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Swedish University of Agricultural Sciences
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Selenium in Swedish sheep production

Sanna Linder

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Selen till svenska får

Sanna Linder

Handledare:	
Supervisor:	Gun Bernes, Department of Agricultural Research for Northern Sweden
Bitr. handledare:	
Assistant supervisor:	Lena Stengärde, Svenska Djurhälsovården
Examinator:	
Examiner:	Mårten Hetta, Department of Agricultural Research for Northern Sweden
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Abstract

Selenium (Se) is an essential trace element that is involved in several physiological functions in mammals. Mostly, Se is bound to proteins, many of them with enzymatic functions. A group of seleno-depending enzymes, glutathione peroxidases (GSH-Px's), are important as antioxidants and protects against free radicals. The activity of GSH-Px is strongly regulated by Se storage in the liver. Deficiency of Se can cause disturbances such as nutritional muscular dystrophy (NMD), lameness, reduced fertility and growth. During pregnancy, Se is efficiently transferred over the placenta and prioritized to the foetus. The bioavailability of Se depends of the origin and inorganic Se in the form of sodium selenite and sodium selenate has lower bioavailability than organic Se in the form of selenium yeast. It is generally known that Swedish soils have low Se contents and for sheep, which mostly have a roughage and pasture based diet, this can cause Se deficiency. In this master thesis, different studies were carried out to investigate the Se status of Swedish sheep and the amount of Se available in feeds. One study contains a set of sheep blood samples taken in 2003-2005 where Se was subsequently quantified. The results suggest that younger ewes have a higher requirement of Se than older ewes. Secondly, a small farm study was performed during the winter 2013/2014 where blood samples were collected at two times, in November and January, from seven ewes. The analyses of Se in whole blood indicate that the ewes' blood levels were within the reference interval 0.1-0.5 mg/L but at the lower end. Although the Se requirement was met, there was an unexpected decrease in blood Se levels from the first to the second sampling, probably due to a reduced consumption of the mineral feed. Data obtained by *Växa Sverige* concerning Se content in Swedish roughage are also presented in this thesis and a smaller market survey summarizes the Se content in the most common mineral feeds for sheep that are available at the Swedish market. According to the results found in this study, one can discuss if the daily recommended intake of Se has to be increased and that the age of ewes also should be taken into account, when planning diets and daily allowances of mineral feed.

Keywords: Selenium, sheep, selenoproteins, NMD, selenium yeast, organic selenium

Sammanfattning

Selen (Se) är ett spårämne som är av stor betydelse för många fysiologiska processer hos däggdjur. Det mesta av selenet är bundet till proteiner och många av dem har enzymatiska funktioner. Glutationperoxidas (GSH-Px) är en grupp enzymer som är beroende av Se för sin funktion. De fungerar som antioxidanter, skyddar kroppen mot fria radikaler och aktiviteten hos GSH-Px påverkas av hur mycket Se som finns lagrat i levern. Selenbrist kan orsaka ett flertal störningar hos får, däribland muskeldegeneration, hälta, reproduktionsstörningar och minskad tillväxt hos lammen. Under dräktigheten transporteras Se mycket effektivt över till moderkakan och fostrets behov prioriteras före tackans. Hur effektivt Se tas upp av kroppen beror delvis på om det kommer från oorganiska källor som natriumselenit och natriumselenat eller från organisk selenjäst. Biotillgängligheten är högre hos organisk Se än hos de oorganiska varianterna. Svenska jordar är väl kända som uttalade selenbristområden och för får, som till största delen äter grovfoder och bete, är risken för selenbrist därigenom stor. I det här arbetet genomfördes ett antal studier för att ta reda på selenstatusen hos svenska får och innehållet i olika fodermedel. En studie innehöll en uppsättning av blodprov från får tagna under åren 2003-2005 och analyserade för Se-innehåll. Resultaten från denna studie tyder på att yngre tackor har ett högre behov av Se än äldre tackor. En mindre gårdsstudie genomfördes vintern 2013/2014 då blodprov från sju tackor togs vid två tillfällen, i november och januari. När Se analyserades i helblod visade det sig att tackorna låg inom referensintervallet 0,1-0,5 mg/l men nära den lägre gränsen. Även om selenbehovet täcktes av fodret sågs en oväntad minskning av Se från första till andra provtagningen, troligtvis på grund av en minskad konsumtion av mineralfodret. Information om seleninnehållet i svenskt grovfoder har erhållits från *Växa Sverige* och presenteras också i det här arbetet. En mindre marknadsundersökning har även gjorts med en sammanfattning av seleninnehållet i de vanligaste mineralfodren på den svenska marknaden. Med hänvisning till resultaten i det här arbetet kan man fundera på om det rekommenderade dagliga intaget av Se bör ökas och att tackornas ålder bör tas med i beräkningen när utfodringen planeras.

Nyckelord: *Selen, får, selenoproteiner, muskeldegeneration, selenjäst, organisk selen*

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Clarifications

Selenoenzymes/selenium depending enzymes – Enzymes that have a selenium ion bounded to it. Selenium is essential for the function of the enzyme.

Selenoproteins – A protein with inclusion of at least one selenocysteine in its sequence.

Seleno containing protein – A protein including a selenium methionine molecule. The selenium is not biologically active in this form

Selenocysteine (Se-Cys) – Cysteine is linked to selenium instead of sulphur as in the normal cysteine. Selenocysteine is the main form of selenium in mammals. .

Selenomethionine (Se-Met) – The amino acid methionine linked to selenium, the predominant form of selenium in plants.

Sodium selenite - Na_2SeO_3, one of the inorganic forms of selenium, commonly used in mineral feed. Naturally occurring in the soil.

Sodium selenate - Na_2SeO_4 is another inorganic form of selenium. Naturally occurring in the soil.

Selenide – All forms of ingested selenium needs to undergo a metabolic transformation to selenide in order to be incorporated into selenocysteine.

Selenium yeast (Se-Yeast) – Yeast that has grown in a medium containing selenium. The most common organic form of selenium used in mineral supplements. Se-Met is the contri-buting source of selenium.

1 Introduction

Imbalance in trace minerals may occur in farm animals, especially ruminants, whose intake of minerals depend largely on the content in the forage and thereby on the soil where the ley grows. Selenium (Se) is an important trace element that has a narrow range between deficiency and toxicity in sheep (Humann-Ziehanka *et al.*, 2013). Sweden, as many other countries in northern Europe, has a low content of Se in the soil (Shand *et al.*, 2012) and animals that mainly consume home produced roughage therefore easily can suffer from Se deficiency if they are not supplemented in an appropriate way. Serious Se deficiency can lead to nutritional muscular dystrophy (NMD), also called white muscle disease, but more common are subclinical symptoms such as weak lambs, reduced feed consumption and pregnancy complications (NRC, 2007).

Supplementation of Se in sheep has been discussed for a long time among veterinarians, advisors and sheep producers. One has discussed if supplementation is necessary, as well as the best time for supplementation and what strategy is the best to use. Also the bioavailability of different Se sources has been mentioned, if organic or inorganic Se sources have an influence on the uptake by the sheep. Sweden has good conditions to produce roughage of high quality and nutritional values due to the long summer days. But even if the roughage is of highest quality, it can have low Se levels due to the low soil concentration. Sheep are kept in groups, mostly with free access to roughage and sometimes supplemented with concentrate a few times per day. If given, minerals are often served in buckets as granulates or as licking blocks and the individual consumption for each animal is very hard to control.

One hypothesis that will be discussed in this report is if it is more effective to provide ewes with adequate Se supplementation to prevent deficiency in newborn lambs instead of treating the lambs with Se. This may be possible because of the good transfer of Se across the placenta to the foetus from the gestating ewe (Ghany-Hefnawy *et al.*, 2007; Davis *et al.*, 2006). It will also diminish stress in both dam and young caused by handling the newborn lambs if no treatment of the lambs is necessary except the normal control of sufficient suckling, maternal care and a watchful eye by the farmer.

This study arose from an interest in the interaction between trace elements and their function and importance for sheep. The aim of the thesis was to collect information related to the impact of Se for sheep health and to look into Se status of Swedish sheep. The purpose has also been to collect information about the function and present recommendations of Se and the importance of a properly planned mineral supplementation for sheep. A simple comparison of mineral supplements has also been made, as well as an overview of the Se content in Swedish forage.

2 Literature review

Selenium (Se) as a non-metal element was discovered by the Swedish scientist Jöns Jacob Berzelius in the early 1800s (Arnér, 2010) but the importance of Se in animal metabolism was first reported in 1957 (Muth *et al.*, 1958). Different forms of Se occur and the ability for animals to utilize it varies. The definition of bio-availability is the amount of an ingested nutrient that can be used for essential physiological functions or storage (Tapiero *et al.*, 2003) and in the case of Se it is much depending on the source. Measurements indicate that the mean value of Se in Swedish soils is 0.30 mg/kg which is very low, according to Shand *et al.* (2012). As sheep diets mostly are based on roughage grown in the local area there is a risk of health disturbances caused by Se deficiency.

2.1 General physiological functions of Se

Selenium is an essential trace element that is present in small amounts in the mammal body. The mineral cannot be formed by the organism so it has to come from dietary sources. Most of the Se in animals is tied to proteins which therefore are called selenoproteins or seleno-containing proteins depending on the amino acid. The definition of a selenoprotein is that selenocystein (Se-Cys) is incorporated in the protein. This incorporation is specific and mediated at a ribosomal level and more than 80% of the protein bound Se is Se-Cys (Ghany-Hefnawy & Tórtora-Péres, 2010). In Se-Cys, cysteine is linked to Se instead of sulphur in the regular cysteine (Figure 1). Most of the selenoproteins have enzymatic functions and therefore they are also called selenoenzymes. These enzymes are involved in different metabolic pathways in the mammal organism (Suzuki & Orga, 2002; Ghany-Hefnawy & Tórtora-Péres, 2010; Hoffman, 2007). Selenium can also be bound into protein in the form of selenium methionine (Se-Met). The formation is called a selenium-containing protein as Se here does not function as a mineral but the methionine can be utilized as normal methionine (Suzuki & Orga, 2002).

If there is a deficiency in Se, it will be directed to prioritized organs such as the brain, pituitary gland, the thyroid gland and adrenal glands and the concentration in blood will decrease. Blood analyses are therefore used as a means of detecting Se deficiency. Blood Se concentrations can for instance be analysed in whole blood, serum, plasma or erythrocytes but the different quantifications will not indicate identical biological scenarios. Whole blood Se specifically reflects the dietary Se intake (Oh *et al.*, 1976). Several laboratories prefer to analyse Se in whole blood because it reflects both erythrocyte and serum Se levels. Normal whole blood concentration in sheep should be 0.1-0.5 mg Se/L (Poppenga *et al.*, 2012). Concentrations of plasma Se is an indication of the Se status at present while Se in erythrocytes reflects the situation about three months ago (Maas *et al.*, 1992).

Ruminants have a lower potential to absorb Se compared to non-ruminants, due to the rumen environment and the activity of rumen microbes that converse Se to an insoluble form. Absorption of Se is approximately 35 % in ruminants and for non-ruminants nearly 85 %. For both categories Se is absorbed in the small intestine (Wright & Bell, 1966).

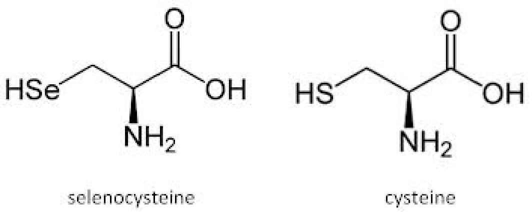

Figure 1. Selenocysteine and cysteine. (bioinformatica.upf.edu)

2.1.1 Glutathione peroxidase

So far, 30 selenoproteins have been found, but the function is known only for 12 of them (Behne *et al.*, 1994). One group is the Se-depending enzymes called glutathione peroxidases (GSH-Px's). This group of enzymes has an anti-oxidative function by reacting with hydrogen peroxide (H_2O_2) and soluble fatty acid hydro peroxides. Currently, four different types of GSH-Px have been identified. They operate through enzymatic catalysts in three steps and thereby protect the body against free radicals (Arteel & Sies, 2001; Brigelius-Flohé & Kipp, 2009). Thyroid cells synthesise H_2O_2, which is a requirement of the biosynthesis of thyroid hormones. This process generates free radicals (Contemre *et al.*, 1996) and GSH-Px protects the cells from these free radicals (Köhrle & Gärtner, 2009). One of the best described physiological processes of GSH-Px is the reduction of H_2O_2 and degradation of lipid and phospholipid hydro peroxides into water and alcohols that do not cause any damage to the animal's body (Allan *et al.*, 1999). The activity of

GSH-Px is strongly regulated by Se storage in the liver, as shown by Chu *et al.* (1993), and an adequate Se status is therefore of great importance.

2.1.2 Transformation of thyroxine (T4) to triiodothyronine (T3)

The thyroid gland has the highest Se concentration of all organs. Selenium is essential for its metabolism, hormone synthesis and conversion of thyroxin (T4), the inactive form of thyroid hormone to the active form triiodothyronine (T3) (Köhrle & Gärtner, 2009). Several Se-depending enzymes, such as the earlier mentoined GSH-Px, iodothyronin deiodinase and thyroperoxidase are involved in local activation and inactivation of thyroid hormones. Studies by Contemre *et al.* (1996) shows that severe Se deficiency generates a marked decrease in the activity of GSH-Px in the thyroid gland, which increases the risk of oxidative cell damage.

Cell necrosis and invasion of macrophages and T-lymphocytes to the thyroid gland has a negative effect on the thyroid function (Köhrle & Gärtner, 2009). In experiments where rats were used, there were indications of Se deficiency increasing necrosis in the thyroid gland and also in the kidneys and liver. (Contemre *et al.*, 1996). In their trials with rats, it was also found that in animals fed a diet creating Se deficiency, plasma GSH-Px was decreased to 11 % of the value in the group of rats that had adequate Se levels. They also mention that Se deficiency increased the thyroid sensitiveness to necrosis. Rats with Se deficiency had a higer epithelial cell necrosis in the thyroid gland and the inflammatory cells were predominantly macrophages.

2.1.3 Immune response

In both humans and animals, Se is known to be important for the immune system, partially by incorporation in selenoproteins. It has been shown that lambs from Se-supplemented ewes have an increased Se concentration in liver and blood at birth and a greater absorption of Se from colostrum (Ghany-Hefnawy *et al.,* 2008). However, Se is utilized by numerous tissues, including those involved in both the innate and adaptive immune response, and injections with Se also increase the antibody production (Behne & Wolters, 1983). In animals with an increased Se intake, both cell-mediated and humoral immune responses increase. However, Se cannot be stored for a longer period and in order to strengthen the immune system, it must be supplied continuously. So far, there are no apparent immune related diseases known to be linked to Se deficiency. Inadequate Se levels may, however, contribute to a less active immune system, which in turn reduces the ability to handle immune-driven disorders such as chronic inflammations, viruses, and allergens (Hoffman, 2007; Hoffman *et al.,* 2007; Beck, 2007). This may explain why sheep predisposed to clinical mastitis have lower concentrations of serum Se compared to healthy sheep in the same herd (Giadinis *et al.*, 2011).

2.2 Inorganic and organic selenium sources

Selenium is a trace element that naturally occurs as inorganic salts in bedrock and as organic components in plants (Shand *et al.*, 2012). Sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) are inorganic forms of Se (Juniper *et al.*, 2009). Selenium methionine represents the organic form of selenium in plant material (Vignola *et al.*, 2009). Another organic source is selenium enriched yeast (Se-Yeast) where Se-Met is the contributing source of Se (Korhola *et al.*, 1986). All forms function as supplementation in the diet, but they differ in bioavailability, retention and absorption. Regardless the form Se is present in, uptake occurs in the small intestine in which Se-Met is absorbed via the methionine transport system, while sodium selenite and selenate are absorbed mainly by passive diffusion (Weiss & Hogan, 2005). Both systems for absorption require a metabolic transformation of ingested Se from selenite, selenate or Se-Met to selenide (Se^{2-}) to be able to be assimilated into Se-Cys which is present in selenoproteins (Figure 2). Inorganic Se is reduced to selenide while organic Se is transformed to selenide by β-lyase. Surplus of Se-Met that has not undergone transformation into selenide can be incorporated into proteins as pure methionine but it is no more a part of the Se metabolism (Suzuki & Orga, 2002).

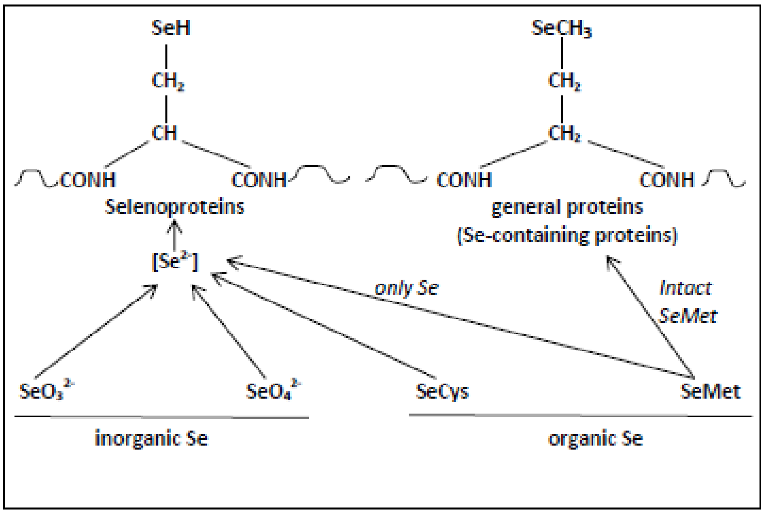

Figure 2. Irrespective of where Se comes from, only the Se-element is incorporated into selenoproteins. Different metabolic pathways transform Se to selenide (Se^{2-}) and further to Se-Cys which is the type present in selenoproteins. Intact Se-Met can be bound in general proteins (Suzuki & Orga, 2002).

In a study performed by Juniper *et al.* (2009), lambs were treated with different amounts of Se-Yeast, sodium selenite or untreated. The aim was to examine the proportion of Se-Met and Se-Cys in total Se of blood, muscle and different organs. The authors could find a linear dose effect of Se concentration in treated lambs, and a significant difference between Se sources was seen in skeletal muscles. When specific organs were analysed, kidneys had the highest Se values and it was not dependent on the dose in the diet. However, in the liver and cardiac muscles, the Se concentrations increased linearly with dose but were not affected by source. A linear increase was also seen in the skeletal muscles and here the Se content was further increased in animals supplemented with Se-Yeast. Irrespective of Se source, Se-Cys was the major form present in the tissue and even if Se-Met only represented a small fraction of total Se, it was a slight increase of Se-Met in kidney, liver and cardiac muscle related to ingested Se-Yeast. Although the Se source had no impact on GSH-Px-activity, there were differences between treated animals and non-treated animals. There was also an unexpected increase in whole blood Se in the untreated group compared to the control group. Juniper *et al.* (2009) assumed that the total mixed ration in the experiment diet was higher in Se than the pasture grazed prior to the study. Increased Se in organ tissue when animals are supplemented probably depends on the binding of Se into selenoenzymes. The higher Se level in skeletal muscles in animals fed Se-Yeast compared to selenite supplemented lambs indicates an improvement in uptake and retention of Se from Se-Yeast.

In an experiment of Qin *et al.* (2007) with wether lambs, the aim was to investigate if Se supplemented from organic or inorganic sources has an influence on Se concentrations in kidney, liver, skeletal muscles and whole blood. The study proceeded over eight weeks and the results showed an increase in tissue Se concentrations in all supplemented groups, compared to non-supplemented animals. Wethers treated with Se from organic sources had higher Se values than those given inorganic Se. In both groups, the highest Se concentration was found in the kidneys, similar to what has been described by others (Juniper *et al.,* 2009; Van Ryssen *et al.*, 1989). There was also an increased GSH-Px activity in both supplemented groups, but GSH-Px activity was higher in wethers with organic Se supplement. At the end of the study, whole blood Se had increased with 132 % and 164 % for inorganic and organic supplemented wethers, respectively. Se concentration in both diets was 0.1 mg/kg feed (Qin *et al.*, 2007).

Since the different metabolic pathways from various Se sources all end up in Se-Cys, it can be useful to see if dietary Se source influences the seleno amino acids present in the tissues. In a study by Vignola *et al.* (2009) 48 lambs were divided into four groups fed different types of Se supplement for 63 days. The groups were supplemented with either sodium selenite (0.30 mg/kg), Se-Yeast (0.30 mg/kg or 0.45 mg/kg) or no supplement, as control. In the end of the study, the animals were slaughtered and samples were taken to analyse Se content in the muscles. Regardless of Se source, supplemented animals had an increase of Se in the muscles and the increase was linear with ingested Se amount. There was also an evident difference depending on type of supplementation with an advantage to Se-Yeast. Seleno amino acids in the muscles also were lower in the control group but here no difference in the concentration was seen between the supplemented groups. In animals supplemented with Se-Yeast, there was an increase in the amount of Se-Met which could not be seen in animals supplemented with sodium selenite (Vignola *et al.*, 2009).

2.3 Selenium contents in Swedish soils

Sweden like other countries in northern Europe is known to have soils poor in Se. Shand *et al.* (2012) made a study to evaluate the Se content in Sweden and used data collected from 1988 to 2007. The plough layer of the arable soils were analysed for Se content and the results indicate elevated Se concentration in areas close to the sea, mainly on the west coast. Also in inland areas located close to alum shale, for example in the Storsjön area in Jämtland the Se content was higher. The lowest Se levels were found in the southeast regions and the inland parts of central and northern Sweden. The arable soils in Sweden and other northern countries are richer in organic matter than soils in more southern countries. Concentration of Se in the soil depends to some extent on the amount of organic carbon, clay content and the pH in the soil. To bind the inorganic Se (sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4)) to organic matter, three scenarios can occur; direct complexion, indirect complexion involving organic-metallic bonds and covalent bindings to organic components (Qin *et al*., 2012).

2.4 Selenium and vitamin E

When Se is mentioned in the scientific literature, it often also concerns vitamin E and how they are linked to each other. Vitamin E works as an important biological antioxidant and is involved in the protection against oxidative damage created by free radicals. Vitamin E occurs naturally in young grass and products of plant origin but decreases during storage. Vitamin E cooperates with the Se-depending enzyme GSH-Px and together they are essential for prevention of several disturbances like muscle weakness after birth, disturbed immune system and reduced growth in lambs (McDonald *et al.*, 2011). There are several forms of vitamin E where α-tocopherol is the most biologically active form. As an antioxidant, vitamin E protects polyunsaturated fatty acids (PUFAs) from oxidation and is especially important for neonates who have a greater sensitivity for oxidative damage (Debier *et al.*, 2005). Vitamin E is mainly stored in the liver, skeletal muscles and in the adipose tissue but it is not stored for a longer period why an adequate regular dietary intake is of importance (McDonald *et al.*, 2007). Liver and placenta contain α-tocopherol transfer protein (αTTP), a cellular binding protein which is involved in the transfer of α-tocopherol between membranes (Debier *et al.*, 2005). Although there is a transfer over the placenta, colostrum is the primary source for newborns to receive vitamin E (Quigley & Drewry, 1998). Plasma concentration in pre-suckling animals is very low but increases after suckling the first colostrum (Debier *et al.*, 2005).

2.5 Ewe diet affects plasma selenium concentration, growth and lamb performance

There is a positive correlation between Se content in the diet and blood Se levels in the animals that consume the feed. In a situation with free access, increasing nutrient requirements lead to increased feed consumption and thereby the sheep automatically get a higher intake of nutrients without any extra supplementation. However, in periods with very high needs, the nutrient concentration of the diet also must be increased (Stowe & Heardth, 1992). Pregnant ewes and their foetuses are particularly sensitive to imbalances in the diet, both concerning macro and micro nutrients. The foetus is provided with nutrients by transfer over the placenta and Se is efficiently transmitted even if the ewe has a low blood Se concentration (Ghany-Hefnawy *et al.*, 2007). Thereby, a sufficient supply to the foetus is ensured, but the ewes' own level of Se will decrease if the diet contains substandard concentrations. Ghany-Hefnawy *et al.* (2007) found in their study a negative correlation in Se concentration between amniotic fluids and plasma of gestating ewes. It was also significant that the Se concentrations in amniotic fluid, maternal plasma and liver decreased over time while there was an increased Se concentration in foetal glands. An evident maternal-foetal relationship in Se concentration over the whole gestation period was also observed and ewes seem to secure foetal requirements prior to their own needs.

Se concentration in foetal liver has occasionally been found to increase in the middle of gestation and thereafter decline in the end of the prenatal period. A possible reason for the decrease is the use of Se in order to increase the activity in selenoenzymes and other selenoproteins in the process of foetal growth (Abdelrahman & Kincaid 1993). An increased Se concentration in the amniotic fluid, liver and plasma of the ewe has been found during early pregnancy, while the increase in foetal organs, especially kidney, liver and the thyroid gland occurs during late pregnancy (Ghany-Hefnawy *et al.*, 2007).

In a study that continued over a period of approximately one and a half year, Davis *et al*. (2006) investigated how ewe Se consumption affects their lambs. They found that ewes with a higher Se intake produced colostrum with higher Se concentration, and the levels increased linearly with dietary Se intake. Ewes that received more than 12 mg Se/kg feed in the diet had higher colostrum Se levels than the control group (0.2 mg Se/kg feed) and ewes provided with 20 mg Se/kg feed produced colostrum with higher Se content than those who received 0.2, 4,8,12 or 16 mg Se/kg feed. Also the plasma Se concentration of the lambs increased linearly with the Se amount consumed by the ewe and was higher at parturition before the first suckling in lambs of "high Se ewes". Likewise, Se concentration in milk produced during the lactation until weaning was influenced by Se in ewe diet and increased linearly with intake. No animals in the study exhibited signs of Se toxicity even if Se was fed in larger amount than the present recommendations (2 mg/kg, NRC 2007). Another effect on the lambs was Se concentration in the testis in ram lambs at weaning. Testicular Se levels at dry matter basis increased linearly with the ewes' dietary Se intake. However, the increase was significant only the first year even if there still was linearly increased testicular Se concentration the second year.

It is widely documented that Se has an influence on reproduction in animals and that Se deficiency can reduce reproductive efficiency and growth as well as general health in young animals (Ghany-Hefnawy *et al.,* 2008). Nutrition transfer to the offspring depends to a large extent on the mothers' nutrient status and therefore it is possible to secure the foetus nutrient status by feeding the ewe a sufficient diet. In a study performed by Gabryszuk & Klewiec (2002) the team saw that the effect of injection of either Se or Se in combination with vitamin E markedly differed between first and second lambing ewes where all had acceptable Se levels before the experiment started. Animals used in the study had a fertility percentage of about 70% and a significant improvement in reproductive performance was seen in the group of second lambing ewes injected with only Se, without vitamin E. In the same group, there was also a significant increase in the daily weight gain of the lambs and the weight at 28 days of age. In the group of ewes that was mated for the first time, the only difference between treatments was an increased lamb live weight at birth, both in the Se and Se+vitamin E supplemented groups. There was also an increased lamb weight at 28 days of age in the group supplemented with only Se but in the group supplemented with both Se and vitamin E there was no increase in lamb weight.

To investigate Se levels of amniotic fluid, colostrum, milk and plasma as well as body weight gain in both ewes and lambs, a study evaluating the effect of Se supplementation was performed by Ghany-Hefnawy *et al.* (2008). Gestating ewes were divided into three groups, one control and the others had either oral supplementation or subcutaneous injections (SC) of Se. The SC group was given 0.1 mg sodium selenite/kg BW in week seven and four before parturition and one week after. Orally supplemented ewes were given 3 mg Se/week of sodium selenite from seven weeks before parturition. Blood samples were taken regularly. There was no difference in ewe body weight, but lambs born by SC ewes had higher body weight than control group lambs, although there was no significant difference between the two supplemented groups. Plasma Se concentration was increased in lambs from supplemented ewes and was highest in the SC group. Lambs raised by supplemented ewes received higher Se levels through colostrum, while it was no significant difference between the types of supplementation. An increased Se content was also seen in the milk the first two weeks after parturition where SC ewes had higher contents than orally supplemented ewes.

2.6 The effect of selenium in lamb diet

Neonate lambs live their first hours on nutrients transferred over the placenta from their time as foetus and postpartum on the colostrum and milk offered by the ewe. If the parturition is difficult, the lamb sacrifices a lot of energy and may not be able to stand and suckle after birth and is thereby deprived the essential colostrum. As mentioned earlier, ewe diet plays a significant role for the nutrients that the lambs receive through the milk early in life. Later on when they have started to eat solid feed, both concentration and source of Se supplemented by either concentrate or mineral feed have been shown to be of importance and affect total Se concentration in lamb tissue (Taylor, 2005; Lawler *et al.*, 2004). In an experimental study performed by Juniper *et al.* (2009) lambs were taken from their dams two hours postpartum. After a supply of 140 ml colostrum substitute they were placed in two groups that throughout the experiment either were supplemented with Se-Yeast or had no Se supplement. The lambs were artificially raised on milk replacer and after weaning on a pellet ration and the study continued for 91 days. The differences in tissue Se-concentrations are shown in Table 1. Moreover, the differences between groups increased over time.

Table 1. *Mean values in mg/L for whole blood and in mg/kg DM for heart, liver, kidney and muscles of lambs on a Se supplemented or non-supplemented diet*

	Treatment		P-value
	Se-Yeast supplement	Control	
Whole blood	0.61	0.23	< 0.001
Heart	10.80	1.28	< 0.001
Liver	22.64	1.35	< 0.001
Kidney	18.96	5.90	< 0.001
Muscle			< 0.001
Longissimus dorsi	7.82	0.30	< 0.001
Psoas major	7.02	0.29	< 0.001

Modified from Juniper *et al.* (2009)

2.7 Deficiency and toxicity

Mineral imbalances are not always as visible as surplus or deficiency of energy and protein, which can be seen in the animals' body condition and weight. Micronutrients are important to prevent different health disturbances but deficiencies and imbalances are often detected when problems already has occurred. In Swedish conditions, supplementation of Se is necessary to meet animals' requirements and too low concentrations in the feed will result in deficiency. It is widely known that health problems associated with Se deficiency is, as earlier mentioned, weak born lambs, nutritional muscular dystrophy (NMD) and reduced fertility. Other possible physical conditions are impaired immune system and disturbances within the endocrine system (Contempre *et al.*, 1996; Köhrle & Gärtner 2009).

NMD is caused by a deficiency in Se and/or vitamin E. Both Se and vitamin E function as antioxidants against free radicals in the metabolic system. Diets deficient in Se or vitamin E allows oxidative damages with the risk of following injuries in physiological processes (Reilly *et al.*, 2012). Vitamin E protects fatty acids against oxidation but a high fat intake increases the risk of development of NMD because of the higher need of vitamin E. Muscle degeneration is most common in lambs and other fast growing animals and sudden muscle activity, such as the first rise after birth or the let out on pasture after the housing season can trigger episodes of NMD (McDowell *et al.*, 1996; Reilly *et al.*, 2012). Muscles with high metabolic activity are most sensitive and the heart is one example. When investigating animals affected by NMD with electro cardio diagrams, some types of abnormalities have been observed which indicate that circulatory failure is one of the fundamental changes caused by Se – vitamin E deficiency (Godwin, 1975). Two types of NMD occur, the acute and the chronic form where the latter is the most common one. Chronic NMD affects skeletal muscles and the lambs become stiff, have difficulties to stand and sometimes they are also unable to nurse (McDowell *et al.*, 1996). Acute NMD is severe and the impact on heart and respiratory muscles can cause death. Lambs suffering from acute NMD are often incapable to get up but can still be alert and interested of the surroundings (Reilly *et al.*, 2012). However, NMD is a consequence of oxidative damages in the muscles owing to deficiency in Se and/or vitamin E, especially with a simultaneous intake of polyunsaturated fatty acids (McDowell *et al.*, 1996). Both ewe and lamb nutrition have a preventive effect on development of the disease when Se is supplemented in sufficient amounts (Davis *et al.*, 2006; Taylor, 2005).

Selenium is also a potentially toxic element and too high consumption can cause poisoning in animals. Toxicity of Se mostly occurs in animals grazing areas with high levels of Se in the soil and consequently also in the pasture and roughage grown in these areas. Unwary supplementation of Se without controlled levels in the rest of the feed can cause overdoses that are toxic for the animals. Toxic Se doses for sheep from dietary sources are over about 5 mg/kg DM, 500 µg/kg in milk or water and approximately 1.25 mg/kg body weight ingested over a longer period (McDonald *et al.*, 2011). Se in organic form such as Se-Yeast has proved to be more toxic than inorganic Se from selenite (Reilly *et al.*, 2012). Selenium toxicity can be expressed as poor wool quality and growth, lameness, oedema of the coronary band, loss of appetite and a poor general condition (McDonald *et al.*, 2011).

The daily requirement of Se for ewes of different age and stages of life is shown in Table 2. Lambs growing 200-250 g/d have a Se requirement of 0.35-0.45 mg/day (NRC, 2007).

Table 2. *Examples of ewe requirement of Se in different stages of life on a roughage based diet. Se mg/day*

	Maintenance	**Maintenance + growth**	**Gestation, two lambs**	**Early lactation, two lambs**
Yearling ewes 60 kg	0.05	0.15	0.21	0.68
Mature ewes 80 kg	0.07		0.15	0.79

Values from NRC (2007)

3 Material and methods

The studies presented in 3.1 and 3.2 were carried out to examine blood values of Se in Swedish sheep. Roughage Se content was compiled (3.3) and a market survey of mineral feeds (3.4) were made in order to show how the Se requirement can be covered by the feed.

3.1 Analyses of data from a vitamin study

To get information of the selenium status in Swedish sheep, data from a study of vitamins and minerals in sheep were used. The trials were performed during the years 2003-2005 in order to investigate the status of vitamins A, D and E in ewes. Also calcium (Ca) and Se blood levels were analysed. Three experiments were designed to study the vitamin status in the ewes; I) supplementation with vitamins from either synthetic or natural sources, II) feeding a hay- or silage based diet, and III) evaluating the impact of lambing early or late in the lambing season. Early lambing was in March/April and late lambing in May/June. In all trials, crossbred ewes (Swedish Finewool* Texel) were used and within each trial they were evenly distributed between the treatments regarding age and weight. Blood samples for Se analysis were taken at three time points; pre mating, in mid pregnancy and late pregnancy (Table 3), and Se in plasma and erythrocytes was quantified. In experiment I there were 56 ewes (15 of them were sampled for Se) with an age of 1-9 years. The average weight in the beginning of the trial was 69.5 kg and after lambing 82.5 kg. In experiment II, 25 ewes were used (10 of them were sampled for Se), aged from 1-7 years. In experiment III, 32 ewes (of which 10 were sampled for Se) in the age of 2-6 years participated. Their weight before mating was on average 73 kg and after lambing 76.5 kg. For the ewes with early lambing (March/April), blood samplings occurred in October, January and February. For the ewes with late lambing (May/June) and those in experiments I and II, blood samplings occurred in December, March and May. In this thesis, the ewes from the three experiments were not separated in the statistical analysis.

Table 3. *Days in average before lambing that blood samplings for Se analysis was taken*

	Pre mating	Mid pregnancy	Late pregnancy
Experiment I	171	72	31
Experiment II	160	70	30
Experiment III	165	74	32

Also the uptake and utilization of vitamin E from synthetic and natural sources in lambs were studied. Thirty ewe lambs were blood sampled, also for Se-concentration, in October and in December. They were 120 days old in the beginning of the trial, had an average weight of 28 kg and were cross breeds of Swedish Finewool*Texel.

The results from all experiments have been presented in a detailed report to the Swedish Board of Agriculture (Bernes & Persson Waller, 2007). Selenium data have here been analysed using the programs MS Excel and NCSS 2000 (NCSS, Kaysville, Utah, USA) to perform Pearson correlations and one way ANOVA analysis of variance.

3.2 Farm study

A farm study was performed the winter 2013/2014 to investigate the selenium status in a Swedish sheep flock. Blood samples were drawn from the jugular vein of seven ewes by a veterinarian and collected in vacuum tubes with EDTA. The samples were sent to a commercial laboratory for analysis (ALS Luleå) for Se quantification. The ewes were cross breeds between Dorset and Gotland sheep. Their age varied from 5 months to 9 years.

The studied farm is located in the south east of Sweden. It is organically certified with a herd consisting of 221 ewes and 10 rams. The sheep are kept outside all year around with access to shelter for protection against wind and weather. Mating occurs 30 December to 22 January and results in a lambing season from 28 May to 15 June. The ewes are one and a half year at their first mating.

On this farm the grazing season starts in mid-April. The pasture includes 40-45 ha natural pastures, 15-20 ha forestland and the sheep also graze the regrowth of 30 ha, of which 4-5 ha is rapeseed and the rest is ley. The leys consist of red clover, lucerne, perennial ryegrass, timothy, grassweed, ribwort plantain, cocksfoot, birds-foot trefoil and white clover. Owing to the organic system regulated by EU it is not allowed to use anthelmintic on a regular basis to control gastrointestinal parasites. Instead it is recommended to collect faecal samples for analysis and deworm only if the sheep are infected. The feeding strategy is free access to roughage. In addition, thin ewes and lambs are given concentrates (Lantmännen Sund Rejäl). Mineral feed (Lantmännen Effekt Lamma m Cu) and salt lick is offered to all animals. According to the farmer, the yearly consumption of minerals is 950 kg for the herd. This gives an average consumption of 950kg/365days/321sheep = 8.1g/animal/day = 0.0081kg×45mg Se/kg minerals = 0.5mg Se/animal/day. One hundred sheep have been added to the number of ewes in this calculation, as an estimate due to that the lambs will consume minerals during some parts of the year.

In the winter 2012/13, 187 ewes were mated. The pregnancy rate was 96.8 % and 294 lambs were born whereof 281were born alive and 13 were stillborn. Two lambs did not survive until weaning. Health disturbances and illness that have affected the animals in the past year include mastitis and pneumonia in ewes. Some of the lambs suffered from the Schmallenberg virus; one lamb was born malformed and 11 lambs were unable to lift their head or to rise. Earlier years there have been various indications of Se deficiency in the herd. Signs have been lambs born weak, muscle weakness and lambs with varying lameness. It has been a problem for several years, but in recent years it has become considerably better. This is believed to be a result of a change in mineral feed to a supplement that contains a combination of inorganic Se and organic Se from selenium yeast. Animals with evident Se deficiency are treated orally with Selevitan® vet, a complex preparation of Se and α-tocopherol (Pharmaxim). The dosage per animal is 5 mg for ewes and 2 mg for lambs and the treatment is repeated three times with four to seven days in between. All lambs in the litter are treated even if not everyone shows symptoms.

3.3 Swedish forage analyses

In order to get an overview of the average Se level in Swedish roughage, data were achieved from the company *Växa Sverige* who works with extension service for dairy farmers. Among other duties, they collect and compile results from feed analyses that dairy farmers have sent to various laboratories. The results shown here are from the years 2011-2013, including all parts of Sweden and illustrate roughages of different cuts and with a variation in legume content. As we did not have direct access to the data base, the data was primarily compiled by *Växa Sverige*. They were also asked to look into correlations between Se and other components in the feeds. Diagrams were made with Se and calcium (Ca), phosphorus (P), magnesium (Mg), potassium (K), copper (Cu), cobalt (Co) and molybdenum (Mo) but they were hard to interpret. The diagrams also included Se levels correlated to ash, organic matter digestibility (OMD), crude protein (CP), neutral detergent fibre (NDF), lactic acid fermentation (LAF) and lignin (ADL).

3.4 Mineral feeds

A market survey was made of the most common mineral supplements for sheep available at the Swedish market. If the nutritional contents were not accessible via internet, the producers were contacted via e-mail to get the current content in their products.

4 Results

4.1 Analyses of data from the vitamin study

Data extracted from the study by Bernes & Persson Waller (2007) has been analysed in this thesis, showing Se content in blood plasma and erythrocytes correlated to different parameters. Table 4 shows the correlation between the ewes' age, the weight before mating, the number of born lambs and Se content in the blood at different times. There was no correlation between the weight of the ewe and plasma Se content before mating. The plasma Se content during pregnancy was however correlated to ewe weight. No correlations were seen between Se content in erythrocytes and ewe weight at any time-point. Age was significantly correlated to plasma Se concentration during pregnancy but not before mating. Plasma Se concentrations during the pregnancy has a tendency to be significantly correlated to the number of lambs born.

Table 4. *Correlations between Se content in plasma (Se P) and erythrocytes (Se E) and some production parameters of the ewe at different time-points. All ewes included (n=35)*

	Age	Numbers born	Weight pre mating	Se P pre mating	Se E pre mating
Nr. born	0.570***				
Weight pre mating	0.751***	0.653***			
Se P pre mating	0.090	-0.064	0.281		
Se E pre mating	0.018	0.082	0.193	0.339*	
Se P mid preg.	0.546***	0.336*	0.562***	0.036	0.234
Se E mid preg.	-0.114	-0.032	0.066	0.398*	0.753***
Se P late preg.	0.625***	0.378*	0.651***	0.354*	0.371*
Se E late preg.	0.100	0.124	0.235	0.211	0.804***

Significance level: $*p \leq 0.05$ $**p \leq 0.01$ $***p \leq 0.001$

When excluding ewes younger than two years old the result becomes different. After excluding the yearlings, there is no significant correlation between weight and Se content (Table 5).

Table 5. *Correlations between Se in plasma (Se P) and erythrocytes (Se E) and ewe weight at different time-points for ewes older than two years.* [a]n=24, [b]n=23

	Weight pre mating	**Numbers born**
Se P pre mating[a]	0.348	
Se E pre mating[a]	0.129	
Se P mid preg.[b]	0.007	
Se E mid preg.[b]	-0.014	
Se P late preg.[b]	0.104	-0.142
Se E late preg.[b]	0.127	-0.027

Significance level: $*p \leq 0.05$ $**p \leq 0.01$ $***p \leq 0.001$

When dividing the ewes into two groups, yearlings and older animals, one can see that younger individuals have lower mean values of Se concentration in plasma than the older ones during pregnancy (Table 6). No difference can, however, be seen in the erythrocytes.

Table 6. *Mean values of Se (mg/kg) in plasma (Se P) and erythrocytes (Se E) at different time-points for yearling ewes and older animals. (Standard error)*

	Yearlings n=11	**Older animals n=24**
Se P pre mating	0.13 (0.01)	0.14 (0.01)
Se E pre mating	0.29 (0.04)	0.32 (0.02)
Se P mid preg.	0.10***(0.00)	0.14*** (0.00)
Se E mid preg.	0.54 (0.04)	0.55 (0.03)
Se P late preg.	0.12*** (0.01)	0.16*** (0.00)
Se E late preg.	0.55 (0.04)	0.60 (0.02)

Significance level: $*p \leq 0.05$ $**p \leq 0.01$ $***p \leq 0.001$

The report by Bernes & Persson Waller (2007) also included examinations of lambs and their vitamin and Se status during the housing period. The weight and the blood Se content in October were significantly correlated, 0.71 ($p < 0.05$) for plasma Se and 0.52 ($p < 0.05$) for the erythrocytes. However, there was no significant correlation between the weight and blood Se content in December, 0.38 and 0.41 for plasma and erythrocytes, respectively.

4.2 Farm study

Results for the sheep in the farm study (Table 7) indicate a decrease in blood Se concentration over the period. At the time of the second blood sampling, the farmer informed that during the autumn, the sheep had shown less interest for the minerals and the amount consumed had dropped. Compared to earlier in the season, when grazing normally leads to a lower intake of mineral feed, the current intake was even lower. The risk that the palatability may change during storage was discussed as the mineral feed was bought in a big amount in order to get a better price.

Results from the Se analyses of blood samples are presented in Table 7. For three individuals, an additional measurement was available from year 2012.

Table 7. *Se concentration for ewes at the studied farm in mg/L whole blood*

Animal	Ewe age (year)[a]	Se status 2012 April mg/L	Se status 2013 Nov. mg/L	Se status 2014 Jan. mg/L
1	5	0.38	0.20	0.19
2	6	0.37	0.22	0.24
3	9	0.30	0.22	0.21
4	2		0.35	0.26
5	5 months		0.31	0.16
6	2		0.20	0.16
7	5 months		0.32	0.18

[a] Ewe age in November 2013

In all individuals except one, the blood Se concentration decreased between the two samplings. For those sampled 2012, the Se concentration was lower 2013/2014.

4.3 Selenium content in Swedish roughage

Results from roughage analyses from the whole country were obtained by *Växa Sverige* and are presented in Table 8 as the mean value of forages containing 0%, 1-50% and 51-100% legumes. Roughage with high content (51-100%) of legumes had higher average Se concentration compared to the other. The diagrams that also were made by *Växa Sverige* regarding correlations between Se and other feed parameters did not show any credible relations except a tendency to a negative correlation between Se content and Cu and Se and Co.

Table 8. *Mean values from the years 2011-2013 of Se mg/kg DM in Swedish roughage with different content of legumes (n= >20)*

Se mg/kg DM	Grass (0% legumes)	Grass/clover (1-50% legumes)	Grass/clover (51-100% legumes)
Mean	0.04	0.04	0.09
Highest value	0.14	0.74	0.39
Lowest value	0.01	0.00	0.01

Most Swedish leys have 1-50 % legumes and therefore this category is more closely presented in Table 9. Comparison over the season implies a slight increase in Se content from 1st to 3rd cut.

Table 9. *Variation in forage content during the season over three years. Mean values from year 2011- 2013 of Se mg/kg DM (n= >20)*

Grass/clover 1-50% legumes	Se mg/kg DM		
	1st cut	2nd cut	3rd cut
Whole Sweden 2011	0.04	0.04	0.05
P10[a]	0.01	0.01	0.02
P90[b]	0.10	0.10	0.10
Whole Sweden 2012	0.03	0.04	0.04
P10	0.01	0.01	0.02
P90	0.06	0.06	0.09
Whole Sweden 2013	0.04	0.03	0.06
P10	0.01	0.01	0.01
P90	0.06	0.08	0.10

[a] P10 is the 10% lowest percentile [b] P90 is the 10% highest percentile

The results from *Växa Sverige* have been divided into regions according to climate, plant growth prerequisites, harvest quantities and qualities (Figure 3). The borders are drawn considering growth conditions and where there is a high density of dairy farms. The division of the country into regions is mainly done to facilitate for the farmers to estimate the optimal time for cutting the ley.

A: Southwestern Götaland (Halland, western and southern Skåne)
B: Southeastern Götaland (northeastern Skåne, Blekinge, Kalmar län, Öland and Gotland)
C: Central Götaland (northern Skåne, central Småland, southern of Västergötaland)
C2: Sjuhärad
D: Northeastern Götaland (Östergötland, northern Kalmar län)
E: Northwestern Götaland/Svealand (coast of Bohuslän, the plains in Västergötland, the area around lake Vänern)
F: Mälaren-Hjälmaren area (Södermanland, Närke, Västermanland, Uppland)
G: Northern Svealand/Southern Norrland (southestern Dalarna, south Gästrikland)
H: Middle part of the Norrland coast and the area around lake Storsjön (Ångermanland, Jämtland)
I: Norhtern Norrland (Västerbotten, Norrbotten)

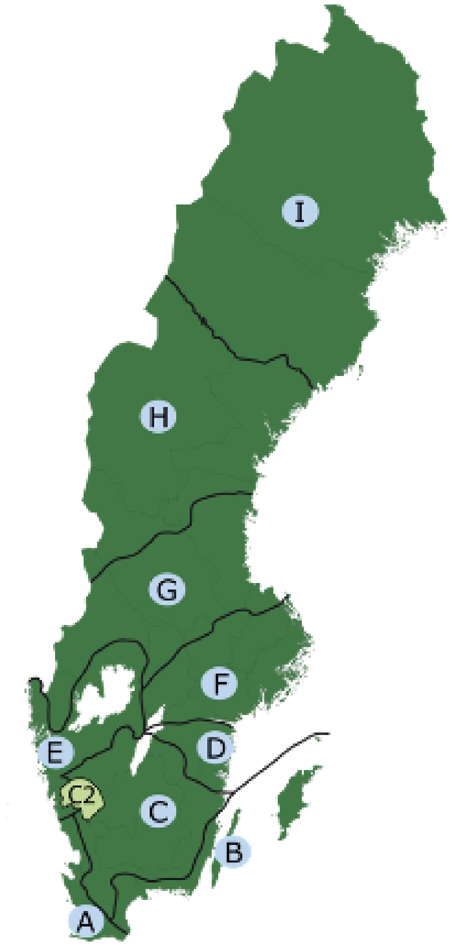

Figure 3. Sweden divided into regions according to prerequisites for roughage production.

Mean values of Se for roughage with 1-50% legumes from the different regions are shown in Table 10. Mean values of the 10 percent lowest (P10) and highest (P90) percentiles indicate a narrow range in Se content.

Table 10. *Average values for Se concentration (mg/kg DM) in forage analyses from different regions of Sweden year 2013*

Mean value Se mg/kg DM	**Grass/clover 1-50% legumes**		
	1st cut	**2nd cut**	**3rd cut**
Region A	0.04[a]	0.05[b]	0.05[b]
P10	0.01	0.01	0.03
P90	0.06	0.07	0.09
Region B	0.05[a]	0.06[c]	
P10	0.01	0.03	
P90	0.10	0.08	
Region C	0.03[b]	0.03[b]	0.06[c]
P10	0.01	0.01	0.02
P90	0.10	0.08	0.10
Region C2	0.05[c]	0.06[c]	
P10	0.01	0.02	
P90	0.17	0.14	
Region D	0.02[c]	0.02[c]	
P10	0.01	0.02	
P90	0.02	0.03	
Region E		0.08[c]	
P10		0.05	
P90		0.10	
Region F	0.03[b]	0.03[c]	
P10	0.01	0.01	
P90	0.05	0.05	
Region G	0.03[c]	0.03[c]	
P10	0.01	0.02	
P90	0.05	0.05	
Region H	0.02[c]	0.02[c]	
P10	0.01	0.00	
P90	0.05	0.04	
Region I	0.03[b]	0.01[b]	
P10	0.01	0.01	
P90	0.04	0.02	

[a] n= >20; [b] n= 10-19; [c] n= 4-9

4.4 Feed calculations and Se contents in products available at the Swedish market

A feed ration (Table 11) was calculated for ewes weighing 60 kg and 80 kg in late gestation, with up to two foetuses. The requirements for MJ, AAT, Ca, P, Se and vitamin E are according to the current recomendations from NRC (2007) and Fodertabeller för idisslare 2003, SLU. The amount of mineral feed was calculated to cover the needs of Se but this amount does not cover the requirement of vitamin E.

Table 11. *Example of a feed ration for ewes in late gestation, based on a good quality roughage with an average Se content for Sweden (0.3 mg Se/kg DM). Nutritional values for the silage is 50% DM, 11,3 MJ/kg DM, 73 g AAT/kg DM, 6.5 g Ca/kg DM, 2.2 P/kg DM and 0.3 mg Se/kg DM.*

	Silage 50 % DM (kg feed)	Effekt Lamma	MJ	AAT g	Ca g	P g	Se mg	Vitamin E mg
Ewe 60 kg Yearling	*requirements*		*16.5*	*117*	*5.1*	*4*	*0.21*	*336*
	3 kg	5 g	**17**	**110**	**10.4**	**3.7**	**0.23**	**152**
Ewe 80 kg	*requirements*		*18.6*	*131*	*5.4*	*4.2*	*0.15*	*448*
	3.5 kg	3 g	**19.8**	**128**	**11.8**	**4.1**	**0.19**	**134**

There are several brands and varieties of mineral feeds available but their content of Se is quite similar. The most common brands made for sheep are shown in Table 12. The total amount of Se in the mineral feeds are 30-50 mg Se/kg, except the special products available without veterinary prescription, containing only vitamin E and Se which have a Se content of 20 mg/kg. Three of the products contain Se-yeast in amounts of 10, 15 or 20 mg of the total Se content. According to the product descriptions, none of the products have a natural source of vitamin E.

Table 12. *Selenium and vitamin E content and source of Se in different mineral and vitamin feeds available at the Swedish market*

	Lant-männen Effekt Får	**Lant-männen Effekt Lamma**	**Svenska foder Deltamin**	**Kvarnby-foder VM Pälsfår**	**Kvarnby-foder VM Köttfår**	**Kvarnby-foder BM Får**	**Jermin Sau/Får**	**Wiromin Får-mineral**	**Lant-männen Vitamin E-Selen[a]**	**Tecnosan E-vitol 5 000[a]**	**Selevitan vet.®[ab]**
Se mg/kg total	30	45	45	45	45	45	30	50	20	20	600
[c]selenite	30	30	45	45	25	45	30	40	20	20	600
[c]se-yeast		15			20			10			
Vitamin E mg/kg	5 000	15 000	4 000	5 000	5 000	3 000	2 000	3 500	10 000	5 000	30 000
[d]from natural source	0	0	0	0	0	0	0	0	0		
Recommended dose/day (g/ewe) according to producer	25	25		20-50	20-50	20-50	20		20	15-20	0.005

[a] only vitamin E+Se supplement [b] veterinary prescription [c] source of total Se (mg) [d] mg of total vitamin E

5 Discussion

Selenium is important for sheep health as it is involved in several physiological functions. There are a number of health disturbances related to Se deficiency where NMD is one of the most severe (Godwin, 1975; McDowell *et al.*, 1996). Because of the general symptoms, it can be difficult to detect early signs of Se deficiency such as poor growth, reduced feed consumption, weak lambs and reproduction difficulties. These symptoms can also be present in other disorders and thereby Se deficiency may be overlooked. Intakes of Se above the minimal recommendations indicate improved Se levels in the tissues and a reduced risk of symptoms associated to Se deficiency (Ghany-Hefnawy *et al.*, 2007). There are a number of studies that highlights the positive effect of supplementing sheep with Se (Juniper *et al.*, 2009; Qin *et al.*, 2007; Vignola *et al.*, 2009). There are also indications of a higher bioavailability for Se-Yeast, especially when analysing skeletal muscles (Juniper *et al.*, 2009). The reason for skeletal muscles to show an increased Se content when Se-Yeast is fed can result from Se being prioritized to the organs if there is a situation of deficiency. Therefore, the skeletal muscles are the last to show an increase of Se when the requirements are fulfilled and the high bioavailability of Se-Yeast increase the possibilities for that.

There is evidence that the lambs' tissue Se concentration is related to the ewes' intake of Se and that Se is efficiently transferred over the placenta and into the milk with a linear correlation with the Se intake of the ewe (Ghany-Hefnawy *et al.*, 2007; Davis *et al.*, 2006). For this reason, it should be possible to secure the Se levels of the lamb by feeding the ewe with sufficient amounts. In this way, the number of lambs in need of extra Se treatment after birth could be reduced. Also, the stress in both dam and young caused by handling of the new-born lambs could be reduced. This is also the most common way to do in Sweden, but still lambs are suffering of Se deficiency some times. As a future improvement, the recommendations can be to increase the daily intake of Se during the whole year instead of providing extra Se only around parturition.

Selenium status in Swedish sheep

The results in the study presented in chapter 4.1 concerning ewe age and its correlations to Se status are interesting because of the differences found between young and older ewes. When all ewes are included in the calculations there is a significant correlation between the weight of the ewe and the concentration of Se in blood plasma. If data from the young ewes are excluded, the significance disappears. In addition, a positive correlation between ewe age and the concentration of Se in plasma was observed and age is increasingly affecting plasma Se concentration. One reason for this can be that the young ewe has an extra requirement of Se to cover her own growth, compared with older ewes. At the sampling before mating, neither the weight nor the age is significantly correlated to plasma Se concentration but at this time point, the young ewe only needs to cover her own Se requirement. When she gets pregnant, the foetus is prioritized and Se is efficiently transmitted over the placenta (Ghany-Hefnawy *et al.*, 2007) and the Se status of the ewe decreases. Further into pregnancy, the age becomes slightly more significant which strengthens this assumption. Older ewes do not grow anymore and are not exposed to such an increased Se metabolism.

The same scenario is seen in the variance analysis of the two age groups (Table 6). Younger ewes have significantly lower plasma Se concentrations than older ewes. One can discuss if the feeding recommendations concerning Se should be increased for the young, first lambing ewes. Farmers who examine their ewes with a pregnancy scanning test usually divide them into different groups according to the number of foetuses they are carrying. The point is to optimise the feeding for each group to maintain the animals' body condition. If the feed rations are the same for all ewes, ewes with several foetuses may end up in a negative nutritional balance at the same time as ewes with only one foetus are in the risk of gaining too much weight, and thereby are exposed to difficulties at parturition. In accordance to findings in this study, it may be necessary to also separate young and older ewes and add higher Se levels in the diet to the younger ewes.

While this study indicates that younger ewes have lower Se concentrations in the blood and are in increased risk of Se deficiency, the opposite has been observed by Gabryszuk & Klewiec (2002). They only observed significant correlations between Se injections, improved reproductive performance and lamb rearing within the three year old ewes and not in the younger ones. In this case it may have been caused by a reduced ability for the older ewes to recover after the previous lambing and also that these young ewes were one year older at their first pregnancy than the ewes in the vitamin study by Bernes & Persson Waller (2007). If their requirement of Se was not re-established, they started the new gestation with too low Se levels and thereby had an evident response on the Se injections. Therefore, it is of high importance to prevent Se deficiency before mating.

Concerning the lambs included in the study of Bernes & Persson Waller (2007) there can be many contributing factors influencing the results. In early autumn the difference in lamb weight most likely is affected by competition during the suckling period. Heavier lambs probably get more milk and thereby more Se, which can cause the positive correlations. During the housing period with a standardized diet, the weight does not affect the Se levels anymore.

The farm study ended up differently than first expected. According to the farmers' experience, the consumption of mineral feed usually increases during the winter compared to the summer when the sheep prefer to graze. Thus, the blood Se levels were expected to increase from November to January but the results show the opposite. The sheep had not been interested in eating the mineral feed they were offered. The results also show that the youngest animals had the greatest decrease in blood Se levels. The reduced consumption could be due to reduced palatability of the mineral feed during storage, or due to an unfavourable placement of the feeding troughs, where only one animal at a time could eat from the trough. Also a mild winter without any snow which engaged the sheep with grazing instead of staying near the feeding place could have affected the consumption. The greater change in the young ewes may be caused by hierarchy within the herd, where the older ewes ate first and the younger did not stay if the herd went on. Also younger individuals, as mentioned above, seem to be more sensitive to inadequate Se supply. Reference values for Se in whole blood are 0.1-0.5 mg/L (Poppenga *et al*., 2012) and these sheep were within the reference interval but close to the lower border, especially at the last sampling.

It would be interesting to study Se need of sheep in different phases of life. To look into this in a more detailed way may lead to new recommendations concerning mineral feeding to young ewes in an attempt to prevent Se deficiency and its consequences. In addition, a comparison of the effect concerning Se-Yeast and sodium selenite could bring information into recommendations about both dosage and type of mineral supplement. In addition to the blood analyses that have been statistically processed in this thesis, National Veterinary Institute (SVA) were contacted with the hope to get access to more data over blood Se analyses but for a number of reasons it was not possible to make use of their data.

The content of Se in Swedish roughage and feed supplements

It is generally known that Swedish soils, and thereby the grass growing here, are poor in Se. When planning the animals' diet it is of importance to know the nutrient contents in the feed that will be used by sending in feed samples for analysis, and based on the result add the nutrients that are lacking. However, to analyse Se is expensive and is not included in the normal roughage analyses. When studying the results summarized from *Växa Sverige*, there are no significant variations between years which makes it possible to rely on the average in the own region; the effect of weather and harvest conditions can be excluded. Depending on the content of other parameters in the feed such as energy, protein and macro minerals, Se can be offered the sheep in concentrates, mineral feed or as a specific Se supply.

The analyses from *Växa Sverige* give an overview of the forage produced in different areas of Sweden. Several of the lowest values estimated from the different regions are close to zero, which indicates a very low Se content in the soil. Referring to the average low Se levels in the leys, a mineral feed containing Se is necessary to cover the animals' requirement, both on pasture and during the housing season. However, the variation in soil Se content between regions is not large enough to make different feeding recommendations necessary. If the analysis database had been fully accessible, it would have been of interest to more in detail study the relationship between the different minerals and trace elements to see if they affect each other. The diagrams that were made indicate however that there is no relationship between Se and other minerals except the slightly negative correlations between Se, Cu and Co. It would have been good to make own statistical correlations with the raw data over Se and the other minerals but unfortunately this was not possible.

The most common mineral products available at the Swedish market are quite similar in their content of total Se. The difference is that some products have included Se yeast as a part of the Se source. The levels of vitamin E are more varied and the product with most vitamin E has more than three times the level than the one with the lowest content. None of the producers have specified in the product declaration if vitamin E is of natural source or of synthetic origin. The recommended dose of mineral feed per day according to the producer is almost the same for all products (~20 g). When a diet for sheep, as for other animals, is formulated, also the macro- and micro minerals must be taken into consideration and the recommended dose of mineral feed may need to be changed. The products may differ in the content of other minerals and must be chosen after analysing the roughage. In the comparison only granulated products are included. There also are minerals in the form of licking blocks, but when using them it is even more difficult to estimate the consumption of each animal. Furthermore, sheep are gourmets and it can be difficult to make them eat the recommended amount, as seen at the farm in the case study. The granulated mineral feeds should be made tastier to tempt the sheep to eat. To make mineral feeds tastier by adding molasses can on the other hand create problems with over consumption. In such cases, there can be a risk of mineral toxicity so there needs to be a balance. The sheep in the farm study were offered one of the products with the highest Se content, including Se yeast, and the highest amount of vitamin E, but they had decreasing Se levels in the blood due to the low consumption. On the other hand, the minerals were not served optimally which probably influenced the results. After the results from the blood samples were known, the farmer changed the feeding strategy and offered the minerals together with a small amount of concentrate and reported an increased consumption by the sheep.

The Se level in the silage used when calculating the feed ration (chapter 4.4) is the average of the data from *Växa Sverige*. In this example, the Se requirement is covered with a mineral amount much lower than the recommendations (3-5 g *vs* ~20 g). Likewise, the sheep in the farm study had a normal mineral consumption of 8 g/day. From that amount, they receive 0.50 mg Se/animal/day which, according to NRC (2007) by far should cover their needs (0.15-0.21 mg/day), but they still had quite low blood Se levels. One can speculate if the recommendations of Se should be increased to reduce the risk of NMD and other health disturbances related to Se deficiency. Another issue is the vitamin E requirement that will not be covered with the amount of minerals that are needed to cover Se. Most of the mineral feeds have low levels of vitamin E. To cover vitamin E, and thereby get a higher amount of Se at the same time, may be a better indicator that the requirements are met.

6 Conclusions

The results from this work have increased the knowledge regarding Se supplementation to sheep. The summary on Se levels in Swedish roughage shows that the content is too low to cover the requirements of sheep. To prevent problems due to Se deficiency, Se needs to be added to the diet for example in a concentrate with sufficient amounts of Se or by a Se containing mineral feed. The study indicated that younger ewes are more sensitive to Se deficiency than older ewes, especially during pregnancy, because of their higher metabolism of Se for maintenance and growth both for themselves and the foetuses. It may be beneficial to introduce special recommendations concerning younger ewes' need of a higher Se concentration in their diet. This might secure that their requirements will be met during pregnancy and also avoid Se deficiency in their lambs. The products on the market are quite similar concerning Se content but one can discuss if the level has to be increased to prevent Se deficiency. Another problem is that the sheep sometimes refuse to eat the mineral feed which can be avoided if the palatability increases. More information is needed, especially concerning Se recommendations to ewes of different ages at the time for gestation.

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