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Organic vs. Inorganic Selenium in Farm Animal Nutrition with Special Reference to Supplementation of Cattle

Kerstin Ortman

SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Abstract

This thesis summarises an discusses the results of five separate trials designed to investigate the differences in the availability and retention of supplements of selenium when they were fed to dairy cattle, suckler beef cows and fattening pigs, either in the inorganic form as selenite or selenate, or in the organic form as selenium yeast.

The selenium yeast supplement caused a higher concentration of selenium in the blood and the tissues of both cattle and pigs and in cows' milk than the inorganic selenium supplement. Long-term supplementation of dairy cows and pigs with selenium yeast did not result in toxic accumulations of selenium in their tissues. No differences were observed between the activity of the selenium-dependent enzyme glutathione peroxidase (GSH-Px) in the erythrocytes of cattle or in the whole blood of pigs after they had been supplemented with either inorganic or organic sources of selenium. There was no difference between selenite and selenate as feed supplements for dairy cattle, and both compounds induced only small increases in the concentration of selenium in milk. Suckler beef calves whose dams were supplemented with selenium yeast had a higher selenium status than calves whose dams were supplemented with selenite. The activity of GSH-Px in platelets could be used as an indicator of the short-term selenium status of cattle, but because of problems with the assay it was concluded that at present the concentration of selenium in plasma is a more reliable indicator. Dairy heifers fed an unsupplemented diet had higher plasma levels of the thyroid hormone thyroxine (T_4) than heifers supplemented with either inorganic or organic forms of selenium, but the levels of triiodothyronine (T_3) in the two groups were not significantly different, No evidence was obtained for the proposal that selenite might have a pro-oxidative effect *in vivo*.

Selenium yeast can be useful as a feed supplement for suckler cows, in ecological dairying and, when fed to dairy cows and fattening pigs, as a method for increasing the intake of selenium by the human population.

Key words: selenium, selenium yeast, selenite, selenate, dairy cattle, beef cattle, fattening pigs.

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En okänd poet ifrån Skara
sin verskonst försökte försvara:
Han sa: på nå't vis
blir det aldrig precis -
men ibland kommer rimmen rätt nära

Henrik Westling

To my surprise

Abstract

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Key words: selenium, selenium yeast, selenite, selenate, dairy cattle, beef cattle, fattening pigs.

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Appendix

Papers I-V

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals I-V.

I Ortman, K. and Pehrson, B., 1997: Selenite and selenium yeast as feed supplements for dairy cows. *J Vet Med. A* 44, 373-380.

II Ortman, K. and Pehrson, B., 1998: Selenite and selenium yeast as feed supplements to growing fattening pigs. *J Vet Med. A* 45, 551-557.

III Ortman, K., Andersson, R. and Holst, H., 1999: The influence of supplements of selenite, selenate and selenium yeast on the selenium status of dairy heifers. *Acta vet. scand.* 40, 23-34.

IV Ortman, K. and Pehrson, B., 1999: Effect of selenate as feed supplement to dairy cows in comparison to selenite and selenium yeast. (Accepted; *J. Anim. Sci.*)

V Pehrson, B., Ortman, K., Madjid, N. and Trafikowska, U., 1999: The influence of dietary selenium as selenium yeast or sodium selenite on the concentration of selenium in the milk of suckler cows and on the selenium status of their calves. (Accepted; *J. Anim. Sci.*).

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Introduction

History and technical use

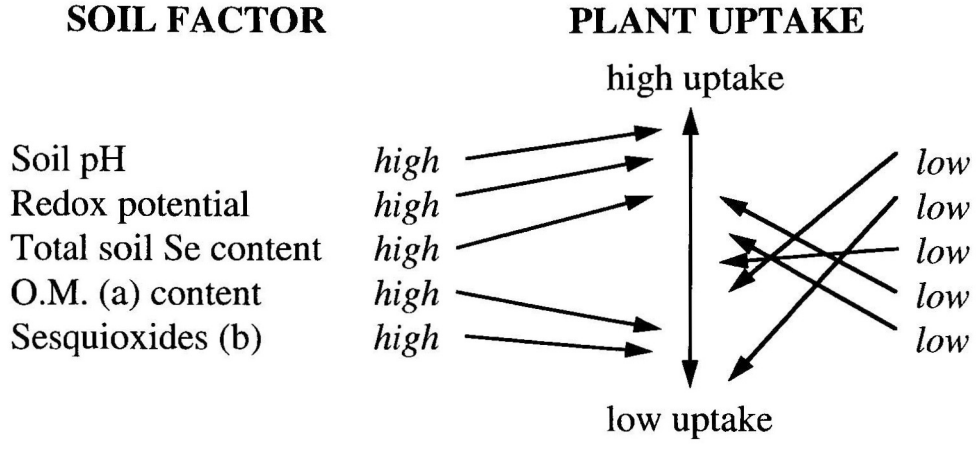
Selenium is an element which was discovered by the Swedish chemist Jöns Jacob Berzelius in 1817. He named the new element selenium after the Greek "Selene" for moon, because he identified selenium in association with tellurium, an element which had been discovered some years earlier and had been named after the Latin "Tellus" for earth (Sunde, 1997).

Selenium is a metalloid which is placed in the periodic system between sulphur and tellurium. It has many physical and chemical properties similar to those of sulphur. In nature, it may be found as elementary selenium and as inorganic or organic compounds. As a semi-conductor, selenium has wide technical uses, for example in photocopiers, photoelectric cells and solar cells. The element is also used as a pigment in plastic and glass products and as an anti-dandruff component in shampoos (Vokal-Borek & Hellsten, 1982).

Global distribution of selenium

Historically, selenium has been considered as much as a toxic agent as an essential micronutrient. One reason for this is that there is a relatively narrow range between dietary levels of selenium which are deficient, essential or toxic. Moreover, selenium is very unevenly distributed in the earth's crust and the availability of the element for plants varies greatly with different soil factors (Fig. 1). This is the reason for the extremely different concentrations of selenium found in feedstuffs and food in different parts of the world e.g. (Mondragón & Jaffé, 1976; Olson & Palmer, 1984; Kumpulainen, 1993; Sanz Alaejos & Diaz Romero, 1995). As a result, disorders due both to selenium deficiency and selenium toxicity have been reported from different regions of the world, although selenium deficiency is a greater global problem than selenium toxicity.

The levels of selenium in feeds and foods are low in the Scandinavian and Baltic countries (Oksanen & Sandholm, 1970; Carlström et al., 1979; Frøslie et al., 1980; Gissel-Nielsen et al., 1984; Pehrson et al., 1997) and in other parts of Europe (Zust et al., 1996), in New Zealand (Andrews et al., 1968), in certain parts of North America (Kubota et al., 1967) and in the central parts of China (Liu et al., 1987), whereas in other parts of the world, for example in some South American countries such as Venezuela (Mondragón & Jaffé, 1976) and certain regions of China (Liu et al., 1987) and North America (Oldfield, 1995) the levels of selenium in feeds and food are high.



(a) Organic matter (b) Iron and aluminium oxides

Figure 1. Simplified diagram over the influence of some different soil factors on the availability of selenium (Se) for plant uptake. In soils, Se uptake by plants is a complex process influenced by these and other factors (from Johnsson et al. 1997).

Selenium toxicity

Selenium was first recognised as a toxic element, causing 'alkali disease' and 'blind staggers' in animals grazing plants rich in selenium (Franke et al., 1934; Draize & Beath, 1935; Rosenfeld & Beath, 1946). Clinical signs similar to those observed in alkali disease have also been reported in human beings (Yang et al., 1983).

Alkali disease

Alkali disease is due to the chronic intake of an excess of selenium (5-40 mg selenium/kg feed). The disease is characterised by loss of condition, changes in the hair-coat - including loss of mane and tail switch - changes in the hoofs at the coronary band, and lameness (Franke et al. 1934; Moxon, 1937). The disease has been recorded in ruminants, pigs and horses in North America, and it has been reproduced experimentally in various species of animal (Miller & Schoening, 1938; O'Toole & Raisbeck, 1995; Panter et al., 1996). It still appears sporadically in horses in the western parts of North America (Raisbeck et al., 1993). The disease has also been described in pigs and horses exposed accidentally to selenium over-supplementation through the feed (Detlef et al., 1995; Hélie et al., 1998).

Blind staggers

Blind staggers is a disease of cattle and sheep in which neurological signs are predominant. It is characterised by blindness, disorientation, dysphagia and paralysis, and it was linked to selenium rich areas by a research group in Wyoming (Draize & Beath, 1935; Rosenfeld & Beath, 1946). Although they are widely cited, it seems that these authors' reports were incomplete and, moreover, the disease has not been reproduced experimentally. These and other factors are the reasons why O'Toole et al. (1996) claimed that blind staggers is not in fact due to selenium toxicity.

Selenium deficiency diseases

In 1957, Schwarz and Foltz demonstrated that selenium is an essential micronutrient, when they found that selenium could prevent liver necrosis in rats depleted of vitamin E and cystine. This was the start of a rapid recognition that selenium-related deficiency diseases could occur in several species of farm animals, and later they were also recognised in human beings.

Ruminants

Nutritional muscular degeneration (NMD)

NMD (also called white muscle disease) was described before it had been recognised that selenium was an essential micronutrient, and at that time it was not linked to selenium deficiency (Vawter & Records, 1947). In cattle and sheep the disease occurs primarily in young animals. The diseased animals have a stiff gait and are unwilling to move. The affected muscles are often swollen and firmer than normal, and muscular tremors may occur. The animals can also become dyspnoeic, with abdominal respiration, if the diaphragm and intercostal muscles are involved. Cases of sudden death also occur and in these cases the heart muscle is involved (Pehrson, 1993; Radostits et al., 1994). In sheep, lambs can be born weak and may die within a few hours, or they may even be born dead, but new-born calves are rarely affected (Andrews et al., 1968). Supplementary selenium can prevent the disease (Muth et al., 1958; Muth et al. 1959; Hartley & Grant, 1961), but other factors, such as a low vitamin E status, sudden muscular activity, and high dietary concentrations of polyunsaturated fatty acids may also be involved in the aetiology of NMD (McMurray et al., 1983; Pehrson et al., 1986; Kennedy et al., 1987).

Other conditions

Later research has identified other clinical conditions which are related to a selenium-deficient diet, for example ill-thrift, reduced growth rate, retained placenta, impaired fertility and mastitis (reviewed by Pehrson 1993). Among these conditions retained placenta in dairy cows is one of the most thoroughly investigated. Several authors have demonstrated a decreased incidence of retained placenta when cows which have been fed a selenium-deficient basic diet have been supplemented with selenium and/or vitamin E (e.g. Julien et al.,

1976; Segerson et al., 1981). It should, however, be noted that selenium could not be considered as a therapeutic agent against retained placenta, and Ishak et al (1983) and Hidiroglou et al (1987) were unable to prove that selenium supplementation had any beneficial effect on the incidence of retained placenta in cows which originally had an adequate selenium status.

Pigs

In pigs, the occurrence of selenium deficiency diseases is closely related to the supply of vitamin E, as was elegantly demonstrated by Hakkarainen and his group in a series of trials (Bengtsson et al., 1978a; Bengtsson et al., 1978b; Hakkarainen et al., 1978). The pathology includes hepatic necrosis, skeletal and cardiac muscle degeneration and microangiopathy (Trapp et al., 1970; Jönsson, 1993). However, in contrast with hepatic necrosis, microangiopathy and muscular dystrophy are considered to be relatively more related to a vitamin E deficiency than to a selenium deficiency (Jensen, 1989). Clinically the vitamin E-selenium deficiency syndrome (VESD) is often characterised by sudden deaths in rapidly growing young animals. The appearance and site of the heart lesions varies; owing to the widespread epicardial and myocardial haemorrhages that sometimes appear the syndrome has been called "mulberry heart disease" (Grant, 1961; Bengtsson et al., 1978b).

Poultry

"Exudative diathesis" is a disease of chickens which are deficient in selenium and/or vitamin E. It is characterised by subcutaneous, and interstitial oedema, which develops into haemorrhages which produce a blue-green discolouration of the skin. Muscular degeneration is also seen (Patterson et al., 1957; Hassan et al., 1990; Sunde, 1997). Poor growth, poor feathering and pancreatic degeneration have been recorded in chickens fed a selenium-deficient diet. The disorder has sometimes been called "pancreatic fibrosis" or "pancreatic atrophy" (Sunde, 1997).

Horses

In horses, NMD has been described in foals (e.g. Schougaard et al., 1972) and, as in ruminants, several different triggering factors are believed to be involved in its pathogenesis (Ronéus, 1985).

Human beings

The fact that selenium is essential for human beings was demonstrated as recently as 1980, when Chen et al. described the selenium-responsiveness of Keshan disease, which is an endemic, often fatal, cardiomyopathy which occurs in some regions of China where the intake of selenium is extremely low. Another endemic condition, Keshin-Beck disease, which is a chronic, degenerative osteoarthrosis occurring in certain rural parts of Asia has also been found in association with a low selenium status (Whanger, 1989). Furthermore, certain forms of cancer (Clark et al., 1996) and cardiovascular

diseases (Aro, 1993; Nève, 1996) have in some studies been associated with a low intake of selenium by people. Moreover, selenium supplementation has been advocated, on more or less good evidence, for many other health defects, including arthritis, cataracts, cystic fibrosis, alcoholism, phenylketonuria, Kwashiorkor, bronchopulmonary dysplasia, haemolytic anaemia, night blindness, sudden infant death syndrome, multiple sclerosis, Down's syndrome, and malaria (reviewed by Foster 1997).

Selenium and immunity

The immune system can be adversely affected by either a deficient or an excessive intake of different nutrients. This is true both for macronutrients, such as protein and fat, and for micronutrients, principally vitamins and minerals (Burkholder & Swecker, 1990). There is strong evidence that selenium has an immunostimulatory effect (reviewed by Larsen, 1993 and Finch & Turner, 1996). Selenium stimulates both the humoral and the cell-mediated immunity in human beings and other mammals, and in birds, and these positive effects have been demonstrated both in selenium-deficient subjects and in subjects fed a diet adequate in selenium. Selenium has often been studied together with vitamin E, but the immune response to selenium seems to be independent of vitamin E status.

Mastitis in cattle

In cattle, most of the studies on the relationships between selenium status and the function of the immune system have concerned mastitis. For example, studies have shown that polymorphonuclear phagocytes from selenium-deficient cows are less able to kill engulfed bacteria of different species (Gyang et al., 1984; Grasso et al., 1990). Erskine and co-workers (1989; 1990) demonstrated that cows supplemented with selenium were more resistant than selenium-deficient cows to experimentally induced *Escherichia coli* mastitis but not to experimentally induced *Staphylococcus aureus* mastitis. More recently, Ali-Vehmas et al (1997) showed that supplementing severely selenium-deficient dairy cows with selenium induced a self-cure of subclinical mastitis. Moreover, Erskine et al. (1987) reported that cows in herds with a high mean somatic cell count (SCC) had significantly lower mean blood selenium concentrations than cows in herds with a low mean SCC. However, Ropstad et al. (1987) found the opposite situation in Norwegian dairy herds. Weiss et al. (1990) concluded that there is little evidence that a dietary intake of selenium exceeding 0.30 mg selenium/kg dietary DM would further enhance the immune defence of cattle against mastitis.

Resistance against viral diseases

Some epidemiological features of Keshan disease, for example its seasonal prevalence, indicate that other factors, such as viruses, may be involved in the aetiology of the disease. In fact, viruses have been isolated from people who have died from Keshan disease (Beck et al. 1994a). A series of experiments

demonstrated that selenium-depleted mice developed heart lesions after they were infected with a Coxsackie virus, whereas mice with an adequate selenium status did not develop the lesions. After passage through the selenium-deficient subjects, the virus was re-isolated and inoculated into mice fed a selenium-sufficient diet; these animals then contracted heart injuries (Beck et al., 1994a; Beck et al., 1994b). In a later experiment it was shown that during its passage through the selenium-deficient mice the virus had mutated from a benign strain of Coxsackie virus to a virulent form (Beck et al., 1995). These series of experiments have recently been reviewed by Levander and Beck (1997). In addition, Beck et al. (1998) demonstrated that the protection afforded by selenium against heart damage was mediated through the selenoprotein glutathione peroxidase (GSH-Px).

It has been proposed that a host's low selenium status might have similar effects on the virulence of other RNA viruses, at least in those from the Picornaviridea family, and possibly also in other species of animals. This effect might have a significant effect on animal and human health, because a majority of viruses are RNA viruses, e.g. enzootic bovine leucosis virus, influenza viruses, swine vesicular disease virus and HIV (Levander, 1997).

Biochemical role of selenium

GSH-Px

Biologically active selenoproteins contain the amino acid selenocysteine in their primary structure, and they have been shown to be regulated physiologically (Burk & Hill, 1993). The first recognised function of selenium was as an integral part of the cytosolic enzyme glutathione peroxidase (cGSH-Px), which was isolated from erythrocytes and was identified as a selenoprotein by Flohé et al. (1973) and Rotruck et al. (1973). It acts as an antioxidant by catalysing the conversion of hydrogen peroxide and organic peroxides to water and alcohols. In addition, later research has demonstrated that cGSH-Px may also have a selenium storage function in the body (Arthur & Beckett, 1994).

The antioxidant defence systems used by mammals consist of several compounds, including for example selenium, vitamin E, vitamin C and betacarotene, which act in varying ways to protect the animals' cells against the free radicals produced endogenously during normal aerobic metabolism (Duthie et al., 1989). The effectiveness of these defence systems is highly dependent not only on an adequate dietary intake of these antioxidants, but also on several other factors such as age, growth rate, level of physical activity and the dietary intake of oxidising agents such as polyunsaturated fatty acids (reviewed by Hakkarainen, 1993). Different antioxidants may act together, so that a deficiency of one can to a certain extent be compensated by an adequate supply of another. Antioxidants may also function synergistically. Vitamin E and selenium have often been studied together and many

experiments have shown that these nutrients can complement one another (Whanger et al., 1977; Hakkarainen et al., 1978; Maas et al., 1984; Pehrson et al., 1990). The biochemical background to this complementary effect is that vitamin E prevents the oxidation of polyunsaturated fatty acids, whereas selenium, as GSH-Px, metabolises the peroxides which have already been formed (MacPherson, 1994). These interacting factors are the main reason why it is so difficult to estimate the nutritional requirements of animals for different essential compounds.

"New" seleno-proteins

For a long time cGSH-Px was considered to be the only functional selenoprotein in mammals. However, during recent years many other selenoproteins have been characterised, either by the purification and sequencing of the protein, and/or by cloning and sequencing the cDNA. Several reviews have been written on this topic (Zachara, 1992; Burk & Hill, 1993; Arthur & Beckett, 1994).

Among these "new" selenoproteins are plasma- or extracellular GSH-Px (eGSH-Px), phospholipid-hydroperoxide GSH-Px (phGSH-Px), selenoprotein P, type 1 iodothyronine deiodinase (ID-1), type 2 iodothyronine deiodinase (ID-2), type 3 iodothyronine deiodinase (ID-3) and selenoprotein W. Moreover, reports of additional selenoproteins are still published (e.g. Behne et al., 1996; Behne et al., 1997; Gladyshev et al., 1998). To a large extent the functions of most of these proteins are still unknown. However, some of the effects of selenium deficiency may be linked to a decreased activity of these other selenoproteins, rather than to a decreased antioxidative capacity due to a reduced activity of cGSH-Px.

eGSH-Px

In 1986 Takahashi et al. reported that eGSH-Px was immunologically distinct from cGSH-Px. This extracellular form has been found in kidney, lung, heart, thyroid and milk, and in human aqueous humour, and it has been proposed that it may be synthesised in the kidney (Huang, 1996). The function of eGSH-Px is not completely clear. The concentration of glutathione, which is its reducing substrate, is very low in extracellular fluids, which suggests that the enzyme may have different functions from cGSH-Px (Sunde, 1997).

phGSH-Px

Phospholipid-GSH-Px is the form that has the least similarity to the other enzymes of the GSH-Px family (Sunde, 1997). It metabolises phospholipid hydroperoxides that are not metabolised by cGSH-Px, and is much more resistant to the effects of selenium deficiency (Weitzel et al., 1990). It is associated with cell membranes, and the protection of these membranes against peroxidation by this enzyme and vitamin E is believed to be the

biochemical basis of the nutritional interaction between selenium and vitamin E (MacPherson, 1994).

Selenoprotein P

Burk and Gregory (1982), identified this selenoprotein in rat plasma, and later, Read et al. (1990) demonstrated that more than 60% of the selenium in rat plasma was in the form of selenoprotein P. This protein has also been isolated from calf serum (Awadeh et al., 1998a) and from human plasma, where it is estimated to account for about 40% of total plasma selenium (Åkesson et al., 1994; Hill et al., 1996). Experiments have indicated that selenoprotein P is synthesised in the liver and kidney and secreted into the plasma. Burk et al. (1991) reported that when selenium-deficient rats were injected with selenium the synthesis of selenoprotein P took precedence over the synthesis of liver GSH-Px and plasma GSH-Px. Selenoprotein P is different from the other known selenoproteins; thus it contains up to ten selenocysteine residues per polypeptide chain, compared with one in the other selenoproteins which have been characterised. It has been suggested that selenoprotein P is a transport protein (Motsenbocker & Tappel, 1982), but it has also been postulated that it may have anti-oxidative functions (Burk & Hill, 1994). Recently Sasakura and Suzuki (1998) reported on an *in vitro* interaction between transition metals (Ag, Cd and Hg), selenide/sulphide and selenoprotein P.

Iodothyronine deiodinases

One of the best characterised of these "new" selenoproteins is ID-1, which was shown to be a selenoprotein independently by Arthur et al. (1990) and Behne et al. (1990). This enzyme catalyses the reaction which converts thyroxine (T_4) to the more metabolically active compound triiodothyronine (T_3), but it also contributes to the degradation of T_3 to diiodothyronine (T_2). Nearly all T_4 is synthesised in the thyroid gland, but most of the circulating T_3 is produced by the deiodination of T_4 by ID-1 in liver, kidney and skeletal muscle. ID-2 is found in brain, pituitary, brown adipose tissue, placenta and skin, whereas the activity of ID-3 is highest in the placenta, skin and central nervous system of adults, and in numerous tissues during fetal development (St Germain & Galton, 1997; Sunde, 1997).

The physiological roles of the iodothyronine deiodinases are not completely understood, but it has been proposed that the three enzymes work in concert, together with the other components of the thyroidal regulation system, to regulate tissue-specific thyroidal hormone levels (St Germain & Galton, 1997). Experiments have shown that in selenium-depleted rats and ruminants there are decreased T_3 levels and increased T_4 levels (Beckett et al., 1987; Arthur et al., 1988; Wichtel et al., 1996). However, the brain and several endocrine organs appear to conserve selenium, which seems to be a mechanism to reduce the effect of selenium deficiency on for example the

metabolism of thyroid hormones (Behne et al., 1988; Buckman et al., 1993). Moreover, the selenoproteins seem to have different priorities in the organism, so that for example, when the supply of selenium is limited the deiodinases are better conserved than GSH-Px (Bermano et al., 1995).

Selenoprotein W

Selenoprotein W was purified from rat muscle in 1993 by Vendeland et al. (1993). It is believed to be similar to a low molecular weight selenoprotein that has been found in lamb muscle and which decreases in concentration during the onset of NMD (Pedersen et al., 1972). It has also been detected in the testis, brain and spleen of rats (Yeh et al., 1997), and in the muscle of monkeys (Gu et al., 1999). The function of selenoprotein W is still unknown.

Selenium availability and requirements

Different methods have been used to assess the availability of dietary selenium and the requirements of people and animals for the element (reviewed by Hakkarainen 1993).

Prevention of disease

The ideal method for determining the requirement for selenium is to determine the amount of the element which can prevent nutritional deficiency diseases, such as NMD or Keshan disease. This might appear to be a reliable method, but it has certain disadvantages. One previously mentioned difficulty is that there are other factors that interact in the aetiology of selenium deficiency diseases, including the intake of vitamin E, the concentration of polyunsaturated fatty acids in the diet and the level of physical activity. Moreover, the less obvious effects of selenium deficiency, such as reduced growth rate, reduced fertility and a suboptimal immune system, are not so easy to monitor.

Functional selenoproteins

Another method of estimating the requirement for selenium is to use the activity of specific selenoprotein enzymes as an indicator. It would seem logical to suppose that the supply of selenium could be considered to be sufficient when the activities of these functional selenoproteins have reached a plateau. This method has been widely used, particularly by using the activity of GSH-Px as the indicator. The activity of this enzyme has been monitored in erythrocytes, plasma, platelets and other body tissues of different animal species and in human beings (Wegger et al., 1980; Levander et al., 1983a; Huang et al., 1995; Nève, 1995). Selenoprotein P has also been used as a marker of selenium requirements or status (Yang et al., 1989; Hill et al., 1996; Persson-Moschos et al., 1998), and so has ID-1 (Behne et al., 1992).

One problem with the use of functional proteins as indicators of selenium status is that, apart from the activity of GSH-Px, no biochemical assays are

available for their routine analysis. Another problem is that the different selenoproteins have different priorities in the body. Behne et al. (1988) showed that there was a hierarchy of requirements between the different selenoproteins and different organs when the supply of selenium was limited; they concluded that the priority order for selenoproteins, at least in the rat, was: 1/ selenoproteins other than GSH-Px in brain, endocrine and reproductive organs, 2/ GSH-Px in these organs, 3/ other selenoproteins in liver, heart and skeletal muscle and 4/ GSH-Px in these latter organs. This order indicates that GSH-Px is not the most important of the selenoproteins. Moreover, one of the specific selenoenzymes, cGSH-Px, is considered to have not only specific metabolic functions in the body but also selenium storage functions (Arthur & Beckett, 1994). The levels of this protein would not therefore be expected to plateau at a certain dietary level, but would continue to increase when dietary selenium levels were more than adequate.

Selenium retention

A third method for the evaluation of selenium requirements and availability is to determine the retention of selenium in blood or serum and other tissues. The method is often used to assess the selenium status of animals and human beings. The disadvantage of the method is that it does not directly reflect the functional part of the selenium in the body. The retention of selenium by tissues is dependent on both the dosage level and the chemical form of the selenium. Thus, organic selenium in the form of selenomethionine is not only used for the synthesis of specific selenoproteins, but is also incorporated non-specifically into body proteins, whereas inorganic selenite and selenate are used mainly for the synthesis of specific selenoproteins (Whanger, 1986; Whanger & Butler, 1988; Behne et al., 1991). Selenium depletion and repletion also has different effects on different tissues. Thus, in pigs, Chavez (1979) demonstrated that the brain appeared to be the organ that was least affected by selenium depletion or repletion, and other researchers have made similar observations in other animal species (Behne et al., 1988). Moreover, dietary factors, for example whether the diet for ruminants is based on concentrates or forages, may influence the degree of selenium retention (Koenig et al., 1997).

Behne et al. (1992) concluded that at a high dietary intake, especially when the selenium is in the form of selenomethionine, the tissue selenium content cannot be considered as a reliable indication of selenoenzyme activities. Thomson (1998) predicted that as the knowledge of the different selenoproteins increases, and as the methods for analysing them become more available, the measurement of the concentration of selenium in tissues will become less important for the determination of selenium status, at least in human beings.

Proposed requirement levels

As mentioned earlier, the requirements of animals for selenium depend on several factors, and in particular upon the dietary supply of vitamin E, and upon the differences in availability between inorganic and organic selenium compounds. It is therefore doubtful whether a single figure for the selenium requirement of any species would be acceptable. However, in a review publication (Anonymous, 1983) it was concluded that it would be reasonable to assume that the selenium requirement for most animals lies in the range from 0.05 to 0.30 mg/kg DM. Pehrson and Johnsson (1985b) concluded that the selenium requirement of young cattle was from 0.10 to 0.15 mg/kg DM. Moksnes and Norheim (1983) concluded that the optimal selenium concentration in feed for sheep was considerably higher than 0.10 mg/kg, and Zachara et al. (1993) proposed that 0.25 mg selenium/kg feed should be sufficient for lambs. In swine Sankari (1985) reported that a dietary intake of 0.10-0.20 mg/kg DM was required. The National Research Council in the USA recommends a minimum nutrient requirement figure for selenium in cattle, sheep and pigs of 0.10, 0.10-0.20 and 0.10-0.30 mg/kg DM, respectively (Sunde, 1997).

In 1989 the Nordic nutrient recommendation for human beings was 30-60 μg selenium/day (Åkesson, 1995). In 1990 a maximum standard figure of 200 μg selenium daily was added to these recommendations (Lennernäs & Becker, 1994).

Selenium supplementation

Methods of supplementation

Different methods have been used for improving the selenium status of farm animals and human beings. In Sweden, the supplementation of commercial feeds for livestock with selenium as inorganic selenite or selenate has been permitted since 1980. The other Nordic countries began to supplement animal feeds with selenium even earlier; in Finland in 1969, in Denmark in 1975, and in Norway in 1979 (Johnsson et al., 1997). In Finland, selenium, as selenate, has also been included in multinutrient fertilisers since 1984. The aim is to improve the selenium status of the human population by increasing the concentration of selenium in foods derived from both animals and vegetables (Varo, 1993). This method of selenium supplementation has also been applied in New Zealand.

Other methods that have been used to increase the selenium status of livestock include injections of long-lasting selenium compounds, the oral administration of intraruminal pellets containing selenium and iron, and drenching with selenium compounds (Wichtel, 1998). In human nutrition certain foods, for example infant formulas, have been supplemented with selenium to prevent deficiencies in groups of the population that might be at particular risk of selenium deficiency (Johnsson et al., 1997).

Organic vs. inorganic selenium compounds

Most of the selenium in grain, soybeans, maize and rice is in the form of selenomethionine (Beilstein & Whanger, 1986; Beilstein et al., 1991). Mammals cannot synthesise selenomethionine but are apparently unable to distinguish between methionine and its selenium analogue, which is therefore incorporated non-specifically into tissue proteins (Burk & Hill, 1993). However, selenomethionine is also used for the synthesis of specific selenoproteins, in which selenium is present as selenocysteine, but the selenium must be reduced to selenide before it can be incorporated into these specific selenoproteins (Sunde, 1997). The proportion of dietary selenomethionine which is incorporated non-specifically into body proteins or into specific selenoproteins may depend on several factors, for example on the total methionine content of the diet (Waschulewski & Sunde, 1988).

Inorganic selenium, either as selenite or selenate, is also used for the synthesis of specific selenoproteins. The absorption of these inorganic selenium compounds has been demonstrated to be as good as or even better than the absorption of selenomethionine (Koenig et al., 1997). However, any excess selenium absorbed as selenite or selenate is excreted, mainly by the urinary route, rather than being stored in the body (Aspila, 1991).

In all specific metabolically active selenoproteins the selenium is present as endogenously synthesised selenocysteine residues (Åkesson, 1995). Although selenocysteine is an organic selenium compound, the metabolism of this selenoamino acid is more similar to the metabolism of the inorganic selenium compound selenite than to the metabolism of selenomethionine (Hakkarainen, 1993).

Since commercial feedstuffs for farm animals in Sweden began to be supplemented with selenium the compound used has been exclusively sodium selenite (Na_2SeO_3). Sodium selenite is also the most common form of selenium used for the supplementation of animal diets throughout the world. Another inorganic selenium compound used to supplement animals and human beings is sodium selenate (Na_2SeO_4). The feeding of supplements of selenite has been questioned because of selenite's possible pro-oxidative properties *in vivo* (Hafeman et al., 1974; Dougherty & Hoekstra, 1982), and it has been suggested that these pro-oxidative properties may result in animals requiring additional anti-oxidative protection.

Organic selenium has also been used for the supplementation of animals and human beings, a common form being selenium yeast products. Selenium yeast is produced when ordinary baker's yeast is grown in the absence of inorganic sulphur after the addition of inorganic selenium (Suomi & Alaviuhkola, 1992). The yeast organisms incorporate selenium into analogues

of sulphur-containing amino acids, thus producing a yeast in which at least 50% of the selenium is in the form of selenomethionine and only a few percent is present as inorganic selenium (Korhola et al., 1986; Kelly & Power, 1995). The advantages of selenium yeast compared with sodium selenite are that it should not have any pro-oxidative properties, and that it is more similar to the natural forms of selenium in feed and food. However, the use of organic selenium compounds for the supplementation of animal feedstuffs has also been questioned, because of the possible risk that selenium might accumulate to toxic levels in tissues and milk. Moreover, the selenium yeast is much more expensive than the inorganic selenium compounds.

Most of the recommendations for the daily intake of selenium have been based on studies in which sodium selenite has been used as the supplement. The recommended dietary intake levels have therefore been assessed without the chemical form of selenium being taken into consideration. In the USA and in the EU the levels of supplementation of feeds for farm animals are defined only in terms of selenite or selenate (Statens jordbruksverk 1993; Anonymous, 1995); as a result the recommendations might be interpreted to exclude organic selenium as a supplement. In contrast, in Japan, sodium selenite is forbidden as a feed additive (Anonymous, 1998).

Aims

The aims of this series of studies were

- To evaluate the differences in blood selenium concentrations, and in the activity of GSH-Px in erythrocytes/whole blood of dairy cattle and growing fattening pigs given long-term supplements of either organic selenium as selenium yeast, or inorganic selenium as sodium selenite,

- To evaluate the retention of selenium in the edible organs of dairy cows and fattening pigs given long-term supplements of selenium as selenium yeast.

- To compare the concentration of selenium in the milk of cows supplemented with either selenium yeast, sodium selenite, or sodium selenate.

- To evaluate the difference in the bioavailability of the two inorganic compounds sodium selenite and sodium selenate when given as feed supplements to young heifers and dairy cows, in terms of the activity of GSH-Px in erythrocytes and the concentrations of selenium in whole blood and plasma.

- To determine whether the metabolism of thyroid hormones is influenced in young cattle fed selenium-deficient rations.

- To study the possibility of using platelet GSH-Px activity as a short-term indicator of selenium status in cattle.

- To evaluate the effect on the selenium status of sucking calves of supplementing their dams with either selenium yeast or sodium selenite.

- To study the possible *in vivo* oxidative effects of dietary supplements of selenite in cattle and pigs.

Materials and methods

Farms and animals

The experiments were carried out with 25 (paper I) and 42 (paper IV) Swedish Red and White dairy cows at the university experimental farm Brogården, with 24 Swedish Red and White dairy heifers at the outlying farm Åkedal (paper III), with 24 cross-bred (Hampshire/ Swedish Landrace/ Yorkshire) fattening pigs at the university experimental farm Bjertorp (paper II), and with 20 Hereford suckler-cows and their calves at the commercial farm Källstorp (paper V). All the farms are situated close to Skara in south-west Sweden.

Selenium supplementation and samplings

The differences between the effects of supplements of inorganic selenium in the form of sodium selenite (papers I-V) or sodium selenate (papers III and IV) and organic selenium as selenium yeast (papers I-V) was studied, as well as the differences between the effects of two doses of organic selenium (papers I and II). The selenium status of the experimental animals was evaluated by measurements of whole blood selenium (papers I-V), plasma selenium (papers III, IV and V), milk selenium (papers I, IV and V), tissue selenium (papers I and II), GSH-Px activity in whole blood (pigs; paper II), GSH-Px activity in erythrocytes (cattle; papers I, III, IV and V), GSH-Px activity in platelets (paper III) and the concentrations of the hormones T_4 and T_3 in plasma (paper III). In addition, samples of heart and liver were collected from pigs (paper II) to evaluate whether histopathological accumulations of lipofuscin pigment could be detected.

The basic feedstuffs contained 0.10-0.13 mg selenium/kg DM for the dairy cows (papers I and IV), 0.10 mg/kg for the fattening pigs (paper II), 0.03 mg/kg DM for the heifers (paper III) and 0.02 mg/kg DM for the suckler cows (paper V). The diet of the pigs described in paper II was supplemented at levels up to a total selenium concentration of 0.20-0.40 mg/kg DM, whereas the cows and heifers in papers I, III and IV were supplemented individually with doses of between 0.75 and 3.0 mg selenium daily, which provided a total selenium concentration in their feed from 0.16 to 0.32 mg/kg feed DM. The suckler cows described in paper V were estimated to consume about 3 mg selenium daily. In papers III and IV one group of animals remained as an unsupplemented control group. The details of the animals' feed and management are described in each paper.

Laboratory methods

The GSH-Px activity in whole blood, erythrocytes and platelets was measured by the method described by Paglia & Valentine (1967).

The selenium in blood, plasma, milk, tissues and feed was determined by hydride-generation atomic absorption spectrophotometry (HG-AAS), a technique which is described in Paper I. However, the concentration of selenium in the feed samples in paper V was determined by a flow-injection HG-AAS technique by the method described by Galgan & Frank (1993)

Statistical methods

In papers I, II, IV and V the data from groups of animals were compared by ANOVA, with subsequent comparisons by Student's t-test, correcting the values in accordance with Bonferroni (Altman, 1993). In papers I and IV, the mean plateau value of the variables which reached a plateau was calculated for each animal and used for comparison between the groups. For the variables that did not reach a plateau during the observation period the difference between the final value and the start value was calculated for each animal and this figure was used in the statistical evaluation. In papers II and V the mean values for each group at each sampling were compared between the different groups. Differences between groups were accepted as significant when $P < 0.05$.

The experiment described in paper III was designed as a randomised block trial. For the activity of GSH-Px in erythrocytes, and for the concentration of selenium in whole blood a regression line was fitted to the data from all the animals, and the value of the mean slope for each group was calculated. For the concentration of selenium in plasma the mean value for each animal during the period when the concentration had reached a plateau was calculated to produce a mean value for each group. Comparisons were then made by means of a two-way ANOVA, followed by multiple comparisons with a 95% simultaneous degree of confidence according to the method of Tukey. The differences between groups for the activity of GSH-Px and the concentration of T_4 in plasma was calculated by using the General Linear Models procedure of SAS (1989).

Results

When dairy and beef cattle, and growing fattening pigs were supplemented with organic selenium as a yeast product their blood selenium concentrations were higher than when they were supplemented with the inorganic compounds selenite (papers I-V) and selenate (papers III and IV). In the studies in which plasma selenium concentration was determined (papers III-V) a similar difference was observed.

The activity of GSH-Px in erythrocytes (cattle) and whole blood (pigs) did not differ significantly between the groups supplemented with either organic or inorganic selenium compounds, with the exception of the suckled calves described in paper V. The calves whose dams were supplemented with selenium yeast had significantly higher activities of GSH-Px in their erythrocytes than the calves whose dams were supplemented with selenite.

The concentration of selenium in milk was significantly higher in the cows that were supplemented with organic selenium than in the cows supplemented with selenite (papers I, IV and V) or selenate (paper IV), but there was no difference between the concentration of selenium in the milk of the cows supplemented with either selenite or selenate (paper IV). Supplements of either selenite or selenate caused only a marginal increase in the concentration of selenium in the milk in comparison with the unsupplemented control group (paper IV).

The selenium status of suckled calves, evaluated in terms of their whole blood and plasma selenium concentrations and the activity of GSH-Px in their erythrocytes, was highly correlated with the concentration of selenium in their dam's milk and blood ($r = 0.59$-0.78; paper V).

It was possible to measure the activity of GSH-Px in bovine platelets and the activity reached a plateau after three weeks of supplementation with selenium (paper III). The activity was higher in the animals supplemented with selenium yeast than in those supplemented with selenite or selenate. There was no significant difference between the selenite-supplemented heifers and the unsupplemented control group, but the selenate-supplemented animals had a higher activity of GSH-Px in their platelets than the controls. However, the method used to isolate the platelets from whole blood and the method used to measure the enzyme activity need to be modified before they can be applied usefully in practice.

In pigs, the concentration of selenium in liver tissue was significantly higher in the animals supplemented with organic selenium than in those supplemented with selenite (paper II). There was an obvious trend for higher concentrations of selenium to be recorded in the samples of liver, heart muscle and skeletal

muscle taken from the cows supplemented with selenium yeast than from the cows supplemented with selenite (paper I). The tissue selenium concentrations were far below toxic levels in both cattle and swine.

The metabolism of thyroid hormones was affected in the selenium-depleted heifers (paper III). The concentration of T_4 was significantly higher in the unsupplemented control group than in the selenium-supplemented groups, regardless of the selenium compound used as a supplement, and there were no differences between selenite, selenate and selenium yeast in this respect. The concentration of T_3 did not differ significantly between the supplemented and unsupplemented animals.

There was no evidence of an *in vivo* pro-oxidative effect of selenite, either in cattle or in pigs.

Discussion

Evaluation of selenium status in cattle

Blood selenium as an indicator

Although selenium has been acknowledged as an essential trace element for almost half a century there are no definitive statements in the literature about what concentrations in the whole blood of cattle should be considered as adequate, marginal or deficient. Nevertheless, blood selenium concentration is widely used a an indicator of the selenium status of cattle. The borderline levels of blood selenium concentration suggested by different authors depend partly on which parameter is used to assess selenium requirements. Most authors appear to agree that concentrations below 30 µg/l whole blood indicate a risk of NMD (Rosenberger, 1978; Pehrson et al., 1986; Radostits et al., 1994), and also that concentrations below 50 µg/l indicate a selenium-deficient state (Counette & Hartmans, 1982; Maas, 1983; Eversole et al., 1988; Andrews, 1992). However, when the borderlines between suboptimal and optimal selenium status are considered the data in the literature are less consistent. Thus, Jensen and Agergaard (1981), Mathis et al. (1992) and Dargatz and Ross (1996) consider that concentrations from 50 to 75 µg/l whole blood are marginal, whereas Ropstad et al. (1987), van Saun (1990) and Fisher et al. (1995) consider that concentrations up to 100 µg/l are marginal. When the effects of selenium on immunity and reproductive functions are taken into account, some authors (Segerson et al., 1981; Swecker et al., 1989; Gerloff, 1992; Stowe & Herdt, 1992) consider that at least 100 µg/l whole blood is required for these functions to be optimal, and Smith et al. (1988), Hogan et al. (1993), Jukola (1994) and Olson (1994) even recommend 200 µg/l to obtain the optimal preventive effect against infectious mastitis[1].

However, Wichtel (1998), has pointed out that most of the trials upon which the recommendations of the highest whole blood selenium values are based have used only small numbers of animals and herds, and furthermore that their designs have differed considerably, especially with respect to the methods of supplementation and the selenium content of the basic diets. As a result, the conclusions which have been drawn from these trials should not be considered to be definitive. It would therefore seem more reasonable to propose that concentrations less than 50 µg/l whole blood should be considered to carry a risk that clinical signs of selenium deficiency diseases might develop, and that concentrations exceeding 100 µg/l should be considered to be adequate.

[1] Some of the results were originally presented as selenium concentrations in serum – for the purposes of the comparisons these results have been transformed to whole blood values by multiplying by 2.

The only group of animals in our cattle trials which did not achieve an adequate selenium status despite receiving selenium supplementation was the sucking calves supplemented with selenite in paper V. After about six weeks of supplementation none of the eleven calves in this group had whole blood selenium concentrations above 100 µg/l, and two of them had concentrations below 50 µg/l and could consequently have run a risk of developing NMD. All the other supplemented animals in our studies were considered to have an adequate selenium status, irrespective of which selenium compound was used to provide the supplement. This observation is in accordance with the general experience from practice that selenium as sodium selenite can effectively protect most categories of animals against selenium deficiency diseases. In general, selenium supplied either as selenite or selenate, or as selenium yeast, is well absorbed and used for the synthesis of selenoproteins (Pierce & Tappel, 1977; Daniels, 1996).

Short-term evaluation

The concentration of selenium in whole blood and the activity of GSH-Px in the erythrocytes of cattle can be used to determine selenium status. However, these parameters are dependent on the life-span of the erythrocytes and there will therefore be a time lag before they respond to changes in the supply of dietary selenium. They are therefore most suitable for determination of long-term selenium status. In contrast, the concentration of selenium in plasma and liver and the activity of GSH-Px in plasma and liver change more quickly in response to changes in the supply of selenium. However, the activity of GSH-Px in bovine plasma (or serum) is very low and constitutes only about 0.5-2% of the total activity in bovine blood (Carlström, 1979; Scholz & Hutchinson, 1979); as a result it is difficult to make use of this parameter for the evaluation of the short-term selenium status of cattle.

Levander (1983b) reported that the activity of GSH-Px in platelets in rats and human beings is a useful parameter for the assessment of their short-term selenium status. The main advantages of using GSH-Px in platelets as an index of selenium status should be that it is a pool of selenium that is biologically active, which readily responds to changes in selenium supply and which is much more easily monitored than liver GSH-Px activity (Levander et al., 1983a). In paper III we showed that it was possible to isolate platelets from bovine blood and to measure their activity of GSH-Px. However, the method needs to be improved before it can be useful in practice. The time difference observed between the changes in GSH-Px activity in platelets and plasma selenium were in our experiment about three weeks. It was concluded that at present plasma (or serum) selenium concentration would be a better parameter for the assessment of short-term selenium status in cattle, owing to the difficulties experienced in measuring the activity of GSH-Px in platelets. However, it must be borne in mind that plasma selenium is not a measurement of the functional selenium.

In human beings and rats, selenoprotein P has been shown to be an possible indicator of short-term selenium status (Burk et al., 1991; Persson-Moschos et al. 1998). Awadeh et al. (1998a) have recently isolated selenoprotein P from bovine serum and this parameter might therefore be applicable to the assessment of the short-term selenium status also in cattle.

Correlations between possibly useful parameters for the evaluation of selenium status

It has been reported that in cattle there is a strong correlation between the concentration of selenium in whole blood and the activity of GSH-Px in whole blood (Scholz & Hutchinson, 1979; Jukola, 1994), and erythrocytes (Wilson & Judson, 1976; Carlström et al., 1979), at least at selenium concentrations that are not extremely high. Much less is known about the correlations between other parameters of potential interest for the evaluation of the selenium status of cattle.

In an attempt to increase knowledge of these relationships we have collected data from different studies done at the department. Individual cows were selected, for which data on the selenium concentrations in whole blood, serum or plasma, and milk, and the activities of GSH-Px in erythrocytes, serum and milk were available. The cows were selected on the basis of their milk selenium concentration and the aim was to collect data from cows in which the milk selenium concentrations were distributed as evenly as possible over as broad a range as possible. Sixty-two cows were selected whose lowest milk selenium concentration was 7.0 µg/l and the highest 33.3 µg/l. To obtain this broad range of concentrations it was necessary to use data from a non-homogenous selection of animals. For example, all the cows with a low selenium status (<12.0 µg selenium/l of milk, n=18) were from Estonian herds, and most of them had received no selenium supplementation. The cows with milk selenium concentrations between 12.0 and 20.0 µg/l (n=32) had been supplemented with selenite and were partly from our experimental herd and partly from Estonian herds. The cows which had milk selenium concentrations above 20.0 µg/l (n=12) were all from our research herd and the majority of them had been supplemented with organic selenium as a yeast product.

The activity of GSH-Px in milk was too low to be used as an indicator of the cows' selenium status. The lowest value was 0.5 µkat/l and the highest was 2.6 µkat/l, which was about 1000 times lower than the activity in the erythrocytes (range: 356-3718 µkat/l). The specificity of the analytical method was considered to be low at enzyme activities below 10 µkat/l because the activity measured could not easily be distinguished from the background activity.

The activity of GSH-Px in serum was also too low to be useful. The lowest and highest values recorded were 2.0 and 14.5 µkat/l, values which were in

accordance with earlier findings (Carlström, 1979; Scholz & Hutchinson, 1979). The highest and lowest whole blood selenium concentrations recorded were 243 and 31 µg/l, and the corresponding values for serum were 117 and 15 µg/l. The correlation matrix for variables other than GSH-Px in milk and serum is shown in Table 1.

Table 1. Correlation matrix for milk, blood and serum selenium (Se) concentrations and the activity of glutathione peroxidase (GSH-Px) in erythrocytes in 62 cows with milk selenium concentrations ranging between 7 and 33 µg/l.

	Milk Se	Blood Se	Erythrocyte GSH-Px	Serum Se
Milk Se	1.00	0.78	0.70	0.71
Blood Se	0.78	1.00	0.96	0.85
Erythrocyte GSH-Px	0.70	0.96	1.00	0.76
Serum Se	0.71	0.85	0.76	1.00

The results are in accordance with earlier findings that in most situations the activity of GSH-Px in the erythrocytes can be used instead of the blood selenium concentration to evaluate a low or normal selenium status in cows (Wilson & Judson, 1976; Carlström et al., 1979; Ullrey, 1987; Counotte & Hartmans 1988). There were also strong correlations between other parameters, in particular between selenium in whole blood and selenium in serum. It must, however, be stressed that the choice of method used to evaluate the selenium status of cattle depends on the clinical situation, i.e. the expected selenium status of the cattle, whether they have been supplemented continuously or discontinuously, and whether their long-term or short-term selenium status is to be evaluated (Wichtel, 1998).

Evaluation of the selenium status of pigs

For pigs, there are few data in the literature suggesting what blood selenium values should be considered as either deficient or adequate. Radostits et al., (1994) considered that a whole blood selenium concentration of 120-300 µg/l

should be considered adequate, and that 5-60 µg/l should be considered deficient. Chavez (1979) considered that whole blood selenium concentrations greater than 100 µg/l were adequate.

One possible reason for the lack of information about pigs could be that their requirements for selenium are very closely related to their intake of vitamin E. Thus, in a series of trials, Hakkarainen and his group found that a dietary supplement of 0.135 mg selenium/kg DM - resulting in a whole blood selenium concentration of about 150 µg/l - was sufficient to protect pigs against VESD when the concentration of vitamin E (dl-α-tocopheryl acetate) in the diet was 5 mg/kg, but that neither of these supplementary levels was adequate when they were given separately (Bengtsson et al., 1978a; Bengtsson et al., 1978b; Hakkarainen et al., 1978). However, Young et al. (1976) showed that pigs survived and showed no signs of selenium deficiency when they were necropsied after nine weeks on a selenium and vitamin E-deficient diet which resulted in serum selenium concentrations between 20 and 50 µg/l, which should be equivalent to whole blood levels not higher than 25-75 µg/l (Sankari; personal communication).

In the experiments described in paper II the lowest mean whole blood selenium concentration (about 160 µg/l) was observed in the group of pigs supplemented with 0.30 mg selenium/kg feed as selenite, and the groups supplemented with selenium yeast had higher mean blood selenium concentrations. It is concluded that all the groups had a selenium status that safely protected the animals from VESD, particularly when the fact that the vitamin E concentration of the diet was 40 mg/kg is taken into consideration. This conclusion is further strengthened by the concentration of selenium measured in the livers of the animals at slaughter, although the concentration in the animals that had been supplemented with selenite was unexpectedly low.

Inorganic selenium in farm animal nutrition

Selenite vs. selenate

According to all the parameters which were used to evaluate the availability of selenium in papers III and IV it is evident that sodium selenate was as efficient as sodium selenite for the supplementation of dairy cattle with selenium. In the literature there are few reports on the use of selenate as a feed additive for ruminants. Podoll et al. (1992) found that selenate had a higher bioavailability than selenite in cattle, and Henry et al. (1988) found the same result in sheep. In contrast, Shepard and Millar (1981) and Serra et al. (1994) observed no significant differences between the availability of selenate and selenite when they were given as oral supplements in trials with sheep. However, in the trial of Podoll (1992), the differences between the blood selenium concentrations of the cattle supplemented with selenite and selenate were very small, and in the trial of Henry et al. (1988) the doses of selenium used were at a toxic level (6 mg selenium/kg DM). It is therefore concluded that in practice selenite and

selenate are equally available when they are given as supplements to ruminants at physiological levels.

Pro-oxidative properties of selenite

Clinical relevance?

Selenite is chemically a pro-oxidative compound (Spallholz, 1994). One reason for suspecting that selenite may also have a pro-oxidative effect *in vivo* has been the reports of increased activities of GSH-Px in blood when selenite has been fed at higher than optimal levels (Oh et al., 1976; Pehrson & Johnsson, 1985b), or at toxic levels (Hafeman et al., 1974). It has been suggested that high doses of selenite induce increased activities of GSH-Px in order to counteract its own pro-oxidative effects. Selenate does not have pro-oxidative properties *per se*, but *in vivo* selenate might be expected to induce the same effects as selenite, because selenate is reduced to selenite before it can be used for the synthesis of specific selenoproteins (Spallholz, 1994). If the pro-oxidative properties of selenite are significant, it would be expected that animals fed selenite (and possibly selenate) would have higher activities of GSH-Px than animals fed organic selenium, which should not have any pro-oxidative properties. However, no such effect was observed in any of our experiments, either in pigs or in cattle. On the contrary, in cattle there was a trend towards higher activities of GSH-Px in the erythrocytes of the animals fed selenium yeast.

Csallany and Menken (1986) found that rats fed a severely selenium-deficient diet supplemented with 0.10 mg selenite selenium/kg feed DM, which is a commonly accepted level of supplementation, developed accumulations of lipofuscin pigment. In the trials described in paper II we studied samples of liver and heart from pigs supplemented with selenite, but did not observe any accumulations of lipofuscin pigment by light microscopy. Although we used a different method from that used by Csallany and Menken, our results indicate that selenite does not have any *in vivo* pro-oxidative effects that are of practical importance at the dosage levels used in our studies.

Off-flavour in milk - a result of the use of selenite?

During recent years there have been increasing numbers of reports about problems with off-flavour in Swedish bulk milk. Some cases seem to have a genetic background, but most commonly the problem is associated with the oxidation of fatty components of the milk (Barrefors et al., 1994). We conducted a trial in a 310-cow herd, managed on an ecological farm where the animals are fed complete mixed rations. The farm had had a problem with off-flavour in the milk more or less constantly during the previous year. In a trial with a cross-over design we replaced the sodium selenite in the mineral feed with an equal dose of selenium as selenium yeast, to test the hypothesis that the pro-oxidative properties of selenite might have contributed to the farm's off-flavour problem. Milk samples from selected cows were tested for

off-flavour weekly and the bulk milk was tested every second day. The study lasted for ten weeks. There was no evidence that organic selenium reduced the off-flavour problem. Moreover, providing the cows with a daily dietary supplement of 1.0-2.5 g vitamin E per cow, which is the most common recommendation for herds with off-flavour problems, also had no effect, either in individual cows, or at the herd level (Pehrson & Ortman, 1998).

Effects of selenite on the nutritional value of the feed

In our experiments we found no evidence of an *in vivo* pro-oxidative effect of supplementing farm animals with selenite at physiological doses. However, it is impossible to exclude the possibility that selenite might have adverse pro-oxidative effects during the storage of feeds. Selenite might oxidise susceptible compounds such as, for example, vitamin E, ascorbic acid and vitamin A (Coelho, 1991, Shurson et al. 1996) and, furthermore, the selenite might be reduced to elementary selenium, which would then not be available for ruminants (Vokal-Borek & Hellsten, 1982). Groce et al. (1973) demonstrated that pigs excreted more selenium when they were fed a stored selenite-containing premix instead of a recently prepared premix. In the trial described in paper I there were indications that selenite mixed and stored in a mineral feed had a lower availability. Before the trial, the cows were estimated to have consumed 6 mg selenium as sodium selenite daily, fed in a mineral feed. During the trial the selenite-supplemented cows received 3 mg selenium daily, but this supplement was not mixed with the other minerals. Thus, during the trial the cows received approximately half the amount of selenium they had received during the period before the trial, but in spite of this their selenium status improved during the experimental period.

Selenate does not have the pro-oxidative characteristics of selenite, and might therefore be preferable as a feed supplement. However, further research is needed to reveal the possible differences between selenite and selenate in their influence on the antioxidant components of stored feedstuffs.

Organic selenium in farm animal nutrition

Depot selenium

The results of our trials and the results described in earlier reports (Conrad & Moxon, 1979; Goehring et al., 1984; Kurkela & Kääntee, 1984; Nicholson et al., 1991; Suomi & Alaviuhkola, 1992; Suoranta et al., 1993; Malbe et al., 1995; Mahan & Kim, 1996; Mahan & Parrett, 1996) show that supplementing cattle and pigs with organic selenium results in higher selenium concentrations in milk, whole blood, plasma and different tissues. This effect is probably due to the non-specific incorporation of selenoamino acids into body proteins (Behne et al., 1991) and may be influenced by the dietary intake of methionine (Sunde et al., 1981; Waschulewski & Sunde, 1988). It is not clear whether this pool of selenium is immediately available for the synthesis of

functional selenoproteins (Thomson, 1998). However, Mahan et al. (1975; 1977) demonstrated that pigs which had a lower tissue selenium content at weaning became selenium-deficient more rapidly after weaning. Moreover, in a recent study in human beings, Persson-Moschos et al. (1998) showed that in individuals who had been supplemented with organic selenium, the decline in the levels of selenoprotein P after the end of a period supplementation was slower than in individuals who had been supplemented with selenate. The authors interpreted these findings by suggesting that the accumulated tissue selenium had been available and used for the synthesis of selenoprotein P during the post-supplemental period.

GSH-Px in erythrocytes and whole blood

There were no statistically significant differences between the activities of GSH-Px observed in the erythrocytes of cattle supplemented with selenium yeast, selenite or selenate (papers I, III, IV and V), with the exception of the calves in paper V. However, in all these experiments there was a tendency for the groups of cows or heifers which were supplemented with selenium yeast to have higher erythrocyte GSH-Px activities than the groups of animals supplemented with selenite or selenate. There were greater inter- and intra-individual variations in the activity of GSH-Px than in the concentration of selenium in blood and plasma, and this greater variation may explain the lack of statistical significance. There are reports in the literature which show that organic selenium compounds can increase the activity of GSH-Px in bovine erythrocytes more than selenite (Pehrson et al., 1989; Malbe et al., 1995), but there are also studies in which no differences in this respect were observed (e.g. Awadeh et al., 1998a) The results of our studies tend to support the suggestion that animals supplemented with selenium yeast may have higher activities of GSH-Px than animals supplemented with selenite or selenate, at least when the basal concentration of selenium in the feed is low. However, the physiological role of the potentially higher activities of GSH-Px in the animals supplemented with selenium yeast is not clear. Arthur & Beckett (1994) proposed that GSH-Px may function not only as an antioxidant, but also as a storage pool of selenium when the supply of selenium is greater than the animal's requirement.

In the trial described in paper II there were no differences between the activity of GSH-Px in the whole blood of pigs supplemented with 0.30 mg selenium/kg feed as selenite or selenium yeast, and pigs supplemented with 0.10 mg selenium/kg feed as yeast. This result is in accordance with the results of Mahan and Parrett (1996), who found that serum GSH-Px reached a plateau in animals fed approximately 0.10 mg selenium/kg feed. Our conclusion is that 0.10 mg selenium/kg feed as selenium yeast is as effective as 0.30 mg selenium as selenite in finishing pig production. The dietary level of selenite selenium required for the activity of GSH-Px to reach a plateau may in fact be lower than 0.30 mg /kg fed, but this was not tested in our trial.

Suckled calf production

There are many different methods which can be used to supplement extensively fed cattle with selenium, including the injection of different selenium compounds, the administration of intraruminal pellets containing selenium and iron, and selenium drenches and selenium fertilisers (Wichtel, 1998). In Sweden, the most commonly used methods have been free access to a selenium-supplemented mineral feed, the injection of selenium (and vitamin E), the oral drenching of cows with sodium selenite once or twice during the last month of pregnancy, and/or the injection of new-born calves with selenite. The results described in paper V clearly show that selenite may not effectively ensure that all sucking calves achieve an adequate selenium status when the selenite is supplied as a free-choice feed supplement to their dams, whereas in most cases selenium yeast should be effective. Therefore, if this method of selenium supplementation is used, the selenium in the mineral feed should preferably be in an organic form. However, even when organic selenium is used, some calves may not achieve an optimal selenium status because some cows may have an inadequate voluntary intake of the mineral feed, as was the case for one cow in the selenium yeast group in our study.

The advantage of using a selenium-supplemented mineral feed compared with injecting or drenching the animals with selenium is that it is much simpler in practice, and that the stress put on the animals is kept to a minimum. Moreover, free access to a dietary source of selenium will keep the blood selenium concentration at a steady level as long as the supplementation lasts, whereas a drench will increase the selenium status of the calves for a maximal period of 2-3 months (Pehrson & Johnsson, 1985a).

Ecological dairy calves - at risk of selenium deficiency?

The results described in paper IV showed that supplementation with selenium as selenite or selenate had only a marginal effect on the selenium concentration of milk, in comparison with an unsupplemented control group. Similarly small effects on the selenium concentration of milk from supplements of inorganic selenium compounds were also observed in the trials described in papers I and V, and in earlier experiments (Conrad and Moxon, 1979; Fisher et al., 1980; Maus et al., 1980; Malbe et al., 1995). In contrast, Fisher et al. (1995) and Awadeh et al. (1998b) found no difference between the concentration of selenium in the milk of cows supplemented with either selenium yeast or selenite. However, in these two studies the concentration of selenium in the milk was initially much higher than in our trials, indicating that the concentration of selenium in the cows' basic feedstuffs could not have been deficient.

In conventional Swedish dairy production the cows' rations often include imported feedstuffs containing high concentrations of organic selenium. As a

result, the concentration of selenium in Swedish milk is relatively high in comparison with the milk from some other, similarly selenium-deficient countries, for example Estonia (Suoranta et al., 1993; Malbe et al., 1995; Pehrson et al., 1997), where almost all dairy rations are based solely on home-grown feed (Pehrson, 1995). However, in Sweden there is an increasing interest in ecological dairy production, and the Swedish Control Association for Ecological Feed Production (KRAV) has established rules for this type of production (KRAV, 1999). KRAV demands that farmers use feed mainly produced locally on the farm, and that the calves must be fed fresh milk; no milk replacers being allowed. As far as their supply of selenium is concerned, the calves on these farms may therefore be comparable to the extensively fed beef calves described in paper V. Ecological farmers should therefore be advised to supplement their cows with organic selenium compounds instead of selenite, to guarantee that the calves have an optimal selenium status.

Concluding remarks

If the reference values for an adequate selenium status for cattle and pigs, used in our trials, are accepted and the animals are supplemented continuously with selenium, the categories of animals which should be supplemented with selenium yeast should probably be limited to suckler calves and possibly cows in ecological dairy production. The effect of organic selenium on milk selenium may also be expected to have a beneficial effect in sucking piglets, as indicated in the studies of Mahan and Kim (1996). Moreover, if the supplementation of livestock with selenium has as a secondary objective to increase the intake of selenium by human beings, then organic compounds of selenium might be preferable to inorganic compounds.

Conclusions

When used as a feed supplement, selenium yeast resulted in higher concentrations of selenium in the blood and plasma of cattle and growing fattening pigs than selenite.

Organic selenium as selenium yeast generally resulted in higher tissue selenium concentrations than the inorganic compound selenite, in both cattle and growing fattening pigs. Even after long-term supplementation with selenium yeast the tissue selenium concentrations remained within acceptable ranges, and the risk of toxic accumulations of selenium in edible organs seemed to be negligible at the recommended levels of supplementation.

There was no difference between the bioavailability of the inorganic compounds selenite and selenate, measured in terms of the concentrations of selenium observed in blood, plasma and milk, and the activity of GSH-Px in erythrocytes, when they were given as feed supplements to dairy heifers and cows.

Supplements of selenite and selenate increased the concentration of selenium in milk only marginally, whereas selenium yeast caused a considerably larger increase in the milk selenium concentration. As a result, suckled calves whose dams are given free access to a selenite-containing mineral feed will have a lower selenium status and have a greater risk of developing nutritional muscular degeneration than calves whose dams are supplemented with selenium yeast. In dairy production the higher concentration of selenium in the milk of cows supplemented with selenium yeast could help to ensure that calves at ecological farms have an adequate selenium status, and it could also help to increase the selenium intake of the human population.

The metabolism of thyroid hormones was affected in heifers fed typical Swedish home-grown feed unsupplemented with selenium. However, although an increase was observed in the concentration of T_4 there was no significant change in the concentration of the metabolically active T_3, indicating that in terms of thyroid hormone metabolism, home-produced Swedish feed is marginal in selenium rather than deficient.

A method was developed for measuring the activity of GSH-Px in the platelets of cattle, and the method seems to be potentially useful for the short-term assessment of selenium status. However, the method needs to be improved before it can be useful in clinical practice. Measurements of the concentration of selenium in plasma or serum are therefore at present more reliable for the determination of the short-term selenium status in cattle.

The pro-oxidative properties of selenite did not appear to have any practical consequences, either in cattle or pigs, when it was used as a selenium supplement at the recommended levels

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