The influence of heat treatment of soybeans on the bio-availability of its selenium in lambs

by

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DECLARATION

I declare that this thesis in my own work. It is being submitted for the degree, Magister Scientiae (Agriculturae) at the University of Pretoria. It has not been submitted before for any degree or examination at any other university

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Date:

Signature:

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LIST OF ABBREVIATIONS

- ADIN :Acid detergent insoluble nitrogen
- CP :Crude protein
- DM :Dry matter
- EU :Enzyme unit
- GSH-Px :Glutathione peroxidase enzyme
- GSH :Reduced glutathione
- GSSH :Glutathione
- GSSeSG :Selenodiglutathione
- H_2O_2 :Hydrogen peroxide
- IDI :type 1 iodothyronine 5'-deieodinase
- IVDOM :*In vitro* digestible organic matter
- kD :kilo Dalton
- NMD :Nutritional muscular dystrophy
- OM :Organic matter
- [Se]Cys :Selenocysteine
- [Se]Met :Selenomethionine
- SH^- :Sulphydril group
- Ser :Serine
- T_3 :3,3'-5-tri-iodothyronine
- T_4 :Thyroxine
- ${}_TRNA_{UGA}$:Transcriptional ribo-nucleotide stopcodon UGA

ABSTRACT

The aim of the present study was to investigate the bioavailability of selenium (Se) from milled soya beans, a plant protein, to lambs after various degrees of heat treatment. Thirty Dohne Merino lambs (ca. 25 kg live weight, 4 months old) were allocated randomly within sex and weight groups into three treatment groups. During the experimental period, they were fed a diet consisting of a large proportion of milled soya beans as their main source of Se and other feeds low in Se to balance the diet. Blood was drawn during this period and analysed for Se and glutathione peroxidase (GSH-Px) activity. Chemical and Se analyses were performed on the feed and soya bean samples as well as on the organs, wool and rumen fluid of the lambs.

Although the ADIN concentration of the soya beans increased with increasing heat treatments, more heat damage could have been inflicted on it to differentiate the different soya bean fractions in comparison to each other. On average, the *in sacco* disappearance of the dry matter (DM), crude protein (CP) and Se was higher for the unprocessed soya beans compared to the two processed soya beans fractions. As a result, the lambs receiving the unprocessed soya beans (control group- C-group) had a significant higher rumen bacterial Se concentration than the lambs receiving the heat-treated soya beans. Despite this higher concentration, the liver Se concentration of this group was statistically significantly lower compared to the two heat-treated groups. This can be interpreted that the heat processing of the soya beans enhanced its Se fraction to be taken up by the liver. However, those differences were biologically not significant. The average liver Se concentration between the lambs receiving the over-processed- (O-group) and those receiving the ideal treated soya beans (I-group), did not differ, indicating that the excessive heat treatment did not influence the amount of Se reaching the lower digestive tract of the O-group. Although the Se concentration of the heart muscle of the O-group was statistically significantly lower than for the I- and C-groups, those differences biologically were not significant. Despite an increase in the erythrocyte GSH-Px activity over the duration of the trial in all three the treatment groups, no significant differences between the three treatment groups were observed at any stage.

It is concluded from this study that the heat treatment of soya beans has not influenced the incorporation and the bioavailability of its Se fraction to any significant degree.

OPSOMMING

Die doel van die huidige studie was om ondersoek in te stel na die biobeskikbaarheid van die Se fraksie van sojabone, 'n plant proteïenbron, na verskillende hittebehandelinge aan die herkouer. Dertig Dohne Merino lammers (ca. 25 kg lewende gewig, 4 maande oud) is ewekansig ten opsigte van geslag en gewig verdeel in drie behandelingsgroepe. Gedurende die eksperimentele periode is die lammers 'n diëet gevoer bestaande uit 'n groot proporsie sojabone as die hoofbron van Se. Die res van die diëetkomponente se Se konsentrasie was laag en is ingesluit om die eksperimentele diëet te balanseer.

Gedurende die eksperimentele tydperk is bloed getrek en geanaliseer vir Se en glutatioon peroksidase ensiem (GSH-Px) aktiwiteit. Chemiese en Se analises was ook

uitgevoer op die voer- en sojaboon monsters sowel as op die organe, wol en bakterië verkry vanuit die rumen vloeistof van die lammers.

Alhoewel die ADIN konsentrasies van die sojaboon fraksies gestyg het met verhoogde hittebehandeling, kon meer hittehebandeling toegepas gewees het om die verskillende sojoboonfraksies meer van mekaar te onderskei. Die *in sacco* verdwyning van die ongeproseerde sojabone se droë materiaal (DM), ru-protein (RP) en Se fraksies was hoër as dié van die hittebehandelde sojabone. Gevolglik het die lammers wat die ongeproseseerde sojabone ontvang het (kontrole groep – C-groep), gemiddeld 'n hoër rumen bakterium Se konsentrasie gehad as die lammers wat die hitte-behandelde sojabone ontvang het. Ten spyte van die hoër konsentrasie, was die Se konsentrasie van die lewer van die groep betekenisvol laer as dié van die hittebehandelde groepe. Hieruit kan moontlik afgelei word dat die beskikbare hoeveelheid Se na die laer spysverteringskanaal (SVK) vermeerder was met die hittebehandeling van die sojabone. Die verskille was egter biologies nie betekenisvol nie.

Aan die ander kant was daar nie betekenisvolle verskille waargeneem in die lewer Se konsentrasie tussen die I- en die O-groep nie. Hierdie waarneming kan suggereer dat die hoeveelheid beskikbare Se wat die laer SVK bereik het, nie benadeel was deur die oormatige hittebehandeling nie. Alhoewel die gemiddelde Se konsentrasie van die hartspier en bloedfraksie aan die einde van die eksperimentele periode stastisties betekenisvol hoër was vir die I-groep vergeleke met die O-groep, was die verskille nie biologies betekenisvol nie. Ten spyte van die verhoogde GSH-Px ensiem aktiwiteit in al drie die behandelingsgroepe oor die behandelingstydperk, was geen betekenisvolle verskille waargeneem tussen die groepe nie.

Vanaf die studie word die gevolgtrekking gemaak dat die hittebehandeling van die sojabone nie die Se fraksie se opname en gevolglik, biobeskikbaarheid, betekenisvol beïnvloed het nie.

INTRODUCTION

Selenium forms part of certain enzymes like the GSH-Px enzyme complex (Rotruck *et al.,* 1973) that are responsible for the removal of dangerous free radicals produced during normal metabolic processes in the cell (Burk, 1990). It also forms part of other enzymes such as type 1 iodothyronine 5'-deiodinase (IDI). This enzyme, found mainly in the liver and kidneys, converts the prohormone thyroxine (T4) to its active T3-form, 3,3'-5 tri-iodothyronine, that is responsible for the normal metabolic processes in the cell (Arthur *et al.,* 1992). Other selenoproteins also exist such as selenoprotein W, present in the muscles of sheep (Yeh *et al.,* 1997) and selenoprotein P (Combs & Combs, 1986). A 15 000 - 20 000-dalton selenoprotein has been observed in the spermatozoa of rats and cattle (Behne *et al.,* 1988). Beilstein *et al.* (1981) furthermore identified selenocysteine in a 10 000-dalton protein isolated from the crude nuclear, mitochondrial and microsomal fractions of ovine liver and cardiac muscle.

Most of the diseases and conditions associated with a Se deficiency in the diet can be associated with the impairment of the above-mentioned enzymes and proteins. Although nutritional muscular dystrophy (McMurray *et al.,* 1983; Pherson, 1993) is the best-known Se deficiency symptom, it is not the only disease associated with an impairment of the GSH-Px enzyme complex (Bryant *et al.,* 1983; Hakkarainen, 1993; Pherson, 1993; Vitoux *et al.,* 1996). Growth impairment, thermogenic response and other diseases associated with an impairment in the thyroid metabolism, such as certain reproductive disorders (Daniels, 1996), can also be influenced by the Se status of the animal. In addition, reproduction is affected directly by a deficiency of Se (Gerloff, 1992). In humans, a condition known as Kaschin-Back disease (Keshan Disease Research Group, 1979) can be prevented by an adequate Se intake.

The economic importance of the mineral in the animal production field is further emphasised by a report of Zachara *et al.* (1989) on the situation in Northern Poland (Bydgoszcz area), where mortality losses among lambs dying of white muscle disease amounted to 30% in one season. However, Se is very toxic with an extremely low safety margin (Sandholm, 1993). It is, therefore, necessary to investigate the bioavailability of this mineral in order to increase the productivity of the animal, but also to avoid toxicity.

There is a number of factors that can influence the bioavailability of dietary Se to the ruminant (Anderson *et al.,* 1983; Combs & Combs, 1986; Mason & Weaver, 1986; Behne *et al.,* 1991; Gerloff, 1992; Vendeland *et al.,* 1992; Planells *et al.,* 1995; Daniels, 1996; Jackson, 1997; Koenig *et al.,* 1997). One such factor is the heat-treatment of protein sources (van Ryssen & Schroeder, 1999; van Ryssen & Schroeder, 2003).

Protein sources such as soya- and fish meal are heat-treated to increase the proportion of available DM and CP reaching the lower digestive tract of the ruminant (Schroeder, 1997). In a study conducted by Reddy & Morris (1993), an increased production in calves was observed due to the heat-treatment of soya beans. However, it was also observed that the heat treatment of protein sources had an effect on the disappearance

of Se from the rumen in the ruminant (van Ryssen & Schroeder, 1999; van Ryssen & Schroeder, 2003) as well as from the lower digestive tract (van Ryssen & Schroeder, 2003). The authors observed that with excessive heat-treatment of the protein source, the percentage of Se in the faeces increased while the rumen undegradable, abomasum degradable nitrogen fraction (UDP-D) decreased (van Ryssen & Schroeder, 1999). The conclusion was made that with excessive heat treatment, the uptake of Se by the ruminant is decreased and possibly also its bioavailability. The incorporation of Se into the body proteins and biologically important proteins, however, was not determined in their study.

It was also observed in other protein sources that the Se fraction of those proteins is not always bio-available to the host animal and can vary between animals (Stowe & Herdt, 1992). In a study conducted by Gabrielsen & Opstvedt (1980), it was discovered by measuring the GSH-Px enzyme activity in chickens that the bioavailability of Se in solvent-extracted soya bean meal was only 17.5% compared to selenite (100%). Other studies also showed that the bioavailability of Se from organic sources, and especially fish- (Gabrielsen & Opstvedt, 1980) and blood meal was very low compared to inorganic sources such as selenite (Cantor & Tarino 1982; Hakkarainen 1993).

It can be concluded that the bioavailability of the different Se sources varies from extremely low in some of the fish meal to extremely high in the case of selenite.

As a result, the aim of this study was to investigate the effect of the heat treatment of plant proteins such as soya beans on the bioavailability of its Se fraction to the host animal. This study should give us a better understanding of the influence of heat treatment on protein sources with regard to their Se bio-availability and whether it is the reason why the bioavailability of Se in certain protein sources, like fish meal, is generally poor (Gabrielsen & Opstvedt, 1980).

CHAPTER ONE

LITERATURE REVIEW

Selenium sources can be divided into organic- and inorganic Se sources. Inorganic Se sources include selenite, selenate and selenide while the organic Se sources includes selenomethionine and selenocysteine (Cantor & Tarino, 1982).

The main form of Se in cereal grains is selenomethionine (Combs & Combs, 1986; Daniels, 1996), while forage plants also contain selenomethionine as well as selenate and other unidentified Se compounds (Combs & Combs, 1986). Selenocysteine is the major form of Se present in animal tissues (Cases *et al.*, 2001). Selenium occurs in the body in both the organic- (selenomethionine and selenocysteine) and inorganic form (selenite, selenate, selenide) as well as in the elemental form (Cases *et al.*, 2001).

1.1 Bioavailability of Selenium

1.1.1 Definition of Bioavailability

Bioavailability can be and is defined in various ways. Fox *et al.* (1981) defined bioavailability as "a quantitative measure of the utilisation of a nutrient under specific conditions to support the organism's normal structure and physiological processes". Jackson (1997), on the other hand, defined bioavailability as the proportion of the ingested nutrient that is used for normal physiological functions as well as storage.

According to Jackson (1997), the utilisation of a supplement depends on the physiological status of the animal. Some of the factors affecting the physiological status of the animal include pregnancy, lactation and growth (Jackson, 1997). Due to these factors, it is possible that the bioavailability of a supplement like Se, measured only by its utilisation, could be higher for one individual than for another.

The following example highlights the difference between the two definitions. Organic Se can replace sulphur in the sulphur containing amino acids like methionine in the muscles where it is "stored" (Whanger & Butler, 1988). This Se, according to the definition of Fox *et al.* (1981), is not bio-available while it does have a bioavailability according to the definition of Jackson (1997). However, the stored Se in the muscles can become available to the animal through protein catabolism and be incorporated into the GSH-Px enzyme complex under certain conditions (Waschulewski & Sunde, 1988). This stored Se is, therefore, available to the animal to be "used" in maintaining the animal's physiological status and is bio-available, as proposed by Fox *et al.* (1981) and Jackson (1997).

As can be seen from the above-mentioned illustration, it is difficult to standardise the term "bioavailability" through the supplement's utilisation by the host, as there are too many factors that can affect its utilisation (Heaney, 2001). In order to eliminate the influence of the physiological status of the experimental animals, the definition of bioavailability as proposed by Jackson (1997) was adopted in this study.

1.1.2 Measurement of Bioavailability

Six approaches exist in determining the bioavailability of nutrients (Heaney, 2001).

- The first approach is the balance method where the absorption of the nutrient is determined by calculating the difference between the intake of the nutrient and its excretion in the faeces, corrected for bacterial interference.

- The second approach is by determining the serum concentration of the nutrient after intake (and absorption).

- The third approach is by urinary excretion. Although the urine can be a major route for the excretion of Se in the ruminant (NRC, 1983), the faeces is the main route (Langlands *et al.*, 1986). Furthermore, Heaney (2001) suggested that this method is not very precise and sensitive in determining the bioavailability of the test nutrient. Care must, therefore, be taken in determining and interpreting the bioavailability of Se using this method, especially for ruminants.

- The fourth approach is by means of a tracer method. The direct absorption and incorporation of the test nutrient is determined through a radioactive or stable tracer. This method appears to be the most optimal approach, although the extrinsic labelling of the test material can have problems in mixing with the non-labelled fraction of the test material.

- The fifth approach is by measuring certain target organs after administration of the test material. According to the author, this method seems to be the ideal method in determining the bioavailability of the test material (nutrient). However, the uptake of the test nutrient including Se is not just a function of the bioavailability of the nutrient, but also of the "need status" of the individual nutrient by the host (Jackson, 1997). The type and physiological status of the animals used in those studies can influence the results of the specific bioavailability study and may not be the same as other bioavailability studies. Results obtained from such studies will as a result not always be a true reflection of the nutrient's bioavailability.

- Lastly, there is the *in vitro* test. For minerals, the solubility of the mineral is mostly tested and then related to their absorbability. This can yield misleading information, as the *in vitro* conditions are not always mimicking the *in vivo* conditions, while solubility does not necessarily mean that the nutrient will be absorbed into the body.

There is no ideal method in determining the bioavailability of Se. Of the six possible methods, two are frequently used (King, 2001). The first method is the absorption, retention and/or tissue retention of labelled Se foods. The second method is the measurement of the GSH-Px enzyme complex activity after supplementation with Se. Selenite is more readily metabolised to the precursors of selenocysteine than the orally consumed organic forms (Sunde, 1990) and is most commonly used as the reference standard in the evaluation of Se bioavailability studies (Gabrielsen & Opstveld, 1980). According to Hakkarainen (1993), a third method can also be used. This method is called a preventive or remedial approach and calculates the bioavailability of the dietary Se from its ability to cure or prevent certain diseases associated with a Se deficiency. Diseases associated with a Se deficiency include pancreatic atrophy in

chickens, myopathes or dystrophy in lambs, pigs, calves and exudative diathesis in chickens (Hakkarainen, 1993).

It was decided for this study that the incorporation of the mineral (Se) into the various organs would be determined, together with the activity of the GSH-Px enzyme complex in determining the Se bioavailability of the heat-treated soya beans.

1.2 Selenium

It is necessary to look at the absorption and metabolism of the mineral (Se) in order to understand the bioavailability of the mineral better and to be able to draw conclusions from the study.

1.2.1 Selenium Absorption and Metabolism

The most efficient absorption of selenite and selenate occurs in the ileum, while selenomethionine is, in a study conducted on rats, rapidly absorbed from all the segments of the small intestine (Vendeland *et al.,* 1992). Essentially no absorption of Se occurs in the rumen and abomasum (Whanger *et al.,* 1977) while van Ryssen & Mavimbela (1999) observed that only a small percentage of the dietary Se was absorbed from the large intestines of the sheep.

Inorganic Se sources such as selenite is absorbed from the gastrointestinal tract via a normal concentration gradient, without a regulatory mechanism (Sunde, 1990). The absorption of selenate however, is also thought to be sodium dependent (Vendeland *et al.,* 1992), while Daniels (1996) suggested that selenate might share the same absorption pathway (sodium mediated) as sulphur. Selenocysteine, an organic Se source is absorbed in the same way as selenite (Sunde, 1990). Reduced glutathione (GSH), which is secreted through the bile into the gut lumen, plays a role in selenite absorption, possibly through the binding of Se to form selenodiglutathione (GSSeSG- Combs & Combs, 1986; Vendeland *et al.,* 1992).

It is of interest to note that the uptake and subsequent metabolism of selenite in the erythrocytes is also dependent upon the GSH concentrations (Gasiewics & Smith, 1977).

Selenomethionine, like the inorganic Se sources and selenocysteine, is absorbed via a normal concentration gradient (Combs & Combs, 1986). However, the *l*-form of selenomethionine is also absorbed against the concentration gradient, using the same active transport mechanism as *l*-methionine (Combs & Combs 1986; Hakkarainen, 1993). According to Deagen *et al.* (1987), no evidence exists to suggest that selenocysteine, an organic Se source, can substitute cysteine and be actively transported as selenocysteine, using the same transport mechanism as cysteine. As a result, it has been observed that the transport of selenomethionine is inhibited by methionine, while no inhibition is observed for the transport of selenocysteine by cysteine (Sunde, 1990). A reason for this lack of substitution of selenocysteine and cysteine might be the differences in the chemical properties between the amino acid and its analogue (Beilstein & Whanger, 1986).

The fate of the absorbed Se will vary according to the form ingested, as well as the overall Se status of the individual (Daniels, 1996) and can be metabolised into one of four classes of selenoproteins (Sunde, 1990 – Diagram 1.1). Krishnamurti *et al.* (1989) observed a rapid uptake of Se as selenite through the hepatic pathway from the plasma, followed by the release of the Se in the system. This released Se appeared to be bound to proteins. Burk & Hill (1993) also observed that selenoprotein P, a selenoprotein was synthesised in the liver and secreted into the plasma. From these observations, it is suggested that the liver may be an important organ where some of the Se metabolism takes place.

The first class of selenoproteins is the selenocysteine proteins. Selenocysteine can be incorporated into Se cysteine proteins (class 1) or be broken down by selenocysteine lyase, releasing elemental Se (Se^0) that can be reduced further to selenide (Se^{-2}, Sunde, 1990). It can also replace cysteine at high dietary concentrations (Wilhelmsen *et al.*, 1985; Behne *et al.*, 1991). However, the substitution of selenocysteine for cysteine in protein synthesis is not a major metabolic pathway, as the chemical properties of the two amino acids differ in too many aspects (Wilhelmensen *et al.*, 1985). Selenocysteine proteins include ß-globulin and yeast proteins (Behne *et al.*, 1991).

Selenate (Se^{+2}) and selenite (Se^{+1}) can also be reduced to selenide following the selenite reduction pathway (Behne *et al.*, 1991). Methylation of selenide leads to the formation of methylated Se species that are excreted in the urine and breath. According to Combs & Combs (1986) the excretion of Se by the lungs only happens at toxic Se intakes. More Se is also excreted from the lungs at lethally high intake concentrations when the source is selenite, compared to selenate in the rat, suggesting that selenate is more slowly reduced to selenide than selenite (Combs & Combs, 1986).

The second class of selenoproteins is the Se-binding proteins. Selenide can be incorporated into seleno-binding proteins (class 2). The Se in this class is so tightly bound to the proteins, through a Se-sulphide linkage, that it cannot be separated from the protein using standard protein purification techniques (Ullrey, 1987). Examples of this class include Se fatty acid binding protein, a 130 kD plasma Se-binding protein and a 77 kD mitochondrial Se binding protein (Combs & Combs, 1986).

The third class of protein has, like the first class, selenocysteine as its substrate. The formation of these proteins, however, differs from that in class 1. Selenite, selenate and selenocysteine firstly have to be reduced to hydrogen selenide and alanine by selenocysteine lyase before it can be incorporated into these proteins (Deagen *et al.*, 1987). The selenide is then used as the Se precursor for these proteins, while serine provides the carbon skeleton (Daniels, 1996). The selenide is incorporated into cysteine to form selenocysteine through a specific stop-codon, UGA, in the mRNA (Daniels, 1996). From this basis, up to 80% of the observed Se in the body (Sunde, 1990) and at least 13 selenoproteins (Daniels, 1996), such as GSH-Px, formate dehydrogenase, selenoprotein-P and glycine reductase are formed (Behne *et al.*, 1991).

The fourth class of selenoproteins is selenomethionine specific proteins. Selenomethionine is similar to methionine, with the only difference that it has an atom of Se instead of sulphur (Sunde, 1990). As a result, selenomethionine can be incorporated into the selenomethionine specific proteins like thiolase and ß-

galaktosidase (Combs & Combs, 1986). It can also be built into the muscle protein, replacing methionine (Sunde *et al.*, 1981; Sunde, 1990; Swanson *et al.*, 1991). As the body cannot distinguish between methionine and selenomethionine, selenomethionine can constitute up to 50% of the total amount of Se in the body (Daniels, 1996). The incorporation of Se into the methionine specific proteins depends on the ratio of selenomethionine to methionine and does not appear to be under any homeostatic control (Daniels, 1996). Selenomethionine can also be degraded by one of two pathways (Waschulewski & Sunde, 1988). The one is the transsulphuration of selenomethionine to selenocysteine. The selenocysteine then can follow the metabolic pathways as described under the first type of selenoproteins. It can also be reduced to the methylated Se species that can be excreted as described, or it can be further reduced to selenide and eventually to Se specific selenoproteins like GSH-Px (Sunde, 1990).

Daniels (1996) sums it up by dividing the different selenoproteins into three groups:

1. Group I contains all the selenoproteins where selenocysteine is built in at its active site (class 3 in the above discussing). There are at least 13 of these proteins and they are metabolically active in the body. An example of these proteins is GSH-Px.

2. Group 2 contains the proteins where Se is non-specifically built into the sulphur amino acids. In this case, Se replaces the sulphur atoms in the two sulphur-containing amino acids, methionine and cysteine (class 1 and 4 in the above-mentioned discussing). According to Daniels (1996), the Se in this class is not metabolically active and it (Se) can be stored in the muscles, hair -or wool follicles, etc.

3. The third class of Se proteins is proteins to which Se binds so strongly that it cannot be separated by conventional methods (class 2 in the above discussing).

A schematic diagram showing the metabolic pathways of Se is presented. The abbreviations used in the diagram is as follows:

[Se]Cys	: Selenocysteine	[Se]Met	: Selenomethionine
Ser	: Serine	$_{T}RNA_{UGA}$	: Transcription ribonucleotid, stopcodon UGA
CH_3	: Methyl		

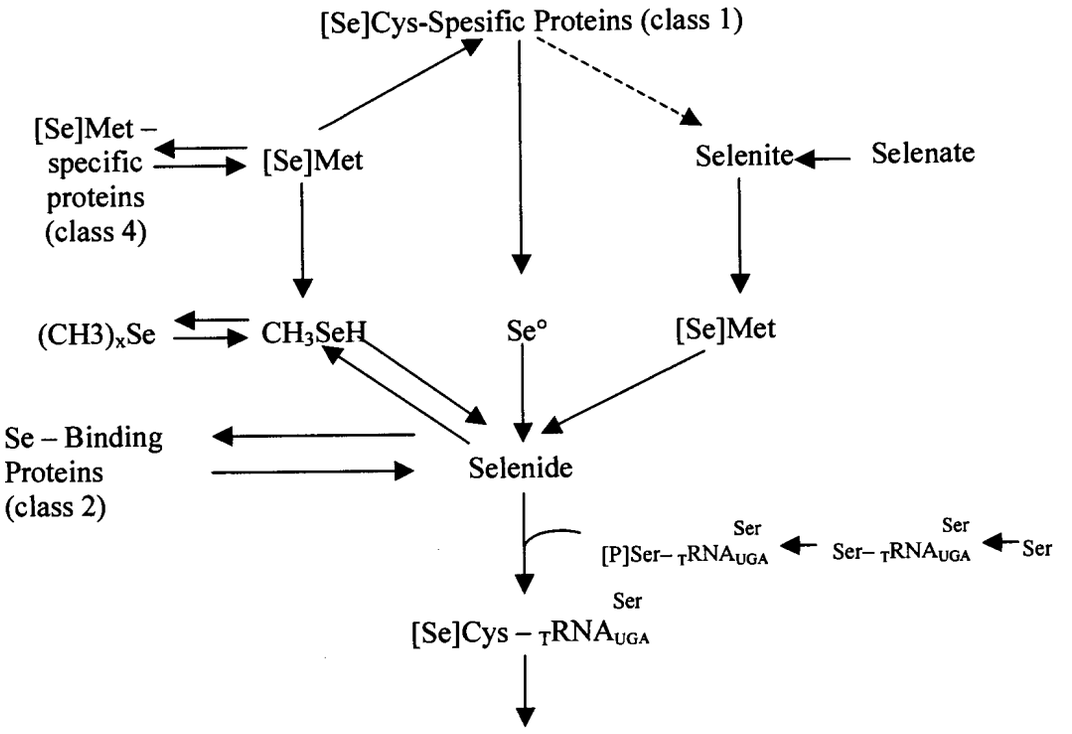


Diagram 1.1: Schematic diagram of the metabolism of Se in the animal body (Sunde, 1990)

From the diagram and according to Sunde (1990) and Daniels (1996), animals cannot synthesise selenomethionine from inorganic Se. This can explain the tendencies observed by several authors (Deagen *et al.*, 1987; Butler *et al.*, 1990; Ekholm *et al.*, 1991) that sodium selenite does not increase the Se concentration of the tissues to the same levels as organic Se. However, Beilstein & Whanger (1986) observed in a study conducted on rats that about 10% of the dietary selenite were recovered as selenomethionine. Further research is necessary in investigating this metabolic pathway in order to completely understand the metabolism of Se.

The metabolic differences between organic and inorganic Se sources also explain the higher effectiveness of organic Se in raising the Se concentrations of whole blood, erythrocytes and blood plasma than the inorganic Se sources (Daniels, 1996). However, inorganic Se (selenite) raises the activity of Se-specific proteins like GSH-Px more efficient than the organic Se sources (Daniels, 1996). An explanation is that inorganic Se only has to be reduced to selenide to provide selenophosphate, the precursor of selenocysteine which is the active form of Se in the selenoproteins (Sunde, 1990). On the other hand, selenomethionine (an organic Se source) has to be transsulphurated to selenocysteine before it can be reduced to selenide (Daniels, 1996).

1.2.2 Functions/deficiency symptoms of selenium

It is not the aim of the present study to investigate the functions and deficiency symptoms of the mineral. As a result, only an overview of the functions and deficiency symptoms of Se will be presented in order to highlight the importance of this mineral in the animal feed industry.

There are at least 13 selenoproteins that contain Se as selenocysteine (Daniels, 1996). Not every selenoproteins' function is however, known and it might be possible that there are still functions of Se that are yet to be discovered (Daniels, 1996).

Selenium forms an integral component of various enzymes like GSH-Px (Rotruck *et al.,* 1973) and IDI (Witchel *et al.,* 1996). It also forms part of some selenoproteins like selenoprotein P and selenoprotein W (Yeh *et al.,* 1997). GSH-Px was the first enzyme associated with Se (Rotruck *et al.,* 1973) and is also the enzyme that is described the most comprehensively of all the selenoproteins.

The GSH-Px enzyme complex plays an important role as a cellular anti-oxidant (Rotruck *et al.,* 1973), thereby protecting the cells against the potential lethal damages caused by free radicals (Behne & Wolters, 1979; Cantor & Tarino, 1982; Burk, 1990; Karlmark, 1993).

Glutathione peroxidase is a selenoprotein (class 3), as described by Sunde (1990). Through the oxidation of GSH to glutathione (GSSH) by peroxides (with GSH-Px as the catalyst), the oxidation of the sulphydril groups (-SH) of many proteins is prevented. The functions of the proteins (that requires the reduced SH^- groups to function normally) are, therefore, not impaired (Beutler, 1989). As a result, GSH-Px by catalysing the reduction of hydrogen peroxide (H_2O_2) and organic peroxides protects the cell proteins against oxidative damage caused by those free radicals (Ullrey, 1987). It also protects the cells against other organic hydroperoxides like lipids, steroids, nucleic acids and prostaglandins to limit lipid peroxidation and cell damages caused by these free radicals (Daniels, 1996). Furthermore, Vitoux *et al.* (1996) observed in humans that the GSH-Px enzyme in the platelets is responsible for a decreased platelet aggregation, decreasing the risk of coronary diseases and arteriosclerosis.

A classical disease associated with a Se deficiency is white muscle disease or nutritional muscular dystrophy (McMurray *et al.,* 1983; Pherson, 1993). Nutritional muscular dystrophy (NMD) is caused by a deficiency of Se in the diet, especially if the diet is high in polyunsaturated fatty acids (McMurray *et al.,* 1983), when the vitamin E-levels are low, with unaccustomed exercise and a rapid growth in young animals (Pherson, 1993).

Selenium furthermore plays an important role in the thyroid metabolism (Wictel *et al.* 1996) via the enzyme type 1 iodothyronine 5'-deiodinase (IDI - Beckett *et al.,* 1987). This hormone is important in that it catalyses the inactive prohormone, thyroxine (T4) to its active T3-form 3,3,5-tri-iodo-thyronine (Beckett *et al.,* 1987) which is important in regulating body temperature, metabolism, reproduction, circulation, and muscle function (Daniels, 1996). Some of the Se deficiency symptoms such as impairment in growth and thermogenic response can be explained by an impairment in the thyroid metabolism (Daniels, 1996).

Selenium might also be required for normal immune response (Jansen van Rensburg, 2001). Droke & Loerch (1989) observed that Se and vitamin E elicited in new feedlot cattle an antibody response against *Pasteurella hemolytica* that could cause pneumonia. Se deficiencies can also cause a decrease in the immune response in the dairy cow, increasing the risk of mastitis and a higher incidence of retained placentas (Gerloff, 1992).

Reproduction can also be improved with the supplementation of Se-to-Se deficient dairy herds (Jukola, 1993). It was observed under *in vitro* circumstances that the maturation, conception and blastocyst production tempo of egg cells could be enhanced by the supplementation of Se (Bowles *et al.*, 1998). Evidence also exists of the presence of a selenoprotein in the sperm of rats (Behne *et al.*, 1988). According to Combs & Combs (1986), a severe dietary Se deficiency can result in reduced sperm production, impaired morphology, motility and viability of the generated sperm. Therefore, it is possible that Se can influence the reproduction and fertility of both sexes directly as well as indirectly through its effect on thyroid metabolism and immune system.

An endemic cardiac disease, Keshan disease, observed in China where low Se soils resulted in low Se intakes, shows the importance of this mineral to humans (Cases *et al.*, 2001). Other diseases that may be caused by a Se deficiency are cancer, cardiovascular diseases, cataracts and Kaschin-Beck disease. The latter is characterized by collagen breakdown and joint deterioration (Keshan Disease Research Group, 1979).

Table 1.1 summarises some of the diseases associated with a Se deficiency together with references.

Table 1.1: Diseases associated with Se

Mechanism	Diseases	References
Through GSH-Px (protecting the cells against the damages caused by the free radicals produced through metabolism and from external sources)	• Nutritional muscular dystrophy	• McMurray *et al.* (1983), Pherson (1993)
	• Liver necrosis	• Hafeman *et al.* (1974)
	• Exudative diathesis	• Noguchi *et al.* (1973); Cantor *et al.* (1975)
	• Decreased incidence of arthritis through decreased prostaglandin synthyses.	• Pherson (1993)
	• Damaged vascular endothelium, capillary bleeding and oedema	• Hakkarainen (1993)
	• General cell damages and premature aging	• Pherson (1993)
	• Coronary diseases through a decreases platelet aggregation	• Bryant *et al.* (1983); Vitoux *et al.* (1996)

Type I iodothyronine 5'eidonase (IDI) converting the prohormone thyroxine (T4) to its active T3 form	• Growth impairment, thermogenic response and other diseases associated through impairment in thyroid metabolism. • Reproduction may in part be influenced through impairment in thyroid metabolism.	• Daniels (1996) • Jukola (1993); Daniels (1996).
Other diseases not necessarily associated through an enzyme.	• Immune response	• Droke & Loerch (1989); Swecker *et al.* (1989); Gerloff (1992); Jansen van Rensburg (2001).
	• Reproduction (female)	• Gerloff (1992); Jukola (1993); Bowles *et al.* (1998).
	• Reproduction (male) maybe through a selenoprotein in the sperm.	• Behne *et al.* (1988)
	• Keshan disease, cancer, cataracts, Kaschin-Back disease (humans)	• Keshan Disease Research Group (1979); Cases *et al.* (2001).

1.2.3 Factors affecting availability/bioavailability of selenium

As can be seen from the discussed functions and deficiency symptoms of Se, it can be concluded that Se is an essential mineral in maintaining the physiological status of the animal. However, Se can become toxic at high dietary intakes (Sandholm, 1993) and is the trace element with the lowest safety margin between an optimal- and toxic intake (NRC, 2001). Therefore, it will be of interest to look at some of the factors that influence the bioavailability of Se in the diet to the host in order to maximise its use and to prevent toxicity symptoms.

The bioavailability of Se from dietary sources depends on a number of metabolic processes, including the absorption of the mineral and its metabolism to the biochemically active forms (Levander *et al.*, 1983).

For the purposes of this discussion, the factors affecting the availability and bioavailability of Se were divided into three groups. The first group of factors is those factors that influence the absorption and excretion of Se from the dietary sources. The second group is those factors that influence the incorporation of the mineral into the different tissues and selenoproteins. However, Se that is incorporated into the tissues can also be incorporated into other selenoproteins and fulfils a more prominent function in maintaining the animal's normal structure and physiological processes. The third group of factors deals with the availability of Se that is incorporated into the

tissues, to be incorporated into other tissues as well as the biologically active components.

1.2.3.1 Factors affecting the absorption and excretion of Selenium

Vendeland *et al.* (1992) observed no evidence of any homeostatic regulation in the absorption of Se. The absorption of Se also does not seem to be the limiting factor determining the bioavailability of Se under normal feeding conditions (Mutanen, 1986). However, Jiménez *et al.* (1997) observed that at restricted feed intakes, the absorption of Se in rats was increased, contradicting the claims that the absorption of Se plays no important role in the homeostatic regulation of the mineral. In the ruminant, the faeces is the main route of excretion (Langlands *et al.*, 1986), although the urine can contribute as well (NRC, 1983). In the monogastric animal, the urine, and not the faeces, is the main route of Se excretion (NRC, 1983). Although it seems that the absorption of Se is not regulated in any homeostatic way there are factors that do influence the amount and chemical form of the absorbed Se as well as its excretion.

- **Rumen milieu**

The microflora in the rumen can alter the chemical form of the ingested Se and as a result, the chemical form that is finally absorbed by the host animal (Henry *et al.*, 1988). This is in particular true for inorganic Se where it is known that Se can be reduced to lesser available forms (selenide or elemental Se) in the rumen (Gerloff, 1992, Serra *et al.*, 1996a) and excreted through the faeces (Langlands *et al.*, 1986).

Furthermore, the reducing capacity of the rumen can influence the absorption of the dietary Se (Gerloff, 1992). Therefore, factors affecting the reducing capacity of the rumen, such as the ruminal pH, will also influence the absorption of Se from the rumen (Gerloff, 1992). One such a factor is the amount of concentrate in the diet. According to the authors, a high concentrated diet would result in a decreased rumen pH and a subsequent increase in the reduction potential of the rumen. The result is a decrease in the efficiency of the Se absorption as more Se, especially inorganic Se (Gerloff, 1992, Serra *et al.*, 1996a) are being reduced to the more unavailable forms like elemental Se. In another study, a monensin supplementation to sheep resulted in an increased blood Se concentration and GSH-Px enzyme activity (Anderson *et al.*, 1983). According to the authors, this was due to a decrease in the rumen methane production and consequently, a decline in the reduction potential of the rumen, resulting in the higher absorption of the dietary Se.

In contrast to the above-mentioned, Koenig *et al.* (1997) observed that the Se absorption (selenite) in the whole tract was significantly higher ($P = 0.053$) for sheep receiving concentrate diets compared to forage diets. According to the authors, the concentrate to forage ratio of the diet is not only instrumental in determining the pH of the rumen and therefore, its reduction potential (Gerloff, 1992), but it will also determine the composition of the microbial populations within the rumen. The predominant microorganisms in the rumens of cows fed high-forage diets are *Prevotella ruminicola* and the *Butyrivibrio* spp., while *Bacteroides amylophilus* and *Selenomonads*, *Lactobacilli* and *Streptococci* spp. dominate when high concentrate diets are fed (Latham *et al.*, 1971, 1972). Evidence exists that the Se metabolism by the rumen bacteria is influenced by the particular species of bacteria present in the

rumen (Hudman & Glen, 1984, 1985). According to Koenig *et al.* (1997), the lower rate of Se absorption observed in sheep fed the forage base diet in their study might have been due to a predominance of *P. ruminicola* that reduced the Se to its unavailable forms. In contrast, *S. ruminantium* and possibly other organisms with a shorter generation time dominated in the concentrate diets and enhanced the availability of the Se by incorporating the Se into the seleno-amino acids of the bacterial protein.

It can be concluded that any factor that causes an increase in the reduction potential of the rumen, such as a decreased ruminal pH, will decrease the absorption of Se from the rumen, especially if inorganic Se is used as the Se source. The composition of the microbial populations in the rumen can also influence the absorption of Se (Koenig *et al.*, 1997). Therefore, factors that influence the microbial populations of the rumen will also influence the amount and chemical form of the absorbed dietary Se.

In summary, it was observed in a study that the retention of dietary Se as selenite was 77 % in pigs compared to only 29 % in sheep (Wright & Bell, 1966), possibly due to the effects of the rumen milieu. The authors, however, admitted that the latter percentage might be an underestimation and that the rumen milieu might not have that big an influence on the bioavailability of Se. However, the role of the rumen milieu in affecting the bioavailability of Se cannot be ignored. Any factor that influences the rumen environment and its microbial population may have an influence on the absorption and thus the bioavailability of Se.

- **Diet composition**

The diet, such as the amount of concentrates, can influence the absorption of Se through its effect on the rumen's microbial population, as well as by influencing the reduction potential of the rumen.

Another way in which the composition of the diet can affect the absorption of Se is by means of the amount of protein in the diet. Serra *et al.* (1996a) observed in sheep given sodium selenite as the Se source, that the Se concentration of the different rumen fractions were influenced by the amount of protein in the diet. In their study, the addition of soya bean meal (and a subsequent increase in the dietary CP concentration) increased both the amount of rumen bacterial- and soluble protein Se concentrations in the rumen, while the amount of free inorganic Se was decreased. The authors postulated that the sulphur amino acids were necessary to incorporate the free Se into the protein fractions of either the rumen bacteria or the soluble protein and that it was supplied by the soya bean meal. Serra *et al.* (1996b) furthermore observed that a lower proportion of Se (as sodium selenite) was absorbed when sheep received high- compared to deficient protein diets. However, no differences were recorded in the absorption of the dietary Se when methionine and cysteine were added to the high protein diets in order to have comparable levels of those amino acids with the control diets. It seems that the dietary protein *per se* was not the factor that influences the absorption of Se, but rather the sulphur amino acids. It was also postulated by Gerloff (1992) that concentrates increase the reduction potential of the rumen in the diet. In the study by Serra *et al.*, (1996b), soya bean meal was added in an attempt to increase the CP fraction of the diet. Therefore, it could also have been caused by the increased reduction potential of the rumen milieu in those diets compared to the control diets.

Inorganic Se can replace sulphur in the sulphur containing amino acids (methionine and cysteine) and is absorbed as selenomethionine or selenocysteine using the same transport mechanisms as its respective amino acids (Serra *et al.,* 1996b). Therefore, any factor which affects the absorption of proteins and especially the sulphur amino acids, such as a deficiency of these amino acids, will affect the absorption of inorganic Se as well. A consequence is that the dietary Se in such diets will have more time in the rumen to be reduced to the unavailable forms (Serra *et al.,* 1996a) and be excreted more easily (Gerloff, 1992). It can be concluded that a diet with an adequate protein content, especially the sulphur amino acids, could result in a higher proportion of inorganic Se being incorporated into the protein fraction of the microbes and Se in the soluble protein. As a result, the amount of free inorganic Se in the rumen will decrease as well (Serra *et al.,* 1996a).

On the other hand with organic Se, it is possible that more Se will be absorbed when methionine is deficient than adequate in the diet (Waschulewski & Sunde, 1988). Most of the organic Se observed in soya beans is in the form of selenomethionine (Yasumoto *et al.,* 1984). The *l*-form of selenomethionine can be actively absorbed, using the same transport mechanism as *l*-methionine (Combs & Combs, 1986; Hakkarainen, 1993). The animal body furthermore cannot distinguish between selenomethionine and methionine (Waschulewski & Sunde, 1988). Consequently, with a methionine deficiency, selenomethionine can be absorbed in the place of methionine and be incorporated into the body proteins (Waschulewski & Sunde, 1988) and possibly the rumen bacteria, also replacing methionine (Hakkarainen, 1993).

The form and type of diet used can also influence the absorption and possibly the bioavailability of the Se. Combs and Combs (1986) observed that selenite was retained and utilised in the GSH-Px enzyme to a lesser extent in rats fed a semi-purified diet than on a corn-soya diet. Mason & Weaver (1986) also observed in rats that the absorption of the extrinsic marked ^{75}Se (as selenite) was significantly less in a solution form than when mixed with soya beans. An explanation, according to the authors is that, in the absence of any food particles, the Se spends less time in the digestive tract, resulting in less time to be absorbed and incorporated into the body proteins. The effect in ruminants and organic selenium sources still has to be clarified.

Generally, the relative proportion of food ingested at a meal also plays a role in the absorption of a mineral. An example is the absorption of calcium where the absorption varies inversely to the logarithm of its load size (Heaney, 2001). As a result, an improvement of up to 80% in the absorbability of the nutrient can occur when the nutrient intake is distributed throughout the day *versus* a single intake (Heaney, 2001). Research performed on Se indicates that, when the dietary Se concentration exceeded the apparent requirements, the urinary Se excretion as a percentage of intake increases (Ullrey, 1987). As a percentage, less Se is absorbed or retained in the body. On the other hand, a Se deficiency seemingly does not have an effect on the absorption of any selenocompound in any intestinal segment of the rat (Vendeland *et al.,* 1992). Behne *et al.* (1991) also observed no homeostatic control in the absorption of organic Se as measured against the concentration gradient. The same is true for inorganic Se, since a tenfold increase in the inorganic Se concentration of the diet did not change the absorption rate of the Se (Diplock & Chaudry, 1988). From the above-mentioned research, it can be concluded that the absorption of the Se is not reduced with

excessive amounts of dietary Se in the diet, but rather that its excretion through the urine and lungs are increased (Combs & Combs, 1986).

Fats (saturated as well as unsaturated) did not influence the absorption of selenite in the rat (Combs & Combs, 1986). Mutanen & Mykkanen (1984) observed that the dietary fat level (4% *vs.* 20%) had only a slight effect on the enteric absorption of Se in chickens. Although information is limited, it seems that fats do not make a significant contribution to the absorption of Se.

- **Chemical form of the selenium**

Organic Se, e.g. selenomethionine, is absorbed more effectively than the inorganic Se sources such as selenite (Vendeland *et al.*, 1992, Daniels, 1996). Together with passive diffusion, selenomethionine can be actively absorbed using the same transport mechanism as methionine. It can also be incorporated into the bacterial protein replacing methionine (Behne *et al.*, 1991). However, no evidence exists to suggest that selenocysteine, an organic Se source, can substitute cysteine or be actively transported as selenocysteine using the same transport mechanism (Deagen *et al.*, 1987) due to the differences in the chemical properties between these two amino acids (Beilstein & Whanger, 1986). Consequently, the absorption of selenomethionine is generally better than selenocysteine.

Inorganic Se sources such as selenite also tend to be reduced more easily in the rumen than the organic Se sources to insoluble Se forms or elemental Se, which are less available in the rumen (Gerloff, 1992) and are excreted more easily in the faeces (Langlands *et al.*, 1986). As a result, more of the organic Se sources are generally absorbed in ruminants compared to the inorganic sources (Wright & Bell, 1966).

Of the inorganic sources, the absorption of selenate is generally more efficient than selenite (Mason & Weaver, 1986; Thomson & Robinson, 1986). A possible reason may be that selenate, together with passive absorption, shares the same absorption pathway as sulphate, while selenite is only absorbed via a concentration gradient (Daniels, 1996). This difference in absorption is noticeable especially at high intakes (Serra *et al.*, 1993b). Cases *et al.* (2001) also observed that selenomethionine and selenate are more diffusible than selenocysteine and selenite that also may contribute to their higher *in vivo* absorption. It is of interest to note that Wason & Weaver (1986) observed in rats using radioactive techniques, that selenate was initially better absorbed than selenomethionine. However, the retention of selenomethionine was better than that of selenate.

It can be concluded that the organic Se sources are generally better absorbed and retained in the body than the inorganic Se sources in the ruminant. The inorganic Se sources are excreted more easily from the body through the faeces (Langlands *et al.*, 1986) and urine (Hakkarainen, 1993) than the organic Se sources. An exception is selenocysteine, an organic Se source, that is absorbed and metabolised more like selenite, an inorganic Se source and as a result, its absorption and excretion simulates more the inorganic Se sources than the organic sources (Beilstein & Wanger, 1989).

- **Vitamin and mineral interactions**

Selenium and sulphur both belong to group VI on the periodic table and have similar chemical and physical properties (Ivaneic & Weiss, 2001). Van Ryssen *et al.* (1998) observed that dietary sulphates reduced the incorporation of Se (selenite) into the rumen bacteria of sheep, possibly reducing the amount of Se that can be absorbed from ruminal bacteria. Ivancic & Weiss (2001) also observed that sulphates reduced the true digestibility of Se. This may be due to the fact that sulphates decrease the rumen pH (Cummings *et al.,* 1995), which in turn, increases the reduction potential of the rumen (Serra *et al.,* 1996a) and subsequently favours the conversion of Se to its unavailable forms (Gerloff, 1992). The result is that an increase in the dietary sulphur can cause an increase in the excretion of Se via the faeces, but not via the urine (Ivaneic & Weiss, 2001). It was concluded that sulphur negatively affects the digestibility and absorption of Se, but not the metabolism of the mineral once it is absorbed (Ivaneic & Weiss, 2001).

Organic Se can, with a deficiency of sulphur, be incorporated into the sulphur-containing proteins of the rumen bacteria due to its similar chemical properties (van Ryssen *et al.,* 1998). These bacterial proteins can be digested in the abomasum and become available to be absorbed as amino acids and be incorporated in the tissues and organs. This can explain the higher Se concentration observed in these tissues at low sulphur diets in a study conducted by van Ryssen *et al.* (1998). Furthermore, an excess of Se in the food can substitute the sulphur in the sulphur containing amino acids and be retained in the body (Combs & Combs, 1986).

It can be concluded that sulphur can influence the absorption of Se, especially the inorganic Se sources, in various ways. In most cases, a low dietary sulphur intake will increase the absorption of Se.

According to a study conducted by Jiménez *et al.* (1997) on rats, a chronic magnesium deficiency decreases their dietary Se (selenite) absorption. Combs & Combs (1986) noted that zinc also decreased the absorption of Se in rats while Planells *et al.* (1995) observed a correlation between the absorption of zinc and magnesium. As a result, Jiménez *et al.* (1997) postulated that magnesium might alter the absorption of Se indirectly through its effect on zinc.

Using the duck as a model, Van Vleet (1982) demonstrated that other interfering minerals influencing the absorption of Se, are silver, cadmium, tellurium, copper, mercury, lead, arsenic, zinc, iron, sulphur and tin alone or in combination, apparently by increasing the enterohepatic circulation of Se (Combs & Combs, 1986). Phytate increased the faecal excretion of Se in young men and apparently decreased the bioavailability of Se (Combs & Combs, 1986). Harrison & Conrad (1984) reported that dietary calcium concentrations can also affect the apparent Se absorption in dairy cows when the Se was provided via natural foodstuffs.

A vitamin C deficiency can decrease the absorption of Se (Combs & Combs, 1986). According to Combs & Combs (1986), when vitamin C was added in the feed at high concentrations, it reduced the inorganic Se to the insoluble and biological inert metallic elemental Se. The result was that less Se was available to be absorbed by the host. However, at normal inclusion levels, vitamin C had no significant effect on the absorption of Se (Combs & Combs, 1986)

The previous Se intake may also influence the absorption of Se. Brown *et al.* (1972) fed rats different levels of Se as Na_2SeO_3 (0, 0.5, 4 ppm) on a low-Se *Torula* yeast-based diet for 33 days before administrating $Na_2{}^{75}SeO_3$ (sodium selenite), either by stomach or intraperitoneal injection. The absorption throughout the range of the $Na_2{}^{75}SeO_3$ was 95-100% of the dose, indicating an absence of a regulatory mechanism for the absorption of selenite. It, therefore, seems that the previous Se intake does not influence the absorption of Se, which is consistent with the conclusion of Burk (1993) that there is seemingly no homeostatic control mechanism regulating the absorption of selenium.

- **Genetic variation**

Genetic variation was reported in Finnsheep in their ability to absorb or retain Se (Sankari & Atroshi, 1983). Sankari, (1993) also observed genetic variations between and within species in their ability to absorb Se. Differences also seem to exist between and with-in species in terms of absorption and incorporating of Se in the body (Gabrielsen & Optsveld, 1980, Douglas *et al.*, 1981).

1.2.3.2 Factors affecting the incorporation of Se into tissues/seleno-proteins/enzymes.

Factors that can influence the incorporation of the absorbed Se into the tissues and different selenoproteins, are as follows:

- **Chemical form**

Selenomethionine is incorporated more readily into the muscle, heart muscle and liver than the inorganic Se sources (Waschulewski & Sunde, 1988). The reason is that selenomethionine can be absorbed and incorporated as its respective amino acid into the body (Waschulewski & Sunde, 1988), as it shares the same absorption- and incorporation mechanism as methionine (Sunde, 1990), especially when methionine is limited in the diet (Waschulewski & Sunde, 1988). The difference of incorporation between inorganic and organic Se into the organs becomes more profound as the Se intake increases. Whanger & Butler (1988) observed in rats that at a concentration of 4.0 mg Se/kg DM, up to 26 times more Se was incorporated into the muscles when selenomethionine was used as the Se source compared to selenite. At a dietary Se intake of 0.2 mg Se/kg DM, the difference was only 2.7 times. For the heart muscle, the increases were 10 and 1.3 times respectively.

On the other hand, the inorganic forms of Se, such as selenite and selenide, are incorporated more readily into the GSH-Px enzyme complex than the organic form, selenomethionine (White & Hoekstra, 1979; Douglas *et al.*, 1981). The latter authors observed that Se in fish (tuna) was only 54% as available to induce GSH-Px activity in the liver and erythrocytes compared to selenite. Noguchi *et al.* (1973) furthermore observed with a direct comparison approach that, in Se-deficient chickens fed diets supplemented with less than 0.1 mg Se /kg DM, selenite was twice as effective as selenomethionine in increasing the activity of GSH-Px in the plasma, liver and heart muscle. However, with higher levels of Se supplementation or a longer duration of supplementation, the biopotency of selenite and selenomethionine were equal (Omaye & Tappel, 1974; Cantor *et al.*, 1975). Cantor & Tarino (1982) furthermore observed

that sodium selenite was more effective in raising the GSH-Px activity in turkeys than selenocysteine. Selenocysteine on the other hand, was more effective than selenomethionine while seleno-ethionine was the least effective of all the Se sources. The ability of selenate to induce GSH-Px activity may also not be as high as selenite (Combs & Combs, 1986).

In general, it can be concluded that organic Se (except selenocysteine) is incorporated at higher concentrations into tissues than inorganic Se. On the other hand, inorganic Se is generally much more available than organic Se in the synthesis of the GSH-Px complex and other biological active selenoproteins.

- **Dietary composition**

The amount of Se in the diet has an influence in the incorporation of Se in the organs and selenoproteins. Behne *et al.* (1991) observed that large intakes of Se only resulted in a relative small increase in the concentration of the selenoproteins and that the levels of those proteins were regulated by a homeostatic mechanism. In earlier studies, Behne *et al.* (1988, 1989) postulated that it appears as if there is a hierarchy of tissue retention of Se when Se depleted rats is supplemented with Se. The authors observed a preferential accumulation of Se in the thyroid, brain, gonads, pituitary and adrenal tissues over tissues like the liver, erythrocytes, heart and muscles. Burk & Hill (1993) also observed that the activity of GSH-Px is less in Se deficient diets than with adequate diets. The authors also observed a differential depression of the selenoprotein synthesis during Se deficiencies. According to the authors and Sunde (1990), it is possible that the available Se can be used for the metabolically most important proteins at the times of Se deficiency, resulting in the preferential accumulation of Se in some of the tissues after repletion.

Studies with chickens showed that a reduced feed- and protein intake resulted in an increased apparent Se bioavailability (from either selenite or selenomethionine) as measured from an increased tissue Se concentration and activity of GSH-Px, together with a reduced incidence of exudative diathesis (Zhou & Combs, 1984). According to Combs & Combs (1986), this apparent increase in the bioavailability of Se might have been mediated through metabolic changes due to a reduced growth rate associated with a reduced protein and/or feed intake. Therefore, the reduced bioavailability of the dietary Se as observed by the authors might only have been a secondary response due the reduced growth associated with those types of diet. According to the authors, it is necessary that bioassays of Se bioavailability studies are to be designed to produce equivalent rates of feed consumption and gain between animals of test and reference groups during the experimental period.

Mutanen & Mykkanen (1984) observed in chickens that the type and level of dietary fats influences the plasma activity of the GSH-Px enzyme complex. According to the authors, maize or sunflower seed oil produced the greatest response at an inclusion level of 4%, while the other types of fats increased the GSH-Px enzyme activity at a 20% inclusion level. No explanation was given and further research is necessary to investigate the mode of action that fats have on the availability of Se for this (and other types of) selenoproteins.

- **Methionine/selenomethionine ratio**

Sunde *et al.* (1981) observed that the dietary methionine concentration is not affecting the activity of the GSH-Px enzyme in the liver, plasma or heart when selenite is administrated to Se-deficient rats. However, sub-optimal dietary methionine concentrations impaired the biopotency of selenomethionine when it was administrated at concentrations below 0.5 mg Se/kg DM. With additional dietary methionine supplementation (0.4% methionine to a diet containing 0.42% sulphur amino acids), the selenomethionine biopotency was equivalent to that of the selenite biopotency in raising the activity of the enzyme.

Selenomethionine follows the metabolic pathways of intact methionine (Waschulewski & Sunde, 1988). When methionine is limiting, selenomethionine will be incorporated into the body proteins in place of methionine, where it will be unavailable for GSH-Px synthesis until those proteins are catabolized (Sunde, 1990). According to the authors, selenomethionine will, under conditions where methionine is adequate in the diet, be degraded by one of two possible pathways. The first pathway is by transsulphuration to selenocysteine, followed by the catabolism of the selenocysteine to selenide by selenocysteine lyase. The second pathway is by the transamination and decarboxylation of the selenomethionine to selenide. In both pathways, the end product is inorganic selenide that can be incorporated into GSH-Px enzyme or one of the other selenoproteins.

It can be concluded that methionine will influence the incorporation of selenomethionine into the tissues just as it will influence the absorption of organic Se. This conclusion can explained the observations made by Waschulewski & Sunde (1988) that, with the addition of methionine in the diet, less selenomethionine from either the diet or stored selenomethionine was stored in the tissues. As a result, the availability of organic Se is increased with sufficient dietary methionine, as more of the Se will be available to be incorporated in biological important selenoproteins like the GSH-Px enzyme complex.

- **Vitamin and mineral interactions.**

Vitamin E can affect the bioavailability of Se in various species including sheep, by promoting its enteric absorption (Combs & Combs, 1986). Cupp (1968) observed that vitamin E increased the post-absorptive incorporation of Se into the GSH-Px enzyme complex, causing a "sink" that drives the enteric absorption of Se through passive diffusion. The same effect was observed with vitamins A and C (Cupp, 1968). It was proposed by the author that antioxidants that increase the intracellular ratio of reduced to oxidised glutathione (GSH: GSSG), would increase the intracellular utilisation of Se increases. According to Combs & Combs (1986), all compounds with antioxidant activities will have the same effect as vitamins A and C in increasing the bioavailability of Se by increasing the post absorptive incorporation of Se into the GSH-Px complex (Combs & Combs, 1986).

Jiménez *et al.* (1997) observed in rats that a chronic magnesium deficient diet reduced the erythrocyte Se concentration while the Se concentration of the plasma and kidney cortices increased significantly. Whole blood Se concentrations were also decreased while the Se concentrations of the heart muscle, plasma and kidneys were increased

significantly. According to the authors, magnesium changes the composition of the membranes and that the observed changes in the Se concentrations might have been related to alterations in the membrane transport system.

Another mineral that can affect the incorporation of Se into the various organs is copper. Hartmann & van Ryssen (1998) observed in lambs a synergistic interaction between the two minerals in both directions. According to the authors, at high supplementation rates, dietary Se increases the hepatic copper concentration and *vice versa*. However, no interactions were absorbed from the whole blood and plasma analyses, while antagonistic interactions were observed from the rumen microbial- and muscle analyses. According to the authors, this relationship between copper and Se in the liver may be related to the sheep's unique ability to accumulate copper in the liver while Combs & Combs (1986) suggested a competitive relationship between copper and Se. According to the latter authors, the two minerals may have the same affinity for binding to carrier and/or storage proteins and that an excess of the one will lead to a displacement and freeing of the other. This freeing could increase the incorporation of the mineral (copper or Se) into other tissues and/or proteins, resulting in a apparent higher bioavailability of the mineral.

The administration of the opposing element (copper or Se) can alleviate toxicity signs when either one of the elements is in excess (Combs & Combs, 1986). According to the authors, this observation might suggest the formation of a metabolically unavailable form of copper and Se. Jensen, (1975) observed at an adequate dietary Se concentration (0.2 mg/kg) for chickens, added copper (800 – 4000 mg/kg) resulted in an increase in exudative diathesis, muscular dystrophy and mortality. When Se was added (0.5 mg/kg), exudative diathesis and muscular dystrophy did not develop while the mortality incidence was comparable with the control group. Their findings also supported the formation of an unavailable Se-Cu adduct.

It can be concluded that a metabolic relationship exists between copper and Se. The hypothesis of competition binding to proteins and the formation of a non-toxic, non-available form of Se and Cu when the two elements are in excess offers a plausible explanation.

In a study conducted by Naganuma *et al.* (1983), zinc affected the distribution of Se in the heart muscle and livers. In the study, the ^{75}Se uptake by the heart muscle and liver was less in mice when injected simultaneously with $Na_2{}^{75}SeO_3$ and $ZnCl_2$ than when $Na_2{}^{75}SeO_3$ was injected alone. No change was observed in the ^{75}Se concentration of the plasma, erythrocytes, lung, brain, kidney, spleen and testes when zinc was co-administrated with $Na_2{}^{75}SeO_3$. Further research is necessary in this regard to investigate the effect of zinc on the incorporation of Se into the organs of the ruminant.

Mykkanen & Humaloja (1984) observed a reduction in the transfer of Se in the body when chickens were fed 1000 mg lead/kg DM three weeks prior to the absorption measurement of ^{75}Se-selenite. This was due to an increase in the retention of the Se in the intestinal tissues. The authors proposed that a long-term exposure of lead might cause the synthesis of proteins that can bind to Se, thereby decreasing its availability to be incorporated into other tissues and selenoproteins. With elevated Se intakes, those binding sites will become saturated and more Se will be available for absorption.

There are other heavy metals that may interfere with the incorporation of Se into the tissues and selenoproteins. According to Ganther & Bauman (1962), arsenic increased the apparent hepatic circulation of Se. As a result, the retention of Se in the tissues was reduced with the treatment of arsenic and as a result, its (Se) toxicity. Se retention was also increased by the treatment of cadmium (Ganther & Bauman, 1962) and mercury (Parizek *et al.*, 1971). Furthermore, Se binds stoichiometrically with mercury, thereby decreasing its (Se) availability for GSH-Px induction (NRC, 1985). Silver intoxication and iron deficiencies may also result in decreased levels of the enzyme in erythrocytes (Beutler, 1989).

The availability of the organic form of Se in terms of the synthesis of GSH-Px depends on its ability to be metabolised to selenide or selenocysteine (Sunde, 1990), with pyridoxine (vitamin B_6) as a catalyst (Beilstein & Wanger, 1989). Therefore, a low dietary vitamin B_6 concentration can decrease the incorporation of organic Se (selenomethionine) into the GSH-Px enzyme complex (Hakkarainen, 1993).

- **Immune response/ Disease**

Increased tissue GSH-Px activity was observed under conditions of increased oxidant stress, such as exposure to ozone or the inclusion of auto-oxidised lipids in the diet (Combs & Combs, 1986). McMurray *et al.* (1983) observed an increase in the activity of GSH-Px when sheep were consuming diets containing high concentrations of linolenic (C18:3) fatty acids. An explanation, according to the authors, could be that the diet produced more free radicals, resulting in a higher incorporation of Se into the enzyme complex. Revis *et al.* (1979) also reported an increase in the activity of the GSH-Px in the muscles from genetically dystrophic mice compared to control mice, suggesting either a GSH-Px induction, or a selective advantage of higher GSH-Px activity in the genetically dystrophic mice. Furthermore, Jornot & Junod (1995) observed that other factors such as oxygen exposure can result in an increase in the formation of GSH-Px. Excess dietary vitamin E has been shown to decrease the activity of tissue GSH-Px in rats (Yang & Desai, 1978), further suggesting that a relative decrease in oxidant stress will lower the need for GSH-Px (Combs & Combs, 1986).

It can be concluded that an increase in oxidant exposure to the host will result in an increase in the activity of the GSH-Px, possibly through an increased incorporation of Se into this selenoprotein. Any factor that will result in such an increase will also cause an increase in the incorporation of Se into the GSH-Px enzyme complex.

- **Genetic composition**

Selenoproteins such as GSH-Px have an endogenously synthesised selenocysteine at its metabolically active site (Daniels, 1996). The stop codon, UGA at the mRNA, is responsible for the formation of this selenocysteine from inorganic Se through selenocysteinyl-tRNA (Daniels, 1996). Selenide is used during this step (Sunde, 1990). The carbon skeleton is provided by serine and through selenocysteine synthetases, it is converted to selenocysteine. For every selenoprotein, there is a specific gene that codes for that protein (Daniels, 1996). Therefore, the Se status of an animal can be considered as being genetically controlled. Furthermore, it was observed

that there is a differential depression of selenoproteins with Se deficiencies, presumably to preserve the metabolically important proteins (Burk & Hill, 1993; Daniels, 1996). This can explain the observations of Sunde (1990) that on Se deficiency diets, the mRNA levels for GSH-Px are almost completely reduced. It was furthermore observed that it was possible to select sheep (Finnsheep) for high and low GSH-Px activities (Sankari & Atroshi, 1983).

In conclusion, it appears that the synthesis of the different selenoproteins is regulated via individual mRNA's at transcriptional or posttranscriptional levels (Sunde, 1990; Burk & Hill, 1993) in response to the availability of Se and other factors such as the chemical form of the Se and oxygen exposure ((Jornot & Junod, 1995). These factors would influence the availability of the absorbed Se (as well as the stored Se in the tissues) to be incorporated into the selenoproteins.

- **Endogenous and exogenous factors of an individual**

There are endogenous factors (physiological status of an animal- Jackson, 1997) as well as exogenous factors (for example feed composition- Waschulewski & Sunde, 1988) that can influence the incorporation of Se into the different body tissues and selenoproteins.

Hill *et al.* (1986) observed that the Se requirements of female rats were lower than that of the males. King (2001) furthermore observed that in pregnant women, especially during the later stages of pregnancy, Se was better conserved than in non-pregnant women through a decrease in their urinary Se excretion. The authors postulated that this better Se conservation was indirectly the result of an estimated 5 kg increase in lean tissue mass during pregnancy. Lean tissue has a Se concentration of 0.2-0.3-mg/kg bodyweight with the result that the increase in body mass could have caused a deficiency of Se in the body, resulting in the observed better conservation of the mineral.

Another endogenous factor is the age of the animal. According to Stowe & Herdt (1992), there is a gradual increase in the Se concentration of the blood of an individual with increasing age. Therefore, comparisons and interpretations should only be made between individuals of the same age.

An example of an exogenous factor is the feed composition of the diet. Waschulewski & Sunde (1988) observed that a methionine deficiency could decrease the incorporation of organic Se into the GSH-Px enzyme complex. When the dietary methionine concentration was adequate, more of the Se (dietary as well as tissue incorporated Se) became available to the enzyme complex. Other exogenous factors include gastrointestinal disorders as this may influences the digestion of the feed particles and the subsequent release of the mineral into the digestive tract (Jackson, 1997).

It can be concluded that the physiological status of an animal, for example pregnancy and exogenous factors like dietary conditions, can alter the availability of the dietary Se to be incorporated into the tissues and/or selenoproteins. This alteration in availability is apparently not caused by changes in the absorption of the Se from the digestive tract, but rather through its utilisation in the body and possibly excretion.

1.2.3.3 Factors affecting the availability of tissue Se for metabolic active selenoproteins.

The factors affecting the incorporation of the absorbed Se into the body proteins (second group of factors) are also affecting the conversion of the "stored" Se from the organs into the metabolically active selenoproteins and will not be discussed in this section. However, as the Se source in our trial is in a natural form (soya beans), with the majority in the form of selenomethionine (Yasumoto *et al.*, 1984), the effect of methionine on especially the organic Se sources will be highlighted.

- **Methionine/selenomethionine ratio**

In a study conducted by Waschulewski & Sunde (1988), the supplementation of methionine deficient diets with methionine resulted in that more Se from selenomethionine was being reused (from the muscle and diet) and catalysed to selenide for biological imported roles like GSH-Px. The authors showed that selenomethionine would be incorporated into the tissues (especially the muscle) when there was a methionine shortage in the diet and that a methionine supplementation made the Se available to be used for biologically active selenoproteins.

According to Behne *et al.* (1991) there appeared to be no homeostatic mechanism controlling the incorporation of selenomethionine into the muscle and liver. This can explain the observations of Waschulewski & Sunde (1988) that selenomethionine had an inability to provide Se for GSH-Px enzyme synthesis over a pro-longed time (14 days), as protein catabolism happened all the time. Protein synthesis would, according to the diagram (Diagram 1.1) and the observations made by Behne *et al.* (1991) incorporate selenomethionine into protein in place of methionine. The Se will consequently not always be available for the formation of selenoproteins. At large intakes of selenomethionine, especially with an insufficient supply of methionine, the risk of chronic Se toxicity could, therefore, be higher than supplying Se in the inorganic form, due to its non-specific incorporation into body proteins (Waschulewski & Sunde, 1988).

Diagram 1.2 is a summary of the factors that influence the bioavailability of Se as discussed.

Table 1.2: Factors that influence the bioavailability of selenium

Site of influence	Factor	Description	References

Absorption of Se	• Rumen milieu	• Reducing capacity (pH, monensin etc.)	• Gerloff (1992); Koenig *et al.* (1997); Anderson *et al.* (1983)
		• Concentrates vs. roughages	• Koenig *et al.* (1997)
		• Purified vs. natural diets	• Combs & Combs (1986); Mason & Weaver (1986)
		• Size of ingestion and Se load	• Ullrey (1987); Behne *et al.* (1991); Diplock & Chaudry (1988); Vendeland *et al.* (1992); Heany (2001).
		• Fats	• Combs & Combs (1986)
	• Chemical form	• Organic vs. inorganic	• Behne *et al.* (1991); Gerloff (1992); Vendeland *et al.* (1992); Daniels (1996).
		• Selenate vs. selenite	• Dreosti (1986); Thomson & Robinson (1986); Hakkarainen (1993); Cases *et al.* (2001).
	• Sulphur amino acids	• Methionine	• Waschulewski & Sunde (1988); Serra *et al.* (1996b)
	• Vitamins and minerals	• Sulphur	• Vendeland *et al.* (1992); van Ryssen *et al.* (1998); Ivanicic & Weiss (2001).
		• Vitamin E	• Hidiriglou (1968)
		• Zinc	• Jiménes *et al.* (1997)
		• Magnesium	• Planells *et al.* (1995).

	• Endogenous & exogenous factors	• Pregnancy, lactation etc.	• Jackson (1997); King (2001).
		• Restricted feed intake	• Jiménez *et al.* (1997).
	• Genetic variation	• With-in species	• Sankari & Artroshi (1983).
		• Ruminants *vs.* non-ruminants	• Sankari (1993)
Incorporation into body proteins/ enzymes etc.	• Chemical form	• Organic *vs.* inorganic	• Noguchi *et al.* (1973); Douglass *et al.* (1981); Waschulewski & Sunde (1988); Whanger & Butler (1988).
		• Selenate *vs.* selenite	• Combs & Combs (1986)
		• Sodium sulphate *vs.* selenocysteine *vs.* seleno-methionine *vs.* seleno-ethionine	• Cantor & Tarino (1982)
	• Vitamins & Minerals	• Piridoxine (Vitamin B_6)	• Beilstein & Whanger (1989)
		• Vitamin E & C and other anti-oxidants	• Combs & Combs (1986)
		• Silver intoxication and iron deficiency	• Beutler (1989)
		• Magnesium	• Jiménez *et al.* (1997)
		• Mercury	• NRC (1985)
		• Copper	• Van Ryssen *et al.* (1998)
		• Sulphur	• White & Somers (1977); van Ryssen *et al.* (1998)

			et al. (1998).
	• Diet	• Sulphur amino acids	• Waschulewski & Sunde (1988); Sunde (1990)
		• Previous/ current status	• Behne *et al.* (1991); Burk *et al.* (1993);
	• Immune response/ Disease		• McMurray *et al.* (1983); Jornut & Junod (1995)
	• Genetic variation		• Burk & Hill (1993); Daniels (1996).
Bioavailability of tissue Se	• Diet	• Sulphur amino acids	• Waschulewski & Sunde (1988)
	• Other factors as discussed under the 2nd group of factors		

It is evident that for organic Se sources (selenomethionine), factors that influence the absorption and metabolism of the CP fraction in both the rumen and lower digestive tract will also influence the bioavailability of the Se fraction of the feed. One such a factor that can cause a change in the absorption of the protein is the heat treatment of protein sources (Schroeder *et al.*, 1997). This factor is also a common denominator in the different protein sources (fish meal – Gabrielsen & Opstveld, 1980 and blood meal – Cantor & Tarino, 1982, Hakkarainen, 1993) which Se bioavailability is generally poor. This factor will be discussed in more detail in the next section.

1.3 The effect of heat processing of protein sources, especially soya beans on the bioavailability of selenium

One of the most important goals in the heat treatment of protein sources is to create a protein fraction that is more resistant to microbial degradation in the rumen than the untreated protein (NRC, 2001). As a result, the proportion of dietary amino acids that are to be absorbed in the lower digestive tract is increased. Therefore, the objective of heat processing is to change the site of digestion of amino acids from the rumen towards the lower digestive tract (Schroeder *et al.*, 1995).

Soya bean meal is a feed high in crude protein and energy and is widely used as a protein source in animal nutrition (NRC, 1985). However, it contains some anti-nutritional factors such as trypsin inhibitors, urease, hemagglutinase, protease

inhibitors, sapponins and goitrogenic factors that can lower its nutritional value to monogastric animals, including calves (< 200 kg body weight - Schroeder, 1997). A high percentage of DM and CP can also be metabolised in the rumen by the rumen microbes to ammonia, lowering the amount of available CP to the animal and a surplus of ammonia in the rumen (Faldet *et al.,* 1992). Soya beans also contain fat, and processing will protect this from ruminal hydrogenation, thereby increasing the amount of unsaturated fatty acids reaching the lower digestive tract (Schroeder, 1997). Heat treatment also increases the palatability of the soya beans (Schroeder, 1997). As a result, more energy and CP will be available to the ruminant in the lower digestive tract from heat processed soya beans, thereby increasing its nutritional value (Reddy & Morris, 1993; Schroeder *et al.*, 1995).

Due to these reasons, soya beans and other protein sources such as cottonseed meal are processed to make the protein less available for ruminal fermentation, but still available to be broken down and absorbed in the lower digestive tract of the ruminant. Heating is the method mostly used to alter the degradability of the protein in the rumen (Schroeder, 1997). This creates cross-linkages between and among peptide chains and carbohydrates (Maillard-reaction). As a result, the solubility of the protein is decreased and it becomes less available in the rumen (Satter *et al.,* 1994). Heat processing over an optimal temperature, however, can cause the protein to be neither fermentable in the rumen, nor digestible in the lower digestive tract (Schroeder *et al.*, 1995) and must be avoided.

Another advantage resulting from the heat treatment is that more minerals may become available to the animal to be absorbed (Schroeder, 1997). This is partly due to the formation of insoluble mineral soaps with the fats in the rumen in the unprocessed form. Calcium especially is affected by this reaction (Schroeder, 1997). The availability of dietary Se can also be influenced by heat treatment of the protein source (Van Ryssen & Schroeder, 2003). These authors observed that the heat treatment of soya beans influenced the disappearance of the mineral in both the rumen and lower digestive tract and that those disappearances were correlated with the disappearances of the CP fraction of the feed. On the other hand, over-processing reduced the disappearance of the Se (and the protein fraction) in the digestive tract in their study. According to Schroeder (1997), excessive heat-treatment can cause the protein to denature and form disulphide bindings with other peptides and/or proteins as well as sugars (Maillard reaction) to such an extent that it cannot be broken down in the abomasum to amino acids and smaller peptides. The result is that more of the protein will be excreted and that its Se absorption and possibly bioavailability will decrease (Van Ryssen & Schroeder, 2003).

Van Ryssen & Schroeder (2003) concluded that the heat processing of protein sources contributes to the factors that affects the disappearance of Se from these protein sources. The authors however, did not measure the effect of heat-treatment on the incorporation of the dietary Se into the tissues and/or selenoproteins and therefore, its bioavailability. As a result, the aim of the present study is not only to determine whether the heat processing of soya bean affects the disappearance of its Se for the host animal, but also its bioavailability.

CHAPTER 2

MATERIALS AND METHODS

2.1: Introduction

The trial was designed to test the effect of heat processing of milled soya beans on the bioavailability of Se in growing lambs. Lambs were fed a diet consisting of a large proportion of soya beans as their main source of Se and other feeds low in Se to balance the diet in other nutrients. The accumulation of Se in the body tissues of the lambs was used to get an indication of the comparative difference in bioavailability of Se in the differently treated soya beans. Glutathione peroxidase enzyme activity in the haemoglobin was also measured.

This research was conducted with the approval and under the supervision of the Ethics Committee of the Faculty of Natural & Agricultural Sciences of the University of Pretoria (EC991209-019).

Processing of soya beans

Unprocessed milled soya beans were roasted to produce a product with an optimal ruminal degradation of protein and DM, to optimise the CP availability in the lower digestive tract (ideal treatment – I-group) of the lambs. This was done at a temperature of 130 ^{0}C for 45 minutes. A second aliquot was over-processed at 150 ^{0}C for 30 minutes with the objective of rendering protein unavailability in the lower digestive tract of the lambs (O-group). Unprocessed soya beans were used as the control (C-group). Temperature and duration of roasting was decided upon based on the studies of Schroeder *et al.* (1995).

Roasting was performed according to the experience of Dr. G.E. Schroeder (Personal communication). A 220 L drum, rotating at 23 revolutions per minute with a dual flame as energy source was used. An internal thermometer was used to measure the temperature inside the soya beans. After roasting the beans were spread open on a canvas to cool down.

The acid detergent insoluble nitrogen (ADIN) analysis was used to get an indication of the proportion of undegradable protein in the soya beans (Schroeder *et al.* 1995). This was also established by incubating the soya beans for 16 hours in the rumen of the sheep, using the *in sacco* technique (Erasmus *et al.*, 1988)

Experimental animals

Thirty Dohne Merino lambs (ca. 25 kg live weight, 4 months old), obtained from the Experimental farm of the University of Pretoria, were allocated randomly within sex (6 wethers: 4 ewes per group) and weight groups into three treatment groups. They were housed individually under roof on the experimental farm at the University of Pretoria. At the onset of the trial, the lambs were weighed and treated with an anthelmintic (Tramisol, containing Levamisole as active ingredient @ 3 mL/10 kg weight) against internal parasites. The lambs were weighed at the onset of the trial and at fortnightly intervals thereafter. Weighing was done on a weighbridge (Duros, type

213A) before the morning feeding of the animals, but the lambs were not starved overnight.

Experimental diets

Each of the experimental diets contained one of the experimental soya bean fractions. Maize starch, salt, urea and lucerne hay formed the balance of the diets. These latter ingredients in the diet were pre-tested and selected to ensure that they contain low concentrations of Se. Lucerne with a low Se concentration was acquired from the Vaalhartz irrigation scheme. The feed composition of the diets is presented in Table 2.1. Urea was included as a precaution, to ensure that sufficient rumen degradable protein would be present in the treatment containing the over-processed soya beans.

Table 2.1: The feed composition of the experimental diets
(DM basis)

INGREDIENT	CONCENTRATION (%)
Soya beans	43.6
Maize starch	10.0
Lucerne hay	45.0
Salt	1.0
Urea	0.4

Feeding procedures

During a two-week adaptation period, the lambs were fed as a group and received lucerne hay (low in Se) and maize starch *ad libitum*. During the experimental period the lambs were fed individually daily. Orts were collected daily and feed intake per sheep was measured. Feed samples were collected daily from each sheep and pooled within treatment. Chemical analyses were performed on these samples.

The lambs had free access to water from troughs, which were cleaned daily.

Blood collection

Two blood samples were collected per lamb from the external jugular through venipuncture using a 20 G needle. This was done on weeks 0 (at the onset of the experimental diet), 6, 9 and 12, when the lambs were slaughtered. One sample per sheep was collected in a 10 mL heparinised vacutainer, the other in a 10 mL vacutainer containing NaK_3EDTA. The tubes were rolled carefully to mix the anticoagulants with the blood without damaging the erythrocytes and were then stored in a freezer pending a Se assay on the heparinised whole blood. GSH-Px enzyme activity was measured on the blood erythrocytes collected in EDTA (Günzler *et al.*, 1974). The blood was put in a freezer at 2-4 ^{0}C and analysed within 48 hours, since GSH-Px activity declines rapidly during storage (Sheppard & Millar, 1981).

Slaughtering and sample preparation

After 12 weeks on the experimental diets, the lambs were slaughtered through cutting the throat and severaging of the spinal cord. The lambs were slaughtered over a period of 3 days, giving an average experimental period till slaughter of 84 days. An equal number of animals per group was slaughtered on each day of slaughter.

The kidneys, livers and hearts were collected. After removing the fat and membranes, the fresh weights of the livers and kidneys were measured. The wet carcass was also weighed. Rumen fluid was collected and squeezed through four layers of cheesecloth to obtain the fluid. Approximately two litres of fluid were collected and stored at –20 ^{0}C. A wool sample was taken over the midrib, 30 cm from the shoulder and from the top line, placed in a paper bag and stored in a wool storage room. In the laboratory the kidney cortices were dissected out and kept for analyses. At specific sites on the liver, samples were cut out and pooled. After removal of the fat, the apex of the heart was collected.

These organs were dried at 60 ^{0}C for 48 hours, milled and stored for Se analysis. (Galgan & Frank (1993) established that oven-drying of the test material below a temperature of 120 ^{0}C does not influence the Se concentration). The wool samples were washed with a commercial washing powder and rinsed several times to remove the soap. The clean wool was then defatted by means of ether where-upon it was analysed for Se.

Isolation of rumen bacteria

This process was conducted with a few deviations from the procedure described by Cecava *et al.* (1990) and Shabi *et al.* (2000): The fluid was allowed to thaw. It was then centrifuged at 1000 g for 10 minutes. The pellets were discarded while the supernatant was again centrifuged at 1000 g for 10 minutes. The supernatant remaining was then centrifuged at 8000 g for 50 minutes (this was equivalent to 20 000 g for 20 minutes, according to the following formula:

(g1)/(g2) = x* original time

where g1 = original force and g2 = actual force (Wilson & Walker, 1995).

The formed pellet was then washed with a phosphate buffer (K_2HPO_4, pH = 7) and centrifuged at 1000 g for 10 minutes to remove trapped plant materials. After this, the remaining supernatant was again centrifuged at 8000 g for 50 minutes. The pellets were dried in an oven at 60 ^{0}C. It was then analysed for Se.

The following diagram outlines the procedure followed in isolating the rumen bacteria from the rumen fluid.

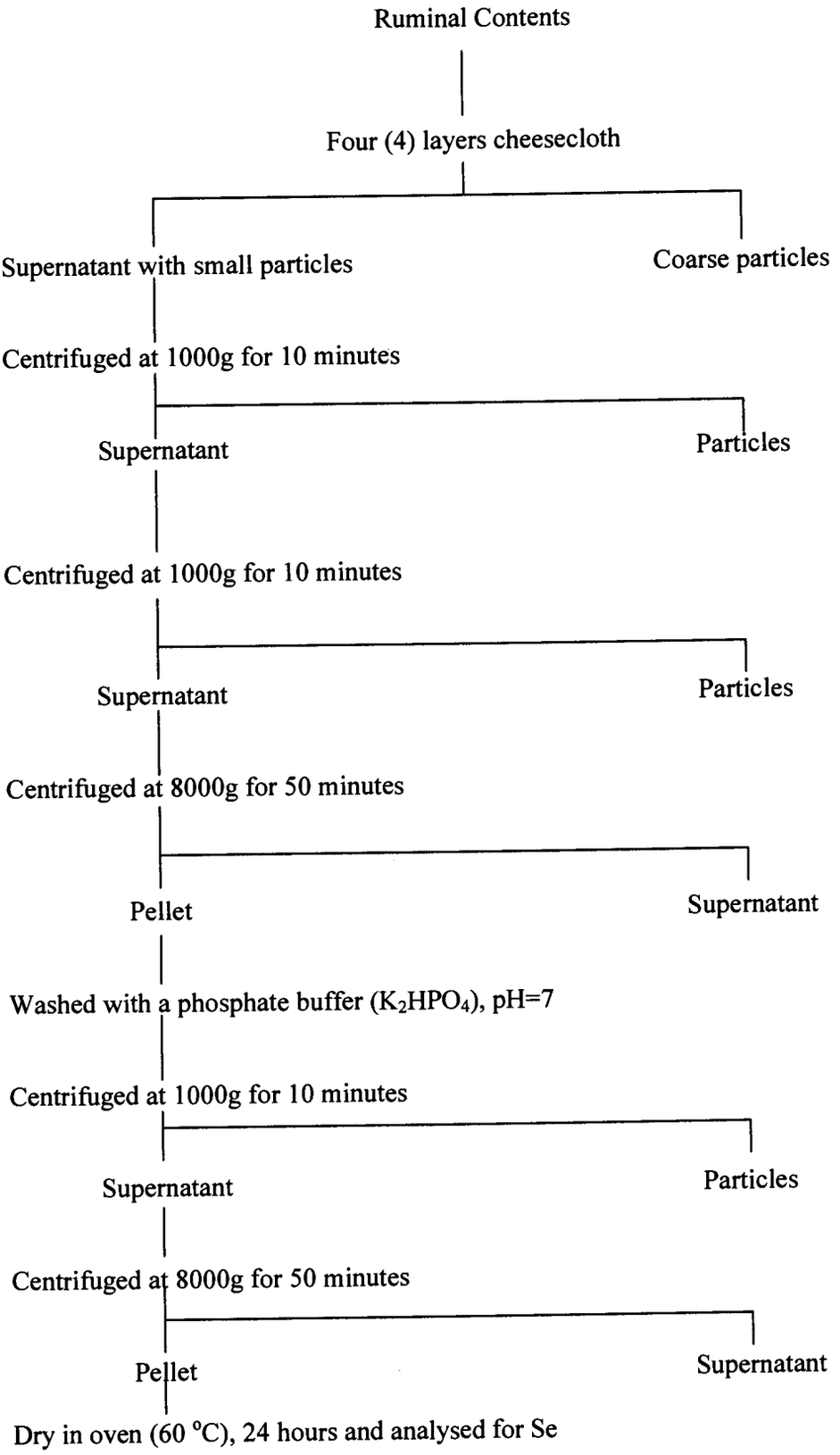


Diagram 2.1 Diagrammatic representation on the procedure to isolate rumen bacteria

2.2: Analytic techniques

Selenium analyses

The Se concentration of the feed, whole blood, wool, kidney cortex, liver and heart muscle was analysed with an atomic spectrophotometer (model 2380), using the following technique:

Between 0.2 and 0.5 g whole blood, 0.1 g of the liver, wool and heart muscle and 0.3 g of the rumen bacteria (DM basis) respectively were weighed out into the test tubes. 0.1g of the kidney cortex samples were taken and diluted with 10 % hydrochloric acid (HCl - 10 mL) due to its high concentration of Se. One mL of this dilution (10 X) was measured out into the test tubes.

Five mL of a digestion mixture consisting of 55% nitric acid (HNO_3) and 72% perchloric acid (HOCl) (4:1 v/v) were added. The reason for this step is that some organic Se products such as selenomethionine, selenocysteine and the trimethylselenonium ions are acid-resistant and an agent (HNO_3) with a high oxidation potential is needed for complete destruction (Verlindin, 1982).

The tubes were put on a programmable digestion block with the following settings:

4 hours at room temperature
1 hour, increasing from room temperature to 100 °C
1 hour at 100 °C
1 hour, increasing from 100 °C to 180 °C
6 hours at 180 °C
2 hours, decreasing from 180 °C to 130 °C
1 hour at 130 °C

During this process, Se IV was converted to the Se VI form. The tubes were then removed from the block for 10 minutes to cool down to room temperature.

Hydrochloric acid (20%, 2.5 mL) was added after which it was heated to 130 °C for 40 minutes, reducing Se VI to Se IV. The remaining Se solutions were made up to 20 mL with 20% HCl for the standards and 10% HCl for the samples.

These Se solutions were put through a hydride generator (Vapor Generation Accessory VGA-77), using 20% HCl as oxidising agent and sodium borohydride ($NaBH_4$) in 0.5% sodium hydroxide (NaOH) solution as a reducing agent (1.2 gram $NaBH_4$ /200 mL 0.5% NaOH).

The gas mixture resulting from the hydride generator was then read by a Perkin-Elmer 2380 Atomic Absorption Spectrophotometer at an absorbency of 196 nm and lamp energy of 16 mA.

Readings were made with reference to standard Se solutions of 2, 5 and 10 parts Se per mL. A bovine liver sample was used as control (National Institute of Standards and Technology, 1577b; Gaithersburg, MD).

The blood Se concentrations in the study were expressed on a weight basis, as it is more accurate than on a volume basis due to differences in viscosity and the amount of red blood cells in the sample (Dr. J.B.J. van Ryssen – Personal communication).

Glutathione peroxidase enzyme activity

The procedure followed in determining the GSH-Px enzyme activity of the red blood cells was in accordance with the recommendations of Dr. S.H. Bissbort (Personal Communication).

GSH-Px activity of the red blood cells was analysed using the Perkin-Elmer Lambda 2 UV/VIS Spectrophotometer at a wavelength of 283 nm and at a temperature of 37 °C. The slit used was 2 nm.

Whole blood was drawn from the external jugular vein into EDTA as the anticoagulant. The EDTA was chosen as the anticoagulant above heparin as Günzler *et al.* (1974) observed that heparin has strong inhibitory effects on the activity of the GSH-Px enzyme. The blood was put in a freezer at 2-4 °C and analysed within 48 hours as the activity declines rapidly (Sheppard & Millar, 1981).

After the blood was allowed to thaw, it was centrifuged at 2500 rpm for 5 minutes. 100 µL red blood cells (erythrocytes) were drawn and mixed with a lyzate (500 µL of a 0.1 M phosphate buffer, pH=7.5 and 20 µL of a 10% Triton-X-100 solution- a non-ionic detergent that lyses the erythrocyte membranes).

500 µL phosphate buffer, 100 µL Dithiothreitol (DTT) solution (20 mg/mL buffer) and 50 µL borohydride $B(OH)_2$ solution (20 µl $B(OH)_2$ in 1 mL buffer) were added into two 2 mL quarts covets on a 37 °C waterbath, together with 1.2 nM t-butyl hydroperoxide (Behne & Wolters, 1983). Günzler *et al.* (1974) observed that by changing the secondary hydrogen peroxide to t-butylhydroperoxide and the reaction temperature from 25 °C to 37 °C, the non-specific catalytic reaction is decreased in the assay. Furthermore, it improves the stability of the substrate. Sodium azide was used to inhibit the activity of catalase, an enzyme that can promote the reduction of hydroperoxides like GSH-Px (Ullrey, 1987). The covets were put into the spectrophotometer for a few seconds to get a zero absorbency. Ten µL of the blood-lyzate solution was drawn and mixed into one covet. The absorbency of the erythrocytes relatives to the mixture in the other covet was measured over a period of 5 minutes. This absorbency was due to the following reaction that had taken place:

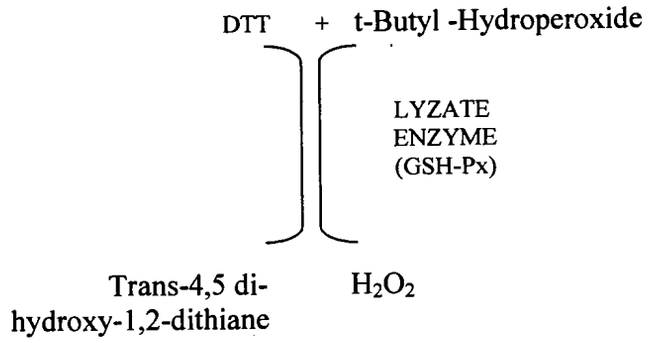


Diagram 2.2: Diagrammatic representation of the chemical reaction in determining the glutathione peroxidase enzyme activity

Trans-4,5 dihydroxy-1,2 dithiane is absorbed at a wavelength of 283 nM. The difference in absorbency between the two covets would, therefore, give the activity of the GSH-Px observed in the erythrocytes. The slope of the reaction of the last 50 seconds was taken as a measurement of the GSH-Px activity.

The haemoglobin values of the blood were analysed using the cyanomethaemoglobin method and averaged 12.0 ± 0.20 g/dL. No significant differences were observed between the treatment groups at any stage. According to Echevarria *et al.* (1988), time or dietary Se concentration does not affect the haemoglobin values of lambs. As a result, this was used as a general value in calculating the activity of the enzyme complex.

Dry matter and ash

The DM and ash analyses were done according to the AOAC (1995) methods.

A ceramic cup was put into an oven at 100 °C for at least an hour to dry completely after which it was allowed to cool down in a desiccator and then weighed. About one gram of a sample was added into the ceramic cup, weighed and put into the oven for 24 hours. Thereafter it was put into a dessicator for half-hour to cool down and weighed. The DM of the sample was calculated as the difference between the ceramic cup and the dry sample at day 2 and the ceramic cup and the sample at day 1 divided by the original mass of the sample.

The ash content was determined by putting the sample (from the DM analysis) into a muffle furnace at 550 °C for four hours after which it was allowed to cool down for two hours (in the oven) and the dessicator. The cup with the ash was then weighed. The ash content of the sample was calculated as the difference of the ceramic cup with the ash and the ceramic cup with the dry sample divided by the original mass of the sample.

To express both analyses as a percentage, the obtained values were multiplied by 100.

***In vitro* digestible organic matter**

The *in vitro* digestible organic matter (IVDOM) of the feed samples was determined using the method of Tilley & Terry (1963), as modified by Engels & Van der Merwe (1967).

The IVDOM analyses consist of two phases. The first phase simulated the rumen digestion, while the second phase consisted of pepsin digestion, simulating the digestion in the abomasum.

In the first phase, artificial saliva was made up into a 2 L flask using the following ingredients:

$NaHCO_3$	9.80 g/L
KCl	0.57 g/L
NaCl	0.47 g/L
$MgSO_{4.7}H_2O$	0.12 g/L
$CaCl_2$	0.04 g/L
$Na_2HPO_{4.12}H_2O$	9.30 g/L

Distilled water was added until a volume of 2 L was reached. After mixing, the flask was put into a water bath (39 ^{0}C) while carbon dioxide (CO_2) was infused through it.

Sifted (1.0 mm) feed samples (0.2 g) were put into the digestion tubes. Two mL of an urea solution (8.68 g urea/ litre H_2O) was added into each digestion tube. The digestion tubes also were put into the water bath in order to reach a constant temperature (39 ^{0}C).

Rumen fluid from a wether consuming lucerne hay was collected and squeezed through 4 layers of cheesecloth. This was mixed with the artificial saliva (1:3 v/v). Twenty mL was added into each digestion tube. CO_2 was infused through the mixture after which the tubes were sealed with a rubber bung. It was kept in the water bath for 48 hours and shaken every 4 hours.

In the second phase (pepsin digestion), HCl was added to each tube until a pH of 2.0 was reached. 3 mL of a pepsin solution (2 g pepsin/ 250 mL distilled H_2O) was added into each tube. Particles were rinsed down with distilled water. The bung were put on while the solutions were kept in the waterbath. The pepsin digestion also lasted for 48 hours with the same procedures followed as already described.

After 48 hours, the solutions were filtrated through a Gooch furnace. This was dried in an oven (100 ^{0}C) for 24 hours after which they were allowed to cool down in desiccators and weighed. They were then ashed for 3 hours and weighed again.

Dry matter and ash analyses were performed on each sample in order to calculate an OM mass of the feed sample.

The calculation of the IVDOM of the feed samples was calculated using the following formula:

$$IVDOM = 100 - \left[\frac{((Kr + \text{undigested residue} - Kr + Ash) - Blank)^* 100}{\text{OM mass of sample}}\right]$$

where Kr = Gooch furnace

Crude protein

Nitrogen analyses were done on the three experimental trial feeds and soya beans, using the Macro Kjeldahl method to obtain CP values (AOAC, 1995). Nitrogen analyses were also performed on the differently heat treated soya bean samples following an acid detergent fibre (ADF) test to obtain the acid detergent insoluble nitrogen (ADIN) values.

A half-gram (DM) of each sample was taken. Ten g sodium sulphate and 0.4 g elemental Se were added together with 25 mL concentrated (98%) sulphuric acid (H_2SO_4) into an Erlenmeyer flask. This was put on a heated oven and allowed to boil for approximately three-quarters of an hour (until the solution was clear). After the solution had been allowed to cool down, 35 mL of the boric acid solution (40 gram H_3BO_3 in 10 mL methyl red and 25 mL methyl blue made up with distilled water to a volume of 1000 mL) was added. 350 mL distilled water, zinc granules and 100 mL sodium hydroxide (45%) were also added after which it was allowed to boil for about 10 minutes until 200 mL distillate remained.

The distillate was titrated with 0.1 N sulphuric acid (H_2SO_4) acid. The values were corrected by the titration of a blank sample.

The percentage nitrogen in the sample was calculated by the following equation:

$$\%\,N = \frac{F \times (\text{Titration} - \text{Blank}) * 100}{\text{Sample mass}}$$

where F = factor associated with the strength of the H_2SO_4.

The percentage crude protein was calculated by multiplying the percentage nitrogen by 6.25.

Mineral analyses

The different feed samples were digested using the following procedure to release the minerals.

One gram of air-dry, milled feed sample was put into tubes. 25 mL HNO_3 (65%) was added after which it was heated. Five mL HOCl was added after about 10 minutes when half of the HNO_3 was boiled away. This solution was allowed to boil for about 40 minutes until only a clear solution remained (HOCl) where-after it was allowed to cool down and made up to 50 mL with distilled water. Each sample was prepared in duplicate and analysed for the different minerals.

- **Magnesium, Calcium and Zinc**

The magnesium, calcium and zinc concentrations were read on the Perkin-Elmer 2380 Atomic Absorption Spectrophotometer that was also used in the reading of the Se concentrations. For the reading of zinc, no further dilution was necessary at a

wavelength of 214 nm. For magnesium and calcium, it was necessary to dilute the solution further with distilled water (120 and 60 times respectively) to get a reading at a wavelength of 285 nm and 423 nm respectively for the two minerals.

- **Manganese and Copper**

The manganese and copper analyses were read on a Varian Flame Atomic Absorption photometer.

Solutions of manganese and copper were made up by putting 1.000 g manganese or copper metal wire or metal wire strip (99.99% pure) in HNO_3 creating one litre solution (1000 µg/mL manganese or copper respectively). From this basis solution, standard solutions of 0.3, 0.5, 2.0 mg/kg manganese and 1, 3 and 5 mg/kg copper were made up in HNO_3.

The lamp currently used for both the manganese and copper minerals was 10 mA. Acetylene was the fuel used in the determination of both minerals, supported by air. The manganese absorbency was read at a wavelength of 279.5 nm, while copper was read at 237.4 nm. In both analyses, a slit width of 0.2 nm was used.

The machine was standardised using the standard solutions made up for both manganese and copper. The absorbency of the minerals was read against the standard solutions absorbency. The concentration was then calculated by the atomic absorbency spectrophotometer using the atomic weight of 54.94 g/mol 63.55 g/mol respectively for manganese and copper.

Phosphorus

Phosphorus (P) readings were done on a Technicon Autoanalyser II Continuous- flow Analytical instrument (AOAC, 1990).

Acid and Neutral detergent fibre

The acid detergent fraction of the feed samples was determined as described by Goering & van Soest, (1970). Two solutions were made up. A pepsin-acid solution was made up by dissolving 4 g pepsin (activity 1:10000) into a heated (42 – 45 °C) HCl solution (0,075 N). About one gram of sample (air-dry, milled through a 1 mm sieve) was weighed and placed into a test tube containing 50 mL of the pepsin-acid solution and incubated in a water bath (42– 45 °C) for 24 hours. The solution was mixed on a regular basis.

After 24 hours, the incubated samples were transferred to the crucibles of the cold extractor unit of the Tecator apparatus by suction and rinsing with hot (50 °C) distilled water. 100 mL of an acid detergent solution (20 g cetyl trimethyl ammonium bromide dissolved into one litre 1 N H_2SO_4) was added to each crucible containing a sample and boiled for an hour.

After an hour, washing and filtering (by suction) removed the acid. The crucibles with the samples were then put in an oven (105 °C) for 24 hours and weighed. After being

weighed, it was ashed at 550–600 °C for 3 hours and weighed again. The percentage acid detergent fibre in the feed samples were calculated using the following equation:

$$\%ADF = \frac{\text{Residue in crucible after drying} - \text{residue in crucible after ashing} * 100}{\text{Original sample mass (corrected for DM)}}$$

The same procedure and calculations were used in determining the neutral detergent fibre (NDF) percentage of the feed samples with the exception of the reagents used. This method is described by Robertson & van Soest (1981). There was also no pepsin-acid incubation of the feed samples in the determination of NDF.

The neutral detergent solution (NDS) used, consisted of 30 g sodium laurel sulphate, 18.61 g EDTA- disodium salt ($Na_2EDTA.2H_2O$), 6.81 g disodium tetraborate ($Na_2B_4O_7.6H_2O$), 4.56 g disodium hydrogen phosphate anhydrous and 10 mL purified 2-ethoxyethanol made up to one litre with distilled water. The pH was kept between 6.9 and 7.1 with HCl.

Acid detergent insoluble protein

The acid detergent insoluble nitrogen (ADIN) analyses were done according to the method described by Goering & van Soest (1970).

The different heat-treated soya bean fractions as well as the complete diets were analysed. Approximately 1 g sieved sample (1 mm circular opening sieve) was put into the test tubes. 50 mL of a heated pepsin-acid solution (4 g pepsin per litre 0.075 M HCl - heated to between 42 and 45 °C) was added.

The samples were put into a waterbath (45 °C) for 24 hours. Every three to four hours the samples were completely mixed in an attempt to completely disperse the sample material by the enzymes.

After 24 hours, the supernatants (pepsin-acid solution) were decanted. The remaining samples were transferred to the dry sintered glass crucibles and placed into the hot extraction unit (Tecator apparatus). 100 mL acid detergent solution (20 g cetyl ammonium bromide/ litre 1M H_2SO_4) was slowly added to the samples while it was heated. This was allowed to boil for 60 minutes after which it was thoroughly washed with hot distilled water.

The samples were dried overnight at 60 °C after which a normal nitrogen analysis was done on the samples as described. The ADIN-value was calculated as follows:

$$\%\ ADIN = \frac{F * \text{titration value} * 100}{\text{Sample mass}}$$

where sample mass is the mass of the sample used in the analyses of the nitrogen and F = the coefficient related to the strength of the sulphuric acid.

Lucerne with a known ADF-value was used as the control in the ADF determinations.

Rumen degradability of the soya beans

The three different heat-treated soya beans samples were analysed for protein and Se degradability in the rumen. The *in sacco* technique as described by Erasmus *et al.* (1988) was used. The air-dry soya beans were milled through a hammer mill with a 4 mm sieve. It was then sifted across a 2.5 mm and a 45 µm sieve to remove the particles bigger than 2.5 mm and the fines respectively. The bigger particles were milled through a 1 mm hammermill and also sieved to remove the fines.

Dacron bags (7 cm x 15 cm, pore size 53 µm) were sown with a double row of stitching and were sealed with a contact adhesive (Ørskov *et al.*, 1980). It was dried at 60 ^{0}C for at least two hours after which it was cooled down in a dessicator and weighed. A lucerne sample with known DM and CP degradability values was used as the control. About 6 g (5 g on a DM-basis) lucerne and of the soya beans samples were placed into each bag and weighed. Dry matter in the bags was calculated by the following formula:

$$DM = (\text{Dry Wt of bag + sample}) - (\text{dry Wt of bag}) \times \frac{DM\%}{100} \quad \ldots\ldots\ldots\ldots\ldots A$$

where Wt = Weight

The bags were tied to a round stainless steel disc (135 g, 8 cm, 2.5 mm thick with 10 evenly spaced drill holes) in the rumens of three wethers. These three wethers were fed lucerne for at least a week before the trial. The three soya bean samples, the lucerne used in the trial and the lucerne used as the control were each analysed on a zero and 16 hours disappearance trial.

All the samples were done in triplicate. Fifteen samples were therefore put into the rumens of the three wethers. After 16 hours, the bags were removed, put into cold water and washed with distilled water. The 0 hour samples were washed in the same way. The samples were then put into an oven (60 ^{0}C) and dried for 48 hours whereupon they were weighed after cooling down in the desiccator. The residual DM in the bags was calculated as follows:

$$\text{Residual DM} = (\text{Dry Wt of bag + residue}) - \text{Dry Wt of bag} \quad \ldots\ldots\ldots B$$

The percentage DM loss from each bag was calculated as:

$$\%\ \text{DM-loss} = \frac{A-B}{A} \times 100$$

The same procedure was followed to calculate the loss of nitrogen in each bag. Nitrogen analyses were done on the samples and on the residues after incubation. Residual nitrogen (N) was calculated as follows:

$$\text{Residual N} = (\text{Dry Wt of bag + Residue}) - \text{Dry Wt of bag} \times \frac{\text{N content of residue}}{100}$$

The percentage nitrogen for each bag was calculated as:

$$\%\ \text{loss of N} = \frac{\text{Nitrogen content of the sample} - \text{Residual nitrogen}}{\text{Original DM of sample}}$$

Statistical Analyses

Data were statistically analysed by variance-analysis, using the GLM (General Linear model) procedure of SAS® (Edition 6) (SAS Institute, Inc, North Carolina, 1990). The Fisher test of SAS was used to establish differences between groups. Significance was set at $P < 0.05$.

CHAPTER 3

RESULTS

3.1 Soya bean- and feed analyses

Lucerne, from the Vaalhartz irrigation settlement near Kimberley, was used in the experimental diets as the roughage component while starch was added as the energy source. Selenium analyses showed that the lucerne contained 12.6 ng Se/g DM and the starch 19.2 ng/g DM. Due to their small percentages in the experimental diets (Table 2.1), the urea and salt supplied negligible amounts of Se to these diets (Table 3.2). The soya beans had an average Se concentration of 327 ng/g DM.

The chemical composition of the soya beans used in the experimental diets is presented in Table 3.1. In addition, Table 3.2 presents the theoretical contribution of the different feed components to the Se concentration of the experimental diets. Table 3.3 presents the ruminal disappearance of the CP, Se and DM fractions of the three heat-treated soya bean samples used in the trial. Figure 3.1 represents the *in sacco* Se and CP disappearance of the different heat treated soya bean fractions at 0 and 16 hours incubation. The data set of Table 3.3 was used to construct the graph in Figure 3.1.

Table 3.1: Chemical composition of the soya beans (DM-basis)

	Control	**Ideal**	**Over-processed**
Se (g/kg)	324.7	324.8	333.3
Dry matter (g/kg)	913	920	924
Ash (g/kg)	75	75	75
CP (g/kg)	564	537	540
***ADIN (% of N)**	1.3%	3.2%	18.0%

*ADIN is expressed as a percentage of the total amount of nitrogen observed in the soya bean fractions

In addition to the above-mentioned chemical analyses of the different soya bean fractions, there was an increase in the colouration (brown) of the soya beans with increasing heat treatments.

Table 3.2: Theoretical contribution of the feed components to the Se fraction of the experimental diets

Feed component	**% Inclusion (DM)**	**Se concentration (ng/g DM)**	**Amount of Se (ng/g DM)**	**Contribution to total diet (%)**
Soya beans	43.6	327.6	142.8	94.9
Lucerne	45.0	12.6	5.7	3.8
Starch	10.0	19.2	1.9	1.3
Salt	1.0	--	--	--
Urea	0.4	--	--	--
Total	100.0	150.4	150.4	100

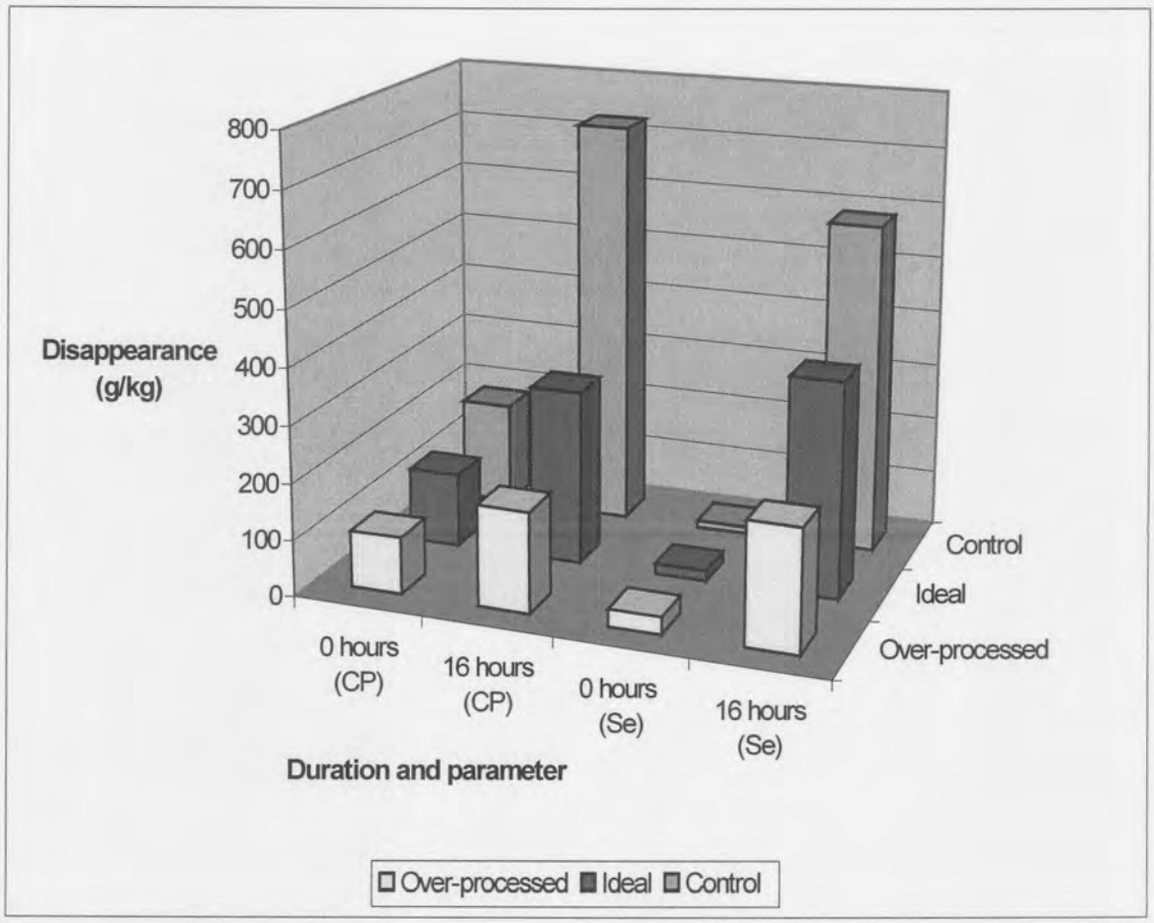


Figure 3.1: Comparative *in sacco* Se and CP disappearance of the different heat treated soya beans at 0 and 16 hours

Table 3.3: The disappearance* of CP, Se and DM in the soya beans after incubation in nylon bags in the rumens of sheep

	DM disappearance (g/kg DM)		**CP disappearance (g/kg CP)**		**Se disappearance (g/kg Se)**	
Soya bean treatment	Incubation time		Incubation time		Incubation time	
	0 hours	16 hours	0 hours	16 hours	0 hours	16 hours
Control	261	793	183	728	12.0	587
Ideal	254	522	131	309	18.9	381
Over-processed	232	404	102	179	30.9	217

* Disappearance of the DM, CP and Se is expressed as proportion (g/kg) of the original amount of DM, CP and Se present in the sample on a DM basis.

The chemical composition of the experimental diets fed to the lambs during the trial is presented in Table 3.4.

Table 3.4: Chemical composition and *in vitro* digestibility of the experimental diets (g/kg DM unless stated otherwise)

	Control	Ideal	Over-processed
Dry matter (g/kg "as is")	906.9	909.4	907.3
Ash	77.5	79.0	79.9
Crude protein	234	239	240
Neutral detergent fibre	355	363	366
Acid detergent fibre	273	277	269
Selenium (ng/g)	140.4	146.9	143.0
Calcium	44	41	42
Phosphorus	35	39	37
Magnesium	19	19	19
Manganese (mg/kg)	27	27	27
Zinc (mg/kg)	21	21	28
Copper (mg/kg)	16	16	17
OM digestibility *(in vitro)*	0.829	0.723	0.676

OM- Organic matter

3.2 Average feed - and soya bean intake and weight of lambs

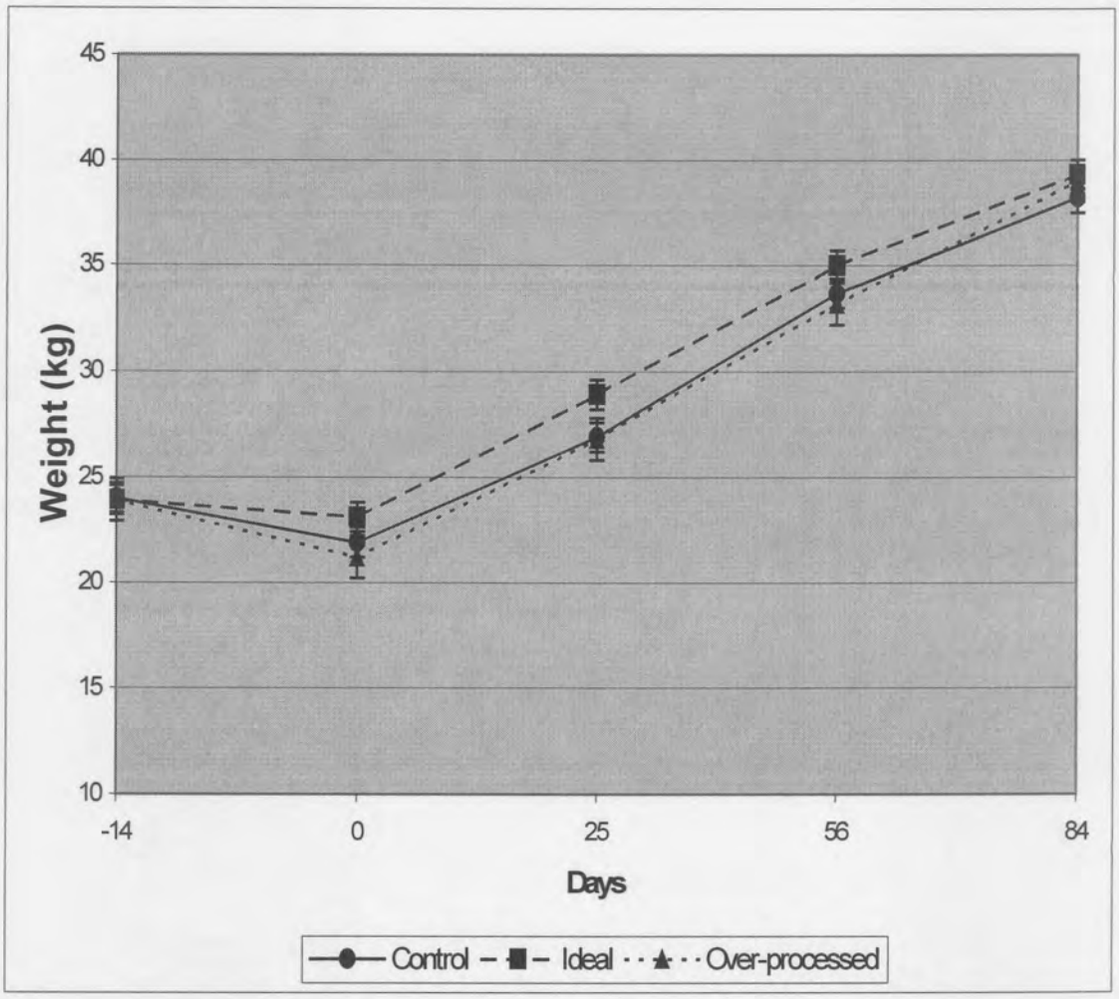
Table 3.5 presents the average feed- and soya bean intake of the three treatment groups per day per lamb.

No significant differences were observed between the intakes of the three treatment groups over the 12-week period (P = 0.80) or at any stage of the trial (Table 3.5). No differences were observed either between the two sexes (P = 0.78; 111.8 ± 1.2 kg *vs.* 111.3 ± 1.4 kg respectively for the wethers and the ewes over the whole trial period).

The soya bean intake during the 12 weeks did not differ significantly (P = 0.76) between the three treatment groups. No significant differences were observed either between the two sexes (P = 0.76) although the ewes' soya bean intake over the whole trial period was slightly higher than that of the wethers (48.3 ± 0.6 kg/ewe *vs.* 48.1 ± 0.5 kg/wether respectively).

Table 3.5: Average feed- and soya bean intakes between the three treatment groups per day per lamb
(g/day ± SD)

Feed and soya bean intake	Duration	Control	Ideal	Over-processed
Feed intake	0-28 days	1265 ± 163	1328 ± 79	1300 ± 83
Feed intake	29-56 days	1393 ± 41	1279 ± 80	1377 ± 65
Feed intake	57-84 days	1335 ± 37	1299 ± 47	1279 ± 80
Feed intake	0-84 days	1330 ± 19	1335 ± 20	1318 ± 19
Soya bean intake	0-84 days	576.8 ± 7.6	575.9 ± 8.0	569.4 ± 7.6



-14 Days mean 14 days before the onset of the experimental period, 0 days means the day when the trial feed were given (start of the experimental period), 25 days means 25 days after the onset of the trial when the trial feed commenced. The same is true for 56 and 84 days.

Figure 3.2: Average weight of the lambs of the three treatment groups for the duration of the trial
(kg ± SD)

Table 3.6: Average weight of the lambs of the three treatment groups for the duration of the trial
(kg ± SD)

Treatment/ Days	-14	0	25	56	84
Control	24.0 ± 0.7	21.9 ± 0.7	26.9 ± 0.6	33.7 ± 0.7	38.2 ± 0.7
Ideal	23.9 ± 0.9	23.1 ± 0.7	28.9 ± 0.6	35.0 ± 0.7	39.3 ± 0.7
Over-processed	23.9 ± 1.0	21.2 ± 1.0	26.8 ± 0.8	33.2 ± 0.8	39.0 ± 0.7

No significant differences were observed between the three treatments during any stage.

Table 3.7 presents the average carcass weight in kilograms (kg) of the lambs in the three treatment groups. The average weight of the kidneys and livers and as a percentage of the carcass weight is also given. All the parameters were measured as wet mass.

No significant differences were observed between any groups for any parameter. There were, however, tendencies to differ between the O- and I-groups in the cold carcass weights ($P < 0.10$), as well as between the O- and C-groups in the liver mass ($P < 0.10$).

Table 3.7: Average carcass, kidney and liver weights (wet basis)

	Control	**Ideal**	**Over-processed**
Carcass weight (kg)	17.6 ± 0.3	17.9 ± 0.3	17.0 ± 0.3
Kidneys (g)	141.2 ± 4.1	131.2 ± 4.1	143.0 ± 4.3
***Kidneys (%)**	0.80 ± 0.02	0.73 ± 0.02	0.84 ± 0.03
Liver (g)	762.5 ± 18.0	758.3 ± 18.8	715.8 ± 18.0
***Liver (%)**	4.3 ± 0.1	4.2 ± 0.1	4.2 ± 0.1

* The wet weights of the liver and kidneys are expressed as a percentage of the cold carcass weight of the lambs. No significant differences were observed between the treatments at any parameter.

3.3 Blood analyses

Average blood Se concentrations during the trial for the three treatment groups are presented in Table 3.8. Day 0 represents the onset of the experimental period after a two-week adaptation period in which all the lambs received the same Se-deficient basal diet. Day 42 represents the 42nd day after the onset of the experimental period. The same implies for days 63 and 84.

Figure 3.3 is a schematic illustration of the change in the average blood Se concentration of the three treatment groups during the trial period. The data set of Table 3.8 was used to construct the graph.

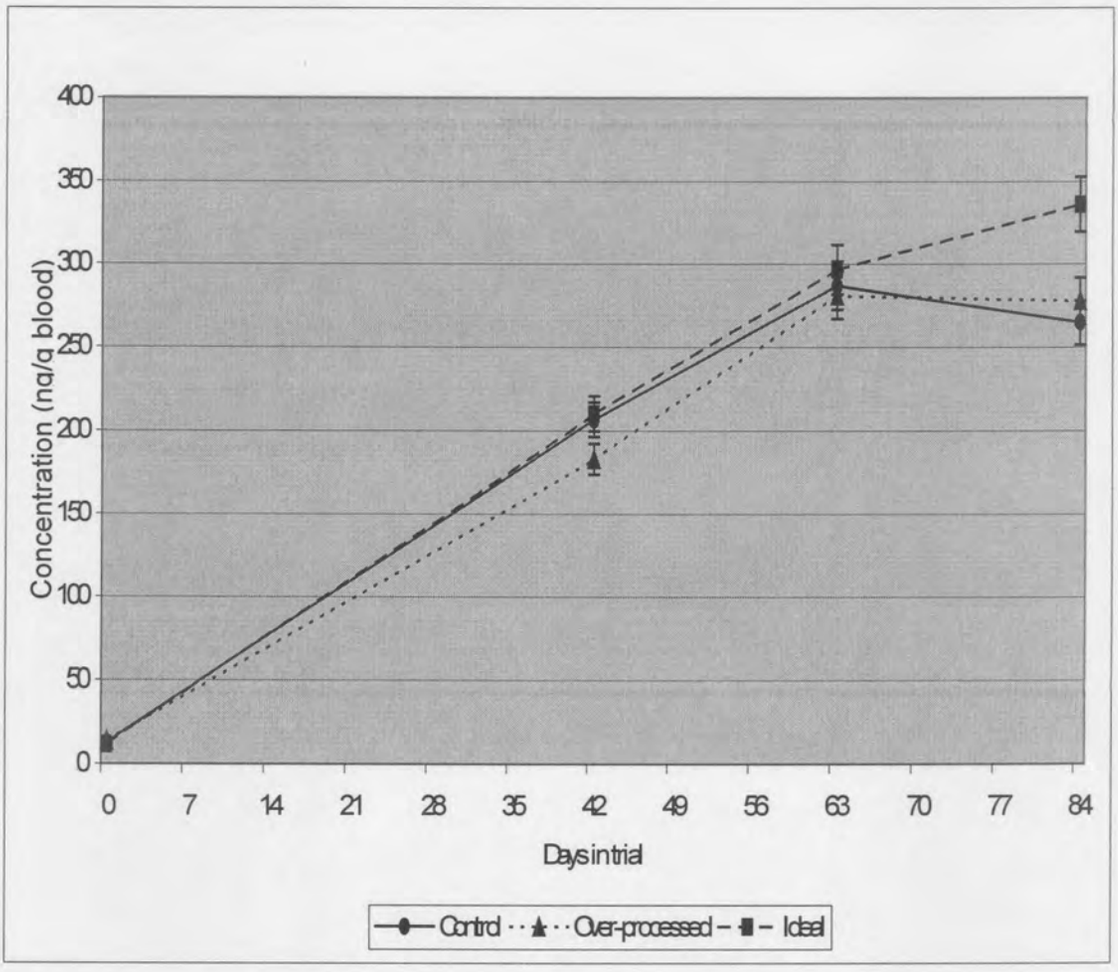


Figure 3.3: The average whole blood Se concentration of the lambs in the three treatment groups over the trial period
(ng/g blood ± SD)

Table 3.8: Average whole blood Se concentration of the lambs in the three treatment groups over the trial period
(ng/g blood ± SD)

Treatment	Day 0	Day 42	Day 63	Day 84
Control	12.8 ± 2.2	206.4 ± 7.0[a]	286.7 ± 7.8	264.8 ± 14.2[c]
Ideal	11.6 ± 2.3	210.0 ± 7.9[a]	296.3 ± 7.8	335.9 ± 13.1[d]
Over-processed	13.6 ± 2.2	182.9 ± 7.0[b]	280.9 ± 7.4	277.8 ± 12.6[c]

Different superscripts within columns show significant differences. The superscripts "a" and "b" present significant differences ($P < 0.05$), while "c" and "d" present highly significant differences ($P < 0.01$).

No significant differences were observed for the blood Se concentrations at the start of the trial period, either between the three treatment groups ($P = 0.82$) or between the two sexes ($P = 0.87$).

The blood Se concentrations of the C- and I-groups were significantly higher than for those receiving the O-group at day 42. No differences were observed between the two sexes ($P = 0.50$) at that stage of the trial. The coefficient of determination (R^2) rose to 0.269 from the beginning of the trial, indicating that the model at that stage accounted for 26.85% of the variance. No significant differences were observed between the C- and I-group ($P = 0.73$).

No significant differences were observed at day 63 ($P = 0.38$) between the three treatment groups. The ewes had, however, a significant higher ($P < 0.05$) blood Se concentration than the wethers at that stage (298.1 ± 6.7 *vs.* 277.8 ± 5.8 ng/g respectively). The coefficient of determination (R^2) further increased from day 42 to 0.450 at day 63, indicating that the model accounted for 45.0% of the variance at that stage between the treatment- and sex groups.

A significant difference ($P < 0.01$) in the blood Se concentration between the three treatment groups was observed at day 84. Significant differences were also observed between the three treatment groups in relation to each other, with the I-group having a significantly higher ($P < 0.01$) blood Se concentration than both the C- and O- groups. No significant differences were observed between the O- and the C-group's blood Se concentration.

No significant differences were observed for the blood Se concentration between the two sexes at this stage (day 63). The coefficient of determination (R^2) continued to rise from four weeks to 0.495, indicating that the model accounted for 49.5% of the variance.

During the 12-week trial period, the blood Se concentration rose in all three groups from approximately 12 ng/g blood to about 260-280 ng/g blood for the C- and O-group and 335.9 ng/g for the I-group. The coefficient of determination also increased from 2.3% at the onset of the trial to 49.5% at the 12^{th} week with the termination of the trial. No sex interactions were observed except at the 9^{th} week when the ewes' blood Se concentration was 7% higher than that of the wethers.

The results of the GSH-Px enzyme activity for the duration of the trial between the three treatment groups are shown in Table 3.9. The enzyme activity is expressed in enzyme units (EU) where 1 EU = 1 ng NADPH oxidised/ g haemoglobin (Hb) per minute at 37 °C. A schematic illustration showing the change of the average GSH-Px enzyme activity of the three treatment groups during the trial period is presented in Figure 3.4. The data set of Table 3.9 in which the weeks were replaced by days, was used to construct the graph.

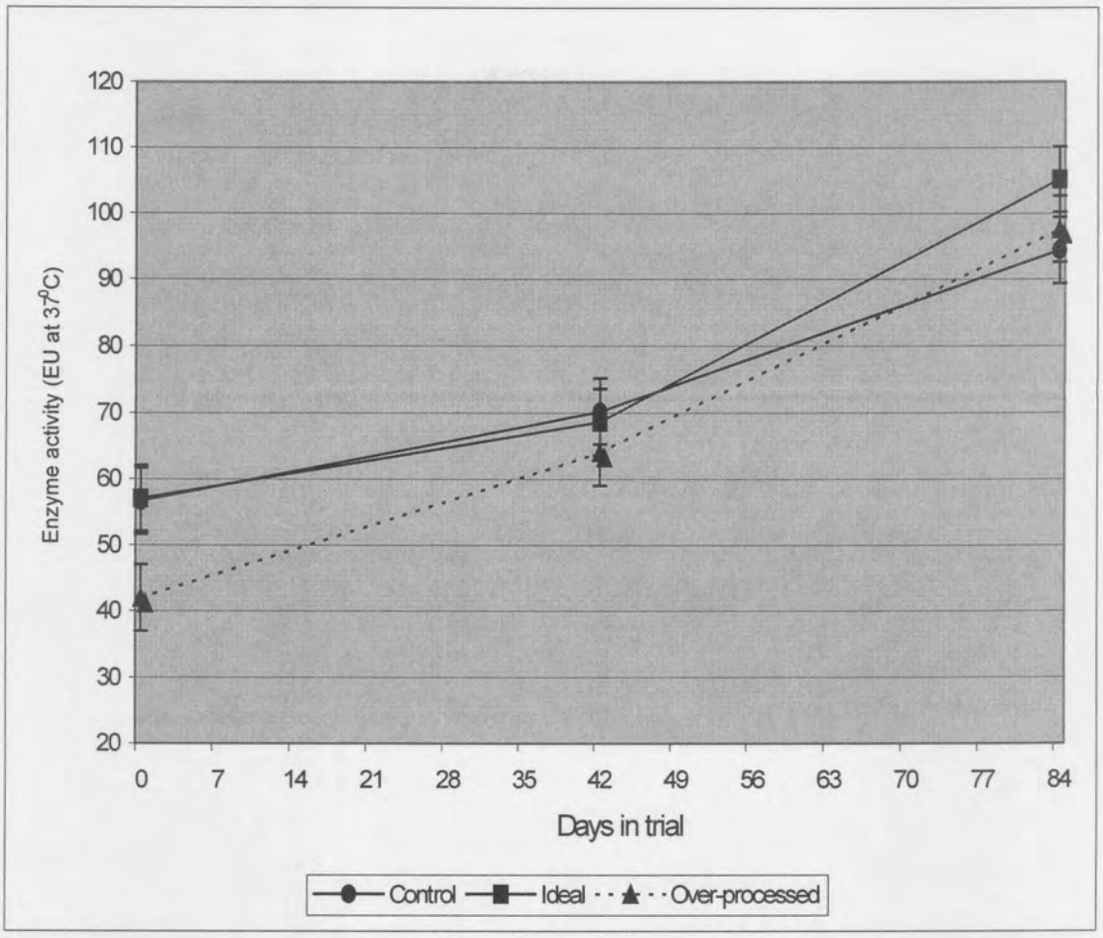


Figure 3.4: The average erythrocyte glutathione peroxidase enzyme activity of the lambs in the three treatment groups over the trial period
(EU ± SD)

Table 3.9: The average erythrocyte glutathione peroxidase enzyme activity of the lambs in the three treatment groups over the trial period
(EU ± SD)

Treatment	Week 0	Week 6	Week 12
Control	56.6 ± 5.7	70.2 ± 4.8	94.4 ± 5.7
Ideal	57.0 ± 6.1	68.6 ± 5.4	105.1 ± 7.1
Over-processed	42.0 ± 6.0	64.0 ± 5.4	97.6 ± 5.7

No significant differences were observed at any stage between any groups or between the two sexes. The coefficient of determination (R^2) was 0.24, 0.04 and 0.12 respectively for weeks 0, 6 and 12, indicating that the treatment and sex model did not have a big influence on the observed results.

3.4 Selenium analyses of the rumen bacteria, liver, heart muscle, wool and kidney cortex

The average Se concentrations of the rumen bacteria, wool and some of the organs of the lambs between the three treatment groups are presented in Table 3.10.

Table 3.10: Selenium concentrations of the ruminal bacteria, liver, heart muscle, wool and kidney cortex for the three treatment groups (ng/g DM ± SD)

	Control	Ideal	Over-processed
Rumen bacteria	2918.2 ± 247.6^{a}	2689.6 ± 231.6^{ab}	2188.2 ± 247.6^{b}
Liver	1327.0 ± 45.2^{a}	1531.4 ± 47.4^{b}	1555.3 ± 45.2^{b}
Heart muscle	1577.6 ± 28.6^{a}	1616.3 ± 30.0^{a}	1480.4 ± 28.7^{b}
Wool	417.5 ± 35.5	507.6 ± 31.3	495.3 ± 31.9
Kidney cortex	6382.0 ± 173.5	5947.5 ± 192.6	5951.6 ± 173.5

Within rows, different superscripts show significant differences ($P < 0.05$)

The rumen bacteria Se concentration of the O-group was significantly lower ($P < 0.05$) than that of the C-group. No significant differences were observed between the C- and I-group ($P = 0.51$) or between the bacterial Se concentration of the I- and O-groups ($P = 0.15$). The coefficient of determination (R^2) was calculated at 0.201, indicating that this model could account for only 20.1% of the variance observed in the rumen bacteria Se concentration.

A significant difference ($P < 0.05$) was observed between the two sexes with the ewes having the higher bacterial Se concentration than the wethers (2706.3 ± 220.0 ng/g DM *vs.* 2491.1 ± 179.0 ng/g DM respectively).

The average liver Se concentration of the C-group was significantly lower ($P < 0.01$) than for both the C- and I-groups. No significant difference was observed between the I- and O-groups ($P = 0.72$). The coefficient of determination was low ($R^2 = 0.383$), indicating that the model only accounted for 38.3 % of the variation observed between the groups.

There was no significant difference observed between the two sexes ($P = 0.69$) with the wethers having a slightly higher liver Se concentration than the ewes (1482.1 ± 34.5 ng/g *vs.* 1460.4 ± 41.0 ng/g DM respectively).

The Se concentration of the heart muscle was influenced by the heat-treatment of the soya beans ($P < 0.01$). The coefficient of determination was higher than for the other tissues ($R^2 = 0.420$), indicating that the model in the present study could explain 42% of the observed variance.

Significant differences were observed for the heart muscle Se concentration between the O- and C-groups ($P < 0.05$) as well as between the O- and I-groups ($P < 0.01$). In both instances the Se concentration of the heart muscle was the lowest in the O-group. There was not a significant difference ($P = 0.36$) observed between the C- and I-groups.

A significant difference ($P < 0.05$) was observed between the two sexes with the ewes having a higher heart muscle Se concentration than the wethers (1599.4 ± 26.0 ng/g *vs.* 1516.8 ± 21.9 ng/g DM respectively).

The Se concentration of the wool did not differ significantly ($P = 0.15$) between the treatment groups or between the two sexes ($P = 0.20$), although the ewes showed higher wool Se concentrations than the wethers (499.0 ± 29.8 ng/g *vs.* 447.9 ± 24.4 ng/g fat free DM respectively). The coefficient of determination was also low (R^2=0.216), indicating that the treatment and sex model only accounted for 21.6% of the observed variance.

However, there was a tendency of a difference ($P < 0.10$) in the wool Se concentration between the C- and I-groups. No significant differences were observed between the C- and O-groups ($P = 0.12$) or between the wool Se concentrations of two processed groups ($P = 0.78$). The average wool Se concentration of the two processed groups was both higher than in the C-group.

The average wool Se concentration of the ewes was higher than that of the wethers (499.0 ± 29.76 ng/g *vs.* 447.9 ± 24.4 ng/g fat free DM). However, differences in the Se concentration of the wool between the two sexes were not significant ($P = 0.20$).

No significant differences were observed between the three heat-treatments for the Se concentration of the kidney cortex. However, there was a tendency ($P < 0.10$) to differ between the C- and O- group's kidney cortex Se concentration. The coefficient of determination was very low ($R^2 = 0.184$), indicating that the model accounted only for 18.4% of the observed variation. There was not a significant difference observed in the Se concentration between the O- and I-group ($P = 0.99$) or between the C-and I-group ($P = 0.11$).

No significant difference was observed between the two sexes ($P = 0.29$), although the average wool Se concentration of the wethers was slightly higher than that of the ewes (6207.9 ng/g *vs.* 5979.5 ng/g fat free DM).

CHAPTER 4

DISCUSSION

As already mentioned in Chapter 1, there are two distinctive ways of defining the bioavailability of an element (Fox *et al.*, 1981 and Jackson, 1997). For the purposes of this study and for reasons already discussed, the definition of bioavailability as defined by Jackson (1997) will be used in interpreting the obtained results.

Henry *et al.* (1988) suggested that the accumulation of Se in the tissues could be used in estimating the bioavailability of Se at high dietary Se concentrations. As a result, it was decided to measure the Se concentration of certain tissues. It was also decided to measure the activity of the erythrocyte GSH-Px enzyme complex after a period of Se-repletion and restoration. The aim was to observe the amount of activity of this enzyme from the various heat-treated soya bean diets in order to draw conclusions on the availability of the Se from the different soya bean fractions to be incorporated into this enzyme.

It is assumed that, due to the small percentages that it occupied in the trial feeds (Table 2.1) together with its low Se concentration, the urea and salt supplied negligible amounts of Se. The Se concentrations of the lucerne and starch used in the feed were also low and would not have contributed much to the Se concentration of the trial feed. As a result, the soya beans in the feed were supplying the majority of Se (94.9 % – Table 3.2). Therefore, differences in the tissue Se concentrations and the erythrocyte GSH-Px activities between the three treatment groups would have arisen almost solely from the Se in the treated soya beans.

Protein sources are moderately heated to increase their nutritional value by decreasing ruminal DM and CP degradability while increasing digestibility in the lower part of the digestive tract (Schroeder, 1997). This change in degradability results through the formation of certain chemical compounds (Maillard reaction) between the reducing sugars and the amino acids present in the protein source (Ljøkjel *et al.*, 2000). On the other hand, overheating can be detrimental as it not only will decrease the degradability of the protein source in the reticulo-rumen, but in the rest of the digestive tract as well (Schroeder *et al.*, 1996). Furthermore, it was observed that the disappearance of Se, in comparison with the CP fraction, was decreased in both the rumen and the lower digestive tract with excessive heat-treatment of soya beans (van Ryssen & Schroeder, 2003). The result of over-heating will, therefore, not only be a decrease in the DM and CP degradability in the total digestive system compared to the moderately heated protein source (Schroeder *et al.*, 1996) but also a decrease in the degradability of the Se fraction (van Ryssen & Schroeder, 2003).

Several authors observed that the ADIN concentration is a good indicator of the UDP-D-fraction of plant proteins and the amount of heat-damage in the plant proteins (Reddy & Morris, 1993) including soya beans (Schroeder *et al.*, 1996).

Demjanec *et al.* (1995) observed in their studies that the amino acid absorption of plant protein sources was maximised in the lower digestive tract of the hosts when the sources were heated in such a manner that between 12-15 % of nitrogen fraction was in the form of ADIN. Using this as norm, Schroeder *et al.* (1996) established that soya

beans have to be roasted at 130 °C for 45 minutes (130/45 treatment) in a 45 L rotating drum to maximise nitrogen absorption in the lower digestive tract. The authors furthermore observed that, when the ADIN concentration of the soya beans was higher than 15%, the digestibility of the nitrogen fraction through the whole digestive system decreased in an inverse relationship to the ADIN concentration. This decrease in nitrogen digestibility is the result of excessive heat-damage of the soya beans. In their trial, excessive heat-damage of the soya beans occurred when the soya beans were roasted at a temperature of 150 °C for 30 minutes (150/30 group). Using this study as norm, it was decided to roast the soya beans according to these specifications in a similar, but bigger (220 L) drum (Dr. G. E. Schroeder, Personal communication).

The increased brown colouration of the soya beans with increasing heat treatment in the present study was possibly the result of formed Maillard reaction products between the reducing sugars present in the soya beans and the protein fraction (Ljøkjel *et al.*, 2000). Soya beans contain up to 1% reducing sugars and 13% nonreducing oligosaccarides (Bach Knudsen, 1997) that could contribute to the Maillard-reaction if it is reduced to monosaccarides during the heating process (Ljøkjel *et al.*, 2000). This increased colouration with increasing roasting temperatures possibly suggests that more heat-damage was inflicted to the over-processed compared to the ideal treated soya bean fractions (Schroeder *et al.*, 1996).

An ADIN-test was performed to confirm the amount of heat-damage objectively in the different heat-treated soya bean fractions. The obtained results showed an increase in the ADIN concentration from 1.3% for the control, unprocessed soya beans to 18.0% for the soya beans subjected to the excessive heat-treatment. The ADIN concentration of the ideal treated soya beans was 3.2% (Table 3.1). These results are lower than the results obtained by Schroeder *et al.* (1996). An interesting observation in the study of Schroeder *et al.* (1996) was that the ADIN-level was 2.13% for the soya beans treated at a temperature of 130°C for 30 minutes (130/30 group), 6.81% for the 130/45 group and above 15% for the 130/60 group. Therefore, the ideal-treated soya beans obtained in the present trial was as effectively heated as the soya beans heated at 130°C for between 30 and 45 minutes in the study by Schroeder *et al.* (1996). A possible explanation could be that a 220 L drum was used in this study, compared to a 45 L drum used in the study by Schroeder *et al.* (1996). It can also be the reason for the lower ADIN concentration obtained in this study for the soya beans subjected to the excessive heat-treatment compared to their study (18.0% *vs.* 23.95% respectively).

The obtained ADIN- concentrations (Table 3.1) suggest that the heat-treatment of the ideal treated soya beans was probably not enough to maximize the amount of dietary amino acid absorption in the lower digestive tract (Demjanec *et al.*, 1995). In retrospect, more heat-damage could have been inflicted on this group of soya beans in order to differentiate it more from the soya beans of the unprocessed group. On the other hand, the ADIN concentration for the over-processed soya beans was above 15%, the norm suggested by Schroeder *et al.* (1996) to indicate the start of excessive heat-damage (Table 3.1). This has suggested a lower bioavailability of the soya protein in the rumen as was supported by the *in sacco* disappearance- (Table 3.3) and IVDOM results (Table 3.4) where the disappearances of the different fractions from the soya beans of the O-group were substantially lower compared to the other groups. However, in retrospect the soya beans of this group could also have been roasted more

to inflict more heat-damage. This could have differentiated this group more from the soya beans of the ideal group.

The percentage DM of the soya beans disappearing *in sacco* in the rumen at 16 hour incubation decreased from 79.3% to 40.4% from the unprocessed- to the over-processed soya bean fractions (Table 3.3). Ljøkjel *et al.* (2000) using soya bean meal in dairy cows obtained comparative results. The disappearance of the CP in the rumen also decreased from 72.8% to 17.9% respectively for the control- and over-processed soya beans (Table 3.3), which was also in accordance with the results obtained by Schroeder *et al.* (1995) and Ljøkjel *et al.* (2000). One of the aims of heat treatment, namely to decrease the amount of DM and CP disappearance in the rumen was fulfilled.

As with the CP fraction, the *in sacco* disappearance of the Se fraction of the soya beans also decreased with increasing heat-treatment (Figure 3.1, Table 3.3). In an earlier study, van Ryssen & Schroeder (1999) also reported a positive correlation between the disappearance of the CP- and Se fractions of soya beans in the rumen with increasing degrees of heat treatments.

The feed- and soya bean intake did not differ significantly between the three treatment groups (Table 3.5) and was with-in the recommendations of the NRC (1985). As a result, the average growth rate of the lambs between the three treatment groups did not differ significantly (Figure 3.2; Table 3.6).

Although the composition of the experimental diets differed in a few aspects (crude protein, selenium- and calcium concentrations – Table 3.4) from the recommendations of the NRC (1985), these differences were across all three the treatment groups and probably would not have influenced the comparable bioavailability results. It can be concluded that the comparable bioavailability results between the treatment groups probably were not affected by the feed parameters. Therefore, differences observed in the tissue Se concentrations and GSH-Px enzyme activity between the three treatment groups would have been the result of a different Se bioavailability of the soya beans between the groups. As a result, conclusions on the bioavailability of Se between the treatment groups can be made based on observed differences in the tissue selenium concentrations and GSH-Px enzyme activity.

Koenig *et al.* (1997) observed with high concentrate diets that more Se is associated with the fluid- than the particulate phases in the rumens of sheep. Based on the ingredient and chemical conditions of the respective experimental diets (Tables 2.1, 3.4), the present study had a higher proportion of concentrates in the diets than in the study of Koenig *et al.*, (1997). Furthermore, the lucerne used in the trial diet had a low Se concentration (Table 3.2). It was, therefore, not expected that the particulate phase would have influenced the Se incorporation into the microbial protein to any significant degree. As a result, it was decided to analyse the bacteria of the rumen fluid for their Se concentration, as the soya beans were basically the sole source of Se to the experimental animals (Table 3.2).

In the present study, no distinction was made between the dietary and endogenous forms of Se in the rumen (van Ryssen *et al.*, 1989), as the aim of the study was to

compare the bioavailability of Se in the soya beans between various heat-treatment groups and not to determine absolute values.

The Se concentration of the rumen bacteria of the C-group was significantly ($P < 0.05$) higher than for the O-group (Table 3.10). The soya beans contained approximately 95% of the dietary Se in the trial feed (Table 3.2). As already discussed, the majority of Se in soya beans is in the form of selenomethionine (Yasumoto *et al.*, 1984). Animals cannot distinguish between these two forms of amino acids with the result that methionine can be replaced by selenomethionine in the body proteins (Waschulewski & Sunde, 1988). Koenig *et al.* (1997) observed that the type of diet also influences the metabolism of Se in the rumen. According to the authors, a more concentrated diet will result in more Se being metabolised into the seleno-amino acid compounds and less being reduced to the lesser available forms like elemental Se. Van Ryssen *et al.* (1989) also observed that the predominant form of Se in the rumen microbes was selenomethionine when the sheep used were fed selenomethionine or high Se wheat.

It was therefore highly possible that the Se from the soya beans was incorporated into the protein structure of the rumen microbes as selenomethionine. The higher Se and CP disappearance in the rumens of the C-group compared to the O-group (Table 3.3) can explain the higher rumen bacterial Se concentration of this group above the C-group (Table 3.10).

No significant differences were observed for the Se concentrations of the rumen bacteria between the C- and I-groups or between the I- and O-groups (Table 3.10). A possibility does exist that the amount of heat-damage inflicted on the soya beans of the I-group was not sufficient to differentiate it from that of the C-group (Table 3.1). As a result, the maximal amount of degradable amino acids reaching the lower digestive tract was not accomplished in this group (Demjanec *et al.*, 1995). As a result, more nitrogen (protein) and possibly selenomethionine (Waschulewski & Sunde, 1988) could have been available to be metabolised in the rumen by the rumen microbes in this group than was anticipated. In addition, more heat-damage could have been inflicted on the soya beans of the O-group (Table 3.1, Schroeder *et al.*, 1995) to differentiate it more from the soya beans of the I-group. This can possibly explain the lack of significant differences observed for the rumen bacteria Se concentration between these two groups.

It is of interest to note that the *in sacco* Se disappearances at 16 hour incubation were 58.8%, 38.1% and 21.7% respectively for the control-, ideal- and over-processed soya bean fractions (Table 3.3; Figure 3.1). In contrast, the rumen bacterial Se concentration of the C-group was only 30% higher than for the two processed groups (Table 3.10). It is therefore suggested that comparatively more Se disappeared in the rumen for the C-group compared to the two heat-treated groups. This is supported by the observation of van Ryssen *et al.* (1989) in an earlier study that the uptake of Se by the rumen microbes of sheep consuming high-Se wheat ranged between 35-43%. High concentrate diets can cause a reduction in the pH of the rumen (Serra *et al.*, 1996a) and subsequently, an increase in the reduction potential of the rumen (Gerloff, 1996). This could have caused an increase in the reduction of the dietary Se to its unavailable elemental forms (Gerloff, 1992), which can be methylated more easily and excreted through the faeces (Langlands *et al.*, 1986). There are also organic Se sources other

than selenomethionine in plant proteins (Combs & Combs, 1986; Daniels, 1996) that is not necessarily being incorporated into the microbial protein structure. It was therefore possible that, although more of the dietary Se was incorporated into the microbial protein of the C-group compared to the heat-treated groups, a bigger percentage of the dietary Se was reduced to their unavailable forms.

Several authors (Combs *et al.*, 1996; Serra *et al.*, 1997) observed that only a small proportion of the bacterial Se from sheep rumen bacteria was available to be absorbed by mice. Peterson & Spedding (1963) furthermore observed that faecal Se consists of an insoluble, inorganic Se fraction as well as a fraction where the Se was incorporated into proteins. According to the authors, it might indicate that not all of the bacterial Se was available to the host. An explanation can be that Se is incorporated into Se analogues of the sulphur amino acids by the rumen bacteria and that it is thereby protected by strong ligands (Hudman & Glen, 1984). Furthermore, other minerals within the bacterial cell also can prohibit the bioavailability of the bacterial Se (Serra *et al.*, 1996b).

From the above-mentioned, it is suggested that the bioavailability of the dietary Se to the host will probably be reduced if it is to be incorporated into the ruminal bacteria. The rumen environment can also reduce the ingested Se into insoluble compounds, thereby lowering its absorption and subsequently its bioavailability (Gerloff, 1992), especially with high concentrate diets (Gerloff, 1992, Serra *et al.* 1996a). The higher *in sacco* Se degradability results (Table 3.3) and bacterial Se concentration results of the C-group (Table 3.10) would suggest that the bioavailability of the Se fraction of the C-group was decreased compared to the two processed groups. However, the tissue Se concentrations of the C-group were not always significantly lower than for the heat-treated groups (Tables 3.8, 3.9, 3.10). More research is necessary on the metabolism of organic Se in the rumen as most of the present knowledge involves inorganic Se.

Blood Se levels are often used to determine the Se status of an individual (van Ryssen *et al.*, 1989; van Ryssen & Mavimbela, 1998). However, Whanger & Butler (1988) observed that the blood Se concentration does not always reflect the whole body Se status, especially if selenomethionine, the predominant Se source observed in plants (Combs & Combs, 1986; Daniels, 1996) and soya beans (Yasumoto *et al.*, 1984) was used. In a study conducted on rats, Whanger & Butler (1988) observed that the organs, especially the muscle, could have a big retention of Se while the blood Se concentration remained relative low. Therefore, the Se concentration of the blood must not be interpreted on it's own, but be viewed together with all the other organs in order to assess the Se status of the host.

The blood Se concentrations of the lambs in all three groups at the onset of the trial were extremely low (Table 3.8), indicating that the lambs were in a Se deficiency state (Puls, 1994). No significant differences were observed between the three treatment groups (Table 3.8) or between the two sexes at that stage. This low blood Se concentration was anticipated as the lambs were obtained from the Hatfield experimental farm of the University of Pretoria, a location where the Se status of unsupplemented sheep is known to be marginally to deficient (Van Ryssen, 2001). No or very little treatment influence was noticeable as is shown by the low coefficient of determination at that stage ($R^2 = 0.023$). This was expected as the lambs received the same Se deficient basal diet during the two-week adaptation period. This low blood Se

concentration was anticipated to ensure that any accumulation of Se in the tissues would reflect the availability of the Se in the experimental diets.

There was an increase in the average blood Se concentration of the lambs in all three the treatment groups throughout the 84-day trial period (Table 3.8, Figure 3.3). The coefficient of determination (R^2) increased from 2.3% at the start of the trial period to 26.9%, 45% and 49.5% respectively at days 42, 63 and 84. Since the soya beans were the main source of Se in the experimental diets (Table 3.2), it can be interpreted that the blood constantly had taken up the Se from the soya beans. Sex did not influence the blood Se concentrations, except for the 63rd day when the ewes had a significant higher (30%; $P < 0.05$) blood Se concentration than the wethers.

It is of interest to note that Butler *et al.* (1990) in monkeys and Deagen *et al.* (1991) in ruminants observed that there was an increase in the Se concentration of the albumin fraction in the blood when the animals were supplemented with selenomethionine compared to selenite. It was therefore possible that, due to the non-specific incorporation of selenomethionine in place of methionine (Waschulewski & Sunde, 1988), the Se was incorporated into the albumin fraction of the blood throughout the duration of the trial. This can explain the rise of the blood Se concentration in all three the treatment groups over the duration of the trial and the subsequent increase in the coefficient of determination.

Although the blood Se concentration of the O-group was significantly lower ($P < 0.05$) than for both the C- and I-groups at day 42, it was not the case at the following measurement at day 63 (Table 3.8). All three groups of lambs received the same amount of feed (and soya beans) during the whole period (Table 3.5) and could not have influenced the observed results. No possible reason can be given, although Behne *et al.* (1988, 1989) observed a hierarchy of tissue repletion when Se deficient rats were supplemented with Se. According to the authors, Se might preferentially be incorporated into some tissues above other tissues. This could have happened here due to the excessive heat treatment of the soya beans from the O-group, resulting in the significant difference at day 42.

At day 84, significant differences ($P < 0.05$) were observed for the blood Se concentrations between the I-group and both the C- and O-groups. The blood Se concentration of both the C- and O-groups showed a plateau or even decreased slightly during the last three weeks of the trial period (between days 63 and 84 - Table 3.8; Figure 3.3). In comparison, the Se concentration of the blood in the I-group rose steeply during the same time period and was, as a result, 21% and 17% higher than the blood Se concentration of the C- and O-groups at that stage. It can be interpreted that the Se from the ideal heat-treated soya beans (I-group) was more available to be taken up in the blood, especially during the last 21 days, than for the other two groups. However, due to the inconsistency of the blood Se concentrations over the trial period without a definite pattern (Table 3.8), there is no substantial proof that the heat treatment of the soya beans has influenced the availability of its Se fraction to be incorporated in the blood.

It is of interest to note that in a study by van Ryssen *et al.* (1989), the blood Se concentration of sheep fed high Se wheat continued to rise for more than 100 days. The blood Se concentration of the I-group, therefore, could have risen to higher

concentrations than was observed in the present study. However, the blood Se concentration of C- and O-groups was not expected to increase further as it was already reaching a plateau (Table 3.8, Figure 3.3). As a result, the observed differences between the three treatment groups could have been emphasised even more with a longer trial period.

The blood Se concentrations of the lambs were adequate in all three groups from the 42nd day onwards (Puls, 1994). Due to the non-specific incorporation of selenomethionine into blood proteins (Deagen *et al.,* 1993), a more conventional diet with a lower soya bean inclusion level could have resulted in lower blood Se results and possibly deficiencies. It is necessary to investigate the effect of heat-treatment of soya beans (and other protein sources) on the bioavailability of Se if normal feed inclusion levels of the protein source is used in the composition of the trial feed.

Selenium is incorporated into the GSH-Px enzyme complex in the red blood cells during erythropoïesis (Douglass *et al.,* 1981). In addition, the life span of erythrocytes in lambs, aged 3-6 months, ranges between 46 and 64 days (Schalm *et al.,* 1975). As a result, the activity of the GSH-Px enzyme measured from the erythrocytes is an indicator of the Se status of the animal over the longer term (Fairweather-Tait, 1997). It therefore was expected that, if there were to be differences between the three treatment groups, it more likely would have been at the end of the trial period.

No significant differences in the GSH-Px activity were observed between the three treatment groups at any stage during the trial period (Table 3.9, Figure 3.4) despite the significant differences observed for the blood Se concentrations at the end of the experimental period (Table 3.8). No sex influence was observed either. Normal adequate GSH-Px activities in the erythrocytes of lambs are in the range of 124 ± 41IU/g haemoglobin (Suzuki *et al.,* 1985). The activity of the GSH-Px enzyme activity observed at the start of the trial period and at week 6, therefore, indicates a Se deficiency in all three the treatment groups.

The GSH-Px activity of the lambs in all three the treatment groups at the end of the experimental period (Table 3.8) were, although marginally low, adequate to the lambs (Suzuki *et al.,* 1985). In comparison, the blood Se concentrations were fairly high (Table 3.8). This marginal deficiency can be explained by the properties of the enzyme as it loses its activity rapidly (Ullrey, 1987). Another possibility may be the form of Se present in the soya beans. According to Hakkarainen (1993) and Douglass *et al.* (1981), sodium selenite increases the activity of GSH-Px the most and prevents and cures deficiency symptoms much more efficient than selenomethionine and other Se sources found in grains, meat- and fish meal. Beilstein & Whanger (1986b) as well as Deagen *et al.* (1991) further observed that, when selenomethionine or high Se wheat was given to rats, the majority of the Se was associated with the haemoglobin fraction in the blood and that it was not incorporated into the GSH-Px enzyme complex. As a result, an increase in whole blood Se concentration could have resulted without an increase in the enzyme activity due to the form of Se present in the soya beans.

Van Ryssen *et al.* (1998) observed that the liver is the most sensitive organ in incorporating dietary Se when inorganic Se is used as the Se source. The authors suggested that the liver more likely would reflect the bioavailability of Se than any

other tissue. The liver is also, according to Froslie *et al.* (1985), a good indicator of the total Se status in animals and is an important organ in the metabolism of Se, converting selenomethionine to selenocysteine (Krishnamurti *et al.*, 1989).

A significant difference was observed between the three treatment groups in general ($P < 0.01$), with the average liver Se concentration of the C-group significantly lower (15%) than for both the O- and the I-groups ($P < 0.01$ – Table 3.10). No significant differences were observed between the liver Se concentration of the two sexes or a sex-treatment interaction, indicating that the heat-treatment of the soya beans had an effect on both sexes. There were also no significant differences between the liver Se concentration of the O- and I-groups (Table 3.10).

These results indicate that the heat-treatment of the ideal- and over-processed soya beans increased the availability of its Se to be taken up and be incorporated in the liver compared to the unprocessed soya beans. It further indicates that there was apparently no difference in the availability of the Se from the two heat-treated groups in terms of its uptake by the liver. Possible explanations could be that, although the Se concentration of the microbial protein was significantly higher (30%) for the C-group compared to the two heat-processed groups, the *in sacco* disappearance of the soya beans for this group was twice the amount observed for the two heat-processed groups. Gerloff (1996) observed that the reducing capacity of the rumen is increased with high concentrate diets. It was therefore possible that more of the dietary Se was being reduced to selenide and other less available seleno-compounds by the microorganisms for the C-group compared to the two heat-processed groups. Furthermore, Serra *et al.* (1997) observed that Se that is incorporated into the microbial protein is not fully available to the host. As a result, even though more Se was incorporated into the rumen bacteria of the C-group, it was highly possible that not all of the incorporated Se was available to the host and that a proportion was excreted.

Although the amount of heat-damage inflicted on the soya bean fraction of the I-group was probably insufficient to maximise the amino acid degradability in the lower digestive system (Table 3.1, Demjanec *et al.*, 1995), its liver Se concentration was significant higher compared to the C-group (Table 3.10). The possibility does exist that at an ideal heat-treatment where the amino acid supply and digestibility is maximised to the lower digestive system could have resulted in even higher liver Se concentrations of the I-group together with more significant differences between the treatment groups.

No significant differences were observed between the liver Se concentration of the O- and I-groups (Table 3.10). As already mentioned, the heat-treatment of the O-group was probably insufficient in order to differentiate it from the I-group (Table 3.1; Schroeder *et al.*, 1996). Therefore, the amount of digestible amino acids and probably selenomethionine – the predominant form of Se in soya beans (Yasumoto *et al.*, 1984) that could have been metabolised by the liver was more than was anticipated in this group, resulting in the lack of significant differences observed for the liver Se concentration between the I- and O-groups.

According to Stowe & Herdt (1992), normal liver Se concentrations range between 1.2 and 2.0 μg/g DM and are not influenced by species or age. The average liver Se concentration of the C-group was 1.3 ug/g DM, while the concentration for the lambs

receiving the heat-treated soya beans was 1.5 μg/g DM (Table 3.10). From these observations, it can be concluded that the Se status of the lambs in all three groups was adequate and that, although significant differences were observed between the C-group and the two processed groups, biologically it was insignificant. However, it must be emphasised that the experimental diets fed to the lambs had a high concentration of soya beans as an ingredient (43.6 % soya beans on a DM-basis – Table 2.1). As a result, as it was possible that a high proportion of the dietary Se was in the form of selenomethionine (Yasumoto *et al.,* 1984), it could have been incorporated into the organs (including the liver) replacing methionine (Waschulewski & Sunde, 1988). A more conventional diet in which the contribution of the protein source in the experimental diets is less might have resulted in the observation of biological significant differences between the treatment groups.

In general, significant differences ($P < 0.01$) were observed for the heart muscle Se concentration between the three heat-treatment groups (Table 3.10). Significant differences ($P < 0.05$) between the C- and the O-groups, as well as between the I- and O-groups ($P < 0.01$) were also observed. In both instances, the average Se concentration of the heart muscle of O-group was lower than the heart muscle Se concentrations of either the C- or I-groups (Table 3.10).

The above-mentioned differences observed imply that the Se fraction of the soya beans of the O- group was probably less available than the Se fraction of the soya beans of either the C- or I-groups to be incorporated into the heart muscle. However, these differences were biological not significant as the Se concentrations of the heart muscle was high in all three the treatment groups and not indicative of a Se deficiency in the lambs (Pherson, 1993).

It is of interest to note that the I-group had a significantly higher heart muscle-, but not liver Se concentration than the O-group (Table 3.10). A possible explanation for this observation is that up to 70% of the Se associated in the muscle can be in the form of selenomethionine in diets supplemented with selenomethionine, while it is only 30% in the liver (Behne *et al.,* 1991). The authors also observed that the non-specific incorporation of the Se into the proteins of the liver is about 2.5 times higher for rats administrated with selenomethionine than for the rats consuming selenite. For the muscle, the incorporation is about 10 times higher. This difference in incorporation is probably due to the higher proportion of methionine in the muscles than the liver (Beilstein & Whanger, 1986a) as well as the relative higher protein turnover of the liver compared to the muscle (Waschulewski & Sunde, 1988). Therefore, the proposed decreased amino acid degradability of the over-processed soya beans resulting from the excessive heat treatment could have influenced the Se concentration of the heart muscle to a higher degree than for the liver. However, further research is necessary to investigate the absorption and incorporation of the different Se sources, especially organic Se sources, by different tissues.

The Se concentration of the heart muscle was significantly higher in this trial for all three groups compared to the results observed by Sunde *et al.* (1981) and Hartmann & van Ryssen (1997). A possibility could be that an organic form of Se was used in the present study, while inorganic Se was used as the Se source by the above-mentioned authors in their studies. With selenite or selenocysteine as Se source, Deagen *et al.* (1987) observed in rats that the Se concentration of the liver was about nine times

higher than the Se concentration of the heart muscle. However, when selenomethionine was used as the Se source, the authors observed that the heart muscle Se concentration of the rats was almost the same as the liver Se concentration. This increase in heart muscle Se concentration when organic Se sources are used may be due to the non-specific incorporation of the dietary Se into the muscle proteins (Waschulewski & Sunde, 1988).

In conclusion, the absorption and metabolism of inorganic Se differs from that of organic Se, with the exception of selenocysteine (Sunde, 1990). Inorganic Se tends to be incorporated into several selenoproteins like GSH-Px more readily than organic Se (Sunde, 1990; Daniels, 1996). On the other hand, organic Se sources tend to be incorporated more into muscles as selenomethionine, especially with a methionine deficiency in the diet (Waschulewski & Sunde, 1988). In studies where comparisons were made between organic- and inorganic Se sources, the Se concentration of the muscles, including the heart muscle, were significantly higher with organic sources than the inorganic sources (Deagen *et al.*, 1987; Whanger & Butler, 1988).

It is suggested that wool may reflect the trace element status of sheep as it can accumulate a number of trace elements at the time of wool follicle development (Lee & Grace, 1988). Selenium is one such a mineral that can be accumulated, especially if the diet fed is low in sulphates (White & Somers, 1977). Zachara *et al.* (1993) observed that the Se concentration of the wool is positively influenced by the dietary Se (as selenite) concentration up to a concentration of 2.0 mg Se/kg DM. Furthermore, Chen *et al.* (1980) observed in humans in China that the hair Se concentrations in areas with an incidence of Keshan disease was less than 12 mg/kg DM, while in non-affected areas, it was more than 20 mg/kg DM. According to a study conducted by Hidiriglou *et al.* (1968), cows with hair Se concentrations ranging between 0.06 and 0.23 mg/kg DM produced calves with white muscle disease, while the cows with hair Se concentrations above 0.25 mg/kg DM did not. It can be concluded that hair- and possibly wool Se concentrations may be useful in indicating the Se status of an individual over the medium- to long term.

The wool Se concentrations of both groups of lambs that received the treated soya beans were higher than for the C-group (Table 3.10). The differences however, were not significant although there was a tendency to differ between the C- and the I-groups ($P = 0.0702$). It can be concluded that the heat treatment of the soya beans had no influence on the incorporation of the Se fraction into the wool fraction.

According to Oster *et al.* (1989), the kidney is not merely a storage organ of Se, but is also involved in the metabolism of Se as well as its excretion. No significant differences were observed between the three treatment groups (Table 3.10), although there were tendencies to differ between the C- and O-groups' kidney cortex Se concentration ($P = 0.0901$) as well as between the C- and I-groups ($P = 0.1067$). In earlier studies, it was observed that the kidney was the least responsive of the organs to increased dietary Se concentrations (van Ryssen *et al.*, 1989; Zachara *et al.*, 1993; van Ryssen *et al.*, 1997). Furthermore, as a percentage of the total body Se pool, the proportion of Se in the kidneys is insignificant. It can be concluded that the kidneys probably had no or very little influence on the bioavailability results obtained in the present study.

CHAPTER 5

CONCLUSION AND FUTURE STUDIES

Conclusion

Although significant differences in the Se concentrations of the tissues have been recorded between the treatment groups in this trial, they were inconsistent and not according expectation. It was expected that the tissue Se concentration of the over-processed soya beans should have been well below that of the ideal treatment group. This was the case in the cardiac muscle, but not in the liver and wool. In fact, in all three treatments, the Se status of the lambs, based on the Se concentrations of the tissues, whole blood and GSH-Px activity was high and only slightly but inconsistently related to treatment. It could be concluded that the heat processing applied in this trial did not affect the Se status of the lambs substantially from a biological point of view. It could be interpreted that the hypothesis was not correct and that heat processing of protein sources does not have a negative effect of practical importance on Se bioavailability.

Future studies

A few areas were highlighted in this study that can be used in future research studies, including the two main aspects responsible for the lack of observed biological differences in the present study and the subsequent rejection of the hypothesis. They include the following:

- ADIN was used as a measurement of the amount of heat-damage in the soya beans. Based on the obtained results from the study and comparable to Schroeder *et al.* (1995), the amount of heat-damage to the over-processed soya beans could have been more. As a result, the Se fraction of this group was probably still fairly available in the lower digestive tract of the host. In addition, more heat-damage could also have been inflicted to the soya beans of the I-group as its ADIN concentration was only 3.2%, substantially lower than the recommendations of 12-15% for soya bean oilcake (Demjanec et al., 1995). More of the Se fraction of this group was probably reduced in the rumen, especially as a high concentrate diet was fed (Gerloff, 1996). For both the heat-treated groups, the amount of Se to the lower digestive tract was probably impaired and therefore comparable to the C-group.

- The Se concentrations of the tissues were high, probably as a result of the high inclusion level of soya beans into the experimental diets (Table 2.1) and the form of Se present in the soya beans (Yasumoto *et al.*, 1984). As a result, the tissues were probably not as sensitive to changes in the bioavailibility of the Se between the treatment groups as would have been when the tissue Se concentrations were lower or even deficient. Behne *et al.* (1991) also observed that large intakes of Se (selenite) only resulted in relative small increases in the selenoprotein concentrations and that those proteins are regulated by a homeostatic mechanism. A more conventional diet with a lower inclusion level of soya beans resulting in lower tissue Se concentrations could have caused different observations and possibly biological significant differences between the treatment groups.

Other areas highlighted include the following:

- Van Ryssen & Schroeder (2003) observed that the disappearance of CP and Se in the rumen and lower digestive tract was correlated for both processed (heat-treated) as well as for non-processed plant proteins. However, the authors also observed that for animal proteins, those correlations were low. A possible reason could be the different chemical forms of Se between plant- and animal proteins. In animal proteins such as fish meal, Se occurs as selenomethionine as well as selenocysteine (Cases *et al.,* 2001) while the predominant source of Se in plant proteins is selenomethionine (Combs & Combs, 1986; Daniels, 1996). Selenocysteine can replace cysteine at high concentrations (Wilhelmsen *et al.,* 1989) but is, unlike selenomethionine, more readily synthesised to selenide or elemental Se (Sunde, 1990). Heat processing, therefore, could have a different effect on animal proteins than on plant proteins. More research is necessary in this regard on animal protein sources including fish meal in determining the effect of heat processing on the bioavailability of its Se.

- Waschulewski & Sunde (1988) observed in rats that stored selenomethionine could be used in maintaining the activity of this selenoprotein if the physiological status of the host changes. To our knowledge, no such work has been done on ruminants. Therefore, further research is necessary to look at the availability of the stored Se (especially from organic Se sources) in the tissues like the heart muscle and blood in ruminants to be used in the metabolic active selenoproteins when the physiological status of the host changes.

- Further research is also necessary in investigating the bioavailability of Se by measuring selenoproteins other than GSH-Px. Awadeh *et al.* (1998) analysed Se from three selenoproteins (Selenoprotein P, albumin and GSH-Px) in cattle given diets of increasing amounts selenite as well as Se yeast. Selenoprotein P contained the largest amount of Se among the serum proteins (31.6 ng/ml), while the smallest contribution was from GSH-Px (4.7 ng/ml). No differences were observed between the treatments for the protein fraction, although the Se of the yeast resulted in higher blood Se concentrations than selenite at the same inclusion levels. This observation was in accordance with the observations made by several authors (Burk & Hill, 1993; Deagen *et al.,* 1993; Daniels, 1996) who observed that selenoprotein P contains about 65% of the Se present in the plasma of various species. Beilstein *et al.* (1981) furthermore identified selenocysteine proteins in the crude nuclear, mitochondrial and microsomal fractions of the ovine liver as well. In addition, Burk & Hill (1993) observed a differential depression of selenoproteins with Se deficiencies, presumably to preserve the metabolic important proteins. In order to accurately make an assessment of the bioavailability of Se, it is necessary to investigate the functions of each selenoprotein and the impact that a Se deficiency/retention would have on each protein/enzyme from different food- and Se sources.

- It is of interest to note that Swanson *et al.* (1991) observed that the activity of the GSH-Px enzyme was not as high when the animals received organic (selenomethionine) *versus* inorganic Se sources for reasons already discussed. However, the activity of the enzyme was maintained for a longer period after supplementation was ceased for the group receiving the organic- compared to the

inorganic Se source. The authors concluded that the organic Se source (selenomethionine) was probably non-specifically replacing methionine in the blood proteins. After the supplementation period, this Se became available through protein catabolism and was being able to maintain the activity of the enzyme complex. However, in another study, Waschulewski & Sunde (1988) observed that after the supplementation of Se was ceased, stored selenomethionine was unable to provide Se for GSH-Px enzyme synthesis in the rat over a prolonged period of time (14 days). Further research is necessary to investigate the effect of stored Se in its incorporation of biologically active seleno-proteins when the physiological status of the ruminant changes.

- Comprehensive research is necessary on the effect of the ruminal conditions on the absorption and incorporation of organic Se sources by the rumen bacteria and subsequently, on its (rumen bacteria Se) availability to the host. Selenomethionine is absorbed and incorporated into the tissues in the same way as methionine (Waschulewski & Sunde, 1988) using the same transport mechanism (Sunde, 1990). It is, therefore, assumed that the form of Se in the rumen bacteria will be in the form of selenomethionine if organic Se (selenomethionine) is being supplied to the host. Its digestibility and availability in the lower digestive tract should therefore simulate that of methionine. Some authors (van Ryssen *et al.*, 1989; Combs *et al.*, 1996; Serra *et al.*, 1996b; Serra *et al.*, 1997) noted that not all of the Se in the rumen bacteria is available to the host. However, to our knowledge, no comprehensive study was undertaken in investigating the absorption and availability of the incorporated Se to the host. In addition, the present knowledge of Se metabolism in the rumen is fairly limited to inorganic selenium. The metabolism of organic selenium in the rumen and its availability to the host animal are areas that need to be focused on in the future.

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