The influence of selenium source in the layer hen diet on egg selenium concentration and egg characteristics

by

Megan Leigh Marshall

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University of Pretoria
Pretoria
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Supervisor: Dr Christine Jansen van Rensburg

Co-Supervisor: Dr Peter Plumstead

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Declaration

I, Megan Leigh Marshall, hereby declare that this dissertation, submitted for the MSc (Agric) Animal Science: Animal Nutrition degree at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at any tertiary institution.

Signature: 

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List of abbreviations

AAS	Atomic absorption spectrometry
ED	Exudative diathesis
ESI-MS	Electrospray ionisation mass spectrometry
FCR	Feed conversion ratio
GFAAS	Graphite furnace atomic absorption spectrometry
HG-AFS	Hydride generation atomic fluorescence spectrometry
HMSeBA	Hydroxymethylselenobutanate
HU	Haugh units
ICP-MS	Inductively coupled plasma mass spectometry
kg	Kilograms
MAS	Malabsorption syndrome
mg	Milligrams
mRNA	Messenger ribonucleic acid
OSe	Organic selenium
ppm	Parts per million
RNA	Ribonucleic acid
SeAM	Seleno-adenosylselenomethionine
SECIS	Selenocysteine insertion sequence
SeHCy	Selenohomocysteine
SePP1	Selenoprotein P
SS	Sodium selenite
SY	Selenoyeast
tRNA	Transfer ribonucleic acid

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Abstract

Selenium is an essential nutrient in both human and animal nutrition and plays an important role in the immune function within cells. Humans and animals obtain selenium nutritionally and the amount is directly related to the selenium content of the food that is eaten. The most effective and responsible way to increase the selenium status of the population is through selenium-enriched foodstuffs, particularly protein-rich food sources such as eggs. Selenium sources differ in their bioavailability to the bird and the amount of selenium deposited into the egg by the hen is directly related, not only to the amount of selenium added to the feed, but the relative bioavailability of the selenium to the bird. Four treatments were formulated, each using a different source of selenium available for use in feeds. The treatments consisted of three organic selenium sources and one inorganic source, namely; selenoyeast (Sel-Plex manufactured by Alltech), hydroxymethylselenobutanate (Selisseo produced by Adisseo), selenium proteinate (B-Traxim from Pancosma) and the inorganic source, sodium selenite (manufactured by Protea). The treatments all used a basal layer mash devoid of any added selenium and selenium was supplemented as follows; 1.2 mg/kg sodium selenite (SS), 1.2 mg/kg selenoyeast (SY), 1.2 mg/kg hydroxymethylselenobutanate (HMSeBA) and 1.2 mg/kg selenium proteinate. One hundred and sixty (160) 42-week old Amberlink hens were randomly assigned so that there were forty hens per treatment. Each hen was caged individually with *ad libitum* access to water and 160 g/day of assigned treatment feed. Eggs were collected daily to record egg production and egg yolk, albumen and shell weights for each treatment were recorded weekly for 21 days. Eggs collected from each treatment were stored either at room temperature or in a fridge for 28 days and yolk pH, albumen pH and albumen height were recorded every seven days. Selenium source had no effect on egg production or the weight of egg shell, albumen or yolk. Selenium concentration in the egg was significantly higher for eggs from the HMSeBA treatment and lowest in the SS treatment. Eggs stored at room temperature had significantly lower egg quality than eggs stored in the fridge, as indicated by lower albumen heights, higher albumen and yolk pH values and lower Haugh Unit scores. Selenium source did not have a significant effect on egg quality for either storage temperature.

CHAPTER 1: INTRODUCTION

Introduction

Selenium is an essential nutrient in both human and poultry nutrition as a crucial component of the immune system and plays an important role in health and productivity (Yoon *et al.*, 2007). Rao *et al.* (2013) reported that selenium not only contributed to the synthesis of immunoglobulins and increased the number of antibodies against Newcastle Disease present in poultry receiving supplementation, but also prevented exudative diathesis and pancreatic degeneration in broilers, layers and breeders. Both of these disorders can cause economic loss on farms and result in the death of affected birds if left uncontrolled. Treatment with selenium supplementation after the symptoms of deficiency have begun to manifest, such as body weight loss and decreased immune status resulting in sickness, does not always result in complete recovery and often prevention of selenium deficiencies from ever occurring is the only viable solution. The mechanism by which selenium is able to increase the antioxidant status of the bird is by incorporation into selenoproteins, specifically through its association with the amino acid cysteine, forming selenocysteine (Rao *et al.*, 2013). The benefits of selenium supplementation in poultry products are most easily seen in the selenium content of the meat or eggs and in the improved shelf life of the meat due to reduced drip loss, particularly in the breast meat (Ševčíková *et al.*, 2006). The selenium content of feed ingredients is dependent on the selenium content of the soil it is grown in, which can be highly variable (Shini *et al.*, 2015). Soil characteristics, such as pH, influences the amount of selenium that is available to the plant (Courtman *et al.*, 2012). In an experiment to determine the selenium content of South African maize, Courtman *et al.* (2012) established that 94% of the samples analysed has less than 50 µg/kg, making them deficient by both human and animal requirement standards. Despite the fact that the eastern region of South Africa's soil has a high selenium content, the soil pH is low which lowers the availability of selenium to the plant, causing low uptake. On the contrary, the western region has soils with favourable pH but a low selenium content and thus there are limited nutrients available for absorption. In general, South African maize is deficient thus in selenium (Courtman *et al.*, 2012) and because it is the major feed ingredient in poultry diets, supplementing animal diets with additional selenium is essential (Payne *et al.*, 2005). Selenium is required for proper immune functions in both humans and animals, especially for the enzyme glutathione peroxidase to function properly (Yoon *et al.*, 2007). The health benefits of good selenium status in humans include cancer prevention and decreased free-radical damage to cells within the body. An acceptable selenium status can be accomplished through selenium-enriched foodstuffs,

particularly protein-rich food sources, such as eggs (Surai & Taylor-Pickard, 2008). The selenium levels in the egg are proportional to the available selenium in the diet fed to the hen (Payne *et al.*, 2005), which emphasises the potential to manipulate the selenium content of table eggs through supplementation of selenium in the diet fed to the hen (Pan *et al.*, 2007). Increasing the selenium content of eggs consumed by humans is a convenient and safe way to increase the immune status of the population, particularly the elderly and those with an already compromised immune system (Navarro-Alarcon & Cabrera-Vique, 2008). Establishing which of the available selenium sources has the largest potential to increase the amount of selenium accumulated in the egg tissue can be economically beneficial as the supplementation will become more efficient and there will be less wastage.

Selenium supplementation in broilers only positively affects feed intake and feed efficiency up to the first 3 weeks post-hatch (Yoon *et al.*, 2007) and supplementing beyond this point is wasteful when attempting to improve broiler growth. It is more efficient to supplement layer hens as the selenium is deposited in the tissue of the table egg, which forms an integral part of most human diets worldwide. In South Africa, maize forms an important part of human diets but it cannot provide enough selenium to meet the requirements for humans nor poultry on its own. In order to determine which of the commercially available forms of supplemental selenium is most beneficial, the level of selenium deposited into the egg will need to be measured.

1.1 Aim and objectives

The aim of this study was to determine the bio-availability of different selenium sources for layer hens and the deposition of selenium in table eggs. Egg quality was also measured to determine if selenium source had an effect on the quality of table eggs stored either at room temperature or in the fridge over a period of four weeks (28 days).

To achieve this aim, the following objectives were outlined:

1. To determine the bioavailability of each selenium source to the hen by analysing eggs collected at the conclusion of the trial for selenium content.
2. To determine the effect of selenium source on the relative weight of the egg components (egg shell, yolk and albumen) every seven days during the experimental period.
3. To determine the effect of selenium source on egg quality (albumen height and pH and yolk pH) of eggs stored at differing temperatures for 28 days.

1.2 Hypothesis

H_0: There is no significant difference in bio-availability of selenium between inorganic and various organic sources for layer hens

H_A: One or more sources of selenium have a significantly higher bio-availability than others for layer hens

H_0: There is no significant effect on egg production or egg quality between inorganic and various organic selenium sources in layer hens

H_A: One or more sources of selenium have a significant effect on egg production or egg quality in layer hens

Chapter 2: Literature Review

2.1 Introduction

Selenium was first discovered by a Swedish chemist named Jöns Jakob Berzelius in 1817 and he classified it as an essential trace mineral in the body (Surai & Taylor-Pickard, 2008). “Essential” refers to the fact that selenium cannot be synthesised by the body and has to be supplied nutritionally and “trace” refers to the minute quantity required, whereas the macro minerals, such as calcium, are required at much higher levels. Selenium derived its name from the Greek goddess of the moon, Selene, and it was not until 1950 that selenium was established by the aforementioned chemist as an essential micronutrient in the diets of mammals (Shini *et al.*, 2015). Selenium is now a known essential trace mineral that is important for adequate growth and development, as well as proper immune function and metabolic processes (Shini *et al.*, 2015), which will be discussed in detail in subsequent chapters.

Selenium, with the elemental number 34, is in group 6 along with sulphur, oxygen and tellurium and thus shares some common chemical properties with these elements (Brandt-Kjelsen *et al.*, 2017).

Selenium has four oxidation states, namely: elemental selenium (Se^{0}), selenide (Se^{2-}), selenite (Se^{4+}) and selenate (Se^{6+}) and these states form a variety of organic and inorganic matrices (Shini *et al.*, 2015). The majority of the global selenium exists as Se^{4+} and Se^{6+}, which are the known inorganic soluble forms of the nutrient. The ultimate source of this selenium is the rocks and soil on the surface of the earth. The organic selenium compounds exist mostly as seleno-amino acids, with selenocysteine (Se-Cys) making up the primary form in animal tissue and selenomethionine (Se-Met) in plant tissue. Shini *et al.* (2015) discussed the chemistry of selenium and focused on its similarities to sulphur. Because it so closely resembles this element, selenium is able to replace sulphur in some proteins and amino acids. However, the two substances are not identical and therefore are not completely interchangeable. Selenium and sulphur have the same oxidation states and share similar electronegativities, along with atomic radii that closely resemble one another (Brandt-Kjelsen *et al.*, 2017). Due to these atomic similarities, these two elements easily bond with one another to form selenylsulphide. Unfortunately, because they are only similar and not identical, the substitution of either selenium or sulphur for the other results in a compound with differing chemical properties, for example H_2Se is a much stronger acid with a pKa of 3.73 than H_2S with a pKa of 6.96. The two elements also differ in their reduction potential and because of this, selenium is more readily reduced than sulphur. Se-Cys was shown to be identical to cysteine, except the sulphur atom was replaced by a selenium that was ionised at the physiological pH of the body. Se-

Cys is a more reactive amino acid than cysteine, indicating that selenium is able to give amino acids a unique new set of chemical properties (Brandt-Kjelsen *et al.*, 2017). This means that a single atom change from sulphur to selenium creates an entirely new compound that may not necessarily fulfil the correct biological function.

In recent decades, there has been interest in exploring organic sources of selenium in poultry diets to replace inorganic ones, for multiple reasons. The main interests stem from the increased bioavailability of the organic sources to the animal and the lower concentration of organic selenium needed to meet the animal's selenium needs compared to inorganic sources (Attia *et al.*, 2010). This would ultimately decrease the cost of the feed, but the complex mechanisms and interactions of selenium need to be understood to avoid deficiencies and toxicities in both the humans and poultry concerned. This literature review explores the functions of selenium, as well as the requirements of selenium and the resulting deficiencies and toxicities associated with incorrect selenium intake as applicable to both poultry and humans. The mode of action of different sources of selenium is also explored and the benefits of using organic selenium sources over inorganic ones.

2.2 The egg industry

There has been a steady increase in global egg production in recent decades, but not homogenously throughout the world (Windhorst, 2011). Between 1990 and 2008, Asia increased their total egg production by 22 million tonnes. This is linked to the large increase in population size and the favourable nutritional content of table eggs. Intensification of the egg industry first began to occur after the second World War, where developed countries increased farm size, the number of hens in each unit and the labour required to run these larger operations (De Boer & Cornelissen, 2002). Windhorst (2011) found that even though less developed countries were able to increase their production at much higher rates than developed countries, their export numbers were far less. This is because the demand for eggs grew faster than even the increased local production could and so more eggs had to be imported. This is beneficial to the developed countries in Europe and North America, who were able to export their excess eggs and gain a larger share in the global table egg export market. However, it was also shown that only 2.6% of the eggs produced in 2008 were traded, in contrast to 15.5% of poultry meat produced that was exported in the same year. This is because whole eggs cannot be deep frozen as the meat could and so it was more favourable for countries to make use of the long shelf life of eggs and consume them as whole fresh eggs instead.

Globally, the battery-cage system is used in most countries as this is the most economically efficient system, which allows for mechanisation of both feed and water systems, as well as egg collection (De Boer & Cornelissen, 2002). In South Africa specifically, traditional battery cage

systems are the preferred laying hen system. However, the conditions laying hens are being kept in became an issue of animal welfare concern, particularly in European countries where a ban on growth-promoting anti-biotics was introduced (Blokhuis *et al.,* 2007). De Boer and Cornelissen (2002) reported that public concern called for more animal-friendly production systems that made use of free-range and deep-litter principles over the traditional battery cages. The issues raised by these concerns included increased ammonia emissions, higher production costs and an increase in the labour required to run these proposed systems. Feed, manure, housing accessories and the animals themselves are known to contribute to the environmental footprint of agriculture through air pollution, pollution of water through runoff and leaching of groundwater to name a few (Xin *et al.*, 2011). Blokhuis *et al.* (2007) recommended that scientists focus their efforts on maintaining the database containing comparisons between different production systems in order to establish the cause of outbreaks of feather pecking and thus design a system that would minimise this behaviour. They also acknowledged that there is no one correct system as both traditional and modified animal-friendly systems have their own unique set of benefits and challenges.

Despite Africa being the second largest continent in the world, it only contributes 3.7% to the global table egg production (Nys *et al*., 2011). This has been attributed to the inefficient scavenging system that resulted from the large proportion of rural communities throughout the continent that make use of little or no input scavenging systems whereby the birds have little to no shelter or enclosures and will scavenge for their nutritional needs. However, research by Grobbelaar *et al.* (2010) showed that indigenous chicken breeds were not as economically beneficial for egg production as the popular strains, such as the White Leghorn. Despite the physiological adaptations that allowed indigenous breeds to thrive in the climate of South Africa and resulted in a lower mortality rate, the more controlled environments of the battery cage system resulted in breeds such as the White Leghorn having higher production percentages and thus higher outputs and higher profits (De Boer & Cornelissen, 2002). According to Grobbelaar *et al*. (2010). the majority of eggs produced for human consumption in South Africa came from 319 commercial enterprises, and established that in order to be profitable, each hen must produce at least 280 eggs per cycle, the daily hen-housed egg production must fall between 75 and 95 percent and chickens must have efficient feed conversion and low mortality rate. South Africa is considered one of the high egg-producing countries in Africa and had just over 26 million laying hens in February of 2019 and produced 430 000 cases of table eggs per week per annum (South African Poultry Association, 2019). This illustrated the constant growth of the table egg industry with a 10.5% increase in laying hen numbers and a 10.6% increase in table egg volume compared to the same time in 2018. As of the census in 2006, there were 1745 known small-scale farmers producing eggs and this number continues to increase each year, but the number of eggs produced by these small-scale farmers annually is not known (Grobbelaar *et al*., 2010).

2.3 Egg formation in the hen

Hens lay cleidoic eggs, meaning the internal environment of the egg is almost completely isolated from the exterior environment and all the nutrients to support the embryonic development of a chick are contained within the egg (Nys *et al*., 2011). The formation of the egg is a series of complex processes that begins with the ovulation of the yolk and results in an entirely formed egg (Roberts, 2004). It is because of the many complex processes involved that problems can occur at various stages and compromise the quality of the egg. Nys *et al.* (2011) explained in detail the various processes involved in formation of a complete egg within the hen.

The egg yolk components are produced by the liver and transported via the bloodstream to the left ovary where the most mature follicle releases a completed egg yolk into the left oviduct. The yolk then moves through the oviduct where the vitelline membrane, the albumen, the shell membrane and the eggshell are sequentially added around the yolk from specialised segments within the oviduct. The entire process is regulated by the ovary and the pituitary gland which secrete steroid and pituitary hormones, allowing movement of the developing egg through the oviduct and the addition of the various egg components in the correct order. Roberts (2004) documented the time it takes for each developmental step (figure 1). Once the yolk is captured by a structure known as the infundibulum, where it remains for 15 minutes and the perivitelline membrane and chalazae are formed and, in the case of breeder hens, this is where fertilisation also occurs. After this, the egg is moved to the magnum and remains for three hours as the albumen proteins are produced and layered around the yolk and membranes. The egg is then moved to the isthmus and the outer and inner shell membrane fibres are added, which takes about one hour. The egg will then pass through the shell gland and for approximately five hours, a process known as “plumping” will occur, whereby water and electrolytes enter the albumen. Plumping is completed after the egg moves into the shell gland pouch and for about 15 hours, the shell will form. After this, the hen will begin to lay her egg. While there were clear similarities between different studies in terms of the proportion of time various segments required, there were still highly variable results that meant full egg formation could take between 22 and 26 hours to complete. Because of this, hens typically lay eggs in what is known as a clutch, which is a number of eggs laid on consecutive days before there is a one-day pause in egg production. There was early evidence by Warren and Scott (1935) that clutch size remained constant throughout the lifetime of a hen and that clutch size was an inherited trait. The incidence of a “double yolk” occurs when two mature yolks are released into the oviduct at the same time and are common in hens that are just entering production, while only seldomly observed in hens that have been in production for a long time (Sherwood, 1958).

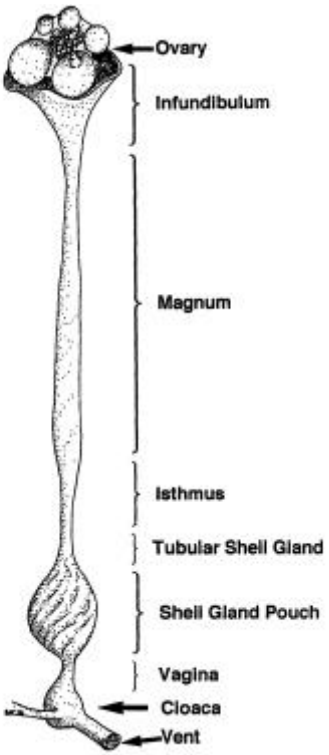

Figure 1 The reproductive tract of the hen highlighting the structures responsible for the formation of the egg (Roberts, 2004)

2.4 Egg quality

Since the intensification of the table egg industry between 1930 and 1940, animal welfare concerns needed to be balanced with consumer preferences and one of the major consumer concerns was egg quality (Singh & Silversides, 2009). Egg quality characteristics have been studied for decades and the application of egg quality has been applied to production systems in order to meet not only the consumer demand, but also their preference for good quality eggs so that the number of eggs sold is maximised (Zita *et al.*, 2009). Egg quality is defined as the characteristics of the egg that will affect the acceptability of the egg to the consumer (Monira *et al.*, 2003).

2.4.1 Measurements of egg quality

Several aspects have been identified that encompass the concept of egg quality, which include; shell thickness and shell strength, yolk and albumen pH, albumen height and yolk colour (Singh & Silversides, 2009).

Simply measuring the weight of albumen, yolk and shells is not sufficient to quantify an accurate measure of egg quality. This is because the weight of individual components as well as the

proportion of the whole egg of each component varied widely between different strains and had many other factors that contributed, such as diet, age of the bird and management system, which could not be quantified and accounted for (Monira *et al.*, 2003).

Roberts (2004) discussed measures of shell thickness as an indicator of egg quality. The thickness of the egg shell was directly related to shell strength, with thicker shells withstanding more compression under controlled conditions before the egg cracked. There was also a correlation between shell weight and eggshell strength; when there was a higher percentage of shell, the breaking strength of the egg was higher.

Studies on albumen thickness to determine egg quality have been referenced for decades as this was one of the first physical measures of egg quality described (Heiman & Carver, 1936). Albumen height is a measure of the viscosity of the thick albumen portion, where a low internal egg quality correlates to a high proportion of thin albumen (Roberts, 2004). The measurement of albumen height was used in conjunction with egg weight to determine an albumen index, also referred to as Haugh Unit, to determine egg quality, where a higher value indicated a higher quality egg. This measurement required that eggs be weighed and then broken open on a flat, even surface where the height of the albumen was measured at a constant distance of 1 cm away from the yolk edge for each repeated measurement. Haugh Units are calculated based on the following formula: Haugh Units = $100 \log (H - 1.7w^{0.37} + 7.6)$ (Silversides & Villeneuve, 1994) where the weight of the egg (w) and measured albumen height (H) are the variables inserted into the equation. A higher albumen height was indicative of a fresher egg because fewer of the proteins have degraded and this then translates into a higher Haugh Unit value. Albumen height is easily observed by the consumer once the egg is broken open, but industry processors are more concerned with the functional properties of albumen, such as heat coagulation and the stable foam formation after whipping, which was tested by whipping the albumen for 80 seconds with a hand mixer and the foam pressed into a beaker where the volume was measured (Silversides & Budgell, 2004).

Albumen pH was identified as an indicator of egg quality because the lower the albumen pH, the fresher the egg (Scott & Silversides, 2000). It was also shown that the longer an egg is stored, the stronger the relationship between albumen height and albumen pH became since storage time of the egg was the only factor affecting the pH increase of the albumen, it was concluded to be a very reliable measure of egg freshness. However, in order to measure albumen pH, the egg must be broken open as there is no non-invasive measurement available.

Beardsworth and Hernandez (2004) detailed the importance of yolk colour as a measure of egg quality. They described how a bright and vibrant yellow would stimulate the appetite and enhance the consumer's enjoyment of the food. They described the Roche Yolk Colour Fan which

was widely accepted in Europe as a good indicator of egg quality. The fan consisted of numbered blades, each of which contained a colour objectively measured and could be reproduced in the yolk. The desired colour score depended on consumer preferences and the population sampled in this research showed a preference for darker yolk colours, which scored 12 or 14 according to the fan. Silversides and Budgell (2004) noted that while yolk colour was commonly used as a measurement of egg quality, it depended almost entirely on the composition of the diet fed to the hen and could easily be manipulated through addition of carotenoids in maize or other feed additives that would have no effect on any other quality parameters. This meant that it was more a descriptor of market preference rather than actual egg freshness and quality.

Although eggshell colour is regarded as an indicator of egg quality by consumers, there was shown to be no link between eggshell colour and the nutritional content of the egg itself (Scott & Silversides, 2000). This means that eggshell colour is not an accurate measure of egg quality and is only selected because of consumer preference.

2.4.2 Factors affecting egg quality

Singh and Silversides (2009) discussed the role that genetics played in determining egg quality in laying hens. It was shown that egg quality varied between breeds and between strains within a single breed. There was potential for these genetic traits to be inherited. They noted that cross-bred hens had higher egg quality, as was shown by higher egg weight, higher albumen height and a higher measure of yolk colour when compared to three purebred commercial breeds. Sherwood (1958) noted that Rhode Island Red breeds had a higher incidence of blood spots in eggs when compared to eggs from the White Leghorn breed. This is in agreement with the research of Zita *et al.* (2009), that also found that genotype affects egg weight and eggshell characteristics. They noted that eggs from white egg laying hens were not as heavy as those from brown egg laying hens and that white eggs had a higher egg shape index than brown eggs. Sherwood (1958) showed that higher producing birds laid eggs with lower albumen thickness and this characteristic was hereditary.

Bird age also affected the quality of eggs laid, where older birds laid eggs of lower quality typically demonstrated by thinner shells and decreased albumen quality because of fewer proteins contained in the albumen (Roberts, 2004). Older birds laid eggs with lower amounts of thick albumen and thus lower albumen height (Sherwood, 1958). This was because the amount of thick albumen in the eggs gradually decreased the longer a bird remained in production.

Longer storage time caused a decrease in egg quality as the albumen height decreased with time and the albumen pH increased with time (Scott & Silversides, 2000). The increase in albumen

pH was due to the deterioration of proteins contained in the albumen and thus decreased the overall quality of the egg (Roberts, 2004). Sherwood (1958) described this as the conversion of thick albumen to thin albumen during storage time, which resulted in a watery albumen and a decreased interior egg quality. Roberts (2004) also found that the vitelline membrane surrounding the yolk lost quality over time and this left the yolk more susceptible to breaking the longer an egg was stored.

The nutrition and diet of the hen could be manipulated in order to control certain egg quality measures (Silversides & Budgell, 2004). Yellow maize, lucerne meal and maize gluten were identified as nutritional additives that could darken the colour of the egg yolks when fed to the hen and chilli peppers could even result in deep orange to red yolks (Sherwood, 1958). Roberts (2004) noted that nutrition did not affect albumen quality as easily as it affected the yolk quality. However, there was still evidence that decreasing the protein content of the diet resulted in lower albumen height and watery albumen. Lowering the protein content of the diet resulted in a decreased egg size (Sherwood, 1958).

Disease was shown to cause decreased egg quality, including increased thinning of albumen, deformed and bleached egg shells and decreased egg size (Sherwood, 1958). There was also a decreased synthesis of albumen proteins in the magnum of the oviduct and fewer proteins were then deposited into the egg (Roberts, 2004). These decreases to quality were commonly observed after an outbreak of Newcastle Disease, infectious bronchitis and coccidiosis in laying hens (Sherwood, 1958; Roberts, 2004).

2.5 Factors affecting egg production

Environmental temperatures affect the production of the hens, who have a preferred temperature range of between 17°C minimum and 25°C maximum and adequate ventilation to ensure the temperature does not exceed 25°C and 50% relative humidity where possible (Dekalb Amberlink Production Guide, 2017). Mashaly *et al.* (2004) found that heat stressed birds, kept at 35°C and 50% relative humidity, had decreased egg production because of their reduced feed intake and body weight during the heat stress period. Heat stress had a deleterious effect on the reproductive system of the hen, as there was a change to the acid-base balance and the duodenal cells had a reduced ability to transport calcium, resulting in thinner eggshells and weaker bone structure over time in the laying hens, causing a decreased productive life.

Evidence that production potential is a heritable trait was demonstrated many decades ago which showed that particular breeds have inherently higher production than others (Warren & Scott, 1935). Grobbelaar *et al*. (2010) found that the Koekoek, Venda, Ovambo and Naked Neck breeds

that are indigenous to South Africa, all produced less eggs per hen per cycle compared to a commercial White Leghorn breed. There are several traits that are typically selected for in modern commercial laying breeds, including predictable production in terms of eggs per hen per cycle, extended laying period and good feathering qualities (Dekalb Amberlink Production Guide, 2017).

Nutrition is an important factor of egg production, as a deficiency of certain trace minerals, such as zinc, copper and manganese, can cause shell deformities in eggs as these minerals are important for shell calcification (Wang *et al*., 2017). These deformities can lower production potential as deformed eggs are often discarded and a hen can be removed from production if she consistently produced eggs with shell deformities. It is known that the nutrients available to the hen will determine what can be deposited into the egg that is laid (Zoidis *et al*., 2014). Manipulating the diet affected certain production parameters and while increasing certain nutrients, such as the lysine content of the diet, did not affect egg production directly, understanding the optimal value for inclusion of 715 mg/hen per day at the starting phases ensured profitable and optimal production throughout the hen's productive lifetime (Novak *et al.,* 2004). Supplementing the hen with anti-oxidants, such as vitamin E, selenium and zinc, will increase their productive lifetime as they are less susceptible to oxidative stress, which is often expressed as inflammation, lethargy and ultimately a reduction in egg production (Wang *et al*., 2017). Vitamin A supplementation was shown to directly increase the weight of eggs laid, without increasing the daily feed intake of the hen (El-Hack *et al*., 2017). This improved production potential of the hen without increasing feed costs and increased the health and reproductive lifetime of the flock, as shown through the increased haemoglobin and lymphocyte concentration in the blood of the hens, indicating better oxygen circulation and immunity respectively.

2.6 Eggs in the human diet

Eggs are a high-quality protein source with at least 12 g of protein contained in a medium (58 g) egg (Menéndez *et al*., 2019) and contain a vitamin and mineral profile unparalleled by any other food source for humans with relatively low energy (326 kJ) and low saturated fat (1.7 g) values compared to other animal products (Griffin, 2016). This means that eggs have one of the highest nutrients to energy ratio of any food. However, consumption of eggs, particularly in more developed countries, have declined as people become more health conscious and associate the potential for cardiovascular disease with the cholesterol content of table eggs (Sahlin & House, 2006). However, the effect that the cholesterol present in eggs has on serum LDL-cholesterol is insignificant compared to other lifestyle factors, such as regular exercise (Griffin, 2016) and body mass index (Menéndez *et al.*, 2019). The total fat content of a medium egg is around 4.6 g, of which only 1.3 g are saturated fatty acids, which is approximately 11% of the saturated fat typically found in other protein-rich foods, such as a beef burger or a sausage roll (Griffin, 2016). This means that eggs

contain a fatty acid profile that provides for the nutritional needs of the body without contributing negatively to health, such as increasing cholesterol values and unwanted body weight gain. Because not everyone is able to consume food in the same way and in the same quantity, eggs provide a nutrient dense option for lower income households to obtain necessary antioxidants, such as vitamin E, carotenoids and selenium and support healthy immune function (Fisinin *et al.*, 2009). An average sized medium egg of about 58 g contains about 1.6 µg of vitamin D and consuming two medium eggs can provide a person with 63% of their daily recommended amount of vitamin D (Griffin, 2016). Vitamin D is known to have many beneficial properties to humans and forms an important part of organ functions, including the lungs, heart, intestines and mammary glands, and is particularly known to decrease the risk of breast cancer occurrence in women (Hossain *et al*., 2019). Vitamin D is an effective anti-inflammatory in cells and can stop the growth of cancerous cells and prevent the spread of inflammatory diseases within the body (Varkaneh *et al*., 2019).

Zoidis *et al.* (2014) emphasised the role of selenium status in the hen and how it affected the rate of selenium deposition in various components within the egg, which gave evidence that eggs could be used to supplement selenium in the human diet. For example, selenium was preferentially deposited in the yolk when compared to the albumen, due to the mineral-binding lipoproteins that are present during the yolk formation process. It was proposed that in times of marginal selenium deficiency, as when there are limited amounts of selenium supplied in the diet, it was preferentially deposited into the yolk. When the selenium in hen diets was adequate, the excess selenium was deposited into the albumen. It is with this knowledge that nutrient contents of the table egg are able to be manipulated through the diet of the hen, that it is possible to increase the concentration of certain nutrients, such as selenium, in the diets of humans consuming these enriched eggs (Brown & Arthur, 2001).

2.7 Functions of selenium

Selenium plays an important role in animal cells, mainly by acting as an anti-oxidant and by forming an important component of certain enzymes. The discovery of selenium's ability to prevent cell damage by minimising oxidation has led many researchers towards understanding the exact role selenium plays within cells and how it is possible to maximise the benefits of selenium supplementation in humans and animals (Surai & Taylor-Pickard, 2008; Shini *et al*., 2015).

2.7.1 Role in enzymes

Enzymes, which are classified as proteins, can be further classified as selenoproteins (SeP) when Se-Cys forms an integral part of the polypeptide chain of the enzyme (Zoidis *et al.*, 2014). The mechanism with which Se-Cys becomes incorporated into the protein is by cotranslational joining to the polypeptide chain. Shini *et al.* (2015) discussed the biological activity of selenium through how it was incorporated into SeP and that Se-Cys and Se-Met both formed sources of selenium for incorporation into these proteins. Selenium was shown to be biologically active because it was incorporated into the catalytically active centre of the SeP through Se-Cys (Surai & Taylor-Pickard, 2008). The role selenium played in these SeP is through their involvement in redox regulation, detoxification and the contribution to immunity through viral suppression, with enzymes being classified as SeP only if the protein was genetically programmed and performed an essential biological function (Shini *et al.*, 2015). This eliminates compounds that are formed through random mutations or compounds that could have detrimental effects on cellular functions.

Selenium has been shown to form part of at least 25 SeP, the most well-researched of these being glutathione peroxidase (GSH-Px) (Surai & Taylor-Pickard, 2008). Glutathione peroxidase is an important antioxidant and helps protect the cells of the body from free-radical damage (Delezie *et al.*, 2014). It is able to destroy peroxides before they cause damage to liposomal membranes (El-Hack *et al.*, 2017). This allows cells to be protected from oxidative stress caused by oxidation products created during cellular metabolic functions. However, it was shown that GSH-Px cannot be used as an indicator of selenium status in all circumstances, particularly when Se-Met is the major form of supplemented selenium, due to the non-specific incorporation of Se-Met into proteins. Yuan *et al.* (2012) further showed that there was no difference between liver and kidney GSH-Px activity between organic and inorganic selenium sources. Because it was well known by this time that a clear difference existed between the metabolic efficiencies of inorganic and organic sources of selenium, other selenium-dependent enzymes needed to be identified for analysis to better illustrate the differences in bioavailability and thus the differences in biologically active selenium. Glutathione peroxidase activity is directly related to the selenium level in the tissues, as the selenium needs to be directly incorporated into the enzyme in order for it to function (Surai, 2000). This means that higher levels of selenium translate into higher levels of GSH-Px activity, but the interaction is only significant for source of selenium and not for dose of selenium. This was demonstrated by Surai (2000) where there was clearly a higher level of GSH-Px activity in birds supplemented with organic selenium sources compared to inorganic sources, even at the same dosage levels. However, the different inclusion levels within one source differed numerically but not significantly. Glutathione peroxidase activity is correlated to the concentration of selenium in the tissue and not simply the

amount of selenium added to the feed, emphasising the difference in efficacy of different sources of selenium in GSH-Px functioning (Zoidis *et al.*, 2014).

Selenium is a catalyst for the production of thyroid hormone (Delezie *et al.*, 2014). Selenium molecules form part of iodothyronine deiodinase, which is responsible for converting triiodothyronine to thyroxine (Shini *et al.*, 2015). Selenium is a structural component of thioredoxin reductase (TrxR) that prevents oxygen and nitrogen free radicals from damaging proteins, nucleic acids and lipids within the body of chickens and humans (Surai & Taylor-Pickard, 2008). When left unrestricted, free radicals inhibit proper tissue functions and cellular metabolism is disrupted. Yuan *et al.* (2012) conducted an experiment using 40-week-old broiler breeders to demonstrate the effects of selenium sources on various selenium-dependent enzymes. The hens were fed their experimental diets for eight weeks, following an eight-week adaptation period on a selenium-deficient basal diet in order to completely deplete the birds of selenium prior to the experiment. The experiment focused on GSH-Px and TrxR activity in the liver and kidney of the breeders. Unlike GSH-Px, there were clear differences in liver and kidney activity of TrxR between organic and inorganic sources of selenium. Organic selenium was shown to be more available by the increased TrxR activity associated with diets containing organic selenium. The enzyme regulations in the offspring of the breeders fed the various diets were similar. This showed that enzyme expression was dependent on the selenium status of the animal and that hens had the potential to transfer increased amounts of selenium to their eggs when they themselves were fed a diet containing higher levels of selenium, known as vertical transfer.

Any enzyme expression is dependent on the production of specific messenger RNA (mRNA) which contains specific DNA instructions for the production of that particular enzyme. mRNA levels can be measured as an indicator of the SeP activity and organic selenium sources show higher levels of TrxR and GSH-Px mRNA, indicating the bioavailability is higher for these organic sources (Yuan *et al.,* 2012).

2.7.2 Role in poultry cellular functions

Selenium has been shown to be an essential nutrient in poultry nutrition, as it forms a crucial component of the immune system and plays an important role in the health and productivity of the animal (Yoon *et al.*, 2007). There are numerous functions that selenium has a direct or indirect effect on, the most important of which are those pertaining to the health of the animal, such as the immune system, and these are linked to functions affecting the production potential of the bird.

Rao *et al.* (2013) reported that selenium not only played a role in the synthesis of immunoglobulins and increased the number of antibodies against Newcastle Disease present in poultry receiving supplementation, but also prevented exudative diathesis and pancreatic degeneration, both of which were associated with selenium deficiencies in poultry. The efficiency of use of selenium is demonstrated in the function of the enzyme GSH-Px, which acts as an antioxidant by destroying free radicals in the body of the bird (Payne & Southern, 2005). This enzyme is known to be produced naturally during normal metabolism of the bird, but requires selenium as a key molecular component (Rao *et al.*, 2013).

2.7.3 Role in human cellular functions

The SePs that contribute to human health and nutrition depend on selenium being present at the active centre of a protein, via a Se-Cys molecule (Rayman, 2012). The insertion of Se-Cys at this active centre is possible because the mRNA communicates the insertion of selenium at the UGA codon during the translation process. This UGA codon site is a nonsense codon, meaning that it does not directly result in a specific protein being formed, but rather indicates to the cells that a selenium molecule needs to be added to the active centre of a protein, most commonly GSH-Px (*Yuan et al*., 2012).

Selenium supplementation has been shown to have the most pronounced effect on humans through their immune system, particularly by increasing the proliferation of activated T cells by as much as 27% compared to groups of people offered a placebo treatment, as SePs are essential for activated T cell function (Rayman, 2012). This is due to an increased cell count of a subset of T cells, known as CD4+ T cells. Selenium is known to prevent oxidative stress and damage in cells (Delezie *et al.*, 2014), making SePs essential for T cells to mitigate the effects of oxidative stress which would otherwise prevent their function and proliferation by suppressing their response to T cell stimulation (Rayman, 2012). However, even with sufficient supplementation, the immune response associated with selenium is often less efficient in elderly patients and those undergoing cancer treatment.

The brain has a selenium requirement that will be met at the expense of other tissue selenium supply. Selenoprotein P (SePP1) is responsible for the delivery of selenium to the brain and binds to a surface lipoprotein receptor, known as apoER2 (Rayman, 2012). SePP1 plays a protective role in neuron survival, by preventing cell death due to oxidative challenge and low levels of SePP1 has been linked to an increased risk of Alzheimer's disease in humans. Although there is ample evidence from *in vitro* animal studies to show that selenium is important for immunity, the information available from purely human studies is scarce (Rayman, 2012).

Surai and Taylor-Pickard (2008) discussed the health benefits of selenium for humans and noted that there was substantial evidence that selenium could prevent cancer and free-radical damage. A review by Navarro-Alarcon and Cabrera-Vique (2008) compiled an overview on the available data for selenium on human health, and concluded that most experiments conducted in the past have shown selenium's potential to decrease the risk of specifically colon and prostate cancer in men. The antioxidant properties of selenium molecules can also prevent cardiovascular disease. The discussion highlighted the importance of optimising the selenium status of the population and how disease prevention and treatment could have a direct link to the selenium content of the diet that was consumed. Supplementing selenium in the diet and increasing selenium consumption was shown to be a convenient and safe way to increase the antioxidant status of elderly people and those with a compromised immune status due to poor health. Selenium is known to be involved in sperm production and the maintenance of healthy pregnancies in humans, therefore a link to reproductive health was indicated. Optimal selenium status in humans could help prevent asthma, cystic fibrosis, arthritis and a variety of brain disorders but it was demonstrated in large human trials that benefits of selenium supplementation were only observed when levels much higher than the needs of basic metabolic requirement were consumed (Surai & Taylor-Pickard, 2008). This was referred to as 'supranutritional inclusion' because the levels surpassed those recommended for basal metabolic functions, listed most commonly as the "recommended levels" in human nutritional guidelines, which will be discussed in detail in a subsequent section. It was also shown through these nutritional experiments that the chemical form of the supplemented selenium greatly affected the physiological functioning. When supplementation was given, retention and utilisation was greatly improved when the chemical form more closely matched the natural form.

2.8 Selenium sources

Selenium occurs naturally in either an organic or an inorganic form and these different forms have all been supplemented into animal feed through various sources (Asadi *et al*., 2017). These sources will be discussed in detail in this section, including the factors contributing to the different bioavailability of each source to the animal consuming it. The sources covered are sodium selenite, seleno-yeast, selenomethionine, hydroxymethylselenobutanate and selenium proteinate.

2.8.1 Sodium selenite (SS)

Selenium was first approved as a dietary supplement in 1974 and the inorganic form was the primary source added to poultry and other livestock feeds (Surai & Taylor-Pickard, 2008). This was due to the lack of information and research into Se-Met at the time. Sodium selenite (SS) was considered the marketable inorganic supplemental source of nutritional selenium and organic sources of selenium could only be marketed as such when the level of SS did not exceed a predetermined percentage of the total selenium present. Surai and Taylor-Pickard (2008) explained that the addition of SS into the livestock feed substantially improved the general health and performance of poultry, but there was evidence that birds of 40 years ago had different metabolic needs to modern birds and so organic sources had to be considered due to problems associated with the use of SS and the small margin of error that existed between the requirement level and the level of toxicity. It was also noted that inorganic selenium had potential pro-oxidant properties that interacted with some viruses and caused a higher replication rate in tissue, rather than inhibition. SS is passively absorbed in the intestine, meaning the uptake is depended on concentration gradients of selenium within the intestinal tract (Surai & Taylor-Pickard, 2008). Once selenite is absorbed by the plant, it is rapidly transformed into Se-Cys or Se-Met and stored in the proteins of the roots, which is contrary to selenate that was shown to be more mobile in the xylem tissue and is translocated throughout the plant tissues that are located above ground (Brandt-Kjelsen *et al.*, 2017). Payne *et al*. (2005) reported that yolk proteins were richer in selenium from SS compared to the proteins in egg white. Sodium selenite metabolism and yolk synthesis both occur in the liver and this resulted in favourable deposition into the yolk of the egg. It was also noted in this trial that SS is needed for Se-Cys synthesis in the body of the bird.

2.8.2 Seleno-yeast (SY)

Seleno-yeast (SY) is an organic selenium source produced by allowing yeast organisms to proliferate in selenium-enriched medium (Asadi *et al.*, 2017). Payne *et al.* (2005) used *Saccharomyces cervisiae* yeast, which was grown in a high selenium medium to produce the SY used in their study on laying hens. Yeast has the ability to permanently incorporate elemental ions from the environment into their cellular structures and it is through this mechanism that SY can be produced for inclusion in animal feeds (Zoidis *et al.*, 2014). Seleno-yeast was shown to be a more bio-available source due to the large amount of Se-Met that was present and was considered to be more environmentally friendly and less toxic for poultry (Invernizzi *et al.*, 2013). Pan *et al.* (2007) draws attention to the anti-oxidant properties of selenium and the higher bio-availability of SY results in higher tissue accumulation of selenium and lower toxicity risk when compared to SS. Surai and

Taylor-Pickard (2008) discussed that SY performed better in animal studies because it contained high levels of Se-Met, which mirrored the natural chemical form of selenium that was supplied through cereal grains. They stated that simple dietary analysis of selenium content was no longer a relevant determinant of selenium availability as the dominant seleno-species influenced the uptake by the animal. Because plants represented the main source of naturally acquired selenium, the growing conditions and soil selenium content were established as key factors that would determine what was naturally available in the diet of the animal, when no supplementation was offered. This led to the emphasis that selenium had to be supplemented in order for the basic metabolic and production needs of the animal to be met. SY has about 63% of the selenium in the form of Se-Met, but this value varies considerably and can fall anywhere between 60 and 80% (Delezie *et al.*, 2014) and in some cases varied between as much as 55 and 80% (Surai & Taylor-Pickard, 2008). The large variation in this amount was attributed to the presence of Se-Cys. Delezie *et al.* (2014) showed in their experiment, using 180 Lohmann Brown hens that were fed one of 10 experimental diets, each containing a different source of selenium at various inclusion levels, that eggs from hens fed SY had higher serum selenium than inorganic sources at any inclusion level. This also led to the conclusion that there is a source and level interaction in selenium supplementation. The control diet was not deficient in selenium and so no deficiency symptoms were present, nor was there any effect on the level of egg production in any of the experimental groups. They showed a linear effect of selenium deposition in eggs for SY sources, whereas the inorganic source plateaued at an inclusion of 0.3 mg/kg. They further discussed that Se-Met within SY is protein-bound and needed to be digested before it could be absorbed in the small intestine. This differed from the pure Se-Met source, which did not have to undergo digestive pathways before being absorbed by the cells of the gut lining. Zoidis *et al.* (2014) discussed how the purity of the yeast strain, the particle size within the SY, the moisture content and the presence of any toxic microbial impurities or contaminants could influence how much Se-Met is contained within the SY.

2.8.3 Selenomethionine (Se-Met)

Selenomethionine (Se-Met) is an analogue of methionine that was shown to possess the potential to be stored in the muscle for later use (Invernizzi *et al.*, 2013). Selenomethionine is the major form of selenium that is present in plant tissues (Zoidis *et al.*, 2014). Surai and Taylor-Pickard (2008) classified Se-Met as an essential amino acid that formed the major nutritional source of selenium for animals that was actively absorbed in the intestine by the sodium dependent methionine transport system. Selenomethionine uptake was shown to follow the same active transport pathway as analogous sulphur species, as it used the same sulphate assimilation pathway for its metabolism

(Brandt-Kjelsen *et al.*, 2017). L-selenomethionine is a free amino acid and is not protein-bound, such as the Se-Met that comprises the majority of SY (Delezie *et al.*, 2014). The tRNA for methionine cannot distinguish between Met and Se-Met and thus is able to use the two molecules interchangeably during protein synthesis (Zoidis *et al.*, 2014). Intensive Se-Met uptake is needed for selenium deposition in eggs and only organic forms of selenium can be taken up in the blood. Se-Met is incorporated non-specifically into proteins, which differed from the way that Se-Cys was genetically coded into the proteins, using the SeCys insertion sequence (SECIS) within the genome itself (Surai & Taylor-Pickard, 2008). Because of the non-specific incorporation, Se-Cys competed with sulphur in the Cys synthesis pathway and this led to the Se-Cys that is commonly present in SY. The absorbed Se-Met can either be incorporated directly into proteins or metabolised by the liver and then used for incorporation into specific SeP (Zoidis *et al.*, 2014).

2.8.4 2-hydroxy-4-methylselenobutanate (HMSeBA)

HMSeBA, also referred to as selenohomolanthionine, was identified in Japanese pungent radish (*R. sativus*) and raised interest as a selenium source because it was less toxic in human cell cultures when compared to Se-Met (Shini *et al.*, 2015). This difference in toxicity was attributed to differences in the metabolism of the two compounds. HMSeBA is an organic selenium source that is bioavailable to all animals and can be added into feed premixes (Sun *et al*., 2017). The proposed pathway of HMSeBA by Shini *et al.* (2015) is less complex than that of Se-Met because it does not share the pathway of methionine incorporation into peptides that Se-Met does. Rather, it was proposed that HMSeBA is only used for SeP synthesis and does not interfere with the metabolic pathways of methionine. It was also proposed that HMSeBA accumulated in the liver and kidneys, contrarily to Se-Met that accumulates in the liver and pancreas, which also contributed to the differences in toxicity levels as HMSeBA does not contribute to pancreatic damage due to high accumulation rates. It is excreted via the kidneys without inducing any damage to the pancreas.

As seen in figure 1, the process of incorporating HMSeBA into SePs generates fewer intermediate products when compared to Se-Met and is therefore a more energetically efficient pathway (Jlali *et al*., 2013. This is shown as Se-Met creates three selenol intermediary metabolites (MMSe, DMSe and TMSe) which all require energy to produce and at least two of these three metabolites must be formed before excretion in the breath or urine is possible. Outlined in figure 1 is the pathway unique to HMSeBA metabolism that shows how a molecule of HMSeBA enters the transsulphuration pathway and is converted to selenohomocysteine. This is juxtaposed to the process that Se-Met must undergo in order to be incorporated into SePs. The molecule of Se-Met has several pathways it can enter other than SeP incorporation, namely; into general protein

synthesis where it is incorporated into the place of methionine in various proteins, or through the methylation pathway where it can be excreted in the breath or urine of the animal. When Se-Met is converted to Se-adenosylselenomethionine, it can then enter the transsulphuration pathway as with HMSeBA. It should also be noted that there is no way for HMSeBA to enter into general protein synthesis, as this can only stem from Se-Met, illustrating how HMSeBA does not interfere with methionine incorporation into proteins. The Se-Met and HMSeBA pathways overlap at the transsulphuration step (figure 1) at which point selenohomocysteine is produced. Following this, the two pathways are identical where the final product is incorporation into SePs or subsequent excretion of excess selenium containing sugars in the urine. Also shown is the ability of HMSeBA to be directly excreted in the urine when levels are in excess of needs, contrary to Se-Met that must also first be metabolised to an extent before it can be excreted. This means that the body is able to efficiently excrete excess HMSeBA as soon as the cellular concentration exceeds the cellular metabolic needs (Tufarelli *et al*., 2016).

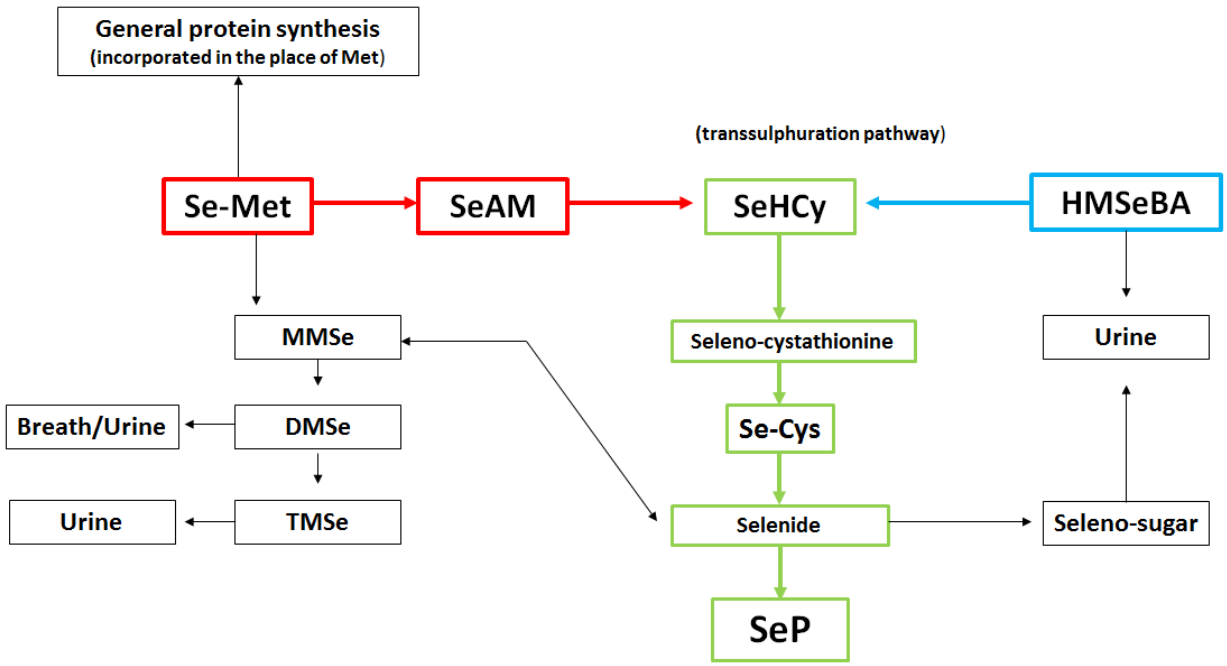

Figure 2 Proposed metabolic pathway of Selenomethionine and hydroxymethylselenobutanate in animal cells (adapted from Shini et al., 2015) (Se-Met: selenomethionine; SeAM: selenoadenosylselenomethionine; SeHCy: selenohomocysteine; HMSeBA: hydroxymethylselenobutan

2.8.5 Selenium proteinate

Selenium proteinate is a relatively new organic source of selenium which uses soybean peptides as a ligand (Leeson *et al.*, 2008). The inorganic selenium is incorporated into the protein of

the soybean, which is then hydrolysed and the resulting product is specifically suited for use in animal feeds (Xu *et al.*, 2014). In an experiment conducted by Leeson *et al.* (2008), egg yolks from birds fed selenium proteinate had higher selenium content than those fed SY or SS. They also showed that selenium proteinate decreased egg deformities when rancid oil was fed to birds, compared to diets containing rancid oil and supplemented with either SS or SY. The increased selenium accumulation in the yolk of selenium proteinate fed birds was due to the di- and tri-peptide ligands that accumulate in the lipid-rich yolk of the egg. Mineral-binding lipoproteins that are deposited during yolk accumulation in the egg-formation process could account for the preferential accumulation of selenium in the yolk when compared to the albumen of the egg. Xu *et al.* (2014) showed that broilers supplemented with selenium proteinate had increased body weight gain and a reduction in detectable intestinal lesions, resulting in a higher level of protection against necrotic enteritis. They hypothesised that selenium proteinate had the ability in broilers to stimulate the production of cytokines that had direct and indirect bactericidal effects on *C. perfringes*, which is known to cause necrotic enteritis. Necrotic enteritis is an enteric disease in poultry that is known to cause huge economic losses through increased mortality rates and depressed growth rates of the birds and the prevention thereof is of great benefit to the industry (Xu *et al.*, 2014).

Leeson *et al.* (2008) showed that selenium proteinate supplementation resulted in less GSH-Px activity in the liver, which translated to lower oxidative stress and a higher anti-oxidant status in the bird.

2.9 Selenium requirements

Even though selenium was established as an essential nutrient, it proved to be very problematic when added to livestock diets, as the margin between adequate supply and toxicity is very narrow, which made supplementation recommendations a challenging task (Shini *et al.*, 2015). This section explores the levels suggested as adequate for poultry and human diets to meet their metabolic needs as well as the challenges associated with developing these recommendations.

2.9.1 Poultry

Payne *et al.* (2005) stated the requirements of laying hens as between 0.05 and 0.08 mg/kg depending on their daily feed intake and this was accomplished in normal soybean-based diets, but in diets that mainly used grains, such as the maize-based diets in South Africa, selenium needs were not necessarily met by the ingredients alone.

It was also shown that selenium acts in combination with vitamin E, meaning that selenium requirements depend on the availability of vitamin E, and *vice versa*, as both of these nutrients prevent unsaturated fatty acid peroxidation within mammalian cells and have a sparing effect on one

another. Shini *et al.* (2015) reported that levels between 0.2 and 0.3 mg/kg of selenium in the diet was enough to prevent deficiency and tolerable levels were reported as 2 to 5 mg Se per kg of feed.

Delezie *et al.* (2014) reported on a concept known as the transfer factor of selenium that was affected by both the source and concentration of the supplemented selenium. The transfer factor was calculated by taking the selenium concentration of eggs and dividing it by the selenium intake of the bird. The highest factor was found in hens that were fed the lowest dose and the factor was higher for organic selenium sources than inorganic ones. These results showed that layers used selenium more efficiently when the concentration in the diet was lower. This further complicated the true determination of selenium requirements for layer hens as oversupply would result in a larger waste.

Yuan *et al.* (2012) showed that maternal nutrition affected the health and performance of the broiler offspring, which means the embryonic needs of the chick must be met by the egg, as the broiler does not obtain any additional nutrients from its mother post-hatch. It was also shown that the selenium supplementation levels in parent stock can persist in the offspring for up to 2 weeks post-hatch. Payne and Southern (2005) and Perić *et al.* (2009) established in their respective trials that selenium supplementation in broilers did not affect performance parameters such as live weight or breast meat yield. However, it did increase the selenium stores in the tissue and thus the size of the selenium pool available for metabolic use. Selenium is needed for optimal growth in poultry and good management practices can prevent deficiency symptoms from showing until the damage is irreversible (Briens *et al.*, 2013). Delezie *et al.* (2014) showed in their trial, using Lohmann Brown layers, that serum selenium concentration had a direct effect on the selenium content of the eggs. There was a linear correlation between the dose of selenium in the feed and the subsequent selenium content of the egg. This showed that a higher selenium status in the laying bird would allow for higher selenium deposition in the eggs that were laid and thus higher selenium reserves available to the developing chick.

Surai (2000) used 100 25-week-old Cobb broiler breeders to determine the maternal transfer of selenium to the antioxidant system of the developing embryo. It was demonstrated that the selenium that accumulated in the egg was transferable to the embryo, shown through the increased amount of selenium present in the liver of day-old chicks. This was a dose-dependent response and showed an increased bioavailability for organic selenium sources. This relationship also held true for the vitamin E status of the offspring. The available selenium had a positive effect on the available vitamin E levels for the chick, as shown by increased vitamin E status in chicks hatched from eggs laid by hens receiving higher selenium diets. Maternal selenium supplementation also showed an increase in GSH-Px activity in the offspring. Combined supplementation with vitamin E did not further

increase the GSH-Px activity beyond that already achieved through selenium supplementation alone.

2.9.2 Humans

Navarro-Alarcon & Cabrera-Vique (2008) reviewed the available data on selenium and its link to human nutrition and health and concluded that the lack of a clear recommended daily level of selenium for human nutrition was due to *in vivo* bioavailability studies being very complex and expensive to conduct. This meant that the available data is limited compared to other vitamins and minerals where more extensive research exists. The potential for selenium toxicity also exists, which will be discussed in detail in subsequent sections, and this has resulted in more conservative recommendations and guidelines with regard to selenium inclusion in human diets. Daily selenium intake is recommended at 60 µg for men and 53 µg for women (Rayman, 2012). However, actual intake is thought to range anywhere from 7 µg to 4990 µg and is influenced by many factors, including selenium content of the soil that the crops were grown in and the availability of the ingested selenium, determined by the selenium source. About 80% of the selenium present in the human body is directly contributed by the food that is consumed and the resulting bioavailability of the ingested selenium depends on the source of the selenium, with organic sources having a higher bioavailability (Navarro-Alarcon & Cabrera-Vique, 2008).

Selenium status in humans was measured using plasma or serum selenium concentration and it was shown that individuals with a baseline concentration of less than 87 µg per litre had a higher risk of cancer (Rayman, 2012). It was also evident that individuals that maintained serum levels of selenium at 135 µg/L had a lower mortality than those with levels below this value during a nine-year study.

There are many strategies that have been implemented all over the world to improve the selenium status of the population, including, but not limited to, Se-enriched fertilisers for crops, selenium supplementation to livestock destined for meat and by-product production and direct multi-micronutrient supplementation (Navarro-Alarcon & Cabrera-Vique, 2008).

2.10 Selenium metabolism

Surai and Taylor-Pickard (2008) explained that inorganic forms of selenium exist in the soils, while both organic and inorganic forms are found in plant and animal tissues. Absorption of both organic and inorganic forms of selenium occurred in the small intestine. However, Shini *et al.* (2015) differentiated that inorganic selenium, such as selenite was passively absorbed across the gut wall, while organic forms were actively transported using a sodium-mediated carrier whose function was

shared with sulphur. Inorganic selenium sources are recognised by the cells of the digestive tissue and can then be incorporated into SePs. However, organic sources, such as Se-Met, are not recognised by mammalian cells as being selenium-containing and due to this, it is absorbed and metabolised based on the methionine needs of the animal. The released selenium is then recognised as an inorganic mineral by the cells and undergoes the metabolic fate of inorganic selenium. If Se-Met is not broken down, it can be incorporated into a variety of non-genetically programmed proteins that contain selenium rather than being excreted rapidly from the body (Rao *et al.*, 2013). This non-specific incorporation of selenium into proteins can cause selenium toxicity and due to this, a metabolic safeguard exists that prevents dietary Se-Met and Se-Cys from being directly incorporated into SePs, which have important biological functions within the body. Dietary selenium must therefore first be metabolised and converted into Se-Met or Se-Cys using a genetically controlled cellular mechanism. The distribution of selenium around the body is mediated by the liver, pancreas, kidney and the brain, with the highest concentration of selenium found in the tissue of liver and kidney samples although when assessed strictly on a mass basis, the muscle contains the highest amount as it comprises the largest proportion of body weight. The only effective mode of transport of significant amounts of selenium through the body is via the blood, using two SePs, namely; SePP1, which has over 50% of circulating selenium in the plasma, and extracellular GSH-Px (Brown & Arthur, 2001). There is evidence that SY supplementation increases the amount of blood Se-Met, but the amount of blood Se-Cys remains constant, which causes a saturation of seleno-enzymes and results in the nonspecific incorporation of selenium into proteins at the expense of sulphur and points towards an alternative selenium transport system that has not currently been adequately researched (Shini *et al.*, 2015). Once selenium has been liberated from the protein through protein turnover, the free selenium is either recycled through enterohepatic circulation or excreted primarily in urine and faeces (Shini *et al.*, 2015).

The mechanism by which selenium increased the anti-oxidant status of the bird is through its incorporation into SeP, specifically through its association with Cys, forming Se-Cys (Rao *et al.*, 2013). Other elements use ionic association which is less specific. It was also shown in and experiment by Briens *et al.* (2013) that muscle selenium decreased between 21 and 42 days of age in broilers, due to the large and quick development of the pectoralis major muscle. Despite this, the digestibility of selenium was stable throughout bird growth. Highly metabolically active tissues had higher levels of Se-Cys than muscles with low metabolic activity. Examples of muscles with high metabolic activity include the liver and the kidney, whereas breast muscle had equal levels of Se-Cys and Se-Met. The Se-Met pool is an indication of the endogenous selenium reserves available to the bird and organic sources of selenium were able to make a larger contribution to this pool due to the unspecific incorporation of Se-Met in the place of methionine in proteins.

Selenium metabolism is also dependent on the levels of available vitamin E (Shini *et al.*, 2015). When vitamin E levels are too low, Se-dependent peroxidases will prevent lipid peroxidation by acting as the primary anti-oxidant and counter to this, vitamin E is able to neutralise oxidants prior to the chain reactions and this prevents selenium losses.

Vitamin A has antioxidant properties and acts by slowing down lipid peroxidation in mammalian cells (El-Hack *et al.,* 2017). The role of vitamin A in determining the egg quality of layers is linked to dietary selenium supplementation. El-Hack *et al.* (2017) used 162 hens that were fed one of nine possible diets, each containing a set level of selenium and vitamin A. Their results showed that increased levels of vitamin A improved feed conversion ratio (FCR), but only until the intermediate supplementation level, where after it plateaued and higher inclusion levels showed no further benefit. They determined that the true interaction of selenium and vitamin A was apparent in heat-stressed birds, where the largest improvement to FCR, egg quality and egg production was evident with higher levels of supplementation. Temperatures above 28ºC caused an increase in tissue mobilisation of various vitamins and minerals and the formation of free radicals due to lipid peroxidation increased, which caused damage to liposomal membranes. El-Hack *et al.* (2017) then determined that excess vitamin A was stored in the liver for later deposition in body tissue, but the metabolic needs of the bird were met using the basal diet and that vitamin A supplementation was not a necessity to sustain egg production under normal commercial conditions, where laying birds were kept between 21 and 24ºC. There was no evidence that suggested any antagonistic relationship between selenium and vitamin A, suggesting that the combined supplementation could improve the overall integrity of body cells, the immune status of the bird as well as the bird's ability to produce eggs in heat stressed conditions.

2.11 Selenium deficiency

Selenium enters the food chain through uptake of inorganic selenium by plants from the soil and in the case of South African soil, where selenium is deficient, it may be necessary to enhance the selenium content of carcass meat and eggs through appropriate dietary supplementation to the animals in order to prevent deficiencies (Pan *et al.*, 2007). The benefit of this supplementation is two-fold, namely to improve the performance and health status of the animal, as well as influencing the quality of the meat and eggs consumed by the human population in a controlled way.

2.11.1 Poultry

Selenium deficiency has been shown to cause impaired growth in broilers and layers, poor development and feathering, reduced egg production and a reduction in egg hatchability, all of which

are associated with economic losses (Brandt-Kjelsen *et al.*, 2017). Due to the large role selenium plays in SeP, a deficiency of selenium leads to inferior enzymatic function, which causes a cessation in some biological pathways and could result in pathologies developing due to compromised immune function (Shini *et al.*, 2015).

Surai and Taylor-Pickard (2008) discussed the role selenium played, along with vitamin E, in mitigating malabsorption syndrome. Malabsorption syndrome was described as an enteric disease in growing broilers that was associated with an avian retrovirus. The role selenium played was preventing the virus from replicating because it inhibited the reverse transcriptase required in the RNA by the virus. Malabsorption syndrome was shown to be present in broilers that were not supplemented with organic selenium sources, which led to the conclusion that selenium deficiency was not the only contributor to malabsorption syndrome, but also the form of selenium that was offered, also referred to as the source.

Selenium deficiency is possible in both humans and animals that consume a plant-based diet where the soil that the plants are grown in is selenium deficient or has low selenium availability (Shini *et al.*, 2015). Yuan *et al.* (2018) discussed how selenium deficiency decreased the efficiency of incorporation of Se-Cys at the UGA codon site, which affected the mRNA translation process. This was due to the necessity of a selenium atom at the active site of any seleno-enzyme, without which the seleno-enzyme is not effective. Without the messenger RNA carrying the correct coding sequence to the mitochondria, there is a decay in the production of GSH-Px within the cells.

Selenium deficiency in poultry is known to cause pancreatic fibrosis and exudative diathesis (ED) (Shini *et al*., 2015). Surai (2002) considered pancreatic fibrosis to be the clearest indicator of selenium deficiency in poultry as it is not complicated by deficiencies of other nutrients. Nutritional pancreatic atrophy exhibited in chicks could be controlled by including vitamin E supplementation at levels more than 15 times the recommended levels. This further emphasised the economic benefit of adequate selenium supplementation, as the alternatives, such as vitamin E, needed to be supplied at such high levels that it became more costly than the initial selenium supplementation that would have prevented the deficiency from the start. It was also noted that exudative diathesis could be observed at hatch, indicating that the lesions were able to develop during the embryonic period and that selenium deficiency in chicks within the egg is possible. Because broilers are such fast growing animals, they are highly susceptible to selenium deficiency as their metabolic needs must be rapidly met by their diet (Huang *et al.*, 2011). Creech *et al.* (1958) outlined that the onset of ED is caused by a deficiency in vitamin E, which can result from a selenium deficiency as the two are metabolised interchangeably and is most commonly noted in birds between one and five weeks of age, making it a disorder of particular importance in broilers. Birds suffering from ED typically showed severe anaemia due to haemorrhaging that resulted from the reduced haemoglobin and total amount of

protein in the body cells. They may also exhibit symptoms such as imbalance or uncontrolled movement, all of which would hinder the bird's ability to eat and thus grow and develop properly.

Se-Met was shown to be four times more effective at preventing pancreatic degeneration in chicks when compared to selenite as a supplementation source, indicating the superior antioxidant potential of organic selenium sources over inorganic ones and the greater potential to mitigate selenium deficiency and its associated disorders in poultry (Shini *et al.*, 2015).

Typical conditions associated with selenium deficiency include pancreatic degeneration, muscular dystrophy and necrotic lesions in the liver, muscle tissue, heart and along the gastro-intestinal tract, all of which are more commonly observed in laying hens and breeder hens as these conditions are the result of long-term deficiencies (Brandt-Kjelsen *et al.*, 2017).

White muscle disease developed when low selenium levels caused impaired immune functioning and was shown to also have a direct effect on the presence of cardiac muscle metamorphism, anaemia and liver bleeding. These conditions have a higher incidence in carcasses of birds that are selenium deficient (Brandt-Kjelsen *et al.*, 2017).

2.11.2 Humans

The essentiality of selenium in human health was only accepted in 1979 and this was because of its overlapping function and close relationship with vitamin E (Navarro-Alarcon & Cabrera-Vique, 2008). This masked the true necessity of selenium for proper immune function, as focus was placed on increasing the vitamin E status of the population rather than the selenium status. The main cause of selenium deficiency in humans is due to the low selenium intake from agricultural products (Brown & Arthur, 2001) and a lack of research on methods of synthetic selenium supplementation in human nutrition (Navarro-Alarcon & Cabrera-Vique, 2008). There is also evidence that selenium supplementation could lead to harmful toxicities, which is explored in more detail in subsequent sections.

Rayman (2012) discussed that during times of selenium deficiency, the synthesis of certain SePs, such as GSH-Px and GPx4, were prioritised over others. This demonstrated the importance of certain enzymes to human health and immune functioning and the body's ability to recognise and synthesise these enzymes accordingly. One important example is that because the brain requires selenium above other tissues, in times of selenium deficiency, irreversible brain damage can occur if selenium cannot be mobilised from other tissues in order to support the needs of the brain. This was shown to correlate with epileptic seizures and a decline in cognitive function, as individuals with lower serum selenium were more likely to display these symptoms. The exact mechanism of selenium partitioning and prioritisation within the human body is not fully understood and warrants

further research. This has led to an increase in research potentially linking selenium deficiency to Parkinson's disease and the role SePP1 may play in preventing or minimising the symptoms caused by this disease. Navarro-Alarcon and Cabrera-Vique (2008) summarised the most common diseases and disorders in humans that have been linked to selenium deficiency. These include endemic Keshan disease in the selenium deficient area of Keshan in China, which affects young children and sometimes women of child-bearing age and manifests itself with symptoms such as enlarged joints and pain comparable to that experienced by individuals suffering from arthritis. The root of this disorder is caused by oxidative damage to cartilage and this results in deformed bone structure.

Rayman (2012) also highlighted the studies on humans where a selenium deficiency, defined as a serum or plasma selenium level below 85 µg/L, was associated with a decreased survival rate in HIV positive individuals. This relationship was not strictly linear and those individuals with more advanced stages of infection showed a higher acute-phase response, causing a more drastic lowering of blood selenium levels. This emphasises the importance of selenium for proper immune function, with particular benefit to those individuals who already have a compromised immune status. These sick individuals have a higher need for bioavailable selenium as their cells have an immediate need for selenium.

2.12 Selenium toxicity

The potential toxicity of selenium was first recognised around 1932 (Brandt-Kjelsen *et al.*, 2017). It was because of this that SS was recommended for selenium supplementation, since it did not increase the level of selenium in tissue significantly when compared to the organic sources of selenium. The initial recommendations were set out to ensure selenium requirements were met, but without increasing the selenium content in muscle and tissue. This was because tissue concentration of selenium was the only measure of selenium status and toxicity was the major concern of all nutritionists, both for humans and livestock.

2.12.1 Poultry

Tissue selenium concentration was considered the immediate selenium status of the animal and the storage of selenium in tissues for later metabolism was not a consideration (Brandt-Kjelsen *et al.*, 2017). When GSH-Px activity was established as a more reliable measure of the bioavailable selenium, more accurate recommended inclusion levels could be developed. It was only after more research was conducted on the toxic levels of selenium, as well as the associated disorders of selenium deficiency, that the benefits of increasing selenium stores in bodily tissues were discovered. Delezie *et al.* (2014) showed that if the bird was in good health, supplemented levels of selenium as high as 0.5mg/kg, coupled with a basal diet that was not selenium-deficient, had no adverse effect on bird performance.

The various sources of selenium and their mechanisms of metabolism has contributed to the limited information on exact toxicity levels for layers (Payne *et al.,* 2005). Payne *et al.* (2005) found reduced egg production and feed intake to be two symptoms of selenium toxicity in laying hens, but they were unable to pinpoint the exact supplementation level that would elicit this response. In this experiment, a level of 0.3 mg/kg supplemented selenium in the feed was not toxic, but this was supplemented as SS, which has been established to be more toxic than organic sources.

DL-selenomethionine was only accepted and approved as an organic source of selenium in 2014 after it was shown that levels up to 1.5 mg of selenium per kg of feed was still safe in chicken feed (Brandt-Kjelsen *et al.*, 2017).

2.12.2 Humans

The definition of a single value for selenium toxicity levels is complicated by many factors, such as selenium source and the subsequent bioavailability of selenium, the exposure time of the person to the selenium source, the interaction of selenium with other metals and the physiological status of the person to name a few (Navarro-Alarcon & Cabrera-Vique, 2008).

However, by examining various studies on the toxic effects of selenium, Navarro-Alarcon and Cabrera-Vique (2008) were able to establish a broad range of selenium concentrations where symptoms of toxicity began to manifest as reduced T_3 levels, hepatotoxicity and an increased risk of cancer due to hydrosulphide oxidation within the cells. This range was stated as levels exceeding 3200 µg to 6700 µg selenium per day. This range is so large because the toxicity level differed greatly between individuals, with some showing adverse effects only at levels more than double those of others. This provides evidence of the many factors that contribute to each individual's response to selenium toxicity.

Selenium toxicity in humans, identified as selenosis and classified as chronic selenium toxicity, has been shown to cause garlic breath, the loss, or brittleness of, nails and hair and nervous system disorders (Rayman, 2012). Other toxic symptoms that are not necessarily associated with chronic toxicity include disruptions to the endocrine system functioning and a disruption in the synthesis of thyroid and growth hormones (Navarro-Alarcon & Cabrera-Vique, 2008).

2.13 Selenium content of maize

The selenium content of feed ingredients is dependent on the selenium content of the soil it is grown in, which can be highly variable (Shini *et al.*, 2015). Sedimentary rocks and shales are known to have the highest selenium concentrations, while igneous or volcanic rocks, sandstone and granite possess significantly lower concentrations of selenium (Brandt-Kjelsen *et al.*, 2017). Although selenium is present in virtually all soils worldwide, the concentration varies anywhere from near zero

to as high as 1250 mg selenium per kg of soil in some areas of Ireland. Surai and Taylor-Pickard (2008) outlined the many factors that influenced the selenium availability to plants. These included the acidity and aeration of the soil that allowed selenium to form insoluble complexes and reduced the availability to the plant. In acidic soils, selenium is present as selenite which has low solubility and low availability for uptake by plants, while alkaline soils oxidise selenium to selenate, a more soluble form of the element, with high availability to plants through soil uptake (Navarro-Alarcon & Cabrera-Vique, 2008). Both selenite and selenate are absorbed by plants through the soil and transferred to cereal grains in the form of Se-Met, the difference being that selenate is more efficiently deposited than selenite. Sulphate competes with selenate for absorption by the sulphate transporter and high sulphur soils or soils with high sulphur fertilisers cause lower selenium absorption by plants. Selenium in the topsoil is leached in areas that experience high rainfall. Selenium bioavailability in the soil plays a larger role in plant selenium content than merely the selenium soil content alone (Brandt-Kjelsen *et al.*, 2017). In an experiment to determine the selenium content of South African maize, Courtman *et al.* (2012) established that 94% of the samples analysed had less than 50 µg/kg, making them deficient by both human and animal requirement standards. The reason for this is that the pH of the soil influenced the amount of selenium that was available to the plant, with high selenium content in the soil of the eastern region of South Africa, but a low soil pH and thus the selenium was not available to the plant. On the contrary, the western region had soils with favourable pH but low selenium content and thus there were limited nutrients available for absorption. It is because of this trend that selenium supplementation in broiler diets has become a common practice, but not always a routine one. Aerobic soils that possess a pH close to 7 have selenate as the predominant form of selenium, whereas soils high in selenite are of a lower pH and lower redox potential (Brandt-Kjelsen *et al.*, 2017).

Plants absorb selenium in the form of selenite and selenate from the soil and this is then converted by the plant to seleno-amino acids, where Se-Met comprises more than 50% of the selenium, particularly in cereals (Surai & Taylor-Pickard, 2008). Selenate was established as more mobile when compared to selenite and thus is more available to plants through the soil (Brandt-Kjelsen *et al.*, 2017).

South Africa is not the only country that experiences low selenium content in their soil; Pan *et al.* (2007) reported that there was a great need in China to establish the benefits of selenium sources as dietary supplements in layer hen feed. It is common practice in China to supplement livestock feed with selenium due to the widely accepted fact that China's soils are selenium deficient (Pan *et al.,* 2007).

2.14 Selenium source affects egg selenium content and egg quality

While there are many documented benefits to selenium supplementation in poultry, the results are not seen in normal growth parameters such as body weight or breast muscle yield, but rather in the selenium content of the meat or eggs and in the improved shelf life of the breast meat due to reduced drip loss (Ševčíková *et al.*, 2006). Egg quality was measured using parameters such as shell thickness, egg weight, albumen quality and yolk weight (Asadi *et al.*, 2017). Selenium supplementation showed no significant difference to egg production, meaning that there was no notable increase to the number of eggs laid by the hens and this was ascribed to the basal diet containing sufficient selenium to meet the production needs of the hens (Invernizzi *et al.*, 2013). Asadi *et al.* (2017) showed that selenium supplementation only improved egg production when the basal diet was lacking in selenium. In their experiment, Invernizzi *et al.* (2013) showed that there was also no notable increase to the amount of feed consumed by the hens, but there was an increase in the weight of the eggs and eggshell. According to this trial, the eggs from the SY treatments showed increased breaking strength when compared to SS sources. This meant that eggs from the SY source had stronger shells than those from the SS source. Overall, this experiment demonstrated a 46.81% increase in egg selenium content for eggs from SY treatments compared to SS treatments. It was also shown that selenium supplementation from either source increased eggshell weight compared to no supplementation. Attia *et al.* (2010) compared organic and inorganic selenium sources in breeder eggs and it was established that higher selenium concentration in the diet would increase the egg weight, with inorganic forms having the largest effect on egg weight when the shell is included and organic sources affecting mostly the egg weight of the internal yolk and albumen. It can be concluded that inorganic selenium sources contribute highly to the egg shell selenium content, whereas organic sources contribute to the selenium concentration in the yolk and albumen of the egg. Attia *et al.* (2010) also found no effect on level of production, as the basal diet was adequate to support laying. However, hatchability increased by 17%, although this was not coupled with a reduced mortality nor increased fertility percentage. Selenium concentration in egg yolks from eggs that originated from organic selenium source diets was higher and this increase was linearly correlated to the level of selenium supplementation.

In a laying hen trial conducted by Delezie *et al.* (2014), the highest level of egg selenium was from the Se-Met treatment, followed by SY and the lowest level was seen in the SS treatment at the same inclusion level. However, all supplemented treatments showed higher levels than the non-supplemented control treatment.

By using Hy-Line laying hens, Payne *et al.* (2005) showed that SY treatments compared to SS treatments increased the incidence of cracked and dirty eggs that were laid, due to less selenium being deposited in the shell and more deposited into the egg contents. This was accompanied by no

change to feed intake between SS and SY treatments and it was demonstrated that SY did not improve the Haugh unit score of eggs stored at room temperature, but did for those stored at 7°C. These results suggested lower egg quality from SY treatments when compared to SS treatments, but the egg weights for the SY treatments were higher than those from SS, which suggested a higher selenium content in these eggs. The whole-egg selenium analysis showed that the SY eggs did have higher selenium content and these eggs also reached peak selenium content sooner than SS eggs. This means that a shorter supplementation period on SY treatments was needed before the beneficial results were observed. This has the potential to reduce feed costs and improve the quality of the eggs.

2.15 Selenium analysis methods in tissue and eggs

Brandt-Kjelsen *et al.* (2017) summarised the most common methods used for selenium speciation and detection of selenium in various matrices. The speciation of selenium was an important milestone in understanding the quantity of selenium that is transferred from the soil into the plants and from adequately supplemented feed into the tissues of the animals consuming it. However, to this day, many questions remain unanswered with regards to the exact turnover of selenium from soil to plants and from feed to animal tissue, but as new methods of analysis are developed, more accurate estimates of turnover rates are established.

Surai and Taylor-Pickard (2008) explained that special analytical procedures were needed to determine the selenium content of eggs and because they contained such low concentrations of selenium, contamination was possible during the collection, storage and handling processes. To minimise this, samples were digested in a mixture of nitric acid and perchloric acid, which minimised the loss of selenium, which is a very volatile element when open digestion processes are used. They discussed the use of graphite furnace atomic absorption spectrometry (GFAAS) as the most common method used to determine selenium content. There was evidence that sample decomposition occurred and this caused background interference unless any residual organic matter was destroyed during the ashing process prior to analysis. Samples also had to be further prepared after the decomposition and ashing and when methods involving hydride generation were used, selenium needed to be present in the Se^{IV} form to be detected at all because this was the only selenium species that effectively formed a hydride. Samples had to be heated with concentrated hydrochloric acid to reduce any Se^{VI} that formed during the digestion and thus prevented irregularities in the results. These explanations emphasised the importance of proper sample handling and collection because selenium analysis is so sensitive and sample contamination is a serious concern to the integrity of the results.

In terms of biological tissues, water-soluble seleno-amino acids have been extracted using water extraction, where driselase was used to release any selenium bound within the cell walls (Brandt-Kjelsen *et al.*, 2017). There have also been reports of *in vitro* digestive techniques used to mimic the gastro-intestinal tract and how bioavailable a selenium source would be under normal metabolic conditions within an animal but these experiments are complex and expensive and the results are highly variable and therefore not conclusive (Brandt-Kjelsen *et al.*, 2017).

Invernizzi *et al.* (2013) individually cracked each egg collected during the experimental period and then homogenised the liquid and subsequently froze the samples. The selenium content was determined on a dry matter basis and the amount of selenium was categorised using a calibration curve that referred to standard solutions. Surai and Taylor-Pickard (2008) explained that atomic absorption spectrometry (AAS) was a low-cost method used to determine selenium content, but it lacked the sensitivity to detect the small amounts of selenium that were present in tissue samples. They recommended the use of inductively coupled plasma with mass spectrometric detection (ICP-MS) which quantified low concentrations of selenium and did not require the selenium to be present in a specific form in order to be detected. The size of the sample needed was much smaller than other techniques and this was advantageous due to the limited quantity of tissue that could be obtained from an egg. Eggs had to be sent intact to the laboratory and often extra volumes of acid needed to be added due to the high fat content of eggs that could have caused charring. It was established through fluorometric methods that egg selenium content varied greatly and was dependent on the dietary intake of the hen. ICP-MS uses the element mass to charge ratio to send signals in order to quantify the selenium and thus allowed accurate quantification because the ionisation is species independent (Brandt-Kjelsen *et al.*, 2017).

Electrospray ionisation mass spectrometry uses the molecule mass to charge ratio to identify the selenium species, as the species and matrix ionisation is very specific and dependent on the species and matrix used in the analysis (Brandt-Kjelsen *et al.*, 2017). This requires that the species be isotopically labelled in order to be identified. Isotope labelling allows a particular selenium species to be followed and its fate through the environment monitored, such as a particular selenium source added to fertiliser, or supplemented into a feed.

Delezie *et al*. (2014) analysed eggs collected from their trial by mineralising whole egg content with HNO_3 in a heating block, with temperatures being adjusted between room temperature and 105ºC over a 3-hour time period. This mineralised the sample and allowed for dilution and comparison to the reference material, which in this case was TORT-2 to determine the selenium content of each sample.

Payne *et al.* (2005) focused on the selenium content of eggs after only a short period of time, in this case only 28 days. They collected sample eggs and stored them at 7.2ºC before the shells were discarded and the whole egg was homogenised in a malt blender and then stored as frozen until selenium analysis was conducted. The process used was a semi-automatic fluorometer and all samples were digested in nitric acid and perchloric acid.

Pan *et al.* (2007) used hydride generation atomic fluorescence spectrometry (HG-AFS) to analyse egg samples for selenium content. Briefly, this required 1.0 g of the sample to be accurately weighed and dissolved in 2 mL of HNO_3 before being transferred into a Kjeldahl flask. The samples were pre-digested at room temperature for 1 hour and 2 mL of $HClO_4$ was added. The sample was then heated and transferred to a volumetric flask and made up to a final volume of 25 mL with ultrapure water. This was then mixed with 1ml of 10% $k_3Fe(CN)_6$ and made to a final volume of 25 mL with 3 mol/L HCl. This treated sampled was injected into an HCl carrier and a volatile hydride was formed, which was atomised by a diffusion flame and finally, the atoms were detected by fluorescent spectrometry. The regression equation obtained by this method is compared to the calibration curve of the standard Se solution.

At present, there is no standard procedure for selenium speciation analysis and, as discussed, there are many accepted methods for sample preparation and separation prior to the detection (Brandt-Kjelsen *et al.*, 2017).

2.16 Conclusion

Eggs are an important dietary source to humans and selenium plays an important function in maintaining the immune system of both humans and poultry. It is because of this that selenium supplementation needs to form an important part of poultry production. There is research to support the assumption that selenium supplementation to hens will increase the selenium that is deposited into their eggs. However, with several different sources of selenium available, the metabolic pathway of each source is not the same and thus each source will differ in their bioavailability to the animal. Selecting the incorrect source could lead to either selenium deficiency or in extreme cases selenium toxicity, although this is less likely as the amount of selenium needed is far greater than what would typically be supplemented in any diet. Because table egg production must remain profitable to the farmer, the cheapest option for selenium supplementation is often the most attractive one for inclusion in the diet, which often leads to the selection of the cheaper, inorganic sources. However, research has shown that organic selenium sources provide better bioavailability and can be included at lower levels with the same effectiveness in the results. But even the organic sources do not all provide equal deposition efficiency within tissues. With a better understanding of the efficacy of deposition into the egg, the most efficient selenium source can be chosen for inclusion in the diet of

the hen. This will ensure immune system support for the hen as well as providing a safe and efficient way to increase the selenium status of the population consuming the eggs. In order to determine this, the amount of selenium deposited into the eggs needs to be measured for each of the different sources and compared in a controlled environment.

CHAPTER 3: MATERIALS AND METHODS

3.1 Birds and housing

One hundred and sixty 43-week old Amberlink hens were purchased from Kuipers Group from their layer farm in Krugersdorp. For this experiment, a climate-controlled house on the University of Pretoria Experimental Farm in Hatfield, Pretoria was used for the duration of the trial. Individual metabolism cages of 84 x 69 x 52 cm were prepared for each hen. Each bird had individual access to feed and water within the cages at all times during the trial. Water was supplied *ad libitum* in water troughs, changed twice daily to ensure clean, cool water was available to the birds. Feed was given twice daily, as 60 g meals, to minimise spillage. Excreta trays were cleaned twice a week to prevent build-up of ammonia in the house. The cages provided a total of 1449 cm^2 space to each bird, which exceeds the 450 cm^2 recommended by the South African Poultry Association Code of Practice for Laying Hens (2012). The wire mesh separating the cages allowed for social interaction between adjacent birds. The birds were caged individually to allow for accurate feeding and collecting of eggs. The cages were raised slightly on one side, using small wooden blocks placed underneath two of the legs of the metabolism crates, to allow eggs to slide to the front of the cages for easy collection and documentation and preventing the hens from standing on the eggs and potentially breaking them.

There were four treatments used in this experiment, which were comprised of four different selenium sources. Three organic and one inorganic source were used to formulate four diets, which differed only in their selenium source. Birds were assigned to these diets as the treatments for this experiment. Details of each diet formulation are discussed in a subsequent section.

A randomised block design was used to assign treatments to cages, with ten replicates of each of the four treatments present in each of the four blocks. This meant that there was a total of 40 hens in each treatment and a total of 160 hens in the entire experiment. Each block consisted of 40 cages and was designed by dividing the house into four areas and assigning entire sets of cages within that area to the corresponding block. The first block was the front left of the house; the second was the front right of the house. The third and fourth blocks were the back left and back right of the house respectively. The blocks did not consider the height of the cages, which meant that there were not equal numbers of bottom, middle and top cages within each block. This was because there were three different heights and ten replicates per block, so having equal numbers was not possible.

Birds were exposed to a 16-hour lighting program, with lights being on from 5 am to 9 pm daily. This allowed for eight hours of consecutive darkness, in accordance with the previous lighting program the birds were housed in. The temperature within the house was maintained between 18°C and 25°C for the duration of the trial, using a SKOV automated temperature system, SKOV being international suppliers of climate and farm management solutions. The temperature was set to 21°C

and allowed to fluctuate 2°C in either direction before interventions from the automated system would begin. The temperature was monitored through thermometers positioned throughout the house, at the front, rear and in the middle. An external thermometer also monitored the outside temperature. Automated air vents allowed for air circulation and ventilation and prevented the house from overheating, as well as circulating fresh air to the birds.

Upon arrival, birds were weighed individually and fitted with an identification tag on their feet. The tag clipped onto the base of the leg easily and without any invasive procedures on the bird. Each tag had a unique number printed on it, which corresponded to the cage number that the bird was assigned to. The selection order of the birds was random and after being weighed and tagged, they were placed into their individual cages with immediate access to water. Once all the birds were placed, they were left undisturbed for 30 minutes to adapt to the new environment. They were then provided with their first portion of adaptation feed.

3.2 Feed formulation and dietary treatments

The layer diets were formulated as a layer mash, using the guideline amounts of nutrients as recommended by Amberlink (Dekalb Amberlink Product Guide, 2017). These guidelines are designed to provide all the basal nutrients and support optimum egg production in the hens. The feed formulation was supplied by the Kuipers Group from whom the hens were purchased. The adaptation and experimental diets were based on this same formulation to ease adaptation of birds. The feed was in mash form, both pre-trial on the farm and during the trial period. Table 3.1 outlines the composition of the diet, used during the adaptation period and for mixing of the experimental diets. The amount of feed to be mixed was determined using the industry standard feeding of 120 g per bird per day (Dekalb Amberlink Product Guide, 2017) with an extra 25% added as a margin of safety to account for spillage and sample removal.

All experimental diets were mixed by SimpleGrow Agricultural Services, located in Centurion, South Africa. The same mixing procedure was followed for each of the experimental diets to ensure uniform nutrient content of each diet. The ingredients were individually weighed out, added to the mixer and mixed for a total of seven minutes.

Table 3.1 Feed ingredient composition (%) of the diet, used for the adaptation period and for mixing of the experimental diets

Ingredient	Formulated (%)
White maize	60.34
Soya oilcake meal	11.44
Full fat soya	8.070
Sunflower oilcake meal	8.070
Limestone grit	6.180
Fine limestone	2.650
Soya oil	1.070
Mono di-calcium phosphate	0.470
Salt (fine)	0.307
Layer premix	0.253
Premix (no selenium or phytase)	0.250
Phytase (600 FTU + maize)	0.250
Antimicrobial aqueous formaldehyde 37%	0.181
DL-Methionine 50%	0.168
Sodium bicarbonate	0.118
Biolysine	0.102
Red Pigment (2%)	0.048
Yellow Pigment (10%)	0.026

The adaptation diet did not contain any supplemental selenium sources, allowing the hens a brief ten-day depletion period from selenium sources other than what was contained in the feed raw materials listed in Table 3.1. A 400 kg batch of the adaptation feed was mixed.

Samples were extracted from the batch of adaptation feed and analysed at ChemNutri Analytical laboratories in Olifantsfontein, South Africa. Table 3.2 shows the analysed values, in percentages, of the nutrients in the adaptation feed.

All feed analysis procedures were conducted as detailed by the official methods of the AOAC (AOAC, 2000). Crude protein was analysed by using a dumatherm to determine the nitrogen content in the sample. This value was then multiplied by 6.25 and the resulting value was calculated as a percentage of the whole sample. Ash content was determined by placing a sample of known weight

in a crucible and then these were placed in a furnace for two hours at 250°C. The temperature was then increased to 600°C for four hours. The samples were cooled for two hours and the placed in a desiccator for 30 minutes. The ashed sample was then weighed and compared to the weight of the initial sample. The weight difference was calculated as a percentage of the initial sample weight. Moisture content was determined by comparing the weight difference of an initial and final sample of the feed, after being dried for 24 hours in an oven at 100°C. The moisture content was then calculated by subtracting the final dried sample weight from the initial sample weight and calculating the percentage difference in the two weights. Crude fibre content was determined by use of fibre bags for filtration of the samples and these were then ashed for six hours at 550°C. The Fat content of the sample was determined by submerging the sample in petroleum ether and then subjected to heat extraction. The resulting sample was weighed and compared to the initial weight of the sample, after which a percentage of the total was calculated. Calcium and total phosphorus content were analysed using a spectrophotometer where the wavelength from the sample was compared to that of a known standard for each nutrient. The selenium content was determined using a method adapted from Matek and Blanuša (1998) and is explained in detail in section 3.6.

Table 3.2 Analysed values (%) for the nutrient composition of the adaptation feed

Nutrient	Analysed content
Crude protein (%)	16.64
Ash (%)	12.98
Moisture (%)	7.85
Crude fibre (%)	4.81
Fat (EE) (%)	4.13
Calcium (%)	3.95
Selenium (mg/kg)	0.64
Total phosphorus (%)	0.44

Three organic sources and one inorganic source of selenium were used in this trial. The seleno-yeast (Sel-Plex, manufactured by Alltech) contained 0.2% selenium. The hydroxymethylselenobutanate (Selisseo, a product of Adisseo) had 2% selenium. The selenium proteinate (B-Traxim, produced by Pancosma) contained 1.1% selenium. The inorganic selenium source, sodium selenite, contained the highest level of selenium at 45%. The desired level of selenium in the diet was set at 1.2 mg/kg. This was based on the research of Payne *et al.* (2005) that showed the level of selenium present in the eggs laid by hens supplemented with selenium at levels of at least 0.6 mg/kg reached their peak after 20 days. Because the adaptation feed already contained about 0.6 mg/kg selenium from the basal ingredients, the addition of 0.6 mg/kg of

supplemented selenium would set the expected selenium content for each experimental diet at 1.2 mg/kg.

In order to accomplish the desired inclusion level of selenium, the percentage of selenium in each product had to be considered and the correct amount to produce the desired final concentration was calculated from this. The percentage of selenium and final amounts of total product included in the experimental diets are shown in Table 3.3.

Table 3.3 Inclusion level of the selenium-containing products into the experimental diets based on the percentage of active selenium contained within each product

Selenium source	Percentage of active selenium in product	Product inclusion in feed (g/kg)
Seleno-yeast	0.20	0.300
Hydroxymethylselenobutanate	2.00	0.030
Selenium proteinate	1.10	0.054
Sodium selenite	45.0	0.0013

For the experimental diets, 140 kg of each treatment was needed for the 21-day experimental period. Due to size limitations of the smaller feed mixer, each treatment was mixed in two batches of 70 kg each and these two batches were then mixed together before being bagged and sealed. The experimental diets differed only in the selenium inclusion, which was mixed with fine maize meal as a carrier. The phytase was also included in the maize-selenium mixture, as the quantity was very small and the maize meal served as a sufficient carrier for the phytase. The selenium-phytase mixture was prepared in a laboratory one day prior to the feed mixing to allow for precise measurement and inclusion. These mixtures were formulated using the specifications in Table 3.4.

Table 3.4 Phytase and selenium inclusion to the maize meal carrier for inclusion into each of the dietary treatments

Ingredient (g)	Seleno-yeast	Hydroxymethyl-selenobutanate	Selenium proteinate	Sodium selenite
Phytase	4.2	4.2	4.2	4.2
Selenium source	21	2.1	3.8	0.09
Maize meal	250	250	250	250

Experimental diets were formulated according to the ingredients listed in Table 3.1 with the maize-selenium mixture added to this, using Spesfeed software to verify the formulation. The

ingredients were systematically weighed out and added to a small 40 kg concrete mixer, with each ingredient being checked off as it was added to ensure no ingredient was left out and all diets differed only in their selenium source. The ingredients were mixed for three and a half minutes on each side, for a total of seven minutes of mixing per batch. The feed was then spread out and five samples were taken from different locations to create a composite sample for analysis.

The feed samples collected prior to bagging were analysed to determine the selenium content for each experimental treatment. Selenium was determined using the adapted ICP-MS and microwave digestion technique explained in section 3.6. Table 3.5 contains the actual selenium content of each experimental diet as it was fed to the hens.

Table 3.5 Formulated and analysed inclusion level of selenium in each experimental diet

Selenium source	Formulated inclusion level (mg/kg)	Analysed inclusion level (mg/kg)
Seleno-yeast	1.2	0.91
Hydroxymethylselenobutanate	1.2	1.2
Selenium proteinate	1.2	1.3
Sodium selenite	1.2	1.3

The formulated inclusion level of selenium was realised in three of the four experimental treatments. Seleno-yeast is known to have a highly variable Se-Met content, with each batch containing anywhere between 60 and 80% (Delezie *et al.*, 2014). This means that accurate formulation of selenium content when using seleno-yeast is very difficult and may explain the reason for the slightly lower selenium value analysed in the seleno-yeast treatment compared to the other treatments.

3.3 Bird feeding

The cages were randomly assigned to a dietary treatment, following a complete randomised block design in the house and experimental diets were fed twice daily to minimise spillage and wastage. 120 g of feed was weighed out each morning, using an ADAM PGW 1502e precision balance, correct to one decimal point, into small plastic containers. In addition to general spillage that occurs as the bird pecks at their feed, hens are known to play with their feed when they are bored and this could lead to excessive spillage on the floor. In order to minimise this, half the ration was fed at 9 am, while the second half was fed at 3 pm. Between feedings, the containers were sealed with a lid and each container was clearly marked with the cage number and feed treatment. Each cage was clearly labelled with the treatment each bird was assigned to, with a bright and

contrasting coloured label, different for each of the five treatments. This allowed for easy identification of which feed was to be fed to each bird.

3.4 Egg collection

Eggs were collected once daily at 8 am and the number of eggs laid per treatment was recorded. Eggs were collected at the same time each day to ensure a 24-hour period between recordings, as not every bird laid at a consistent time each day. Egg production was calculated on a weekly basis for the 3-week duration of the trial. Daily egg production percentage was calculated using the number of eggs collected per treatment on that day (the 24-hour period) divided by the maximum possible number of eggs to be laid that day. In this experiment, it was expected that each hen would lay one egg per day, making the maximum possible number of eggs laid per treatment per day 40 eggs. The weekly egg production percentage was calculated as an average of the seven days production percentage. Once a week, on day 0, 6, 13 and 20, eggs were weighed individually and broken open to measure yolk, albumen and shell weight. Forty eggs were randomly selected on day 0, before feeding of the experimental diets commenced, to provide baseline values.

During the third week of the trial, eggs from day 18 and 19 were collected and sorted into eight groups in order to test the effect of selenium source on the quality of eggs during storage at both room temperature of 25°C and in a fridge at 15°C. The breakout occurred on day 0, day 7, day 14, day 21 and day 28 post-collection with 10 eggs from each treatment being assigned to each day, five stored at room temperature and five stored in a fridge.

On the final day of the trial, one egg from each hen was collected to be analysed for selenium content.

3.5 Preparation of eggs for selenium analysis

After collection, each egg was weighed to determine the whole egg weight. The egg was then broken open and the liquid contents of the egg weighed and recorded. All weights were determined using the ADAM PGW 1502e precision balance, calibrated beforehand to ensure accurate weight recordings. The liquid content of each egg was homogenised for 30 seconds using a hand-held blender. The homogenised samples were sealed in their containers and frozen for 2 days at -20°C. The liquid egg content was then transferred to a Specht Scientific TD 05/10 freeze-drier and left for 7 days to remove all moisture before being weighed to obtain an initial dry weight. The freeze-dried sample was then ground to a fine powder using a glass rod, which was cleaned and dried between each sample to prevent cross-contamination of samples.

3.6 Whole egg selenium analysis

The selenium content of the homogenised liquid egg samples was determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) at the ChemNutri Analytics laboratory in Olifantsfontein, South Africa, using a method adapted from Matek and Blanuša (1998). The homogenised samples were labelled using a heat-resistant marker to identify each individual sample during the microwave digestion process. Two grams of each sample was weighed out into the digestion vessel and 3 mL of HNO_3 added to each of these vessels. The vessels were then sealed and placed into the microwave carousel. The temperature was gradually increased to 180°C over 30 minutes, where after this temperature was maintained for a further 15 minutes. The samples were then left to cool to room temperature before being vented and transferred to labelled 50 mL tubes. Each sample was then diluted to a 50 mL volume with high-purity water. The tubes were then capped and shaken to homogenise the mixture.

Calibration standards and blank standards were created for the ICP-MS analysis using 0.1 mL of 2% HNO_3. The blank standard contained only this liquid, while the calibration standards had 0.1 mL of internal standard added. The internal standard used for this analysis was Yttrium (Y) mass 89. The ICP-MS was calibrated to detect counts per second of isotope ^{78}Se, the selenium isotope with the nominal mass of 78, as the unit of measurement for this analysis.

5 mL of each of the digested samples and prepared blanks were measured out and 0.05 mL of Y internal standard was added and mixed to homogenise.

The ICP-MS cones were conditioned using the prepared blanks for a 20-minute run. The calibration of the machine was then set using the prepared Y internal standards, with the lowest concentration standard first and then increasing in concentration. The lower limit of quantification for this analysis was 0.096 ppm Se (0.096 µg Se/g).

The mixture analysed in the ICP-MS machine consisted of 2.5 mL of sample, 2.5 mL of 2% HNO_3 and 0.05 mL of Y internal standard, which were all mixed prior to insertion into the ICP-MS tray. A total of 40 samples were analysed in each run of the machine. The results obtained from the machine were compared to the calibration curve using the equation:

$y = mx + b$

Where: y = net intensity signal (counts per second of ^{78}Se)

x = selenium concentration (µg Se/g)

m = the calculated slope

b = the calculated intercept

3.7 Egg storage and breakout

Eggs designated for breakout were assigned to two blocks. Block 1 consisted of eggs from day 18 of the trial and block 2 consisted of eggs collected on day 19. The two blocks were cracked one day apart, ensuring the number of storage days remained constant over both blocks. Eggs were cracked on day 0, 7, 14, 21 and 28 post-storage to determine the effect of selenium on the shelf life of eggs.

Ten eggs per treatment were opened each day. Five eggs per treatment were stored at room temperature, with an average temperature of 25.2°C across the 28 days and five eggs per treatment were stored in a fridge, at an average temperature of 14.9°C across the 28-day period.

3.7.1 Egg weight and pH

Each egg was weighed on the day it was collected, as well as day of breakout in order to determine weight lost during storage, using a ME4002 balance from Mettler-Toledo. The pH of the albumen and yolk was recorded for each egg, using the HI98190 Professional Waterproof Portable pH/ORP Meter from Hannah Instruments, which was calibrated using a two-point calibration (pH 4 and pH 7) before each new day of egg breakout.

3.7.2 Haugh units

Each egg was weighed prior to breakout and recorded as the observed weight of the egg. The Haugh Units (HU) were calculated for each egg, using the method described by Silversides & Villeneuve (1994) where each egg was cracked open on a smooth, flat tabletop surface and the albumen height was measured at three locations around the yolk, all 1 cm away from the yolk edge, using an albumen height gauge produced and supplied by Johnson Collab. The albumen meter was calibrated to read the surface of the table as 0 mm. The three albumen height measurements were pooled to create one average albumen height per egg. The HU, which was developed in 1937 by Haugh, is a measure of egg protein quality that is widely used throughout the poultry industry. This measurement considers the height of the albumen, where a higher albumen height indicates better egg quality, and corrects for the weight of the egg. The following formula was used to calculate the HU of each egg sampled (Silversides & Villeneuve, 1994):

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

H = observed height of the albumen in millimetres (mm)

w = observed weight of the egg in grams (g)

3.9 Statistical analysis

To test for significance (P <0.05) in body weight of hens between treatments, the general linear model (GLM) function within Statistical Analysis Systems (SAS, 2019) was used. Least square means were used to test for any significant (P <0.05) difference between blocks or between hens belonging to the sub-block, height (top, middle or bottom).

The data collected from the whole egg selenium analysis was analysed using GLM (SAS, 2019) and the least square means was used to test for the effect of treatment on selenium concentration. The dependent variable tested was the selenium concentration (mg/kg) in the egg. Significance was tested at a level of P <0.05. Significance was tested between blocks within treatments and between treatments.

For the repeated measurements taken on egg breakout data, least square means in the MIXED model were used to test for significance (P <0.05) between the estimate values of the effect, which in this study was the interaction of treatment, temperature and day. The Mixed procedure allows for statistical inferences to be made about data using fitted models created from a variety of mixed linear models. Mixed linear models differ from GLM in that the data is allowed to show correlation and non-constant variability because these non-constants are accounted for in the analysis. (Littell *et al.,* 1996). This gives more flexibility within the model because the analysis is not limited to only the means of the data, but also the variances and covariances. It also accounts for any unbalance in the data that could arise from the unequal number of eggs within each block that originated from either top, middle or bottom cages. The dependent variable considered were egg weight, albumen height, albumen pH, yolk pH and Haugh Units.

CHAPTER 4: RESULTS

4.1 Average hen weight and feed and selenium intake

Hens were weighed on arrival and randomly assigned to treatments and blocks. Each of the four blocks had 10 hens from each treatment for a total of 40 hens per treatment across the whole experiment. An average bird weight was calculated for each treatment and is shown in Table 4.1. The body weights of the birds used in this trial ranged from 1.59 kg and 2.60 kg. All treatments had an average hen weight close to 2 kg with selenium proteinate having the highest average weight numerically (2.066 kg), followed by seleno-yeast (2.045 kg), sodium selenite (2.011 kg) and then HMSeBA with the lowest average weight numerically (1.998 kg). However, there was no significant (P >0.05) difference in body weight between any of the treatments, nor between any of the blocks within each treatment.

Table 4.1 Average hen weight (kg ± SD) per treatment at the start of the trial

Treatment	
Seleno-yeast	2.045 ± 0.178
Hydroxymethylselenobutanate	1.998 ± 0.162
Selenium proteinate	2.066 ± 0.197
Sodium selenite	2.011 ± 0.168

Values did not differ significantly at P< 0.05

The daily feed intake of the hens was recorded, and each hen ate all of the 160 g supplied throughout the adaptation and experimental periods. Any wastage or leftover feed each day was negligible. The selenium intake for the adaptation and experimental periods was calculated using the analysed selenium content of each diet and is shown in Table 4.2.

Table 4.2 Selenium intake (mg/day) per hen from each treatment during the adaptation period and the experimental period

Treatment	**Adaptation period**	**Experimental period**
Seleno-yeast	0.096	0.146
Hydroxymethylselenobutanate	0.096	0.192
Selenium proteinate	0.096	0.208
Sodium selenite	0.096	0.208

Values did not differ significantly at P< 0.05

4.2 Egg production

Daily egg production was monitored and recorded for each treatment. Table 4.3 shows the number of eggs collected for each treatment per day for 20 days, beginning the morning after the experimental feed was first given (denoted as day 1 in Table 4.3) and ending on the final day of trial, 21 days after the experimental feed was first given. Weekly egg production percentages are shown in Table 4.4. There was no significant (P >0.05) difference in egg production between any of the treatments.

Table 4.3 Effect of selenium source on number of eggs produced per day for each treatment over a 21-day period during the experimental period

Day	Seleno-yeast	Hydroxymethyl-selenobutanate	Selenium proteinate	Sodium selenite
1	23	31	22	23
2	31	24	26	32
3	44	40	41	41
4	38	43	42	40
5	41	47	40	38
6	35	30	37	37
7	40	42	44	43
8	38	39	37	39
9	44	44	39	41
10	33	32	34	31
11	33	35	36	37
12	34	34	33	40
13	41	41	44	41
14	43	41	43	41
15	30	32	30	37
16	36	36	35	39
17	46	50	44	44
18	30	30	30	32
19	42	45	42	48
20	43	45	44	42
21	44	43	41	42

Values did not differ significantly at P< 0.05

Large variations in daily egg production were due to egg formation taking either slighter longer or slightly less than 24 hours within a hen. This meant that some days egg numbers were very low as hens took longer than 24 hours to lay their egg and was then followed by a day with much higher egg numbers.

Table 4.4 Effect of selenium source on weekly egg production percentage over a 3-week period during the experimental period

Week	Seleno-yeast	Hydroxymethyl-selenobutanate	Selenium proteinate	Sodium selenite
1	90.00	91.79	90.00	90.71
2	95.00	95.00	95.00	96.43
3	96.79	100.36	95.00	101.43

Values did not differ significantly at $P< 0.05$

4.3 Weight of egg components

Table 4.5 shows the average weight of each egg component (yolk, albumen and shell) across all the treatments, expressed as a percentage of the whole egg weight. On day zero, the eggs collected all weighed between 49.39 g and 62.93 g. On day 7, all the eggs collected weighed between 43.90 g and 66.87 g. On day 14, all eggs collected weighed between 47.12 g and 71.66 g. On day 21, all eggs collected weighed between 48.73 g and 67.99 g. Due to the variation in body weight of the birds, average egg weight between treatments was not considered, but rather the individual components' weight, expressed as percentage of whole egg weight. There was no significant ($P >0.05$) difference observed between component weight percentages across any of the treatments.

Table 4.5 Effect of selenium source on weight of components of egg expressed as a percentage of whole wet egg weight measured weekly over a 21-day period during the experimental period

Day	Treatment	Shell	Albumen	Yolk
0	Baseline	13.83	58.84	27.32
7	Seleno-yeast	12.91	59.34	27.75
	Hydroxymethylselenobutanate	13.40	58.34	28.25
	Selenium proteinate	13.43	58.85	27.73
	Sodium selenite	14.00	58.30	27.70
14	Seleno-yeast	13.15	58.92	27.94
	Hydroxymethylselenobutanate	12.89	59.50	27.60
	Selenium proteinate	12.80	58.95	28.25
	Sodium selenite	12.69	59.18	28.13
21	Seleno-yeast	13.59	57.51	28.89
	Hydroxymethylselenobutanate	13.47	57.76	28.77
	Selenium proteinate	13.86	56.77	29.37
	Sodium selenite	13.70	57.08	29.22

Values did not differ significantly at $P< 0.05$

4.4 Selenium content in eggs

Average selenium content, in mg/kg (equivalent to ppm) in the eggs was calculated per treatment and is shown in Table 4.6. Sodium selenite resulted in the lowest mean selenium content, 0.56 mg/kg. Sodium selenite did not differ significantly (P >0.05) from selenium proteinate, which resulted in a mean value of 0.60 mg/kg, but did differ significantly (P <0.05) from all other treatments. The second highest mean value was observed in the seleno-yeast group, at 0.63 mg/kg and this did not differ significantly (P >0.05) from selenium proteinate, but did differ significantly (P <0.05) from all other treatments. The only treatment to differ significantly (P <0.05) from all other treatment was HMSeBA, which had the highest mean selenium content of 0.70 mg/kg.

Table 4.6 Effect of selenium source on the mean selenium concentration (mg/kg ± standard deviation) in the whole egg reported on a dry matter basis

Treatment	Selenium content in eggs (mg/kg)
Seleno-yeast	0.629[b]
Hydroxymethylselenobutanate	0.699[a]
Selenium proteinate	0.601[bc]
Sodium selenite	0.564[c]

[a-c] Values without common superscripts are significantly different (P <0.05)

4.5 Egg quality characteristics

4.5.1 Albumen height

The mean values for albumen height per treatment on each day are shown in Table 4.7. The data is separated by storage temperature for comparison between eggs stored at fridge temperature (15°C) and those stored at room temperature (25°C). There was a significant (P <0.05) difference in albumen height of eggs between the two temperatures across all treatments for the mean value of each day, between day 7 and day 28. No treatments differed significantly (P <0.05) from each other on day 0. From day 7 to 28, there was a significant (P <0.05) difference across all treatments between fridge and room temperature eggs for the same day. The eggs stored at room temperature had a significantly (P <0.05) lower albumen height than eggs stored in the fridge for the same day for all days except day 0. There was no significant (P >0.05) difference in albumen height observed between any treatments over any of the days.

Table 4.7 Effect of selenium source on albumen height of eggs stored at 15°C and 25°C recorded every seven days over a 21-day period

	Fridge (15 °C)					Room (25 °C)				
Treatment	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Seleno-yeast	8.55	7.21[1]	6.66[1]	6.09[1]	5.71[1]	8.60	5.54[2]	5.04[2]	4.98[2]	4.75[2]
HMSeBA	8.52	7.69[1]	7.02[1]	6.65[1]	5.60[1]	7.96	5.55[2]	4.59[2]	4.88[2]	4.55[2]
Selenium proteinate	8.24	7.02[1]	6.57[1]	6.57[1]	6.00[1]	8.27	5.70[2]	4.86[2]	4.97[2]	4.68[2]
Sodium selenite	8.12	7.57[1]	6.85[1]	6.50[1]	5.68[1]	8.50	5.74[2]	4.85[2]	4.93[2]	4.70[2]
x̄	8.35	7.35[1]	6.72[1]	6.41[1]	5.71[1]	8.59	5.70[2]	4.86[2]	4.91[2]	4.70[2]
SEM (±)	0.1350	0.1350	0.1350	0.1349	0.1349	0.1350	0.1350	0.1350	0.1349	0.1349

[1,2] Values within rows with differing numbers on the same day at different temperatures are significantly different (P <0.05)

HMSeBA: Hydroxymethyl-selenobutanate

SEM: Standard Error of Means

4.5.2 Albumen pH

Table 4.8 shows the average albumen pH values measured for each treatment every seven days for eggs stored either at room temperature or in a fridge. There were no significant (P >0.05) differences to albumen pH noted between the two storage temperatures on any of the days. The average values for eggs stored at room temperature on day 14 were significantly (P <0.05) lower for selenium proteinate than any of the other treatments. This significance did not persist after day 14, nor was it present in any day prior.

Table 4.8 Effect of selenium source on the albumen pH of eggs stored either in a fridge at 15°C or at a room temperature of 25°C measured every seven days for a period of four weeks

	Fridge (15 °C)					Room (25 °C)				
Treatment	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Seleno-yeast	7.66	9.02	9.38	9.50	9.51	7.75	9.18	9.55[a]	9.52	9.50
Hydroxy-methylseleno-butanate	7.67	8.90	9.46	9.52	9.53	7.96	9.21	9.54[a]	9.60	9.52
Selenium proteinate	7.82	8.94	9.48	9.56	9.56	7.96	9.21	8.68[b]	9.60	9.54
Sodium selenite	7.89	9.02	9.42	9.58	9.57	8.07	9.16	9.62[a]	9.60	9.56
x̄	7.80	8.98	9.42	9.51	9.51	7.88	9.19	9.37	9.54	9.48
SEM (±)	0.687	0.687	0.687	0.686	0.686	0.687	0.687	0.687	0.686	0.686

[a,b] Values within columns without common superscripts are significantly different (P <0.05)

SEM: Standard Error of Means

4.5.3 Yolk pH

Table 4.9 shows the average yolk pH values measured for each treatment every seven days for eggs stored either at room temperature at 25°C or in a fridge at 15°C. The eggs stored at room temperature showed significantly (*P* <0.05) higher yolk pH values than those stored in a fridge on day 21 and day 28. There were no significant differences (*P* >0.05) in yolk pH between any of the treatments at either room or fridge temperature.

Table 4.9 Effect of selenium source on yolk pH of eggs stored either in a fridge at 15°C or at a room temperature of 25°C measured every seven days for a period of four weeks

	Fridge (15 °C)					Room (25 °C)				
Treatment	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Seleno-yeast	6.24	6.18	6.39	6.26	6.07[1]	6.24	6.15	6.42	6.35	6.47[2]
HMSeBA	6.11[1]	6.24	6.31	6.25	6.17[1]	6.33[2]	6.18	6.44	6.41	6.58[2]
Selenium proteinate	6.31	6.17	6.32	6.17	6.23	6.24	6.25	6.37	6.50	6.67
Sodium selenite	6.20	6.19	6.32	6.23	6.18[1]	6.19	6.18	6.41	6.39	6.67[2]
x̄	6.31	6.19	6.33	6.23[1]	6.16[1]	6.31	6.21	6.43	6.40[2]	6.58[2]
SEM (±)	0.3013	0.3013	0.3013	0.3011	0.3011	0.3013	0.3013	0.3013	0.3011	0.3011

[1,2] Values within rows with differing numbers on the same day at different temperatures are significantly different (*P* <0.05)

HMSeBA: Hydroxymethyl-selenobutanate

SEM: Standard Error of Means

4.5.4 Haugh Units

Table 4.10 shows the average Haugh Units calculated every seven days for eggs from each treatment stored either at room temperature or in a fridge. The eggs stored at room temperature showed a significantly (P <0.05) lower Haugh Unit score than eggs stored in a fridge from day 7 to day 28. Eggs stored at room temperature from HMSeBA and selenium proteinate treatment groups had significantly (P <0.05) lower Haugh Unit scores than any other treatment on day 0 only.

Table 4.10 Effect of selenium source on Haugh Unit measurement of eggs stored either in a fridge at 15°C or at a room temperature of 25°C measured every seven days for a period of four weeks

	Fridge (15 °C)					Room (25 °C)				
Treatment	**0**	**7**	**14**	**21**	**28**	**0**	**7**	**14**	**21**	**28**
Seleno-yeast	93.07	86.29[1]	82.51[1]	79.90[1]	77.54[1]	92.74[a]	74.13[2]	70.27[2]	71.07[2]	68.66[2]
Hydroxy-methylseleno-butanate	92.93	88.95[1]	84.33[1]	82.59[1]	75.74[1]	90.15[b]	75.03[2]	66.11[2]	70.15[2]	67.57[2]
Selenium proteinate	91.33	83.82[1]	80.72[1]	81.67[1]	78.55[1]	90.92[b]	76.36[2]	69.59[2]	71.06[2]	68.86[2]
Sodium selenite	90.66	87.67[1]	82.87[1]	81.14[1]	76.28[1]	92.57[a]	75.52[2]	70.29[2]	71.29[2]	69.72[2]
x̄	92.04	86.70[1]	82.42[1]	81.01[1]	76.78[1]	92.83	75.83[2]	69.41[2]	70.78[2]	69.06[2]
SEM (±)	0.861	0.861	0.861	0.860	0.860	0.861	0.861	0.861	0.860	0.860

[1,2] Values within rows with differing numbers on the same day at different temperatures are significantly different (P <0.05)

[a.b] Values within columns without common superscripts are significantly different (P <0.05)

SEM: Standard Error of Means

CHAPTER 5: DISCUSSION

5.1 Hen weight

The average hen weight was not significantly different between any of the treatments, indicating that hen weight should not play a significant role in the size of the egg. However, the smallest hen in this study weighed 1.59 kg and the largest hen weighed 2.60 kg. This means that even though the average weight for each treatment was not significant, there were some birds above or below the mean value. The body weights of the birds show a normal distribution. Silversides and Budgell (2004) noted that there was a relationship between egg size and the age of the hen. Older hens laid larger eggs, but all the hens in the present study were the same age and so this was not a factor contributing to any differences noted in the egg sizes. The hens were also all of the same breed and therefore breed differences were not a factor contributing to differences in egg size.

It was noted by Bennon and Price (1940) that there was a correlation between hen body size and egg size within a breed and thus larger hens were able to lay larger eggs with a higher weight. Rahn *et al.* (1975) developed an equation to demonstrate this relationship. Their experiment analysed over 800 eggs from a variety of poultry strains and the results concluded that equation (1) is a satisfactory allometric equation to describe the increase of egg weight as the weight of the hen increases. The power function b is equal to 0.67 and is constant across all breeds and strains, whereas the variable a is dependent on the breed of chicken being considered. This shows that even hens of the same age and breed are capable of laying eggs of differing sizes, depending on their body weight because hens of the same breed will all have a constant value for a and only the body weight will differ between individual hens. Because of this relationship, there was no evidence that any differences noted in the size of the eggs laid by the hens in this experiment were due to the selenium source supplied to them. The duration of the experimental selenium supplementation for this experiment was 21 days and there is no literature to support that this period is long enough to significantly alter the weight of the eggs. It is therefore expected that any difference in egg weight was a function of the hen's body weight.

$$\text{egg weight} = a \cdot (\text{body weight})^{b} \qquad (1)$$

5.2 Egg production

There were no significant (P >0.05) differences in average weekly egg production between treatments. However, a numerical increase was noted over time. This is likely due to the hens

acclimatising to their new environment and feed, as well as experiencing less stress during the daily egg collection process. The hens used in this experiment were 44 weeks old, which is after peak production and so a natural drop in egg production is expected rather than an increase. These findings are in agreement with Jlali *et al.* (2013) who also noted no change in laying rate when different selenium sources were fed. Pavlović *et al.* (2009) noted no change in egg production rate for up to eight weeks after initial supplementation, but began to see an increase in laying percentage after nine weeks. This indicates that longer periods of supplementation may be necessary for any significant increase in egg production to be observed. There are some observations in Table 4.3 of egg numbers greater than 40 collected within a treatment on one day. This could be due to hens laying their eggs later than the usual collection time for that particular day, resulting in her egg counting towards the following day's total. This is supported by lower egg numbers either one day before or one day after days where more than 40 eggs were collected. This means that treatments where more than 40 eggs were collected on one day were due to the timing of the hen's oviposition, rather than any effect of dietary treatment.

5.3 Average weight of egg components

The proportion of egg component weights stabilised in the final week of the experiment, with an increase in yolk and shell percentage across all treatments and a decrease in albumen percentage across all treatments by the end of the third week of collection. Although there was no significant (P >0.05) difference in the percentages of the various egg components between treatments, there were numerical changes. Across all treatments, there was a clear shift in the final week of the experiment. At 21 days, there was an increase in shell weight and yolk weight for all treatments and a decrease in albumen weight across all treatments. This is in contrast to the research of Pappas *et al.* (2005) who noticed a decrease in yolk weight for eggs supplemented with selenium. However, their research focused specifically on the effect of selenium in combination with two oil sources and not the effect of selenium in isolation. Their result could be attributed to the interaction of selenium with the oil source causing the decrease in yolk weight. Zoidis *et al.* (2014) found that selenium was preferentially deposited into the yolk rather than the albumen, due to the mineral-binding lipoproteins present during yolk formation, which could explain the increase in yolk weight over time, as more selenium was able to be deposited after a longer period of supplementation. Across all treatments in the present study, there was also a numerical increase in shell weight over time. This indicates that, regardless of selenium source, there was an increase in the proportional weight of the egg shell. Within treatments, selenium proteinate resulted in the highest proportional weight of egg shell and HMSeBA resulted in the lowest. SY and SS supplemented groups had intermediate values, with SS having slightly higher eggshell percentage than SY. This is in agreement with Gjorgovska *et al.* (2012) and Invernizzi *et al.* (2013) who have

noted that selenium supplementation in hens increased the shell weight of eggs laid. In both of these experiments it was found that the increased weight was due to an increase in the selenium content of the eggshells and a resulting increase in the eggshell breaking strength. Gjorgovska *et al.* (2012) tested one selenium source versus a control with no supplementation and noted that a higher inclusion rate of selenium resulted in a higher eggshell weight. Invernizzi *et al.* (2013) noted that organic selenium sources increased the proportional weight of eggshells more than inorganic sources. The present study considered three different organic sources and was able to rank them based on their ability to increase the eggshell weight. Although differences were not significant, this study showed that after 21 days of supplemental feeding, selenium proteinate, an organic source of selenium, resulted in the highest eggshell percentage and SS, despite being an inorganic source, resulted in a slightly higher eggshell percentage than diets supplemented with HMSeBA and SY.

Ahn *et al*. (1997) noted that egg size did not affect the content of solids in the whole egg. This means that the differing sizes of the eggs broken open and weighed would not affect the proportional weights of each egg component and allowed for a fair comparison between treatments, even if hens laid eggs of differing size and weight.

In order to eliminate bias caused by the differing egg weights as a result of hen body size, each egg component was weighed and converted to a percentage of whole egg weight. This allowed for comparison across all hens, regardless of hen weight and egg size.

5.4 Selenium content of eggs

There were significant (P <0.05) differences noted in the selenium content of eggs between treatments. The HMSeBA treatment showed the highest concentration of selenium in the eggs, which was significantly higher than all other treatments. This is in agreement with the research of Jlali *et al.* (2013) who also observed the highest level of selenium in the egg from hens fed a diet supplemented with HMSeBA. The higher selenium content was likely due to the metabolic pathway of HMSeBA generating fewer intermediate metabolites and thus a more efficient incorporation into the proteins of the egg (Shini *et al.*, 2015). HMSeBA also has more specific protein incorporation than Se-Met and thus does not interfere with methionine pathways. In agreement with the research of Shini *et al.* (2015), HMSeBA was shown to be more efficiently incorporated into SePs and does not interfere with general protein synthesis. The research of Briens *et al.* (2014) highlighted the complete conversion of selenium contained in the HMSeBA molecule to Se-Cys, whereas other sources only have partial conversion and this could contribute to the higher levels of selenium noted in tissues originating from HMSeBA feed sources. At an inclusion rate of 1.2 mg/kg in the feed and an egg content of 0.7 mg/kg, there was a 58% efficiency of transfer between hen and egg.

The SY treatment showed a significantly higher selenium content in the eggs compared to the SS treatment. Although numerically higher, the SY treatment did not result in a statistically significant

increase in egg selenium content compared to the selenium proteinate treatment. This is likely due to SY consisting of varying amounts of selenium and so it is not always known exactly what percentage of Se-Met is contained within the yeast molecules. The actual Se-Met content of a SY source can vary anywhere between 60 and 80% (Delezie *et al*., 2014). The selenium content of the eggs from the selenium proteinate treatment was only numerically higher than the eggs from the SS treatment. This is in agreement with the research of Payne *et al.* (2005), which noted that organic sources of selenium had higher concentrations of selenium deposited into the eggs than inorganic sources. The SS treatment represented the inorganic source in the present stud. This pattern of selenium deposition is supported by numerous studies that also found organic sources of selenium increase egg selenium concentration significantly more than inorganic sources when supplemented at the same level (Payne e*t al.,* 2005; Pan *et al.,* 2007; Invernizzi *et al*., 2013). It is thus widely accepted that organic sources of selenium are more cost-effective as they yield higher egg and tissue concentrations of selenium than the conventional inorganic SS source.

5.5 The influence of different selenium sources on egg quality

5.5.1 Albumen height

Regardless of selenium source or storage temperature, albumen height decreased as storage time increased, with the highest albumen height always observed at day 0 and the lowest albumen height always observed on day 28. However, the rate of albumen height decrease was not linear over time. There was a decrease in the rate at which the albumen height decreased over time. This means that the albumen height decrease decelerated the longer the eggs were stored. The greatest decrease in albumen height occurred between day 0 and day 7 and the smallest decrease occurred between day 21 and day 28 (Table 4.7).

Albumen height is a useful measure of egg quality because it is easily measured and requires very basic equipment. The albumen height, measured in millimetres above a set surface, is a good indicator of freshness of the egg; the higher the albumen, the fresher the egg (Silversides & Budgell, 2004). This is attributed to the components of the ovomucin within the albumen. Albumen height reduces as proteolysis occurs in the ovomucin and the disulphide bonds begin to cleave. These chemical changes begin immediately once the egg has been laid and is evident by the change in albumen height over time observed in all the eggs. The longer an egg is stored, the greater the loss in albumen height. However, storage of eggs in the fridge reduced the rate at which albumen height decreased, indicating that storage of eggs in the fridge is able to preserve the egg quality for a longer period of time, but is not able to prevent it from occurring.

For the eggs stored in the fridge, although not significant (*P* >0.05), eggs from the SY treatment showed the highest numerical average albumen height at day 0. This means that the SY treatment resulted in the highest egg quality immediately post-lay.

Although not significantly (*P* >0.05) different, eggs from the HMSeBA treatment showed the lowest albumen height numerically for eggs stored at room temperature throughout the duration of the trial, despite this treatment having the highest concentration of selenium in the egg contents. This means that there was not a positive correlation between selenium content of the egg and the resulting albumen height of the egg. A difference was expected because Pappas *et al.* (2005) found that selenium supplementation increased albumen height during storage above those measured for eggs from hens not receiving selenium supplementation. Their research also showed that an increased inclusion rate of selenium would yield a further increase in albumen height above treatments with lower inclusion rates. From the present study, it appears there is no effect of selenium source on the albumen height of eggs stored for 28 days post-lay after a supplementation period of 21 days.

Silversides and Budgell (2004) noted in their research that the statistical association between albumen height and albumen pH was only moderate. This suggested that there were factors other than storage time that contributed to the decrease in albumen height over time. Because it was already demonstrated by Silversides and Scott (2001) that albumen pH is almost entirely dependent on storage time, consideration of albumen height as the only factor of egg quality is not reliable. Because of this, albumen pH was also considered when testing for significant effects of selenium source on egg quality.

5.3.2 Albumen pH

Silversides and Budgell (2004) suggested albumen pH as a measure of egg freshness and egg quality. Albumen pH is expected to increase over time and so a lower albumen pH is indicative of a fresher egg and thus an egg of higher quality.

It is noted in Table 4.8 that there is no significant (*P* >0.05) difference in albumen pH between storage temperatures. This means that storage temperature did not have a significant (*P* >0.05) effect on the egg quality over time. However, there is a numerical increase in albumen pH over time, with the lowest pH always observed at day 0. This is in agreement with the research of Scott and Silversides (2000), who also observed an increase in albumen pH as egg storage time increased. They also noted that albumen height and albumen pH had no statistical relationship in fresh eggs, but as storage time increased, the association between these two parameters became stronger. This suggests that albumen pH is a strong indicator of the effects of storage, whereas albumen height measures multiple factors present when the egg is first laid and changes as the storage time increases.

On day 14, there were significant differences observed between treatments in eggs stored at room temperature. The eggs from the selenium proteinate treatment had a significantly lower pH than all the other treatments. This appears to be an anomaly in the data as this significance did not persist after day 14, nor was it present on any day prior to day 14. There is evidence that selenium supplementation lowers albumen pH values over time when compared to eggs from hens not receiving selenium supplementation (Pappas *et al.*, 2005) but the present study shows that selenium source has no significant (P >0.05) effect on albumen pH for eggs stored for 28 days post-lay after a supplementation period of 21 days.

5.3.3 Yolk pH

Like albumen pH, yolk pH increases with storage time and a lower pH is indicative of higher egg quality (Scott & Silversides, 2000). In the current study the selenium proteinate treatment was the only treatment that did not show a significant (P >0.05) difference in yolk pH between eggs stored at 25°C and 15°C on day 28. This suggests that selenium proteinate was able to prevent a significant decrease in yolk pH even in eggs not stored in a fridge and had the highest ability to maintain egg quality over a long period of time. However, there was no significant (P >0.05) difference between any of the treatments at either of the storage temperatures.

There was a (P <0.05) significant difference from day 21 onwards between the average yolk pH of eggs stored at room temperature and eggs stored in the fridge. The eggs stored in the fridge maintained a significantly lower pH from day 21 onwards, indicating that after 3 weeks, the deterioration of egg quality with respect to the yolk pH became significant. While not significant, there was still a numerical difference between the average pH values for eggs stored at room and fridge temperature from day 7 to day 14, with the fridge eggs always maintaining a lower yolk pH than the room temperature eggs.

5.3.4 Haugh Units

Regardless of treatment, eggs stored at room temperature had a significantly lower Haugh Unit (HU) score than eggs stored in a fridge. A lower HU score translates to lower egg quality (Silversides & Budgell, 2004). This shows that storage of eggs in a fridge aids in maintaining egg freshness compared to eggs stored at room temperature. This is expected because HU is calculated using the albumen height and thus is directly related to the albumen height observed. HU will decrease as storage time increases because albumen height decreases as storage time increases (Silversides & Budgell, 2004). The patterns observed in the HU is similar to those observed for albumen height, indicating that the correction for egg weight did not play a large role in changing any significance shown by the data. This is supported by research done by Silversides and Villeneuve

(1994), which showed that egg quality was described equally well by albumen height alone as it was by calculating HU. This called into question the necessity of correcting for egg weight when calculating egg freshness and it was due to this uncertainty that other parameters, such as albumen pH are favoured over albumen height when describing egg freshness. There was no significant (P >0.05) effect of selenium source on the HU value of eggs stored for 28 days post-lay after 21 days of supplemental feeding.

Chapter 6: Conclusions

There was no significant change in egg production across any of the treatments, nor any significant change in the proportion of yolk, albumen or eggshell weights. There were numerical changes across all treatments that suggested that selenium supplementation increased yolk and shell proportions, while it decreased the albumen proportion. Selenium source did not affect egg production percentage. The concentration of selenium deposited in the egg was significantly affected by treatment, with HMSeBA showing the highest concentration above all other treatments. The SS treatment was least effective at depositing selenium into the eggs. This showed that organic sources of selenium were more bioavailable to the hen than the inorganic source when supplemented at the same level.

Selenium source had no effect on the albumen height of the eggs stored up to 28 days post-lay. Storage temperature did have an effect, as eggs stored in the fridge had significantly higher albumen heights, indicating better egg quality, from day 7 to day 28 of storage. The reduction of albumen height was slower for eggs stored in the fridge compared to eggs stored at room temperature. This same pattern was observed in the HU of the eggs, indicating that egg weight and size did not play a role in egg quality maintenance and that albumen height was as reliable at indicating egg quality over time as the HU calculation for at least 28 days post-lay. Neither selenium source nor storage temperature had an effect on the albumen pH of the eggs over any of the days during storage. Selenium proteinate was able to prevent a significant difference in yolk pH between eggs stored at 25°C and 15°C after 28 days compared to other selenium sources.

CRITICAL EVALUATION AND RECOMMENDATIONS

Few significant effects of storage conditions on egg quality were observed. This could be due to the experimental period of three weeks and although this was long enough to observe a significant different in selenium egg deposition, a longer period may be necessary before any change in egg quality is observed.

In order to more accurately compare the egg quality parameters, it would be advised that eggs used as replicates be collected from the same hen over time. This would require eggs collected every seven days to be kept and stored at their assigned temperature and on the 28th day all eggs are broken open and analysed. This would also reduce any variation caused by sampling conditions that may have varied across the different days and eliminate the hen as a factor causing variation. In order for this to be accurate, the selenium content of the eggs would have to be tested and recorded in order to identify a time when the selenium content reached a plateau and was no longer increasing. This is because the eggs would not all be collected on the same day and thus those collected on the 28th day would mean the birds had been receiving the experimental feed for a longer period of time. Unfortunately, due to time and budget constraints this approach was not a viable option during this trial.

The levels and activity of GSH-Px should have been measured in the eggs, particularly in the yolk and albumen separately, as this enzyme is a direct indication of the antioxidant properties of selenium. There were no labs in South Africa that were able to assist with this particular research project. However, there are commercial GSH-Px testing kits available, but these require a lengthy pre-order time and the egg tissue requires special preparation for the analysis to be successful. It was due to these constraints that this test was not conducted in the present experiment.

There is potential for future research on the effect of selenium source on the performance of breeder hen progeny. As shown in this research, there are significant differences in the amount of selenium deposited into the egg as a result of the selenium source and it would be beneficial to expand this research to the next generation to explore the possibility of vertical transfer to the supplemented hen’s progeny. In addition to chick performance, there could be benefits to egg hatchability and chick survivability.

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