# 5 Selenium

In 2008, the FDA answered to different requests of food claims underlining the property of selenium in cancer prevention, using for each proposed claim the following "*scientific evidence supporting this claim is convincing but not yet conclusive"* (FDA, 2008). Therefore, according to the FDA further studies are required to support health claims for selenium dietary supplements concerning prevention of different human cancers, such as urinary, digestive and respiratory tract, prostate, thyroid, brain, liver and breast. We could synthesize that selenium is an immune carrier of debates and the FDA position is that the multi-factorial approach necessary in the cancer prevention is not easily synthesizable in a food claim. The importance of selenium for the animal health and for the antioxidant system has grown in the last 20 years. Moreover, selenium is the unique microelement to be codified in DNA as selenium-cysteine (SeCys), which is a structural part of various enzymes involved in the oxidation system, such as glutathione peroxidase. However, the current debate on the recommended daily allowance of selenium and the various seleno-enzymes involved in different physiologic pathways, makes it difficult to set a unique and universally recognized level in which selenium would be considered as safe and health promoting.

The content of selenium in the food chain is greatly variable, therefore high or low exposures to selenium are of health and economic concerns. If deficiency of selenium is almost endemic and animal intoxication quite possible, the human toxicity of selenium is rare and it can only occur in seleniferous areas where the population has a diet strongly based on local products and not much variable, for instance as reported in the Enshi District, China (Fordyce et al., 2000).

The wide variability of selenium content in foods depends on soil, plant specie and part of the same plants (Underwood & Suttle, 1999). The semi-arid and arid regions deriving from cretaceous shales are rich in selenium as selenate ($Se^{6+}$), which is instead reduced to selenite ($Se^{4+}$) in humid areas under acidic and reducing conditions (Allaway, 1968). Well-aerated and alkaline soils offer a more bioavailable selenium (Spadoni *et al.,* 2007), generally as selenate, because the selenite has a higher capacity to adsorb onto soil particle surfaces (Mikkelsen *et al.,* 1989). The different forms of selenium are generally well absorbed by mammals and the related mechanism seems not dependent by homeostatic control. The issue of selenium bioavailability in animals is related to the biochemical pathways occurring in seleno-enzymes synthesis.

The most prevalent compounds of selenium for animal nutrition are the inorganic form sodium selenite (SS, raw formula: $Na_2SeO_3$,) and sodium selenate (raw formula: $Na_2SeO_4$), and recently it is increasing the use of organic source as direct aminoacids seleno-methionine (SeMet) or cysteine (SeCys) or by other carriers such as selenized yeast (SY), containing about 60% of SeMet (2006/1750/EC).

## 5.1 Metabolism of selenium in ruminants

### 5.1.1 Absorption and excretion pathway

The knowledge on selenium metabolism and absorption is still not well understand for the difficulty to quantify all the various forms of selenium, however studies support for a different absorption between rumen and intestinal tract. The inorganic source metabolized by rumen is selenite, and the ingested selenate is almost all reduced to selenite, then a part of this leaves the rumen to be absorbed in the small intestine. The rumen transforms about 30 to 40% of selenite in not chemically identified insoluble forms which are either badly absorbed or used by the microbial hosts; about 10-15% is used to synthesise seleno-enzymes and the remaining amount is probably passively uptaken by the small intestine (Serra *et al.,* 1994). Vendeland *et al.* (1992), quantified about 35% absorption of selenite in the small intestine of rats.

Selenized yeast contains about 60% of SeMet, a part of which can be directly incorporated into microbial protein synthesised in the rumen bacteria, and 36% of other selenium organic forms (Juniper *et al.,* 2006). The absorption efficiency of SeMet is widely recognized as 30% higher compared to selenite, however an elevated presence of methionine in the diet could reduce the SeMet absorption because of it compete with the same absorption site in the intestine (Weiss, 2005). Despite the complete metabolic pathway of SeMet and SeCys is not still well-known, the intake of SY is useful to increase the content in blood and milk of these seleno-aminoacids. Cows fed with the same amount of selenium from SY compared to SS have a higher blood content of SeMet and SeCys (Phipps *et al.,* 2009 and Juniper *et al.,* 2008), the first used for general protein synthesis, while SeCys is specifically retained and incorporated in seleno-proteins.

The distribution of selenium in the body varies with health status, organ, glutathione peroxidase and other seleno-proteins activity and kind of animal. For instance Lindberg (1968), comparing healthy pigs with subjects suffering nutritional muscular dystrophy (NMD), found that in kidney and liver of healthy animals the concentration of selenium is, respectively, 11.5 and 1.8, while in NMD pigs is, respectively, 2.5 and 0.2 mg/Kg DM.

According to Juniper *et al.* (2006) the urinary and/or faeces excretion of selenium in cow is not significantly different using SS or SY. In that condition, for cows feeding 0.3 mg Se /kg DM from SS and SY, and assuming a DM intake of 20 Kg/head/day, dry faeces and urinary excretion of 5 Kg and 10 L, respectively, the amount of excreted selenium is about 70% of the ingested.

The absorption of selenium in lactating cows can be modified by the amount in the diet of calcium and sulphur: it seems that either high (1.3%) or low (0.5%) presence of calcium reduce the absorption of selenium (Harrison & Conrad, 1984a), such as an increase in the diet of sulphur (as calcium and magnesium sulphate) from 0.2 to 0.4 or 0.7% (Ivancic, 1999).

## 5.2 Selenoproteins

Nowadays, at least 15 proteins containing in their structure selenium have been characterized (table 5), and functionally the most known enzymes can be divided into two distinct families. The first is involved in preventing the excess of oxidation process in cell

tissues (e.g. glutathione peroxidase and thioredoxin reductase), the second is represented by iodothyronine deiodinases which convert thyroxin (T4) in the active physiological form triiodothyronine (T3). The synthesis of seleno-enzymes begins with the cellular reduction of whichever form of uptaken selenium in selenide, then is changed with hydroxyl in a molecule of serine linked to a specific tRNA (UGA codon). The produced SeCys-$tRNA_{UGA}$ complex will be used for the seleno-enzymes synthesis (Weiss, 2005). The most physiologically rapid form of selenium for this synthesis are selenite and selenate, because SeMet, though well absorbed, is retained and used for general protein synthesis, therefore with slow conversion in the SeCys-$tRNA_{UGA}$ complex (Henry & Ammerman, 1995). The glutathione peroxidases' activity is given by the cysteine residuals containing ionized selenol, the latter inactivates hydrogen peroxide and oxygen-rich free radicals acting as a reducing agent, and then it is re-established to selenol by glutathione. There are differences between GPX regarding the number of residual seleno-cysteine (4 residuals in GPX1 and GPX2, one in GPX 3), the structure of sub-units, the tissue distribution and in their sensitivity to selenium depletion. GPX1 and GPX2 in liver and blood plasma fall rapidly in activity during selenium deficiency, whereas GPX3, which is associated specifically with phospholipids of cellular membranes, appears more resistant to selenium depletion and it is believed to have other function like the synthesis of prostaglandins (FAO/WHO, 2004). The activity of GPX is improved by the presence of vitamin E and Klein (2003; 2004) suggests the efficacy of a synergic action of selenium and vitamin E to prevent cancer in human, particularly that of prostate thanks to the role of GPX3.

Thioredoxin reductase is a seleno-enzyme distributed almost in all tissues and contains two seleno-cysteine residuals. It is core component of different redox systems diffused throughout the body with the role of degrading locally excessive production of oxidant elements (Mairrino *et al.*, 1991).

Another fundamental group of seleno-enzymes are the iodothyronines, which have the function to transform throughout the body (type I, II or III) the inactive hormone T4 in the active form T3. The distribution of these enzymes is necessary because of the need of some tissues for an in-house synthesis of T3 respect to the possibility of using an elsewhere preformed T3, and uptaken from plasma. The response to selenium deficiency is different between the iodothyronines and it is quite complex depending also by the iodine status. The concomitant deficiency of iodine and selenium worsen the clinical manifestation of goitre (Colzani *et al.,* 1999; Contempre *et al.,* 1993).

More than half of the total selenium in human plasma (about 60 to 80%) is structural component of selenoprotein P, the function of which is not currently clear but there are some hypothesis and data about its role in antioxidant protection of brain. Koyama *et al.* (2009) states that selenoprotein P, recognized as principal carrier of selenium in brain (Schomburg *et al.,* 2003), reduces the risk of stroke, and acts as antioxidant for brain capillary endothelium (Burk & Hill, 2005). There are at least, other 10 selenoproteins having antioxidant properties. In particular, selenoprotein W seems to be involved in the prevention of muscle diseases and sperm abnormalities (FAO/WHO, 2004).

**Table 5.** List of identified and purified selenoproteins with their body location and possible functions.

| Cod. | Selenoprotein | Principal location | Function |
|---|---|---|---|
| GPX1 | Cystosolic GSH peroxidase (GPX) | Tissue cytosol, RBC | Storage, antioxidant |
| GPX2 | Plasma GPX | Plasma, kidney, lung | Extracellular antioxidant |
| GPX3 | Phospholipid hyperoxide GPX | Intracellular membranes (particularly testes) | Intracellular antioxidant |
| GPX4 | Gastrointestinal GPX | Intestinal mucosa | Mucosal antioxidant |
| ID1 | Iodothyronine 5'-deiodinase type I | Kidney, liver, muscle, thyroid | |
| ID2 | Iodothyronine 5'-deiodinase type II | Central nervous system, pituitary gland | Conversion of T4 to T3 |
| ID3 | Iodothyronine 5'-deiodinase type III | Brown adipose tissue, central nervous system, placenta | |
| TRR | Thioredoxin reductase | All tissues cytosol | Redox/antioxidant |
| Sel P | Selenoprotein P | Plasma | Transport, antioxidant, storage, heavy metal detoxifier ? |
| Sel W | Seleprotein W / Testes selenoproteins | Muscle, testes | Antioxidant/Structural? |

Source: Arthur & Beckett, 1994; Sunde, 1994

## 5.3 Effect of selenium deficiency and excess in humans

Selenium status is correctly determined when both serum/plasma concentration and quantification of selenoproteins activities are considered. Combs *et a.l* (2001) estimate, using as parameter the submaximal seleno-enzymes activity, that people suffering selenium deficiency are about 500-1000 millions worldwide. The official recommended daily allowance of selenium is 40 µg/day (WHO, 1996) but many countries do not meet this level.

A great number of human disease or clinical manifestations have been explained as consequence of minus or major deficiencies or overt intake of selenium, but not all of these have sufficient scientific support. There follows a brief overview.

Endemic and severe selenium deficiencies have been associated with specific juvenile cardiomyopathy and chondrodytrophy pathologies, respectively, the so-called Keshan and Kaschin-Beck diseases. China and eastern Siberia are the principal endemic areas of these diseases because their soils and foods contain a very low amount of selenium: in some mountainous area of China, selenium levels are lower than 125 µg/Kg (Tan *et al.,* 1987) and grains content is lower than 10 µg Se / Kg. The international concentration of selenium in grains ranges between 10 and 550 µg/Kg (FAO/WHO, 2004). The Keshan disease, mainly found in children and women in child-bearing age, is manifested as acute or chronic insufficiency of cardiac function, cardiac enlargement and arrhythmias. In the 1940s the mortality was higher than 80%, but currently, it has been reduced to minus than 30% thanks to better medical care and prevention with SS tablets (Combs, 2001). Beck (1997) discovered the disease is caused by a RNA-virus which increases its virulence in selenium deficient status.

Kaschin-Beck disease is a pathology affecting the epiphyseal, articular cartilage and the epiphyseal growth plates of growing bones, whose clinical manifestation are enlargement of joint (especially of the fingers, toes and knees), shortness of extremities (in the most severe case manifesting as dwarfism). Although the fortification and prevention programs with selenium in endemic areas gave satisfying results, the causes of the disease are not still well-known and it is also probable the role of pre-disposing factor or other agents. Wen Sheng *et al.* (2004) discovered, for instance, that low content of T2 toxin in grains reduces the risk of occurring the kaschin-Beck disease.

Apart from the above described endemic diseases, selenium deficiency is recognized as responsible for many other diseases linked to low activities of selenoproteins. Typically, in developing countries children have lower blood selenium content than their mothers (Lee *et al.,* 1995), so it increases the risk for respiratory morbidity (Darlow *et al.,* 1995). Some authors suppose that the antioxidation activity on phospholipids and cholesterol of GPX4 can reduce the platelet aggregation of oxidised LDL in the artery, reducing the risk of cardiovascular diseases (Néve, 1996; Sattler *et al.,* 1994; Salonen *et al.,* 1988). The epidemiological studies about the association between selenium intake and cardiovascular diseases have mixed findings and disparities which could be explained by the basal selenium status of the population, and the diet content of other antioxidant involved in the protection systems such as vitamin E (Rayman, 2002). The improvement of patients' selenium status seems to have positive effects in pancreatitis, asthma, systemic inflammatory response syndrome and general mood (McCloy, 1998; Shaheen *et al.,* 2001; Angstwurn *et al.,* 1999; Finley & Penland, 1998). Regarding rheumatoid arthritis, Perez *et al.* (2001) showed slight improvement in arm movements and health perception in patients fed with SY.

Hoffmann & Berry (2008) reviewed the role of selenium in immune system, which is considered to be highly damaged during selenium deficiency diseases. A considerable number of studies show selenium influence on immune system depending by the type of antigens and involved tissues, and by the selenium status of treated individuals; in particular, better responses after selenium boosting has been showed in mild-deficient subjects than ones with good level of blood selenium and proteins activity. The fortifying role of selenium in favour of immune system has been especially showed against RNA virus, therefore useful for diseases like Keshan, influenza A and AIDS (Rayman, 2002).

Since the complexity and numerousness of biological pathways and diseases in which selenium is involved, this doctoral thesis cannot develop all the "universally recognized" and supposed selenium health benefits, but it is opportune to describe its interaction with thyroid, already described in the selenoproteins paragraph. The highest concentration of selenium throughout the tissues is in the thyroid gland (Köhrle *et al.,* 2005). Communities well-known to have high incidence of myxedematous cretinism are characterized by low plasma selenium status, low GSHPx activity, and low iodine status (Vanderpas *et al.*, 1993). Medical or nutritional cares by iodine supplyings, particularly if excessive and in presence of low plasma content, tends to induce high peroxidative stress. It is postulated that necrosis and thyroid fibrosis leading to irreversible hypothyroidism result if a concurrent deficiency of selenium limits peroxide destruction by the protective action of thioredoxin reductase (FAO/WHO, 2004). The iodine care of ill

subjects in endemic areas of myxedematous cretinism is recommended to be preceded by assessment and rectification of any observed deficit of selenium (Vanderpas et al., 1993).

The acute toxic exposure to selenium is uncommon and the few registered cases of human intoxication have been generally on exposed workers who inhale aerosol containing high level of selenium (Cu smelters or Se-rectifier plants). The symptoms of acute exposure are hypothension by vasodilation, respiratory distress and a garlic-like odour of the breath due to the exhalation of dimethylselenide (Combs, 2001).

The chronic intoxication of selenium is named selenosis and can occur in seleniferous area, which are characterized by soils and food chain overburden by selenium content. It was discovered in 1960 in Enshi County and Hubei Province in China, where the selenium concentration of soils and coal were 0.008 and 84 g/Kg respectively, and the locally produced foods contained the highest concentration of selenium ever reported: corn and rice had 6.33 and 1.48 mg Se/Kg, respectively. The selenosis symptoms are morbidity, losses of hair and nails, skin lesions (erythema, pain in the extremites, hemiplegia), hepatomegaly, polyneuritis and gastrointestinal disturbances (Combs, 2001). According to the United States Environmental Protection Agency (Poirer, 1994), on the basis of Enshi County experience, the no adverse effect daily dose of selenium is 853 µg, and consequentially WHO (1996) settled the upper safe limit of selenium intake for adults at 400 µg/day.

## 5.4 Selenium status in Europe and Italy

According to available data, the estimated average intake of selenium in Europe (calculated from Rayman, 2008; and WHO, 2004) and Italy (Allegrini *et al.,* 1985) are 43.7 and 43 µg/person per day, respectively. The selenium requirement to guarantee the level of 70 ng Se/ml plasma registered in most healthy adults is 40 µg/day, i.e. the current RDA (WHO, 1996). More than half countries in Europe, anyway, do not meet the RDA of selenium suggested by the Panel on Dietary Antioxidants and Related Compounds (2000), which is 55 µg/day for adults. The reason of low selenium status has already been developed, but it is interesting to underline the strict relation between selenium intake and the import/export policies of some countries. Since 1950 to 1980 the consumption of wheat in the United Kingdom was covered up to 85% by wheat imported from Canada, which is rich in selenium. In the last ten years the importations were drastically reduced and the internal wheat yield covers 80% of the market, however being poor in selenium the UK population is now deficient in

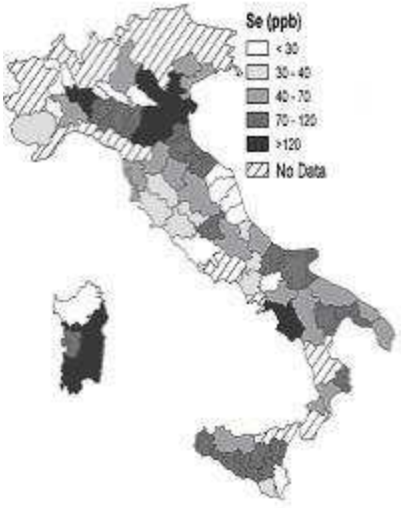


**Figure 5:** Concentration of selenium in wheat of Italian provinces (Spadoni *et al.,* 2007).

selenium. The opposite situation is registered in New Zealand thanks to the importation from Australia (Rayman, 2008).

The knowledge about the Italian population status of selenium is scarce and the most recent investigation is the study of Allegrini *et al.* (1985) in the northern Italian population. In food chain, Panigati *et al.* (2007) evaluated the concentration of selenium in two Italian rice cultivars: the values ranged between 20.1 and 53.0 µg/Kg for white rice and red rice, respectively. The most comprehensive Italian work could be the research on geochemical and pedoclimatic factors affecting selenium bioavailability in Italian agricultural soils (Spadoni *et al.,* 2007). The regions Sardegna, Piemonte, Lombardia, Veneto and Calabria have areas with the highest concentration of selenium in soils (more than 550 µg/Kg), whereas the national content in wheat ranges between 25 and 120 µg/Kg (figure 5).

## 5.5 What is the best recommended daily allowance of selenium for humans?

The recommended daily allowance of selenium is currently under discussion since there are many studies trying to explain that increasing the selenium RDA from 40-55 µg/day to 200 µg/day could be of very healthy effectiveness. This discussion started in 1996 within the Nutritional Prevention Cancer (NPC) trial carried out by Clark *et al.* (1996) in the USA, i.e. the first double-blind placebo-controlled study about selenium in a western population which involved 1312 subjects. People who received 200 µg Se/day showed a reduction of 50 and 37% in total cancer mortality and incidence, respectively, and a reduction of 63, 58 and 46% in the incidence of cancer of the prostate, colon and lung, respectively. These results changed the experimental and economic approach to selenium and open new perspective in the disease prevention by food intake.

The American experience was repeated in Europe by the project Prevention of Cancer by Intervention with Selenium (PRECISE), developed by Denmark, Sweden and UK. The benefits arising from an intake of 200 µg/ day of selenium seem efficient for the immune system activity, due to the increasing production of cytotoxic T-cells and natural killer cells (Kiremidjian-Schumacher *et al.,* 1994). In figure 6 it is clear that 40 µg/day as RDA of selenium to guarantee 70 ng Se/ml plasma in humans (as suggested by WHO, 1996) could be insufficient for GPX activity and for a hypothetical prevention of cancer with selenium.

The great number of physiological pathways in which selenium is involved makes very hard and debatable the decision on its new RDA. Many researchers are currently working to obtain additional results which could help in the formulation of new RDA in the view of cancer prevention and maintenance of good health.

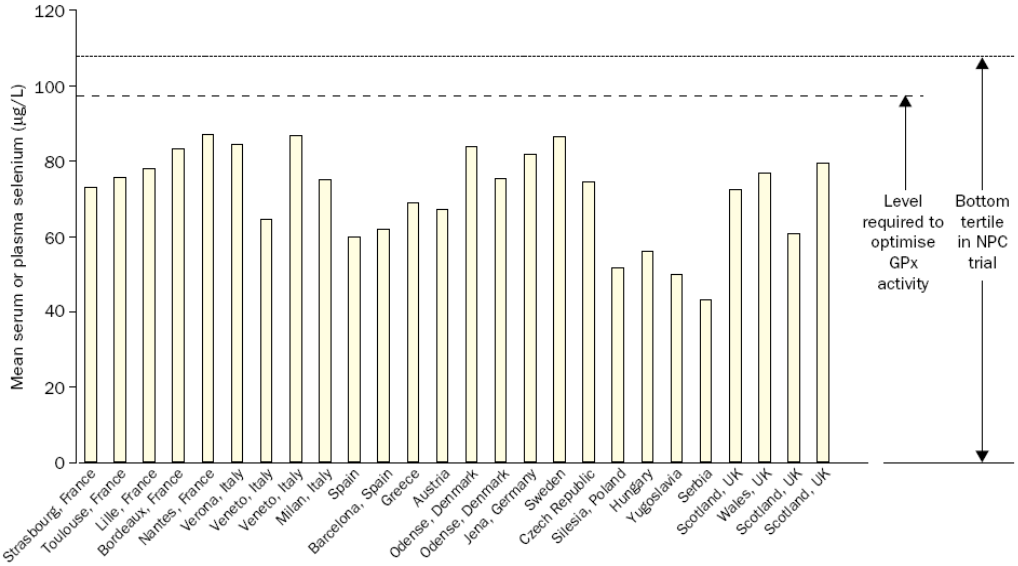


**Figure 6.** Mean levels of selenium plasma contents in Europe compared to the level of subjects participating the Nutritional Prevention Cancer (NPC) trial (Rayman, 2002)

## 5.6 Selenium in dairy cows

Selenium content in dairy cow's milk depends on pasture and mineral mixture used in animal breeding. The concentration in milk can range between 5 and 56 µg/L (Underwood, 1971), whereas if animal are fed 0.3 mg Se / kg DM (NRC, 2001), according to Givens *et al.* (2004) the expected selenium content is 11.6 µg/L. The daily consumption of milk can provide at least 10% of the selenium RDA for adults (Knowles *et al.,* 2004) but a certain margin of improvement is feasible. Since 1970, feeding systems to increase milk concentration have been developed using as feed additive sodium selenite and selenate, selenized-yeast, seleno-amicnoacids or other natural sources. The incorporation of selenium into the different kind of milk proteins and peptides depends strongly by the source of additive and by the way it is administered to the animal. The huge number of publications provides data quite independent and above all there are no studies using sufficient long duration experimental trials. A clear protocol, which can help to develop selenium-fortified milk from feed nutrition, is still unavailable; consequently, market, industry and research have the need to summarize the effect of different source of selenium on animal nutrition and health.

### 5.6.1 Natural source of selenium

The different ability of plants species to accumulate selenium compared to sulphur is divided into three groups: non-accumulators, Se-indicators and Se-accumulators. The best accumulator of selenium are Brazil nuts (0.03 – 512 µg/g) for human diet (Rayman, 2008) and Astragalus species in pastures (Lodge, 1963). A good source of selenium for livestock can be rapeseed, which is a Se-indicator, while cereal crops like wheat, oat, rye and barley are classified as non-accumulators (Fordyce, 2005). The content of the latter is highly

linked to the concentration of soils and it is, therefore, wide variable, from 0.006 to 3.06 mg/Kg DM. In soils with average level of selenium, legumes have less content of this element than grasses but this difference is reduced when the concentration of selenium in soils decline (Minson, 1990). In an experiment conducted in grazing dairy cows in Australia, Heard *et al.* (2007) found a slight difference between autumnal and spring pasture, 65 ± 21.5 and 99 ± 19.0 µg/kg, respectively.

### 5.6.2 Source of selenium for feed fortification and European regulation

The requirement needs of selenium for both humans and livestock cannot be worldwide and regularly satisfied by the natural source of selenium in soils and plants; therefore the use of supplemented selenium in feed for livestock breeding is quite widespread. The use of the inorganic sources sodium selenite or selenate was the first method to increase the content of selenium in animal diets. In the early 1970s, supplementation at 26-30 mg/Kg DM of inorganic selenium in mineral mixture was effective on reduction of the White Muscle Disease (WMD) but did not increase the blood and tissues concentration of selenium compared to lambs receiving no supplements (Underwood & Suttle, 1999). Whereas, the use of selenized block salt seems more efficient for improving the blood and tissues status of selenium (Langlands *et al.,* 1990). Between the 70s and the 90s the elemental selenium was also used in animal nutrition as heavy ruminal pellets, consisting of 95% finely divided iron and 5% of selenium. Earlier studies carried out with this source of selenium, showed that selenium pellet is capable of reducing the incidence of WMD in lambs and maintain good blood concentration of selenium; however, it was useless beyond 12 months after insertion in the rumen and gave high variability within animals (Wilkins & Hamilton, 1980). In beef cattle, 7-56% of selenium petted was regurgitated within few minutes of dosing (Langlands *et al.,* 1989). Contrary, using pellets containing 10% of selenium, Wichtel *et al.* (1994) registered no regurgitation and an improvement in growth. Others contradictories results about the use of selenium pellets are summarized by Underwood & Suttle (1999).

The superior efficiency of organic (principally SY and SeMet) compared to the inorganic sources is demonstrated by many researchers. These sources are easily absorbed and enter rapidly in body circulation as seleno-aminoacids or seleno-general proteins, which can provide good reserve of selenium for the synthesis of SeCys and seleno-enzymes when needed (Weiss, 2005).

The importance of selenium as health promoter is important for the production of quality and/or functional food, the challenge is to tailor the selenium form to be used in supplementing protocol for producing animal (Ceballos *et al.,* 2009). The presence of sulphur is a parameter of choice since the absorption of inorganic selenium is reduced by 20% when 0.2% added sulphur is in the diet, whereas in the same condition SY should be about 50% more available (Ivancic & Weiss, 1999). When using SY, it is important to control the content of methionine in the diet, since the aminoacid competes the same site of absorption (Weiss, 2005).

The current European laws about selenium for zootechnical uses are the regulation 2006/1750/EC and the 96/51/EC directive. The maximum allowed level of selenium is

0.568 mg/Kg DM independently of the source of selenium: the regulation 2006/1750 allows the use of SeMet within the strain CNCM-I-3060 of *Saccharomyces cerevisiae*.

### 5.6.3 Requirements, deficiency and toxicity of selenium in dairy cows

Although not well established, the metabolism and requirement of selenium and vitamin E appear interdependent, therefore, the basal requirement of selenium for dairy cows (as for other species) varies based on the source of selenium and the amount of vitamin E contained in the diet. According to the National Research Council (NRC, 2001), in condition of adequate ingestion of vitamin E the requirement of selenium for dairy cattle is 0.3 mg/Kg DM (this value was established considering different trials in which the supplemented selenium was 0.3 mg/Kg DM and the total selenium in the diet ranged between 0.35 and 0.40 mg/kg DM). The importance of selenium in the diet is particularly clear during gestation for preventing some periparturient disorders and for ensuring the calf an adequate selenium status in the early stage of growth. The selenium content in excretion faeces of dairy cattle ranges from 0.011 to 0.019 mg/Kg DM, whereas the urinary excretion depends by the selenium daily intake, e.g. for lactating cows ingesting 2.5 mg Se/day, the urinary loss of selenium is 0.5 mg/day (Ivancic, 1999). Being these values applicable also to dry cows (Harrison & Conrad, 1984b) and considering the different requirements between the dry and lactation stages, the daily intake of selenium should be 1.75 and 4 mg/day for non-lactating and lactating cows, respectively (NRC, 2001). A value of 6 mg/day, instead, could be more adequate to prevent the incidence of mastitis and retention of placenta (Maus *et al.,* 1980), and to provide a blood and plasma concentration of 0.18 and 0.08 µg Se/ml, respectively (Jukola *et al.,* 1996).

The effect of selenium deficiency or deprivation is extremely variable, since each tissue is subjected to oxidative stress in all stage of life. The oxidative stress by free-radical damage on charge of muscular tissues generates nutritional muscular dystrophy (e.g. White Muscular Disease, WMD), above all in goat compared to calf or lamb (Rammel *et al.,* 1989). The WMD is effectively a degenerative rather than a dystrophy disorders and its incidence varies from sporadic to about 10% in some areas clearly deficient in selenium. Its clinical manifestations are muscular stiffness, arrhythmia, tachycardia and abdominal breathing (Underwood & Suttle, 1999).

Important effects of selenium deficiency in reproduction system are the increase mortality of newborn, retention of placenta (Mace *et al.,* 1963; Trinder *et al.,* 1969), and the reduction of male fertility due to a minor viability of semen (Slaweta *et al.,* 1988). The incidence of mastitis can be reduced with optimal intake of selenium and vitamin E (Weiss *et al.,* 1990).

The chronic toxicity of selenium in livestock (named alkali disease) can occur during ingestion of seleniferous plants, in particular Astragulus spp. for several weeks or months, which could provide from 5 to 40 mg Se/kg DM. The symptoms include sloughing of hooves, lameness, loss of hair and emaciation, lack of vitality (NRC, 2001). When cows are fed 10 to 20 mg Se/kg BW it is highly likely the occurrence of acute toxicity, which is characterized by salivation, respiratory distress, oedema and pulmonary congestion, reflecting circulatory failure and degenerative changes in the heart, liver and kidney (Rosenfeld & Beath, 1964).

The tolerance of selenium is strictly dependent by source of the element, specie and breed of animal. The maximum tolerable dose of selenium is currently settled at 2.0 mg/Kg DM for all the species (NRC, 1980); the maximum allowed levels in feeds are 0.3 and 0.568 mg/Kg DM, respectively in the USA and Europe (NRC, 2001; European Regulation, 2006/1750). Jenkins and Hidiroglou (1986) found that young calves feeding 10 mg Se/Kg DM reduced metabolic activities and diminished their growth rate. The main source of that experiment was the inorganic selenium, whereas Juniper *et al.* (2008), supplementing diets with selenium as SY at level 10-fold and nearly 20-fold higher than those allowed, respectively, in Europe and USA, found no adverse affect for dairy cows in trial lasting from 60 to 90 days.

## 5.7 Carry over of selenium in milk and cheese

The fortified feed with selenium raises the content of this element in milk, however controversial results are registered in literature. Ceballos *et al.* (2009) performed a meta-analysis on 42 studies published between 1977 and 2007, which contain data on carry over of selenium in milk of dairy cows fed basal or supplemented level of selenium. This meta-analysis reports great variability between studies; the average increase in milk concentration of selenium is 0.16 µmol/L when cows were fed supplements. The high variability between results is significantly influenced by continent in which the trials were performed, source of selenium and dose of selenium; instead, study design, production system, previous selenium status, type of production, supplementation protocol and kind of performed analysis are not significantly responsible for variation in data. The meta-regression analysis states that fortified feeds with SY result in higher selenium concentration of milk compared to supplementation with selenite and selenate. Generally, the effect of dose is low in cows receiving less than 3 mg/head of selenium, whichever the source (figure 7).

According to the equations proposed by Givens *et al.* (2004), when diet contains 0.3 mg/Kg DM of selenium, as SS and SY, the relative concentration of milk is 11.6 and 20.1 µg/L, respectively. The slope of the linear regression regarding SY is significantly higher ($P < 0.05$) than that of SS, 0.118 vs. 0.011; therefore, increasing the intake of selenium to the maximum level allowed in Europe (0.568 mg/Kg DM) the concentrations of total selenium in milk is 14.9 and 45.9 µg/L, respectively (figure 8). The dose effect of SY in the diet on milk selenium concentration was linear also in Juniper *et al.* (2006). Carry over of selenium in milk from cow fed SY was about 27% more efficient than SS per milligram of selenium administered in the diet (Knowles *et al.,* 1999).

Many studies agree that selenium supplements do not alter either DM intake or milk yield (Phipps *et al.,* 2008; Heard *et al.,* 2007; Juniper *et al.,* 2006 and Givens *et al.,* 2004). To the contrary, milk yield appeared to be affected by selenium supplement in cows grazing on low selenium pastures (Grace *et al.,* 1997); Phipps *et al.* (2008) reported a positive linear effect in milk production when increasing the amount of SY in the diet.

Since selenium is bioavailable as single aminoacid, peptides or proteins, during cheese-making selenium can transfer efficiently into the casein fraction from whole or skimmed milk. Studies on distribution of selenium in milk and cheese fraction show that

partition of selenium into the casein fraction is higher than 70%, ranging from 71 to 75% (Knowles *et al.,* 1999; Van Dael *et al.,* 1991 and Debski *et al.,* 1987).

The increase of selenium in the diet seemed to change the distribution of copper and zinc in human milk (Bratter *et al.,* 1998), however, in cows fed 25 mg Se/day as yeast selenium supplement for two weeks, neither zinc or copper were differently distributed in milk compared to control (Hoac *et al.,* 2008).

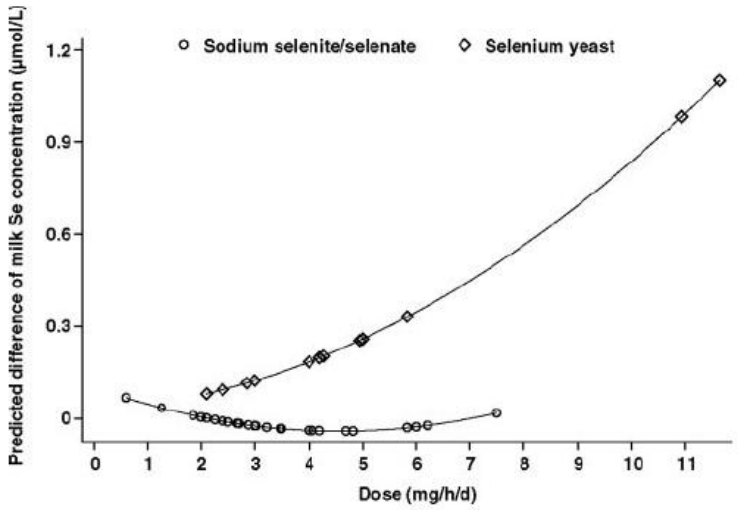


**Figure 7.** Effect of the dose of selenium (mg/head per day) on milk selenium concentration (µmol/L), 75 days after the beginning of supplementation. Symbols indicate the data among the 42 studies studied in the meta-analysis of Ceballos *et al.* (2009).

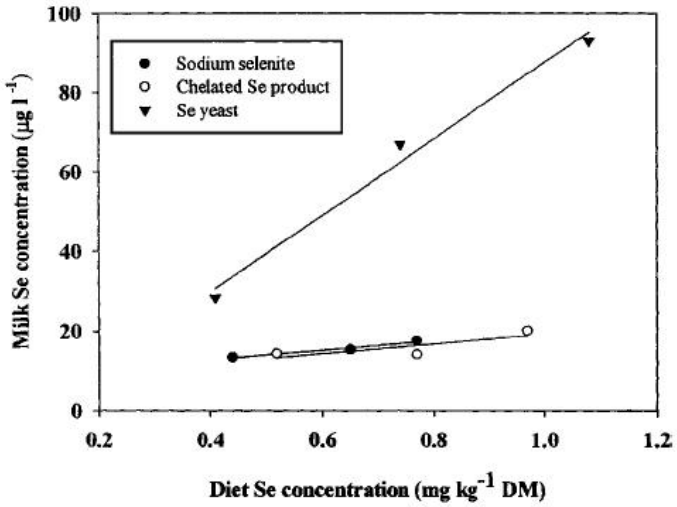


**Figure 8.** Linear regression between selenium concentration in the diet (mg/Kg DM) and milk output (µg/L). Givens *et al.* (2004)

## 5.8 Speciation of selenium species in milk and cheese

The absorption and metabolism of selenium depend by its chemical form, therefore, different sources of supplementation can affect the ratio between selenium species in milk and cheese. Using a high performance liquid chromatography combined with an inductively coupled plasma – mass spectrometry (HPLC-ICP-MS), Phipps *et al.* analyzed the chemical form of selenium present in the bulked blood, milk and cheese from cows fed no supplements (T1), either 0.3 mg Se/Kg DM as SS (T2) and SY (T3), or 0.45 mg Se/Kg DM as SY (T4), in a trial lasting 112 days. As indicated by authors, data offer interesting trends although not supported by statistical analysis because the speciation was performed only in bulked milk due to the financial constraints of their work.

Figure 9, reports the data of speciation in milk by Phipps *et al.* (2009), there was evidence that SeCys was retained by the body since its value in milk remain quite similar between levels of selenium in the diet. Contemporary, the concentration of SeCys in blood from cows of T3 groups (data not showed in figure 9) maintained higher values than T2 during all the experimental period. Therefore, it is quite evident that SeCys is fundamental for selenoproteins activities. To the contrary, SeMet can easily cross the capillary endothelium and be incorporated into proteins instead of methionine, and as suggested by Pherson (1993), in lactating dairy cows SeMet may be preferentially uptaken by mammary gland and used for the synthesis of milk protein. The latter statement could be confirmed by the raise of SeMet in milk from cows fed increasing doses of SY (figure 9).

The distribution of selenium species in cheese (figure 10) confirmed the high content of SeMet in milk protein, being its concentration almost three-fold higher in cheese produced from cows fed the same amount of SY compared to SS, i.e. 157 vs. 57 ng/g (Phipps *et al.,* 2009).

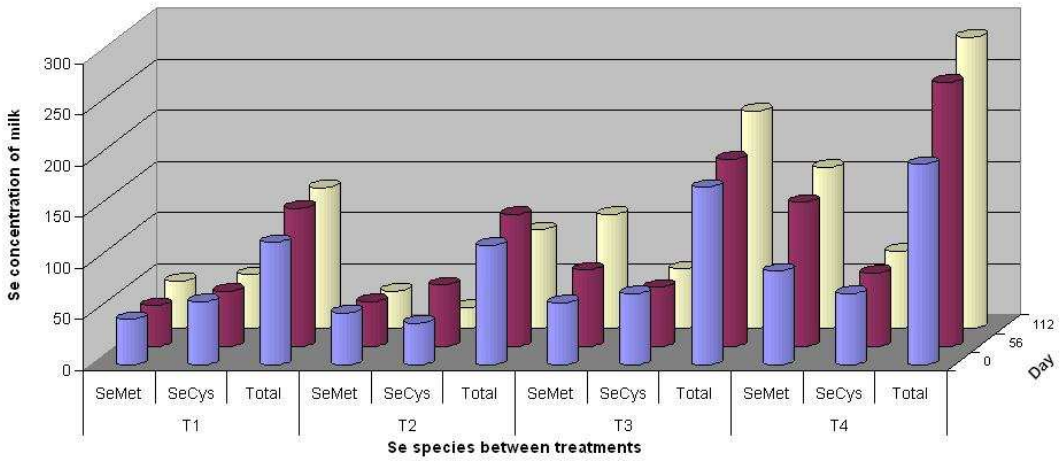


**Figure 9.** Concentration of different species of selenium in the bulked milk from cows fed different level (mg/KG DM) and source of selenium registered after 0, 56 and 112 days in treatment. T1: 0.16 in the basal diet; T2: 0.30 as SS; T3: 0.30 as SY; T4: 0.45 as SY. The species of selenium are SeMet (seleno-methionine), SeCys (seleno-cysteine) and Total (SeMet + SeCys + others). Graph is created using data by Phipps *et al.* (2009).

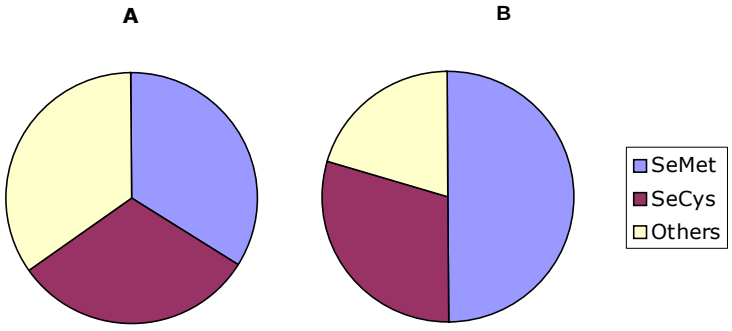


**Figure 10.** Percentage distribution of different species of selenium (SeMet, SeCYs and others) in cheese produced from milk of dairy cows fed 0.3 mg Se / Kg DM as SS (A) or SY (B). Graph is created using data by Phipps *et al.* (2009).