

Selenium concentration of maize grain in South Africa and the effect of three selenium sources on the selenium concentration of eggs and egg quality

by

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DECLARATION

I, Casey-Claire Courtman, declare that the dissertation, which I hereby submit for the MSc. (Agric) Animal Nutrition degree at the University of Pretoria, is my own work and has not been previously submitted by me for a degree at this or any other tertiary institution.

SIGNATURE:

DATE:



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"I can do all things through God who strengthens me." Philippians 4:13



Dedicated to Granny Nell Courtman,

"You are the light of my life."



LIST OF ABREVIATIONS

Availa [®] Se 1	One of the treatment levels such that the Availa [®] Se (a commercial
	Zinc-L-selenomethionine) supplement is supplemented at 130 μ g
	Se/kg DM
Availa [®] Se 2	One of the treatment levels such that the Availa [®] Se supplement is
	supplemented at 260 µg Se/kg DM
Availa [®] Se 3	One of the treatment levels such that the Availa [®] Se supplement is
	supplemented at 390 µg Se/kg DM
DM	Dry matter
Ca	Calcium
EE	Ether extract
GE	Gross energy
GSH-Px	Glutathionine peroxidase
На	Hectare
HCl	Hydrochloric acid
High-Se maize 1	One of the treatment levels such that the High-Se maize is
	supplemented at 130 µg Se/kg DM
High-Se maize 2	One of the treatment levels such that the High-Se maize is
	supplemented at 260 µg Se/kg DM
High-Se maize 3	One of the treatment levels such that the High-Se maize is
	supplemented at 390 µg Se/kg DM
HN0 ₃	Nitric acid
HOCI	Perchloric acid
HU	Haugh unit
Met	Methionine
Ν	Nitrogen
Р	Phosphorus
Se	Selenium
SeCys	Selenocysteine
SeMet	Selenomethionine
S	Sulphur
SS	Sodium selenite



Sodium selenite 1	One of the treatment levels such that sodium selenite is supplemented	
	at 130 µg Se/kg DM	
Sodium selenite 2	One of the treatment levels such that sodium selenite is supplemented	
	at 260 µg Se/kg DM	
Sodium selenite 3	One of the treatment levels such that sodium selenite is supplemented	
	at 390 µg Se/kg DM	
H ₂ SO ₄	Sulphuric acid	



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ABSTRACT

Selenium (Se) is an essential micronutrient for the health and growth of humans and animals. Its functions in the body include its roles as an antioxidant through the Se-containing enzyme, glutathione peroxidase (GSH-Px), and as an activator of the thyroid hormones through Se-containing enzymes. Adequate Se thus strengthens the immune system and protects the body against conditions that compromise it, such as human immunodeficiency virus (HIV) (Surai, 2006; Fisinin et al., 2008a). A survey was conducted where a total of 896 maize grain samples were obtained from all the maize silos throughout South Africa (231 silos) and analysed for Se content. This information was used to compile a regional distribution map of the Se content of maize grain in South Africa. Of the samples analysed, 94% contained below 50 µg Se/kg dry matter (DM) and can thus be classified as deficient from an animal and human nutritional point of view. Maize grain in South Africa is therefore a poor source of Se for animals and humans. The geographical distribution of Se values of maize grain is consistent with that of previous studies on the Se status of herbivores in South Africa, suggesting that plants growing in most of the maize-producing areas of the country contain low concentrations of Se. However, these findings contradict those of the soil Se status in the country as reported by the Agricultural Research Council's Institute for Soil, Water and Climate, which states that the eastern part of the maize-producing areas of the country tends to have adequate to high soil Se levels and the western areas to have low levels. These contradictory results can be explained to a large extent by the varying soil pH throughout the country. Soil pH plays a primary role in the availability of Se to plants. Although the eastern parts of the country tend to have high Se concentration in the soil, it is not available to the maize plant owing to a low soil pH, while in the western parts of the country, where soil pH may be suitable for Se uptake by plants, there seems to be an inadequate concentration of available Se in the soil. Following this survey, a trial was conducted to compare the potential of three Se sources to enhance the Se deposition in albumen and yolk and egg quality, and to establish how soon after commencement of supplementation the Se content in the egg will reach stability. One hundred and fifty Lohmann Brown hens, 64 weeks of age, depleted from Se for two weeks, were randomly assigned to four dietary treatments: 1) a control diet consisting of ingredients selected to contain the minimal amount of Se; and the control diet containing 2) an organic Se source, Availa[®]Se, (Zinpro Corporation); 3) an inorganic Se source, sodium selenite and, 4) an



organic source, high-Se maize. Diets 2, 3 and 4 were formulated to contain 130, 260 and 390 µg Se/kg DM each. These diets were fed to the hens for 12 days. Eggs for Se analysis were collected on days 0, 1, 2, 6, 8 and 12. The eggs were collected on day 10 and 11 for the egg quality analyses. Of the eggs collected, five were analysed immediately for egg weight, eggshell breaking strength, yolk weight, albumen weight, albumen height and Haugh Units (HU). The remaining eggs were subjected to different storage temperatures (4 °C or 22 °C) and different storage periods, either 15 or 38 days. Selenium accumulation was higher in the albumen compared to the yolk for the organic Se supplements (Availa[®]Se and high-Se maize), whilst for the SS and the control treatment, Se accumulated in the yolk rather than the albumen. Availa[®]Se was the most efficient (P < 0.05) at increasing the Se concentration of the albumen (3390 µg Se/kg DM), followed by high-Se maize (2431 µg Se/kg DM) and SS (772 μg Se/kg DM). Availa[®]Se and SS were the equally effective (P<0.05) at increasing the Se concentration of the yolk (1189 and 1107 µg Se/kg DM, respectively), followed by high-Se maize (582 μ g Se/kg DM). Thus Availa[®]Se is an effective Se supplement to be used in layer diets for the enrichment of table eggs. The results suggested that by day 12, steady state in Se content of the albumen has not been reached when the organic Se sources were fed, while the Se content in yolk has reached steady state between days 6 and 8. Sodium selenite was the only Se supplement to increase (P < 0.05) the egg weight and albumen weight of newly laid and stored eggs. Selenium supplementation showed no improvement in the eggshell quality or yolk weight of newly laid or stored eggs. Selenium supplementation is able to improve (P<0.05) the albumen height and HU value of stored eggs, with Availa[®]Se, SS and high-Se maize being equally effective. Refrigeration was effective (P < 0.05) at maintaining the egg quality in terms of improved albumen weight, albumen height and HU values.



CHAPTER 1: INTRODUCTION

Selenium (Se) is an essential micronutrient for the health and growth of humans and animals. Its functions in the body include its roles as an antioxidant through the Se-containing enzyme, glutathione peroxidase (GSH-Px), and as an activator of the thyroid hormones through Se-containing enzymes. Adequate Se thus strengthens the immune system and protects the body against conditions that compromise it, such as human immunodeficiency virus (HIV) (Surai, 2006; Fisinin et al., 2008a). Free radicals produced under normal and stressful metabolic conditions can cause oxidative damage to DNA, proteins and polyunsaturated fatty acids. Thus antioxidant protection is essential for the prevention or reduction in the damage caused by free radicals (Haug et al., 2007). Selenium deficiency is a major health problem for 0.5-1 billion people worldwide and an even larger number of people do not consume enough Se in their diets that is required for optimal protection against cancer, cardiovascular diseases and severe infectious diseases including HIV (Haug et al., 2007; Fisinin et al., 2008a). Dietary Se requirements of humans and animals are between 50 and 300 µg Se/kg DM (Gissel-Nielsen et al., 1984; Elliot, 2006). For a proportion of South African children, Se intakes have been estimated to be below two thirds of recommended dietary allowance (RDA) (Labadarios et al., 2005), which is 10 - 15 µg Se for infants, 20 - 30 μ g for children, \pm 70 μ g for men and \pm 55 μ g for women (NRC, 2000). In South Africa, maize grain is a staple food for humans and a major ingredient in the diets of intensively fed livestock. Therefore, Se in maize could potentially make a substantial contribution to the Se intake of both humans and livestock.

Selenium is not an essential element in plant nutrition, but may be incorporated into plant cells, replacing sulphur (S), because S and Se have similar chemical properties and compete for transporters at plant-root level (Lyons *et al.*, 2004). The Se content of plants is determined by three main factors: the level of Se in the soil, the pH and redox equilibrium of the soil, and plant genetics (Reid & Horvath, 1980; Haug *et al.*, 2007). The presence of Se in the soil is affected by the Se content of the parent rock, the intensity of weathering and leaching, contamination of the soil (factories and the mining of other minerals) and atmospheric deposition of Se (Gissel-Nielsen *et al.*, 1984). Selenium is more available to plants growing in high pH soils compared with low pH soils (Gissel-Nielsen *et al.*, 1984). Therefore, fertilisers that are used to amend soils may have an effect on the uptake of Se by plants, for example, limestone fertilisation increases the soil pH, while superphosphate



decreases it (Gissel-Nielsen *et al.*, 1984). Selenium uptake by plants is also affected by the application of S-containing fertilizers since S and Se compete for root-transporters (Lyons *et al.*, 2004).

The egg has been reported as the ideal method for Se-enrichment of human's diets (Papazyan *et al.*, 2008). The amount of Se supplied in a single egg varies from 20-35 μ g (approximately 50% of the RDA) and this Se is highly available (Papazyan *et al.*, 2008). The form in which Se is supplemented in the hen's diet also has an effect on the amount of Se deposited in the egg. Organic Se supplements show a higher Se deposition in the egg in comparison to inorganic sources (Hassan, 1990; Paton *et al.*, 2002; Payne *et al.*, 2005; Utterback *et al.*, 2005; Skrivan *et al.*, 2006; Pan *et al.*, 2007; Chantiratikul *et al.*, 2008a & b; Leeson *et al.*, 2008; Mohiti-Asli *et al.*, 2008; Chinrasri et al., 2009; Kralik *et al.*, 2009; Pavlovic *et al.*, 2009; Attia *et al.*, 2010; Scheidler *et al.*, 2010). The main form of Se in the organic Se supplements is SeMet and since the main form of Se in the egg is SeMet, and the hen is not able to synthesise this essential amino acid, sodium selenite (SS) or sodium selenate have limited ability to enrich eggs with Se (Surai, 2006; Osman *et al.*, 2010) in comparison to organic sources.

Availa[®]Se (Zinpro Corporation) is a novel, commercial organic Se supplement consisting of 4% Se of which 100% Se is in the form of zinc-L-selenomethionine. According to Chantiratikul *et al.* (2008a) there is a lack of information on the use of zinc-L-selenomethionine in laying hens. Zinc-L-selenomethionine is designed to be highly soluble and to increase the bioavailability of Se (Ward, 2002b) and thus may be an effective means of Se-enrichment of table eggs.

Studies have also shown that dietary Se supplied in organic and inorganic form accumulates to a greater extent in the yolk rather than the albumen of the egg (Robberecht *et al.*, 1987; Paton *et al.*, 2000; Paton *et al.*, 2002; Sheng *et al.*, 2002; Jiakui & Xialong, 2004; Stibilj *et al.*, 2004; Golubkina & Papazyan, 2006; Leeson *et al.*, 2008; Mohiti-Asli *et al.*, 2008; Chinrasri *et al.*, 2009; Hanafy *et al.*, 2009; Kralik *et al.*, 2009; Lipiec *et al.*, 2010; Scheidler *et al.*, 2010). Conversely, Chantiratikul *et al.* (2008b) and Skrivan *et al.* (2006) found that there was a higher Se concentration in the albumen compared to the yolk regardless of Se source. Latshaw & Osman (1975) showed that Se from natural feedstuffs (organic Se) deposited to a greater extent in albumen while Se from SS deposited to a greater extent in the yolk. In a study by Golubkina & Papazyan (2006) in all but one of the eight cases of avian species' eggs analysed for Se, the authors found the Se concentration to be higher in the yolk compared to the albumen.



As soon as the egg is laid its quality begins to deteriorate and the longer the storage time, the more the internal quality deteriorates (Coutts & Wilson, 2007). Selenium supplementation of layer diets has been shown to improve the egg quality of newly laid and stored eggs. Egg weight decreases with increasing periods of storage (Heath, 1977; Silversides & Scott, 2001; Monira et al., 2003; Jin et al., 2011) and this decrease in egg weight is attributed to the decrease of albumen weight over time caused by water-loss from the albumen through the egg shell (Silversides & Budgell, 2004). Some reports have shown that Se supplementation increases the egg weight of newly laid eggs (Rutz et al., 2004; Hanafy et al., 2009) and eggs stored for 14 days (Mohiti-Asli et al., 2008). Fernandes et al. (2008) and Sara et al. (2008) found an improvement in the number of thin-shelled and cracked eggs when organic Se supplements were used compared to inorganic Se supplements. Mohiti-Asli et al. (2008) found that SS or Se-yeast supplementation had no effect on the eggshell breaking strength of newly laid eggs or eggs stored for 14 days at varying temperatures (4 °C, 23-27 °C and 31 °C). With the addition of Se to layer diets the albumen weight of newly laid eggs has been shown to increase (Rutz et al., 2004; Skrivan et al., 2006; Arpášová et al., 2009). Se supplementation has been shown to have no effect on the yolk weight of fresh or stored eggs (Mohiti-Asli et al., 2008). Organic Se supplementation of layer diets increased the albumen height of newly laid eggs (Rutz et al., 2004). Arpášová et al. (2009) found that the albumen height of fresh eggs increased to the same extent with SS and Se-yeast supplementation while Skrivan et al. (2006) found that the albumen height of fresh eggs increased only with Se-chlorella (an organic Se source) supplementation compared to SS and Se-yeast. Payne et al. (2005) showed that SS improved the albumen quality of eggs stored for 28 days at 22.2 °C. Organic Se supplementation has been shown to have no effect on the HU of newly laid eggs (Fernandes, et al., 2008; Chinrasri et al., 2009; Osman et al., 2010) or eggs that have undergone a period of storage (Gravena et al., 2011). Chantiratikul et al. (2008b) found that the supplementation of SS or Zinc-L-SeMet had no effect on the HU of newly laid eggs and similarly Mohiti-Asli et al. (2008) found that SS or Se-yeast supplementation had no effect on the HU of newly laid eggs or eggs stored for 14 days at varying temperatures (4 °C, 23-27 °C and 31 °C). Conversely, it has also been reported that organic Se supplementation showed an improvement in the HU score of newly laid eggs (Rutz et al., 2004; Hanafy et al., 2009) or eggs stored for 14 and 28 days at 4 °C (Gajčević et al., 2009). Skrivan et al. (2006) and Arpášová et al. (2009) found that the HU of fresh eggs increased to the same extent with SS and Se-yeast supplementation. Skrivan et al. (2006) also



found that the only improvement in HU of newly laid eggs above that of a basal diet low in Se occurred when Se-chlorella was supplemented (as opposed to SS and Se-yeast).

The objective of this study was to determine the Se concentration of maize grain in South Africa. This would provide an indication of the contribution of maize grain to the Se intake of humans and animals in the country. In South Africa, humans preferably consume white maize, while yellow maize is used in animal diets (Esterhuizen & Kreamer, 2011). Therefore, a comparison of the Se concentrations in yellow and white maize separately was considered noteworthy. Furthermore, the information was intended to map the geographical distribution of Se concentrations of maize in the country. Through that it was hoped to expand the map of the potential Se status of animals and their feed in South Africa, previously reported by Van Ryssen (2001). A subsequent study was conducted to determine the potential of the Se in maize to enrich table eggs and to improve egg quality compared to Availa[®]Se and SS. It was postulated that Availa[®]Se, which consists of 100% SeMet, would be the best Se source to enrich eggs. Furthermore, since the protein in the eggs is synthesised over a period of just a few days, it was postulated that steady state in the Se concentration of the albumen and yolk would be reached before the end of the 12 day supplementation period. This study was also conducted to compare the efficacy of the three Se sources in terms of their potential to improve egg quality in terms of egg weight, eggshell breaking strength, yolk weight, albumen weight, albumen height and HU of newly laid and stored eggs.



CHAPTER 2: LITERATURE REVIEW

2.1 Selenium

Selenium (Se) is an anionic, non-metallic element in the same chemical family as sulphur (S), oxygen and phosphorus. Naturally occurring Se is mainly present in organic compounds where it replaces the S on the nitro-carbon chain of S-containing amino acids, such as methionine (Met), to form selenomethionine (SeMet).

2.2 Selenium Requirements

Selenium was first identified as an essential nutritional microelement in 1957 in rat (Schwarz & Foltz, 1957) and chick diets (Scott *et al.*, 1957). In general, animals have a dietary Se requirement of 0.1 mg Se/kg in uncomplicated situations, but this requirement increases to 0.3 mg Se/kg when high levels of S or other Se antagonists are present (Mayland, 1994). Wan *et al.* (1988) reported that a Se concentration in forages of 0.1 mg/kg is generally considered adequate for livestock, less than 0.05 mg/kg as deficient and more than 4 or 5 mg/kg as toxic. The latest recommended levels of Se in farm animal feeds in South Africa range from 0.1-0.18 mg/kg on an 'as fed' basis. This is according to Act 36 of 1947, the Fertilisers, Farm Feeds, Agricultural Remedies and Stock Remedies Act. The Se requirement for the maintenance and production of laying hens is 0.05-0.08 mg/kg DM, depending on the hen's daily feed intake (NRC, 1994).

The recommended daily intake of Se is 10-15 μ g for human infants, 20-30 μ g for children, \pm 70 μ g for men and \pm 55 μ g for women (NRC, 2000).

2.3 Selenium metabolism

Most Se in animal tissues is present in two forms (Figure 1). The one form is SeMet and the other is selenocysteine (SeCys) which is found in a variety of proteins such as glutathione peroxidase, iodothyronine deiodinase and selenoprotein P (Levander & Burk, 1996). Selenocysteine and inorganic sources of Se (selenite and selenate) are absorbed passively via a normal concentration gradient from the gastrointestinal tract (Sunde, 1990). Selenomethionine is not regulated by the Se status of the animal and can be regarded as 'an unregulated storage compartment' (Levander & Burk, 1996). This is because the SeMet is



absorbed and incorporated into body proteins via the same pathway as the amino acid Met or it can be absorbed via passive diffusion (Sunde, 1990). Daniels (1996) stated that because the body cannot distinguish between SeMet and Met, SeMet can comprise up to 50% of the total Se in the body. The incorporation of Se into Met-specific proteins depends on the ratio of SeMet to Met and does not appear to be under any homeostatic control (Daniels 1996). Although SeMet is absorbed via a concentration gradient, as are selenite and SeCys, the Lform of SeMet is absorbed against a concentration gradient using the same active transport mechanism as L-Met (Hakkarainen, 1993). Figure 1 shows the metabolic fate of Se in the human body. It is clearly illustrated that the metabolism of inorganic Se follows regulated pathways. Either the inorganic Se is reduced in a stepwise manner to the key intermediate hydrogen selenide (H₂Se) or it is incorporated into selenoproteins after being transformed to selenophosphate and selenocysteinyl tRNA, or it may be excreted in the urine after being transformed into methylated metabolites of selenide (Lobinski *et al.*, 2000).



Figure 1 The metabolic fate of selenium in the human body (Lobinski et al., 2000)

When dietary Se supply is interrupted, turnover of the body's SeMet pool (protein catabolism) is a source of Se to the animal and this Se is in the form of SeCys. Thus Se from SeMet is present in both major tissue components (Figure 2) (Levander & Burk, 1996).



SeCys is the form of Se known to account for its biological activities and is the 'central structural component of specific selenoenzymes', including GSH-Px, iodothyronine deiodinases, thioredoxin reductases, selenophosphate synthetase and others (Arpášová *et al.*, 2009). The SeCys pool in the body is tightly regulated because it is a highly reactive compound and it would react with biological compounds if it were free in the cell (Levander & Burk, 1996).



Figure 2 Relationships of dietary forms (left) to tissue forms (right) of selenium. Excretory metabolites and the transport form are also present in tissues but only in relatively small amounts (Levander & Burk, 1996)

When rats were injected with selenite it was found that most Se was present in tissues as SeCys whilst no SeMet was found in the tissues (Olson & Palmer, 1976; Beilstein & Whanger, 1986; Beilstein & Whanger, 1988), as cited by Whanger, (2003). Sunde (1990) also found that when the Se supplements are orally consumed, selenite is more readily metabolised to SeCys precursors than to organic Se. There is no known pathway of synthesis of SeMet from inorganic Se and thus animals must depend on their diets for this selenoamino acid (Sunde, 1990; Daniels, 1996; Levander & Burk, 1996; Whanger, 2003). This explains why SS does not increase the tissue Se concentrations to the same extent as SeMet. Latshaw & Osman (1975) found that SeMet predominantly accumulated in the albumen while SeCys accumulated in the yolk (a pattern similar to SS). This may show that SeCys is not incorporated into proteins like SeMet but is metabolised into an inorganic Se compound (Latshaw & Osman, 1975). Inorganic Se is more efficient at increasing the activity of Sespecific proteins like GSH-Px compared to organic Se (Daniels, 1996). Whilst SeMet is more readily incorporated into the muscle, heart and liver than inorganic Se sources (Waschulewski & Sunde, 1988), inorganic forms of Se are more readily incorporated into the



GSH-Px enzyme complex (Douglas *et al.*, 1981). In conclusion, organic Se is generally better absorbed and retained in the body compared to inorganic Se.

Very little is known about the mechanism of maternal transfer of Se to the egg in oviparous vertebrates (Unrine et al., 2006). The authors also showed that Se can enter eggs at two levels: vitellogenin (yolk) or ovalbumins (albumen). Vitellogenin is synthesised in the liver and transported to the ovary via the blood where it enters the developing follicle. It is then broken down into lipovitellin and phosphatin by enzymatic cleavage; these are the two main yolk proteins in vertebrates (Unrine et al., 2006). 'The presence of Se in yolk phosphatin indicates that deposition is not dependent on the presence of Cys' (Davis & Fear, 1996), thus inorganic Se can accumulate in the yolk to an appreciable amount and potentially to the same extent as organic Se. Selenium is also transferred to the egg post-ovulation via ovalbumins that are secreted by the oviduct. These ovalbumins are rich in Cys and Met (Unrine et al., 2006). Ovalbumin is the major protein in avian albumen and makes up 60%-65% of the protein in albumen (Huntington & Stein, 2001). Jacobs et al. (1993) conducted a study on the Se distribution in albumen from local supermarket eggs in Wilrijk, Belgium. The authors found that 56% of the total Se content was found in ovalbumin-1 and -2 although their Se concentration is not high $(\pm 500 \text{ ng Se/g})$ in comparison to the other proteins. This is probably because the ovalbumin makes up the majority of egg white proteins. Flavoprotein was found to be the richest Se protein containing 1800 ng Se/g. The Se concentrations of other egg white proteins (lysozyme, conalbumin, globulins and ovomucoid) ranged from 359-1094 ng Se/g.

2.4 The biological importance of selenium

The aim of the following literature study is not to investigate the functions and deficiency or toxicity symptoms of Se, however, a short literature review on these topics will be presented to highlight the biological importance of Se in animal and human nutrition.

2.4.1 Functions

Selenium is essential for healthy functioning of the immune system. Blood *et al.* (1995) also reported that Se is required for antibody production, neutrophil function, proliferation of T and B lymphocytes and cytodestruction of T lymphocytes and natural killer lymphocytes. With increased Se supplementation the spleen and other lymphoid organs



increase in weight relative to body weight (Hegazy & Adachi, 2000; Hussain *et al.*, 2004; Hanafy *et al.*, 2009). Similarly, March *et al.* (1986) and Chang *et al.* (2005) have reported that a Se deficiency has adverse effects on the development of lymphoid organs (especially the spleen and thymus) and thus it resulted in impaired functions of these organs. Selenium is also involved in reducing or preventing the progression of HIV to AIDS (Kupka *et al.*, 2004; Farrell, 2008). Allard *et al.* (1998) reported that deficiencies in antioxidants during HIV infection cause an increased state of oxidative stress and may contribute to immune dysfunction and consequently HIV replication.

Selenium is an antagonist against the toxicity of heavy metals (Mayland, 1994; Watanabe, 2002; Bargellini *et al.*, 2008).

Selenium is the metal cofactor for the enzyme GSH-Px. Selenium is also a component of the enzyme gastrointestinal GSH-Px which is responsible for the action against oxidised lipid absorption and thus probably also preventative against heart diseases and cancer (Surai, 2006).

It is well known that Se plays a role in cancer prevention (Rayman, 2000; Combs *et al.*, 2001; Whanger, 2004; Haug *et al.*, 2007; Fisinin *et al.*, 2008b; Stammer *et al.*, 2008). Selenium may prevent prostate cancer through its ability to switch on cell apoptosis in genetically damaged cells (Waters *et al.*, 2003).

Selenomethionine has radio-protective properties and has been proven to protect against UV-light-induced skin damage in mice and thus it may be considered an anti-aging agent (Schrauzer, 2000; Fisinin *et al.*, 2008).

Selenium is required for the conversion of thyroxine (T_4) to the biologically active triiodothyronine (T_3). Thyroxine is a vital hormone involved in controlling the rate of metabolic processes and influencing physical development, growth and protein turnover, therefore a Se deficiency may affect protein turnover followed by growth retardation (Jianhua *et al.*, 2000). Hanafy *et al.* (2009) reported increased T_3 levels of laying hens when their diet was supplemented with organic Se.

Selenium supplementation improves animal production. In laying hens, supplementation of the diet with organic Se has improved egg production and egg quality (Skrivan *et al.*, 2006; Arpášová *et al.*, 2009; Hanafy *et al.*, 2009; Osman *et al.*, 2010; Scheideler *et al.*, 2010).

Selenium plays a role in fertility. Organic Se supplementation in laying hen diets improved the fertility of hens and hatchability of chicks (lower embryonic mortality) (Hanafy *et al.*, 2009; Osman *et al.*, 2010). The hatchability of chicks was increased through enhanced



protection against oxidative metabolism. This is vital because during incubation, and particularly during the last few days before hatch, the oxidative metabolism of the developing embryo increases as is a normal process relating to growth of the embryo (Freeman & Vince, 1974). Agate *et al.* (2000) have also found improved fertility of hens with organic Se supplements and this is due to a favourable environment for the storage of sperm in the oviduct. This allows the sperm to live longer, increasing their storage time and the average number of sperm holes in the yolk layer (increased sperm penetration). Hanafy *et al.* (2009) also found that supplementing male chicken diets with organic Se increased the ejaculate volume, motility, live sperm % and sperm concentration. The role of Se as a component of antioxidant enzymes is important in sperm cells since sperm are vulnerable to oxidative damage because they contain large amounts of polyunsaturated fatty acids. Thus oxidative defense is vital to the sperm allowing them to maintain flexibility and improving sperm motility (Surai *et al.*, 1998).

Selenium is also required in humans for good sperm quality and the prevention of pregnancy complications in women (Farrell, 2008; Fisinin *et al.*, 2008a). Se supplementation during pregnancy and in the postpartum period reduced thyroid inflammatory activity and the incidence of hypothyroidism (Negro *et al.*, 2007).

Selenium plays an important role in mood regulation and a low Se status has been associated with an increased incidence of anxiety, depression, confusion and hostility (Hawkes & Hornbostel, 1996; Rayman, 2000).

A summary of the importance of some major selenoproteins is given in Table 1.

2.4.2 Selenium deficiency

The role of Se in human health is clear and has been discussed in detail in a number of recent reviews with the main conclusion being that a Se deficiency is recognised "as a global problem which needs solving urgently" (Fisinin *et al.*, 2008a). One of the challenges with Se is that there are few specific deficiency symptoms and thus a Se deficiency may go unrecognised (Farrell, 2008). Van Ryssen & van Malsen (1996) also reported that the Se intake of animals in South Africa is seldom considered and this is probably because typical symptoms of a Se deficiency or toxicity seldom occur nor are they reported. Oldfield (1995) attempted to map the regions of the world in terms of Se status and he concluded that there are problems at both ends of the Se supply spectrum, but that a Se deficiency is more of a widespread problem than toxicity. This is also in agreement with Gissel-Nielsen *et al.* (1984).



Table 1 Known selenoproteins that carry out nutritional functions of selenium (Rayman, 2000)

Selenoprotein	Function	
Selenoprotein	Function	
Glutathionine peroxidases (GSH-Px1, GSH-Px2, GSH-Px3, GSH-Px4)	Antioxidant enzymes: remove hydrogen peroxide and lipid and phospholipid hydroperoxides thereby maintaining membrane integrity, modulating eicosanoid synthesis, modifying inflammation and likelihood of propagation of further oxidative damage to biomolecules such as lipids, lipoproteins and DNA).	
(Sperm) mitochondrial capsule selenoprotein	Form of glutathionine peroxidase (GSH-Px4): shields developing sperm cells from oxidative damage and later polymerises into structural protein required for stability/motility of mature sperm.	
Iodothyronine deiodinases (3 isoforms)	Production and regulation of the level of active thyroid hormone, T3, from thyroxine, T4.	
Thioredoxin reductases (probably 3	Reduction of nucleotides in DNA synthesis, regeneration of antioxidant	
isoforms)	systems, maintenance of intracellular redox state, critical for cell viability and proliferation, regulation of gene expression by redox control of binding of transcription factors to DNA.	
Selenophosphate synthetase	Required for biosynthesis of selenophosphate, the precursor of selenocysteine, and therefore for selenoprotein synthesis.	
Selenoprotein P	Found in plasma and associated with endothelial cells. Appears to protect endothelial cells against damage from peroxynitrite.	
Selenoprotein W	Needed for muscle function.	
Prostrate epithelial selenoprotein	Found in epithelial cells of ventral prostate. Seems to have redox	
(150 kDa)	function (resembles GSH-Px4), perhaps protecting secretory cells against development of carcinoma.	
DNA-bound spermatid selenoprotein	GSH-Px-like activity. Found in stomach and in the nuclei of	
(34 kDa)	spermatozoa. May protect developing sperm.	
18 kDa selenoprotein	Important selenoprotein found in the kidney and a number of other tissues. Preserved in a Se deficiency.	

It has been reported that approximately 70% of land in China are Se-deficient areas (Sheng *et al.*, 2002) and most European countries are Se-deficient (Surai, 2006). In many countries the Se status has declined recently due to the increased use of low-Se cereals and advances in cropping techniques (irrigation, fertilisation and decreased days to harvest) (Elliot, 2006).

In severely Se-deficient areas of China two diseases are associated with a severe Se deficiency in children; Keshan disease (juvenile cardiomyopathy) and Kaschin-Beck disease



(chrondrodystrophy). Such severe deficiencies have not been recorded elsewhere (Mayland, 1994).

2.4.3 Selenium toxicity

Se is an example of *Paracelsus' dictum*, 'the right dose makes the poison'. At levels of less than part-per-million (mg/kg) in the diet it is an essential microelement, whilst at levels of 5 ppm and above it is highly toxic (Oldfield, 1995).

The tolerance of individual animals to toxic Se levels varies considerably. Some animals show tolerance to high levels of dietary Se even after showing symptoms of chronic toxicosis such as hair loss and lameness (Mayland, 1994). Different strains of chickens and pigs have shown different heritabilities for susceptibility to Se deficiency or toxicity. Some of these animals can actually be conditioned to withstand high levels of Se and they adapt to this by increasing the production of methylated Se compounds, which are readily excreted (Mayland, 1994). Sub acute or chronic toxicosis of birds, fish and animals may occur if they are exposed to high Se concentrations (5-25 mg Se/kg) in their diet over an extended period of time (a number of weeks) (Mayland, 1994). Chronic Se toxicosis decreases conception and causes embryo loss in birds and death of foetuses in animals (Mayland, 1994). According to Humphreys (1988), poultry are not usually directly affected, however the hatchability of eggs may decrease and the chicks that do hatch are weak and deformed. Chicks given large oral doses of Se suffer from a number of conditions; disruptions in lipid metabolism, degeneration of the cytoplasm, disturbed permeability of the walls of blood vessels, formation of intraepithelial hyaline and necrosis (Humphreys, 1988). Seven mg/kg-9 mg Se/kg in the diet of hens decreased egg weight and hatchability and 9 mg/kg Se also decreased egg production (Humphreys, 1988). Chickens receiving high dietary Se suffered from depressed growth, decreased egg production, anaemia and stiffness of the tibiotarsal joint (Humphreys, 1988). According to the NRC (1994) Se toxicity of laying hens is said to be at a level of 10 mg Se/kg in the diet.

Selenium toxicity symptoms in humans include; nausea, vomiting, diarrhoea, chills and a breath with the typical odour of dimethylselenide (a garlic odour) (Mayland, 1994). After a few days loss of body hair, loss of some fingernails and pronounced arthralgia (pain) of joints occurred (Mayland, 1994). The maximum dietary intake of Se that is safe for humans, the mean NOAEL (no observed adverse effect level) is 819 µg per day (Whanger, 2004).



In terms of the different forms of Se, SS and sodium selenate appear to be quite toxic, SeMet has moderate toxicity and elemental Se has the least long term toxicity (Mayland, 1994).

2.5 Selenium concentration of plants

Selenium is not essential to the survival of plants; however, it may still be incorporated into plant cells in place of S. This is possible since the chemical properties of Se are very similar to S and S is essential to plants (Passwater, 1999). In this manner selenium enters plants and thus the food chain.

Cereals and forage crops convert the Se they absorb from the soil mainly into SeMet and then incorporate it into plant protein in place of Met because the tRNA^{Met} does not differentiate between Met and SeMet (Schrauzer, 2000). The absorption of Se in feedstuffs, inorganic supplements and selenoamino acids is approximately 70%. The bioavailability of Se in feedstuffs is usually expressed on a percentage basis relative to SS which is assigned a bioavailability of 100%. Selenium in animal by-products (including fish meal) has a low availability (9%-25%) while plant products have a much higher availability (80%) (Mayland, 1994). Approximately 80%-81% of the Se found in maize is in the form of SeMet (Schrauzer, 2000). Other authors have also shown that most of the Se in cereal grains is SeMet (Whanger, 2003).

The Se content of plants is determined by three main factors; the presence of Se in the soil in an available form, the pH and redox equilibrium of the soil and plant genetics (Reid & Horvath, 1980; Haug *et al.*, 2007). The presence of Se in the soil is affected by the Se content of parent rock, the intensity of weathering and leaching, contamination of the soil (from factories or mining of other minerals) and atmospheric deposition of Se (Haug *et al.*, 2007). Depending on the redox potential of the soil, Se exists in various forms that have different bioavailabilities. These different forms of Se include; selenides, elemental Se, selenites, selenates and organic Se (in increasing order of bioavailability) (Oldfield, 1995). Selenium is more available to plants growing in high pH soils compared to low pH soils (Gissel-Nielsen *et al.*, 1984). Fertilisers contribute to the soil pH value and affect the Se concentration of plants either through interaction with Se in the soil or through a dilution effect by increased plant dry matter (DM) yield (Gissel-Nielsen *et al.*, 1984). Limestone fertilisation increases the soil pH whilst superphosphate decreases it. Selenium concentration in plants is also



affected by sulphate application because sulphate competes with selenate for transporters in the plant root (Lyons *et al.*, 2004).

Plant genetics also has an influence of the Se concentration of plants. Selenium accumulator plants, when grown on seleniferous soils, tend to accumulate more Se than other plants, for example, S-rich plants such as the *Brassica* species and other cruciferae (Mayland, 1994). Plants containing high Se concentrations are usually unpalatable and are only eaten if other forage is not available. Non-accumulator plants such as grasses and grains are poor concentrators of Se (Mayland, 1994). The Se concentration within a plant species can also vary widely (Varo, 1993). There are differences between different plant species with regards to rooting depth and genetic traits that affect the absorption and translocation of Se to shoots (Mayland, 1994).

Although the uptake of Se by plants does not always correspond directly to the soil Se content, knowledge of high and low soil Se areas are still useful to help identify areas of possible toxicity and deficiency. Comprehensive analyses of Se concentration in forages have not been mapped for Africa but there have been reports of white muscle disease outbreaks of grazing animals in some areas of South Africa as early as 1959 (Tustin, 1959). Van Ryssen & Van Malsen (1996) reported that the information on the Se content of South African soils is limited. Although a number of studies have identified areas where livestock have a low Se status, the information is not complete and adequate enough to map the country according to Se status of the grazing animal.

If the minimum animal requirements for Se are 100 μ g Se/kg feed, then most of the energy concentrates in South Africa would be Se deficient. The inclusion of protein sources would increase the Se concentration, but only if relatively high levels are included in the diet. Although animal protein sources are high in Se, their inclusions in diets are usually low and uncertainty exists about the bioavailability of Se in these sources (Van Ryssen & Van Malsen, 1996).

2.6 Selenium in maize grain in South Africa

A study was conducted by Van Ryssen & Van Malsen (1996) to determine the Se content of maize meal samples obtained from different locations in Southern Africa. It was generally found that the Se levels were low and showed no distinct regional trend. The authors also stated that a more comprehensive survey would be necessary to establish any



regional variation in South Africa and that this information would be of value in human nutrition.

Location	Number of samples	Selenium (µg/kg DM)
Klerksdorp	4	42
Piet Retief	1	7
Potchefstroom	1	14
Vereeniging	1	12
Lichtenburg	3	24
Umlaas Road	1	13
Pongola	1	11
Dundee	1	15
Douglas	2	20
Harare	6	19
Bethal	4	13

Table 3 Selenium in maize samples originating from different locations in southern Africa (Van Ryssen & VanMalsen, 1996)

The concentration of Se in cereals (such as maize) may vary widely; for example, Moxon & Rhian (1943) found wheat samples in South Africa to contain up to 1500 μ g Se/kg, whilst a wheat sample analysed by Van Ryssen & Van Malsen (1996) contained only 34 μ g Se/kg.

For South African children, Se intakes are estimated to be below two-thirds of the Recommended Dietary Allowance (RDA) (Labadarios *et al.*, 2005). Maize is a staple food in South Africa and a good source of Se to consumers, with humans consuming preferably white maize while yellow maize is used in animal diets (Esterhuizen & Kreamer, 2011). Maize meal and bread are the most commonly consumed staple food in South Africa (Steyn *et al.*, 2007). The Se content of maize is not only of relevance in human nutrition, but it is also important to note that maize makes up a large portion of poultry diets, up to 70% of the diet.

A geographical distribution map of the Se status of grazing herbivores in South Africa was composed by Van Ryssen (2001) (Figure 3). The author found marginal to acute Se deficiencies occurring in the Midlands and mountainous areas of KwaZulu-Natal and in the southern coastal region of the Western Cape. It was also noted that these areas have high



annual rainfall and acidic soils. The Se status of the highland sourveld areas of Gauteng, northern Freestate and Mpumalanga are varied (Van Ryssen, 2001). In other areas the Se deficiency was adequate but localised deficiencies were found (Van Ryssen, 2001). In this particular study a Se concentration of less than 0.1 mg/kg in plant material was classified as marginally deficient and less than 0.05 mg/kg as deficient.



Figure 3 Geographical distribution of the selenium status of herbivores in South Africa (Van Ryssen, 2001)

2.7 Selenium supplementation

Because a large proportion of the world's population does not consume sufficient Se, feedstuffs are routinely supplemented with various Se sources at levels of approximately 0.2-0.3 mg Se/kg DM (Arpášová *et al.*, 2009). In the US, the Food and Drug Administration limits feed production practises to a maximum of 0.3 mg Se/kg added to commercial feeds (Scheideler *et al.*, 2010). There are many methods to increase the Se intake of animals such as injectable products, salt-mix formulations and total-ration formulations with supplemental-



Se (Haug *et al.*, 2007). The level of Se in the human diet can also be increased through production of Se tablets, enrichment of table salt with SS, enrichment of animal products, such as milk and eggs, and foliar sprays of Se on crops (Mayland, 1994). In countries such as Finland and New Zealand where the soil is deficient in available Se, fertilisation of crops is used to increase the Se content of plants (Haug *et al.*, 2007). To overcome the widespread Se deficiency in these two countries, selenate fertiliser has been applied in crop-producing areas since 1980 (Mayland, 1994). Selenate is readily available to the plant root for absorption since it is weakly absorbed by all soil colloids. This method is effective in preventing a Se deficiency and is safe in all regards: handling of the product for mixing with fertiliser, transport and distribution, safe for the grazing animals and safe for humans consuming the animal products (Watkinson, 1983).

2.7.1 Organic versus inorganic selenium supplementation

Although selenite and selenate may be used for selenoprotein biosynthesis, they are not incorporated into body proteins like SeMet (Whanger, 2003). This property of SeMet is advantageous because it allows Se to be stored in the body and to be reversibly released by normal metabolic processes. It is in this manner that more Se is available for critical functions, such as antioxidant protection. Sodium selenite, through its metabolic pathway, can be incorporated into GSH-Px but not into SeMet as a storage protein in liver and muscle (Feeding Times, 2002).

Organic minerals are able to utilise peptide or amino acid uptake pathways (active transport) rather than the normal mineral ion uptake pathways in the small intestine (Close, 1998). This prevents competition that may occur between minerals for the same uptake carriers, therefore organic minerals have a higher bioavailability and are more readily transported and absorbed in the intestine (Close, 1998). Organic forms of Se, such as SeMet, that are absorbed by active transport, are nonspecifically incorporated into proteins in place of Met, whilst inorganic sources, such as SS, are passively absorbed by the body and typically have lower rates of absorption (Bennett & Cheng, 2010). According to Cantor *et al.* (1975) (as cited by Oldfield, 1997) SeMet was four times more effective than selenate or SeCys in preventing pancreatic degeneration, a Se-deficiency symptom, in poultry. This shows the superiority of organic Se over inorganic Se.

Humans and animals are not able to synthesize SeMet in their bodies and thus it must be obtained from the diet (Schrauzer, 2000; Whanger, 2003). Since the main form of Se in



the egg is SeMet, and chickens are not able to synthesise this amino acid, the inclusion of SS in the hen's diet has a limited ability to produce Se-enriched eggs (Osman *et al.*, 2010). The chronic toxicity of SeMet is lower than that of selenite thus also making it the supplement of choice (Schrauzer, 2000; Feeding Times, 2002; Wu *et al.*, 2011).

Selenomethionine and selenate are found in varying concentrations in common feedstuffs but very little selenite is present, yet, SS remains the predominant Se supplement for livestock throughout the world (Ward, 2002a). This may be due to the fact that SS is relatively cheap, it has proved effective in preventing Se deficiencies and resultant diseases and it is the most efficient source at increasing the body's GSH-Px levels: neither Se-yeast nor *Availa*[®]Se (zinc-L-selenomethionine) (zinc-L-SeMet) were able to demonstrate improvement over SS in this regard (Ward, 2002a).

According to Chantiratikul *et al.* (2008a) there is a lack of information on the supplementation of zinc-L-SeMet in laying hens' diets. Zinc-L-selenomethionine is designed to be highly soluble and to increase the bioavailability of Se. Previous studies have shown that zinc-L-SeMet has improved the Se status of horses and improved the plasma Se concentrations in broilers above that of SS (Chantiratikul *et al.*, 2008a). Zinc-L-selenomethionine improves the bioavailability of Se through improved solubility and stability since it is already in a form that is one hundred percent available for absorption and does not require digestion in order to release the selenoamino acids as may be the case in feedstuffs and other Se supplements (Ward, 2002b). Organic sources, or chelates, of minerals have enhanced trace mineral bioavailability by 'binding minerals to organic molecules, allowing the formation of structures with unique characteristics and high bioavailabilities' (Fernandes *et al.*, 2008). There is no variation between batches of zinc-L-SeMet and one hundred percent of the Se in zinc-L-SeMet comes from the raw material zinc-L-SeMet, unlike Se-yeast or common feed ingredients which contain a variety of Se compounds including SeMet (Ward, 2002b).

In human nutrition, SeMet has been shown to have an advantage over selenite in terms of its action against cancer. Whanger (1986) found that the protective effects of selenite in the prevention of tumorigenesis were nullified by vitamin C whilst the chemoprotective action of SeMet was not affected. This suggests that SeMet is more stable in the presence of reducing agents and this may be a factor to consider when dietary levels of vitamin C or other reducing agents are high.



2.8 Selenium in albumen and yolk

It has been well-documented that the deposition of Se in the egg is not only determined by the level of Se in the diet of the hen, but also by the form in which Se is included in the hen's diet. In layer hens the total egg Se concentration has been shown to increase linearly with Se supplementation and Se intake in the diet (Chinrasri *et al.*, 2009; Wu *et al.*, 2011). Apart from the Se deposition of the egg increasing with an increase in dietary supplementation, the Se deposition efficiency increases as the hen ages (Pappas *et al.*, 2005, cited in Fernandes *et al.*, 2008), thus one would expect older hens to have a higher Se deposition in the eggs. A large number of studies have found that increasing the dietary level of Se supplementation increases the Se concentration of the egg and that organic Se supplements are particularly more effective than inorganic sources (Hassan, 1990; Paton *et al.*, 2002; Payne *et al.*, 2005; Utterback *et al.*, 2005; Skrivan *et al.*, 2006; Pan *et al.*, 2007; Chantiratikul *et al.*, 2008a; Chantiratikul *et al.*, 2008b; Leeson *et al.*, 2009; Mohiti-Asli *et al.*, 2008; Chinrasri et al., 2009; Kralik *et al.*, 2009; Pavlovic *et al.*, 2009; Attia *et al.*, 2010; Scheidler *et al.*, 2010).

Studies have also shown that dietary Se supplied in organic and inorganic form accumulates to a greater extent in the egg yolk (Table 4) (Robberecht et al., 1987; Paton et al., 2000; Paton et al., 2002; Sheng et al., 2002; Jiakui & Xialong, 2004; Stibilj et al., 2004; Golubkina & Papazyan, 2006; Leeson et al., 2008; Mohiti-Asli et al., 2008; Chinrasri et al., 2009; Hanafy et al., 2009; Kralik et al., 2009; Lipiec et al., 2010; Scheidler et al., 2010; Wu et al., 2011). Conversely, Chantiratikul et al. (2008b) and Skrivan et al. (2006) found that there was a higher Se concentration in the albumen compared to the yolk. Latshaw & Osman (1975) showed that Se from natural feedstuffs (organic Se) deposited to a greater extent in albumen while Se from SS deposited to a greater extent in the yolk. In a study by Golubkina & Papazyan (2006) in all but one of the eight cases of avian species' eggs analysed for Se, the authors found the Se concentration to be higher in the yolk compared to the albumen. The only exception was the ostrich egg which showed a higher Se concentration in the albumen rather than the yolk. When two organic Se supplements, Se-yeast and Se-enriched bean sprout, where supplemented at 0.3 mg Se/kg DM, it was found that Se from Se-enriched bean sprout accumulated in the yolk while Se from Se-yeast accumulated in the albumen (Chinrasri et al., 2009). This result is interesting since these are both organic sources and according to the literature, one would expect them to accumulate to a greater extent in the yolk. Studies report that the main form of Se in the bean sprout is Se-methylselenocysteine



(Finley *et al.*, 2001; Sugihara *et al.*, 2004) which is a common metabolite in Se-enriched vegetables (Chinrasri *et al.*, 2009). The difference in deposition of these forms of Se in the albumen and yolk may be confirmed by the major forms of organic Se in these sources indicating a different metabolic pathway for Se in Se-yeast and Se-enriched bean sprout. In general, SeMet mainly deposits in the egg albumen while inorganic Se or non-SeMet deposits mainly in the yolk (Cantor & Scott., 1974; Hassan, 1990; Sheng *et al.*, 2002). According to Chantiratikul *et al.* (2008b) these differences could be due to the different organic Se sources and the variation in the amounts or ratio of different selenoamino acids (selenocysteine, SeMet, Se-methylselenocysteine) which have a different metabolism in animals. This may also be due to the fact that albumen has a higher protein content than yolk and a higher Met content than yolk proteins (Scott *et al.*, 1969).

Table 4 Selenium concentrations in eggs sampled from different countries (expressed on a wet weight basis in $\mu g \text{ Se/g}$) showing the higher concentration of selenium in the yolk in most cases (Adapted from Lipiec *et al.*, 2010)

Country of origin	Concentration in albumen	Concentration in yolk
Standard Commercial Eggs		
Slovenia	0.06; 0.07	0.42; 0.35
Portugal	0.17; 0.19	0.33; 0.29
Thailand	0.19 ± 0.03	0.51 ± 0.08
Slovakia	0.09 ± 0.01	0.34 ± 0.05
China (1)	0.03- 0.17	0.38- 1.17
China (2)	0.07 ± 0.02	0.67 ± 0.02
UK (1)	0.085 ± 0.005	0.03 ± 0.003
Enriched eggs		
China (2)	0.17 ± 0.02	1.07 ± 0.03
UK (1)	0.51 ± 0.01	0.25 ± 0.015
UK (2)	0.40; 0.62	0.85; 1.09

In general, Se is believed to be incorporated into egg proteins but the specific speciation of which selenoamino acids, selenoproteins and inorganic Se forms occur in the egg is limited (Lipiec *et al.*, 2010). The only species-specific Se determination of eggs published to date was conducted by Jabubowski *et al.* (2001) using seagull eggs.



2.9 Selenium-enriched eggs

According to Papazyan *et al.* (2008) there is no food of animal origin that is consumed by so many people worldwide, and none served in such a variety of ways than the egg is. It is easily produced and has an excellent nutritional profile. The egg contains a highly digestible and complete set of proteins, containing all the essential amino acids. The egg amino acid profile is similar to the ideal balance of amino acids required by men and women (Papazyan *et al.*, 2008). Thus the egg fits all the requirements for an ideal method for Se-enrichment (Table 2). However, the manipulation of mineral concentration in the egg, in order to produce enriched eggs, is not an easy task. The delivery of these minerals to the egg is controlled by different physiological mechanisms to ensure that toxic doses of these minerals do not affect the developing embryo. For this reason it is almost impossible to increase the level of copper, zinc or iron in the egg in order for it to be considered a valuable source of these minerals (Papazyan *et al.*, 2008).

Golubkina & Papazyan (2006) studied the Se distribution in the eggs of eight avian species; the quail, pigeon, guinea fowl, pheasant, domestic chicken, ostrich, geese and ducks. It was found that the mean Se concentration was 38.7 μ g/100 g regardless of the egg size, even though individual concentrations for yolk varied from 13.0-59.1 μ g/100 g and for albumen 10.2-34.1 μ g/100 g. Thus it is safe to conclude that the amount of Se accessible to the developing embryo is also controlled by egg size. However, it is possible to increase the level of Se in the egg by supplementing layer hens' diets with organic Se in the form of SeMet. The situation with SeMet is different because it can be incorporated into body proteins. Since the main form of Se in the egg is SeMet and the hen is not able to synthesise this essential amino acid, SS or sodium selenate have limited ability to enrich eggs with Se (Surai, 2006; Osman *et al.*, 2010). The amount of Se supplied in a single egg varies from 20-35 μ g (approximately 50% of the RDA) and this Se is highly available (Papazyan *et al.*, 2008).

Today, Se-enriched eggs are produced in more than 25 countries around the world, the top producers are found in the United Kingdom, Russia, Thailand, Ireland, Mexico, Colombia, Malaysia, Australia, Turkey and the Ukraine (Fisin *et al.*, 2008a).


Table 2 Some characteristics of food choice for selenium-enrichment (F	Papazyan <i>et al.</i> , 2	008)
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The food should be	Comments		
A part of traditional meals for the	It would be counterproductive to attempt a change in culturally-based		
population	food habits by introducing a new type of food. Emphasis should be		
	given to the possibilities of changing composition of existing foods		
	such as by selenium enrichment.		
Consumed regularly and in a	Since the objective is to deliver the amount of selenium needed to meet		
moderate amount	RDA it is necessary to choose food which is consumed regularly in a		
	moderate amount. Over-supplementation is unnecessary and		
	undesirable.		
Consumed by the majority of the	This is particularly important given that immune function is more		
population	likely to be compromised in groups such as children and the elderly.		
Affordable	Affordability of food would play a major role in the consumer choice.		
Enriched with other health-promoting	Examples of minerals critical to health that are frequently deficient		
nutrients that are in short supply in the	include iron and iodine. Vitamin E and lutein are also in short supply		
same population	in the human diet. This can give a greater improvement in the diet.		
Supplying a meaningful amount of the	This is an important point that distinguishes true functional foods from		
nutrient (e.g. at least 50% RDA)	products that include 'tag-dressing' amounts of nutrients for		
	advertising purposes.		

2.10 The influence of selenium on egg quality

Quality is a term that defines the properties of any food that has an influence on the acceptance or rejection of this food by the consumer (Kramer, 1951). As soon as the egg is laid its quality begins to deteriorate and the longer the storage time, the more the internal quality deteriorates. However, the chemical composition of the yolk and albumen does not change dramatically (Coutts & Wilson, 2007). There are six main factors affecting internal egg quality: handling and storage, disease, bird age, egg age, temperature and humidity (Jin *et al.*, 2011). According to Williams (1992) flock age is the most important factor determining internal quality of freshly laid eggs. Generally, the older the hens are, the poorer their internal egg quality will be. Eggs that are several days old show weak and watery albumen and the CO_2 loss causes the egg to become more alkaline, thus affecting the eggs' flavour (Coutts & Wilson, 2007). Newly laid eggs are saturated with CO_2 and during storage the CO_2 is lost by diffusion through the eggshell. Most of the CO_2 is lost within a few hours of the egg being



laid (Banerjee *et al.*, 2011). As CO₂ is lost by diffusion, the pH of the albumen rises, causing dissociation of two of the albumen's proteins, lysozyme and ovomucin, which subsequently reduces the viscosity of the albumen (Powrie, 1977). At higher temperatures the loss of carbon dioxide is faster and the albumen quality deteriorates faster (Chukwuka et al., 2011). The best storage results are reported to be in a cool room at 10 °C and at a humidity of 70% as this helps decrease egg water loss (Coutts & Wilson, 2007). Eggs can lose as much of their quality in one day at room temperature than four to five days when kept in a fridge (Coutts & Wilson, 2007). Eggs also contain a high proportion of polyunsaturated fatty acids thus increasing their requirement for antioxidants during storage (Mohiti-Asli et al., 2008). The increased Se concentration in eggs, through Se supplementation of commercial laying hens' diets, improves antioxidant protection of the yolk, prolongs the egg's stability and increases the nutritional value of the eggs (Arpášová et al., 2009). According to Kralik et al. (2009) the supplementation of Se has a positive effect on the activity of GSH-Px enzymes, which reduces the presence of free radicals that cause oxidation of lipids and proteins, and it reduces the activity of some other enzymes, such as polymerase, nuclease and ligase, thus ensuring the preservation of egg freshness.

Egg quality is an important price-contributing factor in table and hatching eggs (Monira *et al.*, 2003; Chukwuka *et al.*, 2011) and according to Chukwuka *et al.* (2011) one of the biggest challenges to the poultry industry is to provide eggs of a consistent quality to the consumer.

2.10.1 Egg weight

Egg weight decreases with increasing periods of storage (Heath, 1977; Silversides & Scott, 2001; Monira *et al.*, 2003; Jin *et al.*, 2011). This decrease in egg weight is caused by a reduction in albumen weight over time due to water-loss from the albumen through the egg shell. There is a high correlation between albumen weight and egg weight, and albumen weight is said to be the main factor determining egg weight (Silversides & Budgell, 2004). Thus it can be expected that with variations in albumen weights, the egg weights will vary correspondingly.

Some studies have shown that supplementation with inorganic or organic Se at levels equal or larger than 0.3 mg Se/kg had no effect on the egg weight of newly laid eggs (Utterback *et al.*, 2005; Chantiratikul *et al.*, 2008b; Şara *et al.*, 2008; Chinrasri *et al.*, 2009; Bennett & Cheng, 2010; Osman *et al.*, 2010; Scheideler *et al.*, 2010). Conversely, other



authors have reported that egg weight of newly laid eggs increases with Se supplementation (Rutz *et al.*, 2004; Hanafy *et al.*, 2009). Rutz *et al.* (2003), Payne *et al.* (2005), Skrivan *et al.* (2006) and Arpášová *et al.* (2009 & 2010) showed that only organic Se supplementation was able to increase the egg weight of newly laid eggs. However, Attia *et al.* (2010) found that the egg weight of newly laid eggs was heaviest with SS supplementation compared to Se-yeast, although Se-yeast was still able to increase the egg weight above that of hens receiving no Se supplementation.

According to Mohiti-Asli *et al.* (2008) Se supplementation is not able to improve the egg weight of eggs that undergo a period of storage. Eggs that were stored for 14 days were heavier when hens were supplemented with SS compared to Se-yeast (60.25 and 58.37 g, respectively). However, these values were not significantly heavier than the control (having no Se supplementation).

2.10.2 Eggshell breaking strength

Eggshell breaking strength is an important quality parameter since cracked eggs are not available for retail sale and a high number of cracked eggs will have a negative impact on profitability (Chukwuka *et al.*, 2011). The soundness of the shell is the consumer's first impression of egg quality and weaker eggshells are more prone to cracks and thus microbial contamination. Poor eggshell quality has been of major economic concern to commercial egg producers. Data from the USA, United Kingdom and Germany show that the incidence of broken eggs is approximately 6%-8% thus causing high financial losses (Coutts & Wilson, 2007). Eggshell breaking strength has been shown to decrease with increasing storage periods (Monira *et al.*, 2003).

The potential of Se to improve the eggshell strength of eggs could thus have important economic implications. Fernandes *et al.* (2008) reported that trace minerals may indirectly affect the eggshell quality through their involvement as cofactors in catalytic enzyme reactions in membrane and shell synthesis, or directly though their interaction with Ca crystal formation. According to Rutz *et al.* (2004), Skrivan *et al.* (2006) and Arpášová *et al.* (2010) the eggshell breaking strength of newly laid eggs did not improve with organic or inorganic Se supplementation. However, Fernandes *et al.* (2008) and Şara *et al.* (2008) found an improvement in the number of thin-shelled and cracked eggs when organic Se supplements were used compared to inorganic Se supplements. Mohiti-Asli *et al.* (2008) found that SS or



Se-yeast supplementation had no effect on the eggshell breaking strength of newly laid eggs or eggs stored for 14 days at varying temperatures (4 °C, 23-27 °C and 31 °C).

2.10.3 Internal egg quality

Unlike eggshell quality, the internal quality of the egg begins to decline as soon as the egg is laid (Chukwuka *et al.*, 2011). It is well known that Se is a powerful antioxidant and one of its most important functions is the protection of lipids against oxidation (Heindl *et al.*, 2010). This is of key importance to egg quality because the quality of eggs inevitably decline over time, however, with Se supplementation, the shelf life of eggs, especially the yolk (which contains a higher lipid content than albumen), is prolonged. The preservation of egg quality using Se supplementation is based on the antioxidant functions of seleno-enzymes in the egg. These enzymes reduce the production of free radicals which initiate uncontrolled oxidation processes that mainly affect lipids and cause peroxidation of the egg lipids (Arpášová *et al.*, 2009). The damage of the lipids can induce further damage of proteins and DNA (Kelly *et al.*, 1998).

The measures of internal egg quality investigated in this study were albumen weight, yolk weight, albumen height and Haugh units.

2.10.3.1 Albumen weight

The albumen weight of an egg decreases with increasing storage time (Silversides & Scott, 2000; Jin *et al.*, 2011). This is said to be due to a transfer in protein from the albumen to the yolk through the yolk membrane and a loss in water through the eggshell (Heath, 1977; Ahn *et al.*, 1999; Silversides & Scott, 2001). Conversely, Akyurek & Okur (2009) showed that the albumen weight did not change with 10 days storage at 5, 21 or 29 °C.

With the addition of organic Se to layer diets the albumen weight of newly laid eggs has been shown to increase (Rutz *et al.*, 2003 & 2004; Skrivan *et al.*, 2006). Arpášová *et al.* (2009) found that the albumen weight of newly laid eggs increased to the same extent with SS and Se-yeast supplementation. No literature could be found on the effect of Se supplementation in terms of improving the loss in albumen weight with storage time.



2.10.3.2 Yolk weight

Egg yolk from a newly laid egg is round and firm. As the egg ages the yolk absorbs water and amino acids from the albumen thus increasing in size (Heath, 1977; Silversides & Scott 2001; Coutts & Wilson, 2007). Similarly, Jin *et al.* (2011) reported that yolk weight increases with increasing storage time and temperature. While Akyurek & Okur (2009) showed that the yolk weight did not change with 10 days storage at 5, 21 or 29 °C.

Selenium supplementation has been found to have an effect on the yolk weight of newly laid eggs. When Se-yeast was supplemented in layer diets the yolk weight was heavier than when SS was supplemented (Rutz *et al.*, 2003). Skrivan *et al.* (2006) and Arpášová *et al.* (2009) found that the yolk weight increased to the same extent with SS and Se-yeast supplementation. Mohiti-Asli *et al.* (2008) found that SS or Se-yeast supplementation had no effect on the yolk weight of newly laid eggs or eggs stored for 14 days at varying temperatures (4 °C, 23-27 °C and 31 °C).

2.10.3.3 Albumen height

The main factor influencing the albumen height is the egg storage time and storage conditions (Silversides & Scott, 2000). Albumen height of eggs is at the highest point when eggs are newly laid and then it starts to decline with increasing storage period (Monira *et al.*, 2003; Jin *et al.*, 2011). Williams (1992) reported that the main factor affecting the albumen height of freshly laid eggs is the age of the bird and the initial albumen height decreased rapidly with increasing flock age. Another factor influencing the albumen height is albumen weight and egg weight. Silversides & Scott (2000) found that albumen height was more closely associated to albumen weight than egg weight, although these correlations were low (r = 0.27 and 0.29, respectively).

Organic Se supplementation of layer diets has increased the albumen height of newly laid eggs (Rutz *et al.*, 2004). Arpášová *et al.* (2009) found that the albumen height of newly laid eggs increased to the same extent with SS and Se-yeast supplementation while Skrivan *et al.* (2006) found that the albumen height of newly laid eggs increased only with Se-chlorella (an organic Se source) supplementation compared to SS and Se-yeast.

In terms of the influence of Se supplementation on stored eggs, Payne *et al.* (2005) showed that SS improved the albumen quality of eggs stored for 28 days at 22.2 °C.



2.10.3.4 Haugh units

The height of the albumen and weight of the egg are measurements used to calculate the HU score on a scale of 0 - 110, the lower the value, the poorer the egg quality. A minimum HU value of 60 is desirable for whole eggs sold to the domestic consumer whilst most eggs leaving the farm should have a HU value of 75- 85 (Coutts & Wilson, 2007). The HU value has been shown to decrease with increasing storage time (Monira *et al.*, 2003; Akyurek & Okur, 2009; Jin *et al.*, 2011). The decrease in HU score is reported to occur at a fairly constant rate of 0.0458 HU/day (Doyon *et al.*, 1986). The HU score also decreases with increasing bird age: according to Doyon *et al.* (1986) the HU score should be on average 102 HU at 20 weeks of age, then dropping to an average of 74 HU at 78 weeks of age.

Organic Se supplementation has been shown to have no effect on the HU of newly laid eggs (Fernandes, *et al.*, 2008; Chinrasri *et al.*, 2009; Osman *et al.*, 2010) or eggs that have undergone a period of storage (Gravena *et al.*, 2011). Chantiratikul *et al.* (2008b) found that the supplementation of SS or zinc-L-SeMet had no effect on the HU of newly laid eggs and similarly Mohiti-Asli *et al.* (2008) found that SS or Se-yeast supplementation had no effect on the HU of newly laid eggs or eggs stored for 14 days at varying temperatures (4 °C, 23-27 °C and 31 °C).

It has also been reported that organic Se supplementation showed an improvement in the HU score of newly laid eggs (Rutz *et al.*, 2003 & 2004; Hanafy *et al.*, 2009) or eggs stored for 14 and 28 days at 4 °C (Gajčević *et al.*, 2009). Skrivan *et al.* (2006) and Arpášová *et al.* (2009) found that the HU of newly laid eggs increased to the same extent with SS and Se-yeast supplementation.

2.11 Rate of Se deposition

It is accepted that SeMet is incorporated into proteins at protein synthesis (Whanger, 2003). Since the protein in albumen is laid down during the formation of the egg (Unrine *et al.*, 2006), it can be assumed that Se in albumen should stabilise within a few days after the commencement of Se supplementation, specifically in the case of SeMet sources. Literature has shown different results for the rate of Se deposition. Latshaw & Osman (1975) collected eggs over a 14-day period to determine how quickly changes in dietary Se affected that of the egg. Changes in Se accumulation in the albumen were rapid and completed by 7 days while changes in the yolk were not completed by 14 days. Ort & Latshaw (1977) found that when



SS was added to the diet there was a lag of 2 to 3 weeks before the Se content of the egg reflected that of the diet and as long as the Se concentration of the diet remained the same, so did that of the eggs. According to Skrivan *et al.* (2006) and Pan *et al.* (2007) dietary Se is gradually transferred to eggs over a 4-week period with the Se concentration of the eggs increasing significantly each week as the experimental period progressed. It has been reported that feeding dietary Se supplements to layers for two weeks is not a sufficient amount of time in order to achieve the maximum level of Se in eggs (De Lange & Oude Elferink, 2005). The reason for this, however, is unclear.

2.12 Hypothesis

The detailed study of available literature shows that a survey of the Se concentration of maize grain in South Africa would be valuable to human and animal nutritionists since maize is one of the staple foods in the country and it comprises a large proportion of animal diets, especially poultry diets. Complementary information, such as soil Se concentration and soil pH, shall be obtained in order to provide possible explanations for resultant Se concentrations in the survey. A large proportion of the world's population does not consume sufficient Se in their diet, in particular, South African children do not have an adequate Se intake and thus strategies to improve the Se concentration through dietary enrichment of foods should be investigated.

Since the egg is a cheap protein source, has a high biological protein value and can accumulate Se effectively, it is seen as an ideal vehicle for the enrichment of human diets. From the literature study it is evident that a large number of studies have compared the efficacy of various Se sources in terms of the deposition of Se in eggs. Extensive comparisons have been made between inorganic and organic Se sources, yet there is little research or available literature on *Availa*[®]Se. Because *Availa*[®]Se is claimed to have a high bioavailability it may be an effective source of Se for the Se enrichment of eggs. From the literature review it is also evident that Se may have a beneficial effect on the egg quality of newly laid and stored eggs.

It is hypothesised that maize in South Africa is a poor source of Se. Thus Se supplementation of layer diets would be critical to hens and it would also be an effective vehicle for the enrichment of human diets. This experiment was conducted to compare different Se sources with a high-Se maize diet. It is hypothesised that high-Se maize, SS and *Availa*[®]Se will be equally available to layer hens in terms of the accumulation of Se in the



albumen and yolk and in terms of their potential to improve egg quality. The three Se sources were fed at increasing inclusion levels (130, 260 and 390 μ g Se/kg DM) and compared to a control diet low in Se, to determine if the increasing levels of Se supplementation have an effect on the Se deposition in the egg and subsequently, an effect on egg quality. The eggs were also stored for different time periods (15 and 38 days) and under different storage conditions (room temperature and in a fridge) to determine if Se, through its antioxidant properties, can preserve egg quality even when under suboptimal conditions (at room temperature for longer periods of time).



CHAPTER 3: MATERIALS AND METHODS

3.1 Selenium Concentration in Maize Grain in South Africa

A total of 896 maize grain samples were obtained from all the maize grain silos throughout South Africa (231 silos) and were analysed for Se at the UP-Nutrilab (Department of Animal and Wildlife Science, University of Pretoria). The samples were collected under the approval of the Grain Silo Industry (Pty) Ltd (Lynnwood Corporate Park, Lynnwood Manor, Pretoria) and supplied for the study through the Southern African Grain Laboratory (SAGL) (www.sagl.co.za). The samples were from the 2008–2009 planting season. A representative maize grain sample was obtained in the following manner: a 10 kg grading sample was drawn for grading purposes with each consignment at the silos and after the grading sample was divided, 500 g of the 10 kg sample was added into a 50 kg bag representing a certain class and grade of maize. When the bag was full, the maize was divided and a 5 kg sample was obtained per silo bin (according to class and grade) and sent to SAGL. To obtain an indication of the geographical distribution of the Se content of maize grain in South Africa, samples were classified in rather arbitrary groups of less than 12 µg, 12-25 µg, 26-40 µg and samples above 40 µg Se/kg DM. However, maize samples were not obtained from all the maize-producing regions of the country because in some areas there are apparently no grain silos, for example Griqualand East, Transkei, Ciskei and KwaZulu-Natal Midlands, except for a silo at Dalton near Greytown.

3.2. Feed and Selenium Supplementation

One hundred and fifty Lohmann Brown hens (15 hens per treatment with 10 treatments), 64 weeks of age, were fed a pre-trial, low Se diet without the addition of Se in the vitamin and mineral premix. This diet was fed two weeks prior to commencement of the trial in an attempt to deplete the selenium reserves in the hens' bodies.

The different diets and water were provided on an *ad libitum* basis. The daily photoperiod consisted of 16 hours light: 8 hours dark. The trial was approved by the Animal Use and Care Committee of the University of Pretoria (EC036-11).

The hens were randomly assigned to four treatment diets: 1) a control diet containing no Se in the vitamin and mineral premix and diets made up of the control diet supplemented with 2) *Availa*[®]Se (Zinpro Corporation), 3) SS and, 4) high-Se maize. Treatments 2, 3 and 4



supplied Se at three different levels, 130, 260 and 390 μ g Se/kg DM. These treatment diets were fed to the hens for a period of 12 days. The highest level of Se supplementation was selected according to the concentration of Se in the high-Se maize, that is 626 μ g Se/kg DM. This concentration multiplied by the percentage of yellow maize in the diet (Table 5) is 626 x 0.66 which will give a highest level of 390 μ g Se/kg DM.

Ingredient	%	Weight (kg)
yellow maize	66.09	660.94
maize gluten 60	6.62	66.19
wheat bran	7.00	70.00
soybean 46	10.00	100.01
L-lysine HCL	0.08	0.78
DL methionine	0.03	0.33
vitamin & mineral premix	0.15	1.50
limestone	8.49	84.88
salt	0.39	3.95
monocalcium phosphate	1.14	11.42
Total	100.00	1000.00

Table 5 Diet composition, on a percentage and weight of total diet basis, as formulated using Format

Raw materials used for the diets were sourced from a local feed supplier with the exception of the high-Se yellow maize that was imported from the USA, and the soya oilcake and yellow maize obtained from Bethal and Davel, respectively, in Mpumalanga. These key ingredients were sourced specifically from the afore-mentioned areas because those regions are known to produce raw materials with a low Se concentration (as can be concluded from the survey of Se in maize in South Africa). Diets were mixed at the University of Pretoria Experimental Farm, Hatfield, Pretoria. Treatment 4 was formulated to contain varying levels of Se by combining two maize sources, one high in Se and one containing zero Se. The high-Se maize was sourced with the assistance of Dr Bret Taylor (United States Department of Agriculture, Agricultural Research Service, US Sheep Experiment Station) from Dubois, Idaho, an area in the USA where maize grain is high in Se (B. Taylor, personal communication).



Treatment	Se Supplementation	Se Concentration (mg/kg DM)	Se Concentration (µg/kg DM)
Control	None	0	0
A1	Availa [®] Se	0.13	130
A2	Availa [®] Se	0.26	260
A3	Availa [®] Se	0.39	390
B1	Sodium selenite	0.13	130
B2	Sodium selenite	0.26	260
B3	Sodium selenite	0.39	390
C1	High Se maize	0.13	130
C2	High Se maize	0.26	260
C3	High Se maize	0.39	390

Table 6 Different treatments and levels of selenium supplementation used in the experiment

The amount of feed per treatment diet required for the 12 day trial was calculated as follows:

15 hens/treatment with a 12 day experimental period, assuming the feed intake is 180 g/day per hen (overestimated). Therefore, 15 x 12 x $180 = 37\ 800\ g = 37.8\ kg$. This figure was rounded up to 40 kg, per treatment diet to be mixed

Two fundamental diets per treatment were prepared in order to mix the 10 different treatment diets, namely, the control diet of 136.5 kg (containing zero Se), 80 kg of a diet containing 0.39 mg Se/kg DM of *Availa*[®]Se, 80 kg of a diet containing 0.39 mg Se/kg DM of SS and 33 kg of a diet containing 0.39 mg Se/kg DM of high-Se maize. The high-Se maize diet was limited to 33 kg since this was the amount of maize that was available for importation from the USA. It was for this reason that only 16.5 kg of the three different levels for the three treatment diets of the high-Se maize could be prepared whilst 40 kg of the other treatment diets were prepared.

Both Se supplemented diets require 0.39 mg Se/kg feed at the highest treatment level. In 40 kg of feed then: 40 x 0.39 is 15.6 mg Se/40 kg feed.

Availa[®]*Se* contains 4 mg Se per 100 g *Availa*[®]*Se*. For the highest treatment level 0.39 mg Se/kg DM was required, thus in 40 kg of feed 15.6 x 100/4 = 390 mg of *Availa*[®]*Se* was required. Thus for the 80 kg diet mix, 780 mg of *Availa*[®]*Se* was required (390 mg x 2).



Sodium selenite (Na₂SeO₃) contains 46% Se, thus there is 46 mg Se in 100 mg SS. In 40 kg of feed 15.6 x 100/46 = 33.9 mg of SS was required. Therefore for the 80 kg diet 67.8 mg of SS was required (33.9 x 2).

Treatment	Se (µg/kg)	Diet weight	Proportions	Weight Composition (kg)	Total 0 Se (kg)	Total 0.39 Se (kg)
A1	130	40	2/3 x 0Se + 1/3 x 0.39Se	26.7 x 0Se + 13.3 x 0.39Se	26.7	13.3
A2	260	40	1/3 x 0Se + 2/3 x 0.39Se	13.3 x 0Se + 26.7 x 0.39Se	13.3	26.7
A3	390	40	1 x 0.39Se	40 x 0.39Se	0	40
						Total: 80
B1	130	40	2/3 x 0Se + 1/3 x 0.39Se	26.7 x 0Se + 13.3 0.39Se	26.7	13.3
B2	260	40	1/3 x 0Se + 2/3 x 0.39Se	13.3 x 0Se + 26.7 x 0.39Se	13.3	26.7
B3	390	40	1 x 0.39Se	40 x 0.39Se	0	40
						Total: 80
C1	130	16.5	2/3 x 0Se + 1/3 x 0.39Se	11 x 0Se + 5.5 x 0.39Se	11	5.5
C2	260	16.5	1/3 x 0Se + 2/3 x 0.39Se	5.5 x 0Se + 11 x 0.39Se	5.5	11
C3	390	16.5	1 x 0.39Se	16.5 x 0.39Se	0	16.5
						Total: 33

 Table 7 Composition and proportions of diets used to mix the different treatment diets

A1, A2 and A3= *Availa*[®]Se supplemented at different levels (130, 260 and 390 µg Se/kg DM, respectively) B1, B2 and B3= Sodium selenite supplemented at different levels (130, 260 and 390 µg Se/kg DM, respectively) C1, C2 and C3= High-Se maize supplemented at different levels (130, 260 and 390 µg Se/kg DM, respectively)

The hens were housed in a controlled-environment house consisting of a two-tier battery system. The hens were placed in individual cages side-by-side in a row. Eggs were collected for Se analyses on days 0, 1, 2, 4, 8 and 12 for the highest level of each treatment only. For the remaining treatment levels and the control treatment, eggs were collected on days 0 and 12 only. These limitations were due to limited space for samples on the block used for Se analyses in the laboratory and due to time restrictions. Only five out of a possible 15 eggs were randomly collected from each treatment-level group on each collection day. Eggs were separated into albumen and yolk and analysed for Se.

Table 8 Se concentration of raw materials used in the formulation of treatment diets

Raw Material	Se concentration (mg Se/kg)	Se concentration (µg Se/kg)
High Se yellow maize from the USA	0.626	626.91
South African maize	0	0
Maize gluten 60	0.049	49.00
Wheat bran	0.065	65.00
Soyabean oilcake	0.020	20.00



3.3. Egg Quality

The eggs were collected on days 10 and 11 for the egg quality analyses. There was a maximum of 30 possible eggs that were available for egg quality analyses per treatment diet on each collection day (if each of the 15 hens were to lay an egg each). Of the eggs collected, five were analysed fresh for eggshell breaking strength, egg weight, albumen height, Haugh units (HU), yolk weight and albumen weight. The remaining eggs were subjected to different storage temperatures, 4 °C or approximately 22 °C (room temperature), and different storage periods, either 15 or 38 days.

Table 9 Storage conditions and time periods of the egg quality analyses

	Fresh eggs		Storage conditions		
Day	0	1	5	3	8
Temperature	NA	4 °C	22 °C	4 °C	22 °C
Number of eggs	5	5	5	5	5

3.4. Laboratory Analyses

3.4.1 Selenium concentration

The Se concentration of raw materials, treatment diets and albumen and yolk samples of the eggs were analysed using the continuous hydride generation atomic absorption method (AOAC, 2000). Samples were read using a Perkin-Elmer 2380 atomic absorption spectrophotometer at an absorbency of 196 nm and lamp energy of 16 mA.

Maize grain samples and feed samples were first ground with a 1 mm sieve using a Retschzm 200 mill. Every tenth maize grain Se sample was analysed in duplicate. This was due to the fact that most of the samples contained zero Se, thus it was decided that it would be feasible to duplicate every tenth sample only, in order to spare time and costs. This was also made possible through the consistent comparison of standard readings in reference to peach leaves (National Institute of Standards and Technology standard reference material 1547. US Department of Commerce, Gaithersburg, MD 20899) that was included in each batch of analyses to verify the accuracy of the Se assays.



Approximately 2 g of albumen, raw materials and treatment diets and 0.5 g of yolk were weighed into test tubes. These samples were weighed on a wet basis and then after completing the DM analyses the values were corrected to a DM value. 5 ml of a digestion mixture consisting of 55% nitric acid (HN0₃) and 72 % perchloric acid (HOCl) (4:1 v/v) were added to the test tubes. This step is critical in the Se analyses because some organic Se products such as SeMet, SeCys and trimethylselenonium ions are acid-resistant and an acid such as HN0₃ with a high oxidation potential is required for complete destruction (Verlinden, 1982). The tubes were then placed on a programmable digestion block with the following settings:

- 1. 4 hours at room temperature
- 2. 1 hour, increasing from room temperature to 100 °C
- 3. 1 hour at 100 °C
- 4. 1 hour, increasing from 100 °C to 180 °C
- 5. 6 hours at $180 \,^{\circ}\text{C}$
- 6. 2 hours, decreasing from $180 \,^{\circ}\text{C}$ to $130 \,^{\circ}\text{C}$
- 7. 1 hour at 130 °C

During this process Se IV was converted to Se VI. Then the tubes were removed from the digestion block for 10 minutes to cool down to room temperature. 2.5 ml of 20% hydrochloric acid (HCl) was added to the test tubes and then the tubes were placed on the digestion block again and heated to 130 °C for 40 minutes, thus reducing Se VI to Se IV. The test tubes were removed from the block and the Se solutions were made up to 20 ml with 10% HCl.

These solutions were put through a hydride generator (Vapor Generation Accessory VGA-77) using 20 % HCl as an oxidizing agent and sodium borohydride (NaBH₄) in a 0.5% sodium hydroxide (NaOH) solution as a reducing agent (that is 1.2 g NaBH₄ / 200 ml 0.5% NaOH).

The gas mixture resulting from the hydride generator was then read by a Perkin-Elmer 2380 atomic absorption spectrophotometer at an absorbency of 196 nm and lamp energy of 16 mA. The reading of all the samples (eggs and feed) were made with reference to standard Se solutions of two, five and 10 parts of Se/ml Peach leaves.



3.4.2 Eggshell breaking strength

The eggshell breaking strength was determined using an Instron machine (model 1011). The Instron machine was calibrated before use as follows:

The compression probe was attached to the machine, ensuring that the washer was working. The machine was switched on 15 minutes before use. The transducer button was pressed to set the machine to 50 kg/500 Newtons (N). The speed was set to 100 mm/minute. The balance knobs were turned to zero and locked and then a 5 kg weight was placed onto the machine. The weight setting was then adjusted to 50 N and the 5 kg weight was removed. The gauge length, compression and break action was set. The egg was placed with the narrower end upright before breaking. Then the eggshell breaking strength was read on the Instron screen in Newtons.

3.4.3 Haugh units

Once the eggs had been weighed and their eggshell breaking strength determined, the eggs were broken onto a flat glass surface and the albumen height immediately surrounding the yolk was measured using a Mastercraft 0 - 200 mm digital Vernier Caliper. The HU (Haugh, 1937) score was calculated according to Doyon *et al.* (1986):

 $HU=100 \log (H - 1.7 w^{0.37} + 7.6)$

Where HU= Haugh unit, H= observed height of albumen (mm) and w= egg weight (g).

The egg was then collected into a beaker from the flat surface and the yolk and albumen were separated and weighed individually.

3.4.4 Dry matter and ash

The DM and ash analyses were completed in accordance with the AOAC (1995) methods. A ceramic crucible was placed into an oven at 100 °C for at least an hour to dry completely after which it was allowed to cool in a desiccator and was then weighed. Approximately 2 g of a sample was weighed into the crucible and placed into an oven for 24 hours. The following day it was removed and placed in a desiccator for approximately 30 minutes to cool down, thereafter it was weighed again. The DM of the sample was calculated as the difference between the crucible and the sample after drying in the oven for 24 hours minus the weight of the dried crucible and sample before it was placed into the oven.



The ash content was determined by placing the sample from the DM analysis into a muffle furnace at 550 °C for four hours. Then the crucible was allowed to cool down in the oven for two hours after which it was placed in a desiccator. The crucible and the ash were weighed and the ash content of the sample was calculated by the difference of the crucible containing the ash with the dry sample, from the DM analysis, divided by the mass of the sample.

Both DM and ash values were multiplied by 100 so that they were expressed as a percentage.

3.4.5 Crude protein

The following analyses were performed on the control diet and the three highest treatment level diets (390 µg Se/kg): crude protein (CP), gross energy (GE), calcium (Ca), phosphorus (P) and ether extract (EE). Due to cost and time restraints, the other diets could not be analysed.

Nitrogen (N) analyses were performed using the Macro Kjeldahl method to obtain CP values (AOAC, 1995).

From each sample 0.5 g (DM basis) was taken. 10 g sodium sulphate and 0.4 g elemental S was added to an Erlenmeyer flask containing 25 ml concentrated (98%) sulphuric acid (H_2SO_4). The flask was placed on a heated oven and allowed to boil for approximately 45 minutes until the solution was clear. After the solution had cooled, 35 ml of boric acid solution (40 g of boric acid in 10 ml methyl red and 25 ml methyl blue made up to a volume of 1000 ml with distilled water) was added. 350 ml distilled water, zinc granules and 100 ml NaOH (45 %) were also added. Then it was allowed to boil for about 10 minutes until 200 ml of distillate remained. The distillate was titrated with 0.1 N of H_2SO_4 . The values were corrected by the titration of a blank sample. The percentage of nitrogen in the sample was then calculated as:

% N = F x (titration - blank) *100/sample mass

Where F is a factor associated with the strength of the H_2SO_4 .

The percentage of crude protein in the diet was calculated as the percentage of N multiplied by 6.25.



3.4.6 Crude fibre

These analyses were completed according to the AOCS Approved Procedure BA 6a-05, ANKOM Technology method 10. A solvent and acid-resistant marker was used to mark the filter bags. The filter bag was then weighed (W_1) and then approximately 1 g of the sample to be analysed (W₂) was weighed into the bag. A heat sealer was used to completely seal the upper edge of the filter bag within 4 mm from the top. One blank bag was also weighed and included in the run to determine blank bag correction (C_1) . The inclusion of a blank bag in the run was used to indicate particle loss. Bags were placed in a 250 ml container with enough petroleum ether to cover the bags and then they were soaked for 10 minutes. The solvent was poured off and the bags were allowed to air-dry. The bags were then placed into the fibre analyser vessel of the ANKOM²⁰⁰⁰. The bags were removed from the machine and placed in a 250 ml beaker with enough acetone to cover and soak the bags for five minutes. Thereafter the bags were removed from the acetone and allowed to air-dry in an oven at 102 °C for four hours. The bags were removed from the oven and placed in a desiccator to cool down to room temperature, after which they were weighed. The entire bag/sample was ashed in a preweighed crucible for two hours at 600 °C. The samples were then cooled in a desiccator and weighed to determine the loss of weight of organic matter (W_3) .

% crude fibre = 100 x $[W_3 - (W_1 \times C_1)] / W_2$

Where W_1 = empty bag weight, W_2 = sample weight, W_3 = weight of organic matter (loss of weight on ignition of bag and fibre) and C_1 = ash corrected blank bag factor.

3.4.7 Gross energy

The gross energy was determined using a water bomb calorimeter, modulator calorimeter (CM-1000). The sample to be analysed was placed in a clean metal crucible and weighed to be approximately 0.5 g. The crucible is placed into the calorimeter and the bomb is allowed to run for approximately five minutes to ignite the sample. Then the resulting GE is shown on the screen of the computer.

3.4.8 Calcium and phosphorus analysis

The following procedure was used on the feed samples so as to release the minerals:



1 g of air-dried, milled feed sample was placed into tubes and 25 ml of HNO_3 (65%) was added. The tube was then heated. 5 ml HOCl was added to the tubes after approximately 10 minutes or when half of the HNO_3 had boiled away. The solution was allowed to boil for a further 40 minutes until only a clear solution remained (HOCl). Thereafter the solution was allowed to cool down and then made up to 50 ml with distilled water.

3.4.8.1. Calcium: Ca concentrations were read on the Perkin-Elmer 2380 atomic absorption spectrophotometer that was also used to read Se concentrations. The prepared solution described above required further dilution with distilled water (60 times dilution) to obtain a reading at 423 nm.

3.4.8.2. Phosphorus: P readings were performed on a Technicon Autoanalyser II Continuous-flow Analytical instrument (AOAC, 1990).

3.4.9 Ether extract

This analysis was completed according to the AOAC Official Method 954.02 (2000).

3.4.10 Statistical analysis

The data obtained from the Se concentration of the albumen and yolk were analysed using the GLM (general linear model) (Statistical Analysis Systems, 2012) for the average effects of treatments and levels over time. Repeated Measures Analysis of Variance with the GLM model was used for repeated day measurements (days 1, 2, 6, 8 and 12). Means and standard deviations were calculated and significance of difference (P<0.05) between means was determined by the Fischers test (Samuels, 1989).

The linear model that was used is described by the following equation:

 $Y_i = u + T_i + e_i$ Where;

 Y_i = variable during the period

 $\mathbf{u} = \mathbf{overall} \ \mathbf{mean} \ \mathbf{of} \ \mathbf{the} \ \mathbf{population}$

 $T_i = effect \ of \ the \ i^{th} \ treatment \ or \ level$

 $e_i = error$ associated with each Y



The data obtained from the egg quality study was analysed using an analysis of variance with the GLM model (Statistical Analysis Systems, 2012) to determine the significance between treatment, level, type of storage and period of storage effects. Means and standard deviations were calculated and the significance of difference (P<0.05) between means was determined by multiple comparisons using the Fischers test (Samuels, 1989).



CHAPTER 4: RESULTS

4.1 Selenium concentration of maize grain in South Africa: a survey

The survey showed that 94% of the analysed samples contained below 50 μ g Se/kg, and thus can be classified as deficient if we assume that the Se requirements of humans and animals are between 50 and 300 μ g Se/kg DM (depending on physiological stage) (Gissel-Nielsen *et al.*, 1984; Elliot, 2006). The survey showed that 46% of the maize samples contained <12 μ g Se/kg DM; 30% were between 12 and 25 μ g Se/kg DM; 12% were between 26 and 40 μ g Se/kg DM; and only 10% of the samples contained >40 μ g Se/kg DM (Table 1).

Yellow maize generally had lower Se values than the white maize. However, yellow and white maize varieties were not distributed evenly in the various regions. For example, only yellow maize varieties have been obtained from silos at Vaalhartz and along the Orange and Vaal rivers. Therefore, it was considered not valid to perform a statistical comparison between the white and yellow maize samples.

Selenium	Yellow maize	e samples	White maiz	e samples	Тс	otal
concentration (µg/kg DM)	No of samples (% of total)	Median (µg/kg DM)	No of samples (% of total)	Median (µg/kg DM)	No of samples (% of total)	Median (µg/kg DM)
<12	240 (58%)	0	176 (37%)	0	416 (46%)	0
12-25	125 (30%)	15	140 (29%)	15	265 (30%)	15
26-40	31 (7%)	30	76 (16%)	30	107 (12%)	30
>40	16 (4%)	55	71 (15%)	59	87 (10%)	59
2 10	10 (1/0)	55	/1 (1570)	57	07 (1070)	57

Table 10 Selenium concentrations ($\mu g/kg$ DM) of yellow and white maize grain samples collected from maize grain silos throughout South Africa

Figure 1 depicts the geographical distribution of Se in maize according to the classes decided upon. Most areas, as mentioned above, are deficient in Se. That is, large portions of central and eastern Gauteng, central and western North West and a narrow strip of the central to western Free State have maize with low Se levels. A large proportion (26%) of the samples contained no Se and originated mainly from large areas of Mpumalanga, the southern region of Limpopo, northern KwaZulu-Natal, central and eastern Free State, as well as an area extending from the Vaalhartz irrigation scheme and along the Orange and Vaal rivers. Maize samples classified as marginally deficient to adequate (>40 μ g/kg DM) were collected from



the north-western Free State and North West near Christiana and the remaining silos in Limpopo.



Figure 4 Regional distribution map to show different categories of selenium concentration (µg/kg DM) in maize grain in South Africa

4.2 Selenium concentration in eggs

The treatment diets were formulated to contain three different levels of Se supplementation, namely; 130, 260 and 390 μ g/kg DM. After completion of the trial the diets were analysed for the actual Se concentration of the feed and orts were also analysed. Small differences were obtained between the intended supplemental Se concentration and the actual Se concentration of the treatment diets (Table 11). Please note that the intended concentration of Se will be used to discuss the results of the experiment.



	Treatment	Intended selenium	Actual selenium	Selenium
		concentration (µg/kg	concentration (µg/kg	Concentration (µg/kg
		DM)	DM) of feed	DM) of orts
Control	No supplementation	0	11.94	23.79
A1	Availa [®] Se1	130	127.80	139.08
A2	Availa [®] Se2	260	286.36	247.17
A3	Availa [®] Se3	390	386.28	349.73
B1	Sodium selenite 1	130	179.46	239.04
B2	Sodium selenite 2	260	389.65	469.26
B3	Sodium selenite 3	390	469.68	644.80
C1	High-Se maize 1	130	198.90	190.78
C2	High-Se maize 2	260	294.38	290.36
C3	High-Se maize 3	390	427.46	558.61

Table 11 Actual selenium concentrations in comparison to the intended selenium concentration ($\mu g/kg DM$) in the treatment diets fed to the hens and in the orts of the diets

The actual Se concentration and other diet specifications (Ca, P, ash, CP, CF, EE and GE) were analysed for each of the highest level (390 μ g/kg DM) of the treatment diets (Table 12).

Table 12 Final diet composition (expressed on a dry matter basis) of the three highest treatment levels (390 μ g/kg DM)

	Control	Availa [®] Se 3	Sodium selenite 3	High-Se maize 3
Se (µg/kg)	10.67	346.61	423.50	382.53
Ca (g/kg)	25.5	63.9	70.4	29.1
P (g/kg)	5.4	5.8	5.8	6.1
Ca:P	4.7:1	11.0	12.1:1	4.7:1
Ash (%)	8.6	11.1	12.1	10.2
Crude protein (g/kg)	136.1	156.3	158.7	165.6
Crude fibre (g/kg)	28.5	31.3	28.8	29.3
Ether extract (g/kg)	38.2	39.0	36.6	39.3
Gross energy (MJ/kg)	16.76	16.56	16.26	16.69

The influence of the different treatment diets on the Se concentration of albumen and yolk on the last day of the 12-day experiment was compared (Table 13). The results showed



that the highest Se concentration in the albumen was achieved using the organic Se supplements and these supplements increased the Se concentration of the albumen to the same (P<0.05) extent ($Availa^{\mbox{\sc B}}Se$ with 2228 µg Se/kg DM and high-Se maize with 2042 µg Se/kg DM). The SS treatment did not increase (P>0.05) the Se concentration above that of the control.

The Se concentration of yolk was the highest with the high-Se maize treatment (1788 μ g Se/kg DM) and this was the only value that was higher than the control (*P*<0.05). The deposition of Se was only numerically higher in the albumen compared to the yolk for the organic Se supplements; whilst, in the case of SS the Se concentration was higher in the yolk compared to the albumen (Table 13).

Table 13 The influence of two organic selenium sources (<i>Availa</i> [®] Se and high-Se maize) and one
inorganic selenium source (sodium selenite) on the selenium concentration (μ g/kg DM ± SE) of
albumen and yolk at 12 days after commencement of supplementation

Treatment	Albumen	Yolk
Control	291 ^a ±53	$547^{a}\pm291$
Availa [®] Se	$2228^{b}\pm 1471$	$914^{a} \pm 425$
Sodium selenite	659 ^a ±139	$1039^{a}\pm503$
High-Selenium maize	2042 ^b ±1027	$1788^b \pm 1383$

Column means with superscripts a-b differ significantly at P < 0.05

Table 14 The influence of different levels of Se supplementation on the selenium concentra	tion (µg/kg
$DM \pm SE$) of albumen and yolk 12 days after commencement of supplementation	

Level (µg Se/kg)	Albumen	Yolk
0	291 ^a ±53	547 ±291
130	$1010^{a} \pm 452$	1156 ±1326
260	1721 ^b ±1072	1263 ±840
390	$2198^{b} \pm 1642$	1322 ± 588

Column means with superscripts a-b differ significantly at P < 0.05



Increasing the level of Se supplementation increased (P<0.05) the Se concentration of albumen but only above 130 µg/kg DM (Table 14). For the albumen the first level of Se supplementation (130 µg/kg DM) showed no difference to the control (P>0.05 and the third level of supplementation (390 µg/kg DM) showed no difference (P>0.05) to the second level of supplementation (260 µg/kg DM). Selenium concentration of the yolk did not increase significantly above that of the control (547 µg/kg DM) (P>0.05).

The effects of the different treatments on the Se concentration of the albumen (Table 15) and yolk (Table 16) over the 12 day period and on a specific day were compared. Due to time constraints and a limited amount of space on the digestion block for the Se analyses, only the highest level of the three treatments were analysed (390 μ g Se/kg DM) and compared over the 12-day period. Figure 5 shows the increase in Se concentration of albumen and further explains the values in Table 15, and Figure 6 shows the increase in Se concentration of yolk and further explains the values in Table 16.

On day 6 the organic Se supplements increased the Se concentration to a greater extent (P<0.05) compared to SS (624 µg Se/kg DM for SS compared to 1517 µg Se/kg DM for the high-Se maize and 1912 µg Se/kg DM for *Availa*[®]Se). The results on day 8 are similar to day 6 with the organic Se supplements increasing the Se concentration of the albumen to a greater extent compared to the SS (699 µg Se/kg DM for SS compared to 1833 µg Se/kg DM for the high-Se maize and 1897 µg Se/kg DM for *Availa*[®]Se). There was no significant difference between the two organic treatments in terms of increasing the Se concentration of albumen on day 8 at P<0.05. On day 12 (P<0.05) the organic Se supplements showed the highest Se deposition in the albumen with *Availa*[®]Se producing the highest Se concentration (3390 µg Se/kg DM), followed by high-Se maize (2431 µg Se/kg DM) and SS (772 µg Se/kg DM).

In the comparison of the individual treatments over time, the Se concentration of albumen for the *Availa*[®]Se treatment increased (P<0.05) from day 2 to day 6 and from day 8 and day 12. The high-Se maize treatment diet increased (P<0.05) the Se concentration of albumen from day 1 to day 2 and from day 2 to day 12.

The results for the yolk Se concentration (Table 16) showed that the only significant differences were found on day 6 where *Availa*[®]Se and SS showed the highest Se deposition in the yolk (1189 µg Se/kg DM and 1107 µg Se/kg DM, respectively). These two values increased the Se concentration of the yolk to the same extent (P<0.05). High-Se maize produced the lowest (P<0.05) Se concentration in the yolk on day 6 (582 µg Se/kg DM).



Day	Availa [®] Se	Sodium selenite	High-selenium maize
1	$210_1\pm11$	220 ± 8	$205_1\pm15$
2	$995_{1} \pm 636$	383 ± 61	$1044_2 \pm 422$
6	$1912^{a}_{2} \pm 155$	$624^b \pm 72$	$1517^{a}_{23} \pm 216$
8	$1897^{a}_{\ 2} \pm 203$	$699^b \pm 43$	$1833^{a}_{34} \pm 119$
12	$3390^{a}_{3} \pm 1796$	$772^{b} \pm 108$	$2431^{c}_{4} \pm 1347$

Table 15 The effect of two organic selenium sources ($Availa^{\otimes}Se$ and high-Se maize) and one inorganic selenium source (sodium selenite) supplemented at a level of 390 µg/kg DM on the selenium concentration (µg/kg DM ± SE) of albumen on a specific day and over the 12 day trial within a treatment

Row means with superscripts a-c differ significantly at P < 0.05Column means with subscripts 1-4 differ significantly at P < 0.05



Figure 5 The effect of two organic Se sources (*Availa*[®]Se and high-Se maize) and one inorganic Se source (sodium selenite) supplemented at a level of 390 μ g/kg DM on the selenium concentration of albumen (μ g Se/kg DM) over a 12-day experimental period. Eggs were analysed for selenium on days 1, 2, 6, 8 and 12, except for the control which was analysed on days 0 and 12 only. The Se concentration between treatments on days with superscripts a-c differ significantly at *P*<0.05

For *Availa*[®]Se and SS there was an increase in the Se concentration from day 2 to day 6 (P<0.05). There was no further increase in Se concentration of the yolk for these two treatments until day 12, thus a maximum level of Se concentration may have been reached at day 6. For the high-Se maize treatment the only significant difference was the increase in Se



concentration from day 6 to day 8. Thus a maximum level of Se concentration may have been reached at day 8.

Table 16 The effect of two organic selenium sources (*Availa*[®]Se and high-Se maize) and one inorganic selenium source (sodium selenite) supplemented at a level of 390 μ g/kg DM on the selenium concentration of yolk (μ g/kg DM ± SE) on a specific day and over the 12 day trial within a treatment

Day	Availa [®] Se	Sodium selenite	High-selenium maize
1	$238_1 \pm 15$	$240_1\pm10$	$258_1\pm 60$
2	$380_1\pm55$	$365_1\pm97$	$397_1\pm45$
6	$1189^{a}_{2} \pm 97$	$1107^{a}_{2} \pm 232$	$582^{b}_{1} \pm 76$
8	$1162_2\pm84$	$1051_2 \pm 149$	$1443_2\pm 636$
12	$1322_2\pm496$	$1278_2\pm763$	$1366_2\pm 613$

Row means with superscripts a-b differ significantly at P < 0.05

Column means with subscripts 1-2 differ significantly at P < 0.05



Figure 6 The effect of two organic Se sources (*Availa*[®]Se and high-Se maize) and one inorganic Se source (sodium selenite) supplemented at a level of 390 μ g/kg DM on the Se concentration of yolk (μ g Se/kg DM) over a 12-day experimental period. Eggs were analysed for Se on days 1, 2, 6, 8 and 12, except for the control which was analysed on days 0 and 12. The Se concentration between treatments on days with superscripts a-c differ significantly at *P*<0.05



4.3 The influence of different selenium supplements on egg quality

In the following section of results the treatments and levels at which they were supplemented into the diet are written as abbreviations with level 1 indicating an inclusion of 130 μ g Se/kg DM, level 2 indicating an inclusion of 260 μ g Se/kg DM and level 3, an inclusion of 390 μ g Se/kg DM. It must also be noted that some of the eggs that were stored for 38 days were found to be black and rotten when they were cracked open for the analyses to be conducted and thus had to be discarded.

4.3.1 Egg weight

A comparison was made between the egg weights of the different treatment diets and levels over 15 and 38 days of storage in a fridge or at room temperature (Table 17). For the average egg weight values on day 0, the only significant difference was that SS eggs were heavier (P<0.05) than $Availa^{\$}Se$ eggs (63.7 and 59.0 g respectively). On day 38 for the refrigerated eggs SS also produced heavier egg weights compared to the control and $Availa^{\$}Se$ (60.6, 54.7 and 56.8 g respectively). The last two treatments were not significantly different at P<0.05.

For the individual egg weights of the newly laid eggs (those weighed directly after the 12-day experiment, i.e., on day 0) only the SS 1 was heavier (P<0.05) than the control (66.1 and 59.7 g, respectively). There was no difference (P>0.05) between the egg weights of the treatment diets on day 15 for the eggs stored at room temperature and in a fridge. Of the eggs stored at room temperature on day 38 only the SS 1 treatment was heavier (P<0.05) than the control (60.5 and 56.8 g, respectively). Of the eggs stored in a fridge on day 38 SS 1 and high-Se maize 2 were heavier (P<0.05) than the control (65.5, 62.7 and 54.7 g, respectively). The SS 1 and high-Se maize 2 treatments increased the egg weight values above that of the control to the same extent different at P<0.05.

A comparison was made between the egg weights over time and under different storage conditions within treatments. The only significant difference for the average values was found for SS which showed a decrease (P<0.05) in the egg weight from 63.7 g on day 0, to 59.4 g, for eggs stored at room temperature on day 15.



Day 0 (Fresh)	Day 15		Day 38	
Storage conditions	Room temperature	Refrigerated	Room temperature	Refrigerated
$59.7_1 \pm 2.67$	59.1 ± 6.74	60.7 ± 6.87	$56.8_1 \pm 5.05$	$54.7_1 \pm 7.84$
58.3 ± 1.16	58.7 ± 0.54	59.2 ± 4.26	$57.0_1 \pm 3.63$	55.7 ± 1.50
58.3 ± 1.01	59.7 ± 4.46	60.8 ± 4.20	$57.4_1 \pm 3.59$	56.6 ± 2.16
60.5 ± 2.59	62.3 ± 3.59	58.8 ± 2.11	$59.5_1 \pm 4.71$	58.0 ± 5.77
$66.1_2 \pm 4.46$	62.6 ± 7.25	65.5 ± 5.88	$60.5_2 \!\pm 5.91$	$65.5_2 \pm 3.76$
$61.1^{a} \pm 3.68$	59.9 ± 3.49	58.4 ± 3.02	$55.2^{b}_{1} \pm 1.65$	56.8 ± 2.94
$63.9^{a} \pm 7.58$	$55.7^{\ b} \pm 0.97$	61.6 ± 5.68	$58.8_1 \pm 6.41$	59.0 ± 6.30
62.1 ± 8.04	55.8 ± 3.22	59.8 ± 1.27	$55.9_1 {\pm}~1.00$	57.6 ± 1.93
57.9 ± 2.51	59.8 ± 6.19	56.7 ± 8.05	$57.8_1\pm 6.70$	$62.7_2 \pm 4.84$
$63.1^{a} \pm 3.35$	60.5 ± 0.38	61.3 ± 1.03	60.2 ± 8.75	$55.1^{b}\pm7.50$
59.7 ± 2.67	59.1 ± 6.74	60.7 ± 6.87	56.8 ± 5.05	$54.7_1 \pm 7.84$
$\mathbf{59.0_{1}} \pm 1.9$	60.2 ± 3.45	59.6 ± 3.50	58.0 ± 3.88	$56.8_1\pm3.53$
$63.7^{a}_{2}\pm 5.51$	$59.4^{b} \pm 5.23$	61.8 ± 5.55	60.1 ± 6.79	$60.6_2\pm5.63$
60.8 ± 5.39	58.6 ± 4.40	59.1 ± 4.91	57.8 ± 5.69	58.8 ± 5.50
	Day 0 (Fresh)Storage conditions $59.7_1 \pm 2.67$ 58.3 ± 1.16 58.3 ± 1.01 60.5 ± 2.59 $66.1_2 \pm 4.46$ $61.1^a \pm 3.68$ $63.9^a \pm 7.58$ 62.1 ± 8.04 57.9 ± 2.51 $63.1^a \pm 3.35$ 59.7 ± 2.67 $59.0_1 \pm 1.9$ $63.7^a_2 \pm 5.51$ 60.8 ± 5.39	Day 0 (Fresh)DayStorage conditionsRoom temperature $59.7_1 \pm 2.67$ 59.1 ± 6.74 58.3 ± 1.16 58.7 ± 0.54 58.3 ± 1.01 59.7 ± 4.46 60.5 ± 2.59 62.3 ± 3.59 $66.1_2 \pm 4.46$ 62.6 ± 7.25 $61.1^a \pm 3.68$ 59.9 ± 3.49 $63.9^a \pm 7.58$ $55.7^b \pm 0.97$ 62.1 ± 8.04 55.8 ± 3.22 57.9 ± 2.51 59.8 ± 6.19 $63.1^a \pm 3.35$ 60.5 ± 0.38 59.7 ± 2.67 59.1 ± 6.74 $59.0_1 \pm 1.9$ 60.2 ± 3.45 $63.7^a_2 \pm 5.51$ $59.4^b \pm 5.23$ 60.8 ± 5.39 58.6 ± 4.40	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{c c c c c c c c c c c c c c c c c c c $

Table 17 Mean egg weights ($g \pm SE$) of different treatment diets weighed after the 12-day experiment (day 0) and again after storage at room temperature (22 °C) or in a fridge (4 °C) for 15 or 38 days

Treatments 1= 130 μ g Se/kg DM, 2= 260 μ g Se/kg DM, 3= 390 μ g Se/kg DM Row means with superscripts a-b differ significantly at *P*<0.05 Column means with subscripts 1-2 differ significantly at *P*<0.05

For the remaining egg weights SS 2 decreased (P < 0.05) from 61.1 on day 0 to 55.2 g for the eggs stored at room temperature for 38 days. For SS 3 the only decrease (P < 0.05) in egg weight in comparison to the fresh eggs was at day 15 for the eggs stored at room temperature (63.9 and 55.7 g, respectively). For high-Se maize 3 the only decrease (P < 0.05) in egg weight in comparison to the fresh eggs was at day 38 for the eggs stored in a fridge (63.1 and 55.1 g, respectively).

4.3.2 Eggshell breaking strength

A comparison was made between the eggshell breaking strength of the different treatment diets and levels, over different storage periods (15 and 38 days) and under different storage conditions (room temperature and refrigeration). Results showed high variation in the eggshell breaking strength of the different treatments and few significant differences (Table



18). For the average values the only significant difference was found on day 15 for the refrigerated eggs where SS had a higher eggshell breaking strength than high-Se maize (40.4 and 30.3 N, respectively).

For the remaining values, for the eggs stored in the fridge for 15 days, *Availa*[®]Se 2, *Availa*[®]Se 3, SS 3 and all three treatment levels of the high-Se maize diet showed lower (P<0.05) eggshell breaking strength values than the control (27.1, 32.4, 28.7, 32.1, 31.2, 26.9 and 44.0 N, respectively). These six values that differed from the control were not significantly different from each other at P<0.05. For the eggs stored at room temperature on day 38 SS 1 had a lower (P<0.05) eggshell breaking strength breaking strength than SS 3, *Availa*[®]Se 2 and high-Se maize 2 (22.4, 40.6, 39.4, 42.7 N, respectively). The latter three values were not significantly different from one another at P<0.05.

A comparison was made between the eggshell breaking strength measured at different storage times and conditions within treatments. For the average values the only difference was for the high-Se maize treatment where the eggshell breaking strength of refrigerated eggs stored for 15 days had lower (P<0.05) values compared to that of the fresh eggs (30.3 N and 42.7 N, respectively).

For the individual values the eggshell breaking strength of SS 1 the highest (P<0.05) value was obtained for eggs stored in a fridge for 15 days (50.2 N). Eggs stored for 15 days at room temperature had lower eggshell breaking strengths in comparison to those stored in a fridge (38.4 N) but these values were not significantly different from one another at P<0.05. The eggshell breaking strength did also not decrease (P<0.05) after 38 days storage in a fridge compared to values obtained on day 15. The eggshell breaking strength of eggs stored for 38 days at room temperature further decreased (P<0.05) to 22.4 N. For the SS 3 treatment there was an increase (P<0.05) in the eggshell breaking strength of eggs stored for 38 days in a fridge compared to eggs stored for 15 days in a fridge (46.2 and 28.7 N, respectively). This result is unexpected and there is no known reason for this increase in eggshell breaking strength. The high-Se maize 3 diet showed a decrease (P<0.05) in the eggshell breaking stored in a fridge after 15 days and eggs stored at room temperature after 38 days (26.9 and 27.7 N, respectively). These two values were not significantly different from one another at P<0.05.



Table 18 Mean eggshell breaking strength values (Newtons \pm SE) measured after the 12-day experiment (day 0)
and again after storage at room temperature (22 °C) or in a fridge (4 °C) for 15 or 38 days

	Day 0 (Fresh)	Day 15		Day 38	
Treatment	Storage conditions	Room temperature	Refrigerated	Room temperature	Refrigerated
Control	38.7 ± 16.02	28.8 ± 9.88	$44.0_1 \pm 10.18$	32.8 ± 12.95	36.9 ± 12.11
Availa [®] Se 1	38.9 ± 12.72	39.1 ± 10.18	41.3 ± 4.40	29.4 ± 10.86	39.2 ± 12.00
Availa [®] Se 2	37.0 ± 12.90	30.8 ± 14.47	$27.1_2 \pm 9.63$	$39.4_1\pm12.72$	32.3 ± 11.78
Availa [®] Se 3	40.9 ± 12.38	32.9 ± 10.67	$32.4_2 \pm 8.26$	30.6 ± 11.70	40.7 ± 12.27
Sodium selenite 1	37.2 ± 20.96	$38.4^{a} \pm 10.94$	$50.2^{a} \pm 10.01$	$22.4^{b}_{2} {\pm}~14.96$	$40.2^a {\pm}~8.36$
Sodium selenite 2	36.6 ± 15.69	32.4 ± 12.47	42.3 ± 8.53	33.4 ± 12.34	29.8 ± 9.94
Sodium selenite 3	31.5 ± 5.31	42.8 ± 15.13	$28.7^{a}_{2} \pm 10.80$	$40.6_1\pm23.09$	$46.2^b\pm14.04$
High-Se maize 1	44.9 ± 14.17	39.5 ± 1.65	$32.1_2\pm16.50$	35.2 ± 12.03	38.3 ± 6.24
High-Se maize 2	35.4 ± 16.45	34.6 ± 18.68	$31.2_2\pm12.08$	$42.7_1\pm4.04$	40.8 ± 9.77
High-Se maize 3	$49.5^{a}\pm8.64$	39.0 ± 9.57	$26.9^{b}_{2} \pm 12.01$	$27.7^{b}\pm3.40$	33.5 ± 0.04
Control _{Av}	38.7 ± 16.02	28.8 ± 9.88	44.0 ± 10.18	32.8 ± 12.95	36.9 ± 12.11
Availa [®] Se _{Av}	38.9 ± 11.84	34.2 ± 11.62	33.1 ± 9.43	33.1 ± 11.85	37.4 ± 11.75
selenite _{Av}	35.1 ± 14.52	37.9 ± 12.80	$40.4_1 \pm 12.93$	32.1 ± 17.88	38.2 ± 12.06
Maize _{Av}	42.7 ^a ± 13.91	37.6 ± 11.38	$30.3^{b}_{\ 2} \pm 12.62$	35.9 ± 9.48	38.3 ± 7.26

Treatments 1= 130 μ g Se/kg DM, 2= 260 μ g Se/kg DM, 3= 390 μ g Se/kg DM

Row means with superscripts a-b differ significantly at P < 0.05

Column means with subscripts 1-2 differ significantly at P < 0.05

4.3.3 Albumen weight

A comparison was made between the albumen weights of the different treatments and levels over different storage periods (15 and 38 days) and under different storage conditions (room temperature and refrigeration) (Table 19). There were no significant differences between the average values of albumen weight between treatments over different storage times and conditions at P<0.05.

On day 0 (fresh eggs) SS 1 produced the heaviest (P<0.05) albumen weight (35.2 g). Sodium selenite 1 had eggs with heavier (P<0.05) albumen weights compared to $Availa^{\$}Se$ 1, $Availa^{\$}Se$ 2 and high-Se maize 2 (30.2, 30.1 and 29.6 g, respectively). These three treatments were not significantly different from one another at P<0.05. For the eggs stored at room temperature for 15 days, no conclusive result was found except for SS 1 which produced



heavier (P<0.05) albumen weights than high-Se maize 1 (33.0 and 28.3 g, respectively). Of the eggs stored in the fridge for 38 days SS 1 produced the heaviest (P<0.05) albumen weight (33.4 g). Sodium selenite 1 produced eggs with heavier (P<0.05) albumen weights compared to the control, $Availa^{@}Se$ 1 and 2, SS 2 and high-Se maize 1 and 3 (27.9, 28.4, 29.1, 28.8, 28.6 and 26.0 g, respectively). Sodium selenite 1 produced eggs with equal (P>0.05) albumen weights compared to SS 3, $Availa^{@}Se$ 3 and high-Se maize 2. No conclusive result was found on day 38 for refrigerated eggs.

A comparison was made between the albumen weight measured at day 0 (fresh) and then after 15 and 38 days storage under different storage conditions within treatments. For the average *Availa*[®]Se values there was a decline (P<0.05) in albumen weight from 31.3 g on day 15 for eggs stored at room temperature to 28.6 g for eggs stored for 38 days at room temperature. For the SS treatment there was a decline (P<0.05) in albumen weight from 33.5 g for newly laid eggs compared to the eggs stored for 15 days at room temperature and for eggs stored for 38 days at room temperature and in a fridge (30.7, 29.7 and 30.6 g respectively). These three values were not significantly different from one another at P<0.05. The albumen weight of eggs stored in a fridge for 15 days did not decline below that of the control. For the average values of the high-Se maize treatment the eggs stored for 38 days at room temperature and in a fridge were lower (P<0.05) than the newly laid eggs (27.9, 29.1 and 32.2 g, respectively). The former two weights did not differ from each other at P<0.05.

For the individual treatment values the SS 2 treatment showed a decrease (P<0.05) in albumen weight from 32.4 g on day 0 to 26.7 g on day 38 for the eggs stored at room temperature. For SS 3 the albumen weight decreased (P<0.05) from 33.2 g on day 0 to 27.7 g after 15 days storage at room temperature and to 28.2 g after 38 days storage at room temperature. These two values were not significantly different from one another at P<0.05. For the high-Se maize 1 treatment the albumen weight decreased (P<0.05) from 34.0 g on day 0 to 28.3 g after 15 days storage at room temperature, to 27.3 g after 38 days storage at room temperature and 28.6 g for eggs stored in the fridge. These three decreases in albumen weight compared to eggs on day 0 did not differ significantly from one another at P<0.05. For high-Se maize 3 the albumen weight decreased (P<0.05) from 33.2 g on day 0 to 26.0 g after 38 days for eggs stored in a fridge.



	Day 0 (Fresh)	Day 15		Day 38	
	Storage	Room		Room	
Ireatment	conditions	temperature	Refrigerated	temperature	Refrigerated
Control	31.1 ± 3.19	$31.2_{13} \pm 4.54$	31.5 ± 5.11	29.4 ± 4.75	$27.9_{23} \pm 5.89$
Availa [®] Se 1	$30.2_1\pm0.58$	$30.7_{13} \pm 1.57$	30.7 ± 3.40	27.9 ± 0.89	$28.4_{23} \pm 1.32$
Availa [®] Se 2	$30.1_1\pm0.39$	$30.8_{13} \pm 1.85$	32.2 ± 3.53	28.5 ± 1.65	$29.1_{23} \pm 1.69$
Availa [®] Se 3	32.7 ± 3.03	$32.4_{12} \pm 3.69$	30.1 ± 1.99	29.3 ± 3.86	$29.4_{13}\pm 3.75$
Sodium selenite 1	$35.2_2\pm3.46$	$33.0_1 \pm 5.51$	34.1 ± 4.66	34.3 ± 3.65	$33.4_1 \pm 1.64$
Sodium selenite 2	$32.4^a \pm 2.29$	$30.7_{13} \pm 2.82$	30.2 ± 2.30	$26.7^b \pm 1.22$	$28.8_{23}\pm 2.90$
Sodium selenite 3	$33.2^a\pm 6.32$	$27.7^{b}_{3} \pm 1.6$	32.2 ± 5.82	$28.2^{b}\pm 6.16$	$29.4_{13}\pm 3.04$
High-Se maize 1	$34.0^{a} \pm 4.87$	$28.3^{b}_{\ 23}\pm1.75$	30.8 ± 0.81	$27.3^{\text{b}} \pm 1.48$	$28.6^{b}_{\ 2\ 3}\pm1.46$
High-Se maize 2	$29.6_1\pm1.98$	$31.5_{13} \pm 4.76$	29.6 ± 5.25	28.6 ± 3.33	$31.8_{12}\pm 2.99$
High-Se maize 3	$33.2^{\rm a}\pm1.63$	$31.3_{13} \pm 1.22$	31.1 ± 0.67	27.9 ± 4.32	$26.0^{b}_{3} \pm 4.59$
Control _{Av}	31.1 ± 3.19	31.2 ± 4.54	31.5 ± 5.11	29.4 ± 4.75	27.9 ± 5.89
$Availa^{ entropye}Se_{Av}$	31.0 ± 2.09	$31.3^{a} \pm 2.54$	31.0 ± 2.94	$28.6^b \pm 2.44$	28.9 ± 2.35
Sodium selenite $_{Av}$	$33.5^{a} \pm 4.25$	$30.7^b \pm 4.15$	$32.2^{a} \pm 4.36$	$29.7^b\pm5.14$	$30.6^{b}\pm3.21$
Maize _{Av}	$32.2^{a}\pm3.63$	30.3 ± 3.24	30.5 ± 3.00	$27.9^{b} \pm 2.63$	$29.1^{b}\pm3.67$

Table 19 Mean albumen weights ($g \pm SE$) of eggs of different treatments after the 12-day experiment (day 0)and after storage at room temperature (22 °C) or in a fridge (4 °C) for 15 or 38 days

Treatments 1= 130 µg Se/kg DM, 2= 260 µg Se/kg DM, 3= 390 µg Se/kg DM Row means with superscripts a-b differ significantly at P<0.05 Column means with subscripts 1-3differ significantly at P<0.05

4.3.4 Yolk weight

A comparison was made between the yolk weights of different treatments over 15 and 38 days and under different storage conditions (room temperature and refrigeration) (Table 20).

For the individual yolk weight values after 15 days storage at room temperature *Availa*[®]Se 3 had a heavier (P<0.05) yolk weight at 18.8 g than *Availa*[®]Se 2 and high-Se maize 1 (both having yolk weights of 16.5 g). After 38 days storage at room temperature the only increase (P<0.05) in yolk weight compared to the control (17.2 g) was the SS 1 treatment at 19.3 g (this was also the heaviest value obtained for yolk weight out of all the treatments). After 38 days storage in a fridge the only increase (P<0.05) in yolk weight compared to the control (16.1 g) was SS 1, SS 3 and high-Se maize 2 (19.3, 18.3 and 18.7 g,



respectively). Again, SS 1 produced the heaviest yolk weight but this was not significantly different from SS 3, $Availa^{\$}Se$ 3 and high-Se maize 1 and 2 (P>0.05).

A comparison was made between the yolk weight measured at day 0 (fresh) and then after 15 and 38 days storage under different storage conditions within a treatment. For the average values the only significant difference was found for the *Availa*[®]Se treatment. Eggs stored for 38 days at room temperature had heavier (P<0.05) yolk weights than eggs at day 0 and eggs stored in a fridge for 38 days (18.1, 16.5 and 16.7 g, respectively). The last two values were not significantly different at P<0.05.

	Day 0 (Fresh)	Day 15		Day 15 Day		y 38
	Storage	Room		Room		
Treatment	conditions	temperature	Refrigerated	temperature	Refrigerated	
Control	16.5 ± 1.97	17.3 ± 2.41	17.8 ± 1.90	$17.2_1 \pm 1.00$	$16.1_{34}\pm 2.07$	
Availa [®] Se 1	16.9 ± 0.68	17.2 ± 1.55	17.3 ± 1.22	17.3 ± 1.45	$16.2_{34}\pm 1.98$	
Availa [®] Se 2	16.6 ± 1.26	$16.5_1\pm0.81$	16.9 ± 2.17	$18.2^{a} \pm 2.27$	$16.1^{b}_{34}\pm1.29$	
Availa [®] Se 3	$16.1^a \pm 0.86$	$18.8^{b}_{2} \pm 1.50$	17.3 ± 1.42	$18.5^{b}\pm1.30$	$17.7_{14} \pm 2.09$	
Sodium selenite 1	17.5 ± 1.22	18.5 ± 1.88	18.4 ± 1.30	$19.3_2 \pm 1.09$	$19.3_1 {\pm}~1.98$	
Sodium selenite 2	17.4 ± 1.30	17.2 ± 1.38	17.0 ± 0.48	18.0 ± 1.44	$17.2_{24} \pm 1.38$	
Sodium selenite 3	17.9 ± 1.62	17.4 ± 0.64	17.7 ± 1.90	18.0 ± 1.23	$18.3_{12} \pm 2.35$	
High-Se maize 1	16.8 ± 2.74	$16.5_1\!\pm1.23$	17.8 ± 1.48	17.3 ± 1.06	$17.8_{14} \pm 0.77$	
High-Se maize 2	17.0 ± 0.92	17.6 ± 0.95	16.9 ± 2.23	18.3 ± 2.45	$18.7_{12} \pm 1.21$	
High-Se maize 3	17.6 ± 1.48	17.3 ± 0.27	18.7 ± 1.11	17.7 ± 2.82	$16.9_{24}\pm 2.87$	
$Control_{Av}$	16.5 ± 1.97	17.3 ± 2.41	17.8 ± 1.90	17.2 ± 1.00	$16.1_1\pm2.07$	
$Availa^{\ensuremath{ extsf{8}}}Se_{\ensuremath{ extsf{Av}}}$	$16.5^{a} \pm 0.96$	17.6 ± 1.59	17.2 ± 1.56	$18.1^{\rm b}\pm1.68$	$16.7^{a}_{13} \pm 1.86$	
Sodium selenite $_{Av}$	17.6 ± 1.31	17.7 ± 1.45	17.7 ± 1.37	18.4 ± 1.33	$18.3_{234} \pm 1.97$	
Maize _{Av}	17.1 ± 1.75	17.1 ± 0.99	17.7 ± 1.73	17.7 ± 1.87	$17.9_4{\pm}~1.68$	

Table 20 Mean yolk weights ($g \pm SE$) of different treatments weighed after the 12-day experiment (day 0) and after storage at room temperature (22 °C) or in a fridge (4 °C) for 15 or 38 days

Treatments $1 = 130 \ \mu g \ Se/kg \ DM$, $2 = 260 \ \mu g \ Se/kg \ DM$, $3 = 390 \ \mu g \ Se/kg \ DM$

Row means with superscripts a-b differ significantly at P < 0.05

Column means with subscripts 1-4differ significantly at P < 0.05

For the individual values, $Availa^{\$}Se\ 2$ eggs had heavier (P<0.05) yolks when the eggs were stored at room temperature compared to eggs stored in a fridge on day 38 (18.2 g and 16.1 g, respectively). Yolk weights of the $Availa^{\$}Se\ 3$ eggs increased (P<0.05) from 16.1 g



for newly laid eggs to 18.8 g after 15 days storage at room temperature and to 18.5 g after 38 days storage at room temperature. The difference in yolk weight between these eggs on day 15 and day 38 was not significant at P<0.05.

4.3.5 Albumen height

A comparison was made between the albumen heights of different treatments over 15 and 38 days and under different storage conditions (room temperature and refrigeration) (Table 21). For the average albumen height values the only significant differences were found on day 0 (newly laid eggs) and day 38 for the refrigerated eggs. On day 0 Availa[®]Se, SS and high-Se maize all had lower (P < 0.05) albumen height values compared to that of the control (6.6, 6.4, 5.9 and 8.0 mm, respectively). The Availa[®]Se, SS and high-Se maize treatments were not significantly different from each other at P < 0.05. On day 38 for the refrigerated eggs Availa[®]Se, SS and high-Se maize all produced higher (P < 0.05) albumen heights compared to the control (4.4, 4.5, 4.4 and 3.5 mm, respectively) but they were not significantly different from one another at P < 0.05.

For the remaining individual albumen height values, on day 0 the control treatment produced the highest (P<0.05) albumen height (8.0 mm). All of the treatments, except for $Availa^{\$}Se$ 1, had a lower (P<0.05) albumen height than the control. After 15 days storage at room temperature SS 1 produced a higher (P<0.05) albumen height than SS 2 and high-Se maize 2 (4.9, 3.9 and 3.7 mm, respectively). Sodium selenite 2 and high-Se maize 2 were not significantly different at P<0.05. After 38 days storage in a fridge all of the values obtained were higher than that of the control (3.5 mm). The only values that were significantly higher (P<0.05) than the control, however, were High-Se maize 1 and 3 (4.9 and 4.0 mm, respectively). No conclusive result was found on day 38 for the albumen height of refrigerated eggs.

A comparison was made between the albumen height measured at day 0 (fresh) and then after 15 and 38 days storage under different storage conditions (room temperature and refrigeration) within a treatment. For the average values for the control treatment the albumen height decreased (P<0.05) from 8.0 mm on day 0 to 4.6 and 4.9 mm for the eggs stored for 15 days at room temperature and in a fridge, respectively. These two values were not significantly different from one another at P<0.05. The albumen height further decreased (P<0.05) from day 0 and day 15 values to 3.2 and 3.5 mm after 38 days at room temperature



and in a fridge, respectively. These two values were not significantly different from on another at P < 0.05. For Availa[®]Se the albumen height was the highest (P < 0.05) on day 0 (6.6 mm) and then decreased (P < 0.05) to 4.3 mm after 15 days storage at room temperature. The albumen height also decreased from (P < 0.05) to 5.2 mm after 15 days storage in a fridge. The albumen height further decreased to 3.0 mm after 38 days storage at room temperature. This was the lowest (P < 0.05) albumen height value obtained for the Availa[®]Se treatment. The albumen height value of eggs stored for 38 days in a fridge (4.4 mm) was not significantly different (P>0.05) to eggs stored for 15 days at room temperature. For the average SS values the albumen height was the highest (P < 0.05) on day 0 (6.4 mm) and then decreased (P < 0.05) to 4.3 mm after 15 days storage at room temperature. There were no significant differences in the albumen height of eggs stored for 15 days at room temperature and in a fridge and eggs stored for 38 days in a fridge at P < 0.05. The lowest (P < 0.05) albumen height value was obtained for eggs stored for 38 days at room temperature (3.0 mm). For the average high-Se maize values the albumen height was the highest (P < 0.05) on day 0 (5.9 mm) and then decreased (P < 0.05) to 4.3 mm after 15 days storage at room temperature. There were no significant differences in the albumen height of eggs stored for 15 days at room temperature and in a fridge and eggs stored for 38 days in a fridge at P < 0.05. The lowest (P < 0.05) albumen height value was obtained for eggs stored for 38 days at room temperature (2.9 mm).

For the individual measurements of the control treatment the albumen height decreased (P < 0.05) from 8.0 mm for the fresh eggs to 4.6 mm and 4.9 mm for the eggs stored for 15 days at room temperature and in the fridge, respectively. The decrease in albumen height between these two storage types after 15 days storage was not significant. The albumen height further decreased (P < 0.05) after 38 days storage to 3.2 mm for eggs stored at room temperature and 3.5 mm for eggs stored in the fridge (these two values did not differ from each other at P < 0.05). For $Availa^{@}Se$ 1 the albumen height was highest at day 0 (7.6 mm). It then decreased (P < 0.05) to 4.3 mm and 4.9 mm for the eggs stored for 15 days at room temperature and in the fridge, respectively (these two values did not differ from one another at P < 0.05). Eggs stored for 38 days in a fridge decreased to 4.3 mm and this value was not significantly lower than the two values obtained on day 15. The lowest (P < 0.05) albumen height was found after 38 days storage at room temperature (2.9 mm). For the $Availa^{@}Se$ 2 treatment the albumen height decreased (P < 0.05) from 6.0 mm for the fresh eggs to 4.4 mm after 15 days storage at room temperature.



	Day 0 (Fresh)	Day 15		Day 38	
Tractment	Storage	Room	Defrigerated	Room	Defrigerated
Treatment	conditions	temperature	Reingeraled	temperature	Kenngerated
Control	$8.0^{a}_{\ 1} \pm 0.91$	$4.6^{b} \pm 0.64$	$4.9^{b} \pm 0.29$	$3.2^{c} \pm 0.79$	$3.5^{c}_{1} \pm 0.55$
Availa [®] Se 1	$7.6^{a}_{12} \pm 0.71$	$4.3^{b}\pm0.88$	$4.9^{b}\pm0.44$	$2.9^{c} \pm 0.31$	$4.3^{b}_{126}\!\pm 0.46$
Availa [®] Se 2	$6.0^a{}_{34} \pm 0.59$	$4.4^{b}\pm0.53$	$5.3^{ac} \pm 0.53$	$3.0^d \pm 0.25$	$4.5^{bc}_{14} {\pm} 0.65$
Availa [®] Se 3	$6.0^{a}_{34} \pm 0.60$	$4.4^{c}\pm0.48$	$5.3^{ab}\pm0.78$	$3.2^d \pm 0.48$	$4.5^{bc}_{14} \pm 0.64$
Sodium selenite 1	$6.7^{a}_{\ 2\ 3} \pm 0.38$	$4.9^{b}_{1} \pm 0.60$	$5.2^{b} \pm 0.53$	$3.1^{\circ} \pm 0.48$	$4.9^{b}_{14} \!\pm 0.94$
Sodium selenite 2	$6.2^a_{\ 34} \pm 0.80$	$3.9^{c}_{2} \pm 0.54$	$5.1^{\rm b}\pm0.47$	$3.0^d \pm 0.30$	$4.6^{bc}_{145} \pm 0.76$
Sodium selenite 3	$6.2^a_{\ 34} \pm 0.74$	$4.2^b\pm0.59$	$4.6^{b} \pm 1.13$	$3.0^{c} \pm 0.56$	$4.0^{b}_{145}\!\pm 0.46$
High-Se maize 1	$6.4^{a}_{3} \pm 0.23$	$4.5^{b} \pm 0.60$	$4.9^{\text{b}} \pm 1.31$	$3.0^{c}\pm0.55$	$4.9^{b}_{234}\pm0.53$
High-Se maize 2	$5.4^{a}_{4} \pm 1.60$	$3.7^{cd}_{\ 2} \pm 0.49$	$4.8^{ab}\pm0.36$	$2.8^{d} \pm 0.20$	$4.1^{bc}_{\ 1\ 3\ 6}\pm 0.40$
High-Se maize 3	$5.8^a_{\ 34}\pm 0.45$	$4.7^{ad}\pm1.63$	$5.2^{a} \pm 0.65$	$2.9^{\rm c}\pm0.93$	$4.0^{bcd}_{256}\pm 0.97$
Control _{Av}	$8.0^{a}_{1} \pm 0.91$	$4.6^{b} \pm 0.64$	$4.9^{b} \pm 0.29$	$3.2^{c} \pm 0.79$	$3.5^{c}_{1} \pm 0.55$
$Availa^{\mathbb{R}}Se_{Av}$	$6.6^{a}_{3} \pm 2.09$	$4.3^{b}\pm2.54$	$5.2^{\circ} \pm 2.94$	$3.0^d \pm 2.44$	$4.4^{b}_{\ 2} \pm 2.35$
Sodium selenite $_{Av}$	$6.4^{a}_{23} \pm 4.25$	$4.3^{\circ} \pm 4.15$	$5.0^{b} \pm 4.36$	$3.0^d \pm 5.14$	$4.5^{bc}_{2} \pm 3.21$
Maize _{Av}	$5.9^{a}_{2} \pm 3.63$	$4.3^{\rm c}\pm3.24$	$5.0^b \pm 3.00$	$2.9^{d}\pm2.63$	$4.4^{bc}_{2} \pm 3.67$

Table 21 Mean albumen heights (mm \pm SE) of different treatment diets measured after the 12-day experiment (day 0) and again after storage at room temperature (22 °C) or in a fridge (4 °C) for 15 or 38 days

Treatments $1=130 \ \mu g \ Se/kg \ DM$, $2=260 \ \mu g \ Se/kg \ DM$, $3=390 \ \mu g \ Se/kg \ DM$

Row means with superscripts a-d differ significantly at P<0.05

Column means with subscripts 1-6differ significantly at P<0.05

The albumen height of the eggs on day 15, at room temperature and in a fridge, and on day 38 in a fridge did not differ significantly from the one another or the control at P<0.05. The lowest (P<0.05) albumen height was found after 38 days storage at room temperature (3.0 mm). For the Availa[®]Se 3 treatment the albumen height decreased (P<0.05) from 6.0 mm for the fresh eggs to 4.4 mm after 15 days storage at room temperature. The albumen height of the eggs on day 15, at room temperature and in a fridge, and on day 38 in a fridge did not differ significantly from the control or one another at P<0.05. The lowest (P<0.05) albumen height decreased (P<0.05) from 6.0 mm for the albumen height decreased (P<0.05) from 6.0 mm for the eggs on day 15, at room temperature and in a fridge, and on day 38 in a fridge did not differ significantly from the control or one another at P<0.05. The lowest (P<0.05) albumen height was found after 38 days storage at room temperature (3.2 mm). For the SS 1 treatment the albumen height decreased (P<0.05) from 6.7 mm for the fresh eggs to 4.9 mm after 15 days storage at room temperature and to 5.2 mm after 15 days storage in a fridge. The albumen height also decreased (P<0.05), compared to day 0, to 4.9 mm after 38 days storage in a fridge. These three deviations from the control were not significantly different from one


another. The lowest decrease (P < 0.05) in albumen height was after 38 days storage at room temperature (3.1 mm). For the SS 2 treatment the albumen height decreased (P < 0.05) from 6.2 mm for the fresh eggs to 3.9 mm after 15 days storage at room temperature. The albumen height of the eggs on day 15, at room temperature and in a fridge, and on day 38 in a fridge did not differ significantly from one another at P < 0.05. The lowest (P < 0.05) albumen height was found after 38 days storage at room temperature (3.0 mm). For the SS 3 treatment the albumen height decreased (P < 0.05) from 6.2 mm for the fresh eggs to 4.2 mm after 15 days storage at room temperature and 4.6 mm after 15 days storage in a fridge. The albumen height also decreased (P < 0.05) to 4.0 mm after 38 days storage in a fridge. These three deviations from the control were not significantly different from one another at P < 0.05. The lowest decrease (P < 0.05) in albumen height was after 38 days storage at room temperature (3.0 mm). For the high-Se maize 1 treatment the albumen height decreased (P<0.05) from 6.4 mm for the fresh eggs to 4.5 mm after 15 days storage at room temperature and 4.9 mm after 15 days storage in a fridge. The albumen height further decreased (P < 0.05) from day 0 to 4.9 mm after 38 days storage in a fridge. These three deviations from the control were not significantly different from one another at P < 0.05. The lowest decrease (P < 0.05) in albumen height was after 38 days storage at room temperature (3.0 mm). For the high-Se maize 2 treatment the highest (P < 0.05) albumen height was obtained on day 0 (5.4 mm). The lowest (P<0.05) albumen height was found on day 38 for eggs stored at room temperature (2.8 mm). Eggs stored for 38 days in a fridge did not decrease (P>0.05) in albumen height in comparison to those stored for 15 days at room temperature and in a fridge. For the high-Se maize 3 treatment the eggs stored for 15 days at room temperature and in a fridge did not decrease (P>0.05) in albumen height compared to day 0. Eggs stored for 38 days in a fridge did not have a different albumen height to those eggs stored for 15 days in a fridge or at room temperature or those stored for 38 days at room temperature (P>0.05). The lowest albumen height was obtained for eggs stored for 38 days at room temperature (2.9 mm).

The average and individual values recorded show that the refrigerated eggs' albumen heights were higher (P<0.05) than the eggs stored at room temperature and the fresh eggs always produced the highest albumen height values (P<0.05).



4.3.6 Haugh units

A comparison of the Haugh units (HU) of eggs stored for 15 and 38 days under different storage conditions (room temperature and refrigeration) between treatments was made (Table 22).

For the individual values on day 0 (fresh eggs) all of the treatments had lower (P<0.05) HU values than the control (89.4 HU) except for $Availa^{\circ}Se$ 1 (87.4 HU) which was equal to that of the control. After 15 days storage at room temperature the SS 1 treatment produced the highest HU value at 66.0 HU. This value was not significantly different from the control (64.6 HU) or from the high-Se maize treatment 1 (65.2 HU) at P<0.05. The SS 2 and high-Se maize 2 treatment showed lower (P<0.05) HU values compared to the control (56.5 and 55.1 HU, respectively). After 15 days storage in a fridge the $Availa^{\circ}Se$ 3 treatment produced the highest HU value at 71.6 HU. The only other value that differed significantly from this treatment was the SS 3 treatment having a lower HU value at 63.0 HU (P<0.05). After 38 days storage at room temperature the control was the SS 1 treatment having a lower HU value at 41.7 HU (P<0.05). After 38 days storage in a fridge the Se treatment having a lower HU value at 41.7 HU (P<0.05). After 38 days storage in a fridge the Se treatment sto show improved (P<0.05) HU scores above that of the control (55.7 HU) were $Availa^{\circ}Se$ 2 and 3, SS 1 and 2 and high-Se maize 1. These treatments were not significantly different from one another at P<0.05.

A comparison was made between the HU measured at day 0 (fresh eggs) and then after 15 and 38 days storage under different storage conditions (room temperature and refrigeration) within a treatment. For the average HU values for the control the fresh eggs showed the highest (P<0.05) HU value at 89.4. The HU value decreased (P<0.05) on day 15 to 64.6 and 67.4 HU for eggs stored at room temperature and in a fridge, respectively. These two values were not significantly different from one another at P<0.05. The HU value further decreased (P<0.05) to 50.7 and 55.7 HU for eggs stored at room temperature and in a fridge, respectively. For the Availa[®]Se treatment the fresh eggs showed the highest (P<0.05) HU value at 80.6 HU. The HU value decreased to 61.9 HU after 15 days storage in a fridge and this value was not significantly different to the HU value obtained for eggs stored for 38 days under refrigeration (64.7 HU) at P<0.05. The HU values for eggs stored for 15 days in a fridge were the highest (P<0.05) of the storage periods and conditions at 70.4 HU. The HU values were the lowest (P<0.05) for eggs stored for 38 days at room temperature (47.6 HU). For the SS treatment the HU value was highest (P<0.05) for the fresh eggs (78.4 HU). The



HU value of the eggs then decreased (P < 0.05) on day 15, at room temperature and in a fridge, and on day 38 for eggs stored in a fridge. These three values did not differ significantly from one another at P < 0.05. The lowest (P < 0.05) HU value was obtained after 38 days storage at room temperature (45.5 HU). For the high-Se maize treatment the HU value was highest (P < 0.05) for the fresh eggs (74.7 HU). The HU value then decreased (P < 0.05) on day 15, at room temperature and in a fridge, and on day 38 for eggs stored in a fridge. These three values did not differ significantly from one another at P < 0.05. The lowest (P < 0.05) HU value was obtained after 38 days storage at room temperature and in a fridge, and on day 38 for eggs stored in a fridge. These three values did not differ significantly from one another at P < 0.05. The lowest (P < 0.05) HU value was obtained after 38 days storage at room temperature (45.3 HU).

	Day 0 (Fresh)	Day 15		Day 38	
	Storage	Room		Room	
Treatment	conditions	temperature	Refrigerated	temperature	Refrigerated
Control	$89.4^{a}_{1} \pm 4.78$	$64.6^{b}_{1} \pm 6.65$	$67.4^{b} \pm 1.67$	$50.7^{c}_{1} \pm 7.67$	$55.7^{\circ}_{3} \pm 2.53$
Availa [®] Se 1	$87.4^{a}_{12} \pm 4.44$	$61.4^{b} \pm 8.33$	$68.2^{\text{b}} \pm 3.31$	$45.9^{c}\pm5.15$	$63.8^{b}_{123} \pm 4.87$
Availa [®] Se 2	$77.6^{a}_{\ 3} \pm 4.37$	$62.8^b {\pm} 6.65$	$71.3^{a} \pm 3.52$	$48.2^{c}\pm3.26$	$65.6^{b}_{12} \pm 6.13$
Availa [®] Se 3	$76.7^{a}_{3} \pm 4.86$	$61.6^{c}\pm3.52$	$71.6^{ab}{}_{1}\pm 6.77$	$48.6^d \pm 5.24$	$64.7^{cb}_{12}{\pm}6.10$
Sodium selenite 1	$79.8^{a}_{\ 2\ 3}\pm3.77$	$66.0^{b}{}_{1} \pm 5.96$	$68.0^b \pm 3.43$	$41.7^{c}_{2} \pm 10.83$	$64.7^{b}_{12} {\pm} 8.30$
Sodium selenite 2	$78.6^{a}_{\ 2\ 3}\pm5.34$	$56.5^{c}_{2} \pm 8.46$	$70.1^{ab} {\pm}~3.30$	$49.1^{c}\pm4.18$	$66.1^{b}{}_{12}{\pm}7.25$
Sodium selenite 3	$76.9^{a}_{\ 3} \pm 5.50$	$62.3^{\text{b}} \pm 6.13$	$63.0^{b}_{2} \pm 11.41$	$45.8^{\rm c}\pm 6.68$	$59.7^{b}_{123} \pm 5.86$
High-Se maize 1	$79.1^{a}_{\ 2\ 3}\pm3.86$	$65.2^{b}_{1}\pm 5.82$	$\mathbf{66.3^b} \pm 12.97$	$48.0^{c}\pm8.11$	$68.6^{b}_{1}\pm 4.16$
High-Se maize 2	$71.0^{a}_{3} \pm 15.45$	$55.1^{b}_{2}\pm 6.81$	$68.0^{a}\pm2.76$	$44.1^{c}\pm6.82$	$58.2^{b}_{\ 2\ 3}\pm 6.64$
High-Se maize 3	$73.8^{a}_{3} \pm 4.67$	$64.0^{ab}\pm14.86$	$69.4^{ab}\pm5.25$	$43.5^{c} \pm 8.77$	$59.8^{b}_{123}\pm11.52$
Control _{Av}	$89.4^{a} \pm 4.78$	$64.6^{b} \pm 6.65$	$67.4^{b} \pm 1.67$	$50.7^{c} \pm 7.67$	$55.7^{\circ} \pm 2.53$
$Availa^{ entropye}Se_{Av}$	$80.6^{a} \pm 6.57$	$61.9^{\circ} \pm 6.03$	$70.4^{b}\pm4.72$	$47.6^{d}\pm4.48$	$64.7^{c} \pm 5.36$
Sodium selenite $_{Av}$	$78.4^{a}{\pm}~4.63$	$61.6^{\rm c}\pm7.60$	$67.0^{b}\pm7.30$	$45.5^{d}\pm7.81$	$63.8^{bc}\pm7.27$
Maize _{Av}	$74.7^a \pm 9.69$	$61.2^{c} \pm 9.60$	$67.8^{\rm b}\pm7.74$	$45.3^{d}\pm7.31$	$62.4^{bc}\pm8.32$

Table 22 Mean Haugh unit values (HU \pm SE) of different treatment diets measured after the 12-day experiment (day 0) and again after storage at room temperature (22 °C) or in a fridge (4 °C) for 15 or 38 days

Haugh unit is calculated as HU= 100 log (H – $1.7w^{0.37}$ + 7.6), H= observed height of albumen (mm) and W= egg weight (g) (Haugh, 1937)

Treatments 1= 130 µg Se/kg DM, 2= 260 µg Se/kg DM, 3= 390 µg Se/kg DM

Row means with superscripts a-d differ significantly at P < 0.05

Column means with subscripts 1-3differ significantly at P < 0.05

For the individual HU values obtained within a treatment, for $Availa^{@}Se$ 1, the HU value decreased (P<0.05) from 87.4 HU for the fresh eggs to 61.4 and 68.2 HU for the eggs



stored for 15 days at room temperature and in the fridge, respectively. The difference in HU values between the two storage types after 15 days storage was not significant at P < 0.05. The lowest (P<0.05) HU value was obtained after 38 days storage at room temperature (45.9 HU). For the Availa[®]Se 2 treatment the HU value decreased (P < 0.05) from 77.6 HU for the fresh eggs to 62.8 HU after 15 days storage at room temperature. The lowest (P < 0.05) HU value was obtained for the eggs stored for 38 days at room temperature (48.2 HU). For the Availa[®]Se 3 treatment the highest (P < 0.05) HU value was obtained for the newly laid eggs (76.7 HU). This HU value was equal (P>0.05) to those of eggs stored for 15 days in a fridge. The HU value then decreased to 61.6 HU after 15 days storage at room temperature. The HU of the eggs on day 15, at room temperature and in a fridge, and on day 38 in a fridge did not differ significantly from one another at P < 0.05. The lowest (P < 0.05) HU value was found after 38 days storage at room temperature (48.6 HU). For the SS 1 treatment the HU value decreased (P<0.05) from 79.8 HU for the fresh eggs to 66.0 and 68.0 HU for the eggs stored for 15 days at room temperature and in the fridge, respectively. The decrease in HU values between the two storage types after 15 days storage was not significant at P < 0.05. The lowest (P<0.05) HU value was obtained after 38 days storage at room temperature (41.7 HU). For the SS 2 treatment the HU value was the highest (P < 0.05) for the newly laid eggs (78.6 HU). The lowest (P < 0.05) HU value was obtained on day 15 and 38 for eggs stored at room temperature (56.5 and 49.1 HU, respectively). These two values were not significantly different from one another at P < 0.05. For the SS 3 treatment the HU value decreased (P<0.05) from 76.9 HU for the fresh eggs to 62.3 and 63.0 HU for the eggs stored for 15 days at room temperature and in the fridge, respectively. The difference in HU values between the two storage types after 15 days was not significant at P < 0.05. The lowest (P < 0.05) HU value was obtained after 38 days storage at room temperature (45.8 HU). For the high-Se maize 1 treatment the HU value decreased (P<0.05) from 79.1 HU for the fresh eggs to 65.2 and 66.3 HU for the eggs stored for 15 days at room temperature and in the fridge, respectively. The difference in HU values between the two storage types after 15 days storage was not significant at P < 0.05. The lowest (P < 0.05) HU value was found after 38 days of storage at room temperature (48.0 HU). For the high-Se maize 2 treatment the highest (P<0.05) HU value was obtained for fresh eggs and eggs stored for 15 days in a fridge (71.0 and 68.0 HU, respectively). These two values did not differ significantly from one another at P < 0.05. The HU value decreased (P < 0.05) to 55.1 HU after 15 days storage at room temperature. This value did not differ significantly from eggs stored for 38 days in a fridge (58.2 HU) at P < 0.05. The lowest (P < 0.05) HU value obtained was for eggs stored for 38 days at room



temperature (44.1 HU). For the high-Se maize 3 treatment the HU value was highest for the newly laid eggs (73.8), however, eggs stored for 15 days at room temperature and in a fridge showed equal (P>0.05) HU values to the newly laid eggs. The HU value of eggs stored for 38 days in a fridge did not decrease (P>0.05) in comparison to those stored for 15 days in a fridge and at room temperature. The lowest (P<0.05) HU value was obtained for eggs stored for 38 days at room temperature (43.5 HU).



CHAPTER 5: DISCUSSION

5.1 Selenium concentration of maize grain in South Africa: a survey

The map of the Se content of maize grain in South Africa, compiled from this survey, agrees to a large extent with Van Ryssen's (2001) map (Figure 3) for these grain-producing areas, albeit the 2001 map does not include much data from North West and central Free State.

When comparing the map of the Se concentration in maize grain (Figure 4) with the map of the soil Se status in South Africa (Figure 7), discrepancies are noted. The soils in large parts of Mpumalanga, northern KwaZulu-Natal and northern Free State, and areas of North West and central to eastern Free State contain high concentrations of Se, while the maize grain originating from these regions contained very little, if any, Se. In an investigation on the Se status of free-ranging livestock and game in the eastern regions of the Free State and Mpumalanga, Van Ryssen (2006) concluded that the Se status of these animals indicated a general Se deficiency, thus supporting the results from the survey on the Se content of maize from these regions.

Although Oldfield (1999) stated that the Se concentration in soils is generally reflected by the Se content of plants growing in those soils, it is suggested that the reason for the discrepancy between the soil Se and maize Se maps in the present study could be the influence of soil pH on the Se uptake of the maize plant. It is well documented that Se in the soil is readily available to plants if the soil pH is in the alkaline range (Reid & Horvath, 1980). According to Figure 8, the general trend is that soils in the eastern parts of the maizeproducing regions of South Africa have a low pH (acidic), while the soils in the western regions tend to have a moderate to high pH. This suggests that although the soils in the eastern parts of the country tend to have a high Se content, the Se is not available to the maize plants owing to the low soil pH. Van Ryssen (2006) suggested that the problem of Se availability to plants is probably exacerbated by the problem of acid rain and sulphur contamination in large areas of Mpumalanga and north-eastern Free State. It should be noted that the contribution of soil fertilisation practices to the availability of Se in the soil have not been established in this study.

According to Figure 8, in the western regions of the maize-producing areas of the country, the soil pH might be suitable for the uptake of Se by plants, but the Se content in the



soil seems inadequately low. In the irrigation areas of Vaalhartz and along the Orange and Vaal rivers leaching of Se from the soils might have contributed to a low Se in those soils, as noted by Van Ryssen (2001). The consequence is that the maize grain samples from these regions also contained very little, if any, Se.



Figure 7 Map of soil selenium status (mg/kg) in South Africa (with permission of the Institute for Soil, Water and Climate, Agricultural Research Council, South Africa)



Figure 8 Map of soil pH of South African soils (with permission of the Institute for Soil, Water and Climate, Agricultural Research Council, South Africa)



5.2 Selenium concentration of eggs

The differences between the actual and intended Se concentrations in the diets used (Table 11) may be due to small variations in sampling, or errors during the mixing of the diets since all 10 diets were mixed by hand and small inclusion levels of Se were used. However, the actual Se concentrations obtained still represented the intended Se concentrations, for example, level 1 was still the equivalent of approximately a third of level 3.

Results showed that the Se accumulation was higher in the albumen compared to the yolk when the organic Se supplements ($Availa^{\$}Se$ and high-Se maize) were fed, but lower when SS was the Se source. This is in agreement with Cantor & Scott (1974); Latshaw & Osman (1975); Hassan (1990) and Sheng *et al.* (2002) who found that SeMet deposits mainly in the egg albumen whilst inorganic Se or non-SeMet sources deposits mainly in the yolk. The difference in Se concentration between $Availa^{\$}Se$ and high Se maize (Table 13 and 15) could reflect the fact the Se in $Availa^{\$}Se$ is suggested to be 100% in the form of SeMet while a portion of the Se in maize constitutes Se compounds other than SeMet (Whanger, 2002). Schrauzer (2000) reported that approximately 80% - 81% of the Se found in maize is in the form of SeMet whilst Olsen & Palmer (1976) found that 50% of the Se in grain was SeMet. Daniels (1996) and Whanger (2003) also reported that the main form of Se in maize is SeMet.

Sodium selenite showed the lowest Se concentration in the albumen and the Se concentration did not increase to a large extent over the 12 day period (Figure 5). The highest Se concentration in the albumen achieved by SS was at day 12 (772 μ g Se/kg DM). The Se content from the organic Se supplements were higher (*P* <0.05) than SS at days 6, 8 and 12. *Availa*[®]Se produced the highest (*P* <0.05) Se concentration in the albumen on day 12 (3390 μ g Se/kg DM), followed by high-Se maize (2430 μ g Se/kg DM). This may be due to the fact that the form of Se in Zn-L-SeMet is completely SeMet whilst the form of Se in High-Se maize is largely SeMet. However, a proportion of the Se is present in other forms that may not be as available as SeMet. The remaining proportion of Se in the maize could be Semethyl-SeMet, SeCys and Se-methyl-SeCys (Heindl *et al.*, 2010). Since there is no known pathway of synthesis of SeMet from inorganic Se, animals must depend on their diets for this selenoamino acid (Sunde, 1990; Daniels, 1996; Levander & Burk, 1996; Whanger, 2003). This explains why SS does not increase the albumen Se concentrations to the same extent as



organic Se (SeMet) since the body is not able to synthesise SeMet from SS. However, it has been found that inorganic Se is more efficient at increasing the activity of Se-specific proteins such as glutathione peroxidase (GSH-Px) compared to organic Se (Daniels, 1996) and this may be valuable in terms of its antioxidant function in the storage of eggs and egg quality (see below). This explanation is also in agreement with Latshaw & Osman (1975) who found that Se in the form of SeCys accumulates to a greater extent in the yolk, a pattern similar to SS rather than SeMet, which is of interest since SeCys is also an organic Se source. This is to be expected since SS is incorporated into SeCys which is the form of Se largely found in GSH-Px (Levander & Burk, 1996). The authors suggested that this may be due to the fact that SeCys is metabolised into inorganic Se compounds and it has been shown on numerous occasions, including in this study, that inorganic Se preferably accumulates in the yolk.

For the Se accumulation in the yolk (Figure 6) the SS showed a similar increase in Se concentration compared to the *Availa*[®]Se over the 12 day period. At day 6 the *Availa*[®]Se showed the highest (P<0.05) Se concentration in the yolk (1188 µg Se/kg DM) with SS close behind that at 1107 µg Se/kg DM. *Availa*[®]Se and SS were able to increase the Se concentration of the yolk to the greatest extent (P<0.05) followed by high-Se maize. The large accumulation of organic as well as inorganic Se in the yolk suggests that the hen may have additional metabolic pathways with which to transfer Se to the egg, and specifically to the yolk (Paton *et al.*, 2002).

For the change in the Se concentration of the albumen over time, SS showed the slowest increase in Se concentration over the 12 days compared to the organic supplements. This agrees with the findings of Bennett & Cheng (2010) who found that in comparison to organic Se supplementation, inorganic Se sources have lower rates of absorption since they are passively absorbed by the body. Organic Se supplements, such as SeMet, are absorbed actively by the body and nonspecifically incorporated into proteins in place of Met thus their absorption rate into body tissue and thus egg protein (albumen) is faster. Payne *et al.* (2005) and Chantiratikul *et al.* (2008b) found that whole-egg Se concentrations increased rapidly with increasing dietary supplementation of Se from day four onwards. Results from this study showed a dramatic increase (P<0.05) in the Se concentration of the albumen after only one day of Se supplementation and $Availa^{@}Se$ after two days of supplementation. Sodium selenite did not significantly increase the Se concentration of the albumen over the 12-day period at P<0.05. The high-Se maize treatment increased (P<0.05) the Se



concentration of the yolk from day 6 to day 8 and then it remained constant until day 12, thus it increased the Se concentration of the yolk at the slowest rate but increased the Se concentration of the albumen at the fastest rate.

It is accepted that the SeMet is incorporated into protein at protein synthesis. Since the protein in albumen is laid down during the formation of the egg, it was assumed that Se in albumen should stabilise within a few days after the commencement of Se supplementation, specifically in the case of SeMet sources. However, it appears as if the incorporation of Se into the albumen has not reached steady state by day 12 and the Se concentration may still increase after 12 days for *Availa*[®]Se and high-Se maize. This could indicate that the proteins have been synthesised well before incorporation into the albumen. No similar studies have been reported in the literature. In the case of the yolk it seems as if rate of accumulation of Se has slowed down between days six and eight, thus approaching steady state. This was evident when both the organic and inorganic sources were fed. There is no apparent reason for the difference in the increase of Se accumulation over time between the albumen and the yolk, but it may be suggested that this is due to different metabolic pathways for inorganic Se (largely deposited in the yolk) and organic Se which is deposited in the albumen.

A Se supplementation level of 130 µg Se/kg DM in the diet did not increase (P<0.05) the Se concentration of the albumen above that of the control (Table 14). But increasing the level of Se supplementation to 260 µg Se/kg DM increased (P<0.05) the Se concentration of the albumen. A further increase to 390 µg Se/kg DM did not show an increase in the Se concentration at P<0.05. For the yolk, there was an increase in Se concentration as the level of supplementation increased, but none of those values were different from the control at P<0.05. This shows that there is room for increasing the Se concentration of the albumen above the recommended Se requirement for layer hens by increasing the level of Se supplementation of the diet. The supplemented levels are well above the recommended Se requirement for the maintenance and production of laying hens, which is 50-80 µg Se/kg (NRC, 1994).

These findings are also significant in terms of the cost of the Se supplement. Sodium selenite and Zn-L-SeMet are synthetic forms of Se and they are a cheaper alternative to Seenriched yeast which, for its production, requires complex and high-technology procedures (Suhajda *et al.*, 2000; Ouerdane & Mester, 2008). Sodium selenite is also substantially cheaper than organic Se sources (Heindl *et al.*, 2010). The cost of *Availa*[®]Se is R22.00 per treated ton of feed giving a concentration of 0.3 mg Se/kg (T. Wiggill, personal



communication) while the cost of SS is only R0.57 per treated ton of feed giving the same Se concentration (S. Steenekamp, personal communication).

Furthermore, it is noteworthy that the Se in maize is also largely available and able to increase the Se concentration of the albumen and yolk. The inclusion of natural feedstuffs, containing a higher Se concentration may be a more practical approach to Se supplementation if these feedstuffs are readily available. However, there is insufficient information with regards to the use of Se-enriched plants in animal nutrition (Chinrasri *et al.*, 2009). When the results from the Se concentration in maize grain survey are taken into consideration there are few regions in South Africa that are able to produce maize with an adequate Se concentration for the enrichment of eggs.

5.3 The influence of different selenium supplements on egg quality

5.3.1 Egg weight

Sodium selenite was the only treatment to increase (P<0.05) the egg weight above that of the control for newly laid eggs. This is in agreement with Attia *et al.* (2010) found that the egg weight of newly laid eggs was heaviest with SS supplementation compared to Se-yeast. When the average egg weight values for each treatment for the newly laid eggs were compared, SS produced heavier (P<0.05) eggs than $Availa^{\otimes}Se$. Also, for the average egg weight values of eggs stored in a refrigerator for 38 days the SS produced heavier (P<0.05) eggs compared to the control and $Availa^{\otimes}Se$. These findings agree with Mohiti-Asli *et al.* (2008) who found that the egg weight was heavier after 14 days storage when hens were supplemented with SS compared to Se-yeast. High-Se maize 2 was also able to increase (P<0.05) the egg weight above that of the control, but to the same extent as SS, only on day 38 for eggs stored in a fridge. The improvement of SS in terms of egg weight above the other treatments is probably related to the enhanced effect of SS on albumen weight (see 'Albumen Weight' below). There is no known explanation for the ability of SS to improve egg weight or albumen weight above organic Se sources.

As expected the newly laid eggs showed the heaviest egg weight and eggs stored in a refrigerator were consistently heavier than those stored at room temperature. This is in agreement with Chukwuka *et al.* (2011) who reported that at higher temperatures the loss of carbon dioxide and water through the eggshell is faster and the albumen quality deteriorates



faster, thus indirectly causing a drop in egg weight due to a decrease in albumen weight (Silversides & Budgell, 2004).

There were few significant effects of different storage periods and conditions on the egg weight. This may be due to the fact that there was high variation within and among treatments, as reflected in the standard errors. It is possible that more than five eggs per treatment per storage period and condition should have been sampled, thus decreasing the amount of variation.

5.3.2 Eggshell breaking strength

A large amount of variation was obtained within and among treatments, as reflected in the standard error of the measurements. No definitive conclusions can be made from these results, except on day 15 for eggs that were stored in the refrigerator. In this particular case, all treatments had an equal effect on eggshell breaking strength (they were not significantly different at P<0.05) but they all produced lower (P<0.05) eggshell breaking strength values compared to the control. The hens used in the experiment were 64 weeks old (and 68 weeks old at the end of the experiment) therefore the eggs the produced by these hens probably already showed a poorer shell quality or strength, thus Se supplementation, in organic or inorganic form, and even above that of the hen's requirements did not improve the eggshell strength. This is in agreement with Fernandes *et al.* (2008) who compared the effects of Se supplementation on eggshell thickness and the percentage of thin-shelled and cracked eggs. The authors showed that there was little improvement in these measurements of eggshell quality and the hens used in the experiment were 67 weeks old (and 83 weeks by the end of the experiment). Fernandes *et al.* (2008) also reported that the age and stage of lay of the hen affects the eggshell structure due to an increase rate of diffusion through eggshell pores.

5.3.3 Albumen weight

The increase in albumen weight for eggs of the SS supplement compared to the organic supplements may be related to the improved egg weight values as mentioned above. Sodium selenite was the only treatment to increase the egg weight above that of the control for newly laid eggs and eggs during storage. These results agree with Silversides & Budgell (2004) who reported that there is a very high correlation between albumen weight and egg weight, and albumen weight will be the main factor determining egg weight.



From the comparisons of the average albumen weight values within treatments over different storage conditions and times it is clear that the newly laid eggs showed the heaviest albumen weight. This is in agreement with Scott & Silversides (2000) and Jin *et al.* (2011). This decrease in albumen weight over time is due to a transfer in protein from the albumen to the yolk through the yolk membrane and a loss in water through the eggshell (Heath, 1977; Ahn *et al.*, 1999; Silversides & Scott, 2001). For the SS treatment, there was no decrease (P<0.05) in albumen weight for eggs stored in a fridge for 15 days compared to the newly laid eggs, while eggs stored for 15 days at room temperature had a smaller (P<0.05) albumen weights. Thus refrigeration is effective at maintaining albumen weight of eggs stored for 15 days when supplemented with SS.

5.3.4 Yolk weight

It was not clear as to which treatment had the most positive influence on yolk weight. What is clear, when we observe the yolk weight values obtained within treatments, is that the yolk weight increased with storage time and yolk weights were heavier when stored at room temperature compared to in the fridge. These results are in agreement with Jin *et al.* (2011) who found that the yolk weight increases with increasing storage time and temperature. This increase in yolk weight occurs because as the egg ages the yolk absorbs water and amino acids from the albumen and thus increases in size (Heath, 1977; Silversides & Scott 2001; Coutts & Wilson, 2007).

Although there were a few significant differences in yolk weight between the treatments, Se supplementation above that of the control had no clear effect on yolk weight of newly laid eggs and eggs stored under different storage conditions and temperatures. This conclusion is in agreement with Mohiti-Asli *et al.* (2008) who found that SS or Se-yeast supplementation had no effect on the yolk weight of newly laid eggs or eggs stored for 14 days at varying temperatures (4 °C, 23-27 °C and 31 °C).

5.3.5 Albumen height

For the newly laid eggs Se supplementation was not effective at increasing the albumen height above that of the control diet, in fact, the albumen heights of all the treatment diets were lower in comparison to the control. It was only when the eggs were stored for 38 days in a fridge that the Se supplements were able to improve the albumen height above that of the



control and *Availa*[®]*Se*, high-Se maize and SS were equally effective at increasing the albumen height above that of the control. This is in agreement with Arpášová *et al.* (2009) who showed that organic and inorganic Se was equally effective at increasing the albumen height above that of eggs with no Se supplementation.

The comparison of changes in albumen height within a treatment over time showed that, regardless of Se supplementation, the albumen height decreased after 15 days and then further decreased after 38 days of storage. The lowest albumen height was always obtained on day 38 for eggs stored at room temperature. Also, the eggs stored in the refrigerator for 38 days maintained albumen height to the same extent as those stored in the fridge for 15 days. Thus refrigeration is effective at improving the albumen height of eggs.

5.3.6 Haugh units

None of the Se supplements were able to improve the HU score of newly laid eggs above that of the control, except for the lowest level of $Availa^{@}Se$ which was equivalent to the control at P<0.05. Results showed that organic and inorganic Se supplementation is able to improve the HU score to the same extent after a storage period of 38 days. This is in agreement with Arpášová *et al.* (2009) who found that organic and inorganic Se were equally effective at increasing the HU above that of eggs with no Se supplementation.

Similarly to albumen height, the comparison of changes in HU within a treatment over time showed that, regardless of Se supplementation, the HU decreased after 15 days and then further decreased after 38 days of storage. The lowest HU value was always obtained on day 38 for eggs stored at room temperature. The eggs stored in the refrigerator for 38 days maintained HU to the same extent as those stored in the fridge for 15 days. Thus refrigeration is effective at improving the HU of eggs. The quality of the eggs (according to HU) worsened with increased storage time for eggs kept at room temperature but for the eggs stored in the fridge, the quality was maintained. This may be of great value to the egg producer since it shows that Se supplementation may improve the HU value and egg quality of eggs during storage, thus improving their shelf life, provided that the eggs are refrigerated. These results are in agreement with Gravena *et al.* (2011) who found that eggs stored for long periods should be kept at about 4°C in order to preserve egg quality. This is important for egg producers and retailers since the eggs that are bought in supermarkets are usually stored at room temperature until they are purchased. Storing eggs in a fridge can prolong their shelf life and improve the egg quality for consumption. All HU values for newly laid eggs and



eggs stored for 15 days in a fridge were above 60 HU which is seen as the minimum desirable value for eggs that are sold to the domestic consumer (Coutts & Wilson, 2007). While most values for eggs stored for 15 days at room temperature and 38 days in a fridge were above 60 HU. All of the HU values for eggs stored for 38 days at room temperature were below 60 and thus not suitable for sale. Most of the HU values were above those required for grade 1 eggs (55 HU) according to the Agricultural Product Standards Act (Act 119 of 1990) (Government Gazette, 2011) except some values obtained on day 38 for eggs stored at room temperature. While all of the HU values for eggs (35 HU) (Government Gazette, 2011).

In most cases of egg quality parameters where improvement is made above that of the control, the first level of Se supplementation (130 μ g Se/kg DM) was sufficient and any higher supplementation did not generally improve the egg quality parameters further. A possible reason for little improvement above that of the control could be because the depletion period which lasted two weeks was not long enough in order to deplete the hen's Se reserves. Therefore the hens' reserves and the control diet may have provided sufficient Se to the hens. Since responses to Se concentration of the diet depend on the Se concentration of the basal (or control) diet (Pappas *et al.*, 2005), any Se in excess of the hen's requirement, or for that of antioxidant protection, will not show any additional benefits.



CHAPTER 6: CONCLUSION

Maize grain in South Africa is a poor source of Se for both human and animal nutrition, with 94% of the samples containing less than requirements for humans and layer hens. Since maize grain is a major component in the diet of a large portion of the human population in South Africa, the low Se concentration in most of the maize grain in the country could contribute to the inadequate intake of Se by many South African children, as pointed out by Labadarios *et al.* (2005). This could compromise their immunity and resistance to health problems such as HIV and tuberculosis. In the South African livestock industry, animal feed consultants, feed suppliers and veterinarians are quite aware of the problem of a Se deficiency, and Se supplementation to livestock is a fairly common practice in the country. This is supported by laboratory services, such as UP-Nutrilab, where the Se status of animals and the Se content of feed are measured. This is useful, since Se is one of the few elements in mineral nutrition where liver and blood concentrations are fairly well correlated with Se intake, and thus the Se nutritional status of the animal, even into the adequacy and the toxic ranges of intakes (Van Ryssen, 2003; Van Ryssen *et al.*, 2011).

Availa[®]Se was most efficient (P < 0.05) at increasing the Se concentration of the albumen, followed by high-Se maize and SS, while all three sources of Se were more or less equally efficient to deposit Se in the yolk. Thus $Availa^{@}Se$ is an effective Se supplement to be used in layer diets for the enrichment of table eggs. The Se enrichment of eggs is a good source of Se for humans and could contribute to the diets of South African children who have an inadequate Se intake, as noted by Labadarios *et al.* (2005). The duration for Se concentrations in the albumen and yolk to reach steady state after commencement of Se supplementation, is unresolved and requires further investigation.

In terms of selenium's influence on egg quality, SS was the only Se supplement to increase (P<0.05) the egg weight and albumen weight of newly laid and stored eggs. Selenium supplementation showed no improvement (P>0.05) in the eggshell quality or yolk weight of newly laid or stored eggs. Selenium supplementation is able to improve (P<0.05) the albumen height and HU value of stored eggs, with $Availa^{\text{@}}Se$, SS and high-Se maize being equally effective. The inclusion of Se in organic or inorganic form in layer diets can be used to maintain egg quality, in terms of albumen height and HU, during storage.

Refrigeration was effective at maintaining the egg quality in terms of improved albumen weight, albumen height and HU values. This is important for egg producers and



retailers since the eggs that are purchased in supermarkets are usually stored at room temperature until they are purchased. Storing eggs in a fridge can prolong their shelf life and improve the egg quality for consumption.

Although SS is not as potent as organic Se sources it should not be disregarded as an effective Se source in layer hens' diets. It is able to improve the Se concentration of eggs (particularly the yolk) which is important for enrichment of human diets as well as for the improvement of egg quality during storage. Sodium selenite is also substantially cheaper than organic Se sources (Heindl *et al.*, 2010).



CRITICAL EVALUATION AND RECOMMENDATIONS

The feed intake per hen should have been measured since the orts was recorded it shows that not all of the available feed was consumed. This may have had an effect on the total amount of Se consumed and therefore, the results.

Few significant effects of different storage periods and conditions on all of the egg quality parameters were found. This may be due to the fact that there was high variation within and among treatments, as reflected in the standard errors. It is possible that more than five eggs per treatment, per storage period and condition should have been sampled, thus decreasing the amount of variation. Unfortunately this was not possible in this experiment due to time and financial constraints.

All the eggs used for the egg quality study should have been weighed on day 0, before they were placed under different storage conditions, since this would have allowed for a covariance analysis to confirm the egg weight results after the eggs had undergone different storage conditions

Measurements of the albumen pH could also have been taken because, as reported by Silversides & Scott (2000), the albumen quality is determined by factors already present in the egg at the time of lay as well as storage time and conditions. These authors also stated that measuring albumen height will give a good indication of the freshness of eggs and differences that are present when the egg is newly laid, while the pH measurement is a better indication of the freshness of the egg over time. It would perhaps also be beneficial to use albumen pH instead of albumen height and HU as a measurement of egg freshness since albumen height may also be biased by hen age and strain (Silversides & Scott, 2001). The hens used for this experiment were of the same age and strain, however, but this would be of importance when comparing results to experiments conducted worldwide.

It is also possible that Se supplementation after 38 days of storage showed no improvement of egg quality parameters because the eggs were already too old, at which point antioxidants such as Se are not sufficient to combat decay. Some of the eggs were found to be black and rotten when they were cracked open for the analyses to be conducted.

A recording of the humidity in the two storage conditions could have been measured since humidity has an effect on egg quality (Coutts & Wilson, 2007; Jin *et al.*, 2011).

The GSH-Px activity should have been measured in the albumen and yolk, since it is through this enzyme that Se functions as an antioxidant in terms of egg quality preservation.



There is the question of whether the two week pre-trial period was sufficient to deplete the hens of their Se reserves. The depletion phase should usually be quite rapid. One can also speculate that the control diet did not contain as low a Se level as we were hoping. Prior to the pre-trial depletion period the hens were fed a commercial layer ration containing Se in the premix in the form of SS. Since Se is supplied in the form of SS, one does not expect any Se storage in the hens' tissues except in GSH-Px and in erythrocytes. The GSH-Px is catabolised when erythrocytes are catabolised (Nicholson *et al.*, 2001) and thus is not available to the hen after that. If Se was only in the form of GSH-Px then the two week depletion period should be sufficient enough. However, if Se was present in the form of SeMet, it may have created higher levels of Se in the tissues, necessitating a longer depletion period.

There is potential for further valuable research on the effect of $Availa^{\otimes}Se$ supplementation and its influence on hatchability and chick quality studies since it accumulates to a great extent in the albumen and yolk of eggs. It is well known that the quality of the hatched chick depends on the egg quality (Fisinin *et al.*, 2008) since, ultimately, the egg contents at the time of lay will be incorporated into the developing embryo. It has been proven that chicks hatched from eggs containing a higher Se concentration have an improved hatchability (Cantor & Scott, 1974; Surai, 2006) and a better start in life (Fisinin *et al.*, 2008). A higher Se concentration in the egg and subsequently a higher Se concentration in the chick's tissues, will improve its antioxidant defense mechanism during the hatching process which is conducive to conditions high in oxidative stress (Surai *et al.*, 2006).



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