Effect of TDS content of water on the selenium status and certain growth parameters of mutton sheep

Kabelo Kgomotsego Odirile Holele

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List of Acronyms:

ACTH	-	Adrenocorticotropin
ADG	-	Average Daily Gain
DM	-	Dry Matter
DMI		Dry Matter Intake
Ewt	-	Final mass
FCR		Feed Conversion Ratio
GFR		Glomerular Filtration Rate
GSH-Px	-	Glutathione peroxidase
L	-	Litre
SAMM	-	South African Mutton Merino
SAS		Statistical Analysis System
Ta	-	Ambient Temperature
TDS		Total Dissolved Solids
TR	-	Treatment
Vit E	-	Vitamin E
WHO	-	World Health Organisation
WI	-	Water Intake
WMD	-	White Muscle Disease
WQC	-	Water Quality Constituent
WQGIS	-	Water Quality Guideline Index System
[ ]	-	Concentration, e.g. [Se] = Selenium concentration

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Abstract

Selenium (Se) is an essential mineral in the animal body for effective metabolism and health reasons. It can also have toxic effects if ingested in amounts exceeding the body's metabolic requirements for extended periods (Underwood and Suttle, 1999). Its processes are not fully understood due to its intricate metabolism and its variable responses in the presence of other heavy metals (Rosenfeld, and Beath, 1964; Diplock, 1970: and Georgievskii, *et al.*, 1982). Arthur and Beckett (1989) and Echevarria, *et al.* (1986) noticed that both the deficiency and toxicity of Se affects a wide range of enzymes and metabolic processes. It may thus affect many pathways due to the changes in hormonal concentrations. Se has been observed in the groundwater of livestock across different regions of South Africa at concentrations exceeding the recommended guideline ranges (Casey and Meyer, 2001: Casey *et al.*, 1998; Department of Water Affairs and Forestry, 1996). The respective water was classified as potentially hazardous in some instances and unacceptable in others. It may help cause several health and reproduction problems in livestock and wildlife (Elsenbroek *et al.*, 2003). The marginal difference between adequacy and toxicity of Se in the animal body led to investigations into the effect of the total dissolved solids (TDS) content of the water on the selenium status and its effect on production parameters of mutton sheep over a ten-week period. The guideline limit for Se in drinking water is 0,05 mg / L (DWAF, 1996) with a target water quality range of 0,02mg / L (WRC, 2004). Se was supplemented at 0,7 mg / L during the trial and TDS was given at 3000 mg / L. Animals not receiving Se were less productive than those that received Se. Those that received both Se and TDS grew as well as those supplemented only with Se, but were more efficient. They seemed to accumulate less Se in the blood. Their excretory mechanisms and the functioning of their homeostatic controls against both Se deficiency, at first, and at a later stage against chronic selenosis, were more suitable. Further research exposing the animals for a longer time period and in an extensive production system would assist in quantifying these results.

Chapter 1. Literature Review

1.1. Introduction

Selenium (Se) has been researched quite extensively. It is both an essential mineral and a relatively problematic toxin. The difference between average daily body requirement and its toxic level, in terms of nutrient intake, is rather fine. Several studies have shown the differences between organic and inorganic Se as well as the differences between the Se present, the available Se and the biological activity of Se (Pehrson, 1993; Rosenfeld and Beath, 1964; Underwood, 1977). Other dietary constituents such as Vitamin E., copper (Cu), sulphur (S), and arsenic (As) also influence Se. Selenium occurring in the water of certain areas can serve as a relatively cheap source of this essential mineral.

Safety guidelines and regulatory standards in terms of Se have been compiled for drinking water in several countries as well as by several international bodies. These guidelines and standards often do not consider variations of available Se in solid foods ingested (linked to the amount of available soil Se, that can vary according to season, land use, region, etc.), the types of feed ingested and the resultant effect on metabolism of Se by the body. Physiological state (lactating, pregnant, etc.), activity level, type and size of animal or bird, health status, etc. are other factors that are also often neglected (Casey, e*t al.,* 1998). The World Health Organisation (WHO) stated 0,05 milligram Se per litre (mg Se / L) as the critical level of toxicity of Se in water.

Moseki (2001) found that the geohydrological characteristics of a subterranean water source were of importance in determining the chemical properties (mineral status) of the drinking water of the said source. Vast differences in water level and geochemical properties could occur in sources as close as 200m of each other. Elsenbroek *et al.* (2003) revealed that the presence of potentially hazardous water constituents have been suspected of causing adverse health effects in livestock. Groundwater may impose a potentially hazardous threat to rural communities sharing the same water source as their livestock (Casey, *et al.,* 1998) if this water contained a relatively high concentration of a potentially hazardous chemical. This chemical may accumulate in the body of the humans as well as their livestock – especially in the kidneys and liver – and may lead to an excessive intake of the mineral by the humans if these animals were milked and slaughtered for consumption purposes. The surveys of Casey, *et al*. (1998) show the relative frequency of occurrence of these practices under some rural communal livestock production systems in South Africa.

Casey and Meyer (1996) noted a difference between guideline levels for water quality constituents (WQCs) of different countries and that this is largely due to environmental differences. Guidelines based on South African conditions are relatively few and differ vastly from one another. The Department of Water Affairs and Forestry (DWAF) of South Africa published guidelines in 1996 that

noted a difference in the effect of a specific WQC of a certain concentration depending on the relevant site-specific factors.

Casey and Meyer (1996) suggest a target range for Se of between 0 and 50 µg Se / L (0 – 0,05 mg / L) drinking water. Between 50 and 75 µg Se / L results in chronic effects and above 75 µg Se / L leads to chronic as well as acute toxicity effects, according to these authors. Van Ryssen (2001) noticed relative variability with regards the Se status of animals in South Africa and the resultant assumptions as to the availability of environmental Se. The high rainfall areas of the KwaZulu Natal Midlands and the southern coastal region of the Western Cape province, both with predominantly acidic soils, resulted in marginal to acute deficiencies. The Gauteng Highveld and Western Cape Metropolitan areas with their medium to high rainfall and sporadic regions of water and air pollution result in sporadic dispersions of areas of deficiency, adequacy and toxicity. The vegetation obtained from the drier regions of the country indicates sufficient Se, but the subterranean water from these areas reveals Se concentrations greater than the allowable concentrations for drinking water (Casey *et al.*, 1998). It is important to note that there is a vast shortage of information regarding Se availability in South Africa.

Se toxicity may occur where plant matter of high Se concentration ([Se]) is consumed. Not many cases of Se toxicity of this manner have been noted in South Africa either due to the relative scarcity of Se-accumulator plants or, if they are present, their relatively unpalatable nature (Van Ryssen, 2001; James *et al.,* 1989). The relative scarcity of provision of supplements in rural communal livestock production systems (Casey *et al.,* 1998) could lead to the occurrence of an abnormal appetite for the animal (pica). The relatively unpalatable Se-accumulator plants may thus become a component of the animal's abnormal diet and could lead to the occurrence of selenosis. The drier regions of South Africa tend to have high subterranean Se concentrations. A large percentage of the rural communal livestock production systems are found in these areas. The threat of occurrence of selenosis is not only possible, but probable.

The tendency of Se to complex with heavy metals may indicate their presence in the groundwater. Some of these heavy metals may exacerbate the effects of Se and may cumulatively result in unproductive farming units (Elsenbroek *et al.,* 2003). Another aspect of rural communal livestock production systems is the frequency of interaction of the animal and owner. They often share the same water source, milk and slaughter the animals regularly for human consumption purposes (Casey *et al.,* 1998). The presence of potentially hazardous water quality constituents in the drinking water may affect the animal, but through the concentration of elements, the more serious the effects may be on the human population sharing the water source and consuming the animal products (milk and meat).

The aim of the trial was to quantify the effects of total dissolved solids (TDS) in water on selenium status and the resultant effect that it may have on the growth rate and certain production parameters of recently weaned mutton sheep.

1.2. Literature

Water quality constituents (WQCs) should not be considered as static requirements. Nutritional studies show that the nutritive requirements of animals vary with age and that this is related to their growth and development patterns. This is thus related to breed, farming system, physiological state, activity level, dietary factors (palatability, dietary proportions, etc.), environment and related factors (Casey, *et al.,* 1998). Selenium is a relatively well-researched mineral. Its processes are still not fully understood due to its intricate metabolism and its variable responses in the presence of other nutrients such as copper (Cu), arsenic (As), sulphur (S), molybdenum (Mo) and Vitamin E (Vit E) (Rosenfeld, and Beath, 1964; Diplock, 1970: and Georgievskii, *et al.*, 1982).

Mayland (1994) and Mayland, *et al.* (1989) outline the values of Se levels in the drinking water of animals. In groundwater [Se] generally ranges from less than 0,1 to 100µg Se / L (0,0001 to 0,1mg Se / L), but can be up to 1000µg Se / L. In drier areas where the groundwater has medium to high Se values the surface water can accumulate soluble salt concentrations due to the evaporation of the surface water. Volcanic eruptions and fossil fuel combustion can increase environmental Se levels. Safe drinking water levels are stipulated by USEPA in the USA as 10µg Se / L.

Selenium occurs in all body cells. It is an important physiological component of erythrocyte glutathione peroxidase (GSH-Px). GSH-Px protects haemoglobin from oxidation. Selenium as an anti-oxidant is a role-player in this process (Whitby *et al.*, 1988). It varies within the body according to tissue type, level of Se and chemical form of Se in diet (Underwood, 1977). According to Georgievskii, *et al.* (1982) the concentration of Se in the body of farm animals varies between 20 and 25 µg / kg body mass (between 0.02 and 0.025 mg / kg live mass, dependent on diet). Selenium is not evenly distributed across body tissues. It is highest in the liver and kidneys, followed by the intermediate concentrations of the pancreas, spleen, heart, muscular tissue, bones and blood (not necessarily in that sequence) and very low in adipose tissue. Underwood (1977) states that the concentration of Se in cardiac muscle is higher than that in skeletal muscle and that it is proportional to the dietary Se concentration.

In poultry, however, increasing the Se concentration to 0,8 mg / kg diet leads to an increase in the Se concentration in the liver and kidney tissues, but not in the concentration of muscle or blood Se concentration, especially if inorganic Se is used (Ross *et al.,* 1972). Increasing the body's Se concentration by feeding organic Se as found in soybean meal, fishmeal, etc. leads to higher increases in blood and muscle tissue Se as compared to the equivalent concentrations from selenite. Se in the blood and tissue cells of animals is in the form of seleno-proteins. If Se is consumed in the form of seleno-proteins it is metabolised more readily than if the same amount is consumed as selenite. The normal [Se]

in the kidneys of mature sheep is about 1 mg / kg dry matter (DM) (Underwood, 1977). It can be as high as 5 – 7 mg / kg DM. Beyond these values, however, the Se excretion tends to adjust according to Se absorption. The normal [Se] in the liver of mature sheep is around 0,1 mg / kg DM. Se in blood is highly correlated with dietary changes over a wide range. The blood of sheep fed a highly toxic Se concentration can be 1,34 – 3,1mg Se / L of blood. The normal Se in blood varies between 0,06 and 0,2 mg / L. A rapid rise in blood Se concentration (blood [Se]) is experienced with an increase in dietary Se. The red blood cells tend to display a higher [Se] than plasma cells.

Underwood (1977) states that [Se] in the hair and feathers of domestic animals increases with an increase in Se consumption and that in the hair of cattle [Se] is a good indicator of both Se deficiency and toxicity. An example is that of a trial in which cows with hair containing between 0,06 and 0,23 mg / kg DM Se was compared to those whose hair had a greater Se concentration than 0,25 mg / kg DM. The cows in the lower range all had calves that were sick or died from White Muscle Disease (WMD), while those whose hair had greater than 0,25 mg / kg DM Se had healthy calves. Twenty four (24) hours after the injection of a subtoxic, subcutaneous dose of radioactive selenate there were no changes in the [Se] of hair, skin, teeth and long bones of rats (Rosenfeld and Beath, 1964). Salbe and Levander (1989) noticed that hair Se turnover rates of rats were slower than those of the major body pools and that nail, hair and erythrocyte Se retention of adult rats were respectively about 50 %, 10 – 20 % and 65 % that of weanling rats. The same authors also questioned the authenticity of plasma Se levels as indicators of Se content of the major body pools. This seemingly arose over the fact that despite all the other indicators of Se levels used in their trial (hair, nail and erythrocytes) plasma values were the only ones that were higher in adult rats as compared to weanling rats. Whitby *et al.* (1988) also question the reliability of plasma Se levels, stating that its reference values vary with age, geographical location and pregnancy status. Diet also has an effect on the prevalence of Se in the milk of lactating animals (Miller, 1970).

1.2.1 Functions of Se

Selenium is necessary for growth and fertility in animals. The deficiency of Se results in impaired reproductive performance (Underwood, 1977; Godwin, 1970). Hens lay fewer eggs and these eggs display reduced hatchability if the hens are Se-deficient. The offspring usually have impaired hair or feather growth as well as body growth. Reproduction in rats is adversely affected by a Se-deficient diet. The supplementation of Se of 0,1 mg / kg DM as sodium selenite in the diets of these rats restores hair growth, body growth and reproductive abilities. The motility of spermatazoa increased almost linearly with dietary Se supplementation ranging from 0,01 to 0,08 mg / kg DM. Ewes that were Se-deficient experienced high embryonic mortality at 3 to 4 weeks after conception. Oestrus, ovulation, fertilisation

and early embryonic development occurred normally. The supplementation of Se prevented this early embryonic mortality at the time of implantation, but Vit E or an antioxidant had no effect.

Selenium is necessary for the prevention of liver necrosis, exudative diathesis, pancreatic fibrosis in poultry, hepatosis dietetica in pigs and WMD in calves, lambs and other species (Underwood, 1977; James *et al.*, 1989; Rosenfeld and Beath, 1964). Complemented by Vitamin E, Se is also known to be an antagonist against the toxicity effects of heavy metals (Mayland, 1994). Underwood (1977) states that Se can reverse the pancreatic fibrosis of chicks within two (2) weeks. Mayland (1994) noted a decrease in embryonic mortality in birds, muscle dystrophy and retained placentae in cows and mulberry disease in pigs when these Se-deficient animals were given Se supplementation. This author also notes that severe Se-deficiency leads to juvenile cardiomyopathy in young humans and seems to also be involved in the occurrence of chondrodystrophic disease in young Chinese children. Se-deficiency increases GSH-Px synthesis and release, increasing plasma GSH-Px (Hill and Burke, 1989). The activity of this GSH-Px may, in cases of severe Se-deficiency, however, be as little as 1 % that of animals with sufficient Se. Arthur and Beckett (1989) noticed that the deficiency of Se affects a wide range of enzymes and metabolic processes. It may thus affect many pathways due to the changes in hormonal concentrations. Ohlendorf (1989) noticed there was difficulty differentiating between the signs of Se and Vit E deficiency.

Selenium is absorbed more efficiently in monogastrics (85 %) as compared to ruminants (35 %). This may be due to the reduction of Se by the rumen microbes to non-available forms (Levander, 1989). Se-retention is, however, about 20 – 25 % of the ingested Se in ruminants compared to about 18 – 20 % in monogastrics (Georgievskii *et al.*, 1982). The GSH-Px activity of Se-deficient animals suddenly exposed to Se increases within the first 3 – 6 hours of receiving the Se, then decreases (Georgievskii *et al.,* 1982). Increasing the Se intake of Se-deficient humans decreased platelet aggregation and improved the activity of GSH-Px (Van der Torre *et al.*, 1989). A Se-deficient animal loses GSH-Px early in the depletion of its body Se reserves (Reiter *et al.*, 1989). This results in widespread changes, affecting hepatic enzyme activities.

Selenium is absorbed against the concentration gradient. It thus employs the use of an energy-consuming mechanism (Georgievskii *et al.,* 1982). Sulphur-amino acids and those of Se are seemingly absorbed by the same mechanism in the same part of the intestine. Selenium also enters the erythrocytes through the use of an active, oxygen-dependent transport system. It also readily crosses barriers of the ovaries and mammary glands. With Se supplementation of rats the Golgi apparatus experienced the most extensive and rapid accumulation of Se. The Golgi apparatus is important for protein modification and secretion. It could thus be responsible for Se extraction and distribution in times of Se-deficiency (Reiter *et al.*, 1989).

Vitamin E and Se seem to complement each other in improving the pregnancy rates of ewes, reducing incidences of birth of weak, premature or still-born calves as well as incidences of retained placentae in cows if injected or fed one month prior to mating and one month before parturition (Underwood, 1977). Pigs also display an improvement in farrowing percentages if given a small dose of both Vit E and Se 2 to 3 weeks prior to mating. Sheep grazing Se-treated pastures and those receiving Se from intra-ruminal pellets show increases in body mass and wool production without increasing wool fibre diameter (Whelan *et al.*, 1994). The dietary Se requirements of most animals is quoted as being between 0,05 and 0,3 mg / kg body mass, depending on the animal species and the level of Vit E in the diet (Ohlendorf, 1989). Georgievskii *et al.* (1982) describe Se as a bio-element. This is due to its (1) presence in basically all tissues; (2) therapeutic effect in certain diseases; (3) stimulating effect on development and wool growth in Se-deficient animals; (4) importance in the retina of the eye and the related photochemical reactions of light perception; and (5) affinity to α-tocopherol.

1.2.2 Se Toxicity

Se toxicity may occur where plant matter of high [Se] is consumed, where other dietary forms of Se are ingested or where Se is otherwise deliberately administered at too high a concentration (James *et al.*, 1989; Ohlendorf, 1989; Rosenfeld and Beath, 1964). Selenium accumulator plants may increase the environmental Se by the uptake of relatively unavailable forms of Se from the soils and converting them to more available forms. The ingestion of these plants will expose the animal to relatively high [Se]. On dying these plants may return this Se to the soils in more available forms. Mayland (1994) found that the faecal Se is almost completely unavailable to uptake by plants thereafter. James *et al.,* (1989) noted that, because high Se containing plants are relatively unpalatable, Se poisoning in this manner is relatively uncommon. Areas receiving less than 50 mm of rain annually and with alkaline soils are usually where high [Se] in the soils may be found. All livestock and humans are susceptible to Se toxicity. Selenite, selenate and organic Se generally result in similar toxicological effects (Mayland *et al.*, 1989). The absorption rates differ between chemical forms.

Several symptoms of selenosis have been noted (Rosenfeld and Beath, 1964; Underwood, 1977; Georgievskii *et al.,* 1982; Ohlendorf, 1989). These include reduced feed consumption, lesions in pigs and horses, emaciation, drowsiness, separation of the hoof, trembling of skeletal muscle, shivering during exercise, anorexia and weakness. Denudation of the spine (hair loss), stiffness and lameness, anaemia, blindness and abdominal pain may also occur. Excessive salivation, grating of teeth and thirst and starvation due to lameness and pain of sloughing hooves and the resultant reluctance to move around are other symptoms that have been associated with Se toxicity.

Toxicity varies according to amounts and chemical forms of Se ingested, duration and continuity of intake, nature of the rest of the diet and to some extent the species of the animal (Rosenfeld and Beath, 1964; Underwood, 1977; McConnell *et al.*, 1970). High protein diets seem to protect against Se toxicity (Underwood, 1977). Se from plant sources is readily available (80 %) as compared to animal sources (9 – 25 %) on a scale where the availability of sodium selenite is taken as 100 % (Mayland, 1994). Sodium selenite is among the more toxic forms of Se. Mayland *et al.* (1989) noticed that selenomethionine – an organic form of Se – is more readily absorbed into the animal body than selenite, selenate or selenocystine.

1.2.3 Interactions of Se

Selenium works closely with Vit E. and both have an antioxidant effect on body tissues. It is very difficult to differentiate the functions completely. They have complementary roles in the prevention of dietary hepatic necrosis and exudative diathesis (Mayland, 1994). They have a synergistic effect on lead poisoning in sheep and antagonise the toxicity effects of heavy metals. Underwood (1977) outlines the effect of Se quite extensively. Selenium is necessary for the prevention of various disease conditions that show variable response to Vit E. administration. These are diseases such as liver necrosis, exudative diathesis, pancreatic fibrosis in poultry, WMD in lambs, calves and other species and hepatosis dietetica in pigs. Selenium works together with Vit E. and is not merely a substitute for Vit E (Diplock, 1970). Godwin (1970) states that Vit E. seems not to influence tissue Se levels, but leads to better growth of lambs.

Se is absorbed in the body against the concentration gradient (Georgievskii *et al.,* 1982). There must thus be an energy-consuming active mechanism that assists in the absorption of Se. Sulphur- and Se-amino acids are seemingly absorbed by the same mechanism in the same part of the intestine in ruminants. This may be responsible for the fact that Se toxicity is reduced where the same amount of Se is provided, but where the [S] is increased. The addition of S to seleniferous soils tends to inhibit the uptake of Se by plants. High protein and sulphate intakes and / or feeding of As, Mercury (Hg) or Cu may alleviate Se toxicity (Mayland, 1994). These suggested quantitative interactions are not too well known and may be difficult under extensive conditions. They are, however, suggested due to the fact that Se has a tendency to complex with heavy metals. Elsenbroek *et al.* (2003) noted, however, in extensive conditions that a selenium-induced copper deficiency may be the cause of calf-diarrhoea and hypothyroidism in cows and consequently adversely affect reproduction due to the complex interactions of high concentrations of Se, Mo, Hg and lead (Pb) in drinking water.

Underwood (1977) noticed that faecal Se excretion increased and retention in body tissues decreased when subacute As injections were given together with Se. Selenium tends to protect against

cadmium (Cd) toxicity. There is a metabolic Se and Hg antagonism. Growth depression and high mortality caused by high silver (Ag) concentrations in the drinking water of poultry can be prevented by Se supplementation. Tungsten (T), As, S, Cu, dietary proteins, ACTH and the fat content of diets are other nutrients that have been noted to have an effect on Se metabolism (Rosenfeld and Beath, 1964; Yu and Beynen, 2001;Georgievskii *et al.*, 1982; Miller, 1970).

Georgievskii *et al.* (1982) found that selenium mostly occurs naturally as admixtures in sulphide-, phosphorite-, molybdate- and sulphur-ore deposits. The addition of As to diets of high [Se] in pigs reduces the deposition of Se in the liver, kidney, hair and muscles by up to 30 % compared to animals only given Se. Respiratory Se elimination is greatly reduced if As is included in diet at subacute level. Arsenic only slightly increases Se excretion via the faecal or urinary route. Rosenfeld and Beath (1964) suggest that if Se excretion is not increased in the presence of As, the Se must be bound in the body tissues in an inactive form. This will, however, mean that the tissues of animals treated with As should have a higher [Se] than untreated animals. This is not always the case. Irrespective of the method of administration of Se or As, the protective effect of As to Se toxicity seems to remain. The mechanisms of protection are, however, unknown (Rosenfeld and Beath, 1964).

The addition of S to the animal diet may reduce the growth depression caused by Se toxicity by up to 40 %, but has little effect on preventing liver damage (Rosenfeld and Beath, 1964). Blood Se levels are inversely proportional to dietary S levels (Mayland *et al.*, 1989). The addition of dietary S in feeds high in [Cu] and [Se] reduces hepatic [Se] and [Cu] (Van Ryssen, 1998). Increasing S intakes led to a reduction in the accumulation of both Cu and Se in the liver of sheep. Since these minerals tend to interact in exacerbating the chances of toxicity, the addition of S may result in a diminishing degree of interaction between Cu and Se. Sodium (Na), S and Se have all been shown to be absorbed in minute or negligible quantities by the stomach in the case of monogastrics and the abomasum and omasum of the ruminant species. The majority of these minerals are instead absorbed at the duodenum with smaller amounts at the ileum and jejunum. Meyer (1992) has shown an effect of sodium chloride (NaCl) on fluoride (F) retention in skeletal tissue. Elsenbroek *et al.* (2003) used NaCl to alleviate against a systemic Se deficiency caused by high concentrations of Se, Mo, Hg and Pb in the drinking water of Bonsmara cows.

In a trial by Rosenfeld and Beath (1964) increasing the dietary protein content from 10 – 30 % eliminated almost all the symptoms of Se toxicity caused by a diet containing 10 mg Se / kg DM, especially if casein was used as the protein source. Lactalbumin, wheat protein, linseed oilmeal, creatine, dried brewer's yeast, torula yeast and dessicated liver are other feeds used that offer considerable protection against selenosis (Mayland, 1994; Rosenfeld and Beath, 1964). Calcium disodium ethylenediaminetetraacetate is also quoted as having a protective effect of between 10 and 50 % on the effects of an intramuscular injection of between 7 and 10 mg of sodium selenate. These authors concluded

that since the selenosis symptoms (growth reduction, liver damage, etc.) were similarly reduced in their trials, true detoxification occurs. This may be through the Se being transformed into selenoproteins and other useful metabolic products that are less harmful to the body than the inorganic form. It may also be through increased Se excretion or conversion to unavailable forms by rumen microbes in the case of ruminants or the normal alimentary tract functions in monogastrics. This Se is thus channeled to more useful processes and assists in improving the efficiency of the body's metabolism.

Selenium has many valence states (Miller, 1970). It can thus be involved in different chemical or biochemical reactions in different forms, thus its inherent complexity of involvement in metabolic reactions (McNeal and Balistrieri, 1989). Regardless of the form of administration to farm animals – both monogastrics and ruminants – the body quite rapidly and efficiently absorbs Se (Georgievskii *et al.*, 1982). It enters the circulatory system and is distributed – though not evenly – to the body's tissues. The net Se absorption is higher in monogastrics (85 %) as compared to ruminants (35 %), as well as in Se-deficient animals as compared to those with adequate anatomic Se levels. Ruminants retain 20 – 25 % of the ingested Se as compared to the 18 – 20 % of monogastrics.

The weight gains of animals with adequate blood Se levels improved when they were supplemented with both Cu and Se, but not when these were provided individually (Mayland *et al.*, 1989). Yu and Beynen (2001) researching the effect of Cu and Se levels on rats, realised that Cu seems to have a protective effect on rats fed low or normal dietary Se. A high [Cu] increased [Se] in the liver and kidneys, but lowered that in the spleen. They conclude that the amount of dietary Se has an effect on the influence of [Cu] on Se metabolism. This may be compared to the results obtained by Hartmann and Van Ryssen, (1997) who noticed that hepatic [Se] increased when both Se and Cu were supplemented to sheep. Hepatic Cu retention and concentration both increased with an increase in dietary Se. Elsenbroek *et al.* (2003) noted both a selenium deficiency and a copper deficiency when Bonsmara cattle were exposed to potentially hazardous constituents in their drinking water from a subterranean water source. Casey *et al.* (1998) suggest the need for a water quality guideline index system (WQGIS) for mineral interactions and requirements based on site-specific factors. They also suggest that the assessment of the risk potential of Se should be done in conjunction with the sources of Se, Cu, Mo and sulphates.

Due to the rather numerous and relatively incompletely understood pathways of the metabolism and interactions of Se, it may be possible that an interaction could exist between TDS and Se. Weeth and Lesperance (1965) studied the effect of various salt and water loads on the renal function of yearling heifers. Using a 1 % NaCl water source to highlight the effects of this amount of TDS on drinking and related factors they noted an increase in saline water consumption. This led to water diuresis, but no apparent increase in total urinary urea, Ca, Na, Cl, and P excretion. They noted however, that dietary protein influences urine urea excretion. The studies of Pierce (1968) on saline waters on sheep over a 13-

month period showed an increase in water intake and a reduction in the concentrations of Na, K and Cl in the plasma of the sheep ingesting the saline water. This author also noted several differences between pen-fed and grazing sheep in terms of the effects of the saline water on their growth, reproductive and health parameters.

No adverse effects were observed on health, DMI or wool production of ewes given access to the saline water in an intensive feeding system. There were indications of a poorer reproductive rate in the case of saline water with a high Cl concentration. In an extensive grazing system the same type of saline water as above was provided. In this case it led to a clear reduction in reproduction, body weight gain of the lambs born, wool production ability of these lambs as well as an increase in diarrhoea and mortality of these lambs. Pierce (1966) showed a decline in DMI and body weight, but no adverse effects on the health of sheep given 1,5 % NaCl. There was no effect observed on the blood plasma concentrations of Na, K, Ca, Mg or Cl. When 2 % NaCl was used there was a significant increase in the blood plasma Cl value. Wilson (1966) also quotes 2 % TDS in drinking water as a critical level that either increased WI with little change in DMI, did not increase WI, but reduced DMI or led to a drop in both DMI and WI. The same author suggests that the acceptability or taste of food or water is a factor in determining the salt tolerance of sheep and that this is related more to TDS concentrations rather than total TDS volume.

Meyer (1992) suggests that livestock seem to refuse saline water at a level below that with which the kidney cannot cope. There exist many synergistic and antagonistic interactions between different minerals and their resultant interaction with the environment. In a survey of subterranean water done by this author, some subterranean water sources were found that contained supposedly toxic levels of certain minerals, but the water from these sources were acceptable to the animals. Other sources seemed to contain adequate levels of the minerals present, but these were unacceptable. If the animals were forced to drink this water they lost condition. Thus the toxic elements should not exclusively be stated to be at a toxic level without the complete analysis of the animal production system. In a trial by Casey *et al.* (1998) there was no significant difference of intake between groups of sheep supplemented with 0 mg, 0,1 mg and 1mg Se / L in their drinking water. Underwood and Suttle (1999) noted that chronic selenosis may occur if animals are exposed to a water source containing between 0,5 and 1 mg Se / L. This was also the Se concentration found in many groundwater sources sampled by Casey *et al.* (1998) and Casey and Meyer (2001) in several areas of South Africa. A concentration of 0,7 mg Se / L drinking water was decided upon for the purpose of the trial.

There are different zones of preference of TDS between small stock and large stock with sheep accepting a higher TDS content in their drinking water than cattle (Wilson, 1966; Casey *et al.*, 1998). Wilson (1966) stated that no significant variation in WI occurred for sheep given water containing up to 1 % TDS, but that both WI and DMI were likely to decline at 1,5 % TDS or higher. A TDS concentration of

3000 mg / L drinking water was used for the trial. It is within guideline ranges (DWAF, 1996), should not adversely affect production (Wilson, 1966; Pierce, 1966; and Weeth and Lesperance, 1965), but may be adequate to act as an alleviator treatment (Meyer, 1992) against the chronic effects of Se inclusion in drinking water at the chosen concentration. This concentration is also frequently observed in groundwater samples across different regions of South Africa (Casey *et al.,* 1998 and Casey and Meyer, 2001).

Chapter 2. Materials and Methods

Twenty four (24) South African Mutton Merino (SAMM) ram lambs were divided into four groups using a complete randomised block design. These four groups were assigned to one of four treatments: TR1, TR2, TR3 or TR4. The water used contained selenium and TDS in different quantities as shown below:

TR1. 0 mg selenium / litre in fresh water (<200 mg TDS / litre)

TR2. 0 mg selenium / litre in high TDS water (>3000 mg / litre)

TR3. 0,7 mg selenium / litre in fresh water

TR4. 0,7 mg selenium / litre in high TDS water.

The experiment consisted of a 2-week adaptation period and a 10-week treatment period. Feed and water were provided on an *ad libitum* basis. The different treatments were administered at the commencement of the treatment period. The feed chosen for the trial is Lucerne grown in an area known to have a minimal amount of Se in the soil. The Se in the Lucerne was negligible (0,011 mg / kg DM) and not sufficient to be considered as a supplementary source of Se. Lucerne was chosen so as to increase the water intake by resulting in a higher urea content in the urine (Weeth and Lesperance, 1965), a resultant higher urine clearance rate and thus an increased dose of the treatment (Ruckenbusch, Y. *et al.*, 1991). This should assist in getting clearer experimental results. Faecal samples were taken for further analysis. The animals were housed in individual open-air pens of 1,5 X 2m. A galvanised steel roof 4m above the ground provided shade for the animals during the hours of most direct overhead sunlight.

Three weeks prior to the start of the adaptation period all the sheep were dosed against internal parasites using 7ml of the commercial product Prodose Red®. They were also given access to Rumevite Cattle Block® to cater for their mineral requirements (Appendix A). Replensol® was used in cases of diarrhoea to replenish the animal's electrolytes that may have been lost as a result of this condition. Two severe cases of diarrhoea occurred in which Replensol® was dosed in conjunction with an injection of Sulfatrim 240®. Again a week prior to the adaptation period the animals were dosed as above against internal parasites and vaccinated against pulpy kidney. Mineral supplementation was discontinued.

The feed bins and water troughs were checked twice a day (09:00 and 17:00). Ambient temperature was also checked at these times (Appendix B). The feed intake in terms of dry matter intake (DMI), water intake (WI) and the ambient temperature (Ta) were taken note of on a daily basis. Several samples of the feed were collected on different dates during the trial, their mass taken and their dry matter content or percentage determined. These were later tested for Se content. The trial tests the effect that a toxic Se dose has on the mass gain, feed intake and water intake of growing sheep and also the possibility of interaction of a high TDS content on these Se effects.

The empty stomach mass of each sheep was measured at the beginning of the adaptation period, the end of the adaptation period, once every 3 weeks for the duration of the treatment period and at the end of the treatment period. The slaughter-out percentages of the animals were measured at the end of the trial when the animals were slaughtered. Whole blood samples were taken at the end of the adaptation period as well as every three weeks until the end of the trial. This was done by jugular venipuncture using lithium-heparinised vacutainers. These samples were tested for their selenium content. Whole blood is a good indicator of selenium status in that it responds to selenium supplementation and this response is a non-linear one (Van Ryssen *et al.,* 1998).

Faecal samples were collected four weeks prior to the end of the trial and on the last day of the trial using faecal collection bags. According to Van Ryssen *et al.* (1999) the amount of Se in the animal's body increases in a non-linear and decreasing rate after the commencement of ingestion. Underwood (1977) states that the excretion of Se in ruminants is mainly in the form of faeces, followed by urinary excretion and a small amount is excreted via exhalation and the skin. This differs slightly from the observations of Mayland (1994). This author noticed that the primary excretion route of Se in ruminants varies according to the method of Se administration and the animal's age. When Se is ingested it is mainly excreted as faeces. When it is given as an injection its main excretory route is as urine. In lambs and calves with an underdeveloped rumen, the main route of excretion of Se is as urine, even if it is ingested. As the rumen develops microbes transform the Se into unavailable forms that are excreted via the faeces. Nearly all the Se in faeces is unavailable to plant uptake. Rosenfeld and Beath (1964) noticed that Se may be eliminated via the respiratory tract in cases of chronic as well as acute selenosis. It can also be excreted as a component of urine, faeces or perspiration.

The sheep, at the initiation of the trial, were weaned about a month before and provided with *Eragrostis tef* hay *ad libitum.* These sheep were weaned from the same farm that the experiment was conducted. Stress due to climatic changes as a result of relocation as well as stress related to transportation prior to the trial was thus non-existent. Weaning stress may, however, have played a role during the trial.

The hypothesis was that an increased dose intake of selenium in water may be partly or fully counteracted by the effect of TDS on increasing the glomerular filtration rate (GFR) and tubular fluid speed, thus increasing renal excretion (Meyer, 1992, Elsenbroek *et al.,* 2003). This may concurrently increase renal Se excretion possibly decreasing the amount of Se retained in the body.

The SAS programme (SAS version 8.2, 2001) was used for all the statistical analyses analysing variance and general linear regression models. An F-test was used to test significance levels using the programme GLM of SAS.

Chapter 3. Results

3. 1. Blood Se Values

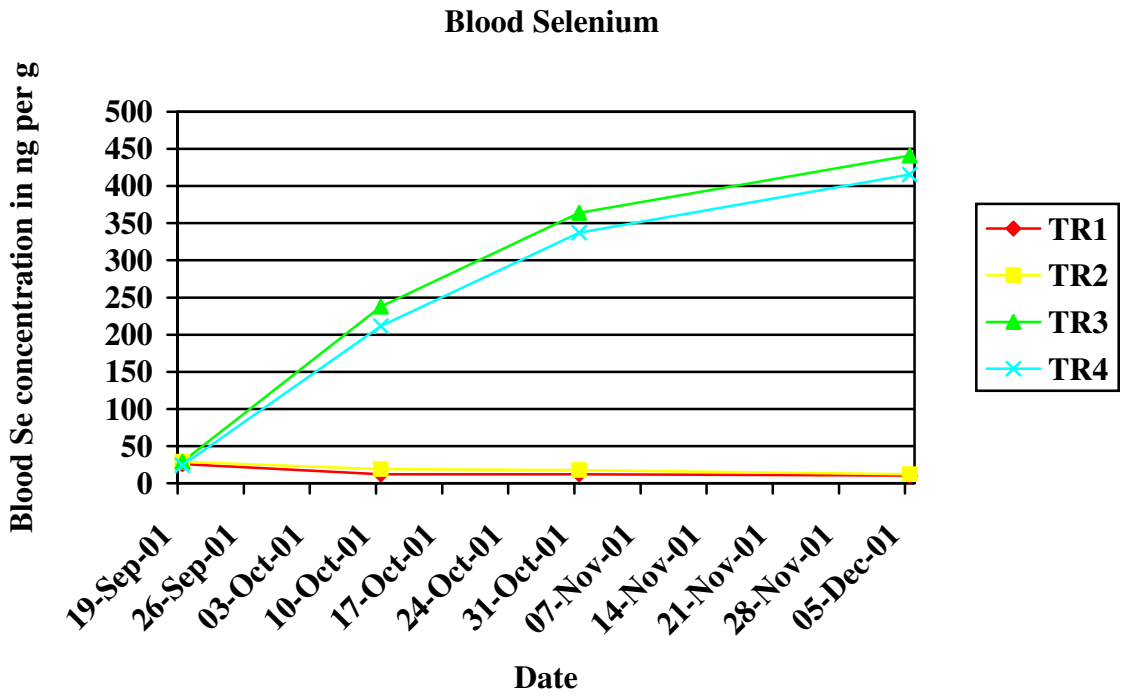

Figure 3.1. The values of selenium in the blood (in ng/g) of the ram lambs throughout the trial.

Figure 3.1. suggests that NaCl in a Se rich water source tends to decrease the rate of accumulation of Se in the blood. The statistical analysis of the LS Means of the blood values indicate that there is a significant difference between TR1 and TR3, TR1 and TR4, TR2 and TR3 and between TR2 and TR4 ($p < 0,05$). The Repeated Measures analysis reveals that there is a significant period effect ($p < 0,05$) as well as a combined effect of both the period as well as the Treatment ($p < 0, 05$). The TR3 (provided only with Se) blood Se values, though consistently above those of TR4, (given Se and the TDS alleviator treatment) were not significantly different.

3.2. Faecal Se Values

The main form of the excretion of Se by lambs under these conditions is by faecal means (Underwood, 1977 and Mayland, 1994). Faecal samples were collected 6 weeks after the commencement of the trial and once again on the last day of the trial. The results are indicated in Figure 3.2. and Table 3.1. below. The graph shows a consistently low excretion of Se by the animals not supplemented with Se (TR1 and TR2). At the collection of the initial faecal samples the amount of Se of the TR3 animals is

higher than that of the TR4 animals. With continued exposure to Se supplementation this picture changes. At the time of the second faecal collection, TR3 values were lower than those of TR4 (both Se and TDS supplementation). The excretion of Se by the TR4 animals seemed to increase much more rapidly than that of TR3 with continued exposure to conditions conducive to selenosis. Table 3.1. below also shows the cumulative excretion effects of Se (Van Ryssen *et al.,* 1999). In all the Treatments the excreted Se in the first sampling was lower than that of the second sampling.

Exposure to Se has a significant effect on the amount of Se excreted ($p < 0,05$). After 6 weeks exposure to trial conditions, TDS and the interaction of TDS and Se did not have a significant effect ($p < 0,05$) on the amount of Se excreted. After 10 weeks TDS and the interaction of TDS and Se had a significant effect ($p < 0,05$) on Se excretion. The Manova Test (SAS, 2001) revealed that time of exposure to the treatment did not have a significant ($p < 0,05$) effect on the Se excretion, but that the interaction of time of exposure and TDS as well as the interaction of time of exposure, TDS and Se had a significant effect ($p < 0,05$).

There was no significant effect of the interaction of time of exposure and Se. The LS Means of both the first and second faecal sampling shows a significant difference ($p < 0,05$) between the excreted Se concentrations of TR1 and TR3, TR1 and TR4, TR2 and TR3, TR2 and TR4, and TR3 and TR4. This also indicates an effect of the interaction of TDS and Se on the amount of Se excreted.

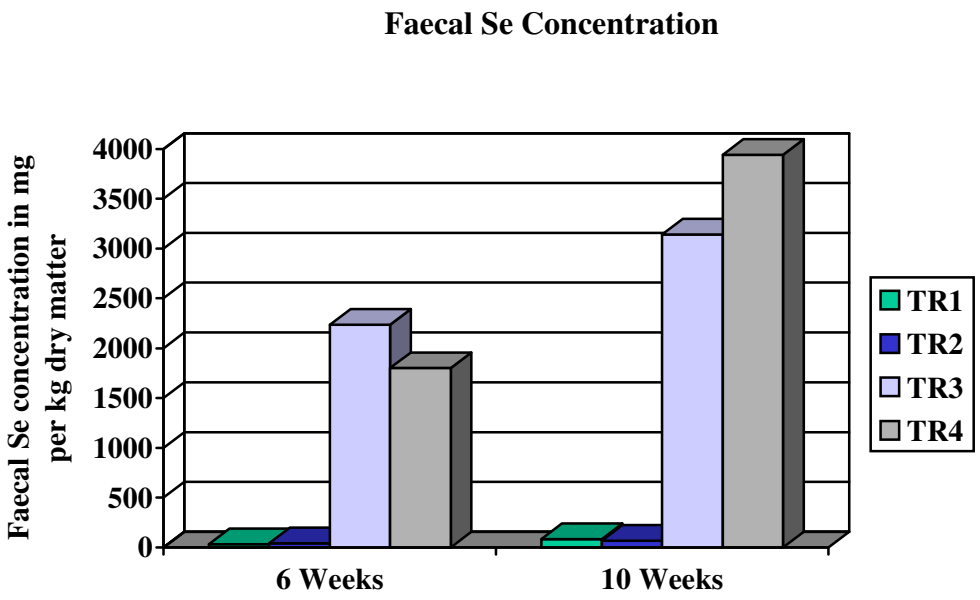

Figure 3.2. The faecal Se concentration as sampled after 6 weeks and 10 weeks exposure to trial conditions.

Table 3.1. LS Means and standard deviations of the faecal Se excreted by the SAMM in their respective treatment groups.

Sampling	TR1	TR2	TR3	TR4
1	29,49 ± 284,55[1]	43,80 ± 284,55[1]	3014,18 ± 449,91[2]	1798,71 ± 259,76[a]
2	84,26 ± 307,45[1]	68,30 ± 307,45[1]	2414,78 ± 486,12[2]	3939,67 ± 280,66[a]

Similar superscripts within a row indicate Means that are not statistically significant at $p < 0,05$.

3.3. Dry Matter Intake (DMI) and Water Intake (WI)

The TR1 and TR2 animals (those that received no Se) showed DMI averages of 62.274 kg and 64.856 kg, respectively. WI stood at 178.4 L and 207.1 L. There is a clearer difference between the water ingested by the two Treatments (16 %), as compared to DMI (4 %). The increased WI of TR2 is in line with studies suggesting that a higher solute concentration in the drinking water of animals leads to a

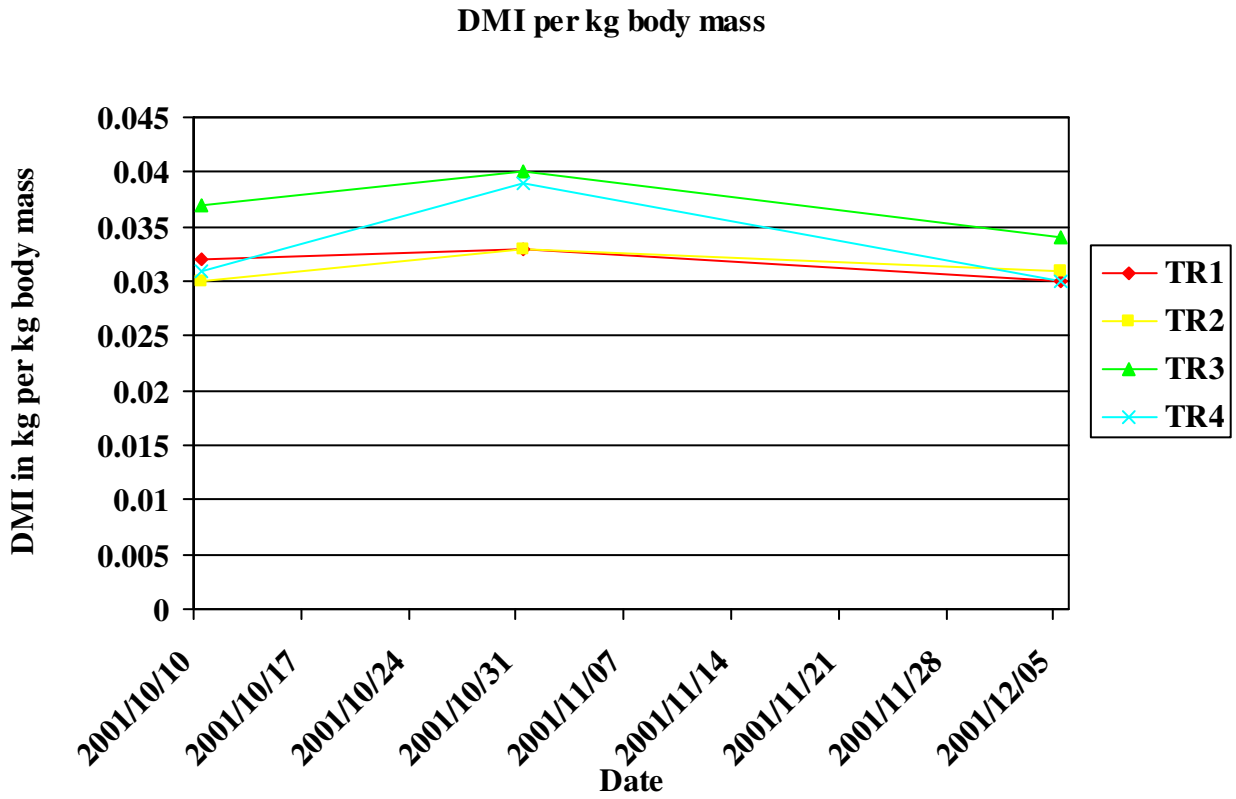

Figure 3.3. The dry matter intake (DMI) of the ram lambs on a kilogram DM per kilogram body mass.

higher WI up to a certain level of solute concentration (Weeth and Lesperance, 1965). On a litre per kilogram body mass basis the WI of TR2 was slightly higher than that of TR1 (Figure 3.4.).TR1 ingested 2.86 L of water per kg feed and TR2 ingested 3.19 L. The average DMI for TR3 and TR4 (the groups that were given 0.7 mg / L Se) were 81.229 kg and 74.854 kg, respectively for the entire trial.

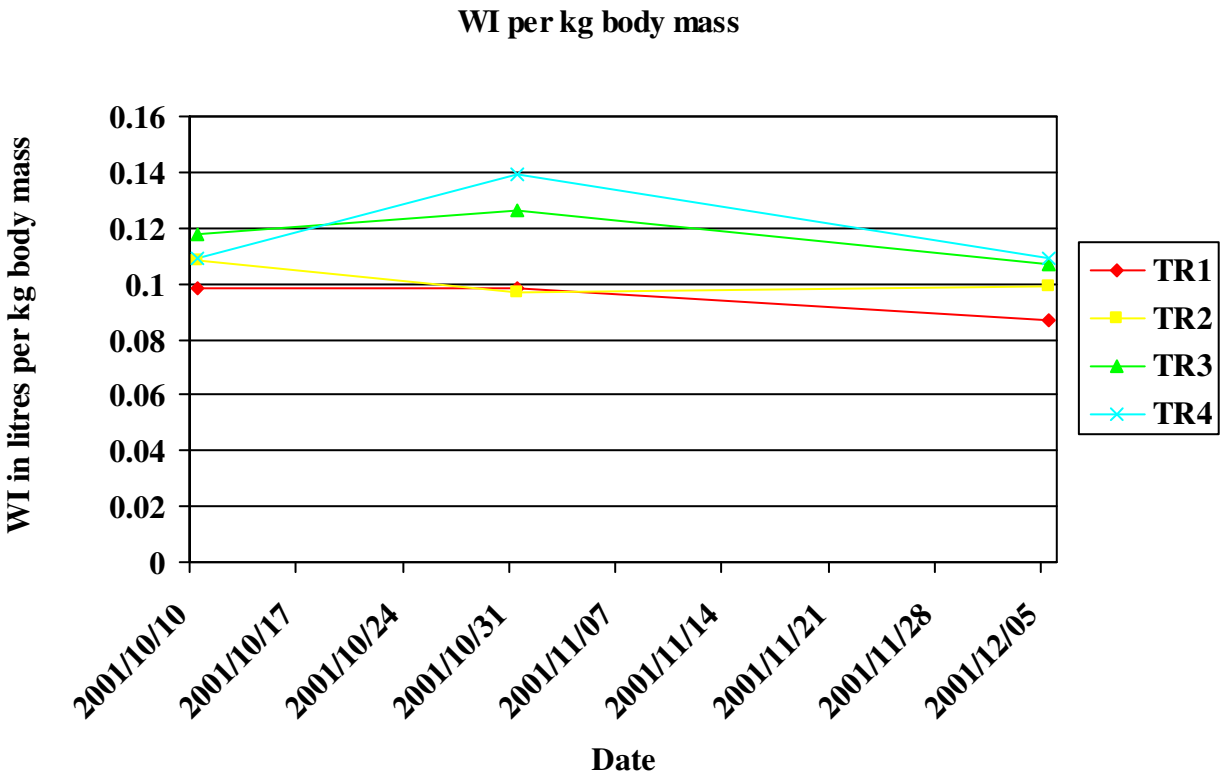

Figure 3.4. The water intake (WI) of the ram lambs throughout the 10-week period (in L per kg body mass).

WI was 255.43 L and 252.33 L. This difference for WI was not significant and disagreed with the expectations that the higher TDS concentration in the water supplied to TR4 animals would result in an increase in thirst. These values of similar WI between TR3 and TR4 animals may indicate that the increased Se ingestion may have had an effect on the influence of a high [TDS] on total water consumption. Further research may verify this hypothesis. The amount of water ingested per kg DM of feed was 3.14 L / kg and 3.37 L / kg, for these two Treatments.

In both the instances of access to Se and no access to Se the groups exposed to a high TDS concentration in their water consumed more water per kg DMI. It is important to note that due to the choice of feed containing low Se levels and no exposure to an alternate feed source the TR1 and TR2

animals may have been exposed to a marginal Se-deficiency. This may be the reason for their poor growth performance (Underwood, 1977, Church and Pond, 1988). Throughout the trial Se had a significant effect ($p < 0,05$) on DMI. This was especially the case from week 3 - 6 of the trial. The Manova test (SAS, 2001) revealed that the length of exposure of the sheep to the trial conditions had a significant effect on the DMI ($p < 0,05$).

In the first third of the trial there was a significant difference between the DMI values of TR1 and TR3, TR1 and TR4, TR2 and TR3 and TR2 and TR4 ($p < 0,05$). These differences were similarly continued into the second third of the trial period. During the final third of the trial there was a significant difference between TR1 and TR3, TR1 and TR4 and TR2 and TR3 ($p < 0,05$). The cumulative DMI over the entire trial period showed a significant difference between the means of TR1 and TR3, TR1 and TR4, TR2 and TR3 and between TR2 and TR4 ($p < 0,05$).

Figure 3.3. on the previous page indicates the difference between the Treatments receiving Se and those not receiving Se in their water in terms of DMI. This signifies the necessity of Se as a dietary mineral. The DMI of TR3 seemed higher than that of TR4, but the difference was not statistically significant. The DMI of TR1 and TR2 didn't differ much either. The difference between DMI measuring at different periods of the trial was significant. This may be due to the fact that these were growing lambs chosen for the trial immediately after weaning, thus ingesting higher volumes per individual with continued increases in body mass. Their DMI per kg body mass increased at first then slowly declined and is in line with the normal DMI patterns of growing animals. However, the average daily gain (ADG) graph, indicates that the second period of the trial resulted in a somewhat contrasting picture to the ones signified by the first (1^{st}) and third (3^{rd}) periods. This could be related to an adaptation mechanism of the animals to the TDS and Se treatments as the TR1animals (the control group) did not show this contrast.

The WI of TR1 and TR2 hovered around 2.5 L / day, while those for TR3 and TR4 were about a litre more per day. The WI of TR3 was less than that of TR4 for the major part of the trial period. On a litre per kg body mass basis WI was similar to DMI in that it initially increased then seemed to slightly decrease. It has been shown that an increase in DMI leads to a corresponding increase in WI (Ruckenbusch *et al.,* 1991). The statistical analysis of WI revealed a significant ($p < 0,05$) Se effect on WI. This was especially significant ($p < 0,0001$) during the 3 – 6 week period of the trial. TDS and the combination of TDS and Se did not have a significant impact on WI. The Manova test shows that the length of exposure to the trial conditions had a significant effect ($p < 0,05$) on the treatment animals as compared to the controls. The combined effect of time of exposure to the trial conditions as well as Se

and also the combined time of exposure, TDS and Se effect also had a significant ($p < 0,05$) effect on the WI of the animals exposed to these trial conditions.

Table 3.2. Least squares means and standard errors for daily water intake (WI) in litres between the Treatments by 3 week periods in SAMM ram lambs exposed to the stated drinking water treatments.

Period	TR1 (0 mg / L Se; <200 mg / L TDS)	TR2 (0 mg / L Se; 3000 mg / L TDS)	TR3 (0,7 mg / L Se; <200 mg / L TDS)	TR4 (0,7 mg / L Se; 3000 mg / L TDS)
1	2.20 ± 0.23[1]	2.48 ± 0.23[1]	2.93 ± 0.23[a]	2.83 ± 0.25
2	2.38 ± 0.23[1]	2.39 ± 0.23[1]	3.32 ± 0.23[a]	3.88 ± 0.25[a]
3	2.38 ± 0.21[1]	2.84 ± 0.21[12]	3.45 ± 0.21[2a]	3.64 ± 0.23[a]

Similar superscripts within a row indicate Means that are not significantly different at $p < 0,05$.

The mean of WI during the 3 – 6 week period was significantly different from that of the 0 – 3 week trial period ($p < 0,05$). The WI of the ram lambs given Se in their water significantly improved in both the 3 – 6 week and 6 – 10 week trial periods ($p < 0,05$). This was, however, not evident when WI was considered on a litre ingested per kg live weight. There was a significant difference between the WI of TR1 and TR3 animals in the first third of the trial ($p < 0,05$). In the second three-week period there was a significant difference between the WI of TR1 and TR3 animals, TR1 and TR4, TR2 and TR3 and the TR2 and TR4 ram lambs ($p < 0, 05$). The WI of the 6th – 10th week showed a significant difference ($p < 0,05$) between TR1 and TR3, between TR1 and TR4, and between TR2 and TR4. When the WI of the entire trial period is analysed a significant difference ($p < 0,05$) is realised between TR1 and TR3, TR1 and TR4, TR2 and TR3, and between TR2 and TR4.

The GLM procedure with class variables of TDS and Se revealed that only Se had a significant effect on WI as compared to TDS and the interaction of TDS and Se. There was no significant difference between the groups that either were or were not given a high concentration of TDS in their water. The Repeated Measures Analysis of variance, however, shows a significant effect of the interaction of Se and TDS in the 3 to 6 week period, as compared to the 0 – 3-week period ($p < 0,05$). The LS means for the interaction of TDS and Se was significant between TR1 and TR3, TR1 and TR4, and TR2 and TR4.

The above outcomes may possibly be explained by the results of a statistical analysis of WI readings taken on a weekly basis. The PROC GLM analysis shows no significant effect of Treatment on the WI of week 1. Week 2 to week 10 of the trial shows a significant impact on WI ($p < 0,05$). An analysis of the LS Means of the WI on a weekly basis reveals no significant difference between the LS Means of the Treatments in the first week. Week 2 shows a significant difference between the WI values of TR1 and TR3 and between TR1 and TR4 ($p < 0,05$). Week 3, week 6, week 7 and week 9 revealed a

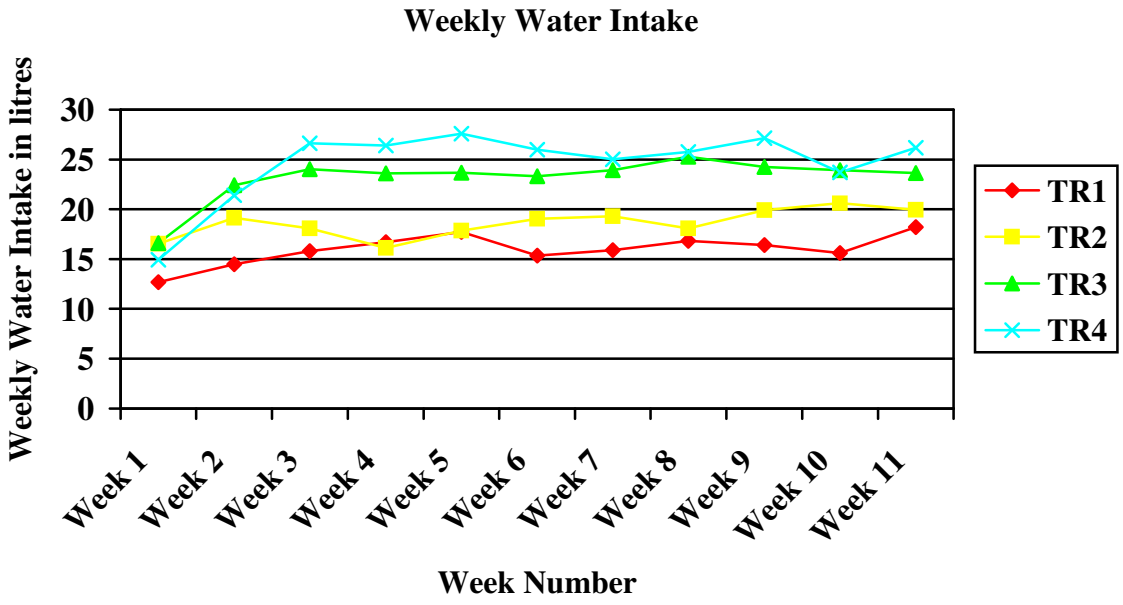

Figure 3.5. The weekly water intake amount of the SAMM ram lambs (in litres).

significant effect on the WI of TR1 and TR3, TR1 and TR4 and between TR2 and TR4. Week 4 and Week 8 showed significant WI differences between TR1 and TR3, TR1 and TR4, TR2 and TR3 and between TR2 and TR4 ($p < 0,05$). Week 5 had significantly different values between TR1 and TR4 and between TR2 and TR4 ($p < 0,05$). Week 10 only showed significant differences between TR1 and TR3 and between TR1 and TR4 ($p < 0,05$).

Table 3.3. Least squares means and standard errors for weekly water intake (WI) in litres between the Treatments received by the SAMM ram lambs exposed to the stated drinking water treatments.

WEEK	TR1 (0 mg / L Se; <200 mg / L TDS)	TR2 (0 mg / L Se; 3000 mg / L TDS)	TR3 (0,7 mg / L Se; <200 mg / L TDS)	TR4 (0,7 mg / L Se; 3000 mg / L TDS)
1	12.67 ± 1.62[1]	16.51 ± 1.62[1]	16.60 ± 1.62[1]	14.91 ± 1.78[1]
2	14.48 ± 1.92[1]	19.13 ± 1.92[1a]	22.43 ± 1.92[a]	21.39 ± 2.11[3]
3	15.79 ± 2.41[1]	18.06 ± 2.41[1a]	24.03 ± 2.41[a3]	26.64 ± 2.64[3]
4	16.68 ± 2.13[1]	16.11 ± 2.13[1]	23.63 ± 2.13[a]	26.41 ± 2.34[a]
5	17.70 ± 2.02[1]	17.84 ± 2.02[1]	23.68 ± 2.02[1a]	27.60 ± 2.22[a]
6	15.34 ± 1.64[1]	19.04 ± 1.64[13]	23.34 ± 1.64[3a]	26.77 ± 1.80[a]
7	15.88 ± 1.68[1]	19.29 ± 1.68[13]	24.03 ± 1.68[3a]	25.72 ± 1.84[a]
8	16.81 ± 1.94[1]	18.06 ± 1.94[1]	25.29 ± 1.94[a]	26.22 ± 2.13[a]
9	16.40 ± 1.66[1]	19.90 ± 1.66[13]	24.26 ± 1.66[3a]	27.15 ± 1.82[a]
10	15.60 ± 2.06[1]	20.60 ± 2.06[a]	23.92 ± 2.06[a]	23.70 ± 2.26[a]

Similar superscripts within a row indicate Means that are not statistically significant at $p < 0,05$.

The Manova Test revealed a significant effect of week on WI. Week 2 was significantly different from weeks 1, 4 and 5 ($p < 0,05$) and was not statistically different from weeks 3, 6 – 10. Weeks 3 to 9 showed some similarities and can be taken as the leveling out of the WI by the respective groups.

3.4. Average Daily Gain (ADG)

The ADG figures of the animals show a slight, but consistent increase in the control (TR1) animals. The growth thus seems consistent with the normal growth curve of recently weaned sheep of this age. All the other Treatments show a drop in ADG in the second period as compared to the first and third periods. The ADG of TR3 and TR4 is significantly higher than that of TR1 and TR2 in the first three weeks, not significant in the 3 – 6 week period, and once again significant in the 6 – 10 week period. The positive effect that Se has on growth parameters is shown here. Both TDS and the interaction of TDS and Se have no significant effect on ADG, whereas time of exposure and the interaction of time of exposure and Se have a significant ($p < 0,05$) impact on ADG. There's a significant ($p < 0,05$) contrast in the ADG values of the 3 – 6 week trial period as compared to the first 3 weeks. There's a significant difference between the ADG values of the TR1 and TR3 animals, the TR1 and TR4 animals and the TR2 and TR4 animals ($p < 0,05$) in the first period. No significant differences were displayed in the 3 – 6 week period. In the final four weeks of the trial the only significantly different ADG values were between TR1 and TR3 and between TR1 and TR4 ($p < 0,05$).

Table 3.4. Least squares means and standard errors for average daily gain (ADG) in grams between the Treatments by 3 week periods in SAMM ram lambs exposed to the stated drinking water treatments.

Period	TR1 (0 mg / L Se; <200 mg / L TDS)	TR2 (0 mg / L Se; 3000 mg / L TDS)	TR3 (0,7 mg / L Se; <200 mg / L TDS)	TR4 (0,7 mg / L Se; 3000 mg / L TDS)
1	65.08 ± 28.84[1]	93.65 ± 28.84[1]	160.32 ± 28.84[a]	192.38 ± 31.59[a]
2	88.89 ± 18.57[1]	76.19 ± 18.57[1]	73.02 ± 18.57[1]	97.14 ± 20.34[1]
3	90.37 ± 19.88[12]	123.81 ± 19.88[2]	164.76 ± 19.88[2a]	161.14 ± 21.78[2a]

Similar superscripts within a row indicate Means that are not statistically significant at $p < 0,05$.

The animals receiving Se in their water (TR3 and TR4) gained mass more rapidly and with a better feed conversion ratio than those not given Se (TR1 and TR2). This trial's TDS content was kept at about 0,3 % (Meyer, 1992). TDS did not have a significant effect on dry matter intake (DMI), water intake (WI), final mass (Ewt), FCR and ADG. Se, on the other hand, significantly improved all of these. This is consistent with other research done on Se in the diet of animals (Oh *et al.,* 1976; Van Ryssen *et al.,* 1999; Van Ryssen and Mavimbela*,* 1999, Underwood, 1977; Whelan *et al.*, 1994).

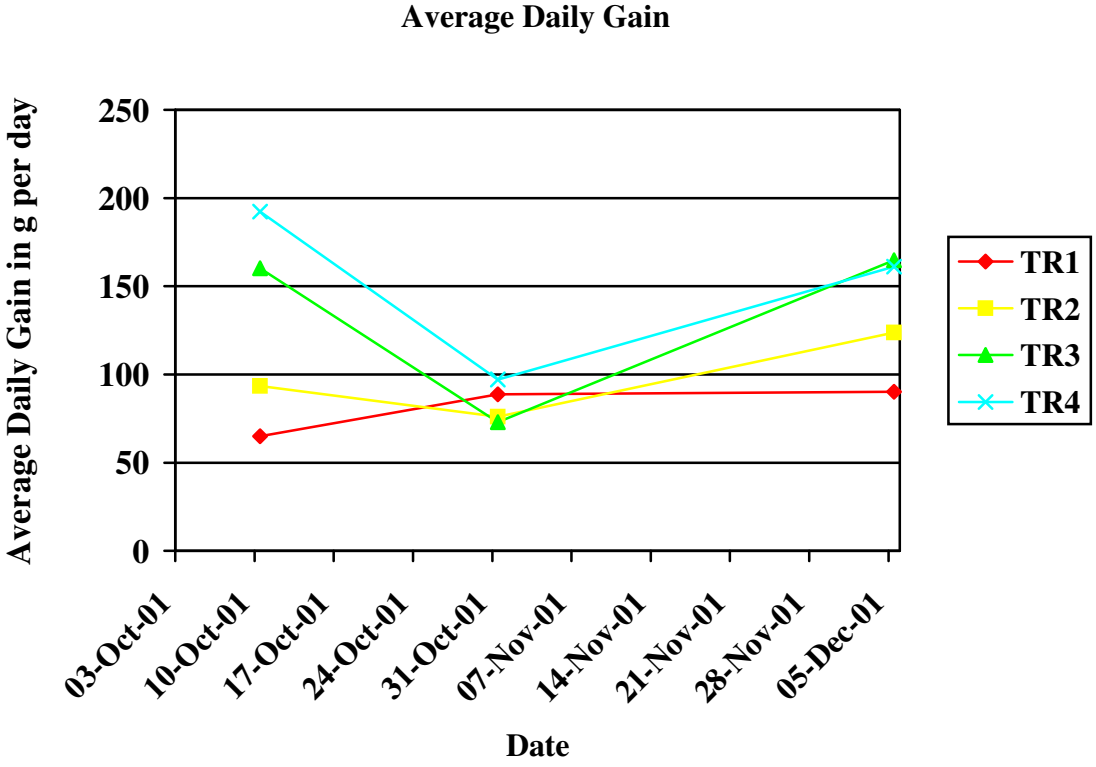

Figure 3.6. The average daily gain (ADG) in grams of the SAMM ram lambs throughout the trial period.

Chapter 4. Discussion

There is a narrow margin between deficiency and toxicity for Se. It is an essential mineral and has to be part of the daily diet. In some instances, however, it may be available in abundance in the drinking water of animals or in excessive supply through the feed. Supplementation of Se through licks or concentrates as a blanket approach to supplementation without considering other possible sources of Se in the farming system (available grazing material, groundwater, etc.) could lead to Se toxicity. Blood is often the most sampled indicator of the mineral status of an animal due to its comparatively simple sampling methods and its relative reliability. It may be advisable to slaughter an animal to sample the kidney, liver and / or thyroid gland for a more complete picture. This is due to the fact that Se often complexes with heavy elements and may seem adequate in the blood, but approaching toxic levels in other organs. Hartmann and van Ryssen (1997) suggest that Se accumulation in the blood of sheep does not increase in a linear fashion and that, when animals are suddenly exposed to Se in their diet, they should be given at least ninety (90) days to allow for the stabilisation of blood [Se]. The kidneys and liver are the body organs of highest [Se] under normal conditions of Se exposure as well as where the animals are exposed to conditions that may induce selenosis (Rosenfeld and Beath, 1964; Underwood, 1977). These organs are also the places where the highest concentrations of other heavy metals may be found.

The blood Se values of TR1 and TR2 were very much similar and far below those of TR3 and TR4. TR3's blood Se levels were consistently above those of TR4. This difference was not significant, however. As the period of exposure as well as the concentration of Se in the drinking water did not result in symptoms of acute selenosis, the period of exposure was most probably not long enough for a significant increase in the body burden of Se. Due to the consistency of TR4 blood Se levels being below those of TR3, it may be assumed that TR3 animals will probably reach toxic Se blood levels before the animals given an alleviator treatment (TR4). The combined effect of TDS and Se showed the existence of an interaction between these parameters. This may suggest that by increasing or decreasing the TDS content of water with a high Se content one may influence the Se-retention by the animal's body. Faecal Se increased throughout the trial for all the Treatments, although this increase was not significant for TR1 andTR2. The amount of Se excreted by TR3 was higher than that of TR4 after six weeks of exposure to 0,7 mg Se / L drinking water, but was lower after ten weeks. This measure is not cumulative and was taken on a per kilogram dry faecal matter.

The faecal Se excretion of TR4 tended to increase at a slow rate at first and then increase more rapidly than that of TR3. The feed provided to the animals was deficient in Se (0,011 mg / kg DM). The lower faecal Se excretion of the TR4 animals as compared to the TR3 animals may be indicative of greater retention of Se by the TR4 group of animals, when exposed to conditions suggestive of Se

deficiency. With continued exposure to the stated trial conditions, the TR4 animals seemed to increase the Se excretion much more rapidly than TR3. The blood Se values of TR4 were not higher than those of TR3 at any time of the trial, suggesting that the retained Se may be stored in the kidney, liver or other organs. As conditions approached Se toxicity the clearance rate of TR4 animals was more efficient than TR3. This may suggest a more efficient metabolic functioning of the body of the animal exposed to high Se in their water and given a TDS alleviator treatment. This may also be the reason for the significant improvement in the health and reproductive status of the Bonsmara cows of Elsenbroek *et al.* (2003) exposed to potentially hazardous water and given a TDS alleviator treatment at 3000 mg TDS / L drinking water. TDS may reduce the negative effects attributed to Chronic selenosis by alleviating whole blood concentration increases.

The combined effect of TDS, Se and time of exposure was significant between TR3 and TR4. This shows an effect of these parameters in combination on faecal Se excretion. The LS Means of both the first and second faecal sampling shows a significant difference ($p < 0,05$) between the excretory Se levels of TR1 and TR3, TR1 and TR4, TR2 and TR3, TR2 and TR4, and TR3 and TR4. The significant difference ($p < 0,05$) between the values of TR3 and TR4, suggests the possibility of an interaction between TDS and Se on faecal Se excretion values. Thus by varying TDS and / or time of exposure to a high Se water source one could influence the amount of Se excreted. This may explain the consistently lower blood Se levels of the TR4 animals as compared to the values of the TR3 animals. It thus seems to affect the susceptibility of animals to a potentially toxic Se water source. It may also be helpful to note that the TDS content of the water used in this trial was 0,3 % and is far below the 1,5 % shown to negatively affect health, reproduction or WI (Pierce, 1966; Pierce, 1968; Wilson, 1966).

TDS seemed to have an effect on the amount of water ingested per kilogram DM ingested. It didn't, under these trial conditions, show any significant effect on WI or DMI. There was no combined effect of TDS and Se on WI. This gives the indication that TDS only affects the amount of water ingested per kg DMI. There also exists a significant interaction ($p < 0,05$) between time of exposure, TDS and Se on the WI values experienced. It may be possible that the varying of one or more of these factors may influence WI. This may lead to a reduction or increase of an ingested amount of Se if the Se in the water is constant. The author is of the opinion that reducing WI in order to reduce the dose of Se ingested may not be a productive solution for continued efficient functioning of the animal's metabolism and may negatively affect production parameters. The effect of an alleviator treatment on both blood and faecal Se values indicates that a possible increased ingestion of the Se-rich water as a result of a higher TDS content may be more beneficial than not providing TDS as an alleviator. The TDS treatment used for this trial was laboratory quality NaCl. At an inclusion rate of 0,3 % of the volume of water (3000 mg per litre) it is a more affordable option than piping in water from elsewhere or drilling another subterranean water

source of which the quality may only be tested after completion. A significant increase in WI was effected by the ingestion of a high Se amount at the 3 – 6 week and the 6 – 10 week periods (both $p < 0,05$) as compared to the 0 – 3 week period. This may highlight the importance of Se on the animal body's metabolism. This may also be a secondary effect of the result of an increase in DMI as effected by Se (Underwood, 1977). There is a significant interaction of TDS and Se as highlighted by the Repeated Measures Analysis of variance in the 3rd to 6th week. This shows an effect of interacting TDS and Se amounts to influence WI for just a while, about a month after initiation of the trial, but this does not seem to continue for too long. This may indicate a heightened glomerular filtration rate as the ram lambs adjust to the treatment and try to excrete more TDS in order to maintain their salt-water balance, thus effecting an increase in WI (Ruckenbusch *et al.*, 1991).

The weekly analysis of WI shows similarities between weeks 1 and 10. This may indicate high stress levels on the ram lambs as these were the weeks of the most intense working hours on the sheep. Week 1 was highlighted by blood sampling, mass taking of the animals and for some animals the changing of the taste of water. Week 10 was when faecal collection bags were placed on the animals, blood samples were taken and the animals were weighed and were separated over a three-day period when they were chosen for slaughter. Weeks 2, 3, 6, 7, 8 and 9 were relatively similar and were highlighted by significant differences between the WI of the animals receiving Se and those not. The picture drawn by this seems to suggest low to normal intakes within the first week of Treatment, followed by a period of adjustment to the conditions, where WI increases and then a period of stability and even a reduction in WI. The controls showed a steady and slight increase in WI from the inception of the trial to the end.

With the above increase in WI in weeks 2 – 5 there was a dramatic drop in ADG for all the animals, but the controls. This may also indicate a metabolic adjustment to the Treatments. An increase in the DMI leads to a subsequent and similar increase in WI (Ruckenbusch *et al.*, 1991). The presence of a high amount of TDS seems to affect the amount of water ingested per kilogram of feed ingested. Se affects both WI and DMI. In relation to these it also improves ADG. The animals ingesting this Se have a higher DMI rate as well as the resultant improvement in mass gain and FCR. The importance of Se as a dietary constituent is shown here. TR4 animals showed similar WI and DMI as compared to the TR3 animals. The TR4 ADG was consistently better than that of TR3. This seems to elicit a more improved body efficiency and may be of interest to feedlot managers. It is important to note that the level of 0,7 mg Se / L and the alleviator treatment were provided for a three month period. It is thus vital that this amount of Se not be exposed to the animals for a time period longer than three months. This is ideal for feedlot managers as they can deliberately increase the Se content of their animals' water to 0,7 mg / L and

provide the TDS treatment simultaneously for a period of three months. Exposure to these treatments for longer than three months is not advised as this may lead to cases of selenosis.

The fact that TDS improves the WI per kg DMI could lead to a more efficient functioning of the metabolism of the animal body with the possibility of an increased glomerular filtration playing a permissive role in the increase in WI. The effect of the supplemented Se and TDS on blood Se and faecal Se contents as shown by Figure 3.1. and Figure 3.2. may highlight this.

The sharing of a potentially toxic water source by humans with the animals that may one day be slaughtered for consumption purposes by these humans may pose a serious threat to the health of the animals and, even more so, the humans consuming the flesh of these animals (Casey *et al.*, 1998, Casey and Meyer, 2001). The use of a 0,3 % inclusion of TDS in the drinking water of the animals could serve as a useful and relatively inexpensive alleviator treatment. It may also improve the metabolism of the animals, especially if the animals may not be adequately supplemented with NaCl. This is very often also a characteristic of communal farming systems, at least in South African conditions (Casey *et al.*, 1998). This treatment of the drinking water of the animals could lead to a reduction in the Se retention ability of the animals and a subsequent reduction in the threat of Se toxicity experienced by both the animals and their human consumers.

Se, as an essential nutrient, is almost always included in livestock supplements (Van Ryssen, 2001). Extension officers and livestock nutritionists often advice farmers as to the supplementation of Se without considering prevailing environmental conditions, possibly exposing the livestock to conditions conducive to selenosis. Mayland (1994) states that excreted Se in faeces is largely unavailable to plants and may not be recycled. Some Se may be excreted in an ionic form in urine (Underwood, 1977) and may add to the environmental burden. Some plants are Se-accumulator plants. When alive or after they die they may avail previously unavailable forms of Se (Mayland *et al.,* 1989). Total environmental burden may be increased unnecessarily if also irreverently supplemented as some Se accumulates and crosses soil-water and soil-plant barriers. Its endocrine disrupting effect is a contentious issue under investigation by the USEPA. Better understanding of its effects, availability and toxicity may warrant further research.

Chapter 5. Conclusion

The beneficial effects of Se on DMI, WI, ADG and FCR have been noted. However, exposure to a potentially hazardous Se source may result in adverse production, reproduction and health aspects. This may not only have a negative effect on the economics of farming, but may also cause a possible health risk to communities that may share a water source with their animals. The fact that an alleviator treatment of 3000 mg TDS / L of drinking water may be used without adverse effects at least over a three month period may assist in attempting to decrease the risk to communities that may be exposed to a high Se water source.

TDS seems to also have an effect on decreasing the blood Se levels of growing sheep. This seems to be as a result of a more efficient metabolic effect on the excretion of Se. Animals given TDS under relatively Se deficient conditions seem to utilise the available Se more efficiently and their faecal excretion of Se is less than those not provided with TDS. As the animal's blood Se increases towards more toxic levels it excretes more Se in the form of faecal Se as compared to animals not given the alleviator treatment. The above results may warrant further analysis of this treatment over a longer exposure period under practical (communal or commercial) farming conditions. It may also be interesting to examine the effects of the alleviator treatment on growing and mature animals in terms of WI, DMI, blood Se levels and other health aspects, as well as a possible long term effect on production (ADG, FCR, etc.) and reproduction characteristics.

There is a growing worldwide concern over the accumulation of Se in the environment. Mayland (1994) stated that nearly all the Se in faeces is unavailable and cannot be taken up by plants. The same author also suggests that the availability of Se from plant sources is greater than those from animal sources. Se-accumulator plants may predispose previously unavailable forms of Se to uptake by other plants and thus increase the available environmental Se. Animals may be able to adapt to potentially hazardous concentrations of Se in their diet by increasing their metabolic production of Se compounds that are readily excreted. These excreta will mainly contain unavailable forms of Se. Selenite and selenate are readily available for plant uptake. These inorganic forms of Se are generally the forms that Se is found in groundwater. If this groundwater is used for irrigation with or without the use of fertilisers and pesticides which may also contain certain concentrations of Se, chances are that the form in which the Se is ingested may induce conditions that could result in high Se concentrations in the environment. These may be readily available for plant uptake and may lead to the environmental accumulation of Se. Pollution of the air, soils and above-ground waters may also result in greater environmental Se levels.

Chapter 6. Further Research and Recommendations

It may be of interest to further study the fields of Se, other heavy metals and TDS with regards their interactions, their effect on water quality, animal production, health and reproduction parameters as well as the consequent effect on the environment and humans. Suggested areas of further research are:

- Short-term exposure of chronic selenosis conditions to cattle (meat and milk), goats (meat and milk), draught animals, poultry and the humans that may consume these.
- Long-term exposure of chronic selenosis conditions to the above groups as well as verification of the efficacy of the alleviator treatment applied.
- Possible application of the alleviator treatment in the extensive, drier regions of South Africa where the aesthetic effects of drinking water is already saline.
- The effect of Se ingestion on the water intake of water with a high TDS concentration.
- The physiology of operation of the alleviator treatments in improving the metabolic efficiency of the relevant animals.
- The interactions of heavy metals in drinking water, forms of excretion and the environmental impact.
- The effect of animal manure for the fertilisation of crops in areas of high heavy metal concentrations in their drinking water on the crops (health and production), cumulative effects of possibly high heavy metal concentrations in both the manure and irrigation water on the crops and the eventual consumers.
- The applicability of the TDS treatment for crop production purposes (where cattle and crops share a water source).
- The ease of application of the TDS treatment for animal management without adversely affecting the focus of the manager.
- The effects of these treatments on the environment.

Chapter 7. References

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Appendix A

Nutritive Contents of Rumevite Cattle Block (25kg). Reg. No. V 10932

Component	Quantity	Unit	Max / Min
Protein	400	G / KG	Min
Percentage protein excluding NPN	89	-	-
Urea	124	G / KG	Max
Fibre	20	″	″
Moisture	140	″	″
Ca	48	″	″
P	3,5	″	Min
S	6,7	″	-
Mg	8	″	-
K	13,2	″	-
Mn	160	MG / KG	-
Cu	40	″	-
Co	0,4	″	-
Fe	200	″	-
I	8	″	-
Zn	160	″	-
Se	0,4	″	-
Vitamin A	20 400	I.U. / KG	-

Appendix B

Prevailing Weather Conditions during the 10 Week Trial Period

Date and Time	Ambient Temperature (°C)	Weather Conditions
19 / 09 09:00	15	Clear sky
17:00	22	
20 / 09 09:00	16	Clear sky
17:00	22	
21 / 09 09:00	19	Clear sky
17:00	24	
22 / 09 09:00	14	Clear sky
17:00	23	
23 / 09 09:00	14	Slightly cloudy
17:00	20	
24 / 09 09:00	16	Clear sky
17:00	21	
25 / 09 09:00	24	Clear sky
17:00	29	
26 / 09 09:00	23	Clear sky
17:00	27	
27 / 09 09:00	18	Cloudy
17:00	17	
28 / 09 09:00	17	Clear sky
17:00	22	
29 / 09 09:00	19	Clear sky
17:00	26	
30 / 09 09:00	20	Clear sky
17:00	24	
01 / 10 09:00	21	Clear sky
17:00	27	
02 / 10 09:00	19	Cloudy
17:00	26	

Date and Time	Ambient Temperature (°C)	Weather Conditions
03 / 10 09:00	18	Clear sky
17:00	25	
04 / 10 09:00	18	Clear sky
17:00	26	
05 / 10 09:00	21	Clear sky
17:00	26	
06 / 10 09:00	22	Clear sky
17:00	26	
07 / 10 09:00	21	Clear sky
17:00	28	
08 / 10 09:00	18	Clear sky
17:00	27	
09 / 10 09:00	15	Cloudy
17:00	21	
10 / 10 09:00	17	Clear sky
17:00	27	
11 / 10 09:00	21	Clear sky
17:00	29	
12 / 10 09:00	19	Clear sky
17:00	29	
13 / 10 09:00	19	Clear sky
17:00	30	
14 / 10 09:00	18	Cloudy with rain
17:00	26	
15 / 10 09:00	20	Cloudy
17:00	27	
16 / 10 09:00	19	Cloudy with rain
17:00	24	
17 / 10 09:00	21	Cloudy with rain
17:00	17	

Date and Time	Ambient Temperature (°C)	Weather Conditions
18 / 10 09:00	14	Cloudy with rain
17:00	22	
19 / 10 09:00	20	Cloudy
17:00	21	
20 / 10 09:00	17	Cloudy with rain
17:00	19	
21 / 10 09:00	14	Cloudy
17:00	19	
22 / 10 09:00	20	Cloudy with rain
17:00	27	
23 / 10 09:00	17	Clear sky
17:00	28	
24 / 10 09:00	22	Clear sky
17:00	29	
25 / 10 09:00	18	Cloudy
17:00	22	
26 / 10 09:00	16	Cloudy with rain
17:00	19	
27 / 10 09:00	20	Cloudy
17:00	22	
28 / 10 09:00	16	Cloudy with rain
17:00	19	
29 / 10 09:00	16	Cloudy
17:00	20	
30 / 10 09:00	22	Clear sky
17:00	28	
31 / 10 09:00	17	Cloudy with rain
17:00	20	
01 / 11 09:00	19	Clear sky
17:00	27	

Date and Time	Ambient Temperature (°C)	Weather Conditions
02 / 11 09:00	18	Clear sky
17:00	26	
03 / 11 09:00	20	Clear sky
17:00	27	
04 / 11 09:00	23	Clear sky
17:00	29	
05 / 11 09:00	26	Clear sky
17:00	32	
06 / 11 09:00	22	Clear sky
17:00	25	
07 / 11 09:00	25	Cloudy with rain
17:00	19	
08 / 11 09:00	16	Cloudy with rain
17:00	18	
09 / 11 09:00	17	Cloudy
17:00	16	
10 / 11 09:00	19	Clear sky
17:00	25	
12 / 11 09:00	21	Cloudy with rain
17:00	17	
13 / 11 09:00	17	Clear sky
17:00	22	
14 / 11 09:00	21	Clear sky
17:00	25	
15 / 11 09:00	22	Cloudy with rain
17:00	18	
16 / 11 09:00	21	Cloudy with drizzles
17:00	19	
17 / 11 09:00	18	Cloudy with drizzles
17:00	21	

Date and Time	Ambient Temperature (°C)	Weather Conditions
18 / 11 09:00	19	Cloudy
17:00	21	
19 / 11 09:00	19	Cloudy with rain
17:00	18	
20 / 11 09:00	21	Cloudy
17:00	18	
21 / 11 09:00	22	Clear sky
17:00	25	
22 / 11 09:00	23	Clear sky
17:00	30	
23 / 11 09:00	20	Clear sky
17:00	28	
24 / 11 09:00	26	Clear sky
17:00	29	
25 / 11 09:00	24	Cloudy
17:00	24	
26 / 11 09:00	20	Cloudy with rain
17:00	21	
27 / 11 09:00	17	Cloudy
17:00	19	
28 / 11 09:00	14	Cloudy
17:00	17	
29 / 11 09:00	19	Cloudy with rain
17:00	21	
30 / 11 09:00	19	Cloudy with drizzles
17:00	22	
01 / 12 09:00	21	Cloudy
17:00	23	
02 / 12 09:00	22	Clear sky
17:00	26	

Date and Time	Ambient Temperature (°C)	Weather Conditions
03 / 12 09:00	24	Clear sky
17:00	20	
04 / 12 09:00	17	Cloudy
17:00	20	
05 / 12 09:00	18	Cloudy
17:00	24	
06 / 12 09:00	24	Clear sky
17:00	28	
07 / 12 09:00	23	Clear sky