

## CHAPTER 3

### MINERALS AND THE IMMUNE SYSTEM

#### 3.1 Introduction

The body's defence mechanism is a function of the mineral status. Optimum nutrition assures optimum tissue integrity, an increased immune response, and other benefits, which alleviate stress. Erosive nutritional imbalances wear out livestock, which then succumb easily to challenges of an otherwise manageable nature.

Skin, its appendages, and the mucous membranes are an effective first line of defence. Adequate protein levels and amino-acid balance in the diet are essential for an intact epithelium. Antibody synthesis and phagocyte activity are, in turn, dependent on several vitamins and minerals, which serve as a second line of defence against infectious agents.

Immuno-competence is the resistance to infectious agents, foreign particles, cells, toxins and neoplastic cells, which ensures the survival of the host. Deficiencies within the host may contribute towards an inadequate response to the above challenges.

Many trace elements influence the immune system of the body (Boyazoglu, 1997), and although common deficiency signs are seen in deficient animals, marginal deficiencies could affect the immune system, without showing any signs of deficiency. It therefore seems that to ensure an adequate defence system, dietary requirements of some minerals are much higher than the levels prescribed in conventional recommendations. This emphasises the importance of preventing trace element deficiencies in the diet.

Some trace elements form part of the anti-oxidant defence capacity of the body to combat reactive oxygen and other free radicals in the body. Laboratory methods have been developed to measure the possible magnitude of the reactive oxygens in the body. Based on enhanced immunocompetence at adequate trace element intakes, a technique has been developed to challenge

the body with a pathogen and measure at what level of mineral intake no further increase in antibody titre production occurs. This would signify an adequate trace mineral intake (Van Rysse, 1997).

### 3.2 Free radicals, antioxidants and immune function

Ordinarily antioxidant defences, which are available intra- and extracellularly are adequate to safeguard against oxidative damage. The balance can however be tipped as a result of overproduction of free radicals or by exposure to sources which overwhelm the antioxidant defences (Bendich, 1990). An active immune reaction stimulates the manifold production of cells, proteins and hormones, which necessitates an immense supply of energy. Metabolic pathways supplying this energy lead to the production of reactive oxygen species. In addition phagocytic cells produce free radicals in the respiratory burst to invalidate foreign organisms (Nockels, 1996).

#### a. Diet

Individual components in the diet can alter the proportions of free radical production in the body. High levels of dietary polyunsaturated fatty acids (PUFA) can be immuno suppressive. The unsaturated double bonds found in these PUFA are primary targets for free radical damage with consequent chain reactions that lead to the formation of lipid peroxides. Lipid peroxides and aldehydes can impede normal cellular function and even lead to the breakdown of oxidised cell membranes. Lipoproteins in the plasma can also be oxidised and the resultant molecules are lymphotoxic (Bendich, 1990)

The fluidity of cellular membranes is partially dependent on the degree of unsaturation of its fatty acids. As the proportion of PUFA increases, so the potential for membrane lipid peroxidation also increases. Lipid peroxidation results in forfeiture of membrane fluidity. Metabolites of lipid peroxidation can furthermore influence immune reaction. Reduced membrane fluidity is directly coupled to a lowered ability of the lymphocyte to react to an immunological challenge (Bendich, 1990).

**b. Stress**

According to Nockels (1996), stress is often a precursor to infection in animals. Throughout a stress period free radicals are produced during corticoid synthesis. Superoxide radicals are produced during the reactions between catecholamines and oxygen. The resultant depletion of antioxidant reserves impedes an effective immune reaction.

**3.2.1 Leukocyte usage of free radicals**

Individual cells of the immune system employ free radicals for the destruction of intrusive pathogens. The two main types of leukocytes, which produce reactive oxygen intermediates, are neutrophils and macrophages (Bendich, 1990). The respiratory burst associated with phagocytosis by leukocytes produces  $O_2^{\cdot-}$ ,  $H_2O_2$ ,  $OH^{\cdot}$ ,  $ClO^{\cdot}$  and possibly singlet oxygen. These free radicals damage the cell walls of target organisms (Hill, 1981).

**3.2.1.1 Neutrophils**

Neutrophils are the most common circulating phagocytic cells in the body. These cells are attracted by chemical signals produced at regions of infection and move in the direction of the signal (chemotaxis). Stimulated neutrophils have the ability to appropriate molecular oxygen and produce reactive oxygen containing molecules (respiratory burst). Free radicals and singlet oxygen, in addition to other reactive molecules, can directly invalidate bacterial pathogens or can react with granulocyte specific enzymes to produce highly toxic compounds (Bendich, 1990). These reactive molecules can however cause mutations, can break down normal cells like other neutrophils, erythrocytes and thrombocytes, can cause inflammation of surrounding tissue, inactivate protected enzymes and inhibit lymphocyte proliferation. Lymphocytes are however guarded against neutrophil oxidative damage by monocytes since they contain catalase. Neutrophils are also capable of breaking down tumour cells (Bendich, 1990).



### 3.2.1.2 Macrophage

Where neutrophils are primarily associated with acute inflammatory reactions, macrophages are associated with chronic inflammation. In contrast to neutrophils, macrophages are central to the development of specific immune reactions. They process antigens for presentation to lymphocytes. Both these phagocytic cells possess the cell membrane associated NADPH oxidation system, which allows the synthesis and secretion of reactive oxygen species. Prostaglandins, leucotrienes, interleukin 1 and interferons are also produced by macrophages (Bendich, 1990).

### 3.2.1.3 Lymphocytes

The leukocytes, which are primarily involved in the production of specific immune reactions, are the T and B-lymphocytes. Individual essential nutrient deficiencies influence many aspects of T and B-lymphocyte function, and are associated with increased infection in domestic animals (Bendich, 1990). It has been confirmed that Se and Cu deficiencies influence T-lymphocytes to a larger extent than B-lymphocytes. As laboratory animals age, the performance of T-lymphocytes is reduced to a larger extent than that of B-lymphocytes. T-lymphocyte membranes are more fluid than B lymphocyte membranes in young mice. As mice age, T-cells lose their fluidity, while B-cells maintain the same level of fluidity. T-cell lipids are more sensitive to peroxidation than B-cell lipids. The ability of T-lymphocytes to form rosettes is significantly inhibited after exposure to oxygen radicals, while B-lymphocyte rosette formation is not influenced to a large degree (Bendich, 1990).

The vitamin C and E content of lymphocytes and mononuclear cells are normally slightly higher than that in the thrombocyte and erythrocytes. When splenocytes of mice receiving standard diets are exposed to substances causing peroxidative damage via the production of free radicals, the multiplication of both T and B-lymphocytes as a result of exposure to mitogens (causative species) is inhibited. In vitro antigen induced antibody production by B-lymphocytes is further

more also inhibited. When additional vitamin E or other antioxidants are added to this culture, the immuno suppression is overcome (Bendich, 1990).

### 3.3 Minerals

#### 3.35.1 Co

In ruminants, Co is needed for the synthesis of vitamin B<sub>12</sub> (cyanocobalamin). Although bacteria produce vitamin B<sub>12</sub>, higher plants or animals cannot synthesise it. Co deficiency in ruminants is therefore a vitamin B<sub>12</sub> deficiency, brought about by the inability of the rumen micro-organisms to synthesise enough vitamin B<sub>12</sub>, when dietary Co is inadequate (Underwood *et al.*, 1999).

Co is essential to mammals in two distinct coenzyme forms of vitamin B<sub>12</sub>, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl) (Ferguson, Mitchell, & MacPherson, 1989). As MeCbl, Co assists a number of methyltransferase enzymes by acting as a carrier of methyl groups, obtained from N<sup>5</sup> – methyltetrahydrofolate (Armstrong, 1989; Dawn *et al.*, 1996), and is thus involved in the build-up of carbon chains. An example would be the methylation of homocysteine to produce methionine via methionine synthase (Armstrong, 1989).

As AdoCbl, Co influences energy metabolism. It facilitates conversion of propionate to succinate by methylmalonyl-coenzymeA (CoA) mutase during the formation of glucose. This reaction is part of the metabolic route for the conversion of carbons from valine, isoleucine, threonine, thymine, and the last 3 carbons of odd chain fatty acids, all of which form propionyl CoA, to the tricarboxylic acid (TCA) cycle intermediate succinyl CoA (Dawn *et al.*, 1996).

Ruminants make extremely inefficient use of dietary Co. The rumen microbes partition Co between active (cobalamins) and physiologically inactive vitamin B<sub>12</sub>-like compounds (corrinoids) that the ruminant can neither absorb nor use. Furthermore, vitamin B<sub>12</sub> is poorly absorbed from the digestive tract (McDonald, Edwards & Greenhalgh, 1988). Seemingly, there is therefore an



interconversion of the two coenzymes. In addition Co is recycled at the tissue level and by way of biliary secretion (Underwood *et al.*, 1999).

In keeping with their reputation of tolerance when compared with sheep, cattle show little or no evidence of MeCbl or AdoCbl dysfunction when subject to severe Co-deprivation (Kennedy, Young, Kennedy, Scott, Molloy, Weir & Price, 1995).

Co-deficiency leads to defective lipid metabolism, which is essential to offset loss of appetite. Consequently, there is an accumulation of triglycerides providing a pool of readily peroxidizable, unsaturated fats. A further metabolic defect, accumulation of homocysteine, initiates a chain of peroxidation, which leads to accumulation of the oxidation product, lipofuscein, depletion of the antioxidant, vitamin E, and damage to mitochondrial structure (Kennedy, McConnel, Anderson, Dennedy, Young & Blanchflower, 1997).

Lack of appetite and weight loss, have been used as parameters to determine the dietary Co and vitamin B<sub>12</sub> requirements of animals. The concentration of vitamin B<sub>12</sub> in liver and plasma is a useful index of the dietary status of an animal, but it includes inactive analogues of cobalamin. The concentration of methylmalonic acid (MMA) in plasma seems to be a more reliable index. Furthermore the Co concentration in tissues has little merit to indicate the vitamin B<sub>12</sub> status of an animal (Van Ryssen, 1997). The Co concentration in feed and that extractable from soil have been used for diagnostic purposes (Van Ryssen, 1997). The mineral concentration of soils is however a poor indicator of mineral uptake by plants and thus their availability to animals.

Disease and parasite resistance may decline during Co deficiency. Furguson *et al.* (1989) reported increased sensitivity to infection by the abomasal parasite *Ostertagia circumcincta* in the Co-deficient lamb. In the same experiment, 7 out of 8 lambs in a severely deficient, unchallenged group succumbed to miscellaneous microbial infections. An account by MacPherson, Gray, Mitchel & Taylor (1987), suggested that immune responses were compromised in Co deficient cattle in response to a challenge by *Ostertagia ostertagii*.

Reductions in the ability of neutrophils to destroy foreign cells in both the ovine (Fisher & MacPherson, 1986) and bovine (MacPherson *et al.*, 1987) have been reported in Co deficiency. It is noteworthy that the reported changes in the ovine neutrophil became evident before plasma vitamin B<sub>12</sub> concentrations had fallen below "normal" and before there was any increase in serum methylmalonic acid (MMA) values. In other words, before the first acknowledged signs of functional deficiency in propionate metabolism. If these results reflect a functional impairment in phagocytic killing ability *in vivo*, then the interpretation of conventional (vitamin B<sub>12</sub>) and new (MMA) biochemical criteria of Co status must be questioned (Suttle, Brebner, Munro & Herbert, 1989).

### 3.3.2 Cu

Cu is an essential element necessary for the routine functioning of all living systems. It is thought to be present in all body cells (McDonald *et al.*, 1988) and is particularly concentrated in the liver, which acts as the main storage organ of the body.

Plasma concentrations of Cu are used to indicate Cu status of animals. Other indices are SOD activity in whole blood, plasma ceruloplasmin activity, and Cu concentrations in various tissues, for example kidney (Van Ryssen, 1997) and liver (Stabel, Reinhardt & Nonnecke, 1991).

Cu-deficiency may be expressed in the conventional ways (swayback, anaemia, diarrhoea and growth retardation). Of greater economic importance, however, is the increased susceptibility to fatal infections. The ideal should therefore be to predict the probability of any individual showing "immune dysfunction" (Suttle, 1988).

Cu is a cumulative poison as the animal body is unable to excrete it efficiently. Continuous ingestion in excess of nutritional requirements therefore leads to an accumulation of the element in the body tissues and eventual toxicity (McDonald *et al.*, 1988). There are various published recommendations for the concentration of Cu in the diet of ruminants. In practice, however, tolerance levels depend on interactions of Cu with other minerals (Underwood, 1977).



Another complicating factor, which would influence the ability of a feed to meet the Cu requirements of ruminants or to pose a risk of Cu poisoning, is the absorbability of the Cu source in the diet (Underwood *et al.*, 1999).

Cu's necessity is expressed primarily as specific cupro-enzymes (Prohaska & Lukasewycz, 1990). In animals there are approximately ten proteins that are commonly accepted as cupro-enzymes. They range in size from small single polypeptides to complex tetramers with up to 8 Cu atoms per mol. Some cupro-enzymes have additional co-factor requirements like Zn, haem Fe and pirroloquinolien guinine. The manifold characteristics of the cupro-enzymes are responsible for the diversity of specialised functions of Cu (Prohaska & Lukasewycz, 1990). When Cu levels in the environment are varied, the levels of a number of these cupro-enzymes are likewise altered. Cu is surpassed only by Zn in the number of enzymes that it activates (Underwood *et al.*, 1999), making it difficult to determine the precise reason why particular abnormalities arise in livestock deprived of Cu.

### **3.3.2.1 The function of Cu in the erythrocyte and O<sub>2</sub> metabolism**

Although Cu is not actually a constituent of haemoglobin it is present in certain other plasma proteins such as ceruloplasmin, which are concerned with the release of Fe from the cells into the plasma. A deficiency of Cu impairs the animal's ability to absorb Fe, mobilise it for the tissues and utilise it in haemoglobin synthesis. Cu is also a component of other proteins in blood. One of these, erythrocuprein, occurs in erythrocytes where it plays a role in oxygen metabolism (McDonald *et al.*, 1988). The element is also known to play a vital role in many enzyme systems; for example, it is a component of cytochrome c oxidase (CCO), responsible for the terminal electron transfer in the respiratory chain in oxidative phosphorylation. Thus, during infection the greatly increased metabolism of cells of the immune system might make them vulnerable to depletion of the energy-generating CCO.



## 3.3.2.2 The function of Cu in the immune system

Cu plays an important role in the immune system, as can be seen during a Cu-deficiency in both man (Kelley, Daudu, Taylor, Mackey, & Turnlud, 1995) and animal. It has been demonstrated that resistance of cattle (Brazle & Stokka 1994) and sheep (Woolliams, Woolliams, Suttle, Jones & Wiener, 1986) to infectious organisms is reduced with a Cu-deficiency. Protection against common pathogens in the Cu-deficient calf (Stabel, Spears & Brown, 1993) and heifer (Arthington, Corah, & Blecha, 1996; Gengelbach, Ward & Spears, 1997) do not, however, seem to be compromised. Stabel *et al.* (1993) suggested that Cu-deficient animals are at a greater risk than non-deficient animals for infection. The authors nevertheless failed to observe a consistent immune response. In immune cells from the Cu-deficient bovine, killing ability is reduced before leukocyte SOD activity declines; this is attributed to lowered intracellular concentration of the superoxide anion (Boyne & Arthur 1986). In the Cu-deficient lamb, prevalence of microbial infection was not increased, but mortality was (Suttle & Jones 1986). Chirase, Hutcheson & Thompson (1991) reported that calves injected with Cu glycinate before shipping had lower dry matter intake (DMI) and body weight (BW) change after a challenge with IBRV than calves not injected with Cu. Serum Cu concentrations have been reported to increase with market and transit stress and after inoculation with IBRV (Orr, Hutcheson, Grainger, Cummins, & Mock, 1990; Chirase *et al.*, 1991; Stabel *et al.*, 1993), possibly to increased Cu availability for immune modulation. Cu levels below optimum in sheep nutrition resulted in lambs having a reduced immunocompetence (Boyazoglu, 1997). Various biochemical and cellular hypotheses exist, which can explain the limitations in the immune system during a Cu-deficiency

### 3.3.2.3 Antioxidant

Changes in the intracellular redox potential and energy metabolism can impair the immune system. Increased lipid peroxidation, as a result of Cu-deficiency, not only influences membrane composition, but can also influence redox potential so that changes in glutathion (GSH/GSSG) occur. This can influence both cell formation and antigen production. In Cu-deficient rats, changes in GSH levels as well as changes in pyridine nucleotide ratios in the brain were found. It was shown that lactate / pyruvate and  $\alpha$ -glycerol phosphate / dehydroxy acetone increases phosphate which can predict a rise in NADH/NAD<sup>+</sup> (Prohaska & Lukasewycz 1990).

Cu may protect tissues from oxidant stress via two distinct pathways, one involving impaired Fe metabolism, the other a Cu-Zn SOD enzyme (CuZnSOD).

Hepatic activity of the Fe-containing, haem enzyme catalase is reduced in Cu-deficiency, despite a rise in tissue Fe concentrations (Taylor, Bettger & Bray, 1988). This rise in tissue Fe concentration promotes free-radical generation and the reduced catalase leads to increased tissue damage by hydrogen peroxide and hydroperoxide (Golden & Ramdath, 1987).

Hepatic defences against another free radical, superoxide, may have been reduced in Taylor *et al.*'s (1988) study by a fall in CuZnSOD, which accompanies liver Cu depletion in all species.

Ceruloplasmin may contribute to antioxidant defences by scavenging free Fe and free radicals (Saenko, Yaroplov & Harris, 1994). There is thus scope for interactions with other nutrients with antioxidant properties, such as Mn, Se and vitamin E (Underwood *et al.*, 1999).

### 3.3.2.4 Immune cells

Immunity can be lowered because of changes in the plasmamembrane due to lipid peroxidation in lymphocyte walls during a Cu-deficiency (Prohaska & Lukasewycz, 1990).

Lymphocytes contain CuZnSOD and cytochrome C oxidase, two Cu dependant factors. They have serruloplasmin receptors and therefore have a



specific mechanism to take up Cu from the blood. Lymphocytes may contain unique Cu-dependent enzymes. During a Cu-deficiency changes in these enzymes may directly influence the immune system. One such enzyme is possibly the sulphhydryl oxidase occurring in the plasma membrane of lymphocytes. This enzyme is important in the formation of IgM and thus B-cell differentiation

There are several reports of dysfunction *in vitro* in immune cells from Cu-deficient ruminants. Xin, Waterman, Hemken & Harmon (1991) indicate that steers given a *Cu-deficient* diet, showed reductions in neutrophil Cu, neutrophil CuZnSOD and neutrophil killing capacity (Suttle *et al.*, 1989). Saker, Swecker & Eversole (1994) reported that Cu lysine-supplemented calves had increased plasma Cu concentrations, monocyte phagocytic activity, and monocyte oxidative burst measurements compared with calves fed the basal diet. Boyne & Arthur (1986) noted that neutrophil phagocytic ability decreased with feed restriction and with Cu-deficiency. Other authors have however, found no impairment of phagocytic ability (Boyne & Arthur, 1981; Jones & Suttle 1981), but the capacity to kill the engulfed organism was invariably reduced (Boyne & Arthur, 1981; Jones & Suttle, 1981; Boyne & Arthur, 1986). Arthington, Corah, & Blecha, 1995) found that Cu-deficiency induced by Mo and S did not affect *in vitro* or *in vivo* measurements of neutrophil chemotaxis.

Reductions in SOD activity in leukocytes from Cu-deficient sheep accompanied increased release of superoxide anion, suggesting that superoxide might accumulate and weaken the phagocyte after the pathogen triggers the respiratory burst (Jones & Suttle, 1981). The physiological role of superoxide may, however, vary between species and with the severity of Cu-deficiency. In addition experimental results may be influenced by the stage of the phagocytic process at which biochemical measurements are made.

Proliferative responses of lymphocytes to mitogenic stimulation *in vitro* are also affected in Cu-deficient ruminants. Cu-supplementation in lambs produced enhanced responses to mitogens (Woolliams *et al.*, 1986). However, other workers (Young, Edwards & Hucker, 1985) could find no effects of Cu depletion

on peripheral blood lymphocyte responses to antigens in lambs. Ward, Gengelbach & Spears, (1997) found that Lymphocyte viability did not differ among treatments, nor did lymphocyte blastogenic responses. The authors concluded that Cu-deficiency had inconsistent effects on immune function in calves.

In severely deficient small laboratory animals, Cu-deficiency can affect the numbers of cells mediating immunity, increasing mast cells (i.e. non-specific immune cells) in muscle (Schuschke, Saari, West, & Miller, 1994) and decreasing some subpopulations of T cells (Mulhern & Koller, 1988). It would appear as if the functions of macrophages are also influenced by a change in Cu-status, seeing that Cu-deficient mice have greater amounts of interleukin-1 (a monokine produced by macrophages) (Prohaska & Lukasewycz, 1990).

Some authors have, however expressed concern about the usefulness of *in vitro* tests and their ability to predict *in vivo* responses (Suttle *et al.*, 1989; Underwood *et al.*, 1999).

### 3.3.2.5 Antibody production

Cu may also be involved in intra and inter molecular disulphide formation in proteins like immunoglobulin (Prohaska & Lukasewycz, 1990). Studies with mice and rats have shown that Cu is important in both antibody and cell mediated immunity and inflammatory reactions (Prohaska & Lukasewycz, 1990; Windhauser, Dappel, McClure & Hegsted, 1991). Ward *et al.* (1997), however, found that the effect of a Cu-deficiency on specific immune functions was minimal and changeable in cattle. In contrast to this, Cerone, Sansinanea, & Auza (1995) found that total levels of IgG and total complement concentrations are restricted by a low Cu status. Kill *et al.* (1991) also reported increased humoral immune response to Cu supplementation. These results show that immune reactions toward an intracellular pathogen may be restricted by a Cu-deficiency (Cerone *et al.*, 1995)



### 3.3.2.6 Neuroendocrine function

Changed neuro-endocrine functions can also not be excluded when considering the effects of Cu-deficiency on immunity. There exists a complex interaction between immune, nervous and reproductive systems. The synthesis of norepinephrine, for example, is dependent on the cupro-enzyme, dopamine- $\beta$ -mono-oxygenase. It is known that the transformation of norepinephrine decreases during an immune reaction. It appears however that norepinephrine transition is increases by a Cu-deficiency. Furthermore it has been shown that norepinephrine levels in the spleen of mice are lower with a Cu-deficiency. Norepinephrine metabolism may thus provide an explanation for the changes in the immune system during a Cu-deficiency

Lymphokines are proteins produced by lymphocytes to serve as molecular communicators among cells of the immune system and are important in the coordination of immune-inflammatory reactions. *In vitro* – studies in Cu-deficient media have shown that splenocytes have a lowered production of interleukin-1, originating from monocytes, and the T-cell replacement factor (interleukin-5). Furthermore there is a decrease in production of interleuckin-2 (a limphokine from type I helper T cells) (Prohaska & Lukasewycz, 1990). Gengelbach *et al.* (1997) found that Cu-supplemented calves had greater plasma tumor TNF concentrations than Mo-supplemented calves at weaning, and Cu-supplemented calves tended to have higher TNF after IRBV inoculation than calves given Mo or Fe. The authors interpreted their findings as evidence of an improved immune response in the groups given Cu and Fe. They suggested that dietary levels of Mo and Cu can alter body temperature and feed intake responses to disease by affecting TNF, and possibly other cytokines (Gengelbach *et al.*, 1997).

Another region of the neuroendocrine system, which may react to changes in dietary Cu, is the hypothalamus. The emission of lutenizing hormone (LH) releasing hormone is greatly increased in the presence of Cu and prostaglandine E<sub>2</sub>. Emission of LH from the pituitary gland has a great effect on gonadal hormone emission, which in turn affects the thymus.

### 3.3.3 Mn

Mn is widely distributed in very low concentrations in the cells and tissues of the animal body, the highest concentrations occurring in the bones, liver, kidney, pancreas and pituitary gland (McDonald *et al.*, 1988). It is essential for the normal development of bone and proper functioning of reproductive processes (Egan, 1972) in both males and females (Underwood *et al.*, 1999). Mn is also important in the animal body as an enzyme activator, activating a number of metalloenzymes, chiefly phosphate transferases and carboxylases (McDonald *et al.*, 1988; Underwood *et al.*, 1999).

Although deficiencies of Mn in grazing ruminants are likely to be rare (McDonald *et al.*, 1988), severe deprivation may impair immunity and central nervous system function (Underwood *et al.*, 1999).

Mn forms part of a SOD enzyme, MnSOD that was first isolated from chicken liver mitochondria (Gregory & Fridovich, 1974). The superoxide dismutases (SOD) protect cells from damage by the free oxygen radical  $O_2^-$ . Malecki & Greger (1996) found that Mn deficiency lowers MnSOD activity in the heart and increases the peroxidative damage caused by high dietary levels of PUFA. Compensatory increases in CuZnSOD suggested overlapping roles for the two forms of SOD and possible interactions between dietary Cu and Mn. Bell & Hurley (1974) related structural changes in liver mitochondria and cell membranes to reductions in the activity of MnSOD. Mitochondria are responsible for the majority of  $O_2$  consumption and may be particularly vulnerable to free radical damage (Leach & Harris, 1997).

Paynter & Caple, (1984) noted that the bulk of the Mn in ovine heart is present as MnSOD, and that this is the predominant dismutase in this tissue and also in muscle. A significant reduction of MnSOD in heart and lung tissue was noted by Masters, Paynter, Briegel, Baker & Purser (1988), in Mn depleted animals. This led them to speculate that in circumstances of oxidant stress (e.g. from PUFA) or depletion of other dietary antioxidants (for example, Cu, Se and vitamin E), Mn deficiency may lead to dysfunction. The effects on MnSOD, following the administration of tumour necrosis factor  $\alpha$  (Wong & Goeddel, 1988),



suggest a need for added protection against oxidative stress associated with the inflammatory responses to some infections.

### 3.3.4 Se

A dietary Se-deficiency in ruminants can cause white muscle disease, a decrease in selenoproteins, forfeiture of glutathione peroxidase activity and suppression of the immune system. Se-supplementation is often necessary as many feeds are produced in areas of the world where there is a Se-deficiency (Pherson, Knutsson & Gyllensward, 1989).

Various tissue concentrations have been proposed as indicative of the Se-status of the animal and to monitor the effects of Se supplementation. The measurement of Se-dependant functions, like glutathione peroxidase activity in serum or isolated blood cells, can also relay valuable information. Glutathione peroxidase activity in the erythrocytes is useful but the analytical technique is difficult to standardise among laboratories (Van Ryssen, 1997). The activity of Se supplements can also be determined by investigating biological and clinical effects (Nève, 1994).

Selenoproteins are present in every cell type. There exists at least 20-30 selenoproteins of which a number were partially or fully described (McKenzie, Rafferty & Beckett, 1998). Deiodinase enzymes and glutathione peroxidase are among these selenoproteins.

#### 3.3.4.1 Cytosolic peroxidase

The first peroxidase to be recognised and investigated in detail is known as cytosolic or GPX1. It is the predominant GPX and source of Se in erythrocytes and liver, and all the Se responsive diseases are accompanied by reduction in blood and tissue GPX1 activities. Se is present in GPX1 in stoichiometric amounts, with 4g atoms Se mol<sup>-1</sup>. The tetrameric enzyme catalyses the reduction of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and of hydroperoxides formed from fatty acids and other substances, according to the general reaction:

### ROOH + 2GSH $\rightarrow$ R-OH + HOH + GSSG

It is questionable whether GPX1 ever becomes a rate-limiting factor in protecting erythrocytes or tissues from peroxidative damage during Se-deficiency, as its activity in erythrocytes and liver can decrease to levels, not far from undetectable, without apparent pathological or clinical changes (Arthur & Beckett, 1994b). Sunde, (1994) proposed therefore that GPX1 is essentially a storage selenoprotein. Seleno-amino acids can, however be incorporated directly and non-specifically into body proteins (Kincaid, Rock, & Awadeh, 1999). Furthermore, its suitability as a vehicle for transferring Se to sites of synthesis of other selenoproteins is questionable (Kincaid *et al.*, 1999). It is more probable to make up a depot for excess Se, while providing a reserve of antioxidant potential, which may be beneficial at times of oxidant stress (Kincaid *et al.*, 1999). Animals can survive without the gene for GPX1 (Cheng, Ho, Ross, Valentine, Combs, & Lei, 1997) suggesting that the enzyme itself is non-essential.

#### 3.3.4.2 Other peroxidases

The plasma or extracellular peroxidase, GPX3, is also tetrameric and synthesised principally in the lung and kidney. Its main functions may possibly be the protection of the renal proximal tubule from peroxidative damage. GPX3 does not have a major extracellular protective function, as glutathione (GSH) concentrations in plasma are too low to support such a role. The gastrointestinal peroxidase (GPX2) may also act locally to protect the intestinal mucosa from dietary hydroperoxides expression characterisation (Chu, Doroshov, & Esworhy, 1993). The peroxidases influencing the immune system are completed by the phospholipid hydroperoxidase form (GPX4), a monomer associated with intracellular membranes. GPX4 is conserved during Se-deficiency, suggesting a rate-limiting importance, which may be accountable for the synergistic relationship between Se and vitamin E (Arthur & Beckett, 1994b).

Activity of GPX1 and also the distribution of the enzyme in the body are dramatically influenced by Se intake (Ganther, Hafeman, Lawrence, Serfass & Ganther, 1975; Lei, Dann, Ross, Cheng, Combs, & Roneker, 1998). Over an



extended period, erythrocyte GPX1 activities increase logarithmically in relation to Se intake, eventually reaching a plateau. Incorporation of selenocysteine into erythrocyte GPX1 occurs at erythropoiesis (Wright, 1965). In the shorter term, there is therefore a lag before the newly 'packaged' enzyme is released into the bloodstream and a further lag before it disappears, when the erythrocyte reaches the end of its normal lifespan. Other GPXs also show contrasting tissue responses to supplementation, earlier plateaux being reached for GPX4 in the thyroid and pituitary than in the liver or heart of young pigs (Lei *et al.*, 1998).

#### 3.3.4.3 Deiodinases

Deiodinases are necessary in the metabolism of thyroxine. Se-deficiency may thus indirectly influence basic metabolic rate and a wide range of physiological processes, with adverse effects on production (Underwood *et al.*, 1999).

#### 3.3.4.4 Glutathione peroxidase activity

An experiment where the efficiency of organic and inorganic forms of Se in grower finishing diets of pigs, was compared, showed that the levels of dietary Se required to maintain maximum serum glutathione peroxidase activity were in the region of 0.05 – 0.10 ppm Se. Although the inorganic form of Se usually leads to higher glutathione peroxidase levels at lower dietary levels, both forms clearly reached maximum glutathione peroxidase activity levels at lower dietary concentrations than which are usually fed to pigs. This study showed that higher dietary levels of Se does not further stimulate production of glutathione peroxidase, and that the activity of the enzyme increases with increase in age of the pig (Mahan & Kim, 1996). Pherson *et al.* (1989) found that fermented Se or selenomethionine was twice as active as selenite in increasing glutathione peroxidase activity in erythrocytes of Se-deficient heifers. This discovery suggests that glutathione peroxidase activity cannot reliably be used as a gauge for Se bioactivity.

Thus the activity of glutathione peroxidase decreases in animals with a Se-deficiency and recovers with Se supplementation. Enzyme activity shows saturation at levels that agree with Se requirements for this important enzymatic function (Nève, 1994).

#### **3.3.4.5 Effect of chemical form of Se on immunity**

Experimental data (Stabel *et al.*, 1991) indicates that addition of organic and inorganic forms of Se increased *in vitro* IgM production in peripheral blood mononuclear cells of mitogen-stimulated cattle. The cells were isolated from animals receiving normal levels of Se in their feed. These results indicate that both inorganic and organic forms of Se increase *in vitro* B cell function.

#### **3.3.4.6 Se as an antioxidant**

Peroxyne, a product of superoxide and sulphureoxide "salpeteroksied", is produced in the skin during exposure to ultraviolet radiation and causes breakage of single strand DNA. Studies with ebselene, a synthetic Se compound which functions as glutathione peroxidase, showed that the enzyme can prevent this breakage. Ebselene also inhibits the pro inflammatory enzymes sulphur oxide synthase and protein kinase C. Furthermore, glutathione peroxidase plays an important role in the synthesis of arachidonic acid metabolites (McKenzie *et al.*, 1998).

#### **3.3.4.7 Se and its influence on the immune system**

Se has, besides its inclusion in the glutathione peroxidase as a selenocystein residue, various effects on the immune system, *inter alia*, the expression of specific and non-specific humoral and cell mediated reactions. The anti oxidative role of GSHPx in the elimination of inorganic and organic hyperoxides plays an important role in cells of the immune system, as well as in various tissues. A principal function of Se in immune cells, especially the phagocytic cells, is to curb excessive production of peroxidative substrates like  $H_2O_2$  (Spallholtz, 1990).



According to McKenzie *et al.*, (1998) there are three mechanisms through which Se-supplementation promotes cellular immunity:

1. Se stimulates the expression of the high affinity IL-2 receptors of T-lymphocytes and increases reactions of T-lymphocytes. Considering that the T-lymphocytes aid the B-lymphocytes in the production of antibodies, this may account for the effect of Se on antibody production (Nicholson, Bush & Allen, 1993).
2. Se prevents damage to immune cells, which may be caused by oxidative stress. The most familiar function of Se is its role as an antioxidant in the GSHPx system. Endogenous production of  $O_2^{\cdot-}$  and  $H_2O_2$ , together with other free radical species during the respiratory burst of phagocytic cells is obligatory for the cytotoxic destruction of invading species. Vitamin E, vitamin C, superoxide dismutase and GSHPx protects the phagocytic cells against self-destruction by free radical peroxidation. If the GSHPx activity in macrophages decreases, there will be a concomitant increase in the release of  $H_2O_2$ . Spallholz & Boylan (1989) have found that macrophages of mice, which have a Se and GSHPx deficiency, indeed produce more  $H_2O_2$  during the respiratory burst. Furthermore it was established that the production of  $O_2^{\cdot-}$  in these cells also was increased. In addition to the antioxidant function of GSHPx in cells, Se and/or GSHPx may also fulfil other functions in the immune system, directly or indirectly related to the increased endogenous release of  $H_2O_2$  (Spallholtz, 1990).
3. Se changes blood platelet aggregation by decreasing the ratio of thromboxin to leucotrine production.

#### 3.3.4.8 The effect of Se on antibody production

The primary antibody reaction and total IgM concentration in both monogastric and ruminant animals are influenced by Se-deficiency. Se-supplementation can also increase serum IgG and the production of secondary antibodies in response to an antigen, which indicates that the helper T-lymphocyte dependent class is also affected by a Se-deficiency (Nicholson *et al.*,

1993). The immunological effects of Se-supplementation on antibody reactions is dependent on both age and type of antigen (Spallholz, Martin, Gerlach, & Heinzerling, 1973; Martin & Spallholz, 1976; Baalsrud & Øvernes, 1986; Jelinek, Ellis, Wroth, Sutherland, Masters & Petterson, 1988; Larsen, Moksnes & Øvernes, 1988a; Reffett, Spears & Brown, 1988a; Reffett, Spears & Brown, 1988b; Droke & Loerch, 1989; Hayek, Mitchell, Harmon, Stahly, Cromwell, Tucker & Barker, 1989; Stabel, Spears, Brown & Brake, 1989; Swecker, Eversole, Thatcher, Blodgett, Schurig & Meldrum, 1989; Ellis, Masters, Hustas, Sutherland, & Evans, 1990; Knight & Tyznik, 1990; Larsen, 1993). According to Baalsrud & Øvernes (1986) and Droke & Loerch (1989) the supplementation of both Se and vitamin E is necessary in horses and cattle to achieve an increased antibody reaction. In lambs, however, no additive effect was attained with supplementation of Se and vitamin E (Larsen *et al.*, 1988a; Reffett *et al.*, 1988b).

Spallholz *et al.* (1973) found that sodium selenite administered by intra peritoneal injection increased the primary immune reaction to ovine erythrocytes. These authors also found that the increase in primary immune reaction in response to ovine erythrocytes was largest when the Se was administered just prior to or simultaneously with the antigen.

Se-deficiency reduced glutathionene peroxidase and immuno-competence. Elevated Se of 0,7 – 2,8 ppm as sodium selenite increased antibody titres seven to 30 times respectively when challenged. The supplementation of Se in calves stressed by weaning, transport and *Pasteurella* haemolytic inoculation resulted in higher serum IgM concentrations and serum antibody titers (Boyazoglu, 1997).

Nemec, Hidioglou, Nielsen & Proulx (1990), found no effect of Se or vitamin E supplementation on IgG<sub>1</sub>, IgG<sub>2</sub>, IgA or IgM antibody levels in response to *Brucella abortus* as antigen. Results of Finch & Turner (1986) also showed that lambs with a marginal Se-deficiency had strong antibody titers in response to a bacterial antigen (*Salmonella dublin*) and that supplementation with Se only marginally increased the immune reaction.



In a study by Reffett *et al.* (1988a) calves receiving two different levels of Se-supplementation received a primary and secondary inoculation with IBR (infectious bovine rhinotracheitis virus), to evaluate the effects of Se-deficiency on the primary and secondary humoral immune response. Serum antibody titres did not differ between treatments 14 days after the challenge but were greater in Se-adequate calves than in Se-deficient calves 14 days after the second IBR challenge.

Nicolson *et al.* (1993) also did not observe large differences between antibody titres of Se supplemented and not supplemented cattle after challenge with ovine erythrocytes and ovalbumine. The results of this experiment support the proposal by Swecker *et al.* (1989) that blood Se levels of more than  $100\text{ }\mu\text{g L}^{-1}$  are necessary for optimum functioning of the immune system.

In the experiment by Swecker *et al.* (1989) calves received diets containing 20, 80, 120, 160 en  $200\text{ mg kg}^{-1}$  Se supplements. Increases in body mass was not affected by Se level of the diet, which suggests that  $20\text{ mg kg}^{-1}$  is sufficient for. IgG titres, in response to chicken egg lysosime, was higher in all groups receiving Se-supplementation greater than  $20\text{ mg kg}^{-1}$ . This data indicates that the immune system has a greater requirement for Se than growth.

Wright, Corah, Stokka & Blecha (1997) used 80 Hereford X Angus calves to evaluate Se and vitamin E combinations. The treatments were as follows: 1) basal diet only; 2) basal + 3 mg of Se/kg; 3) basal + .3 mg of Se/kg + 500 IU of vitamin E; 4) basal + .3 Se/kg + 1000 IU vitamin E; and 5) basal + .3 mg Se/kg + 1500 IU vitamin E. The basal diet was 60% rolled corn, 25% rolled oats, 10% soybean meal, and 5% molasses (as fed basis). Calves were temporarily separated from their dams before weaning and fed their assigned dietary treatments. All calves were vaccinated 17 d before weaning. At weaning, calves were revaccinated and shipped to a commercial feedlot. Antibody titres to IBR and BVD were not affected by treatment, and treatments did not affect serum haptoglobin concentrations. Moreover, treatments did not affect pre- or postweaning gain or transit shrink. The authors concluded that preweaning vitamin E and/or Se-supplementation did not influence postweaning

performance, stress responses, or vaccination responses in beef calves with adequate vitamin E status.

The effect of supplementation on the production of a specific antibody is not necessarily parallel to a change in the total immunoglobulin levels of that same isotype. Different isotypes (e.g. IgM and IgG) are also not necessarily affected in the same manner by supplementation (Reddy, Morrill, Minocha, Morrill, Dayton & Frey, 1986; Larsen *et al* 1988a; Swecker, Thatcher, Eversole, Blodgett & Schurig, 1995). It must thus be accepted that a proposed protocol where supplements are granted, has varying effects on different antibody reactions with different types of antigens (fore example, T-dependant in comparison to T-independent reactions). Different effects can also be observed during different stages of an immune reaction (fore example colostral, as opposed to serum antibodies) (Finch & Turner, 1996). The chemical form of the supplement may also be important: *in vitro* studies on cattle cells have shown that organic Se is more effective than inorganic Se in the stimulation of IgM synthesis (Stabel *et al.*, 1991). A further source of variation may be that animals without real deficiencies are used or that blood Se levels attained are not high enough to show large differences (Nicolson *et al.*, 1993).

#### 3.5.4.9 Se and Lymphocyte Production

Dietary deficiency of antioxidant micronutrients may influence T-lymphocytes to a greater extent than B-lymphocytes. T-cell membranes are more fluid than the membranes of B-cells and T-cell lipids are more sensitive to peroxidation than B-cell lipids. Se deficiencies in isolation or in combination with vitamin E, decreases lymphocyte reaction in response to mitogens (Larsen & Tollersrud, 1981; Turner, Wheatly & Beck, 1985; Larsen, Moksnes & Øvernes, 1988b; Lessard, Yang, Elliott, Rebar, Van Vleet, Deslauriers, Brisson & Schultz, 1991). Adult ruminants are apparently more resistant to the immunological effects of a Se-deficiency on lymphocyte proliferation than are the young. This can be explained in terms of increased availability of rumen microbial Se to lymphocytes (Turner & Finch, 1991).



The effective portion of the T-cell reaction in response to antigens is composed of both stimulation of lymphocytes and activation of phagocytes. In a study by Aziz & Klesius (1985) the mitogen induced production of leukocyte migration inhibiting factor was suppressed in lymphocytes of goats with a Se-deficiency, compared to goats receiving adequate Se. The interleucine-2 production and lymphocyte reaction in response to the mitogen, concavalin A, was however not affected. A decreased production of migration inhibiting factor may also have an effect in circumstances of chronic inflammation, which is associated with macrophage infiltration and activation.

Se-supplementation does not influence the production of IL-1 and IL-2 by macrophages and lymphocytes significantly. This observation suggests that the mechanism(s) responsible for the observed effects of Se on lymphocyte proliferation is independent of the levels of IL-1 or IL-2 (Kiremidjian-Schumacher, Roy, Wishe, Cohen, & Stotzky, 1990). The possible decrease in cell-mediated immunity with a Se-deficiency may be caused by a decrease in interleucine-2 (IL-2) receptor expression, which consequently limits IL-2 mediated cellular immune reactions (Kiremidjian-Schumacher *et al.*, 1990). It is suggested that Se modifies the expression of IL-2 receptors on the cellular surface, which may explain the decreased ability of lymphocytes to react to mitogens and antigens in Se-deficient animals (Larsen, 1993). Studies by Eskew, Scholz, Reddy, Todhunter, & Zarkower (1985) and Koller, Exon, Talcott, Osborne & Henningsen (1986) also showed that changes in the prolific ability of lymphocytes according to Se status, was not as a result of a change in the ability of macrophages to produce IL-1 or to destroy antigens.

Although the production of IL-1 is not influenced by the Se-status, modified secretion or arachidonic acid metabolites by macrophages may have important regulatory implications. GSHPx, and thus Se is necessary in the arachidonic acid cascade for the production of prostaglandines, thromboxanes, leucotrienes and other mediators of inflammatory and immune reactions. Cells with a deficiency of GSHPx and Se have a decreased conversion of the arachidonic acid cyclo oxygenase product, prostaglandin G<sub>2</sub> (PGG<sub>2</sub>), to the alcohol PGH<sub>2</sub>

(Spallholtz, 1990). Maddox, Reddy, Eberhart & Scholz (1991), have shown that there are definite effects of dietary Se on milk eicosanoid concentrations in reaction to *Escherichia coli* infection. Eicosanoids are a family of compounds derived from arachidonic acid and are the principle mediators of inflammation. Increased concentrations of thromboxane B<sub>2</sub>, prostaglandin F<sub>1α</sub>, prostaglandin E<sub>2</sub> and leucotrin B<sub>4</sub> were associated with significantly larger numbers of bacteria in the milk, possibly as a result of decreased destruction of *E. coli* by neutrophils of animals with a Se-deficiency. Increased inflammation and tissue damage, caused by endo-toxins and inflammatory mediators, will induce a higher tissue hydroperoxide concentration. During a Se-deficiency, this may lead to increased activity of cyclo-oxygenase and lipoxygenase enzymes, with consequent higher eicosanoid levels.

Turner *et al.* (1985), Finch & Turner (1989) and Stabel *et al.* (1991) found that the addition of sodium selenite to cell culture of ruminants increased the lymphocyte reaction in response to mitogens. The mitogen induced immunoglobulin production is also increased when serum or pooled plasma of animals with a high Se-status is added to cell cultures. This discovery indicates that the effects of Se transpire in the microenvironment of the cell rather than in the cell itself. Finch & Turner (1989) furthermore also observed that the amount of Se required *in vitro* to increase ovine lymphocyte reaction to the phytohaemagglutinin mitogen, was in the region of 1-10 ng Se/ml. Toxic effects were obtained above 1 g/ml. Similar results were obtained by Larsen *et al* (1988b). Sodium selenite or seleno-methionine at a concentration of 0.1 or 0.5: g/g added to the diet, increased the lymphocyte reaction of lambs in response to mitogens, while 1: g/g seleno-methionine had an inhibitory effect.

Pollock, McNair, Kennedy, Kennedy, Walsh, Goodall, Mackie & Crockard (1994) provided calves with a diet with insufficient vitamin E and Se and supplemented the animals with vitamin E and/or Se to test the possible interacting effects of these micronutrients on cellular immune reactions. *In vitro* lymphocyte proliferation was used to determine the effect of diet on cell-mediated immunity. It was found that this effect was dependent on the type of serum used



to cultivate the cells. These results indicate that the micro nutrient status of both the donor animal and of the cultivating medium must be considered when results of *in vitro* studies on lymphocyte function are interpreted.

#### 3.5.4.10 Se and Phagocyte Function

The production of free radicals is a prerequisite for the destruction of bacterial pathogens by phagocytes. The destructive process is usually limited to intracellular vacuoles, which contain the phagocitised pathogen. The reactive molecules, however, leak from the phagolysosome into the surrounding cytoplasm and intracellular space. These products may damage the cell if they are not detoxified. Neutrophils contain superoxide dismutase, catalase and glutathione / glutathione peroxidase to protect the cell against auto oxidation. During conditions of antioxidant deficiency, the cells may, however, be damaged by the reactive oxygen species (Larsen, 1993).

Se and/or glutathione peroxidase is found in phagocytic and other lymphoid cells. High specific activity of GSHPx was found in bone marrow, spleen and lymph nodes of sheep (Paynter, 1979). Scholz & Hutchinson (1979) found that leukocytes of dairy cows contained 4 times more GSHPx than erythrocytes. This and other examples of Se and GSHPx concentrations in immune tissue and related lymphoid cells demonstrate that Se and GSHPx serve important functions in these cells. The Se-requirement of these cells can be seen when animals are provided with Se-deficient diets. Organs like the liver, heart, kidneys and muscles lose Se relatively quickly, while other tissues, like lymphoid cells retain Se for relatively longer (Spallholz, 1990).

The ability of phagocytes to migrate towards a region of infection or inflammation may also be influenced by an animals Se / vitamin E status. Se-deficiency reduced the random migration and chemotactic reactions of goat neutrophils; either supplementing the animal itself or supplementing the isolated neutrophil *in vitro* (Aziz, Frandsen & Klesius, 1984) could reverse both these reactions. Aziz & Klesius (1986) also found that a Se-deficiency had the ability to limit the production of chemotactic factors, which play a key role in the

recruitment of cells to a region of infection. This decrease in chemotactic factors could be reversed by supplementation with Se.

Dietary deficiency of Se and/or vitamin E impedes macrophage and neutrophil activity. The Se-deficiency however is not associated with a decrease in phagocytosis (Grasso, Scholz, Erskine & Eberhart, 1990), although phagocytosis can be decreased where there is a deficiency of both Se and vitamin E (Turner & Finch, 1990). The intra cellular destruction of yeast and bacteria by macrophages and neutrophils of animals with a Se-deficiency was reduced (Grasso *et al.*, 1990). Grasso *et al* (1990) found that a Se-deficiency decreased the *in vitro* ability of neutrophils to destroy *E. coli* by 30%, and the ability to destroy *Staphylococcus aureus* by 50%. In a study by Serfass & Ganther (1975) neutrophils of rats with a Se-deficiency could not destroy *Candida albicans* after phagocytosis had taken place. Boyne & Arthur (1979) confirmed the fact that Se-deficiency in steers did not affect the ability of neutrophils to phagocytise *C. albicans*, but did affect the ability to destroy the ingested organism. The possible cause, being a decrease in GSHPx activity in the neutrophil, which would allow peroxide and lipid hydroperoxide to accumulate to toxic levels. It was also found that Se-deficient mice, infected with *C. albicans* died faster than mice receiving sufficient Se in their diet (Spallholtz, 1990). The oxidative ability of neutrophils isolated from goats (Aziz & Klesius, 1986) and cattle (Arthur & Boyne, 1985; Grasso *et al.*, 1990) receiving vitamin E sufficient but Se-deficient diets, was decreased. This limitation in function could be repaired by *in vitro* incubation with sodium selenite (Aziz *et al.*, 1984; Aziz & Klesius, 1986) or supplementation of the animal with Se and/or vitamin E (Aziz *et al.*, 1984; Politis, Hidiroglou, Batra, Gilmore, Gorewit & Scherf, 1995).

The finding that Se-deficiency reduces the microbicidal activity of neutrophils (Serfass & Ganther, 1975; Boyne & Arthur, 1979) suggested that low Se-status might be relevant to infectious diseases. Evidence from survey data (Arthur *et al.*, 1979) however suggests that less than 5% of herds will have a sufficiently low Se-status (<0.01 mg Se/l) for microbicidal activity to be decreased.



Considering that Se itself may be toxic and that it produces  $H_2O_2$  and  $O_2^{\cdot-}$  with oxidation of glutathion by selenite, it may be that dietary Se, *in vivo*, is cytotoxic in immune cells (Spallholtz, 1990).

#### 4.1.1.1 Description of the study area

Maputung is an elongated town situated on the southern coast of Mozambique, in southern Africa. The northern border of the town is defined by the Indian Ocean, which borders with Mozambique. It includes a number of hills, including the hills of the KNP together with the hills of the KNP. The town is situated on a hillside, and the surrounding area is characterized by dykes, grassy plains, and a number of hills. The town is situated on a hillside, and the surrounding area is characterized by dykes, grassy plains, and a number of hills. The town is situated on a hillside, and the surrounding area is characterized by dykes, grassy plains, and a number of hills.