METABOLISM OF SELENIUM IN CATS AND DOGS

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ABSTRACT

The main objective of this PhD was to provide fundamental information regarding some metabolic aspects of selenium metabolism in cats and dogs.

The total selenium content of a range of commercially available petfoods was analysed using a fluorometric method. The petfoods contained a wide range of selenium concentrations, with up to 6 µg Se/g DM found in cat foods. Mean concentrations of selenium in dog and cat foods were 0.40 and 1.14 µg Se/g DM respectively. All petfoods analysed met the recommended current minimum dietary selenium requirements.

The use of blood parameters for the assessment of selenium status was investigated in a study in which cats were fed inorganic and organic selenium supplemented at concentrations of up to 2.0 µg Se/g DM for 32 days. Plasma selenium concentrations reflected dietary selenium intakes, however there were no differences between the different levels of supplementation. Whole blood selenium concentrations showed less distinct patterns and were thought to be a more useful indicator of longer term selenium status. Activities of glutathione peroxidase in plasma and whole blood showed no response and the response of cats to supplementation of the different forms of selenium were similar. In the same study, faecal and urinary excretion (µg/kg BW/d) were measured and apparent absorption and retention were estimated during the last seven days of the 32 day trial. Faecal excretion of selenium remained constant whereas urinary excretion of selenium increased with increased dietary intake. The form of selenium had no effect on excretion or apparent absorption however there was a trend in which more selenium was retained in cats fed organic selenium.

A study was conducted with cats and dogs fed high levels (10 µg Se/g DM) of inorganic and organic selenium for 21 days to determine whether there were species differences in their metabolic response. Cats and dogs exhibited the same pattern of response, however cats showed higher plasma selenium levels, lower levels in liver and excreted more selenium compared to dogs. It was concluded from this data that cats and dogs differ in their metabolism of selenium.

The effect of heat processing on the addition of inorganic and organic selenium to petfoods was investigated in cats fed 3.0 µg Se/g DM for 11 days. Apparent absorption was higher in cats fed inorganic selenium added after processing, whilst less selenium of organic origin was excreted in the urine when added after processing.

These preliminary results suggest heat processing may decrease the apparent availability and utilisation of selenium in petfoods.

LIST OF ABBREVIATIONS

λ_{em}	emission wavelength
λ_{ex}	excitation wavelength
%	percent
AAFCO	Association of American Feed Control Officials
ANOVA	analysis of variance
AOAC	Association of Official Analytical Chemists
β	beta
BW	body weight
°C	degrees Celsius
cGSHPx	classical glutathione peroxidase
cm	centimetre
DAN	2,3-diaminonapthalene
DM	dry matter
DNA	deoxyribose nucleic acid
EDTA	ethylenedinitrilotetraacetic acid
FAD	flavin adenine dinucleotide
FDA	Food and Drug Administration
g	gram
GSHPx	glutathione peroxidase
gGSHPx	gastrointestinal glutathione peroxidase
HCl	hydrochloric acid
HIV-1	human immunodeficiency virus
ID	iodothyronine deiodinase
IDI	type 1 iodothyronine deiodinase
IDII	type 2 iodothyronine deiodinase
IDIII	type 3 iodothyronine deiodinase
kcal/kg BW/d	kilocalories per kilogram body weight per day
kDa	kilodalton
kg	kilogram
ME	metabolisable energy
m^2	metres squared
mg	milligram
mg Se/kg	milligrams selenium per kilogram

ml	millilitre
ml/min	millilitres per minute
mM	millimolar
mmol/L	millimoles per litre
mRNA	messenger ribonucleic acid
NADPH	nicotinamide adenine dinucleotide phosphate
ng	nanogram
nm	nanomole
NOAEL	no-observed-adverse-effect level
NRC	National Research Council
pGSHPx	plasma glutathione peroxidase
phGSHPx	phospholipid glutathione peroxidase
ppm	parts per million
rpm	revolutions per minute
Se	selenium
SeEMP	selenium exchangeable metabolic pool
SEM	standard error of the mean
SPS	selenophosphate synthetase
T_2	diiodothyronine
T_3	triiodothyronine
T_4	thyroxine
tRNA	transfer ribonucleic acid
TRR	thioredoxin reductase
µg	microgram
µg/L	micrograms per litre
µg/ml	micrograms per millilitre
µg Se/g DM	micrograms selenium per gram dry matter
U/L	units per litre
µmol/L	micromoles per litre
USA	United States of America

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INTRODUCTION

History and importance of selenium

The history of selenium is somewhat chequered and paradoxical. Initially selenium was thought to be toxic and was identified as a carcinogen, then it was shown to be essential and found to have anticarcinogenic properties. Today, selenium is recognised as an essential trace element with many important biological functions.

The chronological history of selenium research is outlined in Table 1. It is believed that Marco Polo was the first to record a biological effect of selenium in 1295 when he was travelling through China (Polo, 1926; cited by Krehl, 1970; Reilly, 1993). Symptoms in his pack-bearing animals included hoof rot and loss of mane and tail and were attributed to the ingestion of certain local poisonous plants of which the animals were unaccustomed to. It was later recognised that these symptoms resulted from selenium toxicity.

Selenium was officially discovered by Jons Jakob Berzelius, a Swedish chemist, in 1817 (Foster and Sumar, 1997). He identified selenium as a red deposit on the walls of a lead chamber used in the production of sulphuric acid (Reilly, 1993). Selenium was associated with tellurium in this red deposit, and as tellurium was named after the Latin for earth, *tellus*, Berzelius named the element after the Greek for moon, *selene*. It has been suggested that the association with the moon is apt, as like the dark and light sides of the moon, selenium has 'darker' pathological and 'lighter' essential aspects to it (Reilly, 1993). Also, selenium appears to have a predisposition to various patterns (Marier and Jaworski, 1983) that create numerous problems to solve. For this reason selenium has also been referred to as the "maddening mineral" (Krehl, 1970). The initial function of this element following its discovery was to colour glass. Cadmium selenite was used to remove the green tint and to create ruby red coloured glass. Throughout the 19th century selenium was used limitedly for this purpose (Sunde, 1997).

In 1857 symptoms similar to those described by Marco Polo were reported in US cavalry horses in the Nebraska area (Madison, 1860; cited by Krehl, 1970; Ullrey, 1974). In 1907 and 1908 thousands of sheep in the Wyoming area perished from poisoning by an unknown source (Krehl, 1970) and it was not until the 1930s that these symptoms were explained. It was discovered that selenium caused blind staggers and alkali disease - conditions caused by the ingestion of plants containing

large amounts of selenium. Thus, selenium's reputation as a toxic element was established.

The paradox of the role of selenium in animal nutrition began after World War II in parts of Australia, the USA, New Zealand and Northern Europe. During this time feedstuffs became deplete of selenium as a result of a change in livestock management and forage production methods (Marier and Jaworski, 1983). Consequently, livestock contracted the deficiency disease nutritional myopathy, alternatively known as white muscle disease. So, by the mid 20th century, selenium's repertoire included not only the ability to induce toxic effects, but also the potential to cause deficiency syndromes. To further confuse things, several of the symptoms for selenium toxicity were also symptomatic of selenium deficiency.

The discovery in 1943 that selenium was a carcinogenic agent condemned the element to further disrepute. Nelson *et al.* (1943) revealed that rats fed amounts from 5 µg Se/g diet developed cancerous growths in the liver. However several years later Schwarz and Foltz (1957) added to the ambiguity of the role of selenium and claimed that it was an essential trace element. This statement was based on their findings that inorganic selenium effectively protected against necrotic liver degeneration in vitamin E deficient rats, and that a daily intake of 0.25 µg Se per rat provided complete protection. In the same year, additional work in chicks consolidated this finding when small amounts of dietary selenium prevented exudative diathesis (Patterson *et al.*, 1957; Schwarz *et al.*, 1957). Results from these studies confirmed that selenium was indeed an essential element, and with this knowledge a more reputable side of selenium developed.

Work done in the same year continued to produce breakthroughs in the area of selenium research. The first clues as to the biological function of selenium originated in 1957 when Mills discovered glutathione peroxidase (GSHPx) (Mills, 1957), an enzyme that metabolises hydroperoxides, and therefore prevents the oxidative damage to cells which may be caused by these free radicals. However, despite this knowledge of selenium's essentiality, during the late 1950s the selenium requirement of animals was thought to be low and supplementation was considered unnecessary, especially in the presence of vitamin E (Jensen, 1999). In fact, the Food and Drug Administration (FDA), who were still conscious of the carcinogenic effects demonstrated by Nelson *et al.* (1943), prohibited the supplementary use of selenium. This line of thinking later proved to be flawed when Rotruck *et al.* (1973) revealed the significance of GSHPx in relation to the function of selenium. The research showed that GSHPx was actually a

selenoenzyme, containing selenium as a fundamental part of its structure. Thus, through its enzymic actions, selenium functioned as an antioxidant. In addition, further research in the 1960s and 70s demonstrated anticarcinogenic effects of selenium.

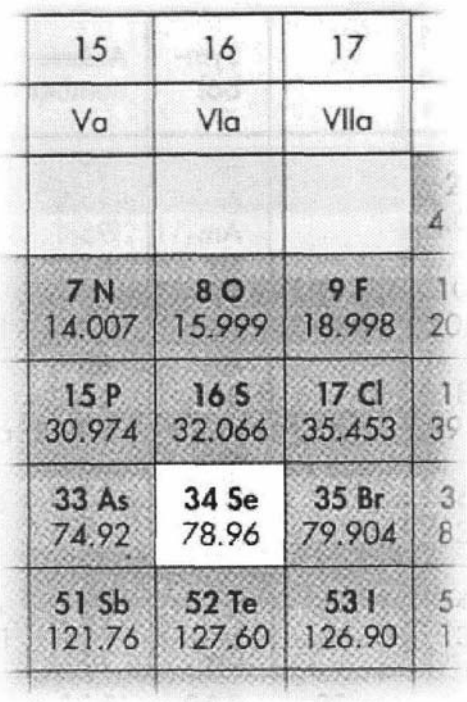
Table 1. The chronological history of selenium research

Date	Event
1295	First biological effect of Se recorded by Marco Polo in China
1817	Se officially discovered by Berzelius
1857	US Cavalry reported similar biological effects to those seen by Marco Polo
19th century	Se used for colouring glass
1907	Reports of extensive number of cases of Se toxicity in the USA
1930s	Cases of Se toxicity reported
1940s	Cases of Se deficiency reported
1943	Carcinogenic effects of Se discovered
1957	Essentiality of Se discovered GSHPx discovered
1960-70s	Anticarcinogenic properties of Se discovered
1973	Biological function of Se discovered – GSHPx is a selenoenzyme

Despite its rather unfavourable beginnings causing toxicity and deficiency syndromes, along with its carcinogenic effects, the importance of selenium for health and normal physiological function is now clearly recognised. The nutritional essentiality of selenium is well established and is reinforced by its antioxidant, anticarcinogenic and antiviral properties. However, the full story of selenium metabolism and function is far from complete, and research regarding its biochemistry and molecular biology continues.

Chemistry of selenium

With an atomic number of 34, selenium is the third element of Group V1A in the Periodic Table. It lies between sulphur and tellurium in Group V1A, and arsenic and bromide in Period 4 (Figure 1). The atomic weight of the naturally occurring isotope is 78.96. Due to its position in the table selenium is classified as a metalloid, which is neither a true metal nor non-metal but shares properties of both. Consequently, selenium has a unique chemistry and biochemistry, which has a strong impact on its biological activity.



Like the other elements in Group V1A, selenium can exist naturally in several oxidation states (+6, +4, -2). It may also exist as volatile species, or analogues of organic sulphur compounds, the properties of which are directly related to their valency and stereochemistry (Foster and Sumar, 1997). Of the 34 electrons, there are 18 in the argon shell, ten 3*d* electrons, and six electrons in the 4*s* and 4*p* orbitals (Sunde, 1997). The +6 and +4 oxidation states are formed when the 4*s* and 4*p* electrons are lost, whereas the addition of two electrons in the 4*p* orbitals forms the −2 oxidation state. Selenium can form bonds with itself up to Se_8.

Figure 1. Selenium in the Periodic Table

Comparison of selenium and sulphur chemistry

Selenium and sulphur share similar chemical properties due to their placement in the same group of the Periodic Table. Both have similar atomic size, bond energies, ionisation potentials and electron affinities (Foster and Sumar, 1997) and their respective electronegativities of 2.44 and 2.48 give them similar chemical reactivity's (Sunde, 1997). They also have comparable radii, with the ionic radii of selenium and sulphur being 2.0 and 1.9 Å, and the covalent radii 1.07 and 1.03 Å respectively, thus the two elements cannot be distinguished on bond length (Sunde, 1997).

However, selenium and sulphur are not interchangeable in biological systems as there are two major differences between the elements under physiological conditions. Firstly, the acid forms of the elements have different strengths, with hydrogen selenide (H_2Se) being much stronger than hydrogen sulphide (H_2S) (Sunde, 2000). Secondly, selenium has a greater reducing potential than sulphur such that selenium tends to undergo reduction reactions to the −2 state (selenides) when metabolised, whereas metabolism of sulphur is directed towards oxidation (+6 state - sulphates) (Prohaska, 1983). Selenium oxides are excellent oxidising agents and oxidation-reduction reactions catalysed by organoselenium compounds account for most of the biological activity of selenium (Prohaska, 1983).

Sources of selenium

Although selenium is not a common element, traces of it occur in nearly all substances. Selenium enters the food chain from soils, the concentration of which varies widely depending on geographical location. It is then absorbed by plants, again to varying degrees due to several factors such as the form and availability of selenium, and the plant species. Animals can obtain selenium directly by ingestion of these plants, or indirectly via selenium-containing dietary components of plant or animal origin, or by dietary supplementation.

Selenium in soils

The occurrence of selenium in soil is important as this is the primary source of selenium in food (Reilly, 1998). The selenium content of soil is firstly a result of the quantity of selenium originating in the parent material; and secondly, dependent on processes occurring during or after soil formation which may subsequently alter this amount (Ganther, 1974). Thus, although the element is widely distributed in rocks and soils, its concentration varies depending on the type of rock and soil, and on the climate (Reilly, 1993).

Selenium usually occurs as a divalent ion in soils, either as selenides, selenites or selenates (Marier and Jaworski, 1983). However soils may also contain elemental selenium, and some selenomethionine.

Soil conditions such as aeration and pH are major factors in determining the availability of selenium for uptake by plants (Jacques, 2001; Table 2). In acidic, poorly aerated or moist soils, it exists in the reduced forms as selenide or elemental selenium (Jacques, 2001). These create insoluble complexes with soil iron hydroxide, thereby making selenium unavailable to plants (Ganther, 1974). In contrast, dry, well-aerated, alkaline soils cause its oxidation to selenate, a form that is soluble and readily available to plants (Allaway *et al.*, 1966). The other form available to plants is selenite, which occurs in acidic, well aerated, and neutral pH soils (Jacques, 2001).

Table 2. Soil type and selenium oxidation state

Soil type	Se oxidation state	Availability to plants
acidic, moist	selenides, elemental Se	unavailable
acidic, dry	selenates	available
alkaline, dry	selenites	available
neutral		

Thus the amount of selenium in soils that is actually available for uptake and utilisation by plants varies substantially and is a result of the total amount of selenium in soil and the soil type, which ultimately determines the form of selenium and therefore its availability.

Selenium in plants

There is some dispute as to whether selenium is essential for plants, as it is not required in some species and only apparently required in others (Raisbeck, 2000). However in suitable conditions plants will take up soil selenite, selenate and selenomethionine (Jacques, 2001). As indicated above, the primary factor influencing the selenium content of plants is the amount of selenium available in the soil for uptake. However, plants also vary in their ability to obtain selenium from soil (Ganther, 1974) and accordingly have been grouped into two categories: accumulator and non-accumulator plants.

Accumulator plants, also known as 'indicator' or 'converter' plants, have the ability to take up large amounts of selenium provided there is a high soil content. It is their occurrence in these seleniferous areas, and lack of occurrence in low selenium environments, that has earnt them the alternative name 'indicator plants'. Accumulator plants can be further divided into primary and secondary indicators depending on whether they appear to require selenium for growth (primary) or not (secondary) (Shamberger, 1983a). Selenium concentrations of between 1000 and 3000 ppm are common in accumulator plants (Underwood, 1971; Table 3). Such high concentrations occur due to the ability of these plants to absorb unavailable forms of selenium from the soil and convert them to available forms. In addition, when these plants die the selenium is returned to the soil making it available to other plants, hence the name 'converter plants'. Examples of accumulator plants include Brazil nuts (Reilly, 1998) and some of the *Astragalus* species (Ganther, 1974). In accumulator plants,

selenium is not usually found in the protein fraction and exists as non-physiologic amino acids such as selenocystathionine and methylselenocysteine (Sunde, 2000). The highest concentrations of selenium in these plants accumulate in the stems or foliage (Lakin and Davidson, 1967). These factors result in potentially high levels of selenium in accumulator plants, thereby creating a toxic threat to grazing animals.

In contrast, selenium in non-accumulator plants is not absorbed at toxic levels even when the plants grow on seleniferous soils (Table 3). Selenium concentrations in these plants are usually less than 50 ppm under normal field conditions (Shamberger, 1983a). Furthermore, any selenium that is absorbed is concentrated in the roots and is therefore relatively inaccessible to grazing animals. When non-accumulator plants take up selenate or selenite, it is converted to the primary form, selenomethionine, in plants (Sunde, 2000), which is then incorporated into plant protein in place of methionine (Jacques, 2001). Such non-accumulator plants include many of the grains and grasses used for nutritional and agronomic purposes (Jacques, 2001).

Table 3. Comparison between accumulator and non-accumulator plants

Characteristic	Accumulator plants	Non-accumulator plants
Soil type grown on:	mainly seleniferous	all types
Se concentration in plant:	up to 1000–3000 ppm	> 50 ppm
Forms of Se stored:	selenocystathionine, methylselenocysteine	selenomethionine
Main areas Se is stored:	stems, foliage	roots
Examples:	some *Astragalus* sp noxious weeds	grains and grasses

Selenium in animals

Animals obtain selenium as the selenoamino acids selenomethionine and selenocysteine, as methylated and non-methylated selenium through food (Foster and Sumar, 1997), or as inorganic selenium through supplementation. Most selenium in animal systems occurs as either selenomethionine or selenocysteine (Levander, 1986).

Selenomethionine is the form of selenium most easily and effectively utilised by both animals and humans as it is incorporated into a variety of proteins in place of methionine. This selenoamino acid can be synthesised by the most common species of plants, marine algae, bacteria and yeast, but it cannot be formed by animals (Jacques, 2001). Thus under natural conditions, selenium is derived primarily from plants and is

transferred to animals via protein bound selenomethionine, with lesser amounts of selenates, selenites and other organic compounds (Allaway *et al.*, 1966). Uptake of selenomethionine is not affected by the selenium status of the animal. Consequently the resulting pool of selenomethionine provides a means of storing selenium for use in times of selenium deficiency (Levander, 1986).

Selenocysteine is the biologically active form of selenium in animal tissues. Its incorporation into proteins such as GSHPx occurs via a specific mechanism which does not involve substitution for its sulphur amino acid analogue, cysteine (Levander, 1986). Selenocysteine-containing proteins are selenium dependent, and reflect dietary intake (Burk and Hill, 1993).

Forms of selenium

Selenium exists naturally as either organic or inorganic forms, or it can be artificially synthesised. The different forms of naturally occurring selenium can be grouped into low molecular weight compounds existing in a free form, and high molecular weight forms of selenium that are present in proteins (Figure 2).

Free forms of selenium

Selenomethionine and selenocysteine are the selenium analogues of methionine and cysteine. Although they are mainly incorporated into proteins they also exist in the free form and have been found as such in plants including onions, clover and ryegrass (Ganther, 1974). There are several organo-selenium compounds associated with accumulator and non-accumulator plant species. These include selenohomocystine (formed from the metabolism of selenomethionine in leaves of *Astragalus*), Se-methylselenocysteine (the primary form of soluble selenium in accumulator plants), selenocystathionine (first discovered in *Astragalus* but also found in other accumulator plants including the 'monkey nut' (Ganther, 1974)), dimethyl diselenide (one of the four volatile species of selenium derived from an *Astragalus* species (Shamberger, 1983b)), and Se-methylselenomethionine (the main form of soluble selenium in non-accumulator plants).

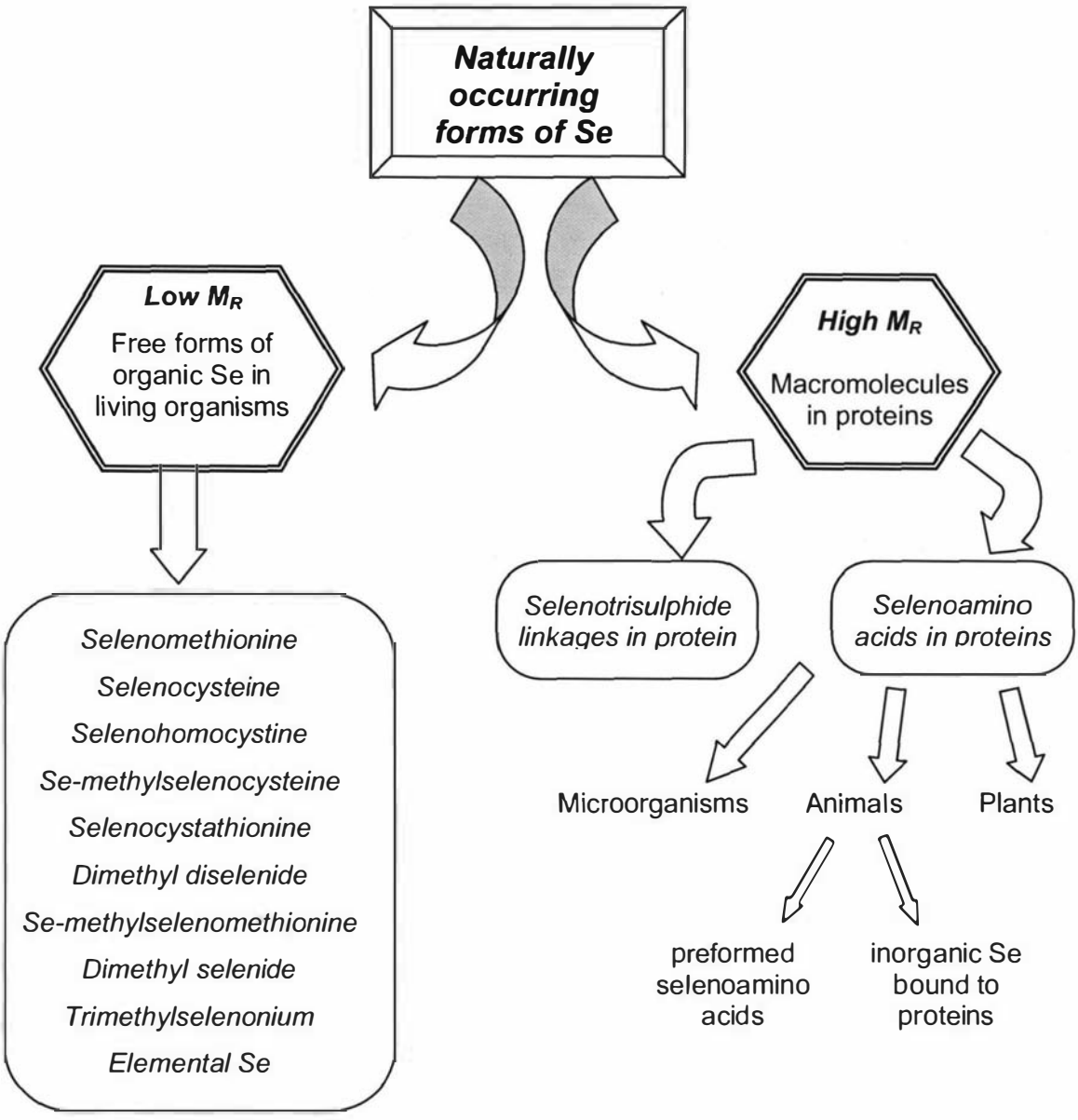


Figure 2. Naturally occurring forms of selenium

Other organo-selenium compounds include the excretory compounds dimethyl selenide, the metabolic excretory product responsible for the garlic odour in the breath of selenium-treated animals (Shamberger, 1983b), trimethylselenonium, and 1β-methylseleno-*N*-acetyl-D-galactosamine, or selenosugar, both urinary excretory products (Suzuki *et al.*, 2005). Elemental selenium is readily formed by reduction of selenites in acid solutions (Allaway *et al.*, 1966) and reduction of selenium salts by microorganisms (Ganther, 1974). It can be reduced to Se^{2-} (selenide) or oxidised to the Se^{4+} (selenite) and Se^{6+} (selenate) oxidation states. Its properties depend on the state of subdivision and its allotropic form (Allaway *et al.*, 1966), of which there are

three: a grey-black metallic hexagonal form, an amorphous white form and a monoclinic red S_8 form (Sunde, 1997). There are six naturally occurring stable isotopes of selenium. These have uses as stable isotopic tracers in studying selenium metabolism, physical studies of selenium-containing proteins using NMR or EPR analysis, and radioactive tracer analysis (Sunde, 1997).

Forms of selenium in proteins

The majority of selenium in plants and animal tissues is closely associated with protein. In broad terms these associations occur in two ways: either by selenotrisulphide linkages, or by association with sulphur in the formation of selenoamino acids (Ganther, 1974).

The first means of incorporating selenium into proteins is by a non-enzymatic reaction of selenious acid with thiols. This reaction creates cross-linkages containing selenium (-S-Se-S-) where the covalently bound selenium is linked to carbon or sulphur.

The other means of incorporating selenium into a protein occurs either when the sulphur atom in an amino acid is replaced by selenium, or when selenium is attached to the sulphur atoms of cysteine residues. The existence of selenoamino acids in plants, micro-organisms and animals has been well established and is described in the previous section.

SELENIUM METABOLISM

Metabolism of selenium varies depending on the species. Selenium metabolism in ruminants is quite different to that in monogastric animals due to the microbial fermentation that occurs in the rumen and therefore will not be discussed here. The following section reviews metabolism in monogastric animals including humans and livestock, whilst what is known of metabolism of selenium in cats and dogs is discussed later in this chapter.

Absorption

The degree to which selenium is absorbed is dependent on the form of selenium ingested. The soluble forms of selenium, which include the major dietary forms selenate, selenite, selenomethionine and selenocysteine, are well absorbed from the gastrointestinal tract. Different forms of selenium are transported across the intestinal mucosa by different mechanisms, and this in turn affects the rate of absorption and the total amount of selenium absorbed.

Using isolated pig jejunum, selenomethionine was found to be transported across the intestinal brush border by active transport which involved a carrier-mediated, Na^+-dependent mechanism for neutral amino acids (Wolffram *et al.*, 1989a). This system requires energy to transport the compound against a concentration gradient from the mucosal to the serosal side of the intestinal membrane. Several amino acids share this absorption mechanism, including methionine (Wolffram *et al.*, 1989a) and its sulphur analogue selenomethionine, and as a result, there may be competition for uptake when several of these amino acids are present. Wolffram *et al.* (1989a) found that when methionine and selenomethionine were present in the same medium, one inhibited the uptake of the other by 90%.

Little is known about the uptake of selenocysteine. Again using pig jejunum, Wolffram *et al.* (1989b) found cysteine transport was inhibited by selenocysteine, as well as lysine and arginine. Consequently, these authors suggested absorption of selenocysteine occurred by a similar method to that of selenomethionine, but with a basic amino acid carrier mechanism instead of the neutral amino acid carrier-mediated mechanism.

Absorption of selenate also occurs by active transport. A Na^+-dependent gradient across the brush border membrane was found to stimulate rapid carrier-mediated transport of selenate in the small intestine of the rat and pig (Wolffram *et al.*,

1986). Selenate is transported into the vesicular lumen of the brush border membrane vesicle rather than just binding to the membrane (Wolffram *et al.*, 1986). Studies by Wolffram *et al.* (1986) revealed a common transport mechanism for sulphate, thiosulphate and selenate in the brush border of pig intestine, therefore as previously described for selenomethionine and selenocysteine, selenate may also have to compete for uptake.

Selenite is absorbed by simple diffusion. In contrast to selenomethionine and selenate, rather than being transported through the brush border membrane, selenite binds to it extensively, possibly resulting from a reaction of selenite with thiol groups in the membrane (Wolffram *et al.*, 1986).

Studies on the site of selenium absorption in monogastric animals have been conducted in pigs (Wolffram *et al.*, 1986; 1988; 1989a; 1989b), rats (Wolffram *et al.*, 1986) and dogs (Reasbeck *et al.*, 1985). Generally in these species absorption of selenium did not occur in the stomach. The site of greatest absorption was the duodenum, followed by the jejunum and ileum. In both humans and monogastric animals, selenium absorption does not appear to be homeostatically controlled (Daniels, 1996).

Using different methods, studies in humans and several monogastric species including rats, chickens, dogs and pigs, have investigated the amount of selenium absorbed in the different forms (Combs and Combs, 1986a). Comparisons between absorption of the different forms of selenium are hard to make due to variations between species, methods used and amount of selenium administered. However apparent absorption of selenium from different foods, inorganic selenium and selenoamino acids, averaged around 70%. In general, selenomethionine is the most efficiently absorbed form of selenium with reports of 83 to 97% absorption in rats and 97% in humans (Combs and Combs, 1986a). Selenate also appears to be absorbed at levels as high as 91% in humans (Van Dael *et al.*, 2001) and under optimal conditions was reported to have a similar rate of absorption to that of selenomethionine (Daniels, 1996). In contrast, absorption of selenite is generally lower and more variable than other forms of selenium, probably due to its passive mechanism of uptake, with absorption ranging from 35 to 59% in humans to 75 to 93% in rats (Combs and Combs, 1986a). Thus selenite is less well absorbed in humans than in rats. Van Dael *et al.* (2001) suggested that variation in the absorption of selenium from selenite is influenced by dietary habits, the result of the interaction of selenite with lumen contents.

There is limited information available regarding the factors affecting selenium absorption, however some reports suggest that absorption of selenite is promoted by the presence of vitamins A, C and E, or a high protein diet (Robinson and Thomson, 1983; Combs and Combs, 1984).

Uptake and transport

Once absorbed, selenium is rapidly taken up by erythrocytes where it is metabolised and released back into plasma. In humans, 50 to 70% of radioactive selenite added to blood was taken up by erythrocytes within 1 to 2 minutes and released back into plasma 15 to 20 minutes later (Shamberger, 1983c). The speed at which this process occurs in erythrocytes has been documented in other species but is thought to be somewhat slower in bovine, avian and ovine erythrocytes (Combs and Combs, 1986b). Most selenium in rat and sheep erythrocytes is associated with GSHPx, however this is not the case with higher primates (National Research Council, 1983b). In humans, selenium as selenomethionine in erythrocytes is incorporated mainly into haemoglobin (Schrauzer, 2000). It has been proposed that the form released by erythrocytes is the selenotrisulfide selenodiglutathione (GSSeSG), however the exact form(s) of selenium released by erythrocytes have not been established (National Research Council, 1983b).

Once released back into plasma, selenium bound to protein to enable transport around the body to tissues and organs as required (Daniels, 1996). This process is not energy dependent or reliant on protein synthesis (National Research Council, 1983b). There are several proteins that selenium binds to including albumin, α- and β-globulins and lipoproteins. The type of protein and the distribution of selenium among them, vary with species, form and dose of selenium (Whanger, 1998). It appears selenium is initially loosely bound to albumin but is later released and bound to, or incorporated into, other plasma proteins including Selenoprotein P and GSHPx (Bopp *et al.*, 1982; Daniels, 1996). After the initial binding to albumin, selenium in mice, rats, dogs and chickens binds to α- and β-globulins (Bopp *et al.*, 1982; National Research Council, 1983b), whereas in humans the major selenium binding proteins in plasma appear to be lipoproteins (National Research Council, 1983b).

Metabolic fate of selenium

Selenium metabolism of both inorganic and organic forms involves conversion to an assumed metabolic intermediate, hydrogen selenide, before reaching its endpoint as either a seleno-containing protein (containing selenomethionine), a selenoprotein (containing selenocysteine), or a methylated excretory product (Figure 3).

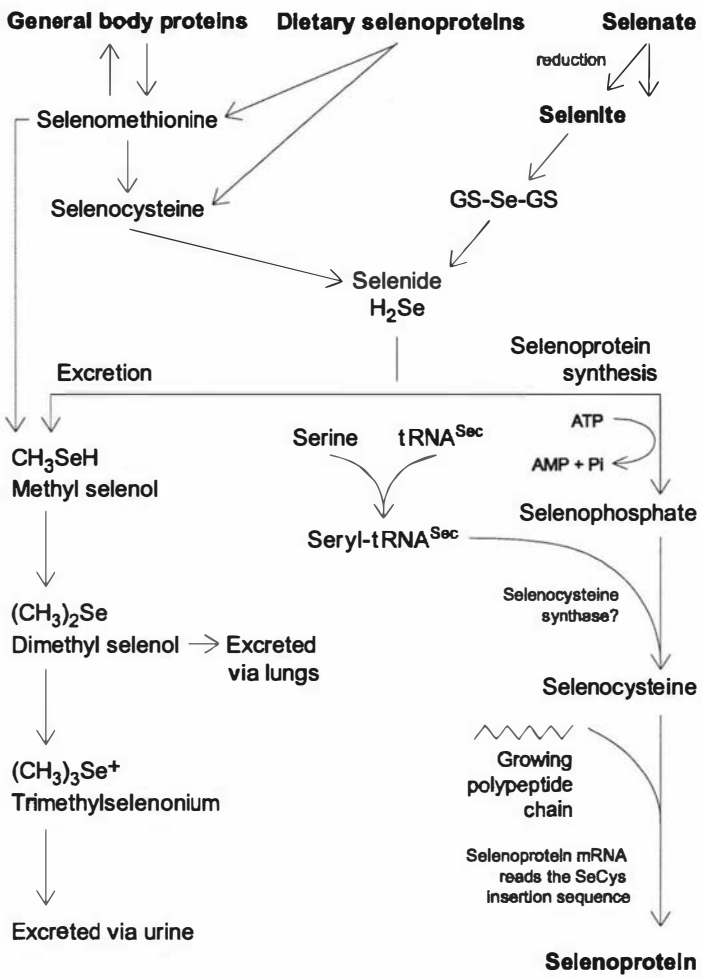


Figure 3. Overview of selenium metabolism (taken from Jacques, 2001)

In humans, following uptake and metabolism of selenium in the erythrocyte and its release back into plasma, selenium then enters one of two proposed metabolic pools depending on its form. The first is the exchangeable metabolic pool (SeEMP), which is involved in the metabolism and synthesis of all functionally important selenocompounds. This pool processes inorganic selenium as selenate and selenite

and therefore includes intermediary products resulting from the reduction of selenite to selenide, methylated compounds derived from selenide, as well as the endogenously formed selenoproteins (Janghorbani *et al.*, 1990; Daniels, 1996). The SeEMP may also contain selenoamino acids resulting from the catabolism of selenomethionine and preformed selenocysteine from Pool 2, the second of the hypothetical selenium pools, however SeEMP does not contribute to Pool 2. The first pool comprises proteins containing selenomethionine that have been formed by non-specific incorporation of the selenonamino acid into general body proteins (Daniels, 1996). Pool 2 has no metabolic role but is thought to provide a means of storing selenium.

Biologically active selenocysteine

Selenium fulfils its metabolic roles in the form of biologically functional selenoproteins which contain selenium as one or more selenocystyl residues within the peptide chain (Wolffram, 1999). Selenium as the amino acid selenocysteine is incorporated into the protein during translation of its primary structure. This is in contrast to many other trace elements which are attached to their respective proteins after translation (Burk *et al.*, 2003). In order to create biologically active selenocysteine, dietary selenium must first be transformed by a series of metabolic processes.

The specific incorporation of active selenocysteine into functional selenoproteins has been well characterised in prokaryotes, but is less well understood in eukaryotes (Patching and Gardiner, 1999). In brief, selenide is used as a substrate for the formation of selenophosphate via selenophospate synthetase (SPS) and selenophosphate then converts $tRNA^{[ser]sec}$-bound serine (seryl-$tRNA^{[ser]sec}$) into selenocysteine (selenocysteyl-$tRNA^{[ser]sec}$) (Driscoll and Copeland, 2003). The $tRNA^{[ser]sec}$ contains a UGA anticodon which thereby enables insertion of selenocysteine into the polypeptide chain of the selenoprotein (Hatfield and Gladyshev, 2002). Other factors required for selenocysteine insertion include a stem loop structure in the untranslated section of the mRNA, and at least two trans-acting protein factors (Gardiner and Patching, 1999; Burk *et al.*, 2003, Driscoll and Copeland, 2003). The latest findings and mechanisms involved in this process are reviewed in detail by Gromer *et al.* (2005). Any selenium as hydrogen selenide not recruited for selenoprotein synthesis, or any selenium catabolised from selenoproteins, undergoes sequential methylation from methylselenol to dimethylselenol and trimethylselenonium to enable excretion via kidneys and lungs (Whanger, 2003).

Metabolism of inorganic selenium

Dietary selenium of inorganic form is used only for selenoprotein synthesis, and because selenium of this origin cannot be stored, any inorganic selenium not utilised in selenoprotein synthesis is methylated and excreted (Jacques, 2001). Thus, dietary selenate or selenite undergoes reduction to the metabolic intermediate hydrogen selenide, however there are differences in the metabolism of these two inorganic compounds in the blood. Selenite is rapidly taken up by red blood cells where it initially combines with glutathione to form the intermediate selenodiglutathione and is then readily reduced by NADPH and glutathione reductase in two steps to form glutathione selenopersulphide followed by hydrogen selenide (National Research Council, 1983b; Whanger, 2003). Selenide is then bound to albumin and taken up by the liver (Shiobara and Suzuki, 1998; Kobayashi *et al.*, 2001). In contrast, selenate is not as readily reduced to selenide (Suzuki, 2005). Reduction of selenate does not occur via thiol groups as with selenite and the mechanism for conversion of selenate to selenide has yet to be determined (Kobayashi *et al.*, 2001). Some selenate is excreted directly into the urine, and the remainder is taken up by the liver directly (Kobayashi *et al.*, 2001). In the liver, both forms of inorganic selenium are metabolised to methylated excretory products or utilised for selenoproteins synthesis in a similar manner as discussed above.

Metabolism of organic selenium

Dietary selenium of organic origin may be utilised for selenoprotein synthesis or excreted in the same way as the inorganic selenium forms. However organic selenium, especially in the form of selenomethionine, has an additional metabolic fate involving its incorporation into general body proteins. This results from the chemical similarities between selenium and sulphur, which enable selenium to replace sulphur in the amino acids methionine, and to a lesser extent cysteine, forming selenomethionine and selenocysteine, as previously discussed.

As with inorganic selenium, in order to facilitate selenoprotein synthesis, organic selenium must first be converted to hydrogen selenide. Providing there is sufficient methionine available, the metabolism of selenomethionine to selenocysteine occurs via the same methionine transamination and transsulphuration pathways as the metabolism of methionine to cysteine (Suzuki, 2005). Thus dietary selenomethionine is activated by adenosylation, demethylated, converted initially to selenocystathionine and then to selenocysteine (Schrauzer, 2000; Whanger, 2003). However there is no

build up of selenocysteine at this point and selenocysteine does not appear to be metabolised by the same metabolic processes as cysteine (Wolffram, 1999). Rather, the selenium contained in the protein is liberated by the enzyme selenocysteine β-lyase and reduced to hydrogen selenide (Daniels, 1996). Selenide is then converted to active selenocysteine for insertion into selenoproteins in the same way as inorganic selenium.

Any selenomethionine not immediately utilised for selenoprotein synthesis is non-specifically incorporated into general body proteins in place of methionine (Schrauzer, 2000; Suzuki, 2005). This occurs in organs and tissues with high rates of protein synthesis such as erythrocytes, liver, kidney, pancreas (Schrauzer, 2000), and in particular, skeletal muscle which contains 40 to 50% of total body selenium (Daniels, 1996). The degree of substitution of selenomethionine for methionine in the proteins depends on the ratio of these two amino acids in the diet. If dietary methionine levels are low, selenomethionine may be used in its place leaving less of the selenoamino acid available for selenoprotein synthesis (Wolffram, 1999). This process is unregulated and effectively acts as a means of storing selenium. Non-specific incorporation of selenoamino acids into protein can be reversed by catabolism during the normal regulated processes of protein turnover, releasing selenium which can then enter the SeEMP and be reutilised or excreted (Shiobara *et al.*, 2000; Suzuki, 2005). However some proteins, such as those in erythrocytes, nails and hair do not undergo protein turnover, and in these cases the selenium in these proteins is retained (Shiobara *et al.*, 2000).

Selenomethionine may also be directly catabolised to the excretory precursor methylselenol via the transamination-decarboxylation pathway without first being converted to hydrogen selenide. This pathway is used to metabolise approximately 90% of methionine and may therefore be a major route for the degradation of selenomethionine (Whanger, 2003). It has also been proposed that this pathway is a means of removing excess selenomethionine (Okuno *et al.*, 2001; Spallholz *et al.*, 2004)

Dietary selenocysteine has the same metabolic fates as selenomethionine and the pathways to selenoprotein synthesis or methylation and excretion via hydrogen selenide are the same for both selenoamino acids. Exogenous selenocysteine cannot be used directly for insertion into selenoproteins, it must first be metabolised to selenide and then active selenocysteine in the same manner as selenomethionine. Selenocysteine may also be incorporated into general body proteins in place of

cysteine (Wolffram, 1999), however this is thought to be a minor metabolic fate as cysteine and its selenium analogue have different chemical properties (Jacques, 2001).

Selenium excretion

Following metabolism to hydrogen selenide, excess selenium of inorganic or organic origin is methylated in a step-wise manner for excretion. Thus selenide is not only a common metabolic intermediate for the metabolism of dietary selenium, it also serves as a checkpoint for utilisation or excretion of selenium (Suzuki, 2005)

Selenium is eliminated from the body via the three major excretory routes of the gastrointestinal tract, the urinary tract, and the lungs. The degree to which selenium is excreted by these pathways is species dependent and also varies according to the chemical form of selenium, amount of selenium ingested, dietary composition and other interacting factors such as arsenic (Shamberger, 1983c; Combs and Combs, 1986b). At normal dietary intakes faecal and urinary excretion are the primary means of elimination with pulmonary excretion becoming increasingly important when higher concentrations of selenium are ingested. Small amounts of selenium are excreted in faeces over a wide range of dietary intakes in monogastric animals, thus faecal excretion of selenium does not appear to be dependent on dose or level of intake (Bopp *et al.*, 1982).

Urinary excretion of selenium is the most important excretory route for monogastric animals at normal selenium intakes and is strongly correlated to dietary intake. Under normal circumstances urinary excretion accounts for 50 to 70% of the total amount of selenium excreted over a wide range of dietary intakes (Daniels, 1996). Excretion of selenium in urine is also affected by form, with lower levels of selenium eliminated in rats fed selenomethionine compared to selenite or selenocysteine (Combs and Combs, 1986b). Selenium excretion via the kidney is dependent on the glomerular filtration rate, therefore renal function is an important factor affecting urinary excretion (Oster and Prellwitz, 1990). This may also contribute to the differences in excretion of different forms of selenium as renal clearance of selenite is higher than that of selenomethionine (Swanson *et al.*, 1991).

Within the normal nutritional range, the major excretory selenium compound in the urine of both rats and humans is 1β-methylseleno-*N*-acetyl-D-galactosamine, or selenosugar B. This urinary metabolite is thought to be produced via an activated form of selenium (glutathione-conjugated selenide) to an activated form of the sugar moiety resulting in selenosugar A (glutathione-conjugated selenosugar), which is then

methylated to produce selenosugar B (Kobayashi *et al.*, 2002; Suzuki *et al.*, 2005; Suzuki *et al.*, 2006a). This appears to be the case for both selenite and selenomethionine (Suzuki *et al.*, 2006b). At higher dietary selenium concentrations trimethylselenonium is excreted, such that the ratio of these two metabolites changes depending on the dose (Suzuki, 2005). It was previously thought that urinary trimethylselenonium increased relative to dietary selenium intake and could therefore be used as an indicator of toxic selenium levels (Whanger, 1998), however studies by Suzuki *et al.* (2005) revealed that although this was they case in young rats, trimethylselenonium was only present as a minor urinary metabolite in adult rats despite the fact these animals displayed greater signs of toxicity. It has been suggested that the selenosugar is produced in the presence of excess selenide when there is sufficient sugar moiety, but when the sugar moiety is insufficient, or when there is an accumulation of methylselenol (the intermediary metabolite of selenoamino acids leading to selenide), more trimethylselenonium becomes the predominant urinary metabolite (Suzuki *et al.*, 2006a).

Elimination of selenium through the lungs becomes significant at high dietary selenium intakes and shows obvious dose dependency (Bopp *et al.*, 1982). When rats were fed potentially lethal doses of selenite, as much as 60% of the dose was exhaled and 70% of this amount was eliminated in the first six hours (Combs and Combs, 1986b). Thus respiratory excretion of selenium is an effective means of eliminating toxic levels of selenium. Pulmonary excretion of selenium also increased when dietary protein and methionine levels were increased (Shamberger, 1983c). Depending on the form of selenium ingested, at least two methylated selenium compounds have been characterised in expired air. Dimethylselenide was produced when mice were fed selenite or selenocysteine, and dimethyldiselenide, along with an unidentified compound, was produced when selenomethionine was ingested (Combs and Combs, 1986b). The primary compound excreted through expired air is dimethlyselenide (Bopp *et al.*, 1982). It is this metabolic compound that has the characteristic garlic odour observed in animals with selenium toxicity (Shamberger, 1983b).

Endogenous losses

A proportion of ingested nutrients may be excreted in the faeces within sloughed off mucosal cells, or via secretion of the nutrient back into the gastrointestinal tract in biliary, pancreatic and gastrointestinal secretions from the various tissues (Ammerman, 1995). The extent of these endogenous losses depends on the animal, nutrient, and form of the nutrient. In order to accurately estimate endogenous losses the use of

isotopes is required. These isotopes are used to label nutrients and act as markers, thereby providing a means to distinguish between exogenous and endogenous sources of nutrients. "True absorption" takes into account endogenous losses and is calculated from the difference between dietary intake and exogenous and endogenous faecal losses (Ammerman, 1995). In humans, endogenous losses of selenium are considered to be significant (Robinson and Thomson, 1983) and should be accounted for when estimating selenium absorption. Stewart *et al.* (1978) found endogenous faecal losses in humans to be approximately half the total faecal output. In ruminants endogenous losses of some minerals can be quite significant (McDonald *et al.*, 2002) and in dairy cows endogenous faecal selenium losses were reported to be 22 to 36% of total faecal excretion (Koenig *et al.*, 1991). Little data is published regarding endogenous faecal selenium losses in monogastric animals, however two balance studies in rats fed selenite for 14 days determined that 86 to 92% of total faecal selenium (10% of dietary intake) was of endogenous origin (Gabler *et al.*, 1997), and in rats fed different forms of selenium for 35 days showed 54 to 94% (8 to 10% of dietary intake) of total faecal selenium was from endogenous sources (Windisch *et al.*, 1998).

Regulation of selenium metabolism

Selenium homeostasis is facilitated via excretion rather than absorption. Selenium is generally well absorbed, regardless of the selenium status of the animal (Wolffram, 1999), which in turn suggests metabolism of selenium is not regulated at the gastrointestinal level. Instead, selenium homeostasis is achieved via changes in urinary excretion (Behne, 1988). As previously mentioned, urinary excretion of selenium is strongly correlated to dietary selenium intake at normal levels. There appears to be a dietary level of selenium above which urinary excretion of selenium increases with increasing intake, and below which only a small amount is excreted in the urine (Behne, 1988). As dietary selenium concentrations increase, so too do the excretory methylated compounds found in urine, and at higher concentrations, in expired air (Whanger, 2003).

At high dietary intakes, regulation of selenium metabolism appears to be affected by chemical form. The levels of selenoproteins found in tissues after ingestion of high doses of selenium are similar to those found at adequate dietary intakes (Patching and Gardiner, 1999). In contrast, less selenium is excreted in urine and therefore more is retained in the body when high levels of selenomethionine are fed compared to selenite or selenate (Behne, 1988). It has been suggested that the deposition of

excess selenium into body tissues that occurs with selenomethionine ingestion is not as well regulated at high dietary intakes due to an inability to differentiate between methionine and selenomethionine (Behne, 1988). However this could also be a means of storing selenium for use in times of selenium deficiency.

Regulation of selenium metabolism via urinary excretion is particularly effective at low dietary selenium concentrations as urinary excretion is decreased in order to conserve selenium in the body. In the long term, the kidney is able to adapt to low dietary selenium intakes by decreasing its renal clearance, which therefore results in low urinary excretion (Robinson *et al.*, 1985).

The level of selenoproteins in various tissues and organs also seems to be well regulated during periods of selenium deficiency and there appears to be a hierarchy in which they are preferentially maintained in accordance with the importance of organ function (Patching and Gardiner, 1999). Thus levels in the brain, reproductive and endocrine organs are preferentially maintained, whereas levels in the liver, heart and skeletal muscle are less important (Behne, 1988). This differential regulation of selenoprotein synthesis is thought to occur at the mRNA level (Patching and Gardiner, 1999).

BIOLOGICAL FUNCTIONS OF SELENIUM

Selenium has a variety of biological roles including acting as an antioxidant, facilitating metabolic processes and providing structural support within cells (Holben and Smith, 1999). These biological functions are exerted through approximately 30 to 40 identified selenoproteins, several of which have been characterised (McKenzie *et al.*, 2002). These characterised selenoproteins include three families, the GSHPx's, iodothyronine deiodinases (ID's) and thioredoxin reductases (TRR's), in addition to several other selenoproteins with lesser known functions (Table 4).

GSHPx's

GSHPx's are a group of enzymes responsible for selenium's role as an antioxidant. Their primary role is to catalyse the reduction of hydrogen and lipid peroxides, thereby preventing production of the cell damaging reactive oxygen species (free radicals) (Surai, 2002). In these reactions, glutathione acts as the reductant to produce water and corresponding alcohols (Gromer *et al.*, 2005). GSHPx's are also involved in the maintenance of the cellular redox state (Surai, 2002) and are known, or are thought to have, several functions associated with the male genital tract. These include acting as antioxidant scavengers, modulators of inflammatory and immune responses, intermediates in signal transduction pathways, and structural component of sperm (Drevet, 2006). There are currently seven distinct GSHPx isoenzymes in humans, and with the exception of GSHPx 5 and GSHPx 7 (Gromer *et al.*, 2005), each selenoenzyme has a single selenocysteine residue within each subunit or molecule (Patching and Gardiner, 1999). Collectively they are found in most cells of the body (Sunde, 2000).

Classical (cellular) GSHPx – cGSHPx (GPx1)

cGSHPx was discovered by Mills in 1957 and was the first selenoprotein to be identified (Sunde, 2000). A tetramer, cGSHPx contains four selenocysteine residues (Patching and Gardiner, 1999). Its enzymatic activity was originally thought to be the only biological function of selenium (Patching and Gardiner, 1999) and as a result cellular cGSHPx was used as, and still continues to be, a functional indicator of selenium status. cGSHPx is found in the cytosol of most cells and is one of several enzymes involved in the detoxification of reactive oxygen species (Patching and

Gardiner, 1999). It therefore has a primary role in the liver and red blood cells where reactive oxygen species are produced during detoxification processes (Patching and Gardiner, 1999). cGSHPx is thought to be associated with regulation of virus production, cellular protection from apoptosis, decreased risk of cancer (Diwadkar-Navsariwala and Diamond, 2004; Gromer *et al.*, 2005).

Table 4. Function and distribution of the primary selenoproteins[1]

Selenoprotein	Location	Function
[ab]*Glutathione peroxidases (GSHPx):*		
Classical (cellular) GSHPx (GPx1)	cytosol of most cells	metabolises hydrogen peroxide functional indicator of selenium status
Plasma (extracellular) GSHPx (GPx3)	synthesised in the kidney, found in plasma and milk	functional indicator of selenium status
Phospholipid hydroperoxide GSHPx (GPx4)	bound to cell membranes, testis, spermatozoa	protects against lipid peroxidation and functions in eicosanoid metabolism
Gastrointestinal GSHPx (GPx2)	gastrointestinal tract	protects against the toxic effects of lipid hydroperoxides
GPx5	epididymis	non-selenocysteine containing isoform
GPx6	olfactory epithelium, embryonic tissues	possible role in olfaction
GPx7	?	non-selenocysteine containing isoform
[b]*Iodothyronine deiodinases (ID):*		
Type I	liver, kidney, thyroid, pituitary	converts T4 to T3
Type II	brain, pituitary, thyroid, muscle, adipose	intracellular production of T3
Type III	brain, placenta, uterus	catalyses deiodination of T4
[b]*Thioredoxin reductases (TRR):*		
TRR1	cytosol	cellular redox regulation
TRR2	mitochondria	
Thioredoxin glutathione reductase	testis	unknown
Others:		
[c]Selenoprotein P	plasma, liver	possible role in transport and oxidant defences
[a]Selenoprotein W	muscle	possible role in muscle metabolism
[a]Sperm capsule selenoprotein	sperm	structural role in sperm function

[1]*taken from:* [a] *Holben and Smith (1999);* [b] *Gromer* et al.*(2005); and* [c] *Daniels (1996)*

Plasma (extracellular) GSHPx – pGSHPx (GPx3)

pGSHPx is synthesised primarily in the lungs and kidneys and is then secreted into the extracellular environment (Patching and Gardiner, 1999). pGSHPx, which also has a tetrameric structure, was purified from human plasma and has also been found in human milk (Holben and Smith, 1999). Activity of pGSHPx in plasma is low compared to other GSHPx's and it has been suggested that this enzyme is either very efficient or has an alternative role (Holben and Smith, 1999). Activity of this glycoprotein is also used as a functional indicator of selenium status (Holben and Smith, 1999).

Phospholipid hydroperoxide GSHPx – phGSHPx (GPx4)

A monomer with only one selenocysteine residue, phGSHPx has a broad substrate specificity including an affinity for membranes (Gromer *et al.*, 2005). It is able to utilise other thiol compounds as a reductant in place of glutathione (Patching and Gardiner, 1999). phGSHPx acts to detoxify lipid peroxides and reduces the hydroperoxides of cholesterol, cholesterol esters and phospholipids in membranes and low density lipoproteins (Holben and Smith, 1999; Patching and Gardiner, 1999). phGSHPx is involved in redox signalling and regulatory processes and forms a structural component of sperm (Gromer *et al.*, 2005). phGSHPx synthesis is preferentially maintained during selenium deficiency (Patching and Gardiner, 1999) and is therefore not as accurate an indicator of selenium status as cGSHPx or pGSHPx (Holben and Smith, 1999).

Gastrointestinal GSHPx – gGSHPx (GPx2)

gGSHPx is another tetramer which is synthesised mainly in the liver and gastrointestinal tract (Gromer *et al.*, 2005), with the incidence in the gut increasing from the crypts towards the gut surface (Florian *et al.*, 2001). gGSHPx has similar physical and enzymatic properties to cGSHPx (Patching and Gardiner, 1999) and its role is to protect against the toxic effects of ingested lipid hydroperoxides (Wingler *et al.*, 1999). It is also thought gGSHPx may be involved in cell growth and differentiation Florian *et al.*, 2001).

GSHPx5

An additional GSHPx has more recently been discovered in the epididymis of mice (Vernet *et al.*, 1996). This isoform does not contain selenocysteine and it is thought it may provide and alternative for other selenocysteine-containing isoforms in sperm (Gromer *et al.*, 2005).

GSHPx6

GPx6 is expressed only during embryonic development and in the olfactory epithelium (Kryukov *et al.*, 2003), near the Bowmans glands and it is therefore thought this selenoenzyme has a function in olfaction (Gromer *et al.*, 2005).

GSHPx7

GPx7 is another non-selenocysteine containing isoform, the function of which has yet to be determined, however it may have a role in the prevention of breast cancer (Gromer *et al.*, 2005).

Thus the GSHPx's act synergistically to provide protection in the parts of the body where oxidative processes occur. Activity of these enzymes is dependent on the amount of selenium in the diet, and the different forms are affected by low levels of selenium to varying degrees. A hierarchy exists in which the GSHPx's are preferentially maintained during selenium deficiency. gGSHPx is retained in the tissues for the longest period of time, followed by phGSHPx, pGSHPx and finally cGSHPx (Surai, 2002).

ID's

ID's are enzymes which implicate the essentiality of selenium for normal growth, development and metabolism as they are involved in the formation and regulation of the thyroid hormone triiodothyronine (T_3) (Holben and Smith, 1999). There are three of these enzymes, each of which have a different structure and sequence and catalyse different reactions (Gromer *et al.*, 2005). Type 1 (IDI), catalyses the conversion of thyroxine (T_4) to T_3. IDI is found in the thyroid, liver, kidney and pituitary gland (Gromer *et al.*, 2005) and is the most susceptible of the three enzymes to decreases in activity as a result of selenium deficiency (Arthur, 1997).

Type 2 iodothyronine deiodinase (IDII) is located primarily in the brain and pituitary gland but has also been found in skeletal and heart muscle, thyroid gland and adipose tissue (Gromer *et al.*, 2005). IDII is involved in the intracellular formation of T_3 within tissues that are unable to utilise circulating T_3 (Arthur, 1997; Holben and Smith, 1999).

The Type 3 enzyme (IDIII) is involved in the deactivation of thyroid hormones (Arthur, 1997). T_3 is degraded to an inactive diiodothyronine (T_2) and T_4 is deiodinated to an inactive reverse T_3 (Patching and Gardiner, 1999). IDIII is found in the brain, placenta and pregnant uterus (Gromer *et al.*, 2005).

TRR's

There are three TRRs known in humans with different tissue distributions and intracellular localisations (Schomburg *et al.*, 2004). The activity of TRRs for the reduction of thioredoxin is dependent on NADPH for the transfer of reducing equivalents, which are received by a flavin adenine dinucleotide (FAD) group attached to the TRR molecule (Sunde, 2000). TRR's regulate redox reactions within the cellular environment, reduce small intracellular molecules and are thought to be important in cell cycling (Sunde, 2000). TRR1 is found in the cytosol and is involved in cellular redox regulation (Sun *et al.*, 1999) and is capable of apoptosis (Anestal and Arner, 2003). TRR2 is found in the mitochondria, with levels highest in prostate, testis, liver, uterus and small intestine, and lower levels found in brain, muscle, heart and spleen (Gromer *et al.*, 2005). Thioredoxin glutathione reductase is a third isoenzyme found in the testis (Schomburg *et al.*, 2004), however its specific function is as yet unknown (Gromer *et al.*, 2005).

Other characterised selenoproteins

Selenoprotein P is an extracellular glycoprotein containing 10 selenocysteine residues (Mostert, 2000). It is produced mainly in the liver, however its mRNA is expressed in most tissues with high concentrations in kidney and heart, and lower concentrations in lung, brain, skeletal muscle and testis (Burk and Hill, 2005). Selenoprotein P and pGSHPx are the only selenoproteins found in plasma, with Selenoprotein P contributing approximately 60 to 80% of the total selenium found in plasma (Arthur, 1997), and approximately 25% of whole body selenium circulates through plasma as Selenoprotein P on a daily basis (Burk and Hill, 2005). It was first

thought that Selenoprotein P was a transport protein facilitating distribution of selenium around the body via the circulation (Arthur, 1997) and this role has since been confirmed (Saito and Takahashi, 2002). It has also been suggested that Selenoprotein P is an antioxidant with important roles in oxidant defence (Burk *et al.*, 2003). In addition, Selenoprotein P is required for sperm development and male fertility (Olson *et al.*, 2005).

Selenoprotein W was first associated with the selenium deficiency disease in sheep, white muscle disease (Sunde, 2000). Selenoprotein W is found primarily in skeletal muscle but also in spleen, testis and the brain (Patching and Gardiner, 1999). Although its exact function is currently unknown, it is thought to have a role in muscle metabolism (Holben and Smith, 1999). Studies in mice embryos showed a high expression of Selenoprotein W in proliferating myoblasts and an immediate response to oxidative stress (Loflin *et al.*, 2006). It was therefore suggested by these authors that Selenoprotein W is involved in muscle growth and differentiation by protecting developing myoblasts from oxidative stress.

There are also several additional selenoproteins whose functions are less well understood.

Sperm capsule selenoprotein contains three selenocysteine residues and is found in the mitochondrial capsule of sperm (Holben and Smith, 1999). It has a structural role associated with the sperm tail and is essential for normal sperm development as it maintains the integrity of the flagella (Patching and Gardiner, 1999).

There are two forms of selenophosphate synthetase in humans but only one of these is a selenoprotein (Holben and Smith, 1999). Selenophosphate is required for the formation of selenocysteine and its subsequent incorporation into selenoproteins. Thus, the selenoprotein, selenophospate synthetase 2, catalyses the reaction involved in selenocysteine synthesis (Stadtman, 1996), thereby providing a means of regulating selenoprotein expression (Surai, 2002).

In addition to the lesser-characterised selenoproteins, there are several other selenoproteins with unknown functions. These include a number of selenium-binding proteins of different sizes (Arthur, 1997). A 14kDa protein binds fatty acids, but it is not known whether there is any function of the bound selenium (Arthur, 1997).

A 15kDa protein, Sep15, occurs mainly in the prostate gland, testes, brain, kidney and liver (Diwadkar-Navsariwala and Diamond, 2004). It was isolated from human T cells (Gladyshev *et al.*, 1998) and it has been suggested that Sep15 is

involved in the quality control of protein transport (Gromer *et al.*, 2005). There is also speculation of a link between Sep15 and the incidence of cancer (Gladyshev *et al.*, 1998; Diwadkar-Navsariwala and Diamond, 2004).

The function of an 18kDa selenoprotein, found in kidney and other tissues, is unknown but appears to be important as it is preserved in times of selenium deficiency (Rayman, 2000a).

DNA-bound spermatid selenoprotein is thought to protect developing sperm (Rayman, 2000a). This 34kDa selenoprotein is found in the stomach and in nuclei of spermatazoa (Rayman, 2000a).

In addition, selenoproteins H, I, K, M, N, O, R, S, T and V have been identified but their functions are also unknown (Gromer *et al.*, 2005).

SELENIUM IN HEALTH AND DISEASE

The essentiality of selenium is reflected by the extent of its biological functions, which are indicative of its importance for the maintenance of good health. However the required dietary levels of selenium fall within a narrow range, outside of which detrimental effects may occur. This section outlines the role of selenium in the health of humans and livestock. Information regarding selenium and the health of companion animals is discussed in a later section.

Dietary intake of selenium in both humans and animals is largely dependent on geographical location and is due to the level of selenium in the soil which is taken up by and incorporated into accumulator plants as previously discussed. Dietary selenium intake in a specific area may therefore also be influenced by the contribution of imported foodstuffs to that area, depending on its place of origin. The range of dietary selenium intakes around the world are shown in Table 5. China and America have the highest dietary selenium intakes, however these countries also contain areas with low soil selenium levels, and these regions have correspondingly low dietary intakes. At the other extreme, New Zealanders have very low dietary selenium intakes (Reilly, 1998).

Table 5. Dietary selenium intakes in different countries[1]

Country	Selenium intake (range: µg/d)
Australia	57-87
Bangladesh	63-122
Canada	98-224
China (low soil Se area)	3-11
China (high soil Se area)	3200-6690
Finland (1974)	25-60
Finland (1992)	90 (mean)
Germany	38-48
Greece	110-220
Mexico	10-223
New Zealand	6-70
Portugal	10-100
Russia	60-80
UK (1978)	60 (mean)
UK (1995)	29-39
USA	62-216
Venezuela	86-500

[1] *taken from Reilly (1998)*

Toxicity

Although selenosis is generally only seen in areas with high soil selenium levels, other cases of selenium toxicity have occurred as a result of accidental or careless over supplementation of animal feeds or pharmaceutical preparations, or as a result of exposure to selenium in industry via inhalation of fumes (Foster and Sumar, 1997).

Evidence from work done in sheep and rats suggests that animals are able to adapt to excess selenium levels by increasing the production of methylated excretory compounds, thereby decreasing selenium storage in tissue and retention in the body (Combs and Combs, 1986c). Rats have also been shown to exhibit adaptive changes in hepatic glutathione metabolism in response to excess dietary selenium (LeBouef and Hoekstra, 1983).

Selenium toxicity in animals

The level of selenium intake at which toxic effects occur depends on several factors including the form of selenium ingested, the degree of intake in terms of duration and continuity, the composition of the diet as a whole, and the species (Foster and Sumar, 1997). In experimental animals toxicity is also affected by mode of administration (National Research Council, 1983a). Hydrogen selenide is the most toxic form of selenium, existing as a gas with an offensive smell (Cooper and Glover, 1974). Of the dietary selenium compounds, selenite is more toxic than selenocysteine, selenomethionine and selenate, which have similar levels of toxicity (Martin and Gerlach, 1972). Organic selenium compounds, in which the sulphur analogues are not normal sulphur metabolites, are less toxic (Martin and Gerlach, 1972).

Livestock:

In grazing animals, acute selenium poisoning, or blind staggers, may result from consuming large quantities of accumulator plants, containing concentrations of around 10,000 ppm within a short time (Moxon and Rhian, 1943). Animals with blind staggers exhibit impaired vision, wander and stumble, are dull and lack vitality, have a rough coat and loose hair, become emaciated, experience soreness and sloughing of the hooves, stiffness and lameness, and death usually results from respiratory failure (Moxon and Rhian, 1943; National Research Council, 1983a; Underwood and Suttle, 1999). Due to the unpalatable nature of the highly seleniferous plants, acute selenium toxicity resulting in death is rare (Shamberger, 1983a), however if they are consumed,

symptoms occur a few hours to a few days after the toxic dose has been ingested (Raisbeck, 2000).

Alkali disease is the outcome of chronic selenium toxicity resulting from the ingestion of grains containing up to 25 ppm over a period of weeks or months (Moxon and Rhian, 1943). Symptoms of alkali disease include dullness and lack of vitality, emaciation, stiffness and lameness, hair loss and sloughing of hooves (Moxon and Rhian, 1943; National Research Council, 1983a)

The minimum lethal dose for cattle has been reported to be 9 mg Se/kg of sodium selenite and in general for livestock, toxic effects of selenium are observed from concentrations of around 5 mg Se/kg (Shamberger, 1983a).

Pigs and poultry:

Growing pigs exhibit similar symptoms of selenium toxicity when exposed to high selenium intakes including hoof lesions, decreased appetite, central nervous system lesions, and impaired development of the embryo in sows (Underwood and Suttle, 1999). A diet containing 10 mg Se/kg DM as selenite decreased the conception rate, and the outcome of those piglets that were viable was poor, with a greater percentage dead, small or weak (Underwood and Suttle, 1999). Work done with pigs demonstrates an effect of dietary composition on the level at which selenium toxicity occurs. When pigs were fed a maize-soybean diet containing 8 mg Se/kg DM as selenite for five weeks, appetite and growth rate were impaired, however this concentration of selenium had no detrimental effect when wheat and oats were added (Underwood and Suttle, 1999). The minimum lethal dose for pigs given a single oral dose was 15 mg Se/kg (Shamberger, 1983a).

Poultry can tolerate seleniferous grain at concentrations of up to 10 mg Se/kg DM without adverse effects (Underwood and Suttle, 1999), and at concentrations below 3 to 5 mg Se/kg toxic effects are not generally seen. However development of the embryo within the egg is affected and hatchability is borderline at 5 mg Se/kg DM (Underwood and Suttle, 1999). Growing chicks have decreased appetite and therefore a slow growth rate when fed excess selenium (Underwood and Suttle, 1999). The minimum oral lethal dose of selenium as sodium selenite appears to vary between species and has been reported to be 0.9 mg Se/kg for turkey poults, 1.7 for broiler chicks and 9.4 for ducks (Surai, 2000).

Laboratory animals:

Symptoms of acute selenium toxicity in laboratory animals include the characteristic garlicky breath associated with excretion of volatile methylated selenium metabolites, as well as vomiting, dyspnea, tetanic spasms and eventual death from respiratory failure (National Research Council, 1983a). The minimum lethal dose of selenium as sodium selenite or selenate in rabbits and rats was 1.5 to 3 mg Se/kg regardless of the mode of administration (Koller and Exon, 1986).

Inhibition of growth during chronic selenium toxicity had been found to occur in laboratory animals fed a normal diet containing 4 to 5 ppm of selenium (National Research Council, 1983a).

Selenium toxicity in humans

There are no accurate criteria to assess the degree of excess dietary selenium levels, and toxicological standards are based on clinical signs of selenosis such as hair or nail loss (Levander and Burk, 1996). The "no-observed-adverse-effect level" (NOAEL) for humans in the western world has been estimated at 350 µg Se/day (Levander and Burk, 1996). The maximum safe upper level of selenium intake in the United Kingdom has been set at 450 µg Se/day (Levander and Burk, 1996). In a study where humans were fed dietary selenium intakes of 3.2 to 6.6 mg Se/day, chronic selenosis resulted, whereas those receiving 750 µg Se/day showed no clinical signs (Koller and Exon, 1986).

Signs of acute toxicity in humans include nausea and vomiting, nail changes, dryness of hair, hair loss, tenderness and swelling of the fingertips, fatigue, irritability and garlicky breath (Scientific Committee on Food, 2000). Additional chronic symptoms, that may occur at intakes of around 3.2 to 6.7 mg Se/day, are hair loss, changes in nail structure, lesions of the skin and nervous system and mottling of the teeth (Sunde, 2000). In a study conducted in a high selenium area of China, the average daily intake was 4.9 mg Se/day. Effects of selenosis included brittle hair with intact follicles, new hair with no pigment, thickened nails, brittle nails with spots and longitudinal streaks, skin lesions on hands, feet, legs, forearm and neck, red and swollen skin that blisters and erupts and neurological disturbances. During the later stages of toxicity numbness, convulsions, paralysis and motor disturbances are experienced (Scientific Committee on Food, 2000).

In addition to toxicity through dietary means, humans may also suffer the toxic effects of selenium via inhalation of selenium fumes from fires or heated metals in an

industrial environment (Combs and Combs, 1986c). Acute toxicity resulting from overexposure by inhalation causes irritation to the mucous membranes of the upper respiratory tract, leading to teary burning eyes, a runny nose, and hoarseness, coughing and sneezing. This is followed by clinical signs of conjunctivitis, rhinitis and bronchitis, with the development of pulmonary oedema after several hours (Combs and Combs, 1986c).

Mechanisms of toxicity

The exact mechanisms of selenium toxicity are unclear, however there have been several suggestions as to the cause of the toxic effects. These include redox cycling of auto-oxidisable selenium metabolites, glutathione depletion, protein synthesis inhibition, depletion of S-adenosyl-methionine (the cofactor for selenide methylation), or replacement of sulphur and reactions with critical sulphydryl groups of proteins and cofactors (Scientific Committee on Food, 2000). The largest group of evidence points towards oxidative stress as the main mechanism for selenium's toxic effects, often caused by metabolites of the parent compound. The step-wise methylation of these selenium metabolites en route to excretion is also thought to assist in their detoxification.

The toxic effects of selenite may be best explained by the production of free radicals during the reaction with glutathione (Surai, 2000). Selenium bound to albumin in the blood is subject to oxidation and yields selenite, which then produces an active reducing agent that is thought to produce reactive oxygen species (Kobayashi *et al.*, 2001). This may explain the differences in toxicity of selenate and selenite.

Although toxic at high doses, selenomethionine does not produce free radicals when reacting with glutathione (Surai, 2000). When investigating the pathway of selenomethionine detoxification in mouse liver, Okuno *et al.* (2001) found that when a single lethal dose of selenomethionine was given orally, the methylated excretory metabolite, trimethlyselenonium ion, was rapidly produced in the liver. In order for this reaction to occur, the presence of an α,γ-elimination enzyme is required in the liver to catabolise selenomethionine to methylselenol. These authors confirmed the existence of a liver α,γ-elimination enzyme, analogous to the bacterial L-methionine γ-lyase enzyme, which played a role in the detoxification of selenomethionine in the mouse.

It is currently thought that several mechanisms are likely to operate among different selenium compounds to exert toxic effects.

Deficiency

Selenium deficiency in animals

Selenium deficiency in animals was first linked to various diseases or conditions in areas of low soil selenium in the 1950s. At the time these reports were of a combined vitamin E and selenium deficiency syndrome, confusion arising from the fact that the metabolism of one is influenced by the other such that when selenium is deficient, there is a greater requirement for vitamin E and vice versa (Fryer, 2002). Such conditions include reproductive impairment, ill thrift, exudative diathesis and pancreatic degeneration in chicks, white muscle disease in calves and mulberry heart disease in pigs (cardiac myopathy) (McCartney, 2005).

White muscle disease is a nutritionally induced muscular dystrophy causing degeneration of the striated muscle in a wide range of animals. Animals with the disease exhibit weakness, stiffness, muscle deterioration and difficulty standing (McDowell *et al.*, 1996). The disease may either occur congenitally, resulting in a stillborn fetus or death soon after birth following sudden physical exertion, or it may develop after birth, usually within three to six weeks, but can occur up to four months of age (McDowell *et al.*, 1996). White muscle disease is mostly associated with lambs and calves due to its economic significance, however similar symptoms have also been detected in foals, pigs, chicks (Levander, 1986). White muscle disease is endemic in some areas of Turkey in which there are low levels of selenium in soil (0.03 ppm) and meadow hay and there have been high lamb mortalities in these areas (Beytut *et al.*, 2002). Lambs suffering from the disease showed weakness, stiffness, difficulty in standing and curvature of the back. When autopsied, widespread lesions with chalky-white necrosis and mineralisation in the heart muscles were found and skeletal muscles were pale and dry in appearance. The diseased lambs also showed significantly lower selenium concentrations in heart, liver and skeletal muscle compared to healthy animals. The disease was also found to be the primary cause of mortality in dairy goat kids aged between 8 and 30 days, farmed on the Mexican plateau (Ramirez-Bribiesca *et al.*, 2001). These animals showed microscopic lesions characteristic of muscular dystrophy, as well as pale skeletal muscle and cardiac white striations. White muscle disease can be prevented by the use of both selenium and vitamin E supplements (Beytut *et al.*, 2002).

Symptoms of exudative diathesis in chicks initially include oedema on the breast, wing, and neck, which later turns into subcutaneous haemorrhaging with symptoms in the form of lesions first seen at six days of age. Growth rate is also affected and

mortality rates are significant (Levander, 1986). Day old chicks depleted of selenium and vitamin E showed low glutathione concentrations and GSHPx activities which were associated with increased susceptibility to lipid peroxidation under oxidative stress (Avanzo *et al.*, 2001). Prevention of exudative diathesis can be achieved by either selenium or vitamin E supplementation via two different mechanisms (Levander, 1986).

In pigs, diets low in selenium and lacking vitamin E cause hepatosis dietetica. This disease, which results in a high mortality rate, causes severe necrotic lesions, the formation of a yellowish-brown colour in body fat, and subcutaneous oedema and is usually evident around three to 15 weeks of age (Levander, 1986).

Ill-thrift occurs in lambs and cattle of all ages and has been a serious condition in New Zealand and Florida (McDowell *et al.*, 1996). The severity of this condition ranges from subclinical growth deficit to rapid weight loss and mortality. The detrimental effects of ill-thrift can be reversed by supplementation with selenium but is not affected by vitamin E supplementation (Levander, 1986).

Today livestock diets are supplemented with selenium so cases of selenium deficiency diseases are less common. Sodium selenite has been the most common form of selenium used for supplementation over the past twenty years. However, sodium selenate has been used increasingly as it is less likely to oxidise other dietary components (Sunde, 2000). Although inorganic selenium is inexpensive as a supplement, it has disadvantages, such as the potential for toxic effects, the possibility for interactions with other minerals or dietary components, a low efficiency of transfer to body tissues such as milk, meat and eggs, and inorganic selenium is not stored which means it has no capacity to supply and maintain reserves of selenium in the body (Surai, 2002). Consequently, organic forms of selenium such as selenium yeasts, are becoming more popular for supplementation as they are considered to be a safer and more effective form of selenium (Mahan, 1994; Schrauzer, 2000; McCartney, 2005).

Selenium deficiency in humans

The endemic cardiomyopathy, Keshan disease, occurs mainly in premenopausal women and children from 2 to 10 years of age (Navarro-Alarcon and Lopez-Martinez, 2000) living in the low soil-selenium areas from northeast to southwest parts of China (Foster and Sumar, 1997). The resulting dietary selenium intakes in these areas have been estimated at 10 µg/d (Tapiero *et al.*, 2003). There are no specific signs or symptoms of Keshan disease, however there are four categories in which certain criteria are used for clinical assessment, acute, subacute, chronic, and latent

(Levander, 1986). In acute cases, heart function is insufficient although the heart itself appears relatively normal. Chronic cases cause moderate to severe enlargement of the heart which develops into an expanded ball shape. Chronic cases also exhibit insufficient heart function to varying degrees. In the latent form of the disease, there is mild enlargement of the heart but function returns to normal. Low selenium status is not the sole cause of this disease, although exactly what does affect it has yet to be determined. Other factors implicated in its occurrence include age, socio-economic status, seasonal variation and viral intervention (Levander, 1986; Foster and Sumar, 1997).

Kashin-Beck disease, also known as enlarged joint disease, is described as "a chronic, disabling, degenerative, generalised osteoarthrosis that involves the peripheral joints and the spine" (Levander, 1986). As with Keshan disease, Kashin-Beck is an endemic condition occurring in northern China, North Korea and eastern Siberia (Foster and Sumar, 1997). It affects children from childhood or puberty until cessation of growth (Tapiero *et al.*, 2003). The disease initially causes limb weakness, symmetrical stiffness and swelling and pain in the fingers. This develops into osteoarthritis of elbows, knees and ankles, and joint enlargement and dysfunction after 30 years of age (Foster and Sumar, 1997). In extreme cases dwarfism may result from epiphyseal impairment (Levander, 1986). As with Keshan disease, selenium is not the only factor in the aetiology of the disease. In addition to selenium deficiency, iodine deficiency, contamination of grains with mycotoxins, and water polluted with organic material and fluvic acid have been implicated in its occurrence, however evidence to support these associations is weak (Sudre and Mathieu, 2001).

Selenium and health

It is thought that low dietary selenium levels may also be associated with the origin of some human diseases and health issues such as cancer, cardiovascular disease and diabetes (Navarro-Alarcon and Lopez-Martinez, 2000). As a result of the essential role selenium has in metabolism and antioxidant defence, an inadequate selenium status decreases the maintenance of optimal health and increases susceptibility to disease. It is thought there are as many as 40 health conditions associated with selenium deficiency, however the strength of evidence to support this varies with each condition (Reilly, 1998). The association may result from low levels of selenium either contributing to the aetiology of the disease process, eg – Friedrich's ataxia (Fryer, 2002) and Keshan disease, or the low selenium status may be a result of

the condition itself which in turn worsens the development of the disease, eg – HIV; (Rayman, 2000a).

Many epidemiological cancer studies, (reviewed by Rayman, 2000a; Schrauzer, 2002; Whanger, 2004; Rayman, 2005) have been conducted over the last 30 years and evidence suggests there is an inverse relationship between selenium intake and cancer mortality. One such study, the Nutritional Cancer Prevention Trial (NCPT) which began in 1983, was carried out to determine whether daily selenium intakes of 200 µg/day of selenised yeast would decrease the incidence of cancer in subjects with non-melanoma skin cancer (Clark *et al.*, 1996). Results showed that although selenium supplementation did not prevent the recurrence of skin cancer, it did decrease the overall cancer morbidity and mortality and significantly decreased the occurrence of secondary cancers such as lung, prostate and colon cancers compared to controls. There is much interest in the use of selenium compounds for cancer prevention. Such compounds include plant-based selenium compounds such as those found in garlic, onion and broccoli; methylated compounds including selenobetaine and seleno-methylselenocysteine; monomethylated compounds including methylseleninic acid and methylselenol; synthetic organoselenium, as well as non methylated selenoproteins (Abdulah *et al.*, 2005). At normal levels of selenium intake there is a reduction in susceptibility to oxidative degeneration, however at dietary selenium concentrations above this level, ie – in the supra-nutritional range, selenium is thought to be beneficial in preventing cancer when given for a continuous period for time (Foster and Sumar, 1997). Methylselenol is thought to prevent cancer at supra-nutritional levels, however there is also evidence to suggest that selenoproteins play an anticarcinogenic role at nutritional levels by reducing oxidative stress and limiting DNA damage. Several of the selenoproteins have implicated roles in the prevention of cancer, including some of the GSHPx's, 15kDa selenoprotein (Sep15), Selenoprotein P and possibly the TRR's (Rayman, 2005). The exact mechanism of selenium's anticarcinogenic action is not yet known, however there have been several suggestions as to how it may occur. These are discussed by Whanger (2004) and Rayman (2005) and include its effects on programmed cell death, reduction of DNA damage and repair of DNA, carcinogen metabolism and the immune system, as well as its role in selenoenzymes, its specific inhibition of tumour cell growth by some selenium metabolites and its ability to inhibit angiogenesis and induce apoptosis of cancer cells. Thus although there is no evidence to suggest selenium prevents cancer *per se,* it is thought the protective effect of selenium against cancer is a consequence of its ability to enhance the immune

response and produce anti-tumourigenic metabolites (Rayman, 2000a). There is a possibility that the anticarcinogenic effects of selenium are greater in men compared to women. In a study by Waters *et al.* (2004), data from studies in seven countries in which selenium status and cancer risk in both sexes were compared directly was assessed. Results indicated a gender difference such that selenium status had a greater effect on cancer risk in men than in women and it was suggested this may be due to sex-based differences in selenium metabolism, tissue distribution and factors that influence tumour biology Waters *et al.* (2004).

There is conflicting evidence to suggest low selenium status is associated with cardiovascular disease, however it is not known whether this association is a cause or a result of the various diseases (Navarro-Alarcon and Lopez-Martinez, 2000). Studies used to determine this relationship were far from standardised and may have involved variation in several factors, such as antioxidant status, which could compromise them (Rayman, 2000a). The mechanism by which selenium deficiency is associated with cardiovascular diseases is unknown, however results that do support this link indicate a low concentration of serum selenium is associated with increased platelet aggregation, low high-density lipoprotein-cholesterol and elevated blood pressure (Foster and Sumar, 1997).

Immune function is adversely affected by selenium status at both extremes. In situations of selenium deficiency immunity is impaired. With adequate to high dietary selenium intakes immunity is boosted, and at toxic levels immunity is again suppressed. Evidence supports the essentiality of selenium for both cell-mediated and humoral immunity (McKenzie *et al.*, 2002). There are three mechanisms by which selenium exerts its immune effects: anti-inflammatory effects; alteration of cell redox state due to antioxidant action; and the production of cytostatic and anti-cancer selenium metabolites (McKenzie *et al.*, 2002).

Selenium deficiency has been associated with viruses and may influence the occurrence, virulence or progression of the infection. In particular, selenium has been shown to play a part in decreasing the effects of human immunodeficiency virus (HIV-1). HIV-1 encodes for one of the GSHPx's and as a result, as the virus replicates, the GSHPx and all its components (selenium, cysteine, glutamine and tryptophan) are depleted, thereby causing the symptoms of AIDS (Foster, 2004). Maintenance of adequate selenium levels in HIV patients increases enzymatic defence and improves general health in addition to establishing immunocompetence and redox control (Tapiero *et al.*, 2003; Sappey *et al.*, 1994). Hepatitis B and C are also influenced by

selenium status and adequate selenium levels appear to prevent progression of the virus to liver cancer (Yu *et al.*, 1997; Tu *et al.*, 2003).

Several selenoproteins are expressed in the thyroid gland including three GSHPx's, IDI, TRR and Selenoprotein P. In addition, the human thyroid contains the highest selenium content per gram of tissue of all organs (Sher, 2001). Thus, deficiency of selenium has a direct effect on thyroid function. An endemic cretinism, myxoedematous cretinism, was found in regions of Zaire in which either iodine, or combined iodine and selenium deficiencies occurred (Kohrle, 1999). The disease results from a progressive involution of the thyroid gland which leads to irreversible hypothyroidism, and when selenium is deficient, necrosis of the gland occurs progressing to fibrosis (Contempre *et al.*, 1996). It was thought that an inflammatory reaction involving macrophages and excess of transforming growth factor-β are involved in this necrotic process (Contempre *et al.*, 1996).

As outlined by Rayman *et al.* (2006), there is much evidence to suggest selenium is important to the brain, and mood has been found to be affected by the level of selenium, whereby deficiency may result in depression, hostility, anxiety and confusion, whereas high levels of selenium were found to improve mood (Rayman, 2000a). However in a more recent larger scale study conducted by these authors, selenium was found to have no effect on mood in a cohort of elderly subjects when supplemented with a selenium-yeast despite significant increases in plasma selenium Rayman *et al.* (2006). It was concluded that this was a valid finding due to the scale of the study, and that discrepancies in results compared to the other published studies were due to differences in subject age, duration of treatment period, form of supplemental selenium and different baseline selenium status.

The number of diseases and conditions associated with low selenium status has prompted consideration of fortification of foods with selenium, especially in low soil selenium areas such as Finland and New Zealand. Daily dietary selenium intakes in these areas during the 1970s were 25-60 and 28 μg/day respectively, levels at which deficiency diseases have been found to occur (Reilly, 1993). Finland introduced the addition of sodium selenate to fertilisers used for cereal production at initial concentrations of 16 ppm, and for feed and hay production at 6 ppm in 1984, however only the lower concentration has been used since 1991 due to concerns of possible toxic effects (Kantola and Vartiainen, 2001). Four years after supplementation began there were significant increases in selenium concentrations of vegetables and dietary selenium concentrations increased four fold (Reilly, 1993), to 100-125 μg/day (Kantola

and Vartiainen, 2001). An increase in selenium concentrations was also seen in human breast milk as a result of the supplementation (Kantola and Vartiainen, 2001). In New Zealand an effort was made to improve selenium status by top dressing with added selenium, drenching and supplementing animal feeds. Selenium status was also improved at this time as a result of the importation of wheat and vegetable products from Australia, and changing dietary habits involving an increase in vegetarianism, increased consumption of fish (high selenium content) and poultry (selenium supplemented) (Thomson and Robinson, 1996).

Although there is a degree of reluctance to introduce fortification of foods with selenium due to safety concerns and potential technological problems associated with processing, there are already some specialised selenium-fortified foods on the market including selenium-enriched infant formulations and sports foods (Reilly, 1998). There is also research available regarding the enhancement of the selenium content in animal produce including milk, eggs, poultry, beef and pork (McCartney, 2005). Milk enhanced with modified amounts of nutrients such as calcium, selenium iron, iodine, vitamin B_{12} and folate can now be produced using on-farm methods (Knowles *et al.*, 2006). In Asia selenium fortification of foods such as selenium-rich green tea, said to help prevent heart disease and ageing, are promoted, along with other foods such as garlic and selenium-rich nuts (Reilly, 1998). Garlic, onion, broccoli and wild leek are selenium accumulator plants (Arnault and Auger, 2006) and therefore have the ability to take up selenium from the soil and produce selenoamino acids. As a primary accumulator, garlic can produce selenium at concentrations greater than 1000 ppm in seleniferous conditions. The health benefits of garlic and onion result from a combination of sulphur, flavanols and selenocompounds. Although there are many seleno compounds in these plants still to be determined, two that have been identified, Se-methyl selenocysteine and γ-glutamyl-Se-methyl selenocysteine, are known to have a role in cancer prevention (Arnault and Auger, 2006). Selenium-enriched garlic has been found to have protective effects against several cancers and cardiovascular disease by decreasing serum cholesterol concentrations, blood pressure and inhibiting platelet aggregation (Arnault and Auger, 2006).

Many dietary components can influence health via genetic means, whereby one, or a combination of processes, such as carcinogen metabolism, hormonal balance, cell signalling, cell cycle control, apoptosis and angiogeneis result in a phenotypic change (Trujillo *et al.*, 2006). Nutrigenomics is defined as "the interaction between nutrition and an individual's genome, or the response of an individual to different diets" and

uses techniques including genetics, microarrays, proteomics and metabolomics (Davis and Milner, 2004). For example, microarray analysis has been used to identify potential molecular targets of selenium (Davis and Milner, 2004). Over 2500 genes responded to selenium treatment in human prostate cancer cells and these were categorised into clusters according to the pattern of kinetics resulting from the way in which they were modulated by methylselenic acid (Dong *et al.*, 2003). The clusters included growth factors, protein synthesis, tumour suppressor/growth inhibitor, signal transduction, cytoskeleton, adhesion/invasion, DNA repair, transcription factor, angiogenesis, apoptosis and cell cycle. This information was then used to develop an integrated scheme of signalling pathways that might explain the action of selenium in blocking cell cycle progression (Dong *et al.*, 2003). Thus, nutrigenomics will become key in areas such as the determination of nutrient requirements, disease prevention and treatment and the testing of functional ingredients. However the ultimate goal of nutrigenomics is to develop foods that can be matched to individual genotypes to enhance health and prevent disease (Trujillo *et al.*, 2006).

ESTABLISHING SELENIUM REQUIREMENTS

Defining requirements

In the past, selenium requirements were established by determining the level of selenium at which deficiency diseases such as Keshan disease were prevented. These values were then considered the minimum amount of selenium required to prevent deficiency. However, providing the lowest level of a nutrient that is necessary does not allow a margin of error for factors that may negatively affect the nutrient status of the animal, and may potentially compromise the animal's health. Therefore, the recommendation of a minimum level as a requirement for dietary intake of a nutrient is not ideal. Estimates of optimum levels of a nutrient should be the basis for determining dietary requirements.

Within the correct range of observed dietary intakes (i.e. the nutritional range) the biological functions of selenium increase and the production of harmful metabolites is minimised (Combs, 1988). It is within this range that the beneficial effects of selenium's actions occur. However there appears to be further health benefits when supranutritional levels of selenium are ingested. At these levels, the activity of the functional selenoenzymes plateau and production of selenium metabolites begins to increase, but in both humans and animals there is evidence to suggest that the anti-cancer and immune enhancement effects of selenium are further promoted (Combs, 1988; Thomson, 2004).

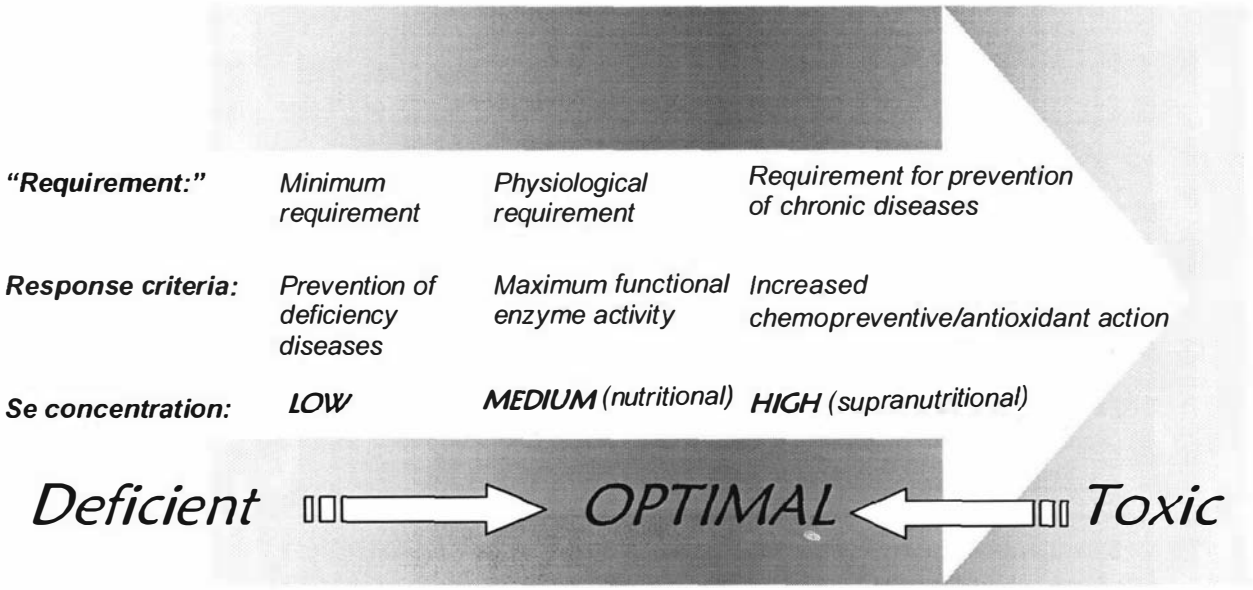


Figure 4. Criteria for defining selenium requirements

As dietary selenium concentrations increase, the production of selenium metabolites also increases, eventually resulting in toxic effects as cells are destroyed. In order for the dietary requirement of selenium to provide maximum health benefits, it would seem that recommended concentrations of dietary selenium intake should fall somewhere between nutritional and supranutritional levels (Figure 4). Although there is a need to generate further evidence to substantiate claims for these increased benefits at higher levels of dietary selenium, data published in the literature suggest recommendations should be set somewhat higher than the current minimum requirements as is the current practice.

Factors affecting selenium reqirements

Several factors influence an animal's need for dietary selenium. These may be inherent to the animal itself, from the diet it is ingesting, and/or from interactions that may occur between the animal and its diet (Figure 5).

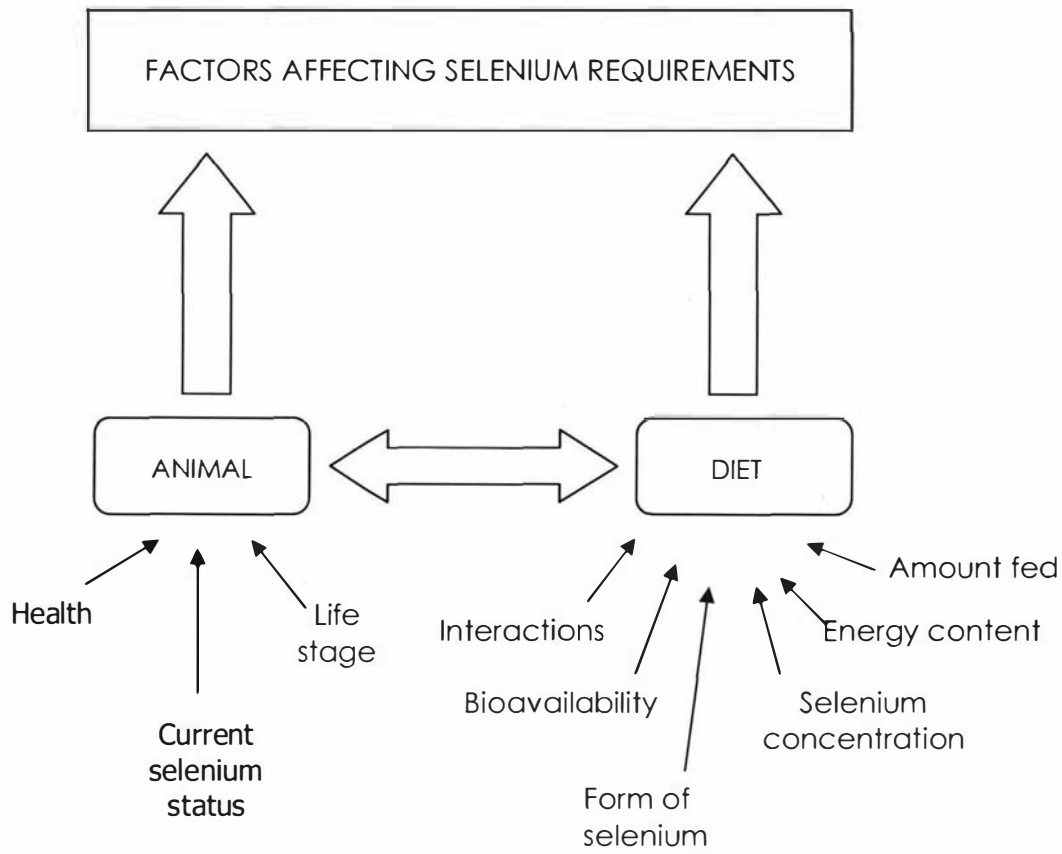


Figure 5. Factors affecting selenium requirements

The amount of selenium required by an animal at any one time is determined by the current selenium status of that animal. This in turn is influenced by its life stage (newborn, growing, adult, pregnant/lactating, geriatric, etc) and health status. Selenium concentrations in the diets themselves are also a factor in the amount of selenium an animal requires. These concentrations may or may not be adequate depending on the amount of that particular food the animal is fed. In order for animals to maintain a healthy weight they are fed according to energy requirements. However if they are fed more or less of a diet with a particular metabolisable energy (ME) content and the selenium is contained within this, they will then require less or more selenium respectively, to compensate for the difference in selenium actually consumed.

Bioavailability

The bioavailability of a nutrient must be taken into consideration when determining its requirement and is crucial to the animal obtaining adequate amounts of that nutrient. The actual concentration of selenium in the diet may be sufficient, however if the diet has a low digestibility or the form of selenium in the diet has a poor bioavailability, the animal will not be able to utilise the whole amount and therefore requirements may not be met.

In general terms, nutrient bioavailability is defined as "a quantitative measure of the utilisation of a nutrient under specified conditions to support the organisms normal structure and physiological processes" (Levander, 1983). There are several factors that may affect selenium bioavailability and these have been outlined by Young *et al.* (1982) and reviewed by Combs and Combs (1986a). Some of these factors include the presence of heavy metals in the diet such as arsenic and mercury, which interact with selenium to change its structure and render it unable to be incorporated into selenoproteins or selenium-containing proteins, thereby reducing its bioavailability (Hill, 1975; Ganther, 1980). High dietary protein appears to decrease bioavailability in some situations (Henry and Ammerman, 1995). The use of heat in processes such as canning and extrusion of petfoods increases shelf life, but decreases the nutritive value of the diet (National Research Council, 1986) and may also effect bioavailability of the nutrient (Young *et al.*, 1982). In addition, bioavailability is largely influenced by the form of selenium present in the diet, as different forms are absorbed, metabolised and utilised by the animal in different ways (Rayman, 2000b).

Estimates of bioavailability may be obtained using different bioassay methods – the preventative approach, such as the prevention of exudative diathesis (Cantor *et al.*, 1975a) and prevention of pancreatic fibrosis (Cantor *et al.*, 1975b); the tissue residual level approach; and the functional assay approach (Combs and Combs, 1986a) involving the use of an enzyme such as GSHPx. There may be discrepancies between results from these methods, as selenium does not exist entirely as the element 'Se', but rather a diverse range of compounds that fill a variety of structural and enzymic roles. The diverse nature of selenium creates a problem when trying to decide upon the most suitable response criteria and parameter(s) to use to determine requirements. Hence estimates should be used with care and kept in context (Young *et al.*, 1982).

Interactions

Interactions between the nutrient status of the animal and that of the diet may also affect selenium requirements. For example, vitamin E and selenium are closely related and have complementary antioxidant roles. There is a mutual sparing effect between the two to prevent deficiency diseases (Maylin *et al.*, 1980), hence a low vitamin E concentration in the animal requires a higher selenium concentration in the diet in order to maintain antioxidant status. Some drugs have also been found to inhibit the action of selenoenzymes such as GSHPx and TRR (Thomson, 2004) thereby creating a greater need for selenium.

Determining selenium status

In order to assess how much selenium an animal needs, one must first be able to accurately assess selenium status. Due to the varied nature of selenium's form and function there are a number of biological parameters used to assess selenium status. These indicators, solely or in combination, are used to assess different aspects of selenium metabolism, as each is representative of a different aspect of selenium metabolism.

Indicators of selenium status are based on the determination of selenium in tissues and biological fluids, and the measurement of selenium-dependent biochemical and functional indices. The most commonly utilised parameters include plasma selenium and GSHPx activity in various tissues. Plasma selenium is an indicator of short-term selenium status and rapidly reflects dietary intake. GSHPx is a

selenoenzyme with redox actions and is used as a measure of functional selenium status. Other parameters used to assess selenium status are summarised in Table 6.

There is much information in the literature regarding the use of these parameters and their applications in humans (Levander, 1985; Neve, 1991; Thomson, 2004) and other animals (Ullrey, 1987). In some situations, clinical symptoms and physiological functions that are influenced by selenium status are used to assess the pathological consequences of selenium (Neve, 1991). However these criteria are highly variable and are dependant on the individual, so they are of more use in situations of extreme selenium exposure and less likely to be of use when defining optimum levels.

Table 6. Parameters used to indicate selenium status in humans and animals

	Parameter	Application	Limitations
Selenium concentration in tissues and biological fluids	Plasma/serum selenium	Short term status	May not reflect body stores at high concentrations
	Whole blood/erythrocyte selenium	Longer term status (weeks)	Doesn't show daily fluctuations
	Nails, hair	Longer term status (months)	
	Urinary selenium	Reflects dietary intake	Useful up to moderate Se intakes - plateaus at high intakes
	Tissues/organs	Reflects Se in body stores	Can be highly variable, may not reflect functional Se
Functional selenium	GSHPx's	Reflects functional Se status at low – moderate levels	Plateaus at higher levels
	Selenoprotein P TRR	Less commonly used selenoproteins	
	T4:T3	ID activities	

SELENIUM AND COMPANION ANIMALS

The importance of nutritionally balanced petfoods should not be underestimated. Worldwide there are 258.1 million cats and 350.7 million dogs owned as pets (Global Market Information Database) and these species are the most popular companion animals in the world (Hendriks, 1999). In New Zealand 36% of households have dogs and 53% have cats (Global Market Information Database) and reasons for owning a cat or dog are varied. As "companions" these animals are not only considered pets, but also members of the family, friends, and even substitute children (Baker and Czarnecki-Maulden, 1991). Cats are primarily owned for their companionship but are also used for breeding and showing. Companionship is the main reason people own dogs, however alongside that, dogs offer protection and have the potential to be trained for specific tasks which provide a service to the community. Such "jobs" include aid to the blind and deaf, use in search and rescue and agriculture, messengers in war, detection of bombs, criminals, drugs and other banned substances (Baker and Czarnecki-Maulden, 1991). Pets may also be used for therapy with emotionally disturbed children, criminally insane prisoners and the sick and elderly in hospitals or nursing homes (Baker and Czarnecki-Maulden, 1991). There are proven health and psychological effects of owning a pet such as decreased loneliness, increased self esteem, increased interaction with others and the development of assertiveness. Thus cats and dogs are of great importance in society and as a result pet owners generally want the best for their animals. With regard to selenium, public demand for the provision of optimal dietary levels of this nutrient in petfoods is fuelled by the popularity of selenium as a health-promoting agent in human diets. It is the antioxidant action of the mineral that is promoted to the general public and makes it an attractive option for inclusion into foods and supplements.

The global market for petfood continues to expand with the increase in pet ownership (Phillips, 2004) and the consumers demand for safety, nutritional adequacy and health promoting effects of the products (Zentek, 2004). It is often expected by pet owners that if health benefits from dietary nutrients can be achieved in humans, the same should be possible for their animals. This creates a demand for "complete and balanced" petfoods that provide not only optimal nutrition but also maximise the animals' health and well-being. For those that formulate petfood diets, this requires knowledge of the requirement an animal has for specific nutrients, and to facilitate this, an understanding of that nutrients' metabolism is needed. Herein lies the

difficulty with selenium, as there is very little information available in the literature specific to companion animals to indicate what these levels might be.

Published data specific to companion animals

Available information regarding selenium metabolism and the role of selenium in health and disease in cats and dogs is limited. Publications date back to the 1930s and these early reports were primarily concerned with naturally occurring or experimentally induced effects of toxicity. Work that followed was sporadic but provided some insight into the various aspects of selenium metabolism. More recent work has focussed on the selenium requirements of cats and dogs, as well as the bioavailability of selenium in petfoods.

Metabolism of selenium in cats and dogs and its distribution in tissues

Absorption of selenium has been investigated in dogs but no information is available regarding cats. Reasbeck *et al.* (1985) performed a gut-perfusion experiment in adult dogs. These authors found that absorption of selenomethionine was significantly greater than that of selenocysteine, whilst selenite absorption was the slowest of the three forms to be absorbed. Approximately 90% of selenium was absorbed as selenite in adult dogs (Furchner *et al.*, 1975).

There have been several studies in dogs and cats that illustrate tissue distribution of selenium. Using radiolabelled selenium (^{75}selenomethionine), Meinhold *et al.* (1975) found significant amounts of ^{75}selenomethionine in the pancreas, liver and kidney of an adult and a growing dog. Boyer *et al.* (1978) fed kittens a diet of commercially canned red tuna, assumed to contain high levels of selenium, (Furr *et al.*, 1976; National Research Council, 1986) for 100 days. The level of selenium in various tissues of the tuna-fed kittens were compared with those of kittens fed a control diet. On average, selenium levels in the brain increased 1.5-fold and in muscle 3-fold, while in blood, bone, kidney and spleen selenium increased by at least 6-fold. The greatest increase in selenium content, of almost 9-fold, was seen in the liver.

Blood selenium levels of control kittens in Boyers' study were the same as those reported in a summary of diagnostic data by Puls (1988) for adequate levels of whole blood selenium in cats. Similarly, levels stated as high by Puls (1988) are the same as those seen in the tuna-fed kittens by Boyer *et al.* (1978) indicating that the data from Puls (1988) may have been obtained from this study. Puls (1988) also gave ranges for

adequate and high levels of selenium in the liver and kidney of cats (ppm, wet weight): liver – adequate: 0.26 to 0.54, high: 2.00 to 4.60; kidney – adequate: 0.77 to 1.14, high: 4.20 to 9.40. However the method of derivation of these estimates was not stated.

Smith *et al.* (1937) studied chronic selenium poisoning in adult cats given 0.1 or 0.25 mg Se/kg from sodium selenite. These authors found a wide distribution of selenium throughout the body tissues, with the highest concentrations in the liver, kidney, spleen, pancreas, heart and lungs. Erythrocytes were found to contain more selenium than plasma. In another study, increased levels of selenium were found in the lungs, kidneys, liver, blood, spleen and heart of adult beagle dogs following inhalation of selenious acid (Weissman *et al.*, 1983). Again, large amounts of selenium were found in the liver, and its significance as a major site for metabolism was suggested. Hepatic liver selenium concentrations in dogs ranged from approximately 1.25 to 3.25 µg Se/g and these levels were found to decrease with age (Keen and Fisher, 1981).

Urinary and faecal excretion of selenium in adult cats was studied by Smith *et al.* (1937). They administered 0.1 to 0.25 mg Se/kg from sodium selenite to cats orally or subcutaneously for up to 188 days. Fifty to 80% of the total intake of selenium was usually excreted in the urine, and from trace levels up to 18% was excreted in faeces. More selenium was excreted in the faeces when it was given orally than when given subcutaneously. A relationship was found between selenium concentration in the urine and the daily dose administered in chronic selenium poisoning (Smith *et al.*, 1937). To determine retention of selenium in the body following chronic selenium poisoning, adult cats were given 0.1 mg Se/kg from sodium selenite per day over a period from 168 to 175 days. Most of the stored selenium was eliminated within two weeks after administration ceased. Small amounts of selenium were found in the urine and some other tissues, especially the liver, for at least a month (Smith *et al.*, 1937).

Selenium in health and disease

Reported signs of toxicity in dogs include refusal of food leading to weight loss, anorexia and stunted growth, nausea and vomiting, diarrhoea, apprehension, respiratory stimulation and cardiovascular changes (Anderson and Moxon, 1942; Rhian and Moxon, 1943; Heinrich and MacCanon, 1957). In more severe cases, nervous disorders and pathological lesions may develop with the liver and spleen being the most affected organs (Rhian and Moxon, 1943), and in extreme cases death may occur

(Anderson and Moxon, 1942). Rhian and Moxon (1943) found that signs of toxicity including weight loss occurred when 7.2 or 10 ppm of selenium was added as sodium selenite to the diet of growing and adult dogs. Sodium selenite given at 20 ppm caused death in a very short time. The minimum lethal dose of sodium selenite administered by intramuscular injection for dogs is 2 mg Se/kg BW (National Research Council, 1976).

Studies in anaesthetised and unanaesthetised adult dogs found that a combination of pentobarbitone and oxygen, as well as oxygen alone, increased the toxicity of selenite (Anderson and Moxon, 1942; Heinrich and MacCanon, 1957). Doses of selenite between 0.1 and 1 mg Se/kg were usually fatal to anesthetised dogs breathing oxygen within seven hours of administration, whereas the same doses were not fatal to dogs breathing room air (Heinrich and MacCanon, 1957). The minimum lethal dose for dogs under barbital depression was found to be between 1.5 and 2 mg Se/kg (Anderson and Moxon, 1942). Consequently oxygen appears to cause increased sensitivity to selenium toxicity in anesthetised dogs (Heinrich and MacCanon, 1957). Conversely, pentobarbital anaesthesia may offer some protection. Heinrich and MacCanon (1957) suggested that central nervous stimulation is responsible for the symptoms of selenium toxicity, and that this central stimulation may be antagonized by pentobarbitone.

Selenium interacts with other substances in the diet such as vitamin E, sulphur amino acids and heavy metals, and in some cases the effects of selenium toxicity are counteracted. When growing dogs were fed 13 ppm selenium, 5 ppm arsenic added to drinking water counteracted or prevented the symptoms of chronic selenium poisoning (Rhian and Moxon, 1943).

In published diagnostic data (Puls, 1988), the minimum lethal dose of selenium for cats is reported to be 1.5 to 3.0 mg Se/kg BW, regardless of route of administration. No references are provided to indicate where these estimates were obtained.

Clinical signs of selenium deficiency in growing dogs include muscular weakness, subcutaneous oedema, anorexia, depression, dyspnea and eventual coma (Van Vleet, 1975). The author also found pathological signs which included extensive muscular degeneration, necrosis in the myocardium and renal mineralisation. Similar lesions have been reported in growing and adult dogs with selenium deficiency (Kaspar and Lombard, 1693; Manktelow, 1963; Van Rensburg and Venning, 1979). The lesions resembled those seen in lambs with white muscle disease (Van Rensburg and Venning,

1979). There have been no reports of selenium deficiency in cats, however Dennis (1982) reported a case of nutritional myopathy in a cat which was primarily attributed to vitamin E deficiency. At the time there was no data available for selenium requirements in cats and it is possible this was a case of white muscle disease associated with selenium deficiency.

Much research has been conducted on the anti-tumourigenic activities of selenium and the use of established cell lines from dogs provides a good model to study selenium-induced inhibition of tumour growth (Fico *et al.*, 1986). A recent study used dogs as a model to look at the effect of selenium supplementation on the occurrence of prostate cancer (Waters *et al.*, 2005). Dogs aged at the physiological equivalent of 62 to 69 year old men were supplemented with selenomethionine or selenium yeast at concentrations of 3 or 6 µg Se/kg on a daily basis for seven months. Their results showed a U-shaped dose response relationship between selenium status as indicated by toenail selenium, and the extent of DNA damage within the prostate. It was also found that the amount of selenium required to minimise DNA damage in the dog paralleled that in men, thus it was concluded that the dog was a good model for prostate cancer in men (Waters *et al.*, 2005).

Hyperthyroidism is a common endocrine disease in cats and it is suspected that selenium plays an important role in homeostasis of the thyroid gland. Foster *et al.* (2001) conducted a study to assess the selenium status of cats in four regions of the world (Edinburgh, Sydney, Denmark and Perth) and compared this with reports of hyperthyroidism in cats from those regions. Plasma selenium concentrations were found to range from 3.95 to 8.70 mmol/l in cats from the four areas. There was no significant difference in the plasma selenium levels of the cats between the four different regions and the authors concluded that selenium status alone did not affect the incidence of hyperthyroidism in cats.

Conversely, hypothyroidism is a common disease in dogs. Low selenium intake impairs the activity of iodothyronine 5'deiodinase which catalyses the deiodination of thyroid hormones. Wedekind *et al.* (2001) studied the effect of varying selenium intake on thyroid hormone metabolism in adult dogs. Dogs depleted of selenium were supplemented with different levels of selenomethionine. The authors measured selenium concentrations and GSHPx activities in serum and red blood cells as well as conducting complete thyroid profiles and antithyroid activities. Thyroid levels were normal, but T_3 increased in accordance with selenium intake and anti-thyroid

antibodies showed no changes related to selenium intake. Their results suggested that selenium deficiency was not a major factor in the aetiology of canine hypothyroidism.

The effect of selenium on immunity in puppies has also been recently studied. Two separate studies have looked at the effect of feeding selenium and vitamin E on the immune function of healthy puppies after vaccination for parvovirus, canine distemper and Taenia hydatigena. Both studies showed the antioxidants were effective immunostimulators and could be used to increase the immune response of puppies (Michalkova *et al.*, 2004; Kandil and Abou-Zeina, 2005).

Comparative nutrition of cats and dogs

As members of the order Carnivora, domestic cats and dogs are often thought of as meat-eaters that have the same nutritional requirements, however cats and dogs are separate species with very different nutritional requirements. The superfamily Feloidea, of which the cat belongs to, are strict carnivores, requiring animal flesh to satisfy their specialised dietary requirements. In contrast, the dog belongs to the diverse Canoidea superfamily which includes not only carnivores, but also herbivores and omnivores. As an omnivorous carnivore, the dog is able to use a variety of plant and animal sources in its diet. Thus, cats and dogs have developed different metabolic adaptations resulting from their evolution on different diets and therefore have different nutritional requirements.

The dependency of cats on a meat-based diet is reflected in their metabolic adaptations. Cats require higher levels of protein compared to dogs as they are unable to down-regulate the catabolic enzymes that metabolise nitrogen (Lowe and Markwell, 1995). Arginine is strictly essential for cats and conditionally essential for dogs, and its absence in the diet results in ammonia toxicity. In cats the requirement for arginine results from a low activity of two enzymes involved in its synthesis (Morris, 2002). Taurine is also an essential amino acid for cats. This is due to the inability of the cat to synthesise sufficient amounts of taurine, coupled with a high metabolic demand for this amino acid due to the use of taurine for conjugation of bile acids. In contrast, dogs are able to synthesise enough taurine to meet their requirements. A dietary source of arachidonic acid is also required by cats as they lack sufficient activity of the enzyme desaturase to convert its precursor, linoleic acid (Lowe and Markwell, 1995). Another essential nutrient for the cat is preformed vitamin A as it is unable to convert ß–carotene to the active vitamin (Lowe and Markwell, 1995). Similarly, niacin cannot be effectively sourced from tryptophan by the cat in the usual way as the intermediate

between the two is utilised by an alternative metabolic pathway. As a result, niacin is not formed in amounts that are sufficient to satisfy the dietary requirements of the cat. In addition, both cats and dogs are unable to synthesise adequate levels of active vitamin D (Morris, 2002) and therefore have a requirement for this vitamin in their diet. Thus there are several nutritional idiosyncrasies of the cat and dog, and these have been reviewed in detail by Morris and colleagues (MacDonald and Rogers, 1984; Morris and Rogers, 1989; Morris, 2002)

Although little is known about the metabolism of selenium in cats and dogs, the species specific metabolic adaptations resulting from their evolution on different diets raises the possibility that there are differences in the metabolism of this mineral. This may have implications for their respective dietary requirements.

Recommendations for the selenium requirements of cats and dogs

The National Research Council (NRC) and the Association of American Feed Control Officials (AAFCO) publish recommendations for the dietary nutrient content of foods for cats and dogs. The NRC provides guidelines for the minimum dietary content of nutrients for growing and adult cats and dogs (National Research Council, 1985; 1986). In these publications, recommendations are expressed as minimum dietary requirements based on information available in the literature at the time of publication. Their recommendations are based on studies that have used highly digestible and bioavailable sources of nutrients and therefore do not take into consideration nutrient bioavailability in typical commercially available petfoods. In addition, recommendations are only available for growth and maintenance and do not account for other life stages or other physiological states. Finally the recommendations made by the NRC do not take into account interactions between dietary ingredients or the heat treatments often required in petfood manufacture.

AAFCO have attempted to expand and improve the NRC recommendations. Using the current NRC requirements as a base, modifications were made according to subsequent knowledge and published information. AAFCO dietary requirement estimates take into account the potential for lower bioavailabilities due to the use of more practical dietary ingredients, instead of the highly purified ingredients used in the studies to determine minimum requirements. In addition AAFCO (2000) provide not only minimum but also maximum dietary requirements for the life stages of growth, maintenance and gestation/lactation.

With regard to the selenium requirements of companion animals, recommendations for dogs are based on a limited number of deficiency studies conducted in dogs. Due to the absence of information on cats, recommendations for these animals are based on data obtained from other species (Table 7).

Table 7. AAFCO and NRC recommended dietary selenium requirements for cats and dogs[1]

	Minimum levels				Maximum levels	
	Growth/reproduction		Adult maintenance			
	Cats	Dogs	Cats	Dogs	Cats	Dogs
NRC - *mg/kg DM*[b]	No data	0.11	No data	No data	No data	No data
µg/kg BW		6		2.2		
AAFCO *mg/kg DM*	0.1	0.11	0.1	0.11	No data	2

[1]*taken from the NRC (1985; 1986) and AAFCO (2000)*

Although both NRC and AAFCO provide estimates of the levels of selenium required by cats and dogs, the values are an approximation of minimum requirements and may not be suitable for the provision of optimal health. The requirements are based on data that are not always species specific, and in addition, there are many gaps that need to be filled relating to the animal in different circumstances. The NRC has recently conducted a thorough review of the literature to more accurately define the nutrient requirements of cats and dogs. The new report, (unpublished at the time of writing), combines the requirements of both cats and dogs in one document. Unlike the previous recommendations, the new publication will account for bioavailability and include safe upper levels. However with regard to selenium requirements, there still remains a shortage of information as much of this data is not specific. Requirements for both cats and dogs are based on levels estimated to be required for kittens and puppies and adjusted for caloric intake. A bioavailability factor, determined from a chick bioassay, was then applied to this value to give the final estimate (see discussion and Table 8 below). Although an improvement on the 1985 and 1986 recommendations, data from the new publication are still largely estimations.

Current work on selenium requirements

A considerable amount of work has been conducted in the last 10 years by Wedekind and colleagues looking at the bioavailability of selenium in petfoods and the requirements of selenium for cats and dogs. Unfortunately much of this work has not been published, or is only available in abstract form. These authors developed a chick bioassay in which to determine the bioavailability of selenium in petfoods and petfood ingredients (Wedekind *et al.*, 1997; 1998). Chicks were fed a selenium-free, purified diet in which the diet or dietary ingredient of interest was included as the only selenium source. Response parameters in chicks fed the test diets were compared with those fed diets containing a known amount of sodium selenite. Results from their work showed low selenium bioavailabilities relative to the amounts of sodium selenite in petfoods and petfood ingredients (Table 8).

Table 8. Bioavailability of selenium in petfoods and petfood ingredients[1]

	Bioavailability
Canned cat diets	17%
Canned dog diets	25%
Dry dog diets	21%
Animal-derived ingredients	28%
Plant-derived ingredients	47%
Canned diets	30%
Extruded diets	53%

[1]*taken from Wedekind et al. (1997; 1998)*

The same research team also conducted requirement studies in cats and dogs and applied these bioavailability factors to the results to create more accurate dietary recommendations. In these studies, as with the chick bioassays, animals were fed a low selenium purified diet, which was supplemented with various concentrations of selenium in the form of sodium selenite or selenomethionine. A breakpoint analysis was applied to the response variable measured to determine the minimum dietary requirement. Their results are summarised in Table 9.

Table 9. Estimates of selenium requirements for companion animals when bioavailability is accounted for[1]

Animal	Response criteria	Break-point (mg/kg)	Applied bioavailability factor	Form of Se supplemented	Recommendation (mg Se/kg DM)
Kittens	Serum GSHPx	0.12	30%	Sodium selenite	0.4
Kittens	Plasma GSHPx	0.15	30%	Sodium selenite	0.5
Adult cats	Serum Se Serum GSHPx RBC GSHPx	0.10	-	Seleno-methionine	0.1
Puppies	Serum Se	0.06	30%	Sodium selenite	0.2
Puppies	Serum Se (Serum GSHPx)	0.21 (0.08–0.13)	-	Sodium selenite	Not definitive
Adult dogs	Serum Se, Serum GSHPx, Erythrocyte GSHPx	0.13	30%	Seleno-methionine	0.43

[1]*taken from Wedekind et al. (1999; 2000; 2002; 2003a; 2003b; 2004)*

Although these chick bioassays provide some indication of the bioavailability of selenium in petfoods and petfood ingredients, the assays are not species-specific and assume that cats and dogs will respond in the same manner as chickens. In addition, as in the requirement studies, the animals were fed a synthetic diet and were in a selenium deficient state. Ideally diets would be comprised of ingredients that would normally occur in a petfood in order to be representative of a more typical situation. Requirements also need to be accurately determined for the different life stages of the animal, as the requirement for kittens or puppies and gestating and lactating females are likely to be different to those of adult cats or dogs.

The most recent study by these authors looked at the effect of dietary selenium concentrations on primary hair growth in dogs as an indicator of selenium requirements. Concentrations of serum selenium and thyroid hormones were also

analysed. Dogs were fed varied concentrations of selenomethionine for 24 weeks and results showed both low (0.04 and 0.09 mg Se/kg) and high (5.04 mg Se/kg) concentrations of selenium decreased hair growth. They concluded from these parameters that dietary selenium concentrations of less than 0.12 mg Se/kg were marginal for a dog (Yu *et al.*, 2006).

OUTLINE OF THESIS

The importance of selenium in mammalian metabolism is reflected in the many biological functions of selenoproteins and is emphasised by the number of diseases and conditions associated with poor selenium status. Because the beneficial effects of selenium are seen within such a narrow range, it is imperative to establish the requirements of this mineral for a given species. The current knowledge of selenium requirements for cats and dogs is limited and an understanding of selenium metabolism in these species is required before dietary recommendations can be made.

The overall aim of this PhD was:

.....to provide fundamental information regarding aspects of selenium metabolism in cats in dogs, including faecal and urinary excretion and estimates of apparent absorption and retention, when supplemented with different levels of inorganic and organic selenium.

In the first study, dietary selenium concentrations in commercially available cat and dog foods in New Zealand were investigated to determine the range of dietary selenium concentrations currently available to these animals, and to ascertain how these levels compared with current dietary recommendations.

In order to determine whether dietary selenium levels are adequate at a given concentration, a means of assessing the selenium status of the animal is required. There was a need to understand the metabolism of the different forms of selenium and the interaction and responses of various biological indicators of selenium status to different circumstances. The second study investigated the metabolism of inorganic and organic selenium in adult cats. Part i determined various indicators of selenium status in response to graded levels of dietary selenium supplements, while in Part ii, faecal and urinary excretion of selenium was examined and used to approximate the degree of absorption and retention of the two forms of selenium at the different levels of supplementation.

Due to their role as companion animals in our society, cats and dogs are often considered equal with similar nutritional requirements when in fact they are different species with correspondingly different dietary requirements. Whether or not there are any differences in selenium metabolism between these animals has yet to be determined, although there is some evidence that cats can tolerate high levels of

selenium without adverse effects. The level at which toxic effects occur in cats and dogs has also yet to be clarified and has implications for the establishment of dietary requirements at a level which promotes optimum health without adverse effects. The response of cats and dogs to high levels of inorganic and organic selenium was compared in the third study.

The level at which selenium occurs in a petfood is not necessarily the amount of selenium that is available to the animal. There are a number of factors that affect the bioavailability of a nutrient and this again has implications for dietary requirements. If a diet contains the recommended amount of selenium but has a low bioavailability the animal is unable to utilise the total amount of selenium in the diet and therefore receives an intake that is less than adequate. Moist and semi-moist commercial petfoods undergo heat processing to prolong shelf life and this has been shown to decrease the overall nutritive value of the diet. In the fourth study the effect of heat processing on supplemental selenium was investigated by determining whether absorption and retention of inorganic and organic selenium was affected in cats when selenium is added before and after processing.

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CHAPTER 2

Total selenium concentrations in canine and feline petfoods commercially available in New Zealand

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ABSTRACT

The aim of this study was to determine the total selenium concentrations in petfoods that are commercially available in New Zealand and to establish whether these concentrations meet the current minimum recommended requirements of selenium in petfoods. Samples (n=89) from petfoods commercially available in New Zealand were analysed for total selenium concentrations using a fluorometric method. Data, expressed on a DM basis, were analysed according to petfood type (dog or cat, and wet or dry), predominant flavour (chicken, seafood, chicken and seafood, beef, meat mix, other), manufacturer and country of manufacture. Fifty percent of petfoods purchased for this study were manufactured in Australia, and the remainder produced in the United States of America (USA), New Zealand or Thailand. Mean total selenium concentrations were similar (0.61 to 0.80 µg Se/g DM) in petfoods produced in Australia, New Zealand and the USA, but higher (mean 3.77 µg Se/g DM; $p < 0.05$) in petfoods produced in Thailand. Petfoods produced in Australia, New Zealand and the USA contained a variety of predominant flavours, whereas petfoods from Thailand contained only seafood flavour. Seafood-based flavours had the highest selenium concentrations in both cat and dog foods. Wet and dry dog foods had a similar concentration of selenium to dry cat foods, but wet cat foods had higher and more variable concentrations of selenium than the others ($p < 0.05$). The mean selenium concentrations in cat and dog foods were 1.14 and 0.40 µg Se/g DM, respectively, and there were no significant differences between manufacturers. Selenium concentrations in petfoods sold in New Zealand appeared to meet recommended dietary requirements, although the range of concentrations was highly variable. Whether these recommendations are adequate for the maintenance of optimal health in cats and dogs has yet to be determined.

INTRODUCTION

The role of selenium in health and nutrition has been well documented in many species including humans and livestock (Koller and Exon, 1986; Foster and Sumar, 1997; Underwood and Suttle, 1999). As a trace element, dietary concentrations of selenium required to maintain good health must remain within a relatively narrow optimum range, the importance of which is reflected in the deleterious effects of too

little selenium, manifesting in deficiency diseases such as Keshan disease (an endemic cardiomyopathy), or the toxic effects of selenosis that result from too much selenium.

Research in humans has shown that, within an optimum range, selenium has many vital roles in maintaining health and wellbeing throughout the life cycle. It is essential for proper functioning of the reproductive system, pregnancy and lactation, and early growth and development of the newborn (Rayman, 2000). Selenium is also required for the normal functioning of thyroid hormones (Holben and Smith, 1999), as the selenoprotein ID regulates the conversion of T_4 to T_3. There is evidence that selenium has an effect on brain function (Rayman, 2000) and mood. Low selenium concentrations are associated with depression and hostility, and improvement in mood has been reported following selenium supplementation (Finley and Penland, 1998). Adequate dietary selenium concentrations are required for optimum immune function (Koller and Exon, 1986; Rayman, 2000). In addition, selenium appears to have a protective effect against progression of some viral diseases in humans (Schrauzer, 2002).

Perhaps the most well known effects of selenium are its anti-carcinogenic actions, which are likely to result from antioxidant, anti-inflammatory and immunostimulant effects (Spallholz, 2003). Increased dietary selenium has been associated with reduced incidence of several types of cancer (Combs and Combs, 1986; Rayman, 2000; Schrauzer, 2002; Stratton *et al.*, 2003; Karunasinghe *et al.*, 2004) and such anti-cancer effects continue to be the subject of intensive research. Several long-term studies are underway to further knowledge in this field (Stratton *et al.*, 2003) and due to the many health-related benefits of dietary selenium in humans, interest in the role of this trace element in nutrition has increased recently.

There is little information available in the literature regarding the role of selenium in companion animals, especially cats. A limited amount of work has been published on various aspects of its metabolism in cats and dogs (Smith *et al.*, 1937; Furchner *et al.*, 1975; Meinhold *et al.*, 1975; Boyer *et al.*, 1978; Keen and Fisher, 1981; Weissman *et al.*, 1983; Reasbeck *et al.*, 1985), and the outcome of extreme levels of selenium intake has been documented in studies investigating toxicity and deficiency in dogs (Anderson and Moxon, 1942; Rhian and Moxon, 1943; Heinrich and MacCanon, 1957; Manktelow, 1963; Van Vleet, 1975; Van Rensburg and Venning, 1979; Puls, 1988). There are no reports of selenium toxicity or deficiency in cats.

Selenium toxicity in dogs is characterised by nausea, vomiting and diarrhoea, refusal of food leading to weight loss, anorexia and stunted growth, loose, coarse hair,

apprehension, respiratory stimulation and cardiovascular changes (Anderson and Moxon, 1942; Heinrich and MacCanon, 1957). In more severe cases, pathological lesions and nervous disorders with symptoms similar to "blind staggers" may develop (Rhian and Moxon, 1943). In extreme cases death may occur (Anderson and Moxon, 1942).

Clinical signs of selenium deficiency include muscular weakness, subcutaneous oedema, anorexia, depression, dyspnea and eventual coma (Van Vleet, 1975). There have also been reports of pathological lesions resembling those seen in lambs with white muscle disease (Manktelow, 1963; Van Rensburg and Venning, 1979). Although these studies provide an indication as to the range of selenium concentrations deemed unsafe, they do not focus on optimum concentrations required by dogs for good health.

The purpose of this study was to determine the total selenium concentrations in petfoods commercially available in New Zealand and to establish whether these meet the current minimum recommended requirements of selenium in petfoods for cats and dogs according to AAFCO (2000) and the NRC (1985; 1986).

MATERIALS AND METHODS

Samples

Petfoods (n=89), comprising 32 dry and 57 wet foods for cats (n=52) and dogs (n=37) were obtained from supermarkets and veterinary clinics in Palmerston North, New Zealand. They were complete petfoods manufactured in New Zealand (Heinz Wattie's, Friskies, Uncle Bens, First Choice, Butch, Effem Foods Ltd; n=29), Australia (Franklins Ltd, Friskies, Uncle Bens, First Choice, Chubpak Australian Ltd, Ralston Purina Co, First Choice; n=45), Thailand (Franklins Ltd, Heinz-Wattie's, Uncle Bens; n=4) and the USA (Friskies, Ralston Purina Co, Hills Pet Nutrition, IAMS Company; n=11). Dried petfood samples were ground, without freeze-drying, to a fine powder using a grinder (Model CG-2; Breville, Oldham, UK). Each ground sample was mixed thoroughly and stored in a plastic bag at –20 °C prior to analysis. Wet diets were freeze-dried for 72 hours, and then ground and stored as described for dry diets.

Chemical analysis

Samples were analysed in quadruplicate using a fluorometric method based on Method 996.16 of the Association of Official Analytical Chemists (AOAC, 2000) and that of Sheehan and Gao (1990). This method involves the oxidative digestion of all forms of selenium to inorganic forms, and the reaction which follows whereby selenite is complexed with 2,3-diaminonapthalene (DAN) to create a fluorophore. With each set of test samples, a reagent blank and six standard solutions containing known amounts of sodium selenite (0.004, 0.008, 0.01, 0.02, 0.03, 0.04 µg/ml in water; Aldrich Chemical Co.) were used to create a standard curve. A commercially available certified reference material of freeze-dried bovine blood (A-13; Analytical Control Services, International Atomic Energy Agency, Vienna, Austria; Pszonicki *et al.*, 1983) was analysed in triplicate as an external control. (See Appendix 1 for full details of the assay).

Statistical analysis

Results are presented as the mean ± standard error of the mean (SEM) and organised by petfood type, predominant flavour, manufacturer, and country. In the first three categories results were further divided into dog and cat foods. Types included wet (moist and semi-moist) and dry (biscuits). Predominant flavours were divided into six categories chosen according to descriptions on the label and were therefore not necessarily a reflection of the primary ingredients, and included chicken, seafood, chicken and seafood, beef, meat mix and other. Manufacturers were assigned a number for identification purposes. Data were analysed using SAS version 8.02 for Windows (SAS Institute Inc, Cary, NC, USA). Within categories 'type' and 'country', results were compared using one-way analysis of variance and Tukey's studentised range *post-hoc* tests to determine between group differences. Within the 'primary ingredient' and 'manufacturer' categories, results were compared using analysis of covariance in a general linear model. Differences between least squares means were determined using a Tukey-Kramer adjustment for multiple comparisons. In all cases, differences were considered significant if $p < 0.05$.

RESULTS

A table of selenium concentrations in all petfoods analysed can be found in Appendix 2. On a DM basis, selenium concentration in wet cat foods was higher than in wet and dry dog foods and dry cat foods ($p < 0.05$; Figure 1), and was the most variable, ranging from 0.16 to 6.12 µg Se/g DM, nine times greater than for wet dog foods (0.16 to 0.81 µg Se/g DM). Overall, selenium concentrations in cat foods were approximately three times that of dog foods (1.14 and 0.40 µg Se/g DM, respectively).

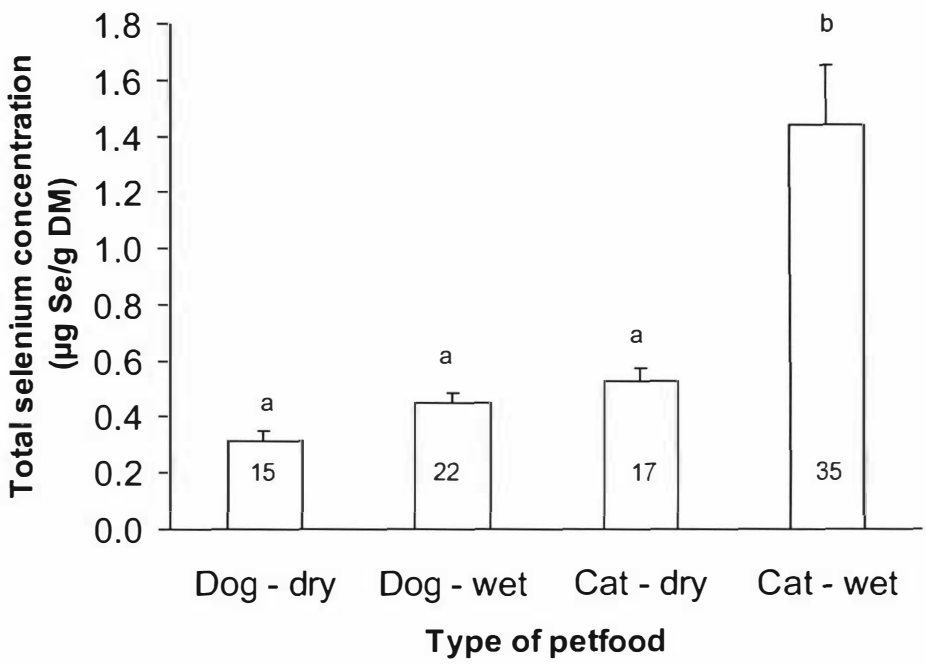

Figure 1. Mean total selenium concentration (± SEM) (µg Se/g DM) of 89 wet and dry foods for both cats and dogs, purchased in New Zealand. [a,b] Bars with different superscripts are significantly different ($p < 0.05$). Numbers within bars denote the number of samples in each group (n).

Seafood was the most common cat food flavour (n=19) and chicken the most common dog food flavour (n=19; Figure 2). The seafood-based flavours (seafood, chicken and seafood) contained the highest concentrations of selenium for both the cat and dog foods. Seafood-flavoured cat foods had the greatest range of selenium concentrations (0.35 to 6.12 µg Se/g DM), which were higher on average compared to all other flavours of petfood ($p < 0.05$).

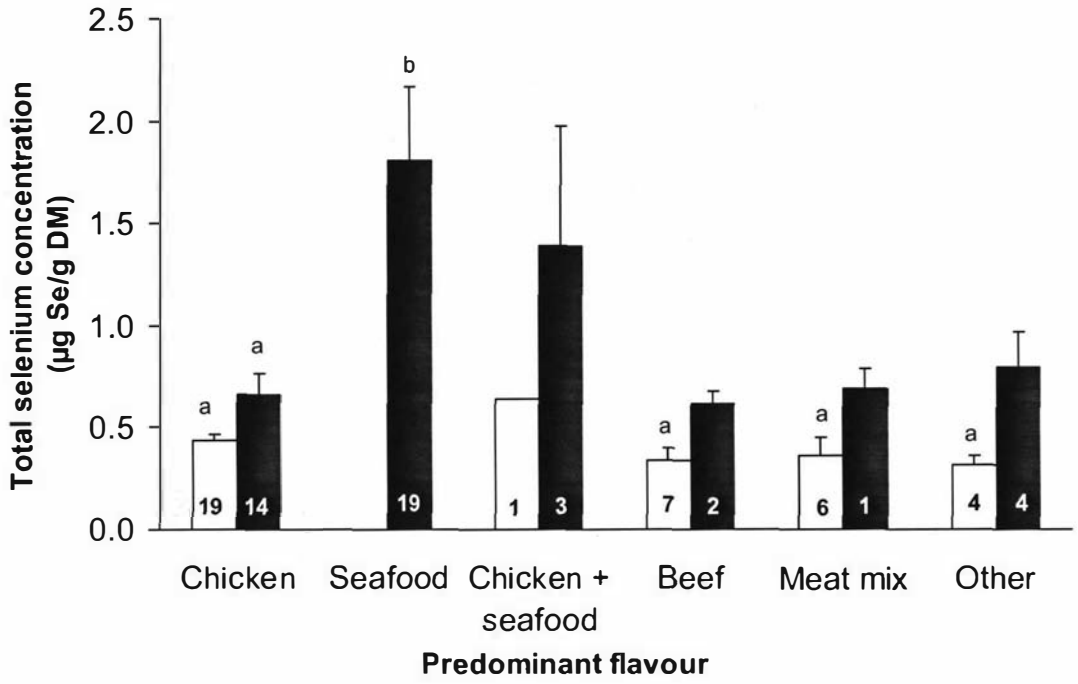

Figure 2. Mean total selenium concentration (± SEM) (µg Se/g DM) of different flavours of dog foods (□) and cat foods (■) purchased in New Zealand. [a,b] Bars with different superscripts are significantly different ($p < 0.05$). Numbers within bars denote the number of samples in each group (n).

Overall there were no significant differences in the concentrations of selenium between manufacturers for dog or cat foods (Figure 3). Despite numerically higher mean values in cat foods, results from the present study showed no significant differences in selenium concentrations between cat and dog foods for any individual flavour (Figure 2) or manufacturer (Figure 3).

Fifty percent of the samples were produced in Australia, 33% in New Zealand, 13% in the USA and 4% in Thailand. Mean total selenium concentrations were similar in petfoods produced in Australia, New Zealand and the USA (0.61 to 0.80 µg Se/g DM), whereas those in petfoods produced in Thailand were much higher (3.77, SEM 0.796 µg Se/g DM; Figure 4). Petfoods produced in Australia, New Zealand and the USA contained all (Australia), or several (New Zealand, USA) of the predominant flavours, the majority of which were chicken. The red-meat-flavoured petfoods were all manufactured in Australia or New Zealand, whereas petfoods from Thailand only contained seafood flavour (Figure 4).

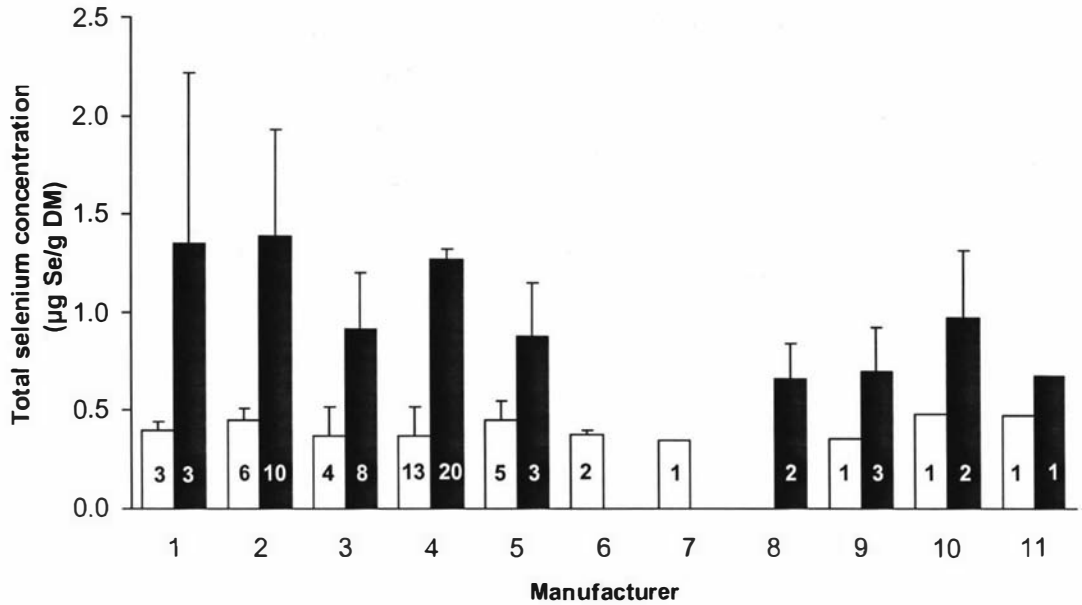

Figure 3. Mean total selenium concentration (± SEM) (µg Se/g DM) of dog foods (□) and cat foods (■) produced by different manufacturers, and purchased in New Zealand. Numbers within bars denote the number of samples in each group (n).

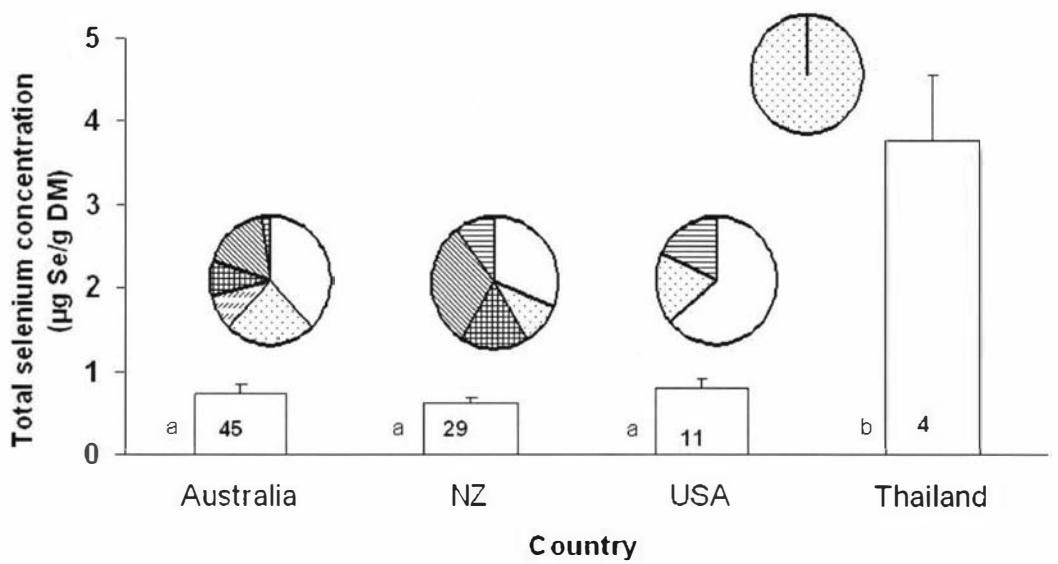

Figure 4. Mean total selenium concentration (± SEM) (µg Se/g DM) of sampled petfoods made in Australia, New Zealand (NZ), United States of America (USA) and Thailand, (bar chart); and comparison of predominant flavours, comprising chicken (□), seafood (⬚), chicken and seafood (▨), beef (▦), meat mix (▧), or other (▤) produced in the respective countries (pie chart). [a,b] Bars with different superscripts are significantly different ($p < 0.05$). Numbers within bars denote the number of samples in each group (n).

DISCUSSION

The present study showed there was a large range in the selenium concentration of commercially available petfoods in New Zealand. The range of selenium concentrations measured was 9.5 times larger in cat foods compared with dog foods. These values were similar to selenium concentrations previously reported by Mumma *et al.* (1986), who measured the toxic and protective constituents, including selenium, on a wet weight basis (µg Se/g DM), of canine and feline commercial diets purchased in Ithaca, New York.

Reference values for selenium concentrations were 0.02 to 1.1 µg Se/g DM for cereals and grains, 0.1 to 2.0 for marine fish, 0.2 to 4.2 for meats, and 0 to 0.6 µg Se/g DM for vegetables (Mumma *et al.*, 1986). Selenium in petfoods can be derived from the ingredients themselves, predominantly grains, cereals and animal tissue, in which some forms of selenium are stored during metabolism. Alternatively, selenium can be added as part of a vitamin/mineral premix supplement. Some types of fish have been found to contain high concentrations of selenium: for example tuna (*Thunnus* spp) has been reported to contain 6.10 µg Se/g DM (Mumma *et al.*, 1986). Although in the present study, petfoods were analysed according to flavours and not actual ingredients, the most common cat food flavour was seafood, particularly in wet cat foods, whereas dog foods and dry cat foods contained very little seafood. Therefore, the higher selenium concentrations in wet cat foods are likely to have resulted from a greater proportion of seafood ingredients found in those diets, which in turn contained high concentrations of selenium. Seafood was the predominant flavour of all the petfoods produced in Thailand that were analysed, and these contained significantly higher selenium concentrations than petfoods produced in the other three countries.

Although the petfoods analysed in this study varied in their selenium concentration, they all met the minimum recommendations (0.10 and 0.11 µg Se/g DM for dogs and cats, respectively) published by AAFCO (2000), as the lowest concentration recorded was 0.16 µg Se/g DM. However, whether AAFCO recommendations are adequate for the maintenance of optimum health has yet to be determined. In determining these values, the selenium requirements for cats and dogs were based mainly on values given by the NRC (1985; 1986). The NRC obtained their values for dogs mainly from deficiency studies conducted in puppies (National Research Council, 1985). With regard to cats, requirements were determined by

extrapolation of the selenium requirements of other species due to the lack of published studies on cats. It is possible that there are unique aspects of selenium metabolism in companion animals, especially cats, a species that has been shown to have several metabolic adaptations (Morris, 2002). In addition, the requirements of an animal for selenium may vary during the different stages of the life cycle, such that a concentration of selenium suitable for a growing animal may not be optimal for maintenance or reproduction. AAFCO has recognised the need for adjustments according to life stages and has attempted to provide these values where possible. However, in many cases information on specific selenium requirements are unavailable.

Chronic toxicity occurred in laboratory animals when fed ≥ 5 µg Se/g DM selenium (National Research Council, 1983). Livestock also produced signs of toxicosis when fed 5 µg Se/g DM for an extended period of time (Osweiler *et al.*, 1985) and in general it seems that this concentration in feeds marks the division between toxic and non-toxic levels (Koller and Exon, 1986). Preliminary results from a study conducted by Wedekind *et al.* (2002) suggested the safe upper limit for dogs was also 5 µg Se/g DM. The present study revealed selenium concentrations in dog foods were generally well below this, however some of the wet cat foods did contain selenium at these potentially toxic concentrations. Although the high seafood component of wet cat foods may have resulted in high concentrations of selenium, some types of seafood have also been reported to have low selenium bioavailabilities (Spungen Douglass *et al.*, 1981), which may explain the lack of toxicity occurring in cats. However, in a study by Forrer *et al.* (1991), serum selenium concentrations in cats were reported to be up to five times higher than in other animal species. Cats also showed the greatest variation in serum selenium concentrations, and were correlated to selenium concentrations in their diet. Thus, it may be that cats are able to tolerate higher levels of selenium than other species, although further studies would be needed to establish this.

Bioavailability of a nutrient is an important factor to consider when assessing dietary requirements. As the minimum requirements for selenium set by AAFCO (2000) and the NRC (1985; 1986) were largely based on data from other species, it would seem that the bioavailability of selenium in petfoods was not accounted for when these recommendations were set. If selenium in the diet has a low bioavailability and the animal receives the minimum amount, there will be less selenium available to the animal than it requires and deficiencies may occur. Results from bioavailability studies (Wedekind *et al.*, 1999; 2000) suggested that the minimum

requirements estimated by the AAFCO and NRC for kittens and puppies are too low. Studies using chick bioassays were conducted in an attempt to account for the bioavailability of selenium in petfoods and petfood ingredients (Wedekind *et al.*, 1997; 1998). The results from those studies suggest that selenium bioavailability was often low in petfoods and the authors recommended that supplementation may be required in many cases. When bioavailability was accounted for, those authors proposed that the recommended dietary intake of selenium for kittens should be 0.5 µg Se/g DM (Wedekind *et al.*, 2003), puppies 0.2 µg Se/g DM (Wedekind *et al.*, 1999), and adult dogs 0.43 µg Se/g DM (Wedekind *et al.*, 2002). If these higher estimates are used as minimum dietary requirements and assuming that the requirements of adult cats are similar to those of adult dogs, the present study suggested that selenium concentrations in 27 (30%) petfoods, 60% of dog foods and 12% of cat foods, did not meet the minimum requirements suggested by these authors.

In summary, selenium concentrations in commercial petfoods sold in New Zealand appeared to meet recommended dietary requirements, although the range of selenium concentrations in petfoods was highly variable. Whether these recommendations are adequate for the maintenance of optimal health in cats and dogs has yet to be determined. Although the bioavailability of selenium in petfoods has been studied in chicks, further studies in cats and dogs are necessary, as are studies on the bioavailability of different forms of selenium found in petfoods, other than selenate. In addition, optimum ranges rather than minimum concentrations need to be established, especially with regard to cats, as they may differ in requirements from other species. Finally, these requirements need to be determined for the different life stages including growth, maintenance, reproduction and old age.

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CHAPTER 3

Metabolism of dietary inorganic and organic selenium in adult cats

Little is known of selenium metabolism in cats. An understanding of metabolism and the metabolic response of the animal to dietary selenium intake is important for the determination of adequate selenium requirements. Part i of this paper investigates plasma and whole blood indicators of selenium status in response to inorganic and organic selenium supplementation, while in Part ii, the degree of faecal and urinary excretion of selenium is quantified in response to the same supplementation. Estimates of absorption and retention are calculated from this data.

CHAPTER 3

Part i

The use of blood parameters for assessing the selenium status of cats fed inorganic and organic selenium

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ABSTRACT

The current study investigated the response of plasma and whole blood selenium concentrations and GSHPx activities to inorganic and organic selenium supplementation in adult cats. A total of 20 adult domestic short-haired cats were fed a control diet containing 0.4 µg Se/g DM, or the control diet supplemented with either sodium selenite or an organic selenium yeast, to give approximate concentrations of 1.0, 1.5 and 2.0 µg Se/g DM over a period of 32 days in two separate studies. Selenium concentrations and GSHPx activities in plasma and whole blood were measured at days 0, 2, 4, 8, 16, 24 and 32. Plasma selenium concentrations reflected dietary selenium intake reaching 7.5 ± 0.1 µmol/L in animals fed the diet supplemented with 1 µg Se/g DM inorganic selenium on day 32 compared to those fed the control diet (4.6 ± 0.5 µmol/L) at 32 days, and 7.1 ± 0.4 µmol/L in cats supplemented with organic selenium on day 32 compared to those fed the control diet (5.6 ± 0.2 µmol/L) at 24 days in cats fed organic selenium. However the level of supplementation (between 1 and 2 µg Se/g DM) had no additional effect. Plasma selenium concentrations in cats fed inorganic selenium continued to increase, whereas the levelling of plasma selenium following supplementation of the organic form may have resulted from selenium that was surplus to the requirements of circulating selenoprotein formation being incorporated into general body proteins and stored. Whole blood selenium concentrations had little value in the determination of selenium status in the present study and may be better suited to longer term studies. Although there were no treatment effects on GSHPx activity, there was some indication of how the different forms of selenium may have been utilised. Further understanding of selenium metabolism and the response of metabolic parameters to dietary selenium intake in cats is still required. This will enable the use of appropriate indices of selenium status, which may then be used to help determine the dietary selenium requirements of cats.

INTRODUCTION

An understanding of the metabolism of any nutrient is important to enable dietary requirements and the adequacy of the diet to be determined. This is especially true for a trace element such as selenium which is an essential part of the diet, but can

have detrimental effects on health at concentrations outside of the required range. In order to determine dietary selenium requirements, biological indicators are commonly used to assess supplementation responses and selenium status of an animal. A variety of parameters have been used for these purposes including whole blood or its components (serum, plasma, platelets, erythrocytes), levels in body organs (liver, kidney, heart, muscle, etc), urinary selenium concentrations, enzyme activities such as GSHPx, and selenium in hair and nails (Levander, 1985; Ullrey, 1987; Gibson, 1989). Each is representative of a different aspect of selenium metabolism and as a result, individual parameters cannot be used to assess overall selenium status. In general, one, or a combination of parameters or indicators are chosen to assess different aspects of selenium status.

The aim of this paper was to determine the responses of a number of blood parameters in cats to dietary inorganic and organic selenium intake, to discuss whether these metabolic parameters are useful indicators of selenium status, and to establish whether there are any differences between different forms of selenium and their level of supplementation.

METHODS

The study reported here was approved by, and conformed to, the requirements of the Massey University Animal Ethics Committee (Anonymous, 2003). Other than the form (inorganic or organic) of selenium used, the study was conducted as two identical trials, with a four month period between each trial.

Animals

A pool of 20 short-haired domestic cats from The Centre for Feline Nutrition (Massey University, Palmerston North, New Zealand) were used for the study, with 16 (eight male, eight female) cats participating in each trial. Cats receiving the inorganic selenium ranged from 13 to 16 months of age and weighed between 2.69 and 4.69 kg (3.34 ± 1.37 kg, mean ± SEM), while cats fed the organic selenium diets ranged from 17 to 20 months of age and weighed between 2.75 and 5.02 kg (3.70 ± 1.72 kg, mean ± SEM). One month before each trial, all cats were fed the control diet *ad libitum* to standardise selenium intake. The control diet used was a commercial moist feline diet (Heinz Wattie's Ltd, Hastings, New Zealand) that had passed a minimum adult

maintenance feeding protocol AAFCO (2000; Table 1) and contained 0.4 µg Se/g DM. In both trials the cats were housed individually in metabolism cages as described by Hendriks *et al.* (1999; Appendix 2) to ensure they received the appropriate diet, and to enable accurate monitoring of dietary intake. Cats were adapted to the metabolism cages for seven days before the feeding of their respective diets and collection of blood samples began. Each cat was fed 70 kcal/kg BW/d to meet its daily energy requirement (National Research Council, 1986) and had access to deionised water at all times.

Treatments

Two different forms of selenium were used to supplement the control diet and throughout this paper they will be referred to as "inorganic" and "organic" selenium. The inorganic supplement used for the first trial was sodium selenite (a 1% premix of sodium selenite and limeflour; Nutritech International Ltd, Auckland, New Zealand), and the organic supplement used for the second was a selenium yeast (Sel-Plex™: containing selenomethionine, selenocysteine, and other selenoproteins and organo-selenium compounds; Alltech Inc, Nicholasville, Kentucky, USA). Each trial contained four treatment groups that corresponded to one of the four levels of selenium inclusion. The four trial diets included the control diet (0.4 µg Se/g DM), and three treatment diets supplemented with the respective form of selenium to obtain total selenium concentrations of 1.0, 1.5 and 2.0 µg Se/g DM. The supplemented diets were freshly prepared on a daily basis.

Sampling

Each cat was allocated to one of four dietary treatment groups balanced according to cat body weight with two male and two female cats in each group. On day 0, a baseline blood sample (3 ml) was taken from each cat by jugular venipuncture using a 23 gauge needle. Blood was collected into 5 ml heparinised tubes and divided into two 1 ml aliquots for the analysis of whole blood selenium concentrations and GSHPx activities. A third 1 ml aliquot was centrifuged for 10 minutes at 3,000 rpm and the plasma removed and stored for the analysis of selenium concentrations and GSHPx activities. All whole blood and plasma samples were stored at –20 ºC prior to analysis. Following the initial blood sample, cats were fed their respective, pre-weighed diets once daily for 32 days. Subsequent blood samples were obtained from all cats on days 2, 4, 8, 16, 24 and 32. Subsamples from each of the four diets were taken daily,

stored at -20 ^{0}C and pooled for analysis over the corresponding six time periods: T1: 0-2d, T2: 3-4d, T3: 5-8d, T4: 9-16d, T5: 17-24d and T6: 25-32d and subjected to total selenium analysis. Food intake was recorded on a daily basis and cats were weighed weekly for the duration of the study.

Table 1. Composition and amino acid profile of the control diet

Composition (g/100g DM)[1]	
Crude protein	52.5
Crude fat	27.7
Ash	9.0

Amino acid profile (g/100g DM)			
Taurine	0.20	Valine	2.80
Aspartic acid	4.39	Methionine	1.14
Threonine	2.23	Isoleucine	1.14
Serine	2.32	Leucine	4.99
Glutamic acid	7.28	Tyrosine	1.95
Glycine	4.06	Phenylalanine	2.50
Alanine	3.65	Histidine	1.62
Lysine	2.83	Arginine	3.04

[1] *DM content = 19.1%*

Chemical analysis

Diet samples were freeze-dried, ground to a fine powder using an electric grinder (Model CG-2; Breville, Oldham, UK) and mixed thoroughly prior to selenium analysis. Diet samples were analysed in quadruplicate and blood samples were analysed in duplicate. Samples having a coefficient of variation between replicates of greater than 10% were reanalysed until variability was reduced below this level. Total selenium concentrations of all samples were analysed using a fluorometric method as previously outlined in Chapter 2 and described in Appendix 1. Plasma and whole blood GSHPx activities were assayed using a Ransel diagnostic kit and controls (Randox Laboratories Ltd, Antrim, UK) on a Roche Cobas Fara II System (Basel, Switzerland).

Statistical analysis

Data were analysed using SAS version 8.02 for Windows (SAS Institute Inc, Cary, NC, USA). Prior to analysis, all data were checked for outliers. Normality was examined by

plotting a frequency histogram, a plot of the residuals, and by calculating tests for normality (Shapiro-Wilk and Kolmogorov-Smirnov) using the residual data. Levene's test for homogeneity of variance was conducted to determine whether variances between group means were equal. When data was normally distributed and variances between groups were equal, a repeated measures ANOVA was performed with the general linear model procedure using the model: parameter of interest = diet + time nested on diet, followed by multiple comparisons using least squared means. When data did not adhere to a normal distribution and/or variances were unequal, the analysis was repeated on ranked data and results from both ranked and unranked analyses were compared. Differences that were common to both tests were reported and the most conservative p value was used. Analyses were performed on both unranked and ranked data for organic plasma selenium (Figure 1B), inorganic and organic whole blood selenium (Figures 2A,B) and combined inorganic and organic plasma selenium (Figure 5A). In all cases the differences found to be significant on unranked data were also significant when analysis was performed on the ranked data. Results are presented graphically as the mean ± SEM. In all cases, differences were considered significant at a probability level of 5%.

RESULTS

All cats remained healthy throughout both trials. Average (± SEM) food intake in trials 1 and 2 were 253 ± 10 g and 293 ± 13 g, respectively, and the average food intake was the same for all groups. The determined concentrations of selenium in the diets in the two trials are shown in Table 2.

Table 2. Selenium concentrations (µg Se/g DM) in diets supplemented with inorganic or organic selenium.

Group	Target level	Actual level[1] (µg Se/g DM)	
		Inorganic Trial	Organic Trial
1	0.4	0.42	0.49
2	1.0	0.98	1.27
3	1.5	1.43	1.70
4	2.0	2.00	2.22

[1]*actual values obtained from the mean of quadruplicate samples pooled over each trial*

Actual concentrations of selenium in the diets prepared for trial 1 (inorganic selenium) were ± 0.02 µg Se/g DM (± 5%) of the target concentrations, whereas the actual concentrations of selenium in diets for trial 2 (organic selenium) were higher than the target concentrations by approximately 0.20 µg Se/g DM (11 to 22%) (Table 2).

Table 3. Dietary selenium intake (µg Se/kg body weight/day) during the collection period in cats fed a control diet (0.4 µg Se/g DM), or control diet supplemented with inorganic or organic selenium to achieve dietary selenium concentrations of 1.0, 1.5 and 2.0 µg Se/g DM) (n=4).

Form	Diet	Se Intake (µg/kg BW/d) mean ± SEM	Pr > F		
			Diet	Form	Diet x Form
Inorganic	Control	6.5 ± 0.2[a]	<0.0001	<0.0001	0.2447
	1.0 µg/g	15.3 ± 0.3[b]			
	1.5 µg/g	22.4 ● 0.5[c]			
	2.0 µg/g	31.3 ± 1.9[d]			
Organic	Control	8.3 ± 0.2[a]			
	1.0 µg/g	21.0 ± 0.9[b]			
	1.5 µg/g	27.3 ± 2.3[c]			
	2.0 µg/g	37.2 ± 1.6[d]			

Differences between diets for each form are indicated by superscripts ($p < 0.05$)

In accordance with the graded level of selenium in the diets, the average dietary selenium intake (µg Se/kg BW/d) between groups was different (Table 3). Average (± SEM) selenium intakes of cats in trial 2 (organic) were slightly higher than those in trial 1 (inorganic; Table 3) as reflected by selenium concentrations in the diet (Table 2).

Plasma selenium concentrations increased over time when cats were fed the inorganic selenium supplemented diets ($p < 0.01$: Figure 1A, Table 4), but were unchanged in control cats ($p > 0.05$). Apart from cats fed 1.5 and 2.0 µg Se/g DM at day 32 ($p < 0.05$), there were no differences in plasma selenium concentrations among cats fed the three different levels of inorganic selenium ($p > 0.05$). Plasma selenium concentrations of cats fed the inorganic selenium supplemented diets were different from the control group from day 16 by up to 1.8 times ($p < 0.05$), and increased up to 14% after 24 days ($p < 0.05$). Plasma selenium concentrations in cats fed organic selenium (Figure 1B, Table 4) did not change over time ($p > 0.05$) and with the exception of cats fed 1 and 1.5 µg Se/g DM on day 32 ($p < 0.05$), there were

no differences among cats fed the three supplemented diets ($p > 0.05$). From day 3 onwards, cats fed the supplemented diets had higher plasma selenium concentrations than cats fed the control diet by up to 1.5 times ($p < 0.05$).

As with the plasma selenium response, there were no differences among the whole blood selenium concentrations of cats fed the inorganic selenium supplemented diets ($p > 0.05$; Figure 2A, Table 4). Whole blood selenium concentrations in cats fed the supplemented diets were 1.3 times higher than controls from day 24 ($p < 0.05$), and in cats fed 2 µg Se/g DM diet, from day 16 ($p < 0.01$). Whole blood selenium concentrations increased over time by up to 26% in cats fed the organic selenium supplemented diets ($p < 0.05$; Figure 2B, Table 4), but there was no change over time in control animals ($p > 0.05$). Apart from cats fed 1 and 1.5 µg Se/g DM on day 32 ($p < 0.05$), whole blood selenium concentrations in cats fed the three organic selenium supplemented diets did not differ during the 32 days ($p > 0.05$). Whole blood selenium concentrations in control animals were 1.2 times lower compared to those in cats fed the 1.5 µg Se/g DM diet from day 16 ($p < 0.05$), and those fed the 2 µg Se/g DM diet from day 24 ($p < 0.05$).

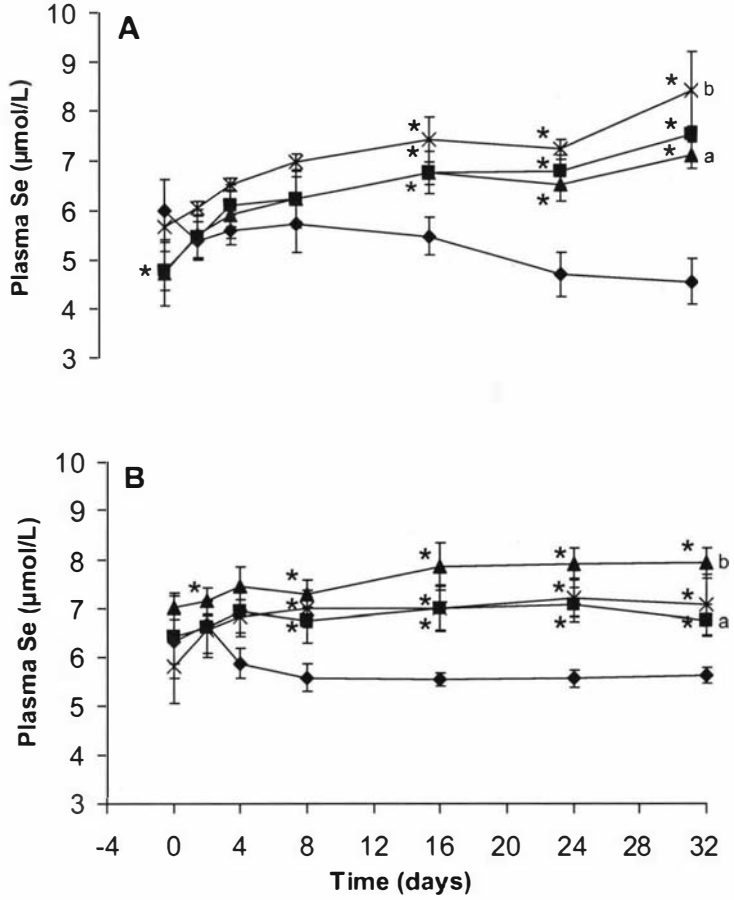

Figure 1. Mean (± SEM) plasma selenium (Se) concentrations (µmol/L) in cats fed a control diet containing 0.4 µg Se/g DM (—♦—), or the control diet supplemented with inorganic (Panel A) or organic (Panel B) selenium to achieve dietary selenium concentrations of 1.0 (—■—), 1.5 (—▲—), and 2.0 (—X—) µg Se/g DM (n=4). At each time point: * different from control; values with different superscripts are different; ($p < 0.05$).

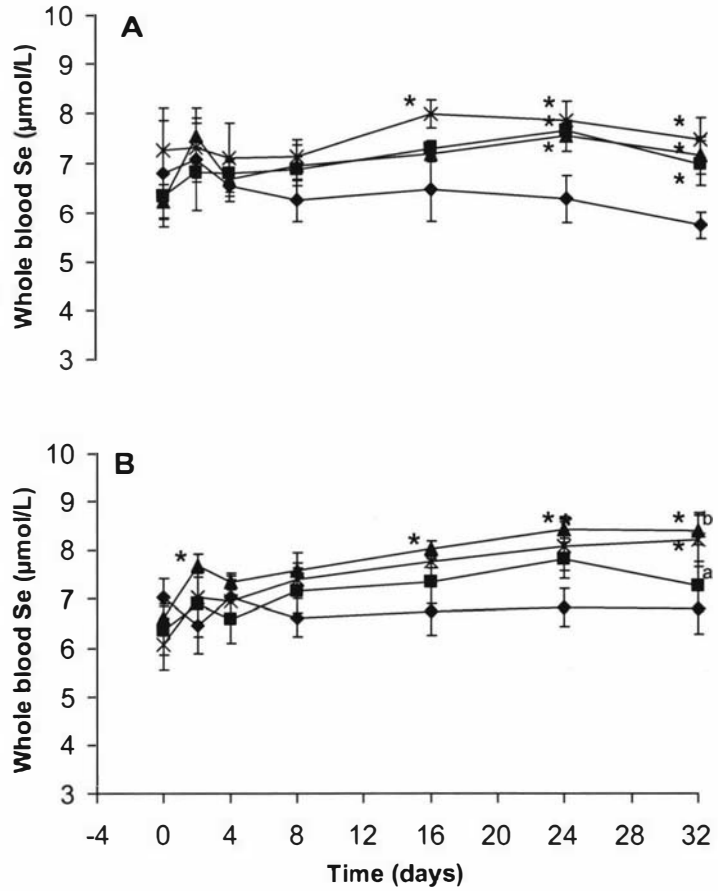

Figure 2. Mean (± SEM) whole blood selenium (Se) concentrations (µmol/L) in cats fed a control diet containing 0.4 µg Se/g DM (—♦—), or the control diet supplemented with inorganic (Panel A) or organic (Panel B) selenium to achieve dietary selenium concentrations of 1.0 (—■—), 1.5 (—▲—), and 2.0 (—X—) µg Se/g DM (n=4). At each time point: * different from control; values with different superscripts are different; ($p < 0.05$).

Table 4. Mean (± SEM) concentrations of selenium (Se) in plasma and whole blood (WB) (µmol/L) in cats fed a control diet (0.4 µg Se/g DM), or the control diet supplemented with three different concentrations of inorganic or organic selenium to give total dietary selenium concentrations of 1.0, 1.5 and 2.0 µg Se/g DM (n=4).

			Time (day)						
Parameter	Form	Diet	0	2	4	8	16	24	32
Plasma Se (µmol/L)	Inorganic	Control	6.0 ± 0.6^{a}	5.4 ± 0.4	5.6 ± 0.3	5.7 ± 0.6^{a}	5.5 ± 0.4^{a}	4.7 ± 0.4^{a}	4.5 ± 0.5^{a}
		1.0 µg/g	4.8 ± 0.4^{b}	5.5 ± 0.4	6.1 ± 0.4	6.2 ± 0.5^{ab}	6.8 ± 0.2^{b}	6.8 ± 0.3^{b}	7.5 ± 0.1^{b}
		1.5 µg/g	4.7 ± 0.7^{ab}	5.5 ± 0.5	5.9 ± 0.5	6.2 ± 0.6^{ab}	6.8 ± 0.3^{b}	6.5 ± 0.3^{b}	7.1 ± 0.3^{bc}
		2.0 µg/g	5.7 ± 0.3^{ab}	6.0 ± 0.1	6.5 ± 0.1	7.0 ± 0.2^{b}	7.4 ± 0.5^{b}	7.2 ± 0.2^{b}	8.4 ± 0.8^{c}
	Organic	Control	6.3 ± 0.5	6.7 ± 0.2	5.9 ± 0.3^{a}	5.6 ± 0.3^{a}	5.6 ± 0.1^{a}	5.6 ± 0.2^{a}	5.6 ± 0.2^{a}
		1.0 µg/g	6.4 ± 0.9	6.6 ± 0.6	6.9 ± 0.5^{b}	6.8 ± 0.5^{b}	7.0 ± 0.5^{b}	7.1 ± 0.4^{b}	6.7 ± 0.3^{b}
		1.5 µg/g	7.0 ± 0.2	7.1 ± 0.3	7.5 ± 0.4^{b}	7.3 ± 0.3^{b}	7.9 ± 0.5^{b}	7.9 ± 0.3^{b}	7.9 ± 0.3^{c}
		2.0 µg/g	5.8 ± 0.8	6.6 ± 0.6	6.8 ± 0.3^{b}	7.0 ± 0.4^{b}	7.0 ± 0.4^{b}	7.2 ± 0.4^{b}	7.1 ± 0.6^{bc}
WB Se (µmol/L)	Inorganic	Control	6.8 ± 1.0	7.1 ± 1.0	6.5 ± 0.3	6.2 ± 0.4	6.4 ± 0.7^{a}	6.3 ± 0.5^{a}	5.7 ± 0.3^{a}
		1.0 µg/g	6.3 ± 0.5	6.8 ± 0.2	6.8 ± 0.3	6.9 ± 0.2	7.3 ± 0.2^{ab}	7.6 ± 0.2^{b}	7.0 ± 0.5^{b}
		1.5 µg/g	6.2 ± 0.3	7.6 ± 0.2	6.7 ± 0.3	6.9 ± 0.4	7.2 ± 0.1^{ab}	7.6 ± 0.3^{b}	7.1 ± 0.4^{b}
		2.0 µg/g	7.3 ± 0.9	7.3 ± 0.6	7.1 ± 0.7	7.1 ± 0.3	8.0 ± 0.3^{b}	7.9 ± 0.4^{b}	7.5 ± 0.4^{b}
	Organic	Control	7.0 ± 0.4	6.5 ± 0.6^{a}	7.0 ± 0.5	6.6 ± 0.4	6.8 ± 0.5^{a}	6.8 ± 0.4^{a}	6.8 ± 0.5^{a}
		1.0 µg/g	6.3 ± 0.5	6.9 ± 0.7	6.6 ± 0.5	7.2 ± 0.5	7.4 ± 0.5^{ab}	7.9 ± 0.4^{ab}	7.3 ± 0.5^{ab}
		1.5 µg/g	6.6 ± 0.3	7.7 ± 0.2^{b}	7.4 ± 0.2	7.6 ± 0.3	8.0 ± 0.2^{b}	8.4 ± 0.3^{b}	8.4 ± 0.3^{c}
		2.0 µg/g	6.1 ± 0.5	7.0 ± 0.5	7.0 ± 0.4	7.4 ± 0.3	7.8 ± 0.1^{ab}	8.1 ± 0.5^{b}	8.2 ± 0.6^{bc}

Within columns for each form of selenium, means with different superscripts are different ($p < 0.05$)

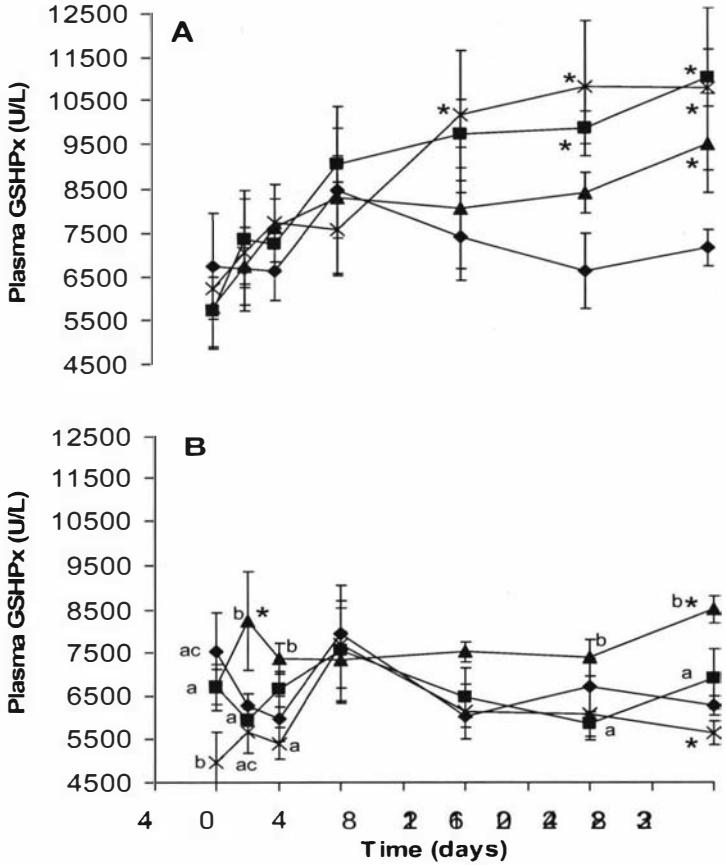

Figure 3. Mean (± SEM) plasma glutathione peroxidase (GSHPx) activities (U/L) in cats fed a control diet containing 0.4 µg Se/g DM (—♦—), or the control diet supplemented with inorganic (Panel A) or organic (Panel B) selenium to achieve dietary selenium concentrations of 1.0 (—■—), 1.5 (—▲—), and 2.0 (—X—) µg Se/g DM (n=4). At each time point: * different from control; values with different superscripts are different; ($p < 0.05$).

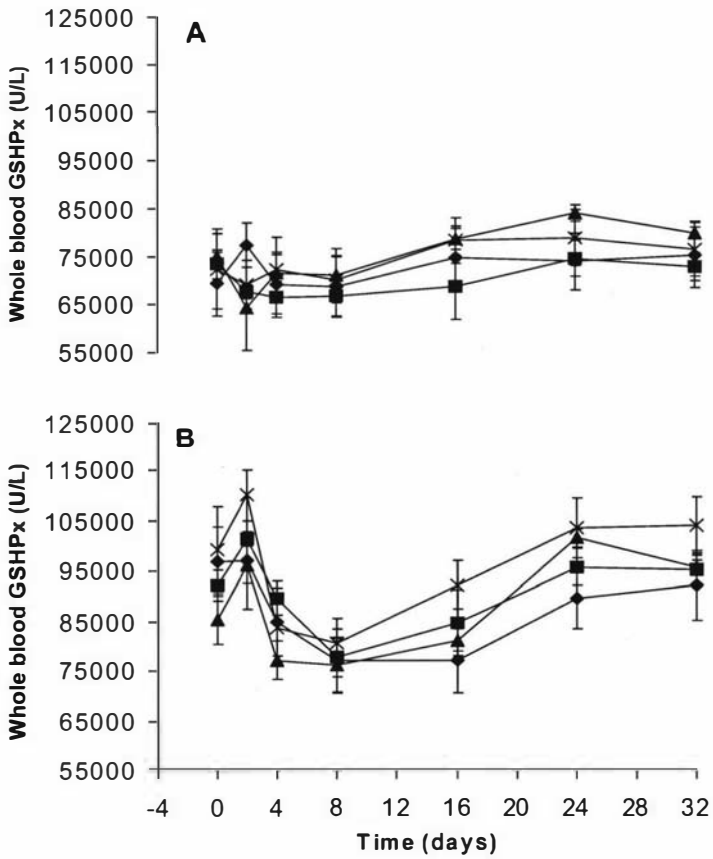

Figure 4. Mean (± SEM) whole blood glutathione peroxidase (GSHPx) activities (U/L) in cats fed a control diet containing 0.4 µg Se/g DM (—♦—), or the control diet supplemented with inorganic (Panel A) or organic (Panel B) selenium to achieve dietary selenium concentrations of 1.0 (—■—), 1.5 (—▲—), and 2.0 (—X—) µg Se/g DM (n=4).

Table 5. Mean (± SEM) glutathione peroxidase (GSHPx) activities (U/L) in plasma and whole blood (WB) in cats fed a control diet (0.4 µg Se/g DM), or the control diet supplemented with three different concentrations of inorganic or organic selenium to give total dietary selenium concentrations of 1.0, 1.5 and 2.0 µg Se/g DM (n=4).

			Time (day)						
Parameter	Form	Diet	0	2	4	8	16	24	32
Plasma GSHPx (U/L)	Inorganic	Control	6735 ±1208	6679 ± 958	6641 ● 673	8463 ± 1886	7403 ± 1003^{a}	6625 ± 865^{a}	7163 ± 418^{a}
		1.0 µg/g	5700 ± 800	7359 ● 1105	7248 ± 406	9054 ± 797	9731 ± 768^{ab}	9869 ± 370^{bc}	11024 ± 677^{b}
		1.5 µg/g	5797 ± 947	6742 ± 419	7635 ± 960	8304 ± 933	8050 ● 1372^{ab}	8399 ± 464^{ac}	9520 ± 1125^{b}
		2.0 µg/g	6217 ● 452	7057 ± 1221	7724 ± 545	7582 ± 1066	10167 ± 1485^{b}	10792 ± 1556^{bc}	10771 ± 1582^{b}
	Organic	Control	7541 ± 891^{ac}	6263 ± 297^{ac}	5962 ± 518^{ab}	7934 ± 610	6028 ± 515	6711 ● 667^{ab}	6277 ± 212^{a}
		1.0 µg/g	6704 ± 530^{a}	5938 ± 136^{ac}	6664 ± 406^{ab}	7555 ± 1158	6470 ± 676	5854 ± 294^{a}	6891 ± 693^{a}
		1.5 µg/g	6711 ● 407^{a}	8230 ± 1135^{b}	7366 ± 353^{a}	7340 ± 651	7520 ± 236	7386 ± 417^{b}	8502 ± 324^{b}
		2.0 µg/g	4965 ± 710^{b}	5683 ± 505^{c}	5140 ± 361^{b}	7696 ± 1366	6133 ± 631	6078 ± 595^{ab}	5651 ± 272^{a}
WB GSHPx (U/L)	Inorganic	Control	69331 ± 7766	77505 ± 5387	69249 ± 7901	68680 ● 7436	74635 ● 7461	73923 ± 6971	75297 ± 8041
		1.0 µg/g	73416 ± 3560	67665 ± 1713	66405 ± 3985	66605 ± 4599	68573 ± 7647	74584 ± 4300	72763 ± 3385
		1.5 µg/g	74928 ± 5785	64175 ± 10066	71581 ± 4471	71135 ± 6355	78705 ± 2508	83958 ± 2027	79868 ± 1518
		2.0 µg/g	72437 ± 9605	69157 ± 5870	72298 ± 7963	70166 ± 5814	78295 ± 5616	78997 ± 6748	76475 ± 6393
	Organic	Control	96960 ± 8020	97262 ± 12437	84993 ● 10519	77075 ● 6008	77051 ± 8087	89571 ± 12747	92217 ± 11959
		1.0 µg/g	92214 ● 7926	101424 ± 7161	89734 ± 8646	77870 ± 7270	84768 ± 7640	95885 ± 13114	95282 ± 6503
		1.5 µg/g	85521 ± 6211	96207 ± 7594	77183 ± 10842	76194 ± 4248	81268 ± 4662	101634 ± 6902	95862 ± 6623
		2.0 µg/g	99379 ± 5677	110044 ± 8364	83850 ● 5529	80581 ± 2859	92293 ± 4435	103556 ± 12226	104074 ± 6806

Within columns for each form of selenium, means with different superscripts are different ($p < 0.05$)

GSHPx activities in plasma showed considerable variation (Figure 3, Table 5). Activities increased over time by up to 50% in cats fed the inorganic selenium supplemented diets ($p < 0.05$; Figure 3A), but there was no change over time in cats fed the control diet ($p > 0.05$). GSHPx activities in cats fed 1.0 µg Se/g DM were 1.5 times higher than in control animals on days 24 and 32 ($p < 0.01$) and activities in cats fed 2.0 µg Se/g DM activities were also 1.5 times higher than in controls on days 16, 24 and 32 ($p < 0.05$). There were no consistent differences among groups or over time for plasma GSPHx activities in cats supplemented with organic selenium ($p > 0.05$; Figure 3B). There were no changes in whole blood GSHPx activities across all groups or over time in cats supplemented with both forms of selenium ($p > 0.05$; Figure 4, Table 5).

No differences ($p > 0.05$) were found in plasma and whole blood selenium concentrations in cats fed the control diets from each trial and data were combined from both trials for statistical comparison. Similarly, as there were no differences ($p > 0.05$) among cats fed the supplemented diets in both trials, (plasma and whole blood selenium concentrations), the three treatment groups in each trial were combined to give one treatment group for inorganic selenium supplementation and one treatment group for organic selenium supplementation. These data were compared with the combined control data from both trials (Figure 5). Initial (day 0) plasma selenium concentrations in cats fed inorganic selenium were lower than those in cats fed the control and organic selenium supplemented diets by 1.2 times ($p < 0.01$; Figure 5A). There was an increase over time in plasma concentrations by up to 33% in cats fed inorganic selenium, and 13% in cats fed organic selenium ($p < 0.05$), with concentrations of selenium from cats fed both forms reaching around 7 µmo/L. Plasma selenium concentrations in cats fed the control diet were lower than those fed organic selenium throughout the 32 day period by up to 1.5 times ($p < 0.05$) and from those fed inorganic selenium by up to 1.4 times from day 8 ($p < 0.05$). Selenium concentrations in whole blood did not differ among cats supplemented with organic and inorganic selenium ($p > 0.05$) except at day 32 ($p < 0.05$) and concentrations in cats fed both forms increased over time by up to 20% ($p < 0.05$; Figure 5B). Whole blood selenium concentrations of cats supplemented with organic selenium were up to 1.2 times higher than controls from day 8 ($p < 0.01$) and cats supplemented with inorganic selenium were higher than controls by a similar magnitude from day 16 ($p < 0.05$).

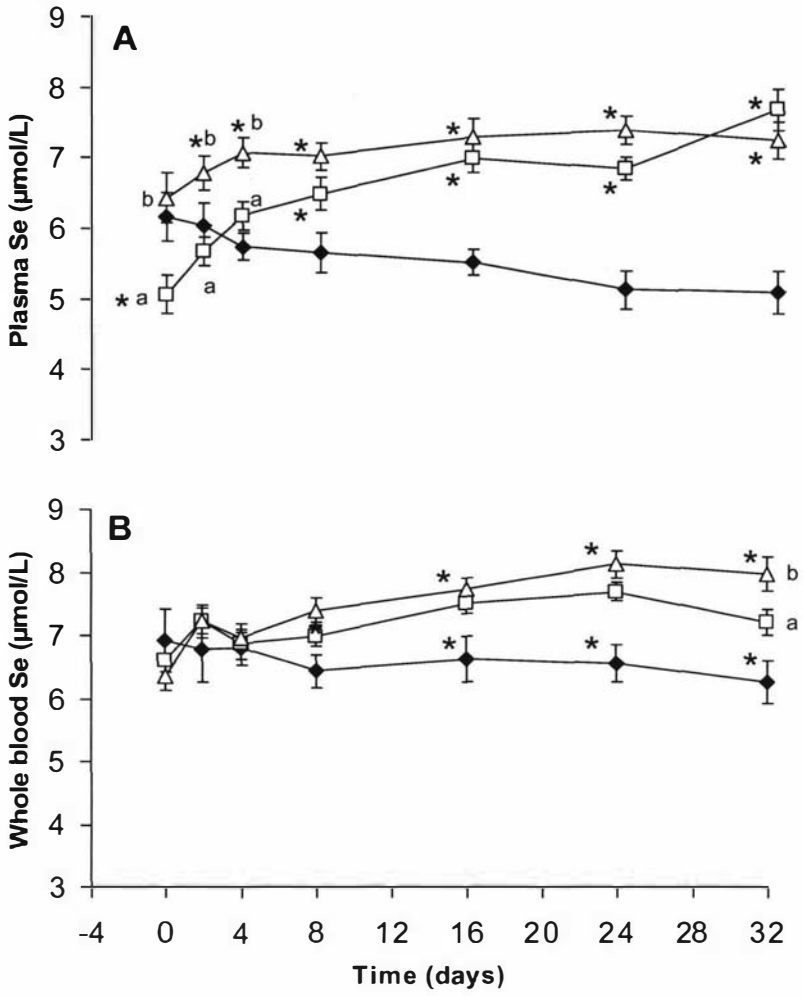

Figure 5. Mean (± SEM) plasma (panel A) and whole blood (panel B) selenium (Se) concentrations (µmol/L) in controls combined from both trials, fed 0.4 µg Se/g DM (—♦—), and treatment groups supplemented with selenium as inorganic selenium (—□—) or organic selenium (—△—) at levels of 1.0 to 2.0 µg Se/g DM (n=4). At each time point: * different from control group; values with different superscripts are different; ($p < 0.05$).

DISCUSSION

There are several different parameters or biochemical markers currently used to determine selenium requirements (Ullrey, 1987). In this study, selenium concentrations and GSHPx activities in plasma and whole blood were used to assess the response of cats to selenium supplementation and to provide information about changes in these key indicators of selenium status in relation to dietary selenium

intake. The two selenium supplements used were an organic selenium yeast (Sel-Plex™) and inorganic sodium selenite. Selenium yeast is manufactured by adding selenium to a medium during growth of *S. cerevisiae* yeast. The yeast utilises the available selenium in lieu of sulphur during aerobic fermentation, resulting in the selenium becoming organically bound to the yeast. At least 90% of the total selenium in the final product should be bound to the yeast to create a reputable organic selenium yeast and the manufacturers of such products conduct specific checks on a regular basis to ensure this is the case (Rayman, 2004). Analysis of the content of selenium in selenium yeasts yields variable results due to different manufacturing and extraction techniques, as well as different methods of laboratory analysis. Most products should contain approximately 60% selenomethionine and no more than 1% of inorganic selenium, whilst a variety of other selenium metabolites and intermediates make up the total selenium content (Rayman, 2004). Sel-Plex has been reported to consist of 62 – 74% selenomethionine, and of the identified species extracted, 83% was selenomethionine, 5% selenocysteine and 0.3% selenite (Rayman, 2004). Thus, for the purposes of this study the selenium yeast supplement will be referred to as "organic selenium" and the sodium selenite supplement as "inorganic selenium".

Unfortunately, due to inter-experimental variation between the two trials, there were differences in dietary selenium concentrations of cats fed the two forms of selenium such that cats fed the organic supplement received slightly higher concentrations of selenium compared to those fed the inorganic form. This accounts for the higher overall concentrations of plasma selenium of cats from all groups fed organic selenium. Despite these discrepancies, some useful conclusions may still be drawn from the data without making direct comparisons between the two trials. Plasma selenium responds rapidly to changes in dietary selenium concentrations (Reilly, 1993). In this study, for both forms of selenium, plasma concentrations were consistently higher in the treatment groups than those of control animals. Therefore it was concluded that plasma selenium concentrations can provide a rapid estimate of dietary selenium intake for both inorganic and organic forms of supplemented selenium in cats. However the amount of selenium in the treatment diets appeared insufficient to elicit differences in plasma concentrations between the three levels of supplemented selenium. This data shows no difference in selenium blood parameter indicators between the chosen supplementation levels. Further investigation is required to determine whether or not plasma selenium concentrations in cats would increase in response to dietary selenium concentrations greater than 2 µg Se/g DM (see Chapter 4).

In general, absorbed selenium is transported in plasma bound to proteins and in humans, selenium in plasma appears as selenoprotein P, GSHPx and bound to albumin (Deagan *et al.*, 1991). Selenium in the selenoproteins GSHPx and selenoprotein P exists as selenocysteine, whereas the chemical form of selenium associated with albumin is selenomethionine (Deagan *et al.*, 1991). Any selenium of inorganic or organic origin not stored or utilised for selenoprotein synthesis is methylated and excreted (Shiobara *et al.*, 1998). Hence regulation of selenium metabolism is required to maintain appropriate levels of selenium in body reserves such that when periodic changes in selenium intake occur, a steady state of selenium can still be maintained and consequently plasma levels of selenium remain constant. A trend to this effect was observed in cats fed organic selenium as plasma concentrations in all treatment groups reached maximum levels after approximately four days, and remained at this constant level for the remainder of the 32 day experimental period. In contrast, plasma concentrations of selenium in cats fed inorganic selenium did not show this plateau and concentrations in the three treatment groups continued to increase during the period of supplementation. Firm conclusions cannot be made comparing selenium concentrations of cats fed the two different supplements due to discrepancies in dietary selenium intake and differences in baseline values. This accounts for the similar value of maximal selenium concentrations reached by cats fed both supplements despite the increase seen by those fed inorganic selenium. These inconsistencies may be accounted for to some degree by assessing the relative change in selenium concentrations from baseline values for each form of selenium. Using this data, the same pattern is observed whereby plasma selenium concentrations in cats fed organic selenium plateaued, whilst those fed the inorganic form appeared to increase. In addition, plasma selenium concentrations in cats fed inorganic selenium were higher than those fed organic selenium after 4 days. The higher selenium concentrations in cats fed the inorganic diet, along with the steady increase in these concentrations over time may indicate an inability of these cats to remove selenium from the plasma for utilisation or excretion, and/or an accumulation of selenium in the plasma resulting from the formation of seleno-compounds. It is possible that urinary excretion was not sufficient to regulate plasma selenium levels in cats fed inorganic selenium. If urinary selenium excretion decreased and dietary selenium levels remained constant, selenium in the body reserves, including plasma, would increase. This seems unlikely as in other species selenium metabolism is well controlled by urinary excretion within normal physiological levels (Kirchgessner *et al.*, 1997). If

urinary excretion was constant, as would be expected in a steady state, plasma selenium levels would also be constant as observed in cats fed organic selenium. The fact that total selenium in plasma increased in cats fed inorganic selenium suggests that increased production of selenoproteins (selenoprotein P and/or GSHPx) may have occurred in these cats, whereas surplus selenium in cats fed organic selenium was incorporated into general body proteins such as liver and muscle and stored. However the differences between time points were not significant and further studies including larger numbers of animals per treatment group, larger differences between supplementation levels and/or longer periods of supplementation are needed to confirm this difference between the two types of supplemented selenium.

Whole blood selenium showed a similar pattern of response to plasma selenium however there were less significant differences between treatment and control animals. This may indicate that the changes seen in whole blood selenium are mainly a reflection of changes in plasma selenium, and that the duration of the present study was insufficient to induce a significant response in whole blood selenium. The lower responsiveness of whole blood selenium concentrations to dietary selenium intake is possibly due to differences of short term selenium supplementation on selenium incorporation into red blood cells on one hand, and plasma selenium on the other. During erythropoiesis selenium is incorporated into red blood cells (Ullrey, 1987), which have an average life span in the cat of 70 days (Liddle *et al.*, 1984). Although whole blood selenium concentrations also reflect dietary intake, the delayed response of selenium incorporation into red blood cells means whole blood concentrations may be a better indicator of longer-term selenium status in cats as they are in other species.

Plasma and whole blood selenium are one measure of total body selenium, and while they may provide useful information on the short and long term selenium status of the animal, they are not perfect. The biological functions of selenium occur through selenoproteins (Holben and Smith, 1999), which comprise several families of redox enzymes: the GSHPx's, ID's and TRR's, as well as approximately 10 other selenoproteins including selenoprotein P (Burk *et al.*, 2003). The physiological requirement for selenium is commonly determined by establishing the level of selenium at which the activity of GSHPx is maximised (Thomson, 2004). In the present study, concentrations of selenium in the control diet (0.4 µg Se/g) were well above the amount of dietary selenium recommended for cats and dogs (0.1 µg Se/g DM) however these concentrations did not show maximal GSHPx activities as indicated by the higher activates seen in cats fed the supplemented diets. It may be that this is

due to a lower bioavailability of selenium in the control diet. Although variation was high, plasma GSHPx in cats fed inorganic selenium showed a similar pattern to plasma selenium. This may explain the increase in plasma selenium levels observed in cats fed inorganic selenium, as GSHPx formation in plasma from selenocysteine of inorganic origin could contribute to the overall plasma selenium concentration, as previously discussed. In contrast, plasma GSHPx activities in cats fed organic selenium did not mirror the response seen in plasma selenium concentrations and showed no particular pattern.

Activities of GSHPx in whole blood were much higher compared to plasma which may be explained by the additional GSHPx present in red blood cells. There were no changes in whole blood GSHPx activities in the cats supplemented with inorganic or organic selenium. This may indicate a delayed response of GSHPx due to the time lag resulting from formation of GSHPx in red blood cells. Cohen *et al.* (1985) showed that in selenium deficient children GSHPx is formed in the presence of selenium in newly synthesised red blood cells, rather than simply being incorporated into existing red blood cells. As a result enzyme activity was not reported to occur for four to five weeks. Whole blood GSHPx did not match the responses seen with plasma GSHPx despite the fact plasma is a component of whole blood. This may be due to the different magnitudes of the two parameters such that the changes occurring in plasma GSHPx activities were too small to detect relative to activities in whole blood. It is interesting to note the marked differences of magnitude between whole blood GSHPx activity from cats in each trial (Figures 4A,B), indicating large normal variation in this parameter of selenium status in cats despite the fact that overall, there was no significant response of GSHPx activities to supplementation of either form of selenium.

It has previously been reported that selenium concentrations in the plasma and serum of cats are up to five times greater than in other species (Forrer *et al.*, 1991; Foster *et al.*, 2001). These two studies investigated plasma and serum selenium concentrations in cats fed commercial feline diets and samples were obtained from cats in Europe and Australasia. The plasma and serum values reported by these authors are similar to those found in the present study, where concentrations ranged from 4.6 to 8.4 µmol/L in individual cats fed the four diets. The observation that cats have higher plasma selenium levels compared to other species was suspected to be due in part to the high dietary selenium concentrations of commercial cat foods. This is demonstrated in the present study which shows higher dietary concentrations of selenium will result in higher plasma or serum selenium concentrations in cats. The

range of selenium concentrations in New Zealand petfoods is approximately 7.5 times higher and 9 times wider in cat foods compared to dog foods (Simcock *et al.*, 2005) with concentrations as high as 6 µg Se/g DM found. Similar levels have been reported in work by Mumma *et al.* (1986) who analysed toxic and protective constituents in petfoods from the USA. However cats still showed higher concentrations of selenium in serum compared to dogs when both were fed the same diet (Wedekind *et al.*, 2003a). It was suggested by the authors that this was due to insufficient regulation of selenium in cats, however additional information including the amount of selenium retained and the amount of selenium excreted in faeces and urine is needed to determine this.

Based on work by Wedekind (1997; 1998; 1999; 2000; 2002; 2003b) the minimum dietary selenium requirement for cats and dogs of 0.1 mg Se/kg (National Research Council, 1985; 1986) is too low. The National Research Council recently reviewed and updated these requirements based on Wedekinds work, however the final version had not been published at the time of writing. Even with this work, there are still many gaps in the knowledge of selenium metabolism in cats and there is a need to establish the specific dietary requirements of selenium for these species, along with appropriate biological parameters to determine dietary adequacy. Results from this study provide some insight as to the response of cats to selenium supplementation as indicated by such parameters. Due to practical constraints, only the minimum number of animals required to elicit a significant difference was used in each group (n=4). It is possible that with greater numbers, the variability seen with some parameters may have been reduced resulting in more obvious and significant differences. Of the parameters used here, plasma selenium was the most useful indicator of selenium levels, reflecting dietary intake of selenium above that of control cats and providing information regarding the short term response of selenium at the lower levels of supplementation chosen. However, there were no differences between different levels of supplementation and it remains to be studied if higher levels of selenium supplementation would induce further changes in selenium blood parameters. Whole blood selenium showed less distinct patterns and may not have been entirely appropriate for use under the conditions of this study, but may be a more useful indicator of long term selenium status. Although differences were not significant in the current study, patterns of GSHPx activities in conjunction with plasma selenium responses, may provide useful insight as to how different forms of selenium are utilised over long term supplementation. Selenium concentrations of 0.4 µg Se/g DM did not result in maximal GSHPx activities which suggests the requirement of selenium for

these animals was not met. It may be that the selenium in the control diet had a low bioavailability, however the level at which maximal GSHPx activities occur in cats warrants further investigation. Responses of cats fed diets supplemented with inorganic or organic selenium were similar, although there was some indication that the different forms are utilised in different ways. A longer period of supplementation may be required to provide further evidence to confirm this. Supplementation of diets with both forms of selenium appeared to be beneficial in raising plasma selenium levels at concentrations of 1 µg Se/g DM, however there appeared to be no additional benefit of increasing dietary supplementation further to concentrations of between 1 and 2 µg Se/g DM.

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CHAPTER 3

Part ii

Metabolism of dietary inorganic and organic selenium in adult cats: apparent absorption, excretion and retention

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ABSTRACT

The current study investigated apparent absorption, faecal and urinary excretion and retention of two different forms of selenium in adult cats. A total of 20 adult domestic short-haired cats were fed a control diet containing 0.4 µg Se/g DM, or the control diet supplemented with either sodium selenite or an organic selenium yeast, to give approximate total selenium concentrations of 1.0, 1.5 and 2.0 µg Se/g DM for a period of 32 days in two separate studies. Twenty-four hour urine collections were pooled between days 0-2, 3-4, 5-8, 9-16, 17-24 and 25-32 to give six time periods. Twenty-four hour faecal collections were also pooled from days 25-32. Pooled urine and faeces from the last time period (days 25-32) were analysed for total selenium content and the results were used to estimate the amount of selenium absorbed, excreted and retained from the supplemented selenium. The form of dietary selenium did not affect the amount of selenium excreted in faeces or urine, or the amount of selenium apparently absorbed. Faecal excretion of selenium was high, and apparent absorption low, compared to published reports in other species. Supplemented selenium was better absorbed than selenium in the canned control diet by a magnitude of two to three times. Faecal excretion by cats fed the supplemented diets remained constant over the range of intakes whereas urinary excretion increased with intake. Thus in cats, as with other species, selenium metabolism appears to be regulated by the kidney within the dietary range of 0.4 to 2.0 µg Se/g DM.

INTRODUCTION

Although well defined in humans, livestock and experimental animals (Bopp *et al.*, 1982; National Research Council, 1983; Levander, 1986; Combs and Combs, 1986b; Foster and Sumar, 1997; Sunde, 2000; Jacques, 2001; Hawkes *et al.*, 2003; Schrauzer, 2003; Whanger, 2003; Suzuki, 2005), there has been little investigation of selenium metabolism in cats and dogs and whether the metabolic pathways in these animals differ from other species. This paucity of information has implications for determining adequate selenium requirements and selenium supplementation for cats and dogs. At present, reported requirements of selenium in cats are based on many assumptions and extrapolation of data from other species (National Research Council,

1985; 1986). This lack of specificity is less than ideal and may prevent the attainment of optimal supplementation and nutrition for these animals.

In an attempt to better understand selenium metabolism a study was conducted in cats supplemented with two forms of dietary selenium to investigate several aspects of selenium metabolism. This paper is the second part of the study and investigates apparent absorption, faecal and urinary excretion and retention of selenium. Thus the aims of the current study were to determine the extent to which supplemented selenium is absorbed and retained, to provide fundamental data regarding the degree of faecal and urinary selenium excretion, and to determine whether the form and level of selenium supplemented has an effect on apparent absorption, retention and excretion in adult cats.

MATERIALS AND METHODS

The experimental protocol for this study has previously been outlined in Chapter 3,i, Materials and Methods, where details on Animals and Treatments can also be found. The materials and methods outlined below for Part ii includes information relevant to this part of the study.

Sampling

Urine samples were collected quantitatively every 24 hours and pooled for each of six time periods: T1: 0-2d, T2: 3-4d, T3: 5-8d, T4: 9-16d, T5 17-24d and T6: 25-32. The last eight days of the 32 day experimental period (T6), were used as the collection period to determine faecal and urinary excretion. Food intake was recorded on a daily basis and subsamples of each of the four diets were taken daily and pooled. Faecal samples were collected quantitatively every 24 hours and pooled by cat, for the collection period. Diet, faecal and urine samples were stored at –20 ^{o}C prior to chemical analysis.

Chemical analysis

Diet and faecal samples were freeze-dried, ground to a fine powder and mixed thoroughly prior to selenium analysis, and analysed in quadruplicate. Hair was separated and removed from the faeces following freeze-drying. Urine samples were

analysed in duplicate. Samples were analysed using a fluorometric method as previously outlined in Chapter 2 and described in Appendix 1.

Statistical analysis

Data were analysed using SAS version 8.02 for Windows (SAS Institute Inc, Cary, NC, USA). In addition to the initial screening of the data for normality, outliers and homogeneity of variance as described in Part i, statistical analysis of data in Part ii was carried out as follows: an initial one way ANOVA was performed on the data with Duncan's multiple range tests to determine overall differences, thus the model for this analysis was: parameter of interest = diet + form of supplemented selenium + the interaction between diet and form, including Types I and III sum of squares, followed by multiple comparisons using Duncan's test. If differences did occur, the ANOVA was repeated with data sorted according to the "By" statement for diet and form in order to determine where differences occurred both within and between groups. For urinary selenium excretion (μmol/L) over the 32 day period (Figure 3), a repeated measures ANOVA was performed with the general linear model procedure using the model: parameter of interest = diet + time nested on diet, followed by multiple comparisons using least squared means. Apparent absorption was estimated by calculating the difference between dietary intake and faecal excretion, and retention was estimated by calculating the difference between dietary intake, faecal and urinary excretion. In addition, apparent absorption, excretion and retention of the supplemented selenium only, was estimated by subtracting the amount of selenium in the control diet from the total amount of selenium in the treatment diets: Apparent absorption, excretion and retention of total selenium in control animals, and supplemented selenium in treatment animals, are expressed as a percentage of dietary intake. Analyses were performed on both unranked and ranked data for all data and the most conservative p value reported. Results are presented as the mean ± SEM. In all cases, differences were considered significant at a probability level of 5%.

RESULTS

The weight of cats during the study and their dietary selenium intakes have been discussed previously (Chapter 3,i, Materials and Methods, Results).

Within groups (level of supplementation), the form of selenium supplemented did not result in any differences in faecal excretion ($p > 0.01$; Figure 1A, Table 1). For each form of selenium supplemented, faecal excretion of selenium by cats fed the supplemented diets was greater than in the control group ($p < 0.001$ inorganic; $p < 0.01$ for organic). With the exception of groups fed inorganic selenium at 1.0 and 2.0 µg Se/g DM, faecal excretion did not differ in cats fed the various levels of supplemented diets ($p > 0.05$).

Table 1. Mean (± SEM) concentrations of selenium (µg Se/kg body weight/day) excreted in faeces and urine, absorbed and retained by cats from days 25 to 32 when fed a control diet (0.4 µg Se/g DM), or the control diet supplemented with three different concentrations of inorganic or organic selenium to give total dietary selenium concentrations of 1.0, 1.5 and 2.0 µg Se/g DM (n=4).

Parameter	Form	Diet			
		Control	1.0 µg/g	1.5 µg/g	2.0 µg/g
Faeces	Inorganic	5.1 ± 0.5^{a}	9.6 ± 0.4^{b}	11.2 ± 1.2^{bc}	13.3 ± 1.9^{c}
	Organic	6.5 ± 0.6^{a}	11.1 ± 1.2^{b}	11.0 ± 0.5^{b}	13.7 ± 2.3^{b}
Urine	Inorganic	2.1 ± 0.1^{a}	5.9 ± 0.6^{a}	$10.6 \bullet 0.5^{b}$	16.9 ± 3.0^{c}
	Organic	$2.0 \bullet 0.1^{a}$	9.3 ± 1.4^{b}	12.4 ± 1.1^{b}	18.7 ± 2.6^{c}
Apparent absorption	Inorganic	1.4 ± 0.4^{a}	5.7 ± 0.7^{b}	11.3 ± 1.6^{c}	18.0 ± 0.1^{d}
	Organic	1.9 ± 0.3^{a}	9.9 ± 1.0^{b}	16.3 ± 2.2^{c}	23.5 ± 1.7^{d}
Retention	Inorganic	-0.6 ± 0.4^{a}	0.5 ± 0.8^{a}	1.3 ± 1.9^{a}	1.7 ± 3.0^{a}
	Organic	-0.1 ± 0.4^{a}	0.6 ± 1.7^{ab}	3.9 ± 2.0^{ab}	4.7 ± 2.1^{b}

Within rows, means with different superscripts are different ($p < 0.05$)

Excretion of selenium in urine was higher in cats fed organic selenium at 1.0 µg Se/g DM compared to those fed the inorganic the inorganic supplement, but there were no differences in urinary selenium excretion of the remaining animals fed the different forms of selenium during the collection period ($p > 0.05$; Figure 1B, Table 1). The amount of selenium excreted in the urine of cats fed the 0.4 and 1.0 µg Se/g DM of inorganic was similar ($p > 0.05$), but lower than the amount of selenium excreted by cats fed 1.5 and 2.0 µg Se/g DM ($p < 0.0001$). Cats fed 2.0 µg Se/g DM of inorganic selenium excreted more selenium in the urine than those fed the 1.5 µg Se/g DM diet ($p < 0.0001$). Cats fed 1.0 and 1.5 µg Se/g DM of organic selenium excreted similar amounts of selenium in urine ($p > 0.05$), and this level was both higher ($p < 0.0001$) and lower ($p < 0.0001$) than cats fed the 0.4 and 2.0 µg Se/g DM diets, respectively.

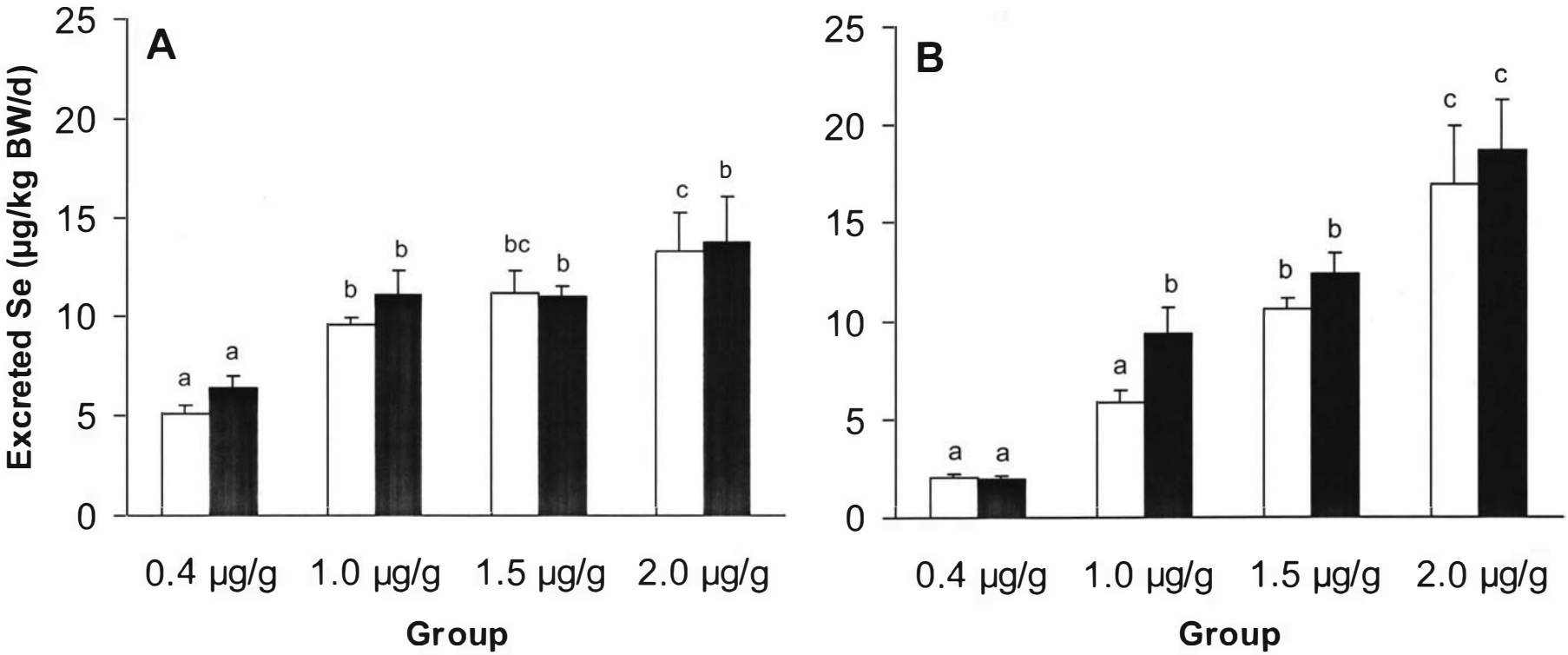

Figure 1. Mean (± SEM) total selenium (Se) concentrations (µg Se/kg body weight/day) excreted in the faeces (Panel A) and urine (Panel B) of cats from days 25 to 32 when fed a control diet (0.4 µg Se/g DM), or the control diet supplemented with three different concentrations of inorganic (□) or organic (■) selenium to give total dietary selenium concentrations of 1.0, 1.5 and 2.0 µg Se/g DM (n=4). Within each form of selenium (inorganic or organic), bars with different superscripts are different ($p < 0.05$).

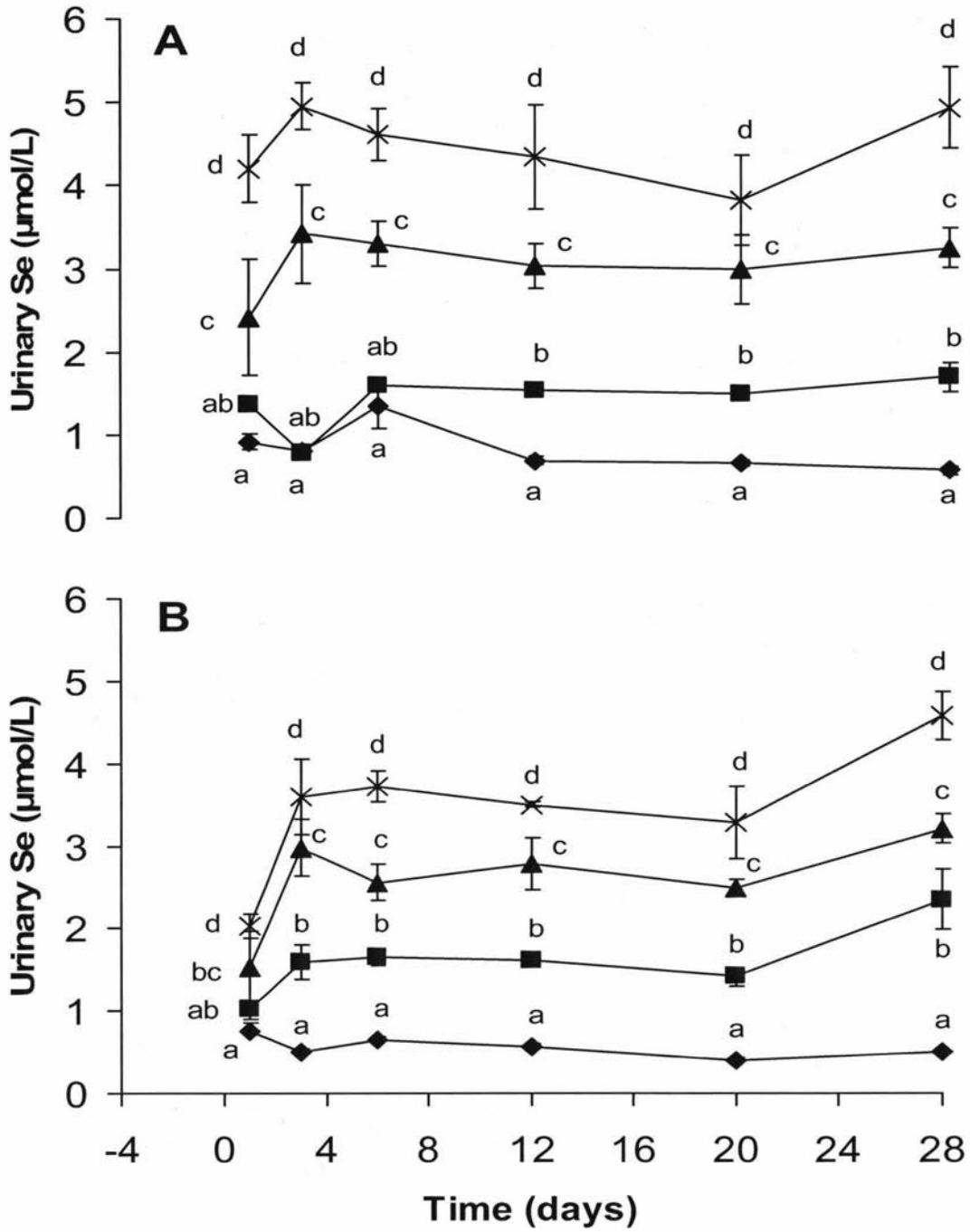

Figure 2. Mean (± SEM) urinary selenium (Se) concentrations (µmol/L) of cats fed a control diet containing 0.4 µg Se/g DM (—♦—), or control diet supplemented with inorganic selenium (Panel A) or organic selenium (Panel B) to achieve dietary selenium concentrations of 1.0 (—■—), 1.5 (—▲—), and 2.0 (—X—) µg Se/g DM (n=4). At each time point, values with different superscripts are different ($p < 0.05$).

Table 2. Mean (± SEM) concentrations of selenium excreted in urine (µmol/L) by cats fed a control diet (0.4 µg Se/g DM), or the control diet supplemented with three different concentrations of inorganic or organic selenium to give total dietary selenium concentrations of 1.0, 1.5 and 2.0 µg Se/g DM (n=4).

		Time (day)					
Form	Diet	1	3	6	12	20	28
Inorganic	Control	0.92 ± 0.10^{a}	0.81 ± 0.04^{a}	1.34 ± 0.27^{a}	0.69 ± 0.05^{a}	0.67 ± 0.04^{a}	0.57 ± 0.04^{a}
	1.0 µg/g	1.38 ± 0.08^{ab}	0.79 ± 0.90^{ab}	1.59 ± 0.06^{ab}	1.55 ± 0.02^{b}	1.50 ± 0.10^{b}	1.70 ± 0.18^{b}
	1.5 µg/g	2.42 ± 0.70^{c}	3.42 ± 0.59^{c}	3.30 ± 0.28^{c}	3.03 ± 0.27^{c}	2.99 ± 0.42^{c}	3.25 ± 0.25^{c}
	2.0 µg/g	4.20 ± 0.41^{d}	4.95 ± 0.28^{d}	4.61 ± 0.30^{d}	4.34 ± 0.62^{d}	3.83 ± 0.54^{d}	4.93 ± 0.49^{d}
Organic	Control	0.76 ± 0.11^{a}	0.50 ± 0.05^{a}	0.64 ± 0.04^{a}	0.56 ± 0.04^{a}	0.40 ± 0.03^{a}	0.49 ± 0.02^{a}
	1.0 µg/g	1.02 ± 0.12^{ab}	1.58 ± 0.21^{b}	1.64 ± 0.11^{b}	1.62 ± 0.10^{b}	1.41 ± 0.12^{b}	2.34 ± 0.37^{b}
	1.5 µg/g	1.52 ± 0.45^{bc}	2.98 ± 0.34^{c}	2.56 ± 0.21^{c}	2.78 ± 0.31^{c}	2.50 ± 0.09^{c}	3.20 ± 0.18^{c}
	2.0 µg/g	2.03 ± 0.14^{d}	3.60 ± 0.47^{d}	3.72 ± 0.19^{d}	3.50 ± 0.03^{d}	3.28 ± 0.44^{d}	4.58 ± 0.29^{d}

Within columns for each form of selenium, means with different superscripts are different ($p < 0.05$)

Over the 32 day experimental period, urinary selenium concentrations reflected dietary selenium intake in cats fed both inorganic and organic forms of selenium (Figure 2, Table 2). In cats fed inorganic selenium, selenium concentrations in urine increased with level of intake from T1 onwards ($p < 0.0001$; Figure 2A). In contrast, urinary selenium concentrations of cats fed organic selenium were initially low, but increased to reflect dietary intake by T2 ($p< 0.0001$; Figure 2B). From T2 onwards, the concentrations of selenium in urine remained constant for the remainder of the study in cats fed both inorganic and organic selenium ($p > 0.05$).

The pattern of excretion in faeces and urine was similar for both forms of selenium (Figure 1, Table 1). Although not significant in all cases, a pattern was observed whereby faecal excretion was higher than urinary excretion in cats fed lower concentrations of selenium (0.4 and 1.0 µg Se/g DM), and urinary excretion was higher than faecal excretion in cats fed the higher concentrations (1.5 and 2.0 µg Se/g DM). Faecal excretion was greater than urinary excretion in cats fed both forms of selenium at 0.4 µg Se/g DM, and in cats fed 1.0 µg Se/g DM of inorganic selenium ($p < 0.001$).

For both inorganic and organic forms of supplemented selenium, apparent absorption increased progressively with dietary intake ($p < 0.0001$; Figure 3A, Table 1). Cats fed 1.0 and 2.0 µg Se/g DM of organic selenium exhibited greater apparent absorption than those fed the inorganic form ($p < 0.01$). Retention of selenium during the collection period in cats fed both forms of selenium was highly variable (Figure 3B, Table 1). Cats fed organic selenium exhibited numerically higher values of retention than those fed inorganic selenium, however possibly due to the inherently high variability of the data obtained, these differences were not significant ($p > 0.05$). Retention also appeared to increase with dietary selenium intake, particularly with regard to those fed organic selenium, however this was only significant in cats fed the 0.4 and 2.0 µg Se/g DM of organic selenium ($p < 0.05$).

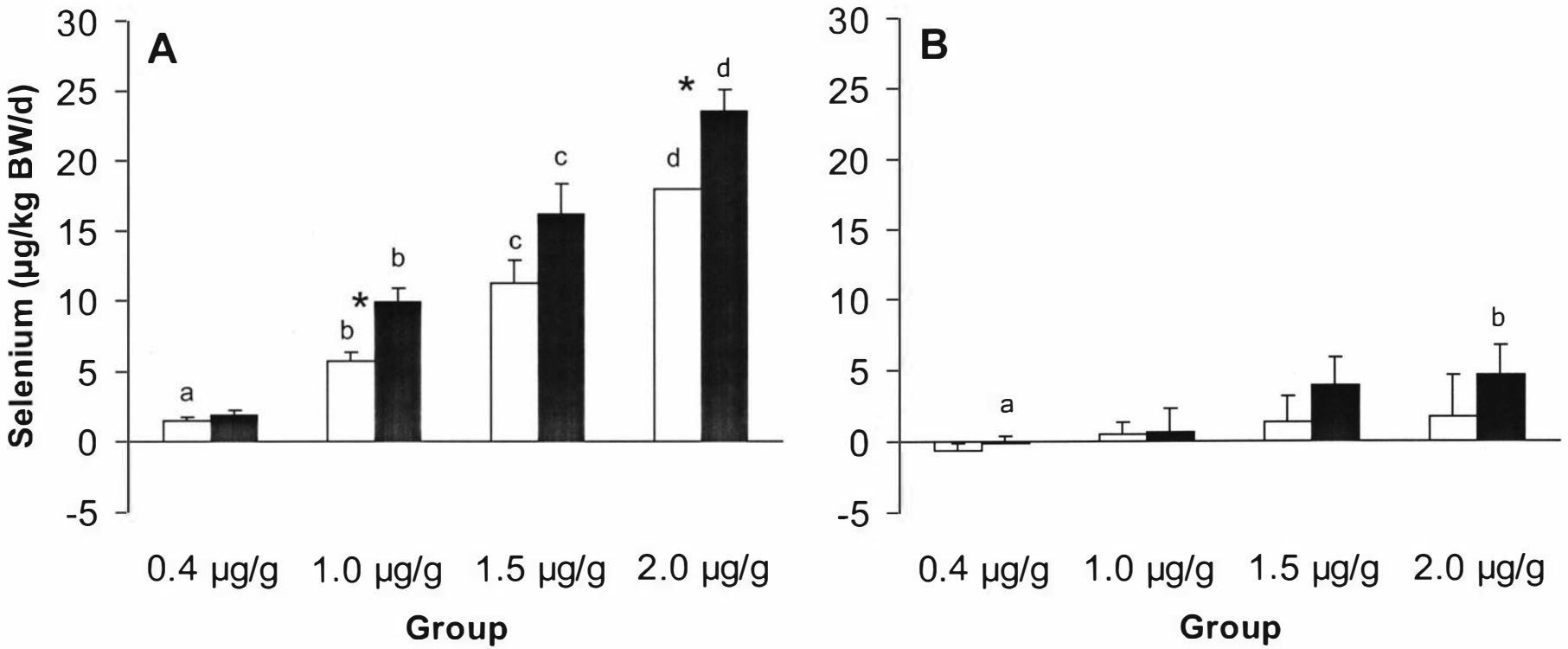

Figure 3. Mean (± SEM) total selenium (Se) concentrations (µg Se/kg body weight/day) absorbed (Panel A) and retained (Panel B) by cats from days 25 to 32 when fed a control diet (0.4 µg Se/g DM), or the control diet supplemented with three different concentrations of inorganic (□) or organic (■) selenium to give total dietary selenium concentrations of 1.0, 1.5 and 2.0 µg Se/g DM (n=4). Within each form of selenium (inorganic or organic), bars with different superscripts are different ($p < 0.05$); * denotes difference between form within a group ($p < 0.01$).

Table 3 shows faecal and urinary excretion, apparent absorption, and retention of total selenium (controls) and supplemented selenium (treatments) as a percentage of dietary intake in cats fed inorganic and organic selenium. The amount of supplemented selenium in treatment groups was calculated by difference from the amount of selenium absorbed, excreted or retained in control animals. Excretion and apparent absorption of selenium in cats fed the control and supplemented diets did not differ between cats fed inorganic and organic selenium ($p > 0.05$). The proportion of selenium excreted in faeces by cats fed the supplemented diets was two to three times lower than in controls, and therefore the proportion of fed selenium that was absorbed was two to three times higher than in controls ($p < 0.001$). Compared to control animals, the percentage of selenium excreted in urine by cats fed the supplemented diets was up to twice as high in cats fed inorganic selenium, and up to 2.5 times as high in cats fed organic selenium ($p < 0.05$). The percentage of selenium retained by cats fed the inorganic and organic selenium supplemented diets was higher than in those fed the control diets. Cats fed organic selenium showed numerically higher retention of selenium than those fed the inorganic form. However for all values variability was high and differences were not significant ($p > 0.05$).

Table 3. Mean (± SEM) percentage of selenium (Se) absorbed, excreted and retained by cats from days 25 to 32 when fed a control diet (0.4 µg Se/g DM), or the control diet supplemented with inorganic and organic selenium to give dietary selenium concentrations of 1.0 to 2.0 µg Se/g DM (Treatments) (n=4).

	Se excreted in faeces[1] (%)		Apparent Se absorption[1,2] (%)		Se excreted in urine[1] (%)		Se retained[1,3] (%)	
	Inorganic Trial	Organic Trial	Inorganic Trial	Organic Trial	Inorganic Trial	Organic Trial	Inorganic Trial	Organic Trial
Controls:	77.7 ± 5.9^{a}	77.3 ± 4.4^{a}	22.3 ± 5.9^{a}	22.7 ± 4.4^{a}	32.1 ± 2.5^{a}	23.9 ± 1.1^{a}	-9.8 ± 6.5	-1.1 ± 4.9
Treatments[4]:								
1.0 µg/g	51.3 ± 6.3^{b}	37.5 ± 7.6^{b}	48.8 ± 6.3^{b}	62.5 ± 7.6^{b}	43.0 ± 4.6^{ab}	56.9 ± 9.5^{b}	5.8 ± 8.8	5.6 ± 12.7
1.5 µg/g	38.4 ± 8.7^{bc}	26.4 ± 5.3^{b}	61.6 ± 8.7^{bc}	73.6 ± 5.3^{b}	54.0 ± 4.1^{b}	54.6 ± 5.4^{b}	7.6 ± 11.7	19.0 ± 9.9
2.0 µg/g	32.5 ± 4.4^{c}	25.2 ± 6.4^{b}	67.6 ± 4.4^{c}	74.8 ± 6.4^{b}	60.3 ± 13.0^{b}	57.7 ± 8.3^{b}	7.2 ± 12.6	17.1 ± 7.9

[1] calculated as a percentage of dietary intake

[2] calculated from the difference between dietary intake and faecal excretion

[3] calculated from the difference between dietary intake, faecal and urinary excretion

[4] values represent apparent absorption, excretion or retention of supplemented selenium only (calculated by difference from the amount of selenium in the control diet).

Within columns, means with different superscripts are different ($p < 0.05$)

DISCUSSION

There is little information available in the literature to indicate typical levels of faecal and urinary selenium loss in cats. In monogastric animals the main route of selenium elimination is via the kidneys (Robinson and Thomson, 1983) and urinary excretion is thought to be the primary means of controlling selenium metabolism (Robinson *et al.*, 1985). In ruminants, excretion of selenium via the faeces becomes more important (Levander, 1986). It is difficult to compare 'normal' levels of faecal and urinary excretion in different species from reports in the literature, as there are many variables that affect excretion. These include the selenium status of the animal, the level and form of supplemented selenium, and the way in which the dose is administered (Robberecht and Deelstra, 1984; Combs and Combs, 1986b).

In the present study, both faecal and urinary selenium concentrations (µg/kg BW/d) increased with dietary intake. Excretion via urine resulted in a progressive increase relating to dietary intake in the four groups. In contrast, total faecal excretion of selenium was higher in cats fed the supplemented diets compared to their respective controls, but there were no increases in excretion within the supplemented groups for each form of selenium. These results may be expected as there is no known gastrointestinal regulation of selenium absorption (Behne, 1988), and faecal selenium is primarily unabsorbed dietary selenium. Small amounts of selenium are excreted in faeces in monogastric animals over a wide range of intakes (Bopp *et al.*, 1982) and the constant level of selenium excreted in the faeces of cats fed the supplemented diets is consistent with reports in humans (Levander, 1986) and animals (Bopp *et al.*, 1982). It would also appear from this comparison of faecal and urinary excretion that excretion of selenium in the faeces is greater than that in urine when cats were fed lower dietary selenium concentrations, but at higher concentrations the opposite may be true. This has possible implications for the way in which selenium is regulated in cats. It may be that at low dietary selenium concentrations conservation of selenium is maximised by reducing urinary loss so that faecal excretion (unabsorbed selenium + endogenous losses) becomes the main source of selenium loss. At higher concentrations regulation occurs primarily by increasing urinary excretion to avoid selenium accumulation that could lead to toxicity. It has already been reported that selenium metabolism is well regulated by urinary excretion in humans and rats (Robinson *et al.*, 1985; Kirchgessner *et al.*, 1997), especially at low dietary intakes. Consequently, if the animal has a sufficient supply of whole body selenium and is in

equilibrium, it would be expected that urinary output would reflect dietary intake in order to maintain the selenium status of the animal as was seen in the present study.

The levels of selenium excreted in the faeces of cats in this study were somewhat higher than have been reported in other species. Cary *et al.* (1973) reported that selenium-depleted rats were fed different levels of selenium up to 0.15 ppm as either selenomethionine or selenite and faecal excretion ranged from 13 to 25% of dietary selenium intake. Other studies in rats fed selenite to the required level for up to 13 days showed total faecal excretion was 11 to 12% of dietary intake (Gabler *et al.*, 1997), while in those fed selenite, selenocysteine or selenomethionine for up to 35 days faecal excretion ranged from 8 to 18% of dietary intake (Windisch *et al.*, 1998). One study conducted in the 1930s looked at the excretion of selenium in cats given varying levels of selenium in daily doses as sodium selenite orally or subcutaneously for up to 188 days. They found low faecal selenium losses, ranging from trace amounts to 18% and this was thought to represent unabsorbed selenium (Smith *et al.*, 1937). In our study, excretion of selenium as a percentage of intake by cats fed the control diet was 77%, and although significantly lower, excretion by cats fed the supplemented diets ranged from 25 to 51%. This implies a significant proportion of selenium in the diets in our study was not absorbed by the cats. In addition, the large amount of selenium excreted by cats fed the control diets compared to the amount excreted by cats fed supplemented selenium is suggestive of a factor related to supplementation. It is possible there is an effect of processing on the availability of selenium in the diet, as the selenium added to the treatment diets in the present study was not subjected to heat processing.

With the exception of the first few days, the pattern of urinary selenium elimination during the study was similar for cats fed both organic (Sel-Plex™ selenium yeast) and inorganic (sodium selenite) forms of selenium, and reflected dietary intake (Figure 2). Urinary concentrations (µmol/L) of inorganic selenium reached maximum levels almost immediately, whereas concentrations of organic selenium in urine were initially similar in all groups and stabilised between days 2 and 4. This may be explained by the differences in metabolism of the two forms of selenium. Inorganic selenium not used for selenoprotein synthesis is excreted in the urine, whilst organic selenium as selenomethionine may also be incorporated into body proteins in place of methionine and stored (Suzuki, 2005). Consequently, inorganic selenium is metabolised and excreted at a faster rate than selenomethionine. There was no true baseline value for urinary excretion of selenium in the present study as the initial

urinary sample was pooled over a 2 day period. Thus the higher initial concentrations of selenium in the urine of cats fed inorganic selenium may have resulted from the rapid absorption and excretion of selenium from the supplement over the 2 day period, whereas, although selenomethionine is also absorbed rapidly, selenium from the organic supplement was still being metabolised and therefore not excreted in the urine to the same extent.

As with faecal data, in order to compare selenium in the urine of cats in the present study with published reports of urinary selenium in other species, the amount of selenium excreted in urine was calculated as a percent of dietary intake. Unfortunately, the significant differences in urinary selenium concentrations of control animals in each trial (inorganic and organic) indicate this urinary data should be considered with caution (Table 3). The reasons for these differences are unknown, as the amount of selenium excreted in the faeces of the same control animals were statistically similar. However the data does show a clear trend that reflects the amount of selenium absorbed. Up to twice as much selenium was excreted in the urine of cats fed inorganic selenium compared to controls, and up to 2.5 times the amount was excreted in cats fed organic selenium. Results from our study show 24 to 32% of ingested selenium was excreted in the urine of cats fed the control diet and 43 to 60% of dietary intake was excreted in cats supplemented with inorganic and organic selenium. In general, from 5 to 50% of the selenium dose given orally or parenterally as selenite was excreted in the urine of rats (Bopp *et al.*, 1982). In cats, 50 to 80% of selenium was excreted in the urine when fed graded doses of selenite for up to 188 days (Smith *et al.*, 1937). In humans, 50 to 70% of the total selenium excreted was found in urine over a wide range of dietary intakes (Robberecht and Deelstra, 1984), or 43 to 86% of the ingested selenium was excreted (National Research Council, 1983). Given the differences between the urinary excretion of control animals in this study and the difficulty standardising variables in order for comparisons to be made between different studies, it would be unwise to draw firm conclusions from this data, but it does contribute to the overall picture of selenium absorbed, excreted and retained in cats in this study.

Absorption may be estimated by measuring the disappearance of the nutrient in question from the gut, or from its appearance in various parts of the body including urine, blood and tissues (Robinson and Thomson, 1983). The metabolic balance technique is commonly used to determine the former (Sandstrom *et al.*, 1993). Absorption is taken as the difference between dietary intake and faecal output, where

the nutrient excreted in the faeces represents the unabsorbed portion of that nutrient (exogenous loss). This is known as "apparent absorption" and is often expressed as a percentage of dietary intake (Ammerman, 1995). The present study estimated the apparent absorption of inorganic and organic supplementary selenium in adult cats. Apparent absorption was derived from faecal data with the aim of also providing basic information as to the amount of selenium excreted in the faeces of cats. Ideally absorption and the concentration of selenium in faeces from only the supplemented form of selenium would have been analysed, however due to the inability to identify and distinguish between different forms of selenium present, only the amount of supplemented selenium absorbed or found in faeces was estimated. This was calculated by subtracting the amount of selenium in the control diet from the amount of selenium in each supplemented diet. Thus, these values reflect the amount of selenium in faeces and are only estimates of apparent absorption. It is assumed that active excretion through the gastrointestinal tract is minimal and/or constant and does not significantly affect the estimate of true selenium absorption.

Although difficult to directly compare as a result of variation in species, method used and amount of selenium administered, apparent absorption of selenium by cats fed the control diet in the present study was low compared to other reported data in which the average value of absorption for different forms and in different foods was 70% (Combs and Combs, 1986a; Van Dael *et al.*, 2001). In contrast, apparent absorption of supplemented selenium by cats in the treatment groups was around two to three times higher than in control animals (48.8 to 67.6% for inorganic, and 62.5 to 60.3% organic; Table 3) and levels were similar to ranges of absorption previously reported in humans (Combs and Combs, 1986a; Whanger, 1998). Although not significant, cats fed organic selenium showed slightly higher apparent absorption than those fed the inorganic form. This trend is consistent with other data which shows that organic forms of selenium are better absorbed than inorganic (Combs and Combs, 1986a).

Low bioavailability of selenium in the canned commercial petfood used in the current study may have resulted in higher faecal excretion rates when compared to other studies where animals were fed either synthetic diets or raw meat. Bioavailability of selenium in canned petfoods, as measured by a chick bioassay, have previously been reported to be low (17 to 30%) by Wedekind *et al.* (1997; 1998). True absorption is often used to define the availability of minerals (McDonald *et al.*, 2002). However this assumes the mineral is able to be utilised by the animal or stored

for future use, and in many situations this is not the case (Ammerman, 1995). With regard to selenium, different forms of the mineral are absorbed and metabolised by different pathways, therefore comparing estimates of bioavailability between forms could prove inaccurate (Ammerman, 1995). Bioavailability and true absorption were not measured in the present study but the estimates of apparent selenium absorption obtained may be used to provide some indication of its potential availability. The low amount of selenium absorbed by cats fed canned petfood in the present study (22%) parallels the low bioavailability estimates found by Wedekind *et al.* (1997; 1998). In addition, apparent absorption of supplemented selenium by cats in the treatment groups was two to three times higher than those of the controls. This is also consistent with other studies in which higher selenium bioavailabilities have been found for plant based petfood ingredients compared to whole petfoods (Wedekind *et al.*, 1998). Thus there appears to be a lower apparent absorption, and therefore potential availability, of selenium in whole petfoods compared to the supplement itself.

There are several factors that may affect selenium bioavailability including the presence of heavy metals (Hill, 1975), high dietary protein (Henry and Ammerman, 1995), and heat treatment of commercially processed canned petfoods (National Research Council, 1986). The high amount of selenium absorbed by cats fed a selenium supplement compared to those fed the control canned diet in the present study suggests processing of canned food may decrease selenium absorption, and consequently, its potential availability. This would also explain the overall high excretion and subsequent low apparent absorption of selenium exhibited by cats in the present study compared to reports in the literature, as the processed canned control diet was fed to both control and treatment animals in the present study.

The amount of selenium retained in the body was estimated by calculation of the difference between intake and faecal and urinary excretion in cats fed the two forms of selenium at different levels (Figure 3B). In making these calculations it is assumed that additional endogenous losses via faeces, urine, hair, nails, skin and lungs are negligible. Selenium loss through hair was calculated using hair growth data from Hendriks *et al.* (1997), and an estimation of selenium content in hair based on a range of values given in humans of 0.36 to 1.20 µg Se/g (Chen *et al.*, 1982; Sun and Hao-zhi, 2000). Losses through hair were estimated to be 0.5% of the dietary intake in cats fed the inorganic supplement and 1.3% in cats fed the organic supplement.

Estimations of retention resulted in large variation within groups with no significant differences, so only considered conclusions can be drawn from this data.

Selenium balance was negative in both groups of cats fed the control diets, and the difference in magnitude of negative balance between these cats may be associated with variation in the original selenium status of the cats in each group, possibly as a result of the different amount of dietary selenium ingested. In contrast selenium was retained in cats fed all supplemented diets. In cats fed the three different levels of inorganic selenium retention increased slightly with increased dietary intake. The increase in retention with increased dietary intake was also seen in cats fed organic selenium although to a much greater extent. This trend is more likely to be a significant effect had the error not been so large, and similar increases in retention due to organic selenium (selenomethionine and selenocysteine) compared to inorganic selenium (selenite) have been previously reported in rats (Cary *et al.*, 1973; Windisch *et al.*, 1998) and pigs (Mahan, 1995). Consequently these results were expected and resembled reports in other species. These results may be explained by the different metabolic fates of the two forms of selenium used. Little retention of selenium occurs when inorganic selenium as selenite is supplemented due to its rapid excretion if not immediately recruited for selenoprotein synthesis. Therefore, assuming the selenium balance of these animals was in equilibrium, minimal formation of selenoproteins would be required in order to maintain body pools at sufficient levels. The remainder of the selenium would be excreted in the urine in order to keep whole body selenium at a constant level.

Alternatively, if retention of selenium in cats fed selenite did increase significantly with increased dietary intake, this may be reflected in the liver, which is the primary site of selenoprotein synthesis, and in the levels of selenium in blood once selenoproteins are released from the liver into circulation. In Part i of this study, plasma selenium concentrations in cats fed inorganic selenium (selenite) appeared to increase at the end of the 32 day period, whereas in cats fed organic selenium (selenium yeast) plasma selenium levels remained constant. There was evidence to suggest an increased production of the selenoprotein GSHPx occurring in the plasma which may explain this. Thus the potential increase in retention of selenium in cats fed inorganic selenium in the present study may be, in part, a reflection of increased GSHPx or other selenoproteins, in plasma. Despite the comparatively large retention in cats fed organic selenium, there was no increase in blood levels of GSHPx. This suggests retention in these animals was mainly due to other factors. Instead, the greater retention seen here may be attributed to the incorporation of selenomethionine from the organic selenium into general body proteins for storage.

The amount of selenium excreted in urine (µg/kg BW/d) was similar in cats fed both forms of selenium. This finding is contrary to what may be expected and is also in disagreement with published reports in which supplemented organic selenium had a greater retention and lower urinary excretion than inorganic selenium (Daniels, 1996). This has been attributed to a greater absorption of organic selenium compared to the inorganic form, which was also found in this study. Other studies have also found organic selenium to be better absorbed than inorganic (Bopp *et al.*, 1982; Windisch *et al.*, 1998). In the present study, as dietary selenium intake increased, whole body selenium retention also increased suggesting that a small proportion of both inorganic and organic selenium was being utilised, perhaps as circulating selenoproteins in order to maintain body pools, whilst a larger amount of organic selenium was being stored as a result of increased apparent absorption.

In summary, when cats were fed dietary selenium concentrations of 1.0 to 2.0 µg Se/g DM, the amount of selenium absorbed and excreted in faeces was not significantly affected by the form of selenium. There were trends to suggest that concentrations of selenium in urine increased with intake, whereas the amount of selenium in faeces remained constant over the range of dietary intakes. Organic selenium appeared to be better absorbed than inorganic selenium, and this was reflected in an increased retention of selenium in the body. Apparent absorption of supplemented selenium in cats fed the treatment diets was two to three times higher than in controls. Further work is required to determine whether absorption, and possibly bioavailability of selenium, is affected by heat processing.

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CHAPTER 4

Comparison of selenium balance and indicators of selenium status in cats and dogs fed high levels of dietary inorganic and organic selenium

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ABSTRACT

Cats and dogs have different nutritional requirements that are suspected to result from metabolic adaptations occurring during the course of evolution. Little is known of the metabolic pathways of selenium in cats and dogs, or whether their requirement for selenium differs from other animals. High levels of selenium have been reported in the plasma of cats with no reports of toxic effects, therefore it has been suggested cats may be able to tolerate higher levels of dietary selenium without adverse effects, compared to other species. This study investigated the response of cats and dogs to high levels of dietary inorganic and organic selenium to determine whether there were differences in the metabolic response of these animals.

In two separate studies, eighteen cats and eighteen dogs were fed a control diet containing 0.6 µg Se/g DM, or the control diet supplemented with inorganic (sodium selenite) or organic selenium (Sel-Plex™ selenium yeast) to give total selenium concentrations of 10 µg Se/g DM for three weeks, of which the last week was the collection period. Selenium concentrations and GSHPx activities in plasma were measured at the beginning and end of the three week period. Faeces and urine were collected daily, pooled for the collection period and used to estimate the amount of selenium absorbed and retained from the supplemented selenium. Plasma and urine samples were obtained from each animal at the end of the collection period for the determination of fractional selenium clearance. A liver biopsy procedure was also conducted at the end of the collection period for the determination of total liver selenium.

Dietary concentrations of 10 µg Se/g DM did not induce physical signs of toxicity in cats and dogs when fed these diets for three weeks and the form of selenium supplemented had no effect on the metabolic response of these animals to high dietary selenium intakes. Both species exhibited the same pattern of response to these dietary levels. However cats had higher concentrations of selenium in plasma, lower concentrations in liver, and greater amounts of selenium in faeces and urine compared to dogs. These results suggest that cats may be more efficient at metabolising and excreting excess selenium than dogs. Thus there is further evidence from this study to suggest metabolism of selenium by cats is different to other species.

INTRODUCTION

As a consequence of their independent evolutionary pathways, it has been proposed that specific metabolic adaptations have developed in cats and dogs to cater for the nature of their respective diets (Morris and Rogers, 1989). It is well established that cats and dogs have different nutritional requirements (Baker and Czarnecki-Maulden, 1991; Lowe and Markwell, 1995), and cats in particular have several nutritional idiosyncrasies not found in other species (MacDonald and Rogers, 1984). Differences in the metabolism of protein and amino acids, carbohydrates, fats and vitamins in these animals and their corresponding nutritional requirements are generally well understood, however knowledge is lacking with regard to mineral requirements. There do not appear to be any known nutritional idiosyncrasies regarding the mineral requirements of cats and dogs, and it is assumed they have similar needs to other species (National Research Council, 1985; 1986). However there is very little data to support this assumption. Although there is little published information on selenium metabolism in these species, cats in particular show several unique peculiarities associated with sulphur-containing compounds. These include a higher requirement of dietary sulphur amino acids than dogs and other animals (MacDonald and Rogers, 1984); an inability to synthesise sufficient taurine, which, when coupled with a high metabolic demand for this sulphur amino acid, makes it an essential dietary component for cats (Morris *et al.*, 1990), and excretion of several sulphur amino acids in their urine (felinine, isovalthine and isobuteine), the biological significance of which has yet to be determined (Hendriks, 1999). These differences in sulphur amino acid metabolism in cats and dogs may also extend to the metabolism of selenium as a result of the chemical similarities of selenium and sulphur, and the shared metabolic pathways of the sulphur amino acids selenomethionine and selenocysteine with methionine and cysteine respectively.

There is some evidence to suggest cats tolerate higher levels of dietary selenium than other species. As previously discussed in Chapter 1, the estimated maximum recommended level of dietary selenium intake for dogs is 2 µg Se/g DM (AAFCO, 2000), and there is no maximum level reported for cats. Subsequent work estimated a safe upper level of dietary selenium intake for adult cats and dogs (Wedekind *et al.*, 2002; 2003), and although data proved to be inconclusive, levels of 5 µg Se/g DM appeared to be excessive when dogs were fed selenomethionine (Wedekind *et al.*, 2002). In humans and livestock chronic effects of toxicity are seen at dietary selenium concentrations of 5 µg Se/g DM (Koller and Exon, 1986), yet levels of selenium in

some cat foods have been found to be as high as 6 µg Se/g DM with no reports of toxicity. There have also been reports of higher blood levels of selenium in cats compared to dogs (Forrer *et al.*, 1991; Foster *et al.*, 2001; Wedekind *et al.*, 2003). In two separate studies, concentrations of selenium in serum and plasma of cats were up to five times greater than in other species (Forrer *et al.*, 1991; Foster *et al.*, 2001). These high levels were thought to reflect high levels of selenium in cat foods, however Wedekind *et al.* (2003) found higher selenium concentrations in cats compared to dogs even when fed similar dietary selenium concentrations. For this reason, the author hypothesised that serum selenium concentrations in cats are not as well regulated as in other species. High blood levels alone are not indicative of inadequate regulation, so without additional information regarding the amount of selenium being retained and excreted, the degree to which selenium is regulated cannot be determined. The high blood selenium concentrations reported in cats provides further evidence to suggest selenium metabolism in the cat differs from other species, as despite these high levels there have been no reports of adverse effects.

The aim of this study was to investigate the response of cats and dogs to high levels of inorganic and organic dietary selenium in order to determine differences between species and the way in which different forms of selenium are metabolised, to gain further understanding of selenium metabolism in cats and dogs.

METHODS

The study reported here was approved by, and conformed to, the requirements of the Massey University Animal Ethics Committee (Anonymous, 2003). The study was conducted as two identical trials run consecutively, one for cats and one for dogs.

Animals

Eighteen short-haired domestic cats (nine males, nine females) and 18 harrier hounds (nine males, nine females) were used for the study. Cats ranged from 1 to 4 years of age and weighed between 2.80 and 4.96 kg (3.69 ± 1.19 kg, mean ± SEM); and dogs ranged from 2 to 8 years of age and weighed between 16.0 and 27.0 kg (22.9 ± 0.6 kg, mean ± SEM) at the beginning of the trial.

One month before each trial, all animals were fed the control diet to standardise selenium intake. The control diet used for both trials was a commercial moist feline diet (Heinz Wattie's, Hastings, New Zealand) that had passed a minimum adult

maintenance feeding protocol (AAFCO, 2000; refer to Chapter 3,i - Table 1 for composition and amino acid profile of this diet) and contained a selenium content of 0.6 µg Se/g DM. Throughout the trial animals were fed to meet their daily energy requirements: 70kcal/kg BW/d for cats (National Research Council, 1986); and 110W x $BW^{0.75}$ kcal/kg BW/d for dogs (National Research Council, 1985), and had access to deionised water at all times.

The trial consisted of a two week adaptation period where the animals were fed their respective treatment diets, and a seven day collection period in which samples were obtained for analysis. During the adaptation and collection periods the animals were housed individually in metabolism cages to ensure they received the appropriate diet, to monitor dietary intake and to enable the separate collection of faecal and urine samples during the collection period. The design of the metabolism cages for cats is described by Hendriks *et al.* (1999; Appendix 3). Metabolism cages for dogs had a metal mesh floor to retain faeces and enable urine to pass through. Urine was then funnelled into a bucket for collection. The floor area of the cages was 0.9 m^2 to meet recommended requirements as described by Bate (1997). Dogs were exercised for 15 to 20 minutes twice a day. All animals were weighed weekly for the duration of both studies.

Treatments

Two different forms of selenium were used to supplement the control diet and throughout this paper they will be referred to as "inorganic" and "organic" selenium. The inorganic supplement used for the first trial was sodium selenite (a 1% premix of sodium selenite and limeflour; Nutritech International Ltd, Auckland, New Zealand), and the organic supplement used for the second was a selenium yeast (Sel-Plex™: containing selenomethionine, selenocysteine, and other selenoproteins and organo-selenium compounds; Alltech Inc, Nicholasville, Kentucky, USA). For each trial there were three groups of six animals, each group assigned to one of the three treatment diets. The three trial diets included the control diet (0.6 µg Se/g DM), and the two treatment diets supplemented with the respective form of selenium to obtain a total selenium concentration of 10 µg Se/g DM. The supplemented diets were prepared fresh daily. Target and actual selenium concentrations in the diets are shown in Table 1.

Sampling

At the beginning of the two-week adaptation period the animals were allocated to one of the three weight-balanced dietary treatments, with three males and three females in each. A preprandial baseline blood sample (5 ml) was taken from each animal by jugular venipuncture using a 23 gauge needle for dogs, and a 25 gauge needle for cats. Blood was collected into heparinised 5 ml tubes and centrifuged for 10 minutes at 3,000 rpm. Plasma was then removed and three aliquots were taken for the analysis of total selenium and creatinine concentrations and GSHPx activities. Following the blood sample animals were transferred to metabolism cages and fed their respective, pre-weighed diets once daily for the duration of the adaptation and collection periods (as described above).

During the collection period food intake was recorded on a daily basis and subsamples of each of the three diets were taken daily and pooled. Faecal and urine samples were collected quantitatively every 24 hours and pooled. Two aliquots of the pooled urine sample were taken for the analysis of total selenium and creatinine concentrations. At the end of the collection period a second preprandial blood sample was taken as described above. Immediately after, a voided urine sample was collected from each animal for the analysis of selenium and creatinine concentrations to determine the fractional clearance of selenium. Following the collection period, a liver biopsy was obtained from each animal for analysis of total selenium concentrations as described below. The specific gravity of each pooled and individual urine sample was measured. Animals were weighed weekly for the duration of the study. All samples were stored at –20 ^{0}C prior to sample preparation and chemical analysis.

Liver biopsies

A 0.7 to 1 g portion of liver was obtained from each cat and dog to measure selenium content. General anaesthesia was induced with ketamine/diazepan following premedication with atropine and acepromacine in cats, and atropine, acepromacine and butorphanol premedication followed by intravenous thiopentone in dogs. Both species were maintained under anaesthesia and analgesia with halothane. A small, preumbilical, midline incision was used to exteriorise a liver lobe and take a wedge biopsy of its border. One or two catgut stitches were used to control haemorrhage of the liver parenquima (Cole *et al.*, 2002). Routine abdominal closure followed. All animals received amoxycillin and ketofen or carprofen for pain relief afterwards. Samples were immediately weighed and frozen.

Chemical analysis

Diet, liver and faecal samples were freeze-dried, ground to a fine powder using an electric grinder (Model CG-2; Breville, Oldham, UK) and mixed thoroughly prior to selenium analysis. Hair was separated and removed from the faeces following freeze-drying. Diet, liver and faecal samples were analysed in quadruplicate while plasma and urine samples were analysed in duplicate. Samples with replicates having a coefficient of variation greater than 10% were subjected to further analysis until variability was reduced below this level. Total selenium concentrations of all samples were analysed using a fluorometric method as previously outlined in Chapter 2 and described in Appendix 1. Plasma GSHPx activities were assayed using a Ransel diagnostic kit and controls manufactured by Randox Laboratories Ltd (Antrim, Northern Ireland) on a Roche Cobas Fara II System (Basel, Switzerland). Plasma and urine creatinine samples were analysed using Roche Creatinine Jaffé method (rate-blanked and compensated) on a Hitachi 912 system.

Statistical analysis

Data were analysed using SAS version 8.02 for Windows (SAS Institute Inc, Cary, NC, USA). In addition to the initial screening of the data for normality, outliers and homogeneity of variance as described in Chapter 3, Part i, statistical analysis of data are as follows: an initial one way ANOVA was performed on all data to determine overall differences, the model for this analysis was: parameter of interest = diet species + the interaction between diet and species, including Types I and III sum of squares, followed by multiple comparisons using Duncan's test. If differences did occur, the ANOVA was repeated with data sorted according to the "By" statement for diet and species in order to determine where differences occurred both within and between groups. When data did not adhere to a normal distribution and/or variances were unequal, the analysis was repeated on ranked data and results from both ranked and unranked analyses were compared. Differences that were common to both tests were reported and the most conservative p value was used. Analyses were performed on both unranked and ranked data for faecal and urinary excretion (Table 5), apparent absorption (Table 7) and retention (Table 8). In all cases the differences found to be significant on unranked data were also significant when analysis was performed on the ranked data. Apparent absorption was estimated by calculating the difference between dietary intake and faecal excretion, and retention was estimated by calculating the difference between dietary intake, faecal and urinary excretion. In addition, apparent

absorption, excretion and retention of the supplemented selenium only, was estimated by subtracting the amount of selenium in the control diet from the total amount of selenium in the treatment diets: In addition, apparent absorption, excretion and retention of the supplemented selenium only, was estimated by subtracting the amount of selenium in the control diet from the total amount of selenium in the treatment diets: Apparent absorption, excretion and retention of total selenium in control animals, and supplemented selenium in treatment animals, are expressed as a percentage of dietary intake.

Fractional clearance of selenium was calculated according to the equation:

$$\text{Clearance (\%)} = \frac{(\text{UrSe} \times \text{PlCr})}{(\text{UrCr} \times \text{PlSe})} \times 100$$

where UrSe = urinary selenium, PlCr = plasma creatinine, UrCr = urinary creatinine and PlSe = plasma selenium.

To account for the ordinal nature of this data, data was transformed to a continuous scale by multiplying the % clearance by plasma selenium. A one way ANOVA was performed on the resulting data as described above. In all cases, differences were considered significant at a probability level of 5%.

RESULTS

All animals remained healthy throughout both trials. Average (± SEM) food intake during the collection period was 335 ± 10 g for cats and 1517 ± 31 g for dogs. The determined concentrations of selenium in the diets in the two trials are shown in Table 1.

Table 1. Selenium concentrations (µg Se/g DM) in diets supplemented with inorganic or organic selenium.

Group	Target level	Actual level[1] (µg Se/g DM)	
		Cat Trial	Dog Trial
1 - Control	0.6	0.59	0.60
2 - Inorganic	10	8.39	8.66
3 - Organic	10	8.56	9.88

[1]*actual values obtained from the mean of quadruplicate samples pooled over each trial*

Actual concentrations of selenium in the diets prepared for the cat trial were up to 1.61 µg Se/g DM (16%) lower than the target concentrations, whereas the actual concentrations of selenium in diets for the dog trial were lower than the target concentrations by approximately up to 1.34 µg Se/g DM (13.4%). The difference in concentration within the same groups in the two different trials were 0.01 µg Se/g DM (1.7%) for the controls, 0.27 µg Se/g DM (3.2%) for the inorganic groups and 1.32 µg Se/g DM (15.4%) for the organic groups.

Concentrations of selenium in plasma were consistently higher in cats compared to dogs both before ($p < 0.0001$) and after ($p < 0.05$) the treatment diets were fed (Table 2). After three weeks on their respective diets, plasma concentrations increased in animals fed the treatment diets compared to those fed the control diet ($p < 0.05$). This increase was similar for dogs fed both forms of selenium, whereas plasma selenium concentrations were higher in cats fed organic selenium compared to those fed the inorganic form ($p < 0.05$). Plasma GSHPx activities did not change in response to the increased dietary selenium intake of different forms in cats or dogs and there were no differences in these activities between species ($p > 0.05$; Table 3).

Table 2. Mean (± SEM) plasma selenium (Se) concentrations (µmol/L) in cats and dogs on day 0 (sample 1) and after three weeks (sample 2) fed a control diet (0.6 µg Se/g DM), or control diet supplemented with inorganic or organic selenium to give total dietary selenium concentrations of 10 µg Se/g DM (n=6).

Sample	Species	Diet	Plasma Se (µmo/L) mean ± SEM	Pr > F Diet	Pr > F Species	Pr > F Diet x Species
1	Cats	Control	5.7 ± 0.2	0.523	<0.0001	0.5755
		Inorganic	5.2 ± 0.2			
		Organic	5.2 ± 0.2			
	Dogs	Control	2.9 ± 0.8			
		Inorganic	3.6 ± 0.3			
		Organic	3.4 ± 0.2			
2	Cats	Control	5.0 ± 0.2 [a]	<0.0001	0.0003	0.3872
		Inorganic	7.8 ± 0.2 [b]			
		Organic	9.1 ± 0.1 [c]			
	Dogs	Control	4.2 ± 0.2 [a]			
		Inorganic	7.2 ± 0.2 [b]			
		Organic	7.8 ± 0.6 [b]			

Differences between diets for each species are indicated by different superscripts ($p < 0.05$)

Table 3. Mean (± SEM) plasma glutathione peroxidase (GSHPx) concentrations (U/L) in cats and dogs on day 0 (sample 1) and after three weeks (sample 2) fed a control diet (0.6 µg Se/g DM), or control diet supplemented with inorganic or organic selenium to give total dietary selenium concentrations of 10 µg Se/g DM (n=6).

Sample	Species	Diet	Plasma GSHPx (U/L) mean ± SEM	Pr > F Diet	Pr > F Species	Pr > F Diet x Species
1	Cat	Control	11007 ± 657	0.9038	0.7702	0.0995
		Inorganic	9493 ± 1892			
		Organic	11691 ± 923			
	Dogs	Control	10689 ± 666			
		Inorganic	11386 ± 461			
		Organic	9445 ± 820			
2	Cats	Control	12611 ± 1654	0.2356	0.1749	0.6463
		Inorganic	13751 ± 1214			
		Organic	11108 ± 647			
	Dogs	Control	11834 ± 947			
		Inorganic	11449 ± 990			
		Organic	10638 ± 1151			

Liver selenium concentrations in animals fed the control diet were similar for cats and dogs ($p > 0.05$; Table 4). Both species had greater concentrations of selenium in the liver when fed the supplemented selenium diets compared to those fed the control diet ($p < 0.001$), and these concentrations were similar in animals fed the two forms of selenium ($p > 0.05$). However within these groups, concentrations of selenium in the liver were higher in dogs compared to cats ($p < 0.001$).

Table 4. Mean (± SEM) liver selenium (Se) concentrations (µg Se/g DM) in cats and dogs after three weeks fed a control diet (0.6 µg Se/g DM), or control diet supplemented with inorganic or organic selenium to give total dietary selenium concentrations of 10 µg Se/g DM (n=6).

Species	Diet	Liver Se (µg Se/g DM) mean ± SEM	Pr > F Diet	Species	Diet x Species
Cats	Control	1.4 ± 0.1 [a]	<0.0001	<0.0001	0.0003
	Inorganic	4.2 ± 1.1 [b]			
	Organic	4.8 ± 0.4 [b]			
Dogs	Control	1.4 ± 0.1 [a]			
	Inorganic	7.5 ± 0.4 [b]			
	Organic	7.9 ± 0.6 [b]			

Differences between diets for each species are indicated by different superscripts ($p < 0.05$)

Although dogs excreted lower amounts of selenium in the faeces and urine compared to cats, the pattern of excretion was the same for both species (Table 5). A greater amount of selenium was excreted in the faeces compared to the urine in control animals ($p < 0.0001$), whereas in those fed the treatment diets, concentrations of selenium excreted in urine were greater than in faeces ($p < 0.0001$). The form of selenium supplemented did not have an effect on selenium excretion in cats ($p > 0.05$), but in dogs fed organic selenium, concentrations excreted in the faeces were slightly higher than in those fed the inorganic form ($p < 0.05$).

For both cats and dogs, the fractional clearance of selenium from the plasma was greater in animals fed the two forms of selenium than in the controls ($p < 0.0001$; Table 6), and within these treatment groups cats demonstrated a greater clearance of selenium than dogs ($p < 0.0001$).

Table 5. Mean (± SEM) faecal and urinary selenium (Se) concentrations (µg/kg body weight/day) in cats and dogs after three weeks fed a control diet (0.6 µg Se/g DM), or control diet supplemented with inorganic or organic selenium to give total dietary selenium concentrations of 10 µg Se/g DM (n=6).

Species	Excretion	Diet	Excreted Se (µg/kg BW/d) mean ± SEM	Pr > F		
				Diet	Excretion	Diet x Excretion
Cats	Faeces	Control	9.4 ± 0.7 [a]	<0.0001	<0.0001	<0.0001
		Inorganic	45.3 ± 4.9 [b]			
		Organic	52.1 ± 3.6 [b]			
	Urine	Control	2.0 ± 0.3 [a]			
		Inorganic	115.1 ± 3.9 [b]			
		Organic	104.8 ± 4.8 [b]			
Dogs	Faeces	Control	6.0 ± 0.5 [a]	<0.0001	<0.0001	<0.0001
		Inorganic	22.1 ± 2.0 [b]			
		Organic	33.6 ± 2.1 [c]			
	Urine	Control	2.3 ± 0.2 [a]			
		Inorganic	73.6 ± 5.1 [b]			
		Organic	67.0 ± 3.3 [b]			

Differences between diets for each species are indicated by different superscripts ($p < 0.05$)

Table 6. Mean (± SEM) fractional clearance of selenium (%) in cats and dogs after three weeks fed a control diet (0.6 µg Se/g DM), or control diet supplemented with inorganic or organic selenium to give total dietary selenium concentrations of 10 µg Se/g DM (n=6).

Species	Diet	Clearance (%) mean ± SEM	Pr > F		
			Diet	Species	Diet x Species
Cats	Control	0.2 ± 0.0 [a]	<0.0001	<0.0001	0.1694
	Inorganic	4.3 ± 0.4 [b]			
	Organic	3.2 ± 0.1 [b]			
Dogs	Control	0.2 ± 0.0 [a]			
	Inorganic	2.7 ± 0.3 [b]			
	Organic	2.4 ± 0.4 [b]			

Differences between diets for each species are indicated by different superscripts ($p < 0.05$)

The form of selenium supplemented had no effect on level of apparent absorption ($p > 0.05$). Apparent digestibility of selenium (selenium ingested – faecal selenium) was considered an estimate of apparent absorption as it was assumed that the excretion through the gastrointestinal tract was minimal and/or constant. Apparent absorption was increased by supplementation of selenium ($p < 0.0001$) to the same degree ($p > 0.05$) in cats and dogs (Table 7).

Table 7. Mean (± SEM) concentrations of selenium (Se) absorbed (µg/kg body weight/day) by cats and dogs after three weeks fed a control diet (0.6 µg Se/g DM), or control diet supplemented with inorganic or organic selenium to give total dietary selenium concentrations of 10 µg Se/g DM (n=6).

Species	Diet	Absorbed Se (µg/kg BW/d) mean ± SEM	Pr > F		
			Diet	Species	Diet x Species
Cats	Control	1.3 ± 0.7 [a]	<0.0001	0.2394	0.4443
	Inorganic	95.0 ± 5.8 [b]			
	Organic	103.1 ± 2.4 [b]			
Dogs	Control	2.0 ± 0.4 [a]			
	Inorganic	92.3 ± 2.9 [b]			
	Organic	96.6 ± 2.0 [b]			

Differences between diets for each species are indicated by different superscripts ($p < 0.05$)

Cats fed the control diet and the treatment diet supplemented with organic selenium showed neither loss nor retention of selenium ($p > 0.05$; Table 8), whereas in cats fed inorganic selenium there was a net loss of selenium ($p < 0.0005$). The balance of selenium in dogs fed the control diet was also in equilibrium. In contrast, dogs fed the treatment diets showed net retention of selenium ($p < 0.0001$), the level of which was greater in animals fed the organic form ($p < 0.0001$).

Table 8. Mean (± SEM) concentrations of selenium (Se) retained (µg/kg body weight/day) by cats and dogs after three weeks fed a control diet (0.6 µg Se/g DM), or control diet supplemented with inorganic or organic selenium to give total dietary selenium concentrations of 10 µg Se/g DM (n=6).

Species	Diet	Retained Se (µg/kg BW/d) mean ± SEM	Pr > F		
			Diet	Species	Diet x Species
Cats	Control	-1.1 ± 0.5 [a]	0.0005	<0.0001	<0.0001
	Inorganic	-20.1 ± 5.9 [b]			
	Organic	-1.7 ± 4.0 [a]			
Dogs	Control	-0.4 ± 0.4 [a]			
	Inorganic	18.7 ± 3.5 [b]			
	Organic	30.6 ± 1.9 [c]			

Differences between diets for each species are indicated by different superscripts ($p < 0.05$)

Table 9 shows faecal and urinary excretion, apparent absorption, and retention of total selenium (controls) and supplemented selenium (treatments) as a percentage of dietary intake in cats and dogs fed inorganic and organic selenium. The amount of supplemented selenium in treatment groups was calculated by difference from the amount of selenium absorbed, excreted or retained in control animals. Thus as a percentage of dietary intake, the amount of selenium excreted in the faeces of cats and dogs fed treatment diets was three to five times lower than the amount of selenium excreted by control animals ($p < 0.0001$). Conversely, the amount of selenium absorbed as a percentage of dietary intake was three to six times higher in the treatment groups than in the controls ($p < 0.0001$). Much less selenium was excreted in the urine of control animals whereas urinary excretion of selenium in animals fed the supplemented diets increased by up to four times. Selenium balance as a percentage of dietary intake for cats showed a net loss, whereas in dogs, selenium was retained in those animals fed supplemented selenium ($p < 0.05$).

Table 9. Mean (± SEM) percentage of selenium (Se) absorbed, excreted and retained by cats and dogs for three weeks when fed a control diet (0.6 µg Se/g DM), or control diet supplemented with inorganic and organic selenium to give dietary selenium concentrations of 10 µg Se/g DM (Treatments) (n=6).

	Se excreted in faeces (%)[1]		Apparent Se absorption (%)[1,2]		Se excreted in urine (%)[1]		Se retained (%)[1,3]	
	Cat Trial	Dog Trial	Cat Trial	Dog Trial	Cat Trial	Dog Trial	Cat Trial	Dog Trial
Controls:	88.1 ± 6.7^{a}	75.3 ± 5.1^{a}	11.9 ± 6.7^{a}	24.7 ± 5.1^{a}	18.8 ± 3.0^{a}	29.1 ± 3.5^{a}	-10.2 ± 5.0^{ab}	-4.3 ± 5.3^{a}
Treatments[4]:								
Inorganic	28.1 ± 3.7^{b}	15.3 ± 1.9^{b}	72.0 ± 3.4^{b}	84.8 ±1.9^{b}	87.2 ± 3.8^{b}	67.0 ± 4.8^{b}	-15.2 ± 4.5^{a}	17.8 ● 3.2^{b}
Organic	29.4 ± 1.8^{b}	22.6 ± 1.3^{b}	70.6 ± 1.8^{b}	77.4 ± 1.3^{b}	71.0 ± 2.0^{c}	52.5 ± 2.3^{c}	-0.45 ± 2.7^{b}	25.4 ± 1.9^{b}

[1] calculated as a percentage of dietary intake
[2] calculated from the difference between dietary intake and faecal excretion
[3] calculated from the difference between dietary intake, faecal and urinary excretion
[4] values represent apparent absorption, excretion or retention of supplemented selenium only (calculated by difference from the amount of selenium in the control diet).

Within columns, means with different superscripts are different ($p < 0.05$)

DISCUSSION

The level of selenium at which toxic effects occur in cats and dogs has yet to be determined, but evidence suggests cats are able to tolerate higher levels without adverse effect. This study investigated the way in which cats and dogs responded to high levels of dietary selenium intake of different forms.

Baseline plasma selenium concentrations of cats were higher than those of dogs when fed the same diets, containing the same selenium concentration. This supports previous findings in which plasma selenium concentrations in cats were higher compared to dogs and other species (Forrer *et al.*, 1991; Foster *et al.*, 2001; Wedekind *et al.*, 2003). The reason for these higher levels of circulating selenium in cats has yet to be determined. However there is evidence from our study to suggest that cats may be more efficient at metabolising and excreting excess selenium, which could account for their apparent ability to tolerate higher levels of selenium compared to other species.

Following dietary supplementation of inorganic and organic selenium, estimates of apparent absorption were similar in cats and dogs, ranging from 70 to 85% of dietary intake. Selenite has been reported to be less well absorbed than selenomethionine due to its passive mechanism of transport through the membrane (Wolffram *et al.*, 1986) in contrast to the active transport employed by selenomethionine (Wolffram *et al.*, 1989). However in the present study, the form of selenium supplemented did not affect the degree of selenium apparently absorbed in either cats or dogs. As expected, plasma selenium levels increased after dietary selenium supplementation but selenium concentrations were higher in the plasma of cats compared to dogs. Plasma selenium concentrations in dogs reflected the amount of selenium absorbed, however concentrations in cats were greater in those animals fed the organic selenium compared to the inorganic selenium supplement, despite a similar estimate of apparent absorption for both forms. Concentrations of selenium in plasma have previously been shown to reflect dietary selenium intake in humans (Reilly, 1993). This was also seen in cats fed up to 2 µg Se/g DM (Chapter 3,i) however results from the current study suggest the relationship between ingested selenium and selenium in plasma is not linear at higher selenium intakes. Plasma concentrations of selenium in cats and dogs after 11 days ingesting 10 µg Se/g DM were 9 µmol/L and 7.8 µmol/L respectively, compared to 7.9 µmol/L which was the highest concentration of selenium in the plasma of cats fed up to 2.0 µg Se/g DM after

16 days. Therefore it may be that blood parameters are not accurate indicators of selenium status in cats and dogs and further study would be needed to clarify this.

The liver is the primary site of selenoprotein synthesis and storage from which selenium is mobilised during times of deficiency, and in which selenium is stored when dietary selenium intake is in excess of requirements (Kirchgessner *et al.*, 1997). Consequently, the increased concentrations of selenium in the liver of animals fed supplemented selenium indicate increased storage of selenoproteins and may indicate an increased production of selenoproteins compared to control animals. One explanation for the lower concentration of selenium in the liver of cats fed supplemented selenium compared to dogs may be that cats are more efficient at removing excess selenium. The efficiency of feline hepatocytes to detoxify foreign compounds by conjugation or breakdown is poor (Hietanen and Vinio, 1973; Larson, 1963; Savides *et al.*, 1984) and this may also have an impact on the differences found between the content of selenium in the liver of cats and dogs and their plasma selenium content under high dietary selenium supplementation. Thus cats may be able to tolerate higher levels of selenium by more efficiently excreting excess selenium through the kidney instead of transforming or incorporating selenium into other compounds in the liver. Unless selenium is retained in the liver as a selenoprotein, it remains in circulation, and in the case of inorganic selenium, is distributed to the kidney were it is methylated and excreted in the urine (Kobayashi *et al.*, 2001). In contrast, organic selenium as selenomethionine is distributed to target organs where it can be incorporated into body tissues and plasma proteins and stored (Suzuki, 2005). This theory would explain the increased plasma levels of selenium in cats following dietary selenium supplementation compared to dogs, and it also agrees with the higher level of urinary selenium excretion in cats compared to dogs.

Excretion of selenium in the urine is dependent on kidney function and urine volume (Oster and Prellwitz, 1990). A low glomerular filtration rate may result in an abnormally low amount of selenium excreted in the urine. Similarly, urine volume is affected by fluid intake and this in turn influences the concentration of selenium excreted in the urine (Neve and Peretz, 1988). Thus the accurate determination of urinary selenium excretion poses several problems and is dependant on the way in which samples are collected, and the interpretation and expression of results (Neve and Peretz, 1988). Typically urine samples are collected over a 24 hour period to minimise variation due to food and selenium intake through the day, and the effect of fluid intake on urine volume (Neve and Peretz, 1988), however these samples have

proven to be difficult to accurately collect and preserve. Creatinine is a metabolic waste product which, due to its large size, is neither reabsorbed nor secreted by the kidney. Therefore the amount of creatinine, which is produced at a constant rate, filtered by the glomerulus is the amount excreted in the urine. As a result, urinary creatinine can be used to asses the completeness of 24 hour sample collections of selenium in urine, and the clearance of creatinine from the plasma is used to determine glomerular filtration rate, and therefore kidney function (Oster and Prellwitz, 1990). However, the use of creatinine in 24 hour collections is also subject to substantial variation (Bingham and Cummings, 1985), and a single-void urine sample expressed as selenium concentration per creatinine urinary content has been proven to be a better indicator of urinary selenium concentration in humans (Hojo, 1982).

In order to accurately assess the excretion of selenium in urine in the present study, fractional clearance of selenium from the plasma to the urine was calculated using plasma and urine creatinine concentrations. Results show a relatively higher clearance in animals fed the supplemented diets compared to those fed the control diet, and a greater clearance in cats compared to dogs. The low fractional clearance of selenium in cats and dogs fed the control diet indicates the kidney is functioning effectively as a modulator of selenium excretion. The increased fractional clearance shown by animals fed the supplemented diets suggests selenium is being increasingly secreted into the renal tubules from blood after excretion through the glomeruli to be later eliminated in the urine. Most selenium as selenomethionine or inorganic forms in plasma is associated with proteins which are too big to pass through the glomerulus and into the filtrate (Robinson *et al.*, 1985). Organic selenium in the form of selenomethionine is able to enter the glomerular filtrate, however, as with other amino acids, it is primarily reabsorbed back into the blood and returned to the body pool where it continues to be metabolised (Boldizarova *et al.*, 2003). Selenium excreted in the urine under normal conditions is mainly free inorganic selenium compounds that are not required for selenoprotein synthesis, or that have been metabolised in blood. Therefore, under normal conditions, very little selenium is excreted by the kidney.

In the previous study (Chapter 3,ii), faecal excretion of selenium was greater than urinary excretion in cats fed lower amounts of selenium, but this was reversed at higher concentrations (up to 2.0 µg Se/g DM) and urinary excretion reflected dietary selenium intake whilst faecal excretion remained constant. The trend was also observed in the present study when cats and dogs were fed 10 µg Se/g DM. This further supports the idea that metabolism of selenium in cats and dogs is well regulated by the kidney within normal physiological levels of dietary selenium intake as

occurs in other species (Robinson *et al.*, 1985; Kirchgessner *et al.*, 1997). At higher dietary selenium intakes, urinary excretion of selenium plateaus and is excreted by alternative pathways via the lungs, and/or accumulates in the body (Whanger, 2003). The level at which this occurs is affected by the filtration capacity of the kidney and the availability of methyl groups for the formation of excretory products (Kirchgessner *et al.*, 1997). However there was no obvious garlicky smell on the breath of the cats and dogs in this study that would suggest excretion of selenium as dimethylselenol via the lungs.

The amount of selenium retained by dogs was as predicted. The balance of selenium was in equilibrium in animals fed the control diet, whereas selenium was retained in those fed 10 µg Se/g DM of inorganic selenium, and a greater level of retention was observed in dogs fed the same concentration of the organic form. We were only able to determine total selenium concentrations and could not distinguish between the different forms, however these results indicate increased selenoprotein formation in dogs fed inorganic selenium, and additional storage of selenium in body proteins in dogs fed the organic form. The results of selenium retention in cats are somewhat unexpected as cats fed inorganic selenium showed a net loss of selenium, whereas the balance of selenium in cats fed the organic form was in equilibrium. These results again suggest efficient excretion of excess selenium, with possible overcompensation, rather than increased storage as occurred in dogs, as excess selenium of inorganic origin is methylated and excreted in the urine. Whole body balance of selenium in cats fed organic selenium also appeared to be in equilibrium which suggests that rather than being incorporated into body proteins and stored, excess selenium of organic origin is also metabolised and excreted. These data suggest metabolism of whole body selenium is much more dynamic in cats compared to dogs, and that selenium excess to requirements is mobilised and excreted to a greater degree in cats than dogs. This can act as a protective mechanism against chronic toxicity.

The differences seen in the response of cats to high levels of dietary selenium compared to dogs may also be connected to the unique requirement of cats for higher levels of sulphur amino acids. As cysteine is an intermediate in the synthesis of taurine (Morris *et al.*, 1990), and because the selenoamino acids follow the same metabolic pathways as their respective amino acids, it follows that selenocysteine and selenomethionine may also be utilised for taurine synthesis. Any selenocysteine used for this purpose would be transaminated to pyruvate, releasing the cysteine

intermediate and selenium (Morris *et al.*, 1990). This may also be a factor contributing to the higher plasma selenium levels in cats compared to dogs. The additional requirement of cysteine for hair growth and felinine production in cats also creates a potential demand for the selenoamino acids, especially if the diet contains low amounts of these amino acids. Thus the higher levels of selenium reported in blood and the absence of any reports of toxic effects in cats may in part be due to utilisation of selenoamino acids for synthesis of other sulphur amino acids in these animals, or increased metabolism of selenoamino acids.

To summarise, the form of selenium supplemented had no effect on selenium absorption, excretion and retention by cats and dogs when fed high dietary levels. Feeding cats and dogs 10 µg Se/g DM for a three week period did cause any apparent physical signs of selenium toxicity. Although the same patterns were observed, there were differences in the level of response these species exhibited to high dietary selenium concentrations. The lower amount of selenium in the liver and the increased concentration of selenium in the plasma of cats compared to dogs are indicative of a reduced capacity for hepatic storage or increased mobilisation of selenium with the possible purpose of eliminating excess selenium, as indicated by greater faecal and urinary excretion in cats compared to dogs. Alternatively, the higher requirement for sulphur amino acids in cats may provide an additional means of utilising the selenoamino acids, thereby preventing toxic effects of selenium from occurring. Results from this study suggest some aspects of selenium metabolism in cats may differ from other species.

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CHAPTER 5

The effect of heat treatment during petfood manufacturing on selenium balance in cats fed dietary inorganic and organic selenium

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ABSTRACT

The bioavailability of a nutrient is an important factor to consider when formulating petfoods as it dictates the dietary requirements of an animal. Commercial petfoods are subject to several methods of heat treatment and these processes decrease the nutritive value of the diet. In this study a preliminary investigation of the metabolic response of cats to supplementation of inorganic and organic selenium added to a commercial petfood before and after heat processing was conducted.

Twenty domestic cats were fed a commercial canned cat food containing 0.5 µg Se/g DM (control diet), or the control diet supplemented with inorganic (sodium selenite) or organic (Sel-Plex™ selenium yeast) selenium to give total selenium concentrations of 3 µg Se/g DM and the following treatment groups: Control, inorganic selenium added before processing (Inorg+), inorganic selenium added after processing (Inorg-), organic selenium added before processing (Org+) and organic selenium added after processing (Org-). Cats were fed the control diet for one month to stabilise selenium levels, and then fed their respective treatment diets for a six day adaptation period and a five day collection period. Blood samples were obtained and diet, faecal and urine samples collected daily and used for the analysis of total selenium concentrations and subsequent estimations of apparent absorption and retention.

Cats fed inorganic selenium added after processing (Inorg-) had a higher apparent absorption compared to those fed inorganic selenium added before processing (Inorg+). Cats consuming selenium in the inorganic form, irrespective of when it was added, excreted the same amount of selenium in their urine as they absorbed. Cats consuming the organic form of selenium absorbed the same amount of selenium but less was excreted in the urine when selenium was added after processing, and hence more of this selenium was retained in the body compared to when selenium was added before heat treatment. This preliminary data indicates that heat processing may be an important factor in decreasing the apparent availability of inorganic selenium and the utilisation of organic selenium in commercial petfoods, which warrants further investigation.

INTRODUCTION

Formulation of a well-balanced petfood requires knowledge of the nutrient requirements of the animal, the composition of ingredients and the bioavailability of nutrients in those ingredients (Dzanis, 1994). As an essential trace element, an adequate intake of dietary selenium is important for the maintenance of optimum nutrition and health, as previously discussed (Chapter 1). Selenium in petfoods originates from the ingredients used to formulate the food, such as grains, cereals, animal tissues, plant and animal by-products (Mumma *et al.*, 1986), and/or it can be added as a supplement. Selenium obtained from plant sources exists as selenate, selenite and selenomethionine, whilst it occurs mainly in the form of selenocysteine when present in animal sources (Sunde, 1997). There is large variation in the selenium content of petfoods (Simcock *et al.*, 2005; Chapter 2) and this may be attributed to the variety and source of selenium in the dietary ingredients. In addition, as there are no enforced regulations governing the inclusion of selenium in petfoods it is left to the discretion of the manufacturer to do so. Historically sodium selenite has been used for supplementation (Sunde, 1997), however organic forms of selenium such as selenised yeasts are believed to be more beneficial due to their increased bioavailability, decreased toxicological risk, ability to increase production in animals (Mahan, 1999) and improved selenium status in both animals and humans (Power, 2005). Previous studies in cats and dogs (Chapters 3,ii and 4) have shown increased retention in animals supplemented with organic selenium compared to those fed the inorganic form.

In order to increase shelf life, improve palatability and attain a certain physical form, unprocessed petfoods are subjected to heat treatment during extrusion, baking, pasteurisation or sterilisation (Hendriks, 1999). Commercial petfoods are highly processed, and heat treatment decreases the nutritive value of the diet (National Research Council, 1986). Processing affects the protein fraction of the diet, alters taurine status in cats, causes loss of vitamins and effects heat-sensitive thiamine (Hendriks, 1999). Losses of up to 50% of natural antioxidants are reported in extruded petfoods on a regular basis (Tucker, 2004). Many selenium compounds are unstable and volatile (Higgs *et al.*, 1972), consequently, it is possible that heat treatment has an effect on the availability of selenium in the diet. The aim of this study was to conduct a preliminary investigation into the effect of heat processing on apparent absorption and retention of inorganic and organic selenium in cats.

METHODS

The study reported here was approved by, and conformed to, the requirements of the Massey University Animal Ethics Committee (Anonymous, 2003).

Animals

Twenty short-haired domestic cats (ten males, ten females) from Massey University's Centre for Feline Nutrition (Palmerston North, New Zealand) were used for the study. Cats ranged from 2 to 8.5 years of age at the start of the trial and weighed between 2.61 and 6.09 kg (mean ± SEM, 4.19 ± 0.23 kg).

One month before each trial, cats were fed the control diet in an attempt to standardise selenium status. The control diet was a commercial moist feline diet (Heinz Wattie's, Hastings, New Zealand) formulated to meet the minimum adult maintenance feeding protocol as determined by AAFCO (2000), with a selenium content of 0.5 µg Se/g DM (see Chapter 3,i - Table 1 for composition and amino acid profile of this diet). Throughout the trial cats were fed to meet their daily energy requirements of 70kcal/kg BW/d (National Research Council, 1986) and had access to deionised water at all times.

The trial consisted of a six day adaptation period where cats were fed their respective treatment diets, and a five day collection period in which samples were obtained for analysis. During the adaptation and collection periods the animals were housed individually in metabolism cages to ensure they received the appropriate diet, to monitor dietary intake and to enable the separate collection of faecal and urine samples during the collection period. The setup of metabolism cages is described by Hendriks *et al.* (1999a; Appendix 2).

Treatments

Two different forms of selenium were used to supplement the control diet and throughout this paper they will be referred to as "inorganic" and "organic" selenium. The inorganic supplement used for the first trial was sodium selenite (a 1% premix of sodium selenite and limeflour; Nutritech International Ltd, Auckland, New Zealand), and the organic supplement used for the second was a selenium yeast (Sel-Plex™: containing selenomethionine, selenocysteine, and other selenoproteins and organo-selenium compounds; Alltech Inc, Nicholasville, Kentucky, USA). Five groups of four cats were used in the study. Each group received the control diet or the control diet with either the inorganic or organic selenium source added, to produce a total

selenium concentration of 3 µg Se/g DM. The supplemental selenium was added to the control diet either by the manufacturer before heat processing in a bulk amount or on a daily basis after heat processing. The five treatment groups were "Control" (heat treated, no additional selenium added); "Inorg+" (inorganic selenium added to control diet then heat treated); "Inorg-" (inorganic selenium added to control diet after heat treatment); "Org+" (organic selenium added to the control diet then heat treated); "Org-" (organic selenium added to control diet after heat treatment.)

Sampling

At the beginning of the adaptation period cats were allocated to one of the five dietary treatment groups, with two males and two females in each group. Cats were matched for body weight to avoid large variations in food intake. Following an overnight fast, a preprandial baseline blood sample (2 ml) was taken from each animal by jugular venipuncture using a 25 gauge needle. Blood was collected into 4 ml heparinised tubes and centrifuged for 10 minutes at 3,000 rpm. Plasma was then removed and an aliquot taken for the analysis of total selenium concentrations. Following blood sampling animals were transferred to metabolism cages and fed their respective, pre-weighed dietary treatments once daily for the adaptation and collection periods (as described above).

During the collection period food intake was recorded on a daily basis and subsamples of each of the five diets were taken daily and pooled. Faecal and urine samples were collected quantitatively every 24 hours and also pooled. At the end of the collection period a second preprandial blood sample was taken as previously described. All samples were stored at $-20\ ^{0}C$ prior to chemical analysis. Animals were weighed weekly for the duration of the study.

Chemical analysis

Diet and faecal samples were freeze-dried, ground to a fine powder using an electric grinder (Model CG-2; Breville, Oldham, UK) and mixed thoroughly prior to selenium analysis. Hair was separated and removed from the faeces following freeze-drying. Diet and faecal samples were analysed in triplicate while plasma and urine samples were analysed in duplicate. Samples with replicates having a coefficient of variation greater than 10% were subjected to further analysis until variability was reduced below this level. Total selenium concentrations of all samples were analysed using a fluorometric method as previously outlined in Chapter 2 and described in Appendix 1.

Statistical analysis

Data were analysed using SAS version 8.02 for Windows (SAS Institute Inc, Cary, NC, USA). Data was initially screened for normality, outliers and homogeneity of variance as described in Chapter 3,i, and all data exhibited a normal distribution. Statistical analysis of data in the present study are as follows. An initial one way ANOVA was performed on all data to determine overall differences. The model for the analysis of plasma selenium was: plasma selenium = diet + time + the interaction between diet and time; for faecal and urinary excretion: excreted selenium = diet, type of excretion + the interaction between diet and type of excretion; and for the remaining parameters (apparent absorption, retention and Table 7): parameter of interest = diet. In each case the model included Types I and III sum of squares, followed by multiple comparisons using Duncan's test. If differences did occur in a model that contained interactions, the ANOVA was repeated with data sorted according to the "By" statement for diet and sample, or diet and type of excretion, in order to determine where differences occurred both within and between groups. Apparent absorption was estimated by calculating the difference between dietary intake and faecal excretion, and retention was estimated by calculating the difference between dietary intake, faecal and urinary excretion. In addition, apparent absorption, excretion and retention of the supplemented selenium only, was estimated by subtracting the amount of selenium in the control diet from the total amount of selenium in the treatment diets: Apparent absorption, excretion and retention of total selenium in control animals, and supplemented selenium in treatment animals, are also expressed as a percentage of dietary intake (Table 7). In all cases, differences were considered significant at a probability level of 5%.

RESULTS

All animals remained healthy throughout the trial. Average (± SEM) food intake during the collection period was 310 ± 16 g/day. The determined concentrations of selenium in the diets are shown in Table 1.

Table 1. Selenium concentrations (µg Se/g DM) in diets supplemented with inorganic or organic selenium.

Group	Target level (µg Se/g DM)	Actual level[1]
1 - Control	0.5	0.48
2 – Inorg+	3.0	2.39
3 – Inorg-	3.0	2.65
4 – Org+	3.0	2.76
5 – Org-	3.0	3.50

[1]*actual values obtained from the mean of quadruplicate samples pooled over the trial*

Food intake was similar in cats from all groups during the trial period ($p > 0.05$). Cats fed the treatment diets ingested more selenium than those fed the control diet ($p < 0.0001$; Table 2). However there were differences in the amount of selenium ingested between cats that received selenium supplementation ($p < 0.05$).

Table 2. Mean (± SEM) dietary selenium intake (µg Se/kg body weight/day) during the collection period in cats fed a control diet (0.5 µg Se/g DM), or control diet supplemented with inorganic (Inorg) or organic (Org) selenium before (+) and after (-) heat processing to give total selenium concentrations of 3 µg Se/g DM (n=4).

Diet	Se Intake (µg/kg BW/d) mean ± SEM	P > F Diet
Control	6.9 ± 0.8[a]	<0.0001
Inorg+	35.2 ± 5.0[b]	
Inorg-	43.9 ± 2.5[bc]	
Org+	37.0 ± 6.7[b]	
Org-	53.3 ± 7.2[c]	

In the initial plasma sample, selenium concentrations of cats fed the Inorg-, Org+ and Org- diets were greater than those in cats fed the control and Inorg+ diets ($p < 0.05$; Table 3). Plasma selenium concentrations increased over the 11 day trial period in animals fed the treatment diets ($p < 0.05$), however this increase was not

significant in cats fed the Inorg+ ($p > 0.05$). Similar concentrations of selenium were found in plasma of all cats fed the treatment diets after 11 days ($p > 0.05$).

Table 3. Mean (± SEM) plasma selenium (Se) concentrations (µmol/L) in cats on day 0 (time 0) and 11 days later (time 1) fed a control diet (0.5 µg Se/g DM), or the control diet supplemented with inorganic (Inorg) or organic (Org) selenium before (+) and after (-) heat processing to give total selenium concentrations of 3 µg Se/g DM (n=4).

Time	Diet	Plasma Se (µmol/L) mean ± SEM	P > F Diet	Time	Diet x Time
0	Control	4.5 ± 0.2[a]	<0.0001	<0.0001	0.1412
	Inorg+	5.3 ± 0.7[ab]			
	Inorg-	5.9 ± 0.3[b]			
	Org+	6.1 ± 0.2[b]			
	Org-	5.8 ± 0.2[b]			
1	Control	4.5 ± 0.4[a]			
	Inorg+	6.7 ± 0.5[b]			
	Inorg-	7.1 ± 0.4[b]			
	Org+	7.6 ± 0.1[b]			
	Org-	7.0 ± 0.2[ab]			

Differences between diets for each sample are indicated by different superscripts ($p < 0.05$)

With the exception of cats fed the Inorg- diet, in which the amount of selenium excreted in faeces did not differ from that excreted by control animals ($p > 0.05$), significantly more selenium was excreted by cats fed the treatment diets compared to those fed the control diet ($p < 0.0001$; Table 4) by up to four times in faeces and up to 20 times in urine. Aside from cats fed the Org- diet, cats fed the different treatment diets excreted similar amounts of selenium in their faeces ($p > 0.05$), whereas the amount of selenium excreted in urine varied between cats fed the different treatment groups ($p < 0.05$). Faecal excretion was greater than urinary excretion of selenium in cats fed the control and Org- diets by approximately 6 and 1.5 times, respectively ($p < 0.01$), whereas urinary excretion was greater than faecal excretion in cats fed the Inorg- diet by 2.4 times ($p < 0.001$). There were no differences in faecal and urinary excretion of cats fed the Inorg+ and Org+ diets ($p > 0.05$).

Table 4. Mean (± SEM) concentrations of selenium (Se) (µg/kg body weight/day) excreted in faeces and urine by cats fed a control diet (0.5 µg Se/g DM), or control diet supplemented with inorganic (Inorg) or organic (Org) selenium before (+) and after (-) heat processing to give total selenium concentrations of 3 µg Se/g DM (n=4).

		Excreted Se (µg/kg BW/d)	P > F		
Excretion	Diet	mean ± SEM	Diet	Excretion	Diet x Excretion
Faeces	Control	7.8 ± 2.2[a]	<0.0001	0.4604	<0.0001
	Inorg+	20.2 ± 3.2[b]			
	Inorg-	13.9 ± 1.1[ab]			
	Org+	17.7 ● 2.8[b]			
	Org-	29.6 ± 1.4[c]			
Urine	Control	1.3 ± 0.3[a]			
	Inorg+	16.4 ± 1.5[b]			
	Inorg-	32.8 ± 2.3[c]			
	Org+	25.5 ± 5.6[cd]			
	Org-	18.8 ± 2.5[cd]			

Differences between diets for each type of excretion are indicated by different superscripts ($p < 0.05$)

Up to 30 times more selenium was absorbed by cats fed the treatment diets compared to those fed the control diet ($p < 0.0001$; Table 5), in which a net loss occurred. Similar levels of selenium were absorbed by cats fed the Inorg- and Org- diets that had the selenium added before processing and those that had selenium added after processing ($p > 0.05$). Cats in the latter group absorbed twice as much selenium than those in the former groups ($p < 0.05$).

Table 5. Mean (± SEM) concentrations of selenium (Se) (µg/kg body weight/day) absorbed by cats fed a control diet (0.5 µg Se/g DM), or control diet supplemented with inorganic (Inorg) or organic (Org) selenium before (+) and after (-) heat processing to give total selenium concentrations of 3 µg Se/g DM (n=4).

Diet	Apparent Se absorption (µg/kg BW/d) mean ± SEM	P > F Diet
Control	-0.3 ± 1.4[a]	<0.0001
Inorg+	15.0 ± 2.5[b]	
Inorg-	30.1 ± 3.0[c]	
Org+	19.3 ± 4.2[b]	
Org-	28.3 ± 4.6[c]	

Differences between diets are indicated by different superscripts ($p < 0.05$)

Retention of selenium was highly variable throughout all groups (Table 6). Selenium was retained when cats consumed diets with organic selenium added after heat processing ($p < 0.05$) whereas cats in all other groups showed similar amounts of retention ($p > 0.05$) negative values indicating no net gain of selenium.

Table 6. Mean (± SEM) concentrations of selenium (Se) (µg/kg body weight/day) retained by cats fed a control diet (0.5 µg Se/g DM), or control diet supplemented with inorganic (Inorg) or organic (Org) selenium before (+) and after (-) heat processing to give total selenium concentrations of 3 µg Se/g DM (n=4).

Diet	Retained Se (µg/kg BW/d) mean ± SEM	P > F Diet
Control	-1.6 ± 1.5[a]	0.0503
Inorg+	-1.4 ± 2.7[a]	
Inorg-	-2.8 ± 4.0[a]	
Org+	-6.2 ± 3.5[a]	
Org-	8.0 ± 3.4[b]	

Differences between diets are indicated by different superscripts ($p < 0.05$)

Table 7 shows faecal and urinary excretion, apparent absorption and retention of total selenium (controls) and supplemented selenium (treatments) as a percentage of dietary intake in cats fed the different diets. The proportion of supplemented selenium in treatment groups was calculated by difference from the amount of selenium absorbed, excreted or retained in control animals. Cats fed the control diet excreted 100% of the ingested selenium in their faeces whereas cats fed the treatment diets excreted 17 to 45%. The proportion of selenium intake excreted in faeces by cats fed both Org diets and the Inorg+ diet was up to 2.5 times less than that excreted by cats fed the control diet, while cats fed the Inorg- diet excreted the least amount of selenium in their faeces ($p < 0.0001$). The opposite pattern was seen for absorption with no apparent absorption of selenium by control animals, similar amounts in cats fed both Org and Inorg+ diets, and a much greater amount absorbed by cats fed the Inorg- diet ($p < 0.0001$). Between 2.2 to 4.6 times as much selenium was excreted in the urine of cats fed treatment diets compared to those fed the control diet ($p < 0.0001$). Urinary excretion of selenium was highest in cats fed the Inorg- and Org+ diets and lowest in cats fed the Org- diet ($p < 0.0001$). Retention of selenium as a percentage of dietary intake was highly variable. Cats fed the Org- diet showed significant retention of selenium ($p > 0.05$) whereas there was no significant retention shown by cats fed the other diets ($p > 0.05$).

Table 7. Mean (± SEM) percentage of selenium (Se) absorbed, excreted and retained by cats fed a control diet (0.5 µg Se/g DM), or the control diet supplemented with inorganic (Inorg) and organic (Org) selenium before (+) and after (-) heat processing to give dietary selenium concentrations of 3.0 µg Se/g DM (n=4).

	Se excreted in faeces[1] (%)	Apparent Se absorption[1,2] (%)	Se excreted in urine[1] (%)	Se retained[1,3] (%)
Controls:	100.3 ± 16.0[a]	-0.3 ± 15.6[a]	17.3 ± 3.2[a]	-17.6 ± 15.4[a]
Treatments[4]:				
Inorg+	46.3 ± 6.2[b]	53.7 ± 6.2[b]	55.1 ● 6.5[b]	-1.9 ± 11.2[ab]
Inorg-	16.7 ± 4.2[c]	83.3 ± 4.2[c]	88.1 ± 8.1[c]	-4.9 ± 11.5[ab]
Org+	38.4 ● 5.5[bc]	61.6 ± 5.5[bc]	80.8 ± 14.7[c]	-19.2 ± 12.2[a]
Org-	44.1 ± 3.7[b]	55.9 ± 3.7[b]	38.3 ± 2.3[ab]	17.8 ± 5.3[b]

[1] calculated as a percentage of dietary intake
[2] calculated from the difference between dietary intake and faecal excretion
[3] calculated from the difference between dietary intake, faecal and urinary excretion
[4] values represent apparent absorption, excretion or retention of supplemented selenium only (calculated by difference from the amount of selenium in the control diet)

Within columns, means with different superscripts are different ($p < 0.05$)

DISCUSSION

The level at which a nutrient is contained in a diet is only as significant as its ability to be utilised. Thus the bioavailability of a nutrient is an important factor to consider when accounting for its requirement in the formulation of a diet. This is a matter of concern when considering the amount of selenium included in petfoods, as bioavailability of this mineral in whole petfoods is reported to be low compared to that of the dietary ingredients (Wedekind *et al.*, 1997; 1998). Some dietary ingredients contain high concentrations of selenium, (eg, tuna; Boyer *et al.*, 1978; Mumma *et al.*, 1986), but have low or comparatively low bioavailabilities compared to other ingredients (Alexander *et al.*, 1983) which decreases their nutritive value. Similarly, in human diets some high-selenium containing vegetables have been reported to lose significant amounts of selenium during the process of cooking (Higgs *et al.*, 1972). There are several factors that affect the bioavailability of a nutrient (Combs and Combs, 1986a; Chapter 1), one of which is the process of heat treating commercial petfoods. In order to determine whether this was the case for selenium, the current study investigated the metabolic response of cats to supplemented inorganic and organic selenium added to the diet before and after heat processing. Due to difficulties in obtaining the same dietary selenium concentrations in each of the treatment diets in the commercial setting used to produce them, the selenium content of the treatment diets was not uniform. However differences were observed that indicate a potential effect of heat processing on supplemented selenium.

Plasma selenium concentrations have been reported to reflect dietary selenium intakes in other species (Reilly, 1993) and to some extent this occurred in cats fed 0.46 and 1 µg Se/g DM in previous studies (Chapter 3,i). Plasma samples were obtained to provide an indication of the level of circulating selenium in cats following ingestion of the treatment diets. As expected, plasma selenium concentrations in cats from all groups were similar following the pre-trial stabilisation period. At the end of the 11-day trial period, plasma selenium concentrations were higher in cats fed the treatment diets compared to the initial sample and concentrations in control animals, although this difference was not significant in cats fed the Inorg+ diet. It is possible the 11 day period in which cats were fed the treatment diets was not long enough to increase plasma selenium concentrations sufficiently, or in a stable manner. Results from a previous study (Chapter 3,i) show plasma selenium levels in cats fed concentrations from 1.0 to 2.0 µg Se/g DM plateau after approximately seven days. In addition,

concentrations of selenium in the plasma of cats fed 2.0 µg Se/g DM were significantly higher than those of the control animals in that study. These values were of a similar magnitude after 11 days as those in the present study, therefore it is unlikely that the trial period was too short, or the differences in dietary selenium concentrations between groups too low.

There is evidence to support the theory that heat processing has the potential to affect the bioavailability of selenium in petfoods. Results from a previous study (Chapter 3,ii) showed apparent absorption of supplemented selenium added to a whole canned diet as a percentage of dietary intake in cats was up to three times greater than in those animals fed the whole canned diet (the control) where no supplementation occurred. A similar finding was observed in a subsequent study in which cats and dogs were fed 10 µg Se/g DM (Chapter 4). In both cases apparent absorption of selenium was higher in cats fed supplemented diets compared to control diets, but it was uncertain at that stage if this was a result of heat processing during manufacturing or endogenous gastrointestinal losses.

In general, excretion of selenium in faeces and urine showed a similar pattern to that shown by cats in a previous study (Chapter 3,ii) whereby excretion of selenium in both faeces and urine was greater in cats fed the higher concentration of selenium. In the previous study, a greater percentage of selenium was excreted in the urine compared to faeces in animals fed higher dietary selenium concentrations which reinforces the idea that the kidney plays an important role in selenium homeostasis (Behne, 1988; Kirchgessner *et al.*, 1997) in cats as it does in other species. If the fact that all cats in the treatment groups in the current study ingested similar dietary selenium concentrations is considered, there are certain discrepancies in these trends which suggest heat treatment may affect selenium utilisation.

The different response of cats fed the inorganic selenium diets suggests a possible effect of heat processing on the ability of selenium to be absorbed. A greater amount of selenium was excreted in the faeces of cats fed inorganic selenium added before processing (Inorg+) and consequently these animals showed a lower apparent absorption of 54% of dietary selenium intake. Although this value is only an estimate and other factors such as endogenous faecal losses were not accounted for, it is low compared to reports in the literature which suggest absorption of inorganic selenium as selenite in rats was 95 to 100% (Behne, 1988) and in humans 91 to 93% (Combs and Combs, 1986b). In contrast, cats fed the Inorg- diet had an apparent absorption of 83%. The reason for this decreased apparent availability of inorganic selenium in cats fed the heat processed diet is unknown. It is possible that selenite, a selenium

salt existing as a divalent ion (Sunde, 1997) may be unstable at the temperatures required for heat processing resulting in breakdown, interactions with other elements and attachment or entrapment to indigestible material, or changes in its structure.

Approximately 60% of inorganic selenium has been reported to be excreted in the urine of humans (Robinson *et al.*, 1997) which is similar to that excreted by cats fed the Inorg+ diet (55%). Although there were differences in the amount of selenium excreted in the urine of cats fed the two Inorg diets, this may be attributed to the way in which inorganic selenium is typically metabolised and utilised (Patterson *et al.*, 1989; Kobayashi *et al.*, 2001), rather than an effect of heat processing. Both groups of animals excreted the same amount of selenium in their urine that they had absorbed. This suggests the majority of the selenium absorbed by these animals was not required for selenoprotein synthesis, and was therefore methylated and excreted in urine. From this response it may be assumed that the overall metabolic balance of selenium was close to equilibrium, and despite the high variability, the retention data also suggests this was the case.

Similar proportions of selenium intake were excreted in the faeces of cats fed the two Org diets and therefore these animals also showed similar apparent absorptions of around 55 to 60%. The slightly higher apparent absorption seen in cats fed the Org- diet was likely to be due to the higher selenium content of that diet. These values were lower than those obtained in the previous study (Chapter 3,ii), where cats fed up to 2.0 µg Se/g DM of the organic form absorbed up to 75% of dietary selenium intake. As with inorganic selenium, organic forms of selenium are also reported to be well absorbed in humans and animals (Bopp *et al.*, 1982) and the reason for the low apparent absorption in the present study is unknown. Selenium of organic origin is also utilised for selenoprotein synthesis, however when there is no further requirement for selenoproteins, rather than being excreted in the urine as occurs with inorganic selenium, excess dietary selenomethionine may be incorporated into general body proteins from which selenium may be released by normal catabolic processes and returned to the metabolic pool when the need arises (Suzuki, 2005). Despite the lower apparent absorption, the response of cats fed the Org- diet reflected this pattern and was the only diet to cause retention of selenium. Twenty to 30% of selenomethionine has been reported to be excreted in human urine (Robinson *et al.*, 1997) and in the current study 38% of selenium was excreted in the urine of cats fed the Org- diet. Thus a smaller proportion of selenium was excreted in the urine relative to the amount

these animals absorbed, and this level of urinary excretion was also low compared to cats fed the other diets.

In contrast, cats fed the Org+ diet excreted 80% of their ingested selenium in the urine, however this value is the result of an unusually high level of excretion of one cat in the group. Without this, the urinary excretion is a much more realistic value of 68%, and as with cats fed the Inorg diets, a similar amount of selenium was excreted in the urine as was absorbed by these animals. The difference between cats fed the Org- and Org+ diets suggests that heat processing is in some way affecting the ability of organic selenium to be incorporated into general body proteins. In a previous study, Hendriks *et al.* (1999b) looked at the effect of heat processing on protein measured by a rat bioassay. The amino acid content of the diet remained unaltered suggesting amino acids were not destroyed by heat processing, however true digestibility of all amino acids measured decreased as heat treatment increased. It was suggested that cross-linking between amino acids and within proteins may have occurred, resulting in a reduced rate of protein digestion and therefore digestibility (Hendriks *et al.*, 1999b). Alternatively, these authors proposed increased endogenous losses of amino acids in the gut may explain the decreased amino acid digestibility after heat treatment. The lower digestibility can also arise from changes in the gut environment that lead to different bacterial utilisation of diets according to its processing, with production of more or less bacterial protein remaining in the gut content and interacting differently with different forms of selenium.

With regard to organic selenium, heat processing did not appear to affect apparent absorption of selenium (despite the low level), therefore the differences seen in excretion and retention of cats fed selenium added before and after processing are not likely to be due to different digestibilities. It may be that the decreased utilisation of selenium by cats fed the Org+ diet compared to the Org- diet results from the effect of heat processing altering the structure of the organic compound such that it is unable to be incorporated into body proteins, and is therefore excreted in urine.

Accurate conclusions cannot be drawn from the results obtained in this study due to discrepancies in dietary selenium concentrations and subsequent dietary selenium intakes. However, the data revealed some trends which may warrant further investigation, specifically the decreased availability of inorganic selenium, and decreased utilisation of organic selenium supplemented in petfoods subjected to heat processing.

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CHAPTER 6

General Discussion

Limitations of the Studies

Future Research

GENERAL DISCUSSION

The essentiality of selenium and its role in maintaining normal metabolic function is well recognised and optimum dietary intakes of this mineral promote good health and prevent disease in both humans and animals. However, little is known of the metabolic pathways of selenium in cats and dogs, or what the requirements of these animals for selenium are.

Analysis of the total selenium content of commercially available cat and dog foods in New Zealand revealed a wide range of concentrations, with much higher concentrations of selenium in moist cat foods compared to the other diets. This was attributed to the inclusion of seafood ingredients which have been found to contain high concentrations of selenium. All petfoods analysed contained concentrations of selenium above the minimum dietary requirements of selenium specified for cats and dogs (National Research Council, 1985; 1986; AAFCO, 2000). However it has been established that these requirements are inadequate as they are based on extrapolation of data from other species. They do not account for the requirement of the animal for the nutrient at different life stages, nor do they consider the bioavailability of the mineral, and values that are given are purely estimates. Mean concentrations of selenium in the petfoods analysed were less than 0.5 µg Se/g DM. This may be considered low when compared to the levels at which the beneficial health effects of selenium are reported to occur in humans (Schrauzer, 2002; Whanger, 2004), especially if the bioavailability of selenium in petfoods is low as previously reported (Wedekind *et al.*, 1997; 1998). In contrast, some moist cat foods contained selenium concentrations that were greater than the suggested safe upper level for dogs (Wedekind *et al.*, 2002) and the recognised level at which toxic effects occur in livestock (Koller and Exon, 1986). Thus there is a need to establish the dietary requirements of selenium for cats and dogs at a level which facilitates optimum health, and to adjust the selenium content of petfoods accordingly.

When carrying out research to establish dietary selenium requirements, the response of the animal to various levels of selenium intake must be quantified and the resulting indicators of selenium status of the animal measured in order to determine which levels provide the optimum response. Due to the diversity of its functions (Combs and Combs, 1986; Arthur and Beckett, 1994; Holben and Smith, 1999; Rayman, 2000), there are various parameters that may be used to measure selenium status. These have been well documented in humans and other animals (Ullrey, 1987;

Diplock, 1993), however there is no information relevant to companion animals. For unknown reasons, GSHPx activities in cats were highly variable and showed no clear pattern in response to supplementation. Dogs and cats fed selenium at 10 µg Se/g DM also showed no apparent response of plasma GSHPx activities to supplementation. GSHPx activities were slightly higher in these animals and this could be attributed to the higher dietary selenium intake. Whole blood selenium concentrations were also variable in cats supplemented with 1.0 to 2.0 µg Se/g DM and there appeared to be a delay in the response of this parameter compared to that of plasma selenium. It is likely this was due to incorporation of selenium into red blood cells (Ullrey, 1987), therefore whole blood selenium concentrations may be better suited for use as a longer term measure of selenium status as in other species. Plasma selenium concentrations appeared to be a reliable indicator of immediate selenium supplementation and reflected dietary selenium intakes rapidly at the supplemented levels of 1.0 to 2.0 µg Se/g DM. Selenium in the plasma of cats from the other studies also showed increases in plasma selenium concentrations with increased dietary selenium intake, however these concentrations did not appear to reflect dietary selenium in a linear fashion. Whether or not there is a significant relationship between dietary selenium intake and plasma selenium levels would need to be determined in a study designed for that purpose, however results from these studies suggest plasma selenium concentrations may not be a reliable indicator of selenium status in cats.

In addition to the use of blood parameters, the metabolic response of cats to selenium intake was determined by measuring the apparent absorption, excretion and retention of the supplement. Excretion of selenium in the faeces and urine of cats from all the studies showed similar patterns and confirms that the important role of the kidney in selenium homeostasis (Behne, 1988; Kirchgessner *et al.*, 1997) is also applicable to cats and dogs. Faecal excretion of selenium as a proportion of intake was much greater in cats fed the control diets in each trial compared to that excreted in urine by these animals. In contrast, faecal excretion of selenium remained relatively constant within trials in cats fed the treatment diets regardless of the level of selenium intake, whereas the amount of selenium in urine increased with increased dietary intake. At very high levels of dietary selenium intake the capacity of the kidney to excrete selenium diminishes (Kirchgessner *et al.*, 1997) and an alternative excretory pathway is recruited to prevent build up of the harmful metabolites that may cause toxic effects. This pathway involves methylation of selenium compounds to form dimethylselenol which is excreted by the lungs and has a garlic odour that may be

smelt on the breath (Shamberger, 1983). There was no evidence of this garlic odour, nor any other physical signs of toxicity in the cats and dogs fed 10 µg Se/g DM, therefore it may be assumed that this level of dietary selenium intake was not toxic to these animals. However the time period in which they received these diets was relatively short and it is possible that toxic effects may have developed over a longer period.

In general, apparent absorption of selenium by cats from all studies appeared to be influenced by the amount of selenium ingested as occurs in humans (Whanger, 1998), with higher apparent absorption seen in animals with higher dietary selenium intakes. There was a trend in which cats fed the organic supplement showed higher apparent absorption than those fed the inorganic form, and although there were no clear differences, often as a result of variability, this was reflected in the amount of selenium retained by these animals. This may be expected, as due to their different metabolic pathways, organic selenium in the form of selenium enriched yeasts are more bioavailable and less toxic than inorganic forms and have the advantage of being able to be stored and reversibly released during times of selenium deficiency (Rayman, 2004).

There is some evidence to suggest that cats may be able to tolerate higher levels of selenium better than other species (Forrer *et al.*, 1991; Foster *et al.*, 2001). Although there is little known regarding the metabolism of selenium in cats and dogs, cats in particular show several unique peculiarities of metabolism associated with sulphur-containing compounds (Morris, 2002). In the study conducted with cats and dogs fed high levels of selenium (10 µg Se/g DM), both species exhibited the same response, however the degree of magnitude of this response differed between them. Cats had higher plasma selenium concentrations, lower concentrations of selenium in the liver and excreted greater amounts of selenium in faeces and urine compared to dogs. These findings suggest cats may be more efficient at metabolising and excreting higher levels of selenium. Alternatively, the different response of cats to high dietary selenium intakes compared to dogs may be related to the increased requirement of cats for sulphur amino acids (Hendriks, 1999). Further study would be needed to investigate these hypotheses however the results of this thesis provide additional evidence to indicate species differences in selenium metabolism. This may be another idiosyncrasy to add to the list of unique metabolic characteristics of cats.

In addition to the nutritional peculiarities inherent in cats, special nutritional considerations may arise as a result of the manufacturing process petfoods are

subjected to. Heat treatment of commercial petfoods is primarily used to increase shelf life and achieve a certain physical form (Hendriks *et al.*, 1999), however it can have a negative effect on the nutritive value of the diet (National Research Council, 1986). Investigation of the effect of heat treatment on apparent absorption and utilisation of supplemented inorganic and organic selenium in cats revealed a decrease in the apparent absorption of inorganic selenium and an apparent decrease in the utilisation of organic selenium. These preliminary results give cause for concern as they suggest heat processing may affect the bioavailability of selenium, and if a diet contains the minimum recommended concentration of selenium but has a low bioavailability, the animal will effectively receive an inadequate dietary intake. These findings warrant further investigation into the effect of heat processing on the inclusion of selenium in petfoods and illustrate the need to account for mineral bioavailability when formulating petfoods.

As with other trace elements, it can be expected that nutritional requirements for selenium may occur within a narrow range, outside of which adverse effects may be observed. The lack of associated conditions resulting from inadequate concentrations of selenium in petfoods, be they deficient or toxic levels, suggests there is no reason to be concerned about the current selenium status of petfoods in New Zealand. However, the previous thinking of providing just enough of a nutrient to prevent adverse effects has become outdated and has been superseded by the increased knowledge of how nutrients may provide and optimise health. An animal or human may be provided with the minimum amount of a nutrient to function without apparent adverse effect, however the full potential of that nutrient is often not realised. Moreover, there is usually a fine line between any beneficial effects and the onset of toxic effects. This division needs to be established for each species, each form of dietary selenium, and the time period over which certain amounts of selenium consumption may cause toxic effects. The NRC are soon to release a new publication of nutrient requirements for cats and dogs in which the latest research has been considered and applied. Unfortunately in the case of selenium, there has been little progress in the determination of selenium requirements specific to cats and dogs. The work that has been done by Wedekind and colleagues on bioavailability has been incorporated (Wedekind *et al.*, 1998; 2003; 2004), however much of these data are still extrapolated and are not species specific, thus a complete picture is still unavailable.

The research carried out in this thesis appears to indicate that in cats, selenium supplementation at less than 1.5 µg Se/g DM is insufficient to maintain body stores of selenium as indicated by plasma selenium concentrations and the amount of selenium retained in the body. However there appears to be no additional benefit of increasing selenium status when supplementing at levels between 1.5 and 3 µg Se/g DM as indicated by these parameters. Providing supplementation at higher levels (10 µg Se/g DM) appeared to reverse any beneficial effect in cats by increasing excretion to the extent of leading to a negative balance of selenium and consequently, depletion of body selenium stores. Thus it would seem from these results that a potentially suitable level of selenium supplementation for cats may be found around 1.5 µg Se/g DM. However the selenium in these studies was added after processing, and therefore may have a greater bioavailability than the same amount added to a diet subjected to heat processing. In addition, the form of selenium supplemented would need to be accounted for, as although organic selenium is retained to a higher degree than inorganic selenium, the organic form is far less toxic than the inorganic form due to its stability within compounds. More research is needed to confirm these finding in cats and to determine suitable levels of selenium supplementation for dogs.

In summary, results from the studies in this thesis have provided an insight into the metabolism of selenium in cats and dogs and contributed fundamental data for future utilisation. More work is needed to further understand selenium metabolism in companion animals in order to determine optimum dietary levels that will convey the health benefits afforded by selenium to other species.

LIMITATIONS OF THE STUDIES

Results from the research described in this thesis have been compromised to some degree by practical constraints and technical difficulties. Animal studies are expensive by nature. They are also costly in terms of animal welfare and a balancing act is requested from scientists, especially those working with domestic species valued by the public. At the time the study was designed it was considered that given the data available, four animals would be sufficient to provide valuable data and/or to show useful trends. However, a significant amount of individual variation was found in some of the experiments. Although no animal data was excluded from these studies at

any time, the considerable variation may have masked any significant differences, and a greater number of animals would have been beneficial to clarify this.

All animal nutritional studies are dependant on the animals willingly ingesting a designated amount of food so that the different concentrations of dietary nutrients in the food are translated into different total nutrient intakes. In addition, ideally, a selenium-free diet should have been used to generate baseline data and allow treatments comparisons. Originally a purified diet was fed in the attempt to achieve this but palatability was poor and many of the animals did not ingest their required daily food intake. This significant variation in food intake compromised the expected differences in selenium intake between the different treatment groups. Hence a low-selenium, commercial canned petfood, with proven palatability was used instead.

Difficulties were encountered in achieving accurate and consistent selenium concentrations in the treatment diets. Diets were prepared on a daily basis by homogenising the control diet and adding the appropriate amount of the supplement to it. To minimise variation, for each study the base petfood used to create all diets was obtained from the same batch, as were the selenium supplements, and the task of preparing the diets was conducted by the same person in the same manner every day. Despite these precautions some variation did still occur. One single batch of each diet containing enough food for the duration of both trials would have eliminated such variation. However, this could have introduced other complications, such as the loss of palatability during long term storage or selenium losses over time. Therefore, although not ideal, but accounting for the importance of palatability and overall food consumption for the success of this research, it was decided to prepare the diets with as much care as possible on a daily basis.

Similar difficulties were encountered in trying to achieve equivalent selenium concentrations in each of the four treatment diets in the heat processing study (Chapter 5). Although care was taken to initially incorporate the selenium into the liquid gravy mix in order to increase homogeneity, there were differences in the selenium concentrations of the different treatment diets. This may be attributed to the fact that, in order to minimise disruption of the manufacturing process, it was necessary to use different batches of the diet for each form of supplemented selenium. Previous work has shown that concentrations of selenium vary considerably in the same petfood produced in different batches (Todd, unpublished data).

Faecal and urine samples were pooled over various time periods during the collection periods of each study because of costs. Pooled samples provide an average

value for each of the time periods and it was assumed that changes during the pooling period were minimal. Whereas samples obtained at a specific time on a daily basis provide more accurate information regarding the pattern of elimination and daily variability, they may be affected by such factors as how much water the animal has consumed immediately before sampling and when it last ate. The disadvantage of pooled samples is the inability to determine exactly when an event, such as a maximum or minimum value, may occur, as this could be masked within the pooled time period. In addition, if a problem occurred with part of a sample within the pooled period it may affect the result of the overall sample. While validation of this pooling method would have been desirable, it was considered that valuable information could still be gained using pooled samples without exceeding the budget.

Estimations of apparent absorption and retention were obtained from balance studies conducted in this thesis using dietary intake, faecal and urinary excretion data. These estimates did not provide entirely accurate values as endogenous faecal losses were not accounted for. In order to calculate true absorption, the use of isotopic marker techniques is required to account for loss of the nutrient through intestinal secretions and mucosal cell sloughing. There are two types of isotopes available, radioactive and stable. Radioactive isotopes can be produced for many elements. They are cheap, easy to measure and can be added to the diet for labelling purposes at trace levels thereby maintaining the total content of the element in the diet (Sandstrom *et al.*, 1993). The disadvantage of using a radioisotope is its safety, with the potential for ionising radiation to occur. ^{75}Se is a radioisotope of selenium and has a half-life of 120 days (O'Dell, 1985).

Stable isotopes occur naturally in the body and are a safer option because they do not break down. There is no time constraint for their use under experimental conditions, however there is a limited availability of stable isotopes and they are also expensive. In addition, large amounts of the isotope must be used to enable accurate analysis which alters the total amount of the nutrient in question and potentially disrupts the system (O'Dell, 1985). The cost of analysing stable isotopes is also considerable with regard to both time and money. Stable isotopes of selenium include ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se and ^{82}Se with natural abundances of 0.87%, 9.02%, 7.58%, 23.5%, 49.8% and 9.19% respectively, and the cost of production increases as the natural abundance decreases (O'Dell, 1985).

Stable isotope techniques that may be used to assess mineral metabolism and bioavailability have been developed as an alternative to radioisotopes. These methods

are outlined by Fairweather-Tait and Dainty (2002), and include isotope balance techniques using faecal and urinary monitoring, where the isotope is used as a marker to calculate absorption and retention and double isotope and compartmental modelling techniques to determine endogenous losses, plasma appearance kinetics and urinary appearance. Thus isotope use could provide a more accurate means of conducting balance studies and may also be used to assess bioavailability. Unfortunately we were unable to find a source of radioisotopes and we did not have the equipment or the finances to utilise stable isotopes at the time these studies were conducted.

However, the trends which emerged from these data provided very useful information and should be followed by further research.

FUTURE RESEARCH

Some of the data resulting from these studies has provided a preliminary understanding of the metabolism of selenium in the cat and dog and has produced trends which warrant further investigation.

It would be worthwhile conducting a study to further investigate the response of both cats and dogs to various levels of selenium supplementation using a variety of short and long term parameters, to ascertain their appropriateness for the assessment of selenium status in various situations. For example, in other species, plasma selenium concentrations are routinely used as an indicator of short-term selenium status, however results in this thesis suggest plasma selenium may not reflect dietary selenium intake in a linear fashion at higher concentrations in cats. Therefore it would be useful to determine the species-specific response of selenium supplementation to both short term (eg – plasma and urinary selenium) and long term (eg - selenium in organs, blood, nails, hair) parameters, as well as the response of functional parameters (eg – TRR and thyroid hormones, GSHPx, Selenoprotein P).

The use of analytical procedures for isolating the different forms of selenium, combined with the use of isotopic markers would both be invaluable tools for understanding selenium metabolism in cats and dogs. The metabolic pathways of specific forms of selenium supplemented to selenium-free diets may be established, with concentrations of the various forms determined in the different metabolic pools of tissue and blood. In addition, more accurate balance studies could be conducted using similar methods to incorporate endogenous losses, making the determination of

bioavailability more accurate. Information from the above studies could then be utilised to conduct requirement studies for cats and dogs, ie – the appropriate parameters may be chosen to look at the metabolic responses of cats and dogs of various ages and life stages to various levels and forms of dietary selenium, whilst accounting for bioavailability.

These tools may also be used to help determine the level at which toxic effects of selenium occur in cats and dogs. In this thesis, symptoms of toxicity were not apparent after a three week supplementation period when cats and dogs were fed 10 ppm. Analysis of some of the urinary metabolites such as selenosugar and trimethylselenonium, and the ratio of these, may provide a more accurate picture of how excess selenium is being metabolised, and in conjunction with other parameters such as hair growth, and labelled selenium retained within the tissues, may give insight as to how these animals tolerate high levels of selenium and at what stage this becomes toxic. Longer term studies should also be carried out to determine chronic effects of selenium toxicity.

Results from this thesis suggest cats may be more efficient at metabolising and regulating higher levels of dietary selenium by increasing urinary loss of selenium to a greater degree than dogs. This may also be investigated further using the urinary metabolites and isotopes to determine the rate and extent of excretion resulting from the ingested selenium.

Potential differences in sulphur amino acid metabolism between cats and dogs may also be worth investigating. There is little information available on sulphur amino acid metabolism in cats, and it is not known why they require greater sulphur amino acid levels than other species. Therefore the link between sulphur amino acid metabolism and that of selenium metabolism in cats is somewhat speculative. A study in which cats are fed a low methionine and cysteine diet supplemented with selenium looking at resulting levels of each amino acid and rate of selenium incorporation in the liver and other tissues may provide some insight as to whether or not there is a link here. There is also potential for investigating the enzymes required for the different metabolic pathways and whether or not these are similar for the sulphur amino acids and their respective selenium counterparts.

Nutrigenomics is rapidly becoming a key area of research in which selenium will no doubt feature. There is evidence that selenium has an effect at the nuclear level causing changes in DNA and gene expression. This has important implications for increasing the understanding of selenium metabolism, the determination of selenium

requirements and treatment of diseases such as cancer on both species and individual levels.

In conclusion, the starting point for future research should be the accurate quantification of the response of cats and dogs to selenium supplementation in order to understand its metabolism, and thus enable the establishment of adequate dietary selenium concentrations for these animals. Once this is achieved, it may be possible to then investigate additional factors such as sulphur amino acid metabolism in companion animals, and ultimately with the use of new technologies and techniques, establish specific individualised dietary selenium requirements for the maintenance of optimum health and prevention of disease, tailored to suit the animals specific requirements.

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APPENDICES

APPENDIX 1

Fluorometric analysis of selenium for petfoods, urine, blood and faecal samples

This assay is a modification of the methods described by the AOAC official method 996.16 (2000) and that of Sheehan and Gao (1990).

Principle

This method of fluorometric analysis is based on the oxidative digestion of selenium to inorganic forms, and the reaction which follows, whereby selenium in the Se^{4+} valence state is complexed with DAN to create a fluorophore which enables measurement to occur.

Method

The digestion system used included 3 aluminium blocks, each 50 x 10 x 5 cm with 76 holes of 16 mm diameter, set on a 60 x 30 cm hotplate. The tubes used were 12 ml Kimax culture tubes with a screw cap and PTFE-faced rubber liner, 16 x 100 mm, rinsed then furnaced prior to use. This system was contained within a fume cupboard suitable for handling perchloric acid.

Routinely, samples are digested in 0.5 ml of a nitric acid-perchloric acid mixture (4:1 by volume) for 60 minutes or until nitric acid has evaporated. The presence of perchloric acid in the oxidation mixture prevents loss of selenium. The temperature of the hotplate is 100 ^{0}C when tubes are first put in the blocks, and is gradually increased to 190 ^{0}C during the digestion period. Digestion of this sample/acid mix yields selenate (Se^{6+}). Following digestion samples are removed from the block and allowed to cool, whilst the hotplate is also cooled to 110 to 150 ^{0}C. Selenate is then reduced to selenite (Se^{4+}) with the addition of 0.5 ml of 0.1 M HCl. Tubes are then returned to the hotplate and heated for 30 minutes with the temperature remaining between 110 and 150 ^{0}C. After this time tubes are removed from the hotplate and without delay, 2 ml of 20 mM EDTA, followed by 0.5 ml of DAN are added to the tubes which are then capped and incubated for 30 minutes in a 60 ^{0}C water bath. EDTA prevents interference from metal ions, whilst the DAN forms a complex with the digested mixture to create a piazselenol, or fluorophore, which can then be measured. The piazselenol is extracted by addition of up to 3 ml of cyclohexane followed by a 10 minute extraction period using a shaker capable of creating a vortex to all tubes. The

supernatant cyclohexane layer is transferred to vials and the total selenium content determined using a scanning fluorescence detector (Waters™ 474) and a separation module (Alliance™ Waters™ 2690: Alphatech Sytems Ltd & Co, Parnell, Auckland), which is used without a column as a spectrophotometer (λ_{ex} 375 nm, λ_{em} 525 nm). Cyclohexane (100%) is used as a buffer with the flow rate set to 1 ml/min. Samples (100 µl) are injected with a run time of 30 seconds. The temperature is maintained at 10 ^{0}C. Data are quantified using Millenium^{32} version 3.05.01.

Quality Assurance

Each solid sample was analysed in quadruplicate due to its heterogeneous nature, and each liquid sample was analysed in duplicate. With each set of test samples a reagent blank and 6 selenite (0.4 µg/ml: Aldrich Chemical Co.) calibrating standard solutions were used to create a standard curve (0 – 0.04 µg). A commercially available CRM of freeze-dried bovine blood (A-13: Analytical Control Services, International Atomic Energy Agency, Austria; Pszonicki *et al.*, 1983), analysed in triplicate was used as a control. Performance of the assay was assessed using methods and calculations according to Roper *et al.* (2001), Miller and Miller (1986) and Wernimont (1985).

Performance

Results of the assay were reproducible using four replicates for each sample. Standard curve linearity for selenium concentrations was ≤ 400 µg/L (5.1 µmol/L). For a typical standard curve: range r^2 = 0.996, y = 1566406x + 4162.8. For a 100 µl sample, the limit of detection, calculated from mean + 3 standard deviations of the blank fluorescence value, was 14 µg/L (0.177 µmol/L) or 1.4 ng of selenium per tube. The level of precision of the assay was set as ≤ CV of 10% of the mean. This was assessed using a Chi-squared test and results were within the required degree of precision. Comparison of known and calculated values of the CRM showed no bias in the assay. Within and between-assay variations were determined using the mean square values obtained from a one way ANOVA of CRM results. Within and between-assay variations were calculated to be 6.2% and 4.7% respectively.

The fluorometric assay described here proved to be a suitable method for the analysis of selenium in petfood, faecal, urine and blood samples. The greatest source of error appeared to be achieving homogeneity of the diet and faecal samples. This

problem was minimised by the use of quadruplicate replicates, such that the level of error of the assay was set at 10%.

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APPENDIX 2

Selenium concentrations in commercial petfoods

Petfood type	Flavour	Selenium concentration (µg Se/g DM)
Dog food - dry (n=15)	Crunchie chicken	0.448
	Moist chunky beef rings	0.335
	Beef and vegetable	0.249
	Meaty	0.188
	Chicken, barely and rice	0.252
	Beef, vegetable and marrowbone	0.226
	Chicken	0.596
	Chicken, rice, kelp and garlic	0.202
	Gourmet beef, chicken, garden vegetables	0.238
	Real beef	0.192
	(No given flavour)	0.180
	Chicken, pasta, garden vegetables	0.273
	Chicken and rice	0.351
	Chicken	0.481
	Chicken	0.476
Dog food - wet (n=22)	Chunky chicken	0.361
	Meat lovers	0.407
	Beef nuggets	0.681
	Tasty chicken	0.522
	Lamb and vegetable	0.328
	Chicken	0.518
	Chicken prime cuts	0.809
	Chicken and rice	0.189
	Chicken and marrowbone	0.385
	Chicken, rice and vegetables	0.348
	Beef	0.335
	Select meats	0.437
	Chicken and rice	0.433
	Chicken and liver pate	0.157
	Shredded chicken and seasonal vegetables	0.492
	Succulent chicken with seafood	0.636
	Chicken	0.449
	Chicken	0.583
	Casserole with lamb	0.748
	(No given flavour)	0.397
	Garlic and rice	0.346
	Beef, rice and vegetable	0.342
Cat food - dry (n=17)	Chicken and rice	0.245
	Prawn, tuna, salmon and sardine	0.346
	Seafood and chicken	0.273
	Chicken	0.463
	Fish	0.503
	Chicken	0.554
	Chicken and rabbit	0.454
	Ocean fish	0.504
	Shredded chicken, prawn flavoured crisps and vegetables	0.580
	Salmon and tuna	1.136

	Turkey and barley	0.594
	Salmon and rice	0.374
	Chicken	0.623
	Chicken	0.677
	(No given flavour)	0.627
	Seafood mornay with cheese	0.518
	Tasty chicken and liver	0.478
Cat food - wet	Jellied chicken	0.483
(n=35)	Seafood cocktail	3.086
	Hearty beef	0.557
	Oceans bounty	1.409
	Chunky chicken	0.231
	Beef and venison	1.118
	Chicken and turkey	0.705
	Beef and lamb	0.916
	Tender chicken and veal	0.588
	Ocean fish tuna	6.118
	Flaked fish and shrimp	2.582
	Chunky chicken feast	1.408
	Chicken in gravy	0.798
	Salmon	1.196
	Chicken and tuna	1.657
	Jellimeat	0.647
	Beef and lamb	0.740
	Chicken and turkey	0.155
	Chicken and rabbit	0.696
	Ocean fish platter	1.256
	Salmon and cheese	3.746
	Sardine, salmon, chicken and vegetable	2.237
	Tuna	1.113
	Tender beef terrine	0.676
	Fish and calamari risotto	4.081
	Chicken and venison	1.083
	Tuna whitemeat	3.303
	Chunky seafood	0.816
	Chicken	0.532
	Seafood	1.406
	Chicken	0.712
	Chicken	0.472
	Seafood delight	0.841
	Turkey	1.313
	Chunky chicken	1.605

APPENDIX 3

Set up of metabolic cage and urine/faeces collection system for cats

From: Hendriks WH, Wamberg S and Tartellin MF (1999). A metabolism cage for quantitative urine collection and accurate measurement of water balance in adult cats (Felis catus). *Journal of Animal Physiology and Animal Nutrition,* 82: 94-105.

The metabolism cage was made from a polyethylene plastic bin (0.8 x 0.8 x 1.1 m) with a partially covered galvanized steel barred door fitted to the front (Figure 1a). A collection system allowing the separate collection of uncontaminated faeces and urine consisted of two plastic trays (0.15 x 0.30 x 0.45 m) which fitted inside each other (Figure 1b). The top tray had a 1 mm stainless steel wire mesh floor whereas the bottom tray was made from solid plastic. When fitted, a 2 cm space between the bottom of the two trays ensured separate collection of the urine. The two collection trays were securely positioned in the rear left-hand corner on the floor of the metabolism cage under a 5^0 slope and a 3^0 tilt such that urine collected in the corner of the bottom tray. Faeces were retained on the wire mesh of the top tray while urine passed through the mesh and collected in the corner of the bottom tray.

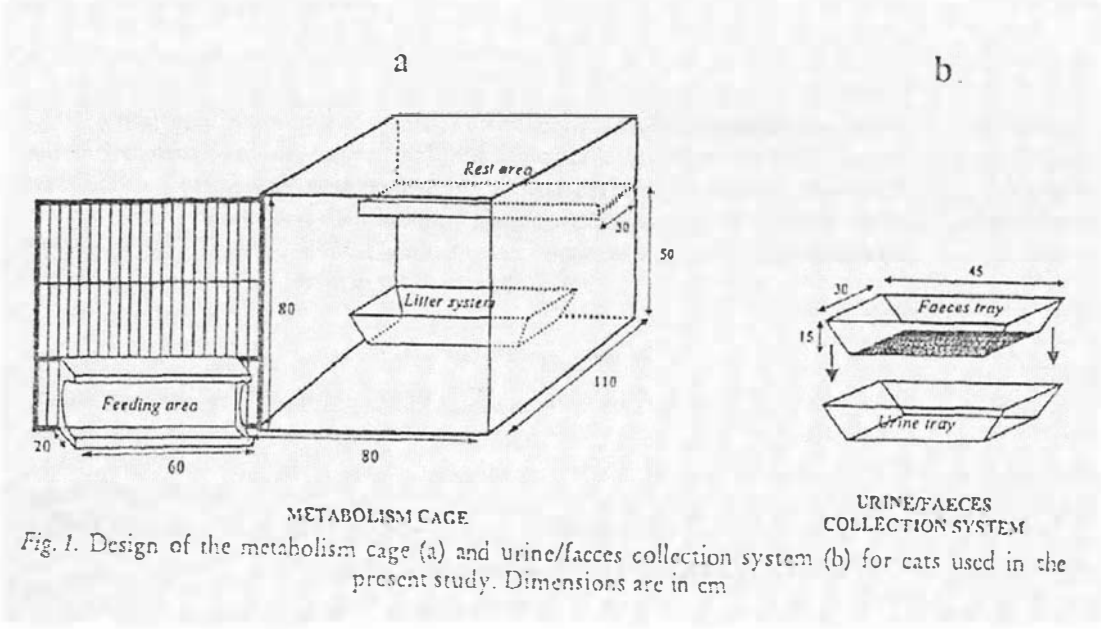

Fig. 1. Design of the metabolism cage (a) and urine/faeces collection system (b) for cats used in the present study. Dimensions are in cm

APPENDIX 4

List of Publications

Todd SE, Hendriks WH. (2005) Comparative selenium metabolism in cats and dogs. Proceedings of Alltech's 21st Annual Symposium, Lexington, Kentucky. May 22-25. Edited by TP Lyons and KA Jacques. Nottingham University Press, Nottingham. Pp 389 – 397.

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