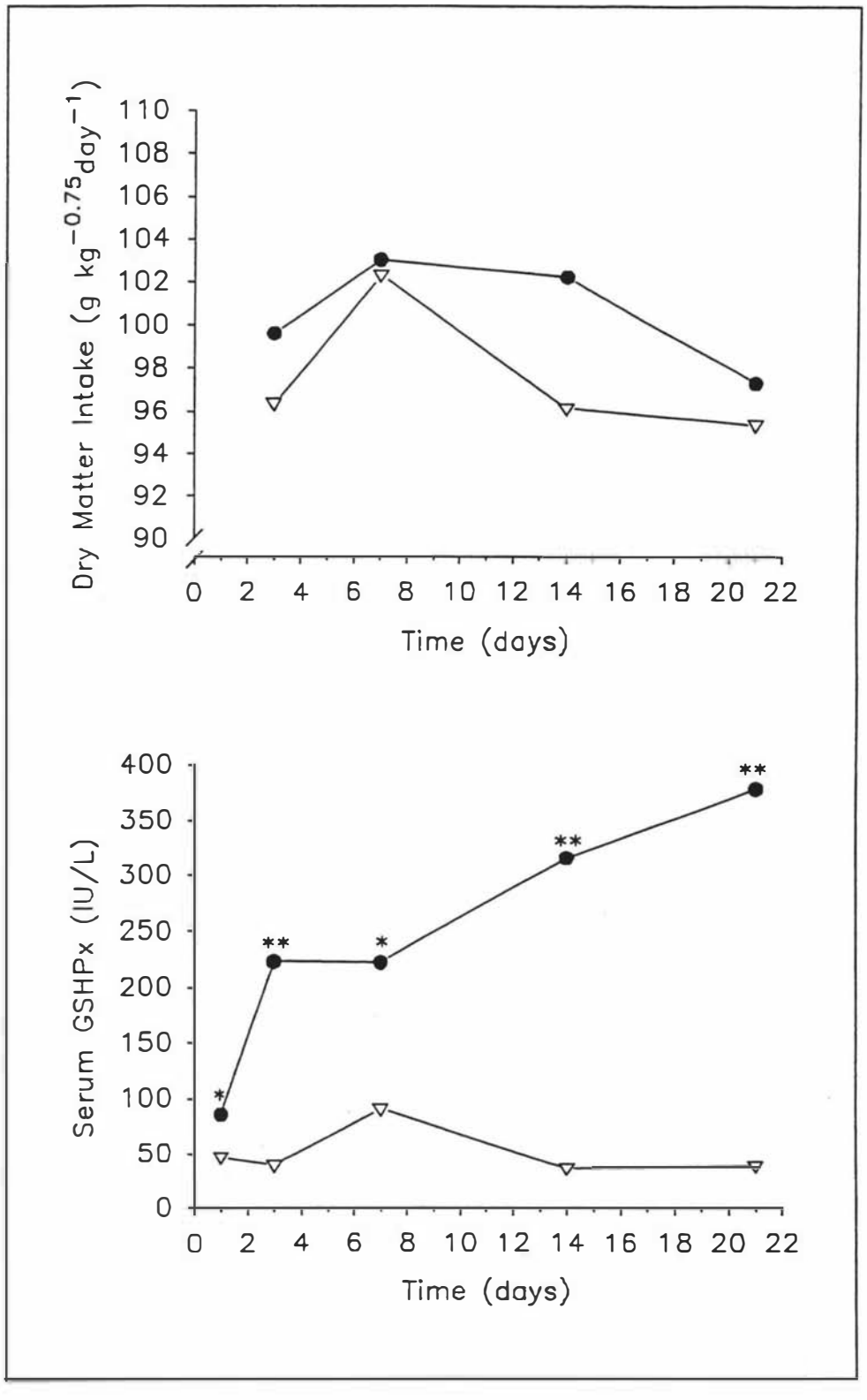

Figure 9.1. **The effect of oral treatment with sodium selenate (●) or deionised water (▽) on the least squares means for daily dry matter intake and activity of glutathione peroxidase (GSHPx) in serum for Angora goat kids. Pooled SE on day 14 for dry matter intake and serum glutathione peroxidase were 4.3 and 10, respectively. Probability: * = $P < 0.05$; ** = $P < 0.01$.**

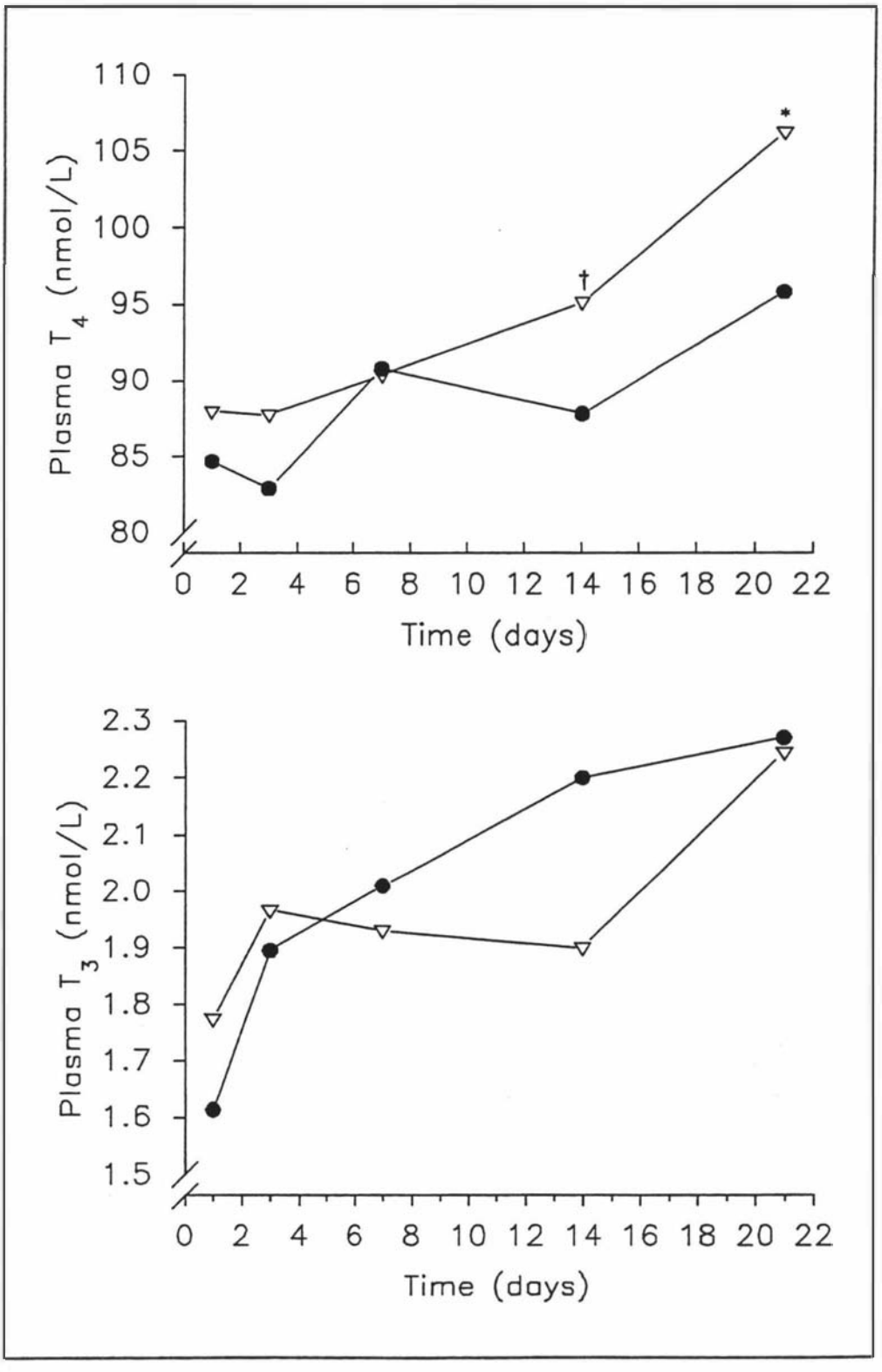

Figure 9.2. **The effect of oral treatment with sodium selenate (●) or deionised water (▽) on the least squares means for concentrations of thyroxine (T_4) and triiodothyronine (T_3) in plasma for Angora goat kids. Pooled SE on day 14 for thyroxine and triiodothyronine were 2.5 and 0.17, respectively. Probability: † = $P < 0.10$; * = $P < 0.05$.**

Discussion

This study has shown that selenium supplementation of kids does not affect intake of a selenium-deficient diet during the first 3 weeks after treatment. This finding differs from results reported for rats (Ewan, 1976) and chicks (Bunk and Coombs, 1980) and suggests that increased appetite is unlikely to be the primary mechanism involved in the growth response to selenium supplementation in Angora goats (see Chapter 7).

The serum activity of glutathione peroxidase increased rapidly in response to supplementary selenium, whereas the decrease in plasma concentration of thyroxine was more gradual, presumably reflecting increased peripheral metabolism of thyroxine by 5′-deiodinase (Arthur *et al.*, 1993). This suggests that alteration of both peroxide and thyroid hormone metabolism could be involved in short-term responses to selenium supplementation. In chicks (Bunk and Coombs, 1980), feed intake was increased before detectable changes in liver or plasma glutathione peroxidase activity, suggesting an effect of selenium on appetite distinct from its effect on peroxide metabolism.

Production responses to supplementation with selenium may be mediated via the thyroid axis, as suggested by studies in calves (Chapter 6) and sheep (Donald *et al.*, 1993). The temporal relationships between treatment and plasma thyroxine concentration in the present study indicate that responses should be expected to occur no sooner than 3 weeks post-supplementation. This is supported by a previous study using Angora kids (Chapter 7), in which a growth response was noted only during the third month of supplementation with selenium.

The observed increase in serum glutathione peroxidase activity after supplementation with selenium agrees with previous findings in calves (Thompson *et al.*, 1980) but contrasts with the findings of Hussein and Jones (1982) who concluded that, in goats, glutathione peroxidase activity in plasma or serum was of no value as an indicator of the selenium status. This disagreement may in part be due to differences in the selenium status of the experimental animals as the selenium intake for the goats in the latter study was not presented.

This study has shown that selenium supplementation of kids does not affect short-term intake of a selenium-deficient diet. However, supplementation increased the serum activity of glutathione peroxidase and decreased the plasma concentration of thyroxine. It is concluded that increased appetite is unlikely to be the primary mechanism involved in the response to selenium supplementation in Angora goats.

CHAPTER 10

General Discussion

General Discussion

Selenium, cobalt and copper deficiencies are the major production-limiting trace element deficiencies of ruminants which occur in New Zealand. Trace element supplementation of grazing ruminants is used extensively throughout New Zealand, most often on the advice of veterinarians (Clark, 1986). Failure to use effective trace element supplementation when needed and using supplementation in the absence of an established need, are still frequently identified problems which can cause loss to pastoral farming operations (Clark, 1986). Despite considerable research effort towards improving the diagnosis and prevention of selenium deficiency in recent years, the occurrence of trace element deficiency remain disconcertingly unpredictable. Basic research concerning the biological function of selenium in pastoral agriculture has been lacking and thus our understanding of the role of selenium in grazing ruminants has been rudimentary at best. The purpose of this sequence of studies was to further our understanding of the production-limiting effects of selenium deficiency in grazing ruminants. In addition, the potential roles for selenium in antioxidant function, thyroid hormone metabolism, somatotropic function and appetite were investigated.

The antioxidant role of selenium and its relationships with vitamin E and polyunsaturated fatty acids have been studied primarily in the context of selenium-responsive myopathy (Rice and Kennedy, 1988) and reproductive disease (Hurley and Doane, 1989). Despite selenium intakes which are typically one tenth of those recommended overseas (National Research Council, 1988), the incidence of white muscle disease in New Zealand calves is low (Ellison, 1992). Similarly, retained placenta, poor uterine involution, metritis and reproductive inefficiency have yet to be confirmed as selenium-responsive conditions in New Zealand herds (Ellison, 1992). A deficiency of vitamin E appears to increase the requirements for selenium in certain situations but it has been argued that a deficiency of vitamin E is unlikely to occur in New Zealand cattle due to their pasture-based diet (Rammell, 1983).

These observations have led to doubt regarding the validity of the classical antioxidant model as a mechanism for the sub-clinical syndromes associated with selenium deficiency.

The findings presented in this thesis (Chapter 4) provide baseline data for α-tocopherol, selenium and polyunsaturated fatty acid concentrations in the serum and feed of cattle managed under conditions typical of spring-calving dairy herds. The pre-calving α-tocopherol concentration for heifers fed pasture hay fell into the range considered deficient (less than 2.0 mg/L), suggesting that New Zealand dairy cattle can become depleted of vitamin E. Despite this observation, the data indicate that the unsaturated fatty acid challenge to cattle on pasture is not great under normal conditions and that vitamin E concentrations in feed and serum tend to change in synchrony with the concentration of polyunsaturated fatty acids. Thus, the mechanism involved in the selenium-responsive disease of grazing ruminants is unlikely to be the same as that established for myopathy of housed calves in the United Kingdom (Rice and Kennedy, 1988).

It appears that the effects of selenium deficiency depend on a variety of management factors and that it is unwise to extrapolate results from studies performed in intensively managed herds where pasture is not the principal feed. The results of the clinical trials presented in this thesis (Chapter 3) provide support for the reference ranges currently recommended for cattle in New Zealand. Although these reference ranges are much lower than those quoted for overseas cattle (Smith *et al.*, 1988; Gerloff, 1992), they appear to be appropriate for predicting responses to supplementation in herds where fresh pasture is the predominant feed.

The discrepancies between New Zealand and overseas recommendations for selenium supplementation may arise from differences in dietary oxidant challenge or other dietary factors that interact with selenium requirements such as the intake of vitamin E. Alternatively, differences in the prevalence of disease and degree of microorganism challenge could be involved. The incidences of nutritional myodegeneration, retained placenta and mastitis in overseas herds are generally much higher than those experienced in New Zealand (Ellison, 1992).

Important differences exist between the approaches to defining reference ranges for blood selenium used in New Zealand and overseas. American research workers appear to be influenced greatly by reports from a limited number of individual herds where a reduction

in disease incidence (primarily mastitis) has been reported at very high levels of supplementation (Smith *et al.*, 1988; Gerloff, 1992). There appears to be no attempt to incorporate the large volume of negative experimental results in the quoted reference ranges for blood selenium in cattle in the United States. In general, the overseas studies are highly variable in design, especially with respect to supplementation methods and the selenium content of the basal diets. No attempt has been made to assess the more important effects of selenium deficiency on sub-clinical disease; well-designed studies of the impact of supplementation on growth rate and milk production in overseas herds are rare.

In contrast, research workers in New Zealand have used a methodical approach to the development of reference ranges for trace elements in livestock. The results of a large number of response trials with similar design from throughout New Zealand have been collated and "reference curves" have been generated. Such curves allow prediction of the probability and magnitude of responses to supplementation incorporating all valid field data (Clark *et al.*, 1985). Using this method, positive and negative results are given equal emphasis. For example, data for 39 trials involving supplementation of calves from throughout New Zealand were analysed by Fraser and Wright (1984). Reference curves for blood selenium were derived from statistical analysis of the resulting growth rate responses to supplementation. These trials have provided the basis for recommended blood selenium reference ranges for growing cattle, and these recommendations can be easily modified if necessary as subsequent results are incorporated in the database. Reference curves for milk production responses to selenium supplementation are currently being developed as trial results come to hand (Ellison, 1992). The validity of the trace element reference ranges for cattle in New Zealand should not be challenged based simply on the data derived from overseas publications. Indeed, overseas researchers would be well-advised to look to New Zealand for a more objective approach to the development of reference ranges for trace elements in livestock.

The effect of selenium on the voluntary feed intake of ruminants has been ignored as a potential mechanism to explain poor growth of selenium-deficient animals. This is surprising as there have been reports of a direct effect of selenium supplementation on feed

intake in non-ruminant species (Ewan, 1976; Bunk and Coombs, 1980). Selenium supplementation of Angora goat kids did not affect short-term intake of a selenium-deficient diet (Chapter 9). However, changes in the activity of glutathione peroxidase in serum and thyroid hormone concentration in plasma occurred soon after supplementation. It is concluded that increased appetite is unlikely to be the primary mechanism involved in the response to selenium supplementation in Angora goats. The same is likely to be true for sheep and cattle.

Growth rate responses to selenium supplementation of calves on pasture commonly occur in the absence of signs of muscular degeneration, which suggests the existence of a mechanism that does not involve abnormal tissue peroxidation. The discovery that type I iodothyronine 5′-deiodinase is a selenium-dependent enzyme (Berry *et al.*,1991) provided the impetus for examination of the effect of selenium deficiency on thyroid hormone metabolism and growth (Chapters 6 and 7). The effects of selenium deficiency on thyroid hormone metabolism, based on studies of rats (Arthur *et al.*, 1990), are summarised in Figure 10.1. The data presented in Chapter 6 suggest that selenium-responsive growth retardation in grazing cattle may be mediated by alterations in thyroid hormone metabolism. It was initially hypothesised that selenium supplementation of ruminants alters type I and type II 5′-deiodinase activity leading to increased peripheral and pituitary concentrations of triiodothyronine (Figure 10.2a). This in turn leads to increased secretion of growth hormone by somatotrophs (Koenig *et al.*, 1987) and enhanced growth rates, as proposed by Arthur *et al.* (1990). It was also hypothesised that concurrent iodine status influences plasma and pituitary triiodothyronine concentrations and that the effect of selenium on growth rate is dependant on iodine status (Figure 10.2a). However, differences in plasma growth hormone secretion (basal or stimulated by thyrotropin-releasing hormone) were not observed following supplementation with selenium. This is in agreement with the findings of Arthur *et al.*(1990) in rats and suggests that the effects of selenium are not mediated via the pituitary secretion of growth hormone. No evidence was found for an interaction between selenium and iodine supplementation in the growth of calves or goats (Chapters 6 and 7), in agreement with the results of Donald *et al.* (1993) in sheep. This finding suggests that the hypothyroidism induced by selenium deficiency differs from that induced by iodine deficiency and that the effects of selenium deficiency on growth cannot

be ameliorated by simply improving iodine status. Thus, the model proposed in Figure 10.2a may not be appropriate for explaining the effects of selenium supplementation in ruminants moderately deficient in selenium and iodine.

Alternative hypotheses to explain the effects of selenium on growth are presented in Figure 10.2b-d. The trend to increased concentrations of insulin-like growth factor-I after selenium supplementation (Chapter 6) suggests that somatomedins may be involved. Selenium-dependant changes in thyroid metabolism in the pituitary or peripheral tissues could lead to alterations in the endocrine or paracrine production of insulin-like growth factor-I, secretion of insulin-like growth factor-II, the number of somatotropic receptors or the peripheral concentration of insulin-like growth factor-binding proteins (Figure 10.2b). Other than the data on insulin-like growth factor-I presented in Chapter 6, no information is available on these aspects of somatotropic function in selenium-deficient mammals.

Alternatively, changes in type I 5′-deiodinase activity may be a simple biochemical response to altered selenium intake which is not causally related to changes in growth rate or lactation. In support of this, infusion of selenium-deficient rats with triiodothyronine restored plasma triiodothyronine concentrations but did not increase growth rate compared to rats infused with saline (Thompson *et al.*, 1995). This result suggests that decreased plasma triiodothyronine concentration is not the cause of impaired growth in selenium-deficient rats but it does not exclude the possibility that impaired 5′-deiodination at intracellular sites such as the pituitary may be involved.

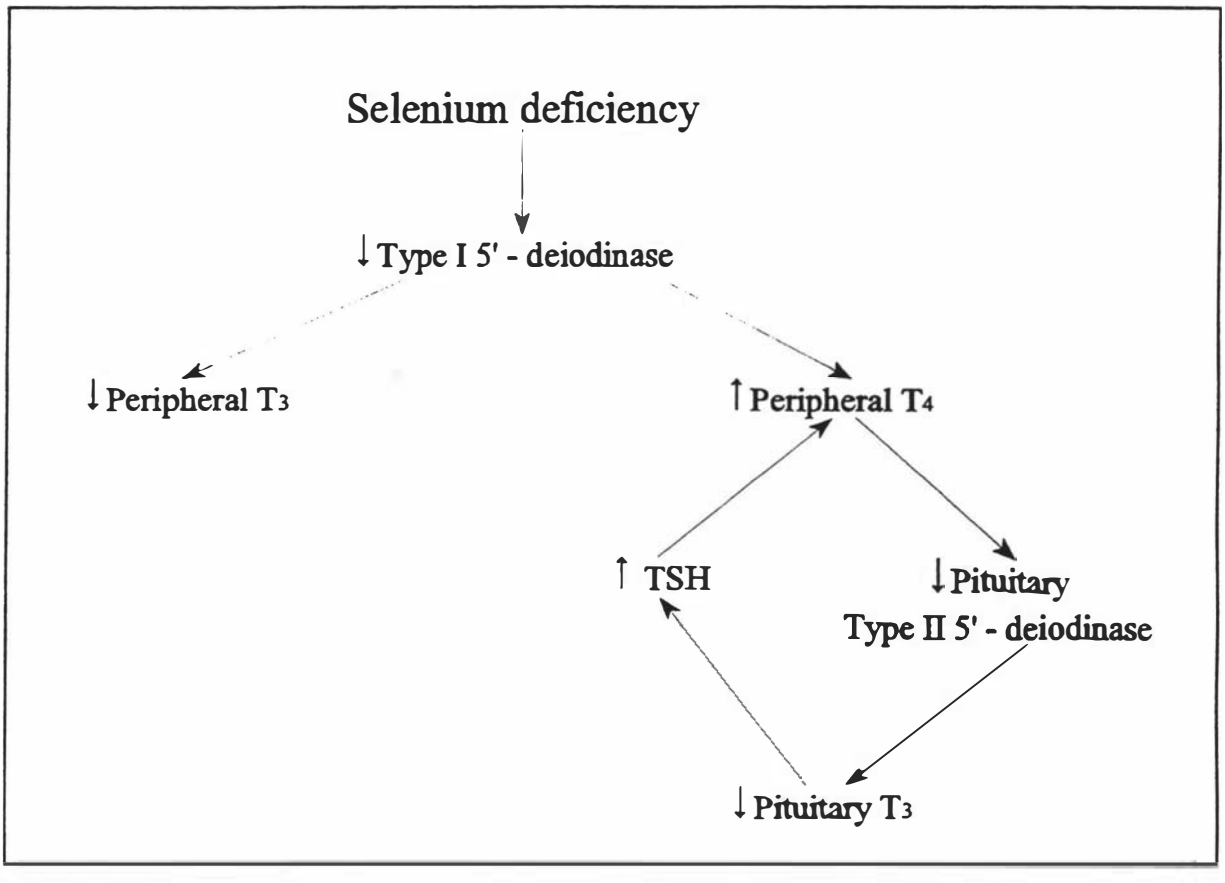

Figure 10.1. **Effects of selenium deficiency on the metabolism of thyroxine (T_4), triiodothyronine (T_3) and the secretion of thyrotropin-releasing hormone (TSH) from the pituitary.**

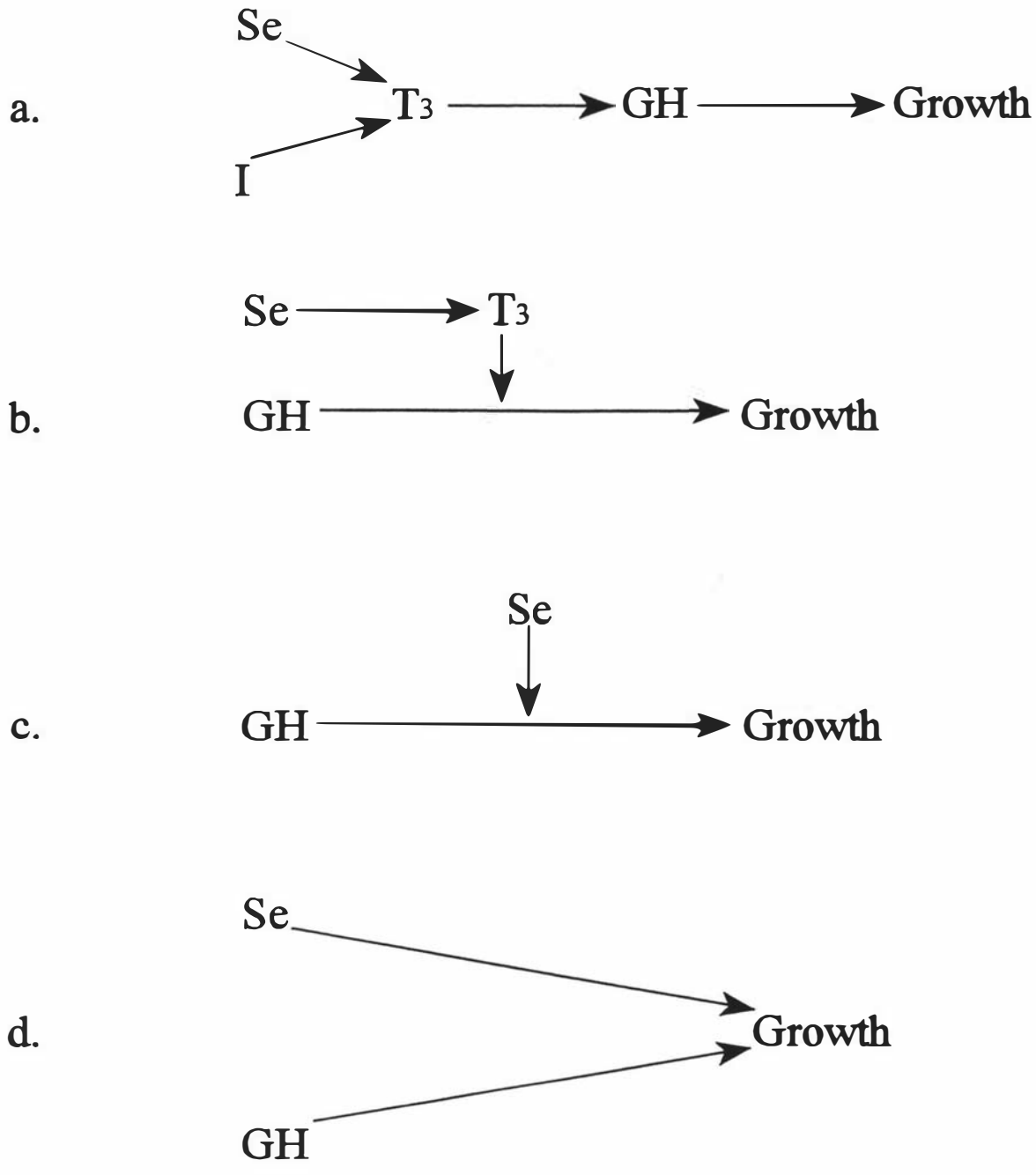

Figure 10.2. **Selenium and somatic growth in ruminants: hypotheses concerning the relationships between selenium (Se), triiodothyronine (T_3), growth hormone (GH) and growth.**

Other selenoproteins with presently unknown functions could influence body growth and lactation. Selenoprotein P is the major selenoprotein in rat serum (Read *et al.*, 1990) and it is hypothesised that it could be responsible for some of the metabolic functions of selenium that cannot be attributed to glutathione peroxidase. Such novel selenoproteins could influence growth and lactation either via the somatotropic axis (Figure 10.2c) or via mechanisms currently not recognised (Figure 10.2d).

Effects of selenium deficiency other than growth retardation may be mediated by changes in thyroid hormone metabolism. Type II 5′-deiodinase activity in brown adipose tissue has been shown to be lower in selenium-deficient rats when compared to control animals (Arthur *et al.*, 1991b). The production of triiodothyronine in brown adipose tissue of neonatal animals may be important in resistance to cold exposure (Arthur *et al.*, 1991b). However, selenium or iodine status did not influence the thermoregulatory ability of newborn lambs in one study (Donald *et al.*, 1994b).

The thyroid-modulating effects of selenium deficiency may also occur in adult cattle. It has been suggested that alterations in 5′-deiodinase activity in mammary tissue may play a role in mediating the galactopoietic response of dairy cattle to bovine somatotropin (Capuco *et al.*, 1989). It is plausible that similar changes in mammary 5′-deiodinase activity could result from selenium supplementation of deficient cows, contributing to the increased milk production observed following treatment (Chapter 3).

The proposed biological functions of those selenoproteins with known roles are summarised in Figure 10.3. The effect of selenium status on immune function (Spallholz *et al.*, 1990) was not specifically investigated during the course of these studies, other than indirectly through recording of disease incidence and somatic cell counts in milk. The results of the clinical trials reported in Chapter 3 indicate that somatic cell counts are influenced by selenium supplementation of grazing cows. However, the low incidence of clinical mastitis and reproductive failure recorded in the present studies suggests immune function is not greatly impaired in moderately selenium-deficient cattle. This aspect of selenium function must be further studied before conclusive recommendations can be made.

Recommendations for Future Research

The specific function of selenium that is the cause of impaired growth and milk production in ruminants grazing selenium-deficient pasture has not been conclusively identified. However, the effects of selenium supplementation on thyroid hormone deiodination and insulin-like growth factor-I (Chapter 6 and 7) and cell counts in milk (Chapter 3) point to relationships that require further study and are described below.

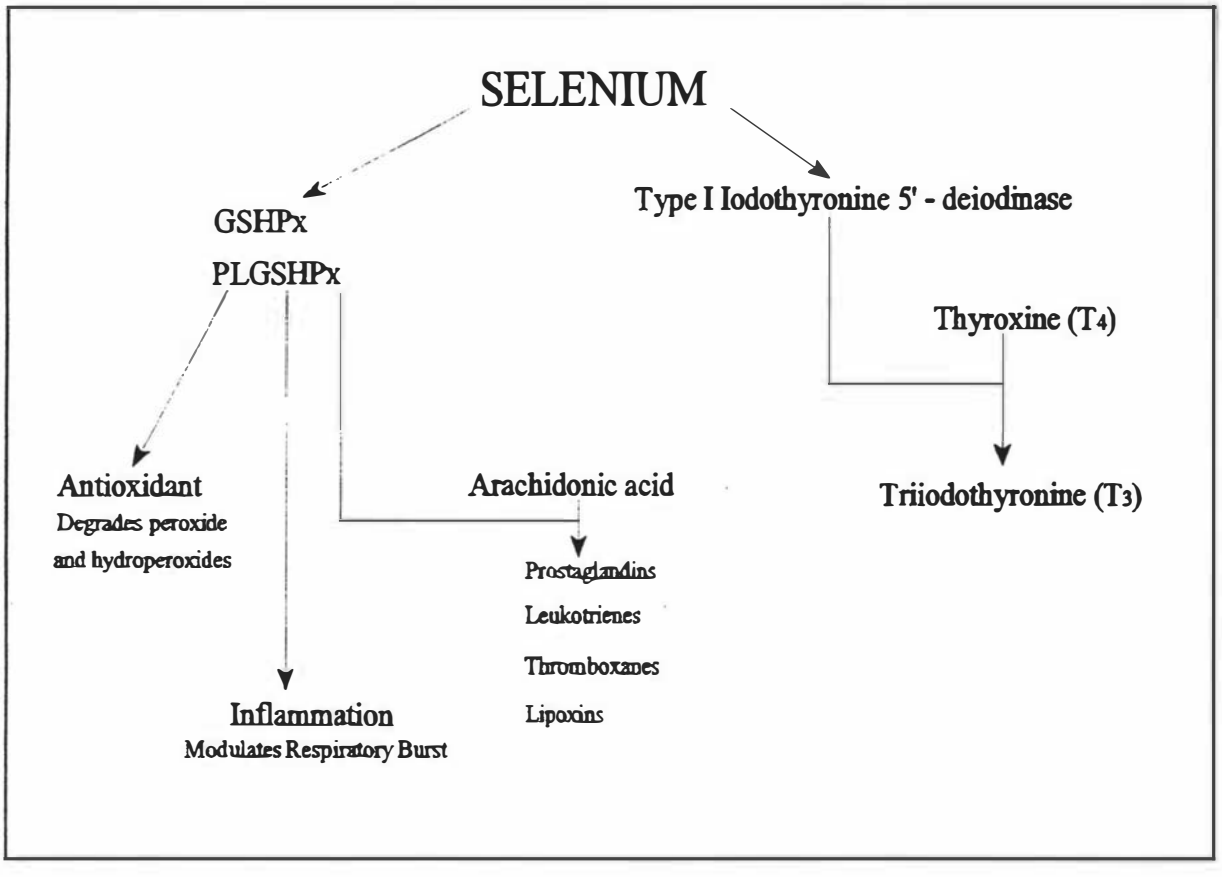

Figure 10.3. The biological function of the selenoenzymes glutathione peroxidase (GSHPx), phospholipid glutathione peroxidase (PLGSHPx) and iodothyronine 5'-deiodinase.

Selenium deficiency and thyroid function

If reduced peripheral concentration of triiodothyronine is the cause of the impaired growth observed in selenium-deficient calves then treatment of deficient calves with exogenous triiodothyronine should result in improved growth rates. This hypothesis could be pursued through the use of the hormone infusion techniques employed by Thompson *et al.* (1995) in rats. Infusion of selenium-deficient calves with triiodothyronine using an osmotic mini-pump would be preferable as non-continuous administration of triiodothyronine in sheep resulted in depressed plasma concentrations of triiodothyronine (Donald *et al.*, 1994a).

Alternatively, restoration of type I 5′-deiodinase activity and triiodothyronine availability at specific intracellular sites, rather than the in the extracellular pool, may be responsible for the enhanced growth following selenium supplementation. Selenium-dependant alterations in deiodinase activity and triiodothyronine in various endocrine organs including the pituitary should be examined in ruminants.

Selenium and somatotropic function

Selenium may influence growth rate via receptors or mediators of growth hormone and this would not necessarily be reflected in altered plasma growth hormone concentration. Endocrine or paracrine insulin-like growth factor-I, insulin-like growth factor-II, somatotropic receptors or insulin-like growth factor-binding proteins could be involved. The impaired growth induced by zinc deficiency in rats has recently been shown to be associated with decreased expression of the hepatic insulin-like growth factor-I and growth hormone receptor genes (McNall *et al.*, 1995). Similar studies could be conducted in selenium-deficient rodents and ruminants.

The effect of chronic administration of bovine growth hormone (McCutcheon *et al.*, 1991; Bass *et al.*, 1991) and insulin-like growth factor-I (Cottam *et al.*, 1992) are techniques that have been employed in the study of the somatotropic function of ruminants. Comparison of the somatogenic effects of these hormones in ruminants with differing selenium status may indicate whether selenium deficiency causes a specific endocrine defect in the somatotropic axis.

Selenium and the immune response

The increasing importance of milk quality means that the effects of selenium on somatic cell counts and the incidence and severity of intramammary infections should be investigated in more detail. The results of the clinical trials reported in Chapter 1 indicate that somatic cell counts are influenced by selenium supplementation of grazing cows. It has been proposed that the selenium requirements for optimal immune function may be greater than that for other functions of selenium (Bendich *et al.*, 1986). Collection of survey data relating bulk milk selenium concentration to somatic cell counts and the incidence of clinical mastitis may be a useful first step in assessing the importance of selenium in immune function in dairy cattle in New Zealand.

Selenium and neonatal morbidity

Associations between selenium status and neonatal disease have been alluded to by researchers studying thermogenesis and resistance to cold exposure (Arthur *et al.*, 1991b). Selenium may also play a role in resistance to neonatal enteric infections. Information on the selenium status of neonatal ruminants in New Zealand is presently not available.

New trends in nutritional management

Nutritional management of livestock in New Zealand will continue to evolve, with an increased diversity of feed sources and less reliance on perennial ryegrass. New pasture species and the introduction of significant quantities of concentrates and by-products to the ruminant diet will offer new challenges and opportunities in the prevention of micronutrient deficiencies. Research leading to non-pharmaceutical methods of prevention should be encouraged.

Concluding Remarks

This research provides new insight concerning the unique role of selenium in the productivity of New Zealand's grazing ruminants. The validity of the current selenium reference range for adult dairy cattle in New Zealand was confirmed and it was shown that the administration of a high dose of selenium yielded no additional benefit in growth or milk production above that obtained after administration of the currently recommended dose.

It was important to acquire baseline data for α-tocopherol, selenium and polyunsaturated fatty acid concentrations in the serum and feed of cattle managed under conditions typical of spring-calving dairy herds. The information obtained suggests that abnormal peroxide metabolism is unlikely to play an important role in the impaired lactational performance noted in selenium-deficient cows. This lack of oxidant challenge may explain why postpartum reproductive dysfunction was not observed in the selenium-deficient cattle in these studies, in contrast to overseas findings.

The effects of selenium deficiency on thyroid hormone metabolism in calves and goat kids were examined in some detail. This work demonstrated a clear relationship between selenium status and plasma thyroid hormone concentrations and provides research workers with a plausible hypothesis of the effects of selenium on ruminant productivity which must be examined further. Interactions between selenium and iodine were unimportant under conditions of moderate deficiency. The principal questions arising from this thesis relate to precisely how somatotropic function responds at a cellular level to changing selenium status and whether the alterations demonstrated in thyroid hormone metabolism are directly involved.

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Addendum

Quality control procedures for the analysis of glutathione peroxidase activity and the concentrations of selenium and α-tocopherol in blood, serum and herbage

Glutathione peroxidase activity

Low, medium and high standards were included in each assay run. These were derived through stepwise dilution of a frozen control stabilised with 10% glycerol. The control consisted of pooled EDTA blood with a glutathione peroxidase activity of 10 kIU/L. Interassay coefficients of variation for the glutathione peroxidase assay were 2.11, 2.41 and 2.03%, respectively for the low, medium and high standards.

Fluorometric determination of selenium concentration in blood and serum

Values for each batch were standardised using a control (127 nmol/L) included in each daily batch. At least three other controls were included in each batch. Low (232 nmol/L) and high (595 nmol/L) standard were derived from frozen pools. A commercially prepared freeze-dried control (660 nmol/L) was also included as an external standard. Interassay coefficients of variation were 4.6, 3.3 and 3.06%, respectively for the low, high and external standards.

Fluorometric determination of selenium concentration in herbage

Two pasture controls derived from frozen pools were included in each batch of samples. The selenium concentrations for the low and high pools were 0.018 ± 0.003 and 0.126 ± 0.008 mg/kg, respectively. Values for the standards remained within 2 standard deviations of the mean over the period of the herbage assays. In addition, a commercially available standard is utilised periodically as an external standard to test the validity of the assay quality control standards.

Alpha-tocopherol concentration in serum and herbage

A sample of known α-tocopherol concentration (5.1 ± 0.7 mg/L) was included at the beginning and end of each assay run. If the reading for either sample was outside of 3 standard deviations from the mean, the batch was rejected. Values for the standards remained within 2 standard deviations of the mean over the period of the α-tocopherol assays. Recovery of α-tocopherol was estimated from the standard included at the beginning, mid-point and end of each batch. Recovery of α-tocopherol varied from 90% to 105%.