Production performance and meat quality of Ross 308 broilers as influenced by the source and duration of dietary zinc and selenium supplementation

By

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Declaration

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

Signature: ……..

Date: ……...

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“Every minute, every moment. Where I've been and where I'm going. Even when I didn't know it or couldn't see it. There was Jesus.” – Zach Williams.

Abstract

Broiler production has become one of the most highly productive segments in the food production sector owed to the rapid change in consumer demand for affordable protein sources. The use of organic trace minerals has taken preference over the use of conventional inorganic sources, not only due to a higher efficacy and retention in the animal body, but also because of lower quantities needed in comparison to inorganic counterparts. However, due to the relative expense of organic trace minerals, higher inclusions of inorganic products are considered to be more cost-effective. The objective ofthis study was to investigate growth performance, carcass and breast meat quality characteristics of broilers fed inorganic or organic sources of zinc (Zn; $ZnSO_4$ or Availa®Zn) and selenium (Se; sodium selenite (SS) or Availa®Se) relative to the duration of supplementation (0–32 days of age or 21–32 days of age) to construct a 4 x 2 factorial design. A three-phase feeding programme was implemented during the course of the trial; starter (0–10 d), grower (10–21 d) and finisher (21–32 d). A total of 1 920 one-day-old male Ross 308 broilers were randomly allocated to 1 of 8 dietary treatments (T). The control diet (T1) was formulated using 100 mg/kg Zn as $ZnSO_4$ and 0.30 mg/kg Se as inorganic SS. The level of mineral supplementation was constant throughout the experimental treatments. Organic minerals were supplemented on an *iso* basis in T2, T3, and T4. Treatments 6, 7 and 8 contained 100% inorganic sources during the starter (0–10 d) and grower (10–21 d) period, whereby organic sources partially replaced inorganic sources during the finisher period (21–32 d). The treatments were as follows: T1 (100% $ZnSO_4$, 100% SS; 0–32 d); T2 (40% Availa®Zn, 100% SS; 0–32 d); T3 (100% $ZnSO_4$, 50% Availa®Se; 0–32 d); T4 (40% Availa®Zn, 50% Availa®Se; 0–32 d); T5 (100% $ZnSO_4$, 100% SS; 21–32 d); T6 (40% Availa®Zn, 100% SS; 21–32 d); T7 (100% $ZnSO_4$, 50% Availa®Se; 21–32 d); T8 (40% Availa®Zn, 50% Availa®Se; 21–32 d), with 12 replicates each. Growth performance was evaluated at 7, 10, 14, 21, 28 and 32 days of age. Carcass and meat quality characteristics were evaluated after slaughter on day 32 and 34. Assessing the growth performance; body weight (BW), body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) over the total feeding period, none of the treatments performed different from the control (P>0.05). Mineral source and duration of supplementation influenced 7-day body weight (P<0.10). The broilers utilised their feed more efficiently (P<0.05) when fed inorganic minerals in the starter phase (T1 and T6). However, broilers obtained better (P<0.05) feed efficiency results when inorganic sources were partially replaced with organic sources during the finisher phase (T5 and T7). Similarly, the partial supplementation of organic Zn and Se improved (P<0.05) FCR during the last week of the study compared to birds receiving organic Zn and Se from the start of the trial. Mineral source was shown to have an effect on FCR during the starter and grower phases (P<0.05). No treatment differences were observed on carcass characteristics

measured in the study (P>0.05), although mineral source showed to yield heavier carcasses (P<0.10) when partially supplemented in organic form. The duration of supplementation showed to improv hot-carcass weight (P<0.05) when organic minerals were supplemented during the finisher phase on an *iso* basis. Breast meat pH, colour, drip and cook loss were comparable to the control group post-slaughter. Organic Zn supplementation from the start (T2) of the trial lowered the ultimate meat pH (P<0.10), whereby the combination of organic Zn and Se increased the ultimate meat pH (P<0.05). The duration of supplementation showed to be promising when organic minerals partially substituted inorganic sources in the finisher phase, indicating the potential to produce chicken meat on a least-cost basis while improving feed efficiency in later stages. Overall, birds fed diets partially supplemented with more bio-available sources of organic Zn and Se showed better results.

Keywords: Zinc, selenium, trace minerals, broiler production, meat quality, carcass characteristics, drip loss, cook loss.

List of abbreviations

%	percentage
°C	degrees Celsius
ANOVA	Analysis of Variance
BW	body weight
BWG	body weight gain
Ca	calcium
CCW	cold carcass weight
CP	crude protein
d	day
DM	dry matter
EE	ether extract
FCR	feed conversion ratio
FI	feed intake
g	gram
h	hour
HCW	hot carcass weight
kg	kilogram
LWB	live body weight
mg/kg	milligram per kilogram
NRC	National Research Council
p. major	*pectoralis* major
p. minor	*pectoralis* minor
Se	selenium
SS	sodium selenite
SY	selenium yeast
t	ton
WHC	water-holding capacity
Zn	zinc
ZnAA	zinc amino acid
ZnO	zinc oxide
$ZnSO_4$	zinc sulphate

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Chapter 1
Introduction

During the last century, discoveries and innovation in the field of meat science has led to ground breaking changes in meat and poultry production (Beerman, 2009). Selecting for superior broiler production characteristics while maximising production profitability has led to remarkable success in improving economically important traits. Although, increasing growth velocity and muscle mass in meat-type chickens could have resulted in product quality trade-off (Webb & Casey, 2010). Meat is considered an essential raw material for further processing (Baker & Bruce,1995).

In the past, broiler production focused on the carcass grading system as opposed to implementing carcass quality evaluations. At present, broiler meat production focuses on the sensory attributes of raw materials which are important for secondary processing. Producers, retailers, and consumers have different perceptions on the subject of meat quality. Ensuring a final product of exceptional quality and profitability has given rise to further processing industries, much-driven by consumer patterns. The proportion of prime carcass cuts has become the focus to support such modernised patterns.

Genetic variation among meat-producing birds has resulted in both fast- and slow-growing broilers. Over the past few years, meat quality expectations have substantially increased due to upward trends of further processed market forms (Fletcher, 2002). Water-holding capacity (WHC) is a factor of significant concern to the industry, as substantial weight loss from broiler carcasses and portioned cuts post-slaughter affect the quality and yield of processed products (Pearce *et al.*, 2011). Raw meat with a low WHC limits the processing of products due to a reduced yield (Otto *et al.*, 2004), as the loss of muscle water can affect the overall eating quality, resulting in cooked meat with tough eating characteristics (Morrissey & Kerry, 2004).

In recent years, organic trace mineral utilisation in animal nutrition has become increasingly widespread due to higher bioavailability, which has resulted in prolonged tissue retention (Oliveira *et al.*, 2014; Lopes *et al.*, 2017; Sałek *et al.*, 2020), lower toxicity, and antioxidant properties (Ibrahim *et al.*, 2019). Organic microminerals are complexed with organic ligands, typically being an amino acid, peptide, or protein, that assist elements in entering the digestive tract without interacting with other dietary components (Star *et al.*, 2012). Since organic minerals are absorbed and retained more easily in the bird's body, they are utilised more effectively, have a lower excretion rate, and can reduce the aspect of environmental waste (Saenmahayak *et al.*, 2012).

Numerous authors have reported improved meat quality, with emphasis placed on drip and cook loss, when broilers were fed either organic Zn- or Se-enhanced diets (Downs *et al.*, 2000a; Liu *et al.*, 2011; Oliveira *et al.*, 2014; Bakhshalinejad *et al.*, 2019; Ibrahim *et al.*, 2019; De Grande *et al.*, 2019, 2020). However, chelated trace minerals are typically more expensive than inorganic trace mineral sources while higher inclusions of inorganic products are considered to be more cost-effective. Nonetheless, ultimately organic microminerals may be more beneficial when included in poultry diets.

Aim and objectives

The aim of the study was to evaluate different Zn and Se sources and the duration of supplementation as a possible strategy for improving performance and meat quality in broilers. The objective of this study was to investigate overall broiler growth performance, carcass and breast meat quality characteristics of broilers fed inorganic or organic sources of Zn and Se, supplemented over two durations of time.

Hypotheses

H0: Zinc and selenium mineral source will have no significant effect on broiler performance, carcass and meat quality parameters.

H1: Zinc and selenium mineral source will have a significant effect on broiler performance, carcass and meat quality parameters.

H0: Duration of organic trace mineral supplementation (0–32 days of age vs 21–32 days of age) will have no significant effect on broiler performance, carcass and meat quality parameters.

H1: Duration of organic trace mineral supplementation (0–32 days of age vs 21–32 days of age) will have a significant effect on broiler performance, carcass and meat quality parameters.

Chapter 2
Literature review

2.1 Broiler production, consumption and consumer trends in South Africa

It is no new phenomenon that broiler production has intensified in recent decades, solely to meet the demands of an ever-growing global population (Mottet & Tempio, 2017). Production costs of poultry meat in comparison to other animal proteins are much lower due to a shorter production cycle and an accelerated growth rate. At present, more emphasis is being placed on sensory features since the modern-day broiler has reached a level of mass production in which characteristics such as appearance, texture, juiciness, wateriness, firmness, and tenderness could be considered as modern meat features, which were not taken into consideration in the past (Mir *et al.*, 2017).

Consumers have altered the pattern of consumption towards further processed products and portioned cuts as opposed to whole bird sales (Petracci & Baéza, 2011; Barbut, 2015; Bowker, 2017) and, in turn, meat quality features have become acceptability parameters whereby consumers base their buying decisions on (Mir *et al.*, 2017). Poultry meat is more acceptable in accordance with religious and cultural beliefs, classifying it as one of the most suitable forms of edible animal protein sources (Petracci *et al.*, 2013b, 2019). The naturally high nutrient content of poultry meat, besides having a low caloric value, satisfies the need for a healthy lifestyle supported by a wholesome diet (Le Bihan-Duval *et al.*, 1999).

Increasing the pressure of genetic selection towards producing heavier birds has altered muscle tissue compositions that may have led to an upsurge of modernised meat quality issues such as wooden breast, white stripping, and spaghetti meat, diminishing the overall sensory quality of poultry meat produce (Cai *et al.*, 2018; Petracci *et al.*, 2019). Mir *et al.* (2017) stated that broilers have highly heritable meat quality traits (h^2 = 0.35–0.81) which makes it possible for genetic selection to improve broiler growth performance, however, such improvements may have been carried out at the expense of the quality of broiler meat with emphasis being placed on product yield losses, as discussed in this review.

Comparing annual producer prices of different protein sources in 2019, the average price of poultry meat on a rand per kilogram basis compared to beef (A2/A3 class) and pork were R22.89/kg, R44.98/kg, and R25.13/kg, respectively, clearly indicating the affordability of broiler meat. Broiler production for slaughter in December 2018 experienced an annual growth of 6% compared to 2017 (SAPA, 2018). Broiler meat production in September 2020 was on average 148 700 t, 170 t more than the predicted forecasting model for September 2020 (SAPA, 2019). The per capita consumption of beef in 2018 was 17,29 kg/annum, after poultry

at 38,52 kg/annum, signifying poultry as a fundamental source of protein consumption in South Africa (SAPA, 2018).

The nutritional value of food has become a crucial part of a consumer's lifestyle orientating towards increased consciousness and well-being, influencing product selection. The evolution of portioned and processed product markets depend on improved carcass quality, higher breast meat yield and superior body composition (Le Bihan-Duval *et al.*, 1999). Given the rise in the consumer's level of income, the increase in meat purchases compared to grain in previous decades point out a directional movement towards pre-cooked and ready-made foods. A decrease in store-bought raw food sales (56%) as compared to the steady increase in pre-cooked/prepared food sales (75%) indicates a decline in fresh produce purchases (Barbut, 2015). The interaction of the production chain and that of consumer lifestyle is aimed to satisfy the demands for convenience and health concerns.

2.2 Avian muscle composition and physiology

The mass of skeletal muscles utilised to produce meat for consumer consumption ranges from 35 to 60% of total body weight in livestock species (Listrat *et al.*, 2016). Meat is considered of good nutritional quality due to its high protein content governed by essential amino acids and polyunsaturated fatty acids. The body of a broiler contains an average of 200 muscles connected to the bone via tendons, consisting of three types of tissues: smooth, skeletal and cardiac (Tondeur & Simons, 2019).

Skeletal muscles uphold voluntary locomotion while facilitating body carriage and structural soundness. After hatching, the number of fibres within the muscle is fixed as myofibres undergo hypertrophy to increase in muscle cell size as opposed to number. Postnatal muscle growth takes place with increased fibre diameter as a broiler matures (Grashorn, 2010; Listrat *et al.*, 2016). The framework of skeletal muscles is made up of 90% muscle fibres, also known as myofibres, whereby connective and adipose tissues make up the remaining segment largely influencing organoleptic meat qualities (Listrat *et al.*, 2016).

The organisation of skeletal muscles on a macroscopic scale is distributed into three connective tissue layers: epimysium, perimysium, and endomysium as illustrated in Figure 2.1. The epimysium envelopes the entire muscle, aiding in structural support; it is the outermost layer of the muscle structure (Grashorn, 2010). The perimysium allocates the muscle into myofibre groups, otherwise known as fasciculi, whereby the endomysium surrounds each myofibre individually (Listrat *et al.*, 2016). On a microscopic scale, elongated myofibres contain polynucleated muscle cells (myocytes) located underneath the sarcolemma, also known as the cellular membrane connecting the individual surrounding

myofibres (endomysium). Microscopic fibre bundle arrangements occupy the entire macroscopic intracellular muscle fibre volume (Listrat *et al.*, 2016).

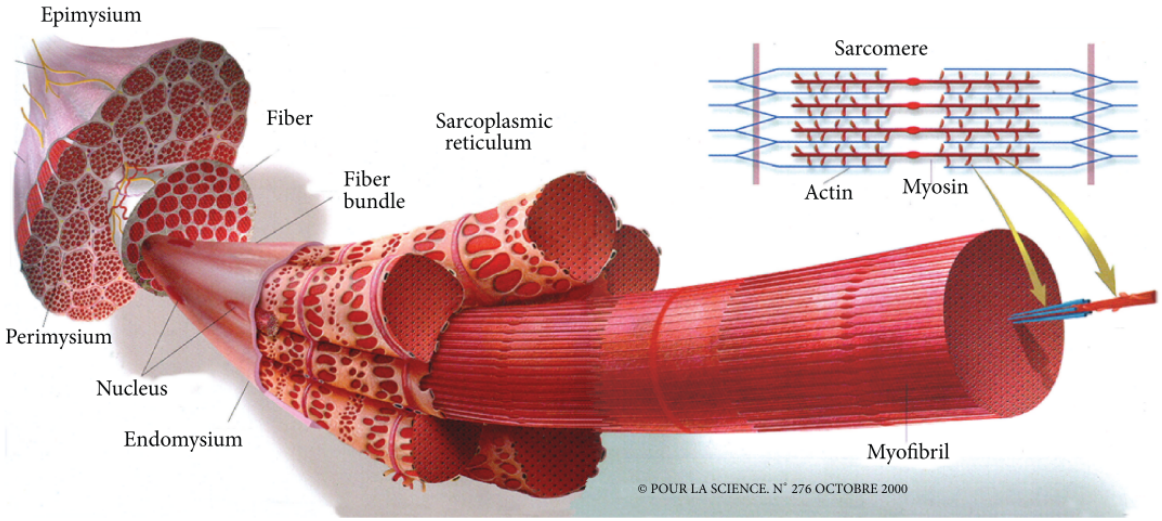

Figure 2.1 The muscle fibres and connective tissues constructing skeletal muscles (adapted from Listrat *et al.*, 2016)

Small contractile subunits assemble the myofibre structure known as myofilaments or myofibrils (Clark & Harding, 2017). These myofibrils extend throughout the whole muscle fibre at a diameter of approximately 1 µm (Listrat *et al.*, 2016; Clark & Harding, 2017). Sarcomeres are the contractile functional units present in myofibrils. X-ray diffraction and electron microscopy have enabled a better understanding of muscle physiology and contractile mechanisms of skeletal muscles (Craig, 2017). Alternating bands, dark (A-band) and light (I-band) as illustrated in Figure 2.2, construct each myofibril observed by longitudinal electron microscopy planes (Listrat *et al.*, 2016). Sarcomeres are enclosed by two Z-lines, comprised of structural α–actinin proteins, which divide the I-bands. Thin myofibrils (I-band) consist of actin and regulatory muscle contractile proteins, troponin and tropomyosin, which connect to the Z-line. Thick filaments (A-band) are comprised of myosin which anchors to the centre of the sarcomere by the elastic protein, titin (Clark & Harding, 2017).

According to Listrat *et al.* (2016), myosin plays a key role in the dephosphorylation of adenosine-triphosphate (ATP), the main energy provider for muscle contraction. Glycogen granules are the main energy source of myocytes, apart from lipid droplets, stored in the sarcoplasm and the cytoplasm of myofibres. The sarcoplasm stores soluble proteins, enzymes regulat glycolytic pathways, and contains myoglobin. Myoglobin is the fluid that transports

oxygen to the mitochondria and provides meat with its distinctive reddish colour (Listrat *et al.*, 2016).

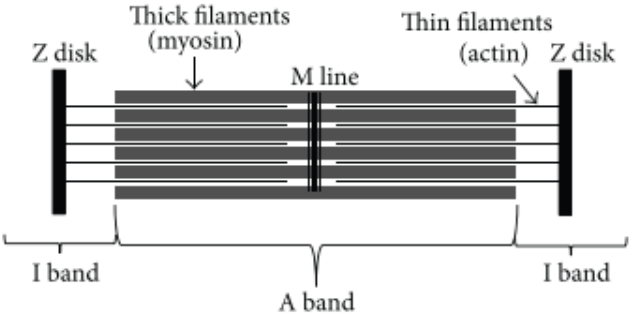

Figure 2.2 The sarcomere (adapted from Listrat *et al.*, 2016)

2.3 Myofibres

Myofibres can be grouped according to the rate of muscle fibre contraction (Listrat *et al.*, 2016). According to Clark & Harding (2017), the speed of contraction is related to the catalyst activity of the myosin ATPase head present within the thick filaments. The ATPase enzyme group is responsible for harnessing energy required for cellular reactions by the hydrolysis of a phosphate bond within ATP. As muscle ATP requirements differ heavily, hence the contraction rate among different fibre types, skeletal muscle fibres can be subclassified into slow (type I) and fast-twitching (type II) groups depending on the amount of fuel required contraction (Clark & Harding, 2017).

Energy availability necessitates the ground by which muscle fibres utilise different types of metabolism (Clark & Harding, 2017). Slow-twitching fibres are resilient against exhaustion, associated with endurance and undergo oxidative metabolism. These muscles are more efficient in ATP production as they are associated with a higher number of mitochondria, blood vessels and myoglobin content (Clark & Harding, 2017; Tondeur & Simons, 2019). Type I muscle fibres generate 30 ATP molecules through glycolysis, the Kreb's cycle and the electron transport chain, highlighting a large demand for oxygen. On the contrary, muscle glycogen and phosphocreatine enable fast-twitching fibres to generate 2 ATP molecules anaerobically used for short, extensive and quick actions that tend to utilise glycogen stores more rapidly causing fatigue relatively faster (Clark & Harding, 2017). Type II fibres lack myoglobin and appear white due to reduced oxygen requirements (Listrat *et al.*, 2016).

Physiologically, given the location of the breast and thigh muscle within the broiler body, myofibre proportion and type could account for muscle functionality. Thigh muscles require a sustainable long-term oxidative ATP generating pathway to aid in broiler locomotion. Having no locomotive influence on the bird, the breast muscle is composed of white fibres

exclusively, while red fibres are found within the thigh muscle constantly used to find feed and water (Tondeur & Simons, 2019).

Overall, organoleptic and sensory meat quality attributes of broiler skeletal muscles are greatly influenced by the framework of myofibres and interior reserved fat. Work cited by Listrat *et al.* (2016) and Tondeur & Simons (2019) mention higher levels of saturated fat associated with oxidative fibres, clarifying why broiler thigh portions have more texture and flavour compared to breast fillets. It is further believed that glycolytic fibres exhibit lower intramyocellular lipid content (Listrat *et al.*, 2016), emphasising the value of understanding muscle composition properties when considering broiler meat quality.

2.4 Muscle contraction

Avian muscle composition and physiology, as discussed in the section above, is a crucial concept to comprehend before gaining knowledge of how a muscle contracts. Broilers are continuously in search of food and water, interact with each other, stretch their legs, and flap their wings. It is a natural behaviour that requires muscles and the contraction thereof. The overlapping patterns of myosin and actin protein filaments define skeletal muscles with a striated appearance (Listrat *et al.*, 2016; Clark & Harding, 2017; Sweeney & Hammers, 2018).

In short, muscle contraction takes place as a force produced by sarcomeres caused by actin and myosin protein filaments overlapping, while maintaining the same dimensions (Craig, 2017; Pollard *et al.*, 2017). Specialised muscle cells with motor and sensory nerves operate as stretch receptors (Pollard *et al.*, 2017). These cells are messengers that send information to the spinal cord, enabling output coordination of the motor neuron. The motor neuron stems from the spinal cord and brainstem connecting to target cells inside of the muscle by what is known as an axon (Kuo & Ehrlich, 2015; Pollard *et al.*, 2017). Therefore, a motor unit consists of a motor neuron along with its target muscle cells (Pollard, *et al.*, 2017).

A large synapse, known as the synaptic cleft (Figure 2.3), is located directly after the terminal axon of the motor neuron. The postsynaptic membrane of the muscle fibre is referred to as the motor endplate. Therefore, the neuromuscular junction (NMJ) consists of three parts: the motor neuron ending, synaptic cleft and the postsynaptic region of the muscle fibre (Kuo & Ehrlich, 2015). The role of the NMJ is to connect skeletal muscles with nerves, aiding in the exchange of neural signals. The synaptic cleft forms junctional folds within the postsynaptic membrane of the muscle fibre, specifically to accommodate acetylcholine receptor sites (Kuo & Ehrlich, 2015). Acetylcholine (ACh) is a neurotransmitter that aids information transfer between the nerve and muscle (Pollard *et al.*, 2017). Synaptic vesicles located in the terminal axon of motor neurons store ACh (Figure 2.3). Acetylcholine is released from the terminal

axon end, diffusing across the synaptic cleft, and binding to the motor endplate. This only takes place once an action potential stimulates the fusion of the motor neuron terminal nerve membrane and ACh vesicles (Pollard *et al.*, 2017).

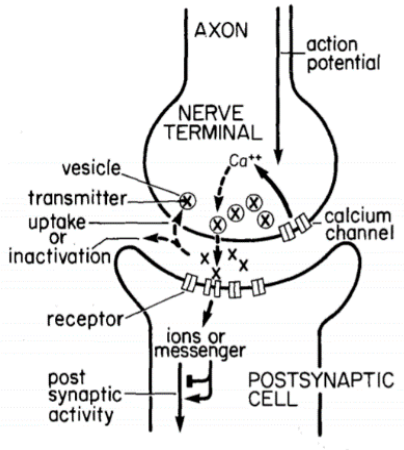

Figure 2.3 The chemical process of the neuromuscular junction (adapted from Hall & Sanes,1993)

According to Feher (2017), neuromuscular transmission is the process whereby the action potential on the motor neuron is converted to a different action potential on the muscle fibre. Furthermore, intracellularly the action potential spreads across the surface of the muscle into the T-tubules (Kuo & Ehrlich, 2015). T-tubules are excitable sarcolemma invaginations that continue across the cell, containing ion channels (Calderón *et al.*, 2014). The sarcoplasmic reticulum (SR), situated in skeletal muscles, is the modification of the smooth endoplasmic reticulum (SER) found in smooth muscles (Szent-Györgyi, 1975). The overall mechanism of the SR is regulating calcium ion (Ca^{2+}) release (Feher, 2017). The SR is a network of horizontal tubules that wrap around myofibrils (Feher, 2017). The SR and T-tubules are in proximity as they form structural triads. Triads consist of one regular sarcolemma invagination, otherwise T-tubule, surrounded by two terminal cisternae (Al-Qusairi & Laporte, 2011).

The excitation-contractile coupling (ECC) process in skeletal muscles initiates with an action potential leading to a series of events that initiate muscle contraction (Calderón *et al.*, 2014). The depolarization of the sarcolemma, taking place at the triads, is coupled to the opening of cation channels (Sperelakis, 2012). During depolarization, the membrane potential becomes less negative. This is due to the opening of ACh receptors stimulating the activation of other voltage-gated sodium channels (Kuo & Ehrlich, 2015). The configurational change acts as an ion channel allowing sodium ions (Na^+) to diffuse past the synaptic cleft of the postsynaptic membrane, into the sarcoplasm. Na^+ ions enter the sarcoplasm in greater quantities, as several sodium-potassium pumps are activated, and as stated previously

manifests a less negative membrane potential (Gehlert *et al.*, 2015). Once a nerve impulse has ended, the enzyme acetylcholinesterase causes the ACh receptors to close. This enzyme hydrolyses ACh into choline and acetate within synaptic areas (Taylor *et al.*, 2009). Repolarization re-establishes the extra- and intracellular ionic gradient as potassium ions (K+) are pumped inward, restoring a negative membrane potential.

Two key proteins namely the T-tubule dihydropyridine receptor (DHPR) and the SR ryanodine receptor (RYR) mediate triad junction transduction (Sperelakis, 2012). Muscles reside in a relaxed state when sarcoplasmic calcium (Ca^{2+}) ion concentration is low. X-ray diffraction confirmations indicate a few nanometres between myosin heads and the actin backbone filaments (Pollard, *et al.*, 2017). In the relaxed state, troponin and tropomyosin inhibit myosin-binding sites on the backbone of actin filaments. The significance of Ca^{2+} ions to maintain skeletal muscle integrity is overwhelming as it plays a key role in muscle contraction, intracellular processes and muscle plasticity (Gehlert *et al.*, 2015).

Sarcolemmal calcium channels (DHPRs) are assembled at triads and move due to the action potential depolarization and in turn, cause the RYR1 to open. Following the excitation phase, the sodium voltage-gated channels trigger the opening of T-tubule calcium voltage-gated channels (Kuo & Ehrlich, 2015). The release of Ca^{2+} ions from the SR is stimulated by the action potential moving down the T-tubules (Gehlert *et al.*, 2015). Calcium binds to troponin, one of the regulatory protein molecules constructing the actin filament (Kuo & Ehrlich, 2015). Tropomyosin, being the second regulatory protein, together with troponin in the relaxed muscle state prevents myosin heads from binding to actin (Sweeney & Hammers, 2018). Yet, once calcium binds to troponin, tropomyosin undergoes a structural change and realigns itself away from the myosin-binding sites on the actin backbone (Sweeney & Hammers, 2018). The actin-myosin complex consequently forms a cross-bridge linkage.

The cross-bridge between actin and myosin pulls the Z-lines towards one another with the help of ATP. Myosin heads hydrolyse ATP to induce what is referred to as a 'power stroke' in which adenosine di-phosphate (ADP) and inorganic phosphate (Pi) are released (Sweeney & Hammers, 2018). During the power stroke, myosin heads pull actin along its length. Therefore, the sliding movements of myosin and actin cause shortening of the sarcomere, and systematic contraction of the muscle takes place (Sweeney & Hammers, 2018).

2.5 Muscle water content

Muscles are highly structured and compartmentalised as reviewed in previous sections, highlighting the organisation of water within the muscle is distributed *in vivo* (Pearce *et al.*, 2011). Lean muscle consists of about 75% water (Honikel, 1987), 20% protein, 5%

lipids, 1% carbohydrates, and 1% vitamins and minerals (Pearce *et al.*, 2011). In living animals the sarcolemma stores water within cells maintained by numerous membrane pumps (Grashorn, 2010). Water relocates to the sarcoplasm after slaughter due to myofibrillar shrinkage where it is kept until the muscle pH falls.

The onset of rigor mortis is defined by a steady decline in ATP (de Fremery & Pool, 1960), enabling ions and water to freely move and diffuse through the sarcolemma into the extracellular spaces (Grashorn, 2010). Once the myofibrils have undergone shrinkage the intracellular sarcoplasmic space expands, allowing water to pass to the extracellular spaces. Intra-myofibril muscle water constructs most of the water found within the muscle matrix, as myofibrils construct 80 – 90% of the muscle volume (Offer *et al.*, 1989; Listrat *et al.*, 2016). The extra-myofibril spaces further hold 15% of muscle water (Pearce *et al.*, 2011); these regions are the sarcoplasmic spaces between the muscle fibres and fasciculi (Pearce *et al.*, 2011).

Water within the muscle matrix exists in three forms (Figure 2.4). The first form is protein-associated water, otherwise known as bound water, tightly bound to charged hydrophilic groups situated on the backbone of protein chains. Accordingly, Zayas (1997) depicts approximately 10% of the total bound water in the muscle being situated within the protein filament framework. The second form of water found within a muscle is known as immobilised water, either bound by steric effects within the muscle structure via hydrogen bonds (Barbut, 2015), or to other macromolecules (Pearce *et al.*, 2011; Bowker, 2017). Immobilised water can relocate to other compartments due to alterations in the protein isoelectric point (pI), meat pH and muscle cell conformation that weakens the attachment of water enabling movement.

According to Bowker (2017), immobilised water can be lost in the form of drip or purge due to its formation into free water. The last form of muscle water is free water which is held by capillary forces (Bowker, 2017). Capillary forces bind free water inside of the myofibrillar structure and sarcoplasm (Warner, 2014). The decrease of immobilized water and the increase of free water causes drip loss as stated by Bowker (2017). Free water usually amounts to 50 – 60% of the water found within the muscle, emphasising the major importance of keeping this form of water within the final product (Barbut, 2015).

A reduced water-holding capacity is accompanied by the onset of *rigor mortis* (Lawrie & Ledward, 2006). This is further explained by the amount of bound water within the muscle structure remaining constant, yet the diffusion and movement of free water reflecting the formation of actin–myosin cross-linking complexes. Henceforth, proteolysis produces more

water over time during cytoskeletal myofibrillar aging (Grashorn, 2010), and processors should be meticulous about conserving as much muscle water postharvest as possible.

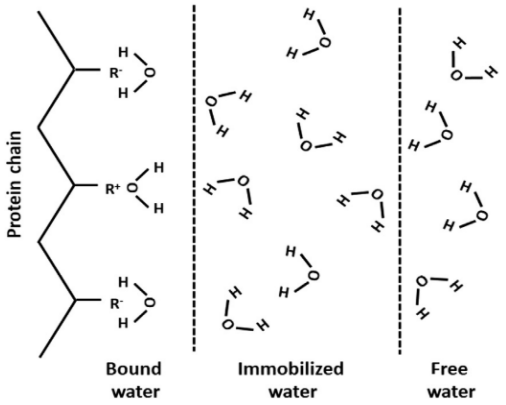

Figure 2.4 The three forms of myowater: 1) Bound water – immobile, 2) Immobilised water – moveable, 3) Free water – affects water loss (adapted from Bowker, 2017)

2.6 Conversion of muscle to meat

The current concept of muscle conversion into meat states that for a reasonable length of time the storage of muscle is required to develop meat organoleptic qualities (Ouali *et al.*, 2006). The timing of biochemical and metabolic responses postharvest causes muscle extensibility before permanent stiffening. These changes influence the quality parameters of flavour, juiciness, shelf life (Warner, 2016), appearance and colour (Tondeur & Simons, 2019).

Ripening is the process whereby the muscle is transformed into meat through the gradual depletion of muscle energy (Grashorn, 2010). Tenderness relates to the texture, being the ease of shear or the hardness of meat, which is a function of intramuscular WHC (Pearson & Dutson, 1994; Mir *et al.*, 2017). Meat tenderisation is directly influenced by both intrinsic and extrinsic factors. Consequently, Bowker (2017) states that intrinsic bird factors under intense commercial alteration have given rise to side-line WHC problems in further processed products. Age, sex, species, and muscle type are intrinsic factors, while electrical stimulation, chilling temperature, storage type, and duration are extrinsic factors (Pearce *et al.*, 2011).

Rigor mortis sets the basis for meat tenderisation by employing antemortem biochemical changes (Grashorn, 2010). In respiring, living muscles oxygen is available to cells through the bloodstream and ATP formation is through glycolysis and the oxidative phosphorylation pathway (Warner, 2016). De Fremery & Pool (1960) states that in respiring muscles a high ATP level is maintained using organic compound oxidation. After slaughter, the phosphate-bond energy reservoir, creatine phosphate (CP), synthesises ATP with the help of ADP and a hydrogen ion (de Fremery & Pool, 1960; Warner, 2016). Yet, CP becomes quickly exhausted, and once it drops to 75% of its initial concentration ATP decline commences. The demand for ATP continues, triggering an irreversible metabolic switch from aerobic to anaerobic glycolysis

to obtain a new means of energy (ATP) supply (Lawrie & Ledward, 2006; Tondeur & Simons, 2019). The anaerobic shift is stimulated by blood removal (exsanguination), causing oxygen supply towards tissue to die down. The loss of blood supply fails to maintain body heat causing the temperature to drop. Oxygen and CP depletion converts glycogen into ATP, forming the by-product lactic acid during anaerobic glycolysis (Bowker, 2017). Warner (2016) and Bowker (2017) both express that anaerobic ATP generation is less efficient than aerobic ATP generation, and thus, the required muscle energy levels are not sufficient.

The chemical event leading up to *rigor mortis* onset is the disappearance of muscle ATP (de Fremery & Pool, 1960; Lawrie & Ledward, 2006). Additionally, calcium ions (Ca^{2+}) released from the sarcoplasmic reticulum trigger the complex formation of actin-myosin (Paredi *et al.*, 2012). In the absence of blood circulation, byproduct formation of anaerobic glycolysis, namely lactic acid (LA) and hydrogen-ions (H), accumulate which causes the pH of respiring muscle to drop from 7.0 to 5.5 – 6.0 post-mortem (de Fremery & Pool, 1960). Lactic acid produced in living muscles during anaerobic glycolysis is reconverted to glucose, whereas postharvest LA remains a waste product causing pH decline (Warner, 2016). According to Paredi *et al.* (2012), the process of LA accumulation consequently leading to pH decline can be referred to as muscle acidification. The author further highlighted the complications of such acidification on the loss of WHC in meat.

The drop in pH continues until glycogen is depleted or the ultimate pH is reached, inactivating enzyme breakdown (Lawrie & Ledward, 2006; Bowker, 2017). Therefore, energy metabolism links both postharvest pH decline and *rigor mortis* development (Bowker, 2017). As the pH is lowered due to hydrogen ion build-up, the electrostatic repulsion between actin and myosin is reduced (Pearce *et al.*, 2011). The decline in pH is not only due to hydrogen ions from LA accumulation, but also, nucleotides like ATP and ADP hydrolysis (Warner, 2016). The microenvironment of muscle fibres is subjected to undergo conformational change as a result of cellular homeostasis disruptions (Bowker, 2017). Various membrane pumps (Na^{+}, K^{+}, Ca^{2+}) are interrupted following the depletion of ATP. In addition, due to an increase in ionic strength, muscle fibre cells are unable of overlooking disruptive conditions. The binding and holding of water within the muscle is therefore vulnerable to weakening membrane integrity and permeability (Bowker, 2017).

The iso-electrical point (pI) is the pH at which muscle proteins have an overall net zero charge (Mir *et al.*, 2017). Reactive groups located on proteins slowly reach the pI where the reactive groups attract one another, exposing or concealing water binding sites within the conformity of proteins (Haque *et al.*, 2016). It can be said that the WHC of a muscle is greatly under the influence of postmortem pH decline and the net electrical charges of the muscle

proteins (Bowker, 2017; Mir *et al.*, 2017). As the pH of the muscle reaches the pI, the electrostatic repulsion between actin and myosin is reduced (Pearce *et al.*, 2011; Bowker, 2017). The protein filaments can move closer to each other, lessening the ability of water to be stored within the muscle. The pI's of actin (5.1) and myosin (5.4), according to Bowker (2017), represent the pH at which WHC of muscles is at its lowest. Thus, the charges on the reactive groups at any pH above the pI of the protein filaments are stronger and may be able to bind water more effectively than the reactive groups at the pH associated with the pI.

In broiler muscles, *rigor mortis* is completed between three to eight hours after slaughter without electrical stimulation (Tondeur & Simons, 2019). The level of ATP determines the time of *rigor mortis* onset, which is steadily lowered by surviving ATPase myosin activity (Lawrie & Ledward, 2006). The conversion of muscle to meat is a critical aspect relating to WHC when considering the movement of water inside of the muscle matrix postharvest. Honikel *et al.* (1986) explain the movement of water after slaughter initiates from the intra-myofibrillar to the inter-myofibrillar space (Offer *et al.*, 1989; Pearce *et al.*, 2011; Bowker, 2017). As the permanent crosslink formation between actin and myosin complex, it reduces space for water to reside inside the muscle matrix (Pearce *et al.*, 2011). As the space for water is reduced so is the WHC of the muscle, forcing water from the inter-myofibrillar space into either the inter or extra fascicular spaces (Honikel *et al.*, 1986; Mir *et al.*, 2017), as seen in Figure 2.5.

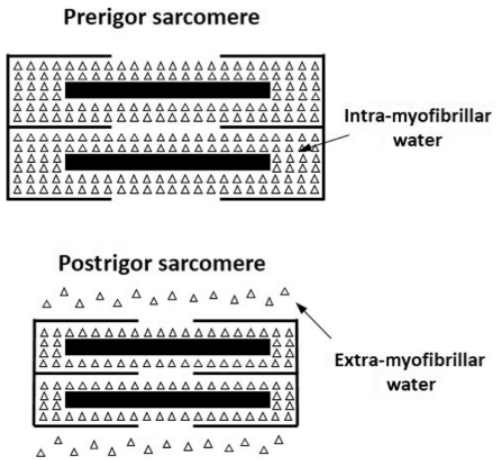

Figure 2.5 Movement of water from intramyofibrillar to extramyofibrillar space (adapted from Bowker, 2017)

2.7 Quantifiable broiler meat properties

2.7.1 Water-holding capacity

Water-holding capacity (WHC) is one of the most important functional properties of meat quality, as it indicates the ability of muscle proteins to bind and hold water (Northcutt *et al.*, 1994). Scientific literature uses a variety of terms, but for the purpose of this review, WHC will be referred to. The moisture content of meat has a direct impact on the quality thereof

affecting tenderness, juiciness and appearance which overall signifies a value-added product of economic significance (Northcutt *et al.*, 1994; Mir *et al.*, 2017). Consumer expectations represent the drive for producing broiler meat of higher quality, as purchasing decisions are based on product presentation. Desired outcomes of higher production yields place great responsibility on producers to satisfy the supply and demand of consumers of the future.

Water-holding capacity is a factor of significant concern to the industry, as substantial mass from broiler carcasses and portioned cuts are lost, affecting the quality and yield of further processed products (Pearce *et al.*, 2011). When measuring the maximum amount of water retained within a muscle after additional ingredients have been added to raw meat, it is referred to as water-binding capacity (Warner, 2014; Mir *et al.*, 2017). Functional ingredients are added to poultry meat during processing to obtain higher quality products with flavour and aromas that alter the functionality of broiler meat. According to Petracci *et al.* (2013a), functional ingredients can be grouped according to their working mechanisms, being improving flavour, appearance, functionality (WHC), or shelf life (Barbut, 2017). Several publications provide additional background on functional ingredients and their usage in meat (Petracci *et al.*, 2013a; Barbut, 2017); since this subject is beyond the scope of this review.

Raw meat with a low WHC limits further processing of products due to a reduced yield (Otto *et al.*, 2004), lowering the visual appeal of raw meat due to excess moisture loss in packages relating to reduced juiciness that affect palatability. Product judgment is under selective control by the buyer and becoming more specialised in grasping consumer attention in stores. Unfortunately, the irreversible effect of postharvest moisture loss from raw meat is inevitable due to protein filament cross-linkage; therefore, innovative packaging enhances the appearance of raw meat with relevance to many preservation benefits. Soaker pads are often used when raw meat is packaged which removes the unpleasant appearance of purge from raw poultry meat (Mane, 2016).

Tenderness, a texture-related element, is regarded as the most important characteristic of consumer preferences (Lee *et al.*, 2012). Yield loss from raw meat is related to water binding and its ease of movement inside of the muscle structure. As stated by Warner (2014), water purge from skeletal muscles is inversely related to WHC. When water is able to tightly bind to muscle proteins, a swelling effect is exerted, giving the meat a firm structure (Mir *et al.*, 2017). Moreover, an improved WHC in raw meat leads to greater marinade uptake, increased product yield (Bowker, 2017), and greater juiciness (Lipiński *et al.*, 2011). On account of rapid oxidative and enzymatic tissue breakdown rates occurring after slaughter, further processed product quality and raw meat shelf-life is significantly influenced by the amount of moisture lost from meat surfaces (Downs *et al.*, 2000a).

The terms pale, soft, and exudative (PSE) meat, as well as dry, firm, and dark (DFD) meat are two major quality issues potentially associated with earlier development of *rigor mortis* that define muscle characteristics compared to normal meat, influencing the yield of fresh meat cuts and further processed products (Warriss, 2000, 2010). Dry, firm, and dark meat is characterised as having a high WHC, whilst PSE meat tends to have a lower WHC as cited by Lipiński *et al.* (2011). Pale, soft, and exudative meat has been observed during acute heat stress exposure before slaughter, whereas DFD occurs during chronic heat stress periods (Gonzalez-Rivas *et al.*, 2020).

As discussed previously, skeletal muscles reach an ultimate pH of approximately 5.5 after slaughter, influencing the ability of proteins to tightly bind muscle water. Myofibril shrinkage and longitudinal skeletal muscle contraction are major aspects forcing the water out of the muscle (Lawrie & Ledward, 2006). Sarcomere length, pH, osmotic pressure, ionic strength, and the progression of *rigor mortis* alter cellular and extracellular components within the muscle structure (Northcutt *et al.*, 1994; Mir *et al.*, 2017). The leaky appearance of membranes and cellular components from fresh meat can be referred to as drip loss, whereas, cook loss is the loss of moisture in the form of liquid or steam during heat treatment (Vujadinović *et al.*, 2014). Drip and cook loss are measurable parameters relating to the WHC of raw and cooked meat that have a significant economic impact.

A standardised drip loss measurement technique is of utmost importance to obtain reliable results, yet no technique has been recognised nor proposed for poultry, and therefore, literature is difficult to compare according to the principle of WHC parameters (Torres Filho *et al.*, 2017). Dos Santos *et al.* (2019) defines drip loss as a method to measure WHC without any externally applied force. Woelfel *et al.* (2002) obtained standard drip and cook loss values for normal breast fillets as 3.32% and 21.02%, respectively, while for pale, soft, and exudative (PSE) breast fillets those values increased to 4.38% and 26.39%, respectively.

Objective WHC measurements reviewed by Warner (2014) include natural or external applied forces, whereas Petracci & Baéza (2011) characterise the analysis of poultry meat into chemical and physical traits. Both authors conclude the gravitational drip loss method, also known as the Honikel method, as the preferred method to measure WHC. This is due to its simplicity and relative accuracy compared to press methods and centrifugation analyses. According to Petracci & Baéza (2011), the press method is not reliable as a proposed standardised procedure of measuring WHC as results from published papers are not comparable due to variation in force (Warner, 2014). Centrifugation applies an external force to relative sample sizes that result in relatively large variation caused by varying sample sizes

and force application (Petracci & Baéza, 2011); further having a very limited use (Warner, 2014).

Cook loss is the loss of moisture in the form of liquid or steam and measured through the application of heat, expressed as a percentage of the pre-cooked weight (Warner, 2014; Vujadinović *et al.*, 2014). This method has the greatest correlation to sensory traits, one being juiciness, and is the most used method apart from a conventional oven.

2.7.2 pH and colour

The primary structure of a protein is assembled by a linear arrangement of amino acids, attached via peptide bonds, further folded into three-dimensional secondary and tertiary structures. Moreover, the quaternary structure represents different amino acid chain arrangements extending from the protein molecule backbone which could be charged. Many authors have reported a direct correlation between colour and pH of meat (Warriss, 2000; Woelfel *et al.*, 2002; Lipiński *et al.*, 2011; Mir *et al.*, 2017), and thus the one cannot be reviewed without the other.

Meat acidification potentially quantifies the quality of meat to the extent of influencing meat colour and WHC (Warriss, 2000). The pH value directly influences meat quality parameters relating to consumer acceptability such as drip loss, colour, and shear force (Salim *et al.*, 2011). According to Warner (2014), the perceiving colour of meat is determined by the chemical state and concentration of myoglobin, the dominant pigmentation of meat, along with birefringence and texture. Myoglobin content possibly influences the optical properties of meat appearance as it is an essential contributor to oxygen-binding abilities within respiring muscles (Mir *et al.*, 2017).

Postmortem pH decline renders reactive protein groups in a neutral state, creating expensive water losses as there is a reduction in the number of charges available to bind water. Myoglobin may potentially be lost, along with muscle water, in the form of drip or purge reducing the quantity of myoglobin within meat (Swatland, 2004). Furthermore, Swatland (2004) and Barbut (2015) explain that myoglobin levels determine the type of light escaping from meat. For instance, the bulk of light reflected to the observer is red together with small amounts of yellow and orange, although at the same time green and blue light are absorbed by myoglobin, while the remainder is scattered into the meat.

Colour is the main contributor to a consumer's intent to purchase and is not necessarily linked to product flavour. The varying states of myoglobin comprise the uniformity of meat colour perceived by the observer due to oxidation of complexed iron molecules at the centre of the haem group within myoglobin: red-oxymyoglobin, purple-deoxymyoglobin, and brown-

metmyoglobin (Barbut, 2015; Frelka, 2017). The accumulation of metmyoglobin on the surface of chicken meat impairs product appearance and decreases the probability of that product being purchased (Warner, 2014).

Meat with a high postmortem pH (≥ 6) transmits light into its depth, appearing darker in colour as the path through the muscle travelled is relatively longer, weakening the scattering of light and increasing the selective absorbance by myoglobin (Swatland, 2004, 2008). Meat with a lower postmortem pH (≤ 5.5) scatters light towards the observer, coming forth as paler than usual. Thus, there is a strong scattering of light when the pathway through the muscle is shorter, resulting in less selective absorption by myoglobin. Mir *et al.* (2017) support this statement by characterising minimal protein denaturation with a closed, translucent appearance structure due to the absorption of light instead of scattering.

Meat colour can be evaluated and reported in numerous ways with the help of colour scales, fans and state-of-the-art equipment. The International Commission on Illumination describes the background influence on colour appreciation. The CIE L*a*b* method is one of the most commonly used systems as values for lightness (L*), redness (a*), and yellowness (b*) evaluate colour deviations potentially influenced by the physical and chemical state of meat (Le Bihan-Duval *et al.*, 1999). CIE L*a*b* also allows the calculation of hue and saturation (chroma) which emphasises the red portion of the spectrum (Petracci & Baéza, 2011; Warner, 2014).

The L* value, as discussed by Barbut (2015), ranges from 0 (black) to 100 (white), expressing lighter coloured meat with high L* values closer to 100. The a* scales from -60 (green) to +60 (red), and b* spans from -60 (blue) to +60 (yellow). Large positive a* values indicate more red-coloured muscle, whereas more yellow-coloured meat is indicated by higher b* values (Le Bihan-Duval *et al.*, 1999; Tang *et al.*, 2007). Multiple authors suggest the quality of chicken meat being normal with a pH range of 5.9 to < 6.1 (Van Laack *et al.*, 2000; Woelfel *et al.*, 2002; Zhang & Barbut, 2005), PSE with a pH range of ≤ 5.8 (Van Laack *et al.*, 2000; Woelfel *et al.*, 2002; Zhang & Barbut, 2005), and DFD meat with a pH range of > 6.1 (Zhang & Barbut, 2005).

Breast meat colour variation is a relative indication of meat pH, and vice versa, shown by Allen *et al.* (1998) in shelf life, WHC, marinade pick-up, and drip and cook loss parameters. Lighter breast fillets had lower initial pH values (5.8 vs 6.02), along with increased drip (5.88% vs 3.34%), and cook (34.4% vs 32.9%) losses compared to darker than normal breast fillet. Petracci *et al.* (2004) obtained similar results where paler breast fillets (L*>56) were characterised as having a lower ultimate pH value (5.77 vs 5.89 and 6.04; $P \leq 0.01$), and a higher percentage of cooking loss (23.84 vs 21.13 and 18.84%; $P \leq 0.01$) compared to normal

and darker breast fillets. The cut-off range of pH and colour reported by Barbut (1997) and Woelfel *et al.* (2002) categorises meat into three different groups: PSE (pH <5.7; L* >53), normal meat (5.7< pH <6.1; 46< L* <53), and DFD (pH>6.1; L*< 46). According to Petracci *et al.* (2004), the limit values based on L* are used to characterise breast fillet colour as being dark (L*<50), normal (50≤ L* ≤56), and pale (L*>56). However, the authors emphasise these ranges should be set according to specific circumstances, as these values are influenced by seasonal effects, bird age, live weight, and pre-slaughter stress.

Woelfel *et al.* (2002) characterised PSE meat with a lower WHC compared to paler fillets as the incidence of paler fillets was identified based on a threshold range (L*>54). Woelfel *et al.* (2002) suggested a region between 50 and 55 whereby the pH approached a plateau, possibly indicating a pH and L* value that identifies protein damage and increased water loss. Furthermore, approximately 47% of measured fillets had L* values above 55 that were not categorised as PSE fillets, suggesting the potential to have a reduced WHC and being paler in colour. As hypothesised by the authors, expressible moisture, drip, and cook loss were significantly (P<0.001) and negatively correlated with pH, and positively correlated with L* values (3 h and 24 h) postharvest.

The remarkable alteration of market-form by consumers driving the poultry industry during recent years highlights the lack of attention given to product quality and appearance. More emphasis should be placed on the quantifiable properties of meat, as most economic relevance lies within these parameters.

2.8 Dietary strategies to improve broiler meat quality

Postharvest enzymatic and oxidative breakdown of tissue components proceeds vastly during processing and storage of fresh broiler products, which causes expensive moisture losses and, ultimately, economical deficits. Numerous dietary components have been included in poultry diets to reduce moisture loss in the form of drip and cook loss. However, the use of trace minerals in recent broiler nutrition research could act in accordance with amelioration strategies to improve broiler meat quality. Research is limited on the topic of trace mineral sources and relative supplementation strategies to reduce such moisture losses in broiler production.

The bioavailability of organic trace mineral supplements has been deemed higher than that of inorganic trace minerals (Oliveira *et al.*, 2014; Lopes *et al.*, 2017; Sałek *et al.*, 2020), evidenced by better meat quality (Salim *et al.*, 2008; Bakhshalinejad *et al.*, 2019). Literature has proven organic zinc (Zn) and selenium (Se) as a positive component in cell membrane integrity postmortem, facilitating improved WHC and cooking losses, enhancing overall broiler

meat quality features (Choct *et al.*, 2004; Rajashree *et al.*, 2014; Saleh *et al.*, 2018; Ibrahim *et al.*, 2019). However, one limitation of using chelated trace minerals is the increase in expense relative to inorganic trace mineral sources. The commercial demand for inorganic product supplementation is substantiated by higher inclusion levels which are considered to be more cost-effective in feed formulation at least-cost (Costa *et al.*, 2010; Lopes *et al.*, 2017).

Ultimately, organic micromineral supplementation during different production stages may be more beneficial when attempting to improve broiler meat quality characteristics on a least-cost basis, which inspired the focus for this literature review on Zn and Se trace minerals concerning meat quality in broiler production.

2.8.1 Selenium

Selenium is classified as an essential trace mineral having numerous biological roles which assist in improving immunity, health (Marković *et al.*, 2018a; Bakhshalinejad *et al.*, 2019), productivity, performance efficiency, and meat quality (Bakhshalinejad *et al.*, 2019). Thus, it plays an essential role in producing well-nourished broilers. Selenium has become a relevant topic of interest to customers due to lifestyle changes focused on wellbeing with the consideration of broiler meat to be one of the main sources by which humans obtain Se.

Owing to the potential geographical variability of soil Se status, the composition of Se-deficient feedstuff constructing animal diets has globally become an observable fact (Downs *et al.*, 2000a; Van Ryssen, 2001). As the progressive utilisation of land for crop growth expanded, the risk of selenosis in animals had been reduced, leaving raw feed materials in a Se-deficient state (Oliveira *et al.*, 2014; Cemin *et al.*, 2018; Bakhshalinejad *et al.*, 2019). Therefore, it is a common practice to supplement broiler diets with Se to prevent inconsistent broiler performance (Deniz *et al.*, 2005), such as exudative diathesis in broiler chicks (Combs & Combs, 1984; Ibrahim *et al.*, 2019), as well as thigh and breast lesion prevalence (Hassan *et al.*, 1990).

Selenium plays a crucial role in the oxidation defence system, forming an integral part of the antioxidant enzyme glutathione peroxidase (GSH-Px), which defends and protects cells and membranes from oxidation (Oliveira *et al.*, 2014; Marković *et al.*, 2018a). This Se-dependent enzyme is responsible for scouting out potentially damaging hydrogen peroxides and organic hydroperoxides formed during oxidative stress (Combs & Combs, 1984; Downs *et al.*, 2000a; Puvača & Stanaćev, 2011). Perić *et al.* (2009) describe cell membrane damage as being a main defect of oxidation, as it reduces the integrity of membranes and allows the seepage of intracellular fluid. On the basis thereof, Rajashree *et al.* (2014) have demonstrated that Se has the potential to improve meat quality by improving the WHC of broiler breast fillets by reducing expensive tissue water loss.

The advancement of different Se supplements incorporated into broiler diets to reduce possible performance shortcomings and deficient disease syndromes is a widespread practice. The National Research Council (NRC, 1994) recommendations for Se supplementation is 0.15 mg/kg, which according to numerous authors is relatively low for genetically selected modern broiler strains (Cemin *et al.*, 2018; Ibrahim *et al.*, 2019). Organic Se is bound to various amino acids (Surai, 2002), essentially cysteine (Se-Cys) and methionine (Se-Met), whereby Se-Met accounts for the majority of Se in Se-enriched yeasts (Downs *et al.*, 2000a). Organic Se has been identified as being more active (Marković *et al.*, 2018a), having a higher bioavailability, increasing Se tissue retention (Oliveira *et al.*, 2014; Silva *et al.*, 2019), and maintaining broiler meat quality (Puvača & Stanaćev, 2011) in comparison with inorganic Se sources (Surai, 2002; Marković *et al.*, 2018b).

Inorganic Se; sodium selenite (SS) and sodium selenate, tend to function as a pro-oxidant reducing the availability and efficacy of Se (Downs *et al.*, 2000a; Surai, 2002; Deniz *et al.*, 2005). This form of Se complexes with other dietary components that hinder their absorption leading to increased environmental waste of inorganic minerals (Costa *et al.*, 2010; Saenmahayak *et al.*, 2012). Organic Se (Se-Met) commonly used in the broiler industry is easily absorbed by erythrocytes, employing active mechanisms similar to that of methionine, whereas inorganic Se is absorbed by simple diffusion, rendering the mineral less retainable (Oliveira *et al.*, 2014). It has been found that organic Se in the form of Se-yeast or Se-Met when compared to inorganic SS reduces the amount of water that seeps from the tissue measured as drip loss (Downs *et al.*, 2000a; Choct *et al.*, 2004; Perić *et al.*, 2009; Jiang *et al.*, 2009; Wang *et al.*, 2011).

A discussion of Se would be incomplete without reference to the interrelationship of the latter with vitamin E, which induces a sparing effect for the other (Puvača & Stanaćev, 2011). Vitamin E acts as the first line of defence against cellular peroxide damage, followed by Se as the second defensive structure forming part of GSH-Px (Surai, 2002). This is due to the inability of vitamin E to prevent all metabolic peroxide destruction. Selenium and vitamin E shortcomings have been demonstrated to cause damages in the antioxidant systems, influencing the redox state of the breast muscle (Avanzo *et al.*, 2001).

Downs *et al.* (2000a) evaluated the effects of different Se sources supplemented at a level of 0.30 mg/kg on broiler carcass, meat, and drip loss parameters. An increase ($P<0.05$) in drip loss was observed when birds received SS treatment diets, whereas improved drip loss results were associated with organic Se-Met supplemented diets. The author further states that inorganic Se may have contributed less to muscle tissue integrity, suggesting organic Se supplementation may potentially reduce raw meat drip losses, attributable to higher Se tissue

retention. Ibrahim *et al.* (2019) found significant improvements in drip and cooking losses (P<0.05) when broiler diets were supplemented with high levels of Se-Met compared to inorganic SS supplemented groups. These observations are consistent with those reported by Upton *et al.* (2008) suggesting oxidative mechanisms along with an increase in moisture losses from processed breast meat associated with SS supplementation. Oliveira *et al.* (2014) reported significant reductions in cooking losses (P<0.05) when birds received treatment diets supplemented with 0.60 mg/kg compared to 0.15 mg/kg (15.87% vs 21.92%).The findings of Bakhshalinejad *et al.* (2019) reported significant (P<0.05) improvements of both drip and cooking losses in treatment groups fed organic Se at a level of 0.30 mg/kg.

On the contrary, Silva *et al.* (2019) observed no difference (P>0.05) between dietary treatments in drip loss when inorganic SS was replaced with Se-yeast at different ages, although numerically lower cook loss values were observed when organic Se was incorporated into the treatment diets. The results obtained by this author showed that supplementing organic Se in broiler chickens throughout the entire period (1 d to 42 d) was not necessary, which indicated the potential of phase supplementation of organic Se while maintaining broiler quality and formulating costs. A study by Deniz *et al.* (2005) noticed organic Se at 0.30 mg/kg improved FCR in addition to significantly reducing whole carcass drip loss (P<0.01) in comparison to the control and inorganic Se supplemented groups. On the contrary, performance results by Silva *et al.* (2019) showed to be comparable across treatment groups by which inorganic Se was replaced by an organic Se source. Results by Oliveira *et al.* (2014) were in agreement with other researchers (Deniz *et al.*, 2005; Bakhshalinejad *et al.*, 2019), revealing Se source nor level of supplementation influencing portioned carcass yields.

Supplementing Se as a more readily available organic source may hold the potential to alleviate meat quality moisture losses while protecting cellular components and membranes from excessive cellular damages. Literature highlights the potential benefit of organic Se as being more prominent than inorganic sources with reference to the biochemistry of muscle tissue.

2.8.2 Zinc

Zinc, after iron, being the second-most abundant trace mineral vital for living organisms, is acknowledged for supporting skeletal development and maintenance (Sałek *et al.*, 2020), normal immune function (Cunningham-Rundles, 1993), antioxidant effects (Oteiza, 2012), meat colour maintenance (Zakaria *et al.*, 2017), skin quality (Salim *et al.*, 2010), and overall meat quality (Salim *et al.*, 2008). Organic forms of Zn, Zn methionine (Zn-Met) or Zn amino acid complex (ZnAA), are characterised by having a better bioavailability and

absorption than inorganic Zn supplements (Świątkiewicz *et al.*, 2001; Star *et al.*, 2012; Yu *et al.*, 2017; Sałek *et al.*, 2020).

Zinc source (organic vs inorganic) greatly influences the rate of absorption within the digestive tract of a broiler. Organic Zn supplements are more bioavailable due to the mineral being attached to an organic ligand in the form of amino acids, peptides, or proteins (Star *et al.*, 2012; Kwiecień *et al.*, 2017; De Grande *et al.*, 2020). The importance of more bioavailable Zn sources incorporated into broiler diets are not only to benefit the animal, but also to protect the environment from possible declining conditions (Zakaria *et al.*, 2017).

Inorganic Zn sources commonly used in the industry are zinc oxide (ZnO) or zinc sulphate ($ZnSO_4$). Zinc sulphate has a higher bioavailability when compared to ZnO (Wedekind & Baker, 1990; Pesti & Bakalli, 1996). The use of organic Zn sources as opposed to inorganic ZnO or $ZnSO_4$ is a common practice in the broiler industry (Zakaria *et al.*, 2017; Kwiecień *et al.*, 2017). The recommended level of Zn supplementation according to the NRC (1994) is 40 mg/kg irrespective of the source, yet diets are often formulated well above these recommendations. Burrell *et al.* (2004) found that feeding a diet with total dietary Zn levels of 110 mg/kg optimised broiler BWG compared to a negative control diet. The author explains the higher dietary Zn requirement to be related to an accelerated growth rate of the birds.

A proper balance of Zn in feed mixtures needs to be revised due to the variable contents of concentrated poultry diets. Disproportionate supplementation of inorganic Zn into broiler diets potentially leads to excessive environmental pollution due to the relatively low efficacy of this element. Previous studies reported increased Zn excretion with greater Zn dietary levels (Mohanna & Nys, 1999). Burrell *et al.* (2004) observed a reduction in total Zn excretion levels when broilers were fed organic Availa®Zn complex compared to $ZnSO_4$. In nature, Zn is naturally bound to phytate, a major antagonist reducing the bioavailability and absorption of this element to animals (De Grande *et al.*, 2020). Lower isomers of phytic acid (InsP6 and InsP5) appear to have depressing effects on Zn (Humer *et al.*, 2015). Additionally, the use of phytase catalyses the stepwise hydrolysis of phytate, improving the digestibility and absorption of trace minerals (Sebastian *et al.*, 1998; Star *et al.*, 2012). Świątkiewicz *et al.* (2001) reported improved broiler performance and tibia Zn content in birds fed diets supplemented with phytase.

Zinc forms part of the copper-zinc superoxide dismutase enzyme, which functions as an antioxidant, protecting cells against harmful oxygen radicals (Oteiza, 2012). Zinc deficiencies are generally a cause of oxidative stress; therefore, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) are key antioxidative stress markers measured in the plasma to evaluate oxidative stress (De Grande *et al.*, 2020). A study conducted by De

Grande *et al.* (2020) showed that ZnAA supplementation, as opposed to inorganic $ZnSO_4$, appeared to alleviate oxidative stress supported by a reduction in MDA levels in the plasma at the end of the starter phase (day 10). The author also observed a significantly lower plasma GPx activity at slaughter age (day 36) when broilers were fed ZnAA, suggesting a lower requirement for antioxidant activity.

Sunder *et al.* (2008) assessed the stress index in birds as the ratio between heterophils and lymphocytes and found a significantly ($P \leq 0.05$) wider ratio in diets without Zn supplementation. At an inclusion level of 40 mg/kg of inorganic Zn, the ratio declined significantly ($P \leq 0.05$), suggesting Zn supplementation was useful in reducing stress in young broilers. Overall, it is a common practice to supplement broiler diets with Zn to prevent associated Zn-deficiency syndromes such as reduced growth, impaired immunity, and feathering, as well as the occurrence of skin lesions (Star *et al.*, 2012).

Scientific studies have reported improved growth performance at a total dietary Zn level of 110 mg Zn/kg (Burrell *et al.*, 2004), and 90 mg Zn-Gly/kg (Feng *et al.*, 2010). Earlier studies reported no effect on growth performance by either Zn level or source (Rossi *et al.*, 2007; Sunder *et al.*, 2008; Saenmahayak *et al.*, 2012; Star *et al.*, 2012; Zakaria *et al.*, 2017). However, broiler diets without Zn supplementation negatively impacted body weight gain ($P<0.01$) and feed conversion ($P<0.05$) according to Burrell *et al.* (2004). In the study conducted by Saenmahayak *et al.* (2012), similar to growth performance, the dietary treatments did not affect ($P>0.05$) carcass yield, nor carcass portion yields. On the contrary, Jahanian & Rasouli (2015) found significant improvements in body weight gain ($P<0.01$), and carcass meat yields ($P<0.05$) during the period in which inorganic Zn was substituted by Zn-Met. Świątkiewicz *et al.* (2001) found similar improvements, whereby the level of dietary Zn supplements had a significant ($P<0.01$) effect on broiler performance, apart from phytase addition. When evaluating the relative bioavailability of Zn source on all the assessed parameters in broilers receiving dietary treatments without phytase, the author reported ZnAA remained a better supplement than $ZnSO_4$.

Previous studies by Zakaria *et al.* (2017) stated no meat quality characteristics except for shear force was affected by Zn source or level. The shear force of breast meat from broilers fed inorganic ZnO (3.53 kg/cm^3) was higher ($P<0.05$) compared to broilers fed a combination of ZnO and organic ZnMet (4.14 kg/cm^3; Availa®Zn). A lower shear force value indicates meat of a higher quality associated with more tender and juicy characteristics. This lack of Zn supplementation effect on meat quality characteristics is similar to Saenmahayak *et al.* (2012); however, a significant ($P<0.05$) increase in fillet drip loss was noted when birds were fed

organic Zn 24 h postharvest, along with a significant (P<0.05) increase in redness (a*) after 28 d of storage.

The ability of Zn to bind myoglobin, which increases meat oxygenation, may explain the outcome on meat colour. Liu *et al.* (2011) obtained a numerical increase in redness (a*) of breast meat, lower shear force values in the thigh muscle (P<0.10), reduced drip loss in the breast and thigh muscles (P<0.90), and higher 24 h pH values (P<0.05) when broilers fed diets containing Zn compared to the control diet with no supplemental Zn. However, there was no significance (P>0.10) between these parameters among Zn level (60, 120, 180 mg/kg) or source in the dietary treatments. On the contrary, Salim *et al.* (2011) found significant improvements in WHC (P<0.05) when broilers were fed either 40 mg/kg or 80 mg/kg of organic Zn.

Rossi *et al.* (2007) concluded organic Zn supplementation enhanced carcass appearance as Zn contributes to skin quality maintenance by way of minimising skin tearing. A study conducted by Downs *et al.* (2000a) showed that a combination of ZnAA (40 mg/kg Zn/kg diet) and vitamin E (48 IU vitamin E/kg diet) supplementation may prove synergistic effects at decreasing cellulitis in broiler chickens. Cellulitis (infectious process; IP) is a pathological condition resulting from dermal/subdermal bacterial colonisation caused by skin damages that triggers an immune response and forms a plaque-like accumulation at the gateway to the injury (Downs *et al.*, 2000a, 2003). This leads to extensive economical losses because it requires the elimination of immune cell proliferation prior to processing, beyond reducing meat quality, and whole carcass condemnation. Underlining the promise of nutritional manipulation through the use of ZnAA and vitamin E complex to decrease the occurrence of such pathological conditions (Downs *et al.*, 2003). Previous studies reported immunological responses with Zn supplementation at 80 mg/kg (Sunder *et al.*, 2008) and an increase in IgA at 90 mg Zn-Gly/kg (Feng *et al.*, 2010).

The supplementation of Zn, irrespective of its form, does hold promising outcomes on broiler performance and meat quality characteristics as discussed by the literature. However, organic Zn being more bioavailable to the bird enables more readily utilisation of the element in an efficient manner, not only on performance but also on final product presentation and quality.

2.9 Conclusion

It has become evident from the literature studied that Zn and Se have the potential to influence broiler production explicitly owing to the form in which these microminerals are included into poultry diets. Acting as a positive component in cell membrane integrity post-

mortem, Zn and Se could facilitate improving WHC and decreasing cooking losses (Choct *et al.*, 2004; Rajashree *et al.*, 2014; Saleh *et al.*, 2018; Ibrahim *et al.*, 2019). Traditionally, inorganic trace mineral sources are included in poultry diets; however, the use of this mineral forms complexes with other dietary components obstructing their absorption, leading to increased environmental waste of inorganic minerals and hindering broiler performance (Costa *et al.*, 2010; Saenmahayak *et al.*, 2012).

Properties influencing quantifiable aspects of meat quality are indispensable for processors with the aim of profitable secondary processing. The substitution of inorganic with organic trace minerals has been suggested, not only due to the higher efficacy of the minerals but also because of lower quantities needed in comparison to inorganic counterparts. However, due to the relative expense of complexed organic trace minerals higher inclusions of inorganic products are considered to be more cost-effective. Research is limited on the topic of trace mineral sources and relative supplementation strategies to reduce such moisture losses.

Taking into consideration the above-mentioned problem statement, the necessity to incorporate organic forms of microminerals into poultry diets employing a duration of supplementation on environmental excretion, broiler performance, and meat quality highlights the important issue for this current research focus. There is paucity of research on improving broiler meat quality at least-cost basis by implementing periodical supplementation of trace minerals. This study aimed to evaluate different Zn and Se sources and the duration of supplementation as a possible strategy for improving performance and meat quality in broilers.

Chapter 3
Materials and methods

3.1 Introduction

The objective of this research was to evaluate overall broiler performance (FI, BW, FCR, mortality) and meat quality characteristics of breast meat (pH, colour, drip and cook loss) of broilers fed inorganic or organic sources of Zn and Se throughout the duration of the trial (0 - 32 d), or during the finisher phase (21 - 32 d). The study was conducted at the Hillcrest Experimental Farm, the University of Pretoria, in the large 96-pen poultry house at the broiler unit. Use of animals and approval for all experimental protocols was granted by the Animals Ethics Committee (AEC) of the University of Pretoria (NAS231/2020).

A completely randomised design involving a 4 x 2 factorial arrangement with four combinations of mineral sources supplemented on an *iso* basis (inorganic Zn and inorganic Se, organic Zn and inorganic Se, inorganic Zn and organic Se, organic Zn and organic Se) and two durations of supplementation (0–32 days of age and 21–32 days of age) were used to assign pens to various treatments. The house consisted of 12 blocks (A-L) in total, six blocks on each side to account for environmental effects such as spatial differences among and within the research facility. Each block was made up of eight pens, allowing all eight dietary treatments to be replicated 12 times (Appendix 2). The research facility was environmentally controlled and divided into two separate parts with 48 pens on each side, forming 96 pens in total. Treatment allocation per pen and blocking design are shown in Appendix 1 and 2.

3.2 Birds and housing

The poultry research facility was disinfected a week before chick arrival to ensure a sterile environment for placement of day-old chicks. Strict biosecurity measures were in effect using disinfectant foot baths and gumboots assigned to the research house to prevent the entry of outside pathogens. Personnel entering the house were not allowed to visit other poultry farms or research houses. The drinking lines were flushed before placement to ensure clean, fresh water upon arrival and both sides of the 96-pen broiler house were pre-heated to 36ºC. Before placement, chicks were randomly selected and placed into crates whereby each bird received either a pink or blue neck tag containing a specific number range designated to a pen. The colour of the neck tags was alternating between the pens and rows within the house allowing identification of incorrectly placed chicks. The birds were weighed to obtain placement weights for each individual pen.

A total of 1 920 vaccinated male day-old Ross 308 broiler chicks were obtained from National Chicks Hatchery (Lynnwood Ridge, 0040) with an average body weight of 40 g from 42-week-old breeders. The chicks were randomly distributed into 96 pens allowing placement of 20 chicks per pen with a stocking density of 9 birds/m^2. The pen dimensions were 1.5 m x 1.5m (±2.25m^2). The surface of the pens was evenly covered with clean wooden shavings and each pen was equipped with a tube feeder and a drinking line with six nipples during the 32-day trial period. Each pen had an allocated bin to retain treatment diets of known weight used to fill feeders. The nipple lines were elevated weekly to facilitate correct drinking behaviour and space amongst broilers within a pen.

During the first seven days of the trial, additional bell drinkers, pan feeders, and chick paper were placed into each pen to stimulate and maximise feeding and drinking behaviour. Chick care for the first week of the trial involved monitoring chick behaviour, house and litter temperatures, lighting, and ventilation twice daily, which were adjusted accordingly. The birds were exposed and reared according to the lighting and temperature practices from the Broiler Management Handbook (Aviagen, 2018). During the first seven days, 23 hours of light and one hour of darkness were applied, whereafter 18 hours of light and six hours of darkness were set for the remainder of the trial. The house temperature was initially set at 36 ºC, whereafter it was gradually dropped to 22 ºC and kept constant for the remainder of the trial according to the management guide (Aviagen, 2018). Birds suffering from illness or disabilities were culled by cervical dislocation and weighed along with mortalities by trained personnel.

3.3 Dietary treatments

A three-phase feeding programme was implemented during the course of the trial: a crumbled starter (0–10 d), a pelleted grower (10–21 d), and a pelleted finisher diet (21–32 d). The birds had *ad libitum* access to feed and water during the 32-day trial period. A standard maize–soya broiler diet was formulated for the study with dietary compositions shown in Table 3.1.

The dietary treatments (T) were formulated to meet or exceed the NRC (1994) requirements and mixed using a 1.5 t fountain blender at Simple Grow Agricultural Services feed mill (M26 Knoppieslaagte 385-Jr, 0157). A large batch of basal diet was mixed, whereafter the batch was divided into aliquots of eight dietary treatments. A maize mineral-carrier premix was created beforehand using one kilogram of ground yellow maize containing different sources of Zn and Se correlating to each of the treatments. The carrier-premix was slowly added on top of the basal diet while blending took place. Inorganic treatment diets (1, 5, 6, 7, and 8) were mixed together and divided into relevant quantities for individual treatments for the starter and grower dietary phases. Thereafter, T (2, 3 and 4) were mixed

individually. For the finisher phase diets, treatments which corresponded in mineral content were mixed together after a basal batch was blended.

Feed samples from each phase of treatment diets were collected and analysed for proximates, total Zn, and Se levels. Zinc sulphate ($ZnSO_4$) and sodium selenite (SS; Na_2SeO_3) served as conventional inorganic sources obtained from Nutroteq (Sunderland Ridge, Centurion, 0157). The mineral availability of $ZnSO_4$ and SS was 35% and 4.5%, respectively. The organic counterparts added into the diets were on an *iso* basis in the form of metal amino acid complexes namely, zinc-methionine (Availa®Zn; 12%) and zinc-L-selenomethionine (Availa®Se; 4%) supplied by Zinpro (Zinpro Performance Minerals, Zinpro Corporation). Zinc sulphate was placed in a desiccator while weighing took place in the laboratory as it is known to absorb moisture from its surroundings (Cao *et al.*, 2000).

The treatment descriptions are indicated in Table 3.2. This study did not investigate different mineral inclusion levels, therefore; the levels remained constant throughout the entire trial. The control diet (T1) consisted completely of inorganic sources formulated to meet the trace mineral requirements of broilers using 100 mg/kg Zn as $ZnSO_4$, and 0.30 mg/kg Se as inorganic SS. In short, organic minerals replaced inorganic minerals on an *iso* basis keeping the inclusion levels of Zn and Se constant. Treatment 2 contained 60 mg/kg of $ZnSO_4$, 40 mg/kg of Availa®Zn (60% inorganic and 40% organic), and 0.30 mg/kg of SS. Treatment 3 was formulated with 100 mg/kg of $ZnSO_4$, 0.15 mg/kg of Availa®Se, and 0.15 mg/kg of SS (50% inorganic and 50% organic). As for Treatment 4, $ZnSO_4$ and SS were partially replaced with Availa® minerals at 60 mg/kg of $ZnSO_4$ and 40 mg/kg of Availa®Zn (60% inorganic and 40% organic), along with 0.15 mg/kg of Availa®Se and 0.15 mg/kg of SS (50% inorganic and 50% organic).

The composition of T5, T6, T7, and T8 were identical to the above-mentioned treatment descriptions only differing in the duration of organic source supplementation during the finisher phase, constructing the 4 x 2 factorial. None of the treatments consisted of 100% organic minerals as these sources partially substituted inorganic minerals to add up to the fixed inclusion level of 100 mg/kg of Zn and 0.35 mg/kg of Se. To further describe the duration factorial; T2, T3, and T4 were formulated to contain both inorganic and organic sources of Zn and Se in the starter, grower, and finisher periods. The inorganic Zn and Se minerals were partially replaced (*iso* basis) by organic (Availa®) counterparts in T5, T6, T7 and T8 during the finisher period. These treatments contained inorganic Zn (100% $ZnSO_4$) and Se (100% SS) sources during the starter (0–10 d) and grower (10–21 d) period whereby organic sources partially replaced inorganic sources during the finisher period (21–32 d).

Table 3.1 Nutrient composition (%) of the starter, grower, and finisher diet

Feed ingredient (%)	Starter (0–10 d)	Grower (10–21 d)	Finisher (21–32 d)
Maize	56.93	64.52	64.93
Soya oilcake (44%)	30.10	23.80	23.10
Full-fat soya	4.70	5.00	5.00
Sunflower oilcake	3.00	3.00	3.00
Limestone	1.67	1.13	1.02
Soya oil	0.50	0.59	1.30
Monodicalcium phosphate	1.35	0.58	0.35
Salt	0.16	0.15	0.15
Choline chloride	0.05	0.04	0.04
Sodium carbonate	0.23	0.17	0.17
Lysine (78%)	0.29	0.31	0.30
DL-Methionine (98%)	0.30	0.29	0.28
Valine	0.01	0.02	0.03
Threonine (98%)	0.15	0.07	0.07
Vitamin–mineral premix[1]	0.30	0.25	0.20
Cycostat (Robenidine 6.6%)	0.05	0.05	0.05
Zinc bacitracin	0.05	0.05	0.05
Syncra[2]	0.10	0.10	0.10
Phytase	0.02	0.02	0.02
Calculated nutrient values (as is basis)			
Metabolisable energy (MJ/kg)	11.47	12.05	12.30
Ash, %	6.43	4.67	4.31
Crude protein, %	21.91	19.65	19.35
Crude fibre, %	3.01	2.95	2.94
Fat (EE), %	4.14	4.40	5.10
Moisture, %	10.40	10.67	10.65
Phosphorus, %	0.63	0.45	0.40
Calcium, %	1.09	0.72	0.65

[1]Vitamin–mineral premix excluding zinc and selenium
[2]Syncra = direct-fed microbial supplemented at a rate of 0.1 kg/t of feed in each basal diet

Table 3.2. Treatment description of inclusion levels of Zn and Se source (%) in the starter, grower, and finisher period

Treatment	Feeding period	Zinc (%)		Selenium (%)	
		Inorganic[1]	Organic[2]	Inorganic[1]	Organic[2]
T1 (Control)	Starter	100	-	100	-
	Grower	100	-	100	-
	Finisher	100	-	100	-
T2	Starter	60	40	100	-
	Grower	60	40	100	-
	Finisher	60	40	100	-
T3	Starter	100	-	50	50
	Grower	100	-	50	50
	Finisher	100	-	50	50
T4	Starter	60	40	50	50
	Grower	60	40	50	50
	Finisher	60	40	50	50
T5	Starter	100	-	100	-
	Grower	100	-	100	-
	Finisher	100	-	100	-
T6	Starter	100	-	100	-
	Grower	100	-	100	-
	Finisher	60	40	100	-
T7	Starter	100	-	100	-
	Grower	100	-	100	-
	Finisher	100	-	50	50
T8	Starter	100	-	100	-
	Grower	100	-	100	-
	Finisher	60	40	50	50

T1 (inorganic Zn, inorganic Se, 0–32 d); T2 (organic Zn, inorganic Se, 0–32 d); T3 (inorganic Zn, organic Se, 0–32 d); T4 (organic Zn, organic Se, 0–32 d); T5 (inorganic Zn, inorganic Se, 0–32 d); T6 (organic Zn, inorganic Se, 21–32 d); T7 (inorganic Zn, organic Se, 21–32 d); T8 (organic Zn, organic Se, 21–32 d)

[1]Inorganic Zn and Se supplemented on top of the basal diet in the form of zinc sulphate and sodium selenite, respectively

[2]Organic Zn and Se supplemented on top of the basal diet on an *iso* basis in the form of Availa®Zn and Availa®Se (Zinpro Performance Minerals, Zinpro Corporation), respectively

3.4 Chemical analyses

Representative samples of each dietary treatment from the starter, grower, and finisher were collected for singular analyses at ChemNutri Analytical (Cedar Lake Industrial Park, Olifantsfontein, 1665). All analyses were determined using the official methods of the AOAC. Samples were analysed for dry matter and ash (method 942.05; AOAC, 2000), moisture content (method 943.01; AOAC, 2000), crude fibre (method 962.09; AOAC, 2000); crude protein (method 988.05; AOAC, 2000), crude fat (method 920.39; AOAC, 2000), and Zn by Optical Emission Spectrometry (ICP-OES). Lastly, Se analyses were determined by Optical Mission Spectrometry (ICP-MS) at SGS (Brakenfell, Cape Town, 7560).

3.5 Measurements

3.5.1 Production performance

Broiler performance for body weight (BW) and feed intake (FI) was measured weekly (at 7, 14, 21, and 28 days), and at the beginning and end of each feeding phase (on day 10, 21, and 32). The birds were placed into portable crates of known weights by which the total weight was divided by the total number of birds in the pen after which they were placed back into their pens. On day 31, individual bird weights were measured, which enabled accurate bird selection within one standard deviation from the mean and served as the final body weight measures in the study.

Average feed intake per bird was measured by weighing the residual feed in the feeder and bin from each pen and subtracting it from the total amount of feed supplied, dividing it by the total number of birds in the pen. Body weight gain (BWG) and feed conversion ratio (FCR) were calculated from the production parameters, and the FCR corrected for mortalities (FCRmc).

$$\text{FCRmc} = \frac{(\text{Feed intake/pen})}{(\text{Body weight gained/pen} + \text{weight of mortalities/pen})}$$

3.5.2 Carcass characteristics

Due to personnel and facility capability restrictions, there were two sampling days (day 32 and day 34) during which three blocks from each house (1 and 2) were slaughtered to account for spatial and slaughter-day variation. Four birds per pen with a body weight closest to the treatment mean were selected and marked using treatment-specific colour leg bands attaching the neck tag to the shank of the carcass (Appendix 3).

To account for the slaughter of one block at a time, hourly intervals of staggered feed withdrawal for 12 hours were constantly maintained for each processing day. In this way, blocks were slaughtered an hour apart from each other. To exclude any possible influences of treatment variation, one bird from each treatment within a block, eight birds in total, were placed into portable live bird crates and transported to the meat laboratory situated on the University of Pretoria's Experimental Farm in Hillcrest. A total of 384 broilers were slaughtered.

The slaughter and dissection procedures were set up to simulate commercial processing practices. Immediately after arriving at the abattoir the birds were weighed to obtain live body weight (LBW), after which each bird was rendered unconscious by electrical stunning and placed into bleeding cones, followed by severing of the jugular vein. Each bird was bled (≥ 90s), scalded (2.5–3min at 53–55°C), de-feathered, and manually eviscerated. This process was time-sensitive and maintained to allow the slaughter of eight birds every 15 minutes, taking 60 minutes to slaughter one block (32 broilers) within a house. This allowed enough time for breast meat sampling and quality evaluations to take place the following day as described in Section 3.5.3.

After manual evisceration, the carcasses were weighed to obtain hot carcass weight (HCW). The leg band, accompanied by the neck tag, was removed from the shank and re-attached following a small incision into the wing using the same treatment-specific colour band. After the meat pH was measured, the carcasses were placed inside a cooler at ca. 3 – 4°C for 24 hours whereafter the cold carcass weight (CCW) was recorded. The dressing percentage was calculated by dividing the HCW by the LBW.

3.5.3 Meat quality evaluations

At 24 hours post-chilling, the breast muscles (*pectoralis* major and *pectoralis* minor) were removed from the carcass, deboned, and weighed together to calculate the breast yield relative to final body weight, whereafter the breast muscles were used for further analyses (Honikel, 1987; Berri *et al.*, 2005; Mazzoni *et al.*, 2015). The right breast fillet was used for pH and cook loss measurements, while the left breast fillet was used for colour and drip loss evaluations, as illustrated in Figure 3.1. The fillet portions were marked with the same number and sent to different analysing stations after portioning. In addition, the neck tag accompanied the right fillet, while the treatment colour band accompanied the left fillet, establishing a point of identification for further meat quality measures.

3.5.3.1 pH

The pH was measured on the cranial area of the right p. major using a portable pH meter (Hanna-HI98190, Hanna Instruments, Romania) and probe (Hanna-FC2323, Hanna

Instruments, Romania) equipped with a stainless-steel blade electrode for meat pH (FC099, 33mm, Hanna Instruments, Romania) calibrated at pH 4.0 and 7.0. The meat pH was measured at 15 min (pH_{15}) post-slaughter by inserting the probe directly into the breast muscle and 24 h (pH_{24}) post-slaughter by using a drop of deionised water at the same incision to improve the contact of the pH probe and the muscle (Qiao *et al.*, 2002; Petracci & Baéza, 2011). The probe was rinsed with deionised water after every four fillets and wiped clean using paper towelling.

3.5.3.2 Colour

The raw left fillet, without the skin, was prepared before colour measurements were taken by ensuring the muscle fibres were orientated in a horizontal direction. Meat colour was measured by the International Commission on Illumination (CIE) system 24 h after slaughter (Petracci & Baéza, 2011). The colour features were expressed as lightness (L*), redness (a*), and yellowness (b*) by a Konica Minolta Colorimeter (CR-400 Chroma Meter, Konica Minolta). The measurements were taken on the medial surface free of any discoloration or defects by placing the colorimeter upright to the surface of the breast fillet and recording the colour attributes (Petracci & Baéza, 2011; Córdova-Noboa *et al.*, 2018). The colorimeter was calibrated using a Minolta calibration plate (reference number 15233058), illuminate source C (Y = 85.7, x= 0.3166, y = 0.3242) before the onset of measurements, and cleaned with alcohol wipes after each fillet was measured.

3.5.3.3 Drip loss

Drip loss samples were taken 24 h post-chilling using a plastic stencil (± 6 cm x 5 cm x 2 cm) to obtain a sample weight of 50–55 g from the medial surface of the left p. major (Petracci & Baéza, 2011). The samples were marked using double-sided tape labelled with a number relating to the neck tag code and treatment band colour as described previously. All samples had a horizontal fibre direction as they were placed in covered plastic boxes on sieved plastic mesh inside a cooler at ca. 4–5°C . Each sample was wiped using paper towelling before being weighed at 0, 24, 48, and 72-hours post-sampling to measure drip loss, expressed as a percentage of the initial weight (Honikel, 1998).

$$\text{Drip loss (\%)} = \frac{\text{Sample weight after drip} - \text{Initial sample weight}}{\text{Initial sample weight}} \text{x}100$$

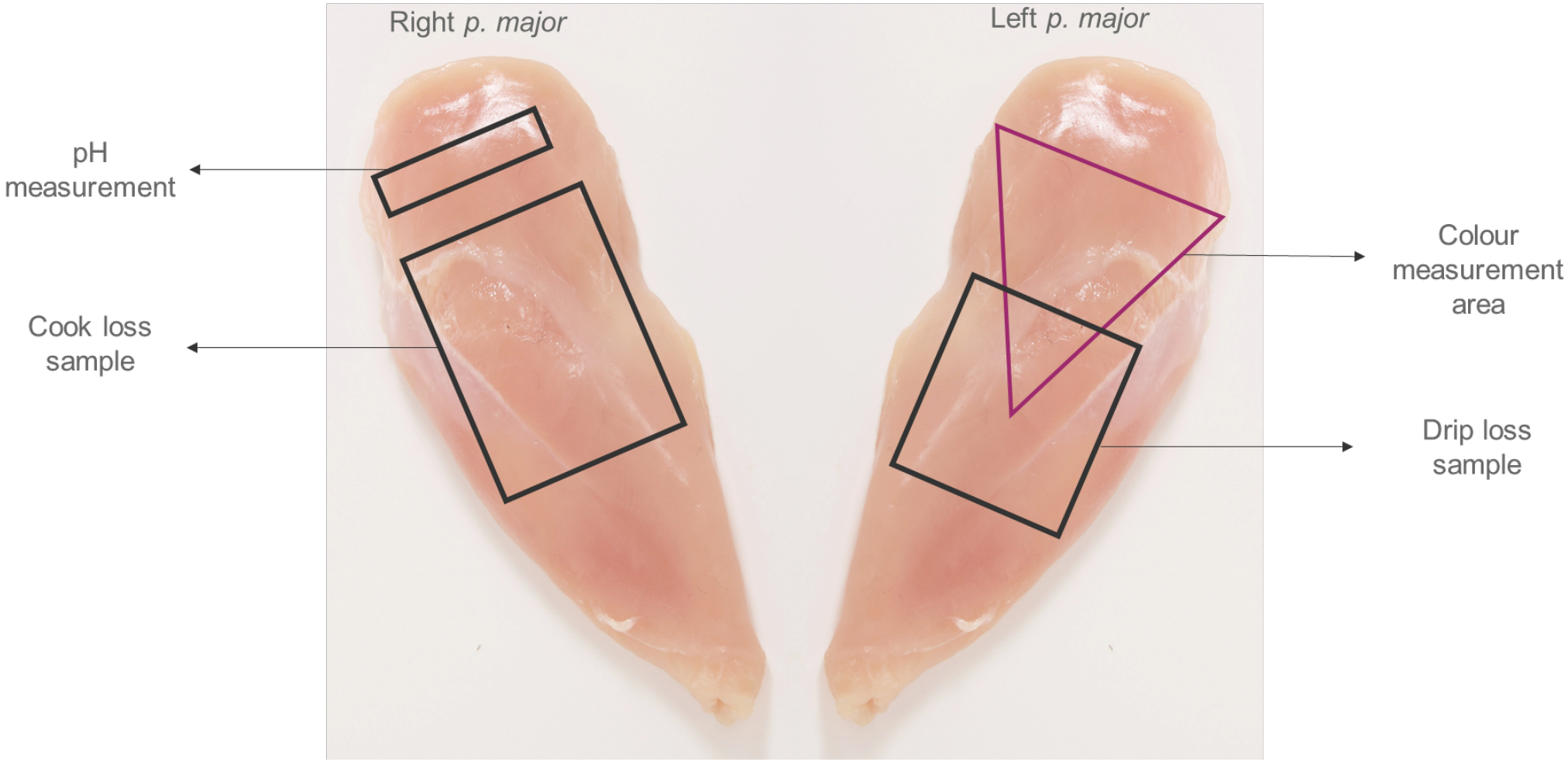

Figure 3.1. The sampling diagram of the right and left pectoralis major (adapted from Petracci *et al.*, 2013b)

3.5.3.4 Cook loss

Cook loss samples were taken from the medial area of the right breast fillet (p. major) after portioning using a plastic stencil (± 7 cm x 5 cm x 2 cm) to obtain a sample weight of 70–75 g. Each sample was weighed before being vacuum-sealed together into transparent plastic bags, marked accordingly using masking tape, and placed in a heated water bath at 75–80°C where the samples were cooked for 25 minutes until reaching an internal temperature of approximately 75°C (Honikel, 1998; Petracci & Baéza, 2011; Zakaria *et al.*, 2017). After cooking, each sample was removed and allowed to cool down for 25–30 minutes until reaching a temperature of approximately 25°C. Thereafter, the samples were removed from the transparent bags, lightly blotted using paper towelling to drain excess liquid, and reweighed to measure cook loss (%) using the equation from Honikel (1998).

$$\text{Cook loss (\%)} = \frac{\text{Weight of uncooked sample} - \text{Weight of cooked sample}}{\text{Weight of uncooked sample}} \text{ x } 100$$

3.6 Statistical analyses

The experimental data was subject to a one-way analysis of variance (ANOVA) in accordance with a completely randomised blocking design (Appendix 2) of seven dietary treatments with the Proc Mixed model using the Statistical Analysis System (SAS, 2021) for average effects. Treatment 5 was excluded from statistical analyses such as it was identical to T1, which served as the control in the study. Means and standard error were calculated. The pen mean served as the experimental replicate and the overall level of significance between means was set at $P<0.05$, while tendencies were stated when $P\leq0.10$ and determined by Fischer's test (Samuels, 1989). The main effects for seven dietary treatments with 12 replicates for performance, carcass, and meat quality parameters were tested. Mortality data were analysed using a Chi-square (Snedecor & Cochran, 1980). Analysis of Variance (ANOVA) with the mixed model was used for a repeated week or period measures. The linear mix model used to analyse the one-way ANOVA is described by the following equation:

$$Y_{ijkl} = \mu + T_i + W_j + H_k + B(H)_l + TW_{ij} + e_{ijkl}$$

where Y_{ijkl} = variable studied during the period

μ = overall mean of the population

T_i = effect of the i^{th} treatment

W_j = effect of the j^{th} week or phase

H_k = effect of the k^{th} house

$B(H)_l$ = effect of the l^{th} block in house

TW_{ij} = effect of the ij^{th} interaction between treatment and house

e_{ijkl} = error associated with each Y

All dietary treatments without the control were subjected to a 3 x 2 factorial between the fixed effects of mineral source ($ZnSO_4$, Availa®Zn, SS, or Availa®Se) and duration of supplementation (0–32 d or 21–32 d) to obtain main effects using the Proc Mixed model of the Statistical Analysis System (SAS, 2021). The linear mix model used to analyse the two-way ANOVA is described by the following equation:

$$Y_{ijklm} = \mu + D_i + S_j + H_k + B(H)_l + W_m + DS_{ij} + DW_{im} + SW_{jm} + DSW_{ijm} + e_{ijklm}$$

Where Y_{ijklm} = variable studied during the period

μ = overall mean of the population

D_i = effect of the i^{th} duration

S_j = effect of the j^{th} source

H_k = effect of the k^{th} house

$B(H)_l$ = effect of the l^{th} block in house

W_m = effect of the m^{th} week or phase

DS_{ij} = effect of the ij^{th} interaction between duration and source

DW_{im} = effect of the im^{th} interaction between duration and week or phase

SW_{im} = effect of the im^{th} interaction between source and week or phase

DSW_{ijm} = effect of the ijm^{th} interaction between duration, source and week or phase

e_{ijklm} = error associated with each Y

Chapter 4
Results

4.1 Experimental diets

The experimental treatments were fed as starter, grower, and finisher mixed diets by which the formulated (Table 3.1) and analysed (Table 4.1) diet will be reviewed in this section. The crude protein content of the starter, grower, and finisher diets was within range of the formulated values as presented in Table 4.1. The starter diet consisted of higher levels of protein to enable optimal growth compared to the finisher diet, with 20.99% and 19.19%, respectively. The dry matter (DM) content of a diet is the moisture-free, usable content remaining after a sample has been dried in the laboratory oven. The DM of the starter, grower, and finisher diets were 89.29, 89.84, and 90.19%, respectively. The ash content (%) of the starter, grower, and finisher diets happened to be relatively lower than the formulated values. Ash is an indicator of the total inorganic (mineral) content of the feed; however, does not specify the exact quantity of each mineral. Therefore, mineral analysis of Zn and Se for each treatment was carried out as shown in Table 4.2. The analysed Zn values in the treatment diets ranged from 125 to 145 mg/kg of total Zn when formulated to total 100 mg/kg of Zn. These values are expected and within an acceptable range.

Table 4.1 Analysed nutrient composition (%) in the starter, grower, and finisher diets (as is basis)

Nutrients	Starter (0–10 d)	Grower (10–21 d)	Finisher (21–32 d)
Ash, %	4.68	3.93	3.73
Crude protein %	20.99	19.26	19.19
Crude fibre, %	3.53	3.57	3.47
Fat (EE), %	4.02	4.43	4.98
Moisture, %	10.71	10.16	9.81

Table 4.2 Zn and Se analysed values in the starter, grower, and finisher diets

Treatment	Zn[1] (mg/kg)	Se[2] (mg/kg)
Starter		
1, 5, 6, 7	127.07	0.95
2	145.44	0.75
3	126.22	0.67
4	125.34	0.69
Grower		
1, 5, 6, 7	139.88	0.51
2	132.41	0.66
3	125.53	0.64
4	143.27	0.65
Finisher		
1	134.43	0.78
2, 5	134.84	0.65
3, 6	135.09	0.52
4, 7	134.16	0.50

T1 (100% ZnSO4, 100% SS; 0–32 d); T2 (40% Availa®Zn, 100% SS; 0–32 d); T3 (100% ZnSO4, 50% Availa®Se; 0–32 d); T4 (40% Availa®Zn, 50% Availa®Se; 0–32 d); T5 (40% Availa®Zn, 100% SS; 21–32 d); T6 (100% ZnSO4, 50% Availa®Se; 21–32 d); T7 (40% Availa®Zn, 50% Availa®Se; 21–32 d)
[1]Zn inclusion level irrespective of source = 100 mg/kg
[2]Se inclusion level irrespective of source = 0.30 mg/kg

4.2 Production performance

4.2.1 Body weight

4.2.1.1 Weekly average body weight

The treatment effects on the weekly average body weight of broilers from 0–32 d are shown in Table 4.3. All chicks had similar initial body weights ranging from 39.48 g to 40.92 g. There were no significant differences (P>0.05) observed in weekly body weight measurements between treatments over the total feeding period. A trend (P=0.0715) was observed during the first week of production whereby birds fed various dietary treatments had differences in body weight gained. Treatment 6 differed from T2, T4, and T5, where T7 tended to differ from T1, T2, T4, and T5, with T3 observed with no significant difference compared to other treatments. This indicated T1 to be different from T7, having the heaviest BW. Although this finding was not significant, broiler BW is of economic importance having an effect on slaughter weight. This observation has no reasonable explanation, as these treatments were identical in dietary composition and mineral source during the first week of the trial.

The effect of different Zn and Se sources and duration of supplementation on the weekly average body weight of broilers from 0–32 d are shown in Table 4.4. On day 7, there was a tendency (P=0.0731) towards heavier body weights when birds were fed either organic Se along with inorganic Zn, or organic sources of Zn and Se together. The mineral source had no further influence on broiler body weight after the first week of the trial. Broilers fed treatments containing inorganic minerals showed a tendency to be heavier (P=0.0899) than the birds receiving organic mineral diets during the first week of the study. The duration for which organic sources were introduced into the diets had no further effect (P>0.05) on body weight after the first week. No significant (P>0.05) interactive effects were observed on weekly body weight measurements.

4.2.1.2 Body weight for the starter, grower, and finisher periods

The treatment effects on the average body weight of broilers for the starter, grower, and finisher periods are shown in Table 4.5. During the starter, grower, and finisher periods, no significant (P>0.05) treatment effects were observed on broiler body weight. However, in the starter period, birds fed T5 had the second-lowest body weights (285.6 g), apart from T4 (284.5 g), but yielded the heaviest (P>0.05) birds during the finisher period (2166 g). Broiler body weight was affected neither by source nor duration of supplementation during the starter, grower, or finisher periods as seen in Table 4.6. No significant (P>0.05) interactive effects were observed for body weight during any of the feeding periods.

4.2.1.3 Weekly and cumulative body weight gain

The treatment effects on weekly and cumulative average body weight gain of broilers from 0–32 are presented in Table 4.7. Results from this study indicate BW gain from broilers

fed organically supplemented diets (*iso* basis) were comparable with birds receiving the control diet. There were no weekly changes observed from day 21 in BW gain among treatments when organic partially replaced inorganic sources. Similarly, cumulative BW gain of the entire feeding period was not influenced (P>0.05) by any of the dietary treatments. Weekly and cumulative BW gain was affected (P>0.05) neither by source nor duration of supplementation (Table 4.8). Replacing inorganic sources of Zn and Se with organic forms during the last two weeks of the trial showed to have no influence on broiler BW gain. No significant (P>0.05) interactive effects were observed for BW gain.

Table 4.3 The treatment effects on weekly average body weight (g) of broilers from 0–32 days of age[1]

Treatments (T)	Day 0	Day 7	Day 14	Day 21	Day 28	Day 32
T1 (Control)	39.48	176.3^{z}	510.8	1065	1805	2143
T2	40.47	174.5^{yz}	506.3	1055	1798	2137
T3	40.34	178.0^{xyz}	503.3	1051	1774	2110
T4	40.51	175.3^{yz}	506.0	1049	1802	2134
T5	40.92	174.3^{yz}	508.1	1057	1813	2166
T6	39.92	181.6^{xz}	514.4	1067	1804	2133
T7	39.73	182.4^{x}	510.0	1056	1783	2128
Standard error	0.387	2.328	4.983	9.789	15.264	18.152
P-value	0.1341	0.0715	0.7741	0.8281	0.5807	0.5228

T1 (100% $ZnSO_4$, 100% SS; 0–32 d); T2 (40% Availa®Zn, 100% SS; 0–32 d); T3 (100% $ZnSO_4$, 50% Availa®Se; 0–32 d); T4 (40% Availa®Zn, 50% Availa®Se; 0–32 d); T5 (40% Availa®Zn, 100% SS; 21–32 d); T6 (100% $ZnSO_4$, 50% Availa®Se; 21–32 d); T7 (40% Availa®Zn, 50% Availa®Se; 21–32 d)
$^{x\text{-}z}$Column means with different superscript indicate a tendency of treatments to differ (P<0.10)
[1]One-way ANOVA to determine differences between dietary treatments

Table 4.4 The effect of different Zn and Se sources and duration of supplementation on weekly average body weight (g) of broilers from 0–32 days of age[1]

Effects	Day 0	Day 7	Day 14	Day 21	Day 28	Day 32
Source[2, 3]						
Org Zn + InO Se	40.70	174.4^{y}	507.2	1056	1806	2152
InO Zn + Org Se	40.13	179.8^{x}	508.8	1059	1789	2122
Org Zn + Org Se	40.12	178.8^{x}	508.0	1053	1793	2131
Standard error	0.284	1.742	3.450	6.567	10.425	11.624
P-value	0.2676	0.0731	0.9452	0.8111	0.4908	0.1861
Duration[4]						
0–32 d	40.44	175.9^{y}	505.2	1052	1792	2127
21–32 d	40.19	179.4^{x}	510.8	1060	1800	2143
Standard error	0.232	1.423	2.817	5.362	8.512	9.491
P-value	0.4486	0.0899	0.1625	0.2607	0.4712	0.2586

^{x,y}Column means with different superscript indicate a tendency to differ (P<0.10)
[1]Two-way ANOVA to determine the main effects of mineral source and duration of supplementation
[2]Inorganic Zn = zinc sulphate; inorganic Se = sodium selenite; organic Zn and Se = Availa®
[3]InO = inorganic; Org = organic
[4]Supplementation throughout trial = 0-32 d; supplementation only during finisher phase = 21-32 d

Table 4.5 The treatment effects on average body weight (g) of broilers for the starter, grower, and finisher period[1]

Treatments (T)	Starter (0–10 d)	Grower (10–21 d)	Finisher (21–32 d)
T1 (Control)	287.5	1065	2143
T2	285.1	1055	2137
T3	286.4	1051	2110
T4	284.5	1049	2134
T5	285.6	1057	2166
T6	293.1	1067	2133
T7	293.5	1056	2128
Standard error		12.120	
P-value		0.7507	

T1 (100% $ZnSO_4$, 100% SS; 0–32 d); T2 (40% Availa®Zn, 100% SS; 0–32 d); T3 (100% $ZnSO_4$, 50% Availa®Se; 0–32 d); T4 (40% Availa®Zn, 50% Availa®Se; 0–32 d); T5 (40% Availa®Zn, 100% SS; 21–32 d); T6 (100% $ZnSO_4$, 50% Availa®Se; 21–32 d); T7 (40% Availa®Zn, 50% Availa®Se; 21–32 d)
[1]One-way ANOVA to determine differences between dietary treatments

Table 4.6 The effect of different Zn and Se sources and duration of supplementation on average body weight (g) of broilers for the starter, grower, and finisher period[1]

Effects	Starter (0–10 d)	Grower (10–21 d)	Finisher (21–32 d)
Source[2, 3]			
Org Zn + InO Se	285.3	1056	2152
InO Zn + Org Se	289.8	1059	2122
Org Zn + Org Se	289.0	1053	2131
Standard error		7.844	
P-value		0.1767	
Duration[4]			
0–32 d	285.3	1052	2127
21–32 d	290.7	1060	2143
Standard error		6.405	
P-value		0.7326	

[1]Two-way ANOVA to determine the main effects of mineral source and duration of supplementation
[2]Inorganic Zn = zinc sulphate; inorganic Se = sodium selenite; organic Zn and Se = Availa®
[3]InO = inorganic; Org = organic
[4]Supplementation throughout trial = 0-32 d; supplementation only during finisher phase = 21-32 d

Table 4.7 The treatment effects on weekly and cumulative average body weight gain (g) of broilers from 0–32 days of age[1]

Treatments (T)	Weekly					cum.[2]
	0–7	7–14	14–21	21–28	28–32	0–32
T1 (Control)	136.5	334.5	550.0	725.1	321.0	2102
T2	133.7	331.1	546.2	743.7	338.7	2096
T3	137.5	323.7	545.0	719.2	335.6	2069
T4	134.7	330.0	543.3	743.5	332.7	2093
T5	133.3	332.3	544.7	746.0	344.8	2124
T6	141.3	332.8	552.4	727.9	313.7	2092
T7	142.7	326.9	541.9	727.2	320.1	2087
Standard error			8.036			17.992
P-value			0.5266			0.5365

T1 (100% $ZnSO_4$, 100% SS; 0–32 d); T2 (40% Availa®Zn, 100% SS; 0–32 d); T3 (100% $ZnSO_4$, 50% Availa®Se; 0–32 d); T4 (40% Availa®Zn, 50% Availa®Se; 0–32 d); T5 (40% Availa®Zn, 100% SS; 21–32 d); T6 (100% $ZnSO_4$, 50% Availa®Se; 21–32 d); T7 (40% Availa®Zn, 50% Availa®Se; 21–32 d)
[1]One-way ANOVA to determine differences between dietary treatments
[2]Cumulative body weight gain

Table 4.8 The effect of different Zn and Se sources and duration of supplementation on weekly and cumulative average body weight gain (g) of broilers from 0–32 days of age[1]

Effects	Weekly					cum.[2]
	0–7	7–14	14–21	21–28	28–32	0–32
Source[3, 4]						
Org Zn + InO Se	133.5	331.7	545.5	744.8	341.7	2110
InO Zn + Org Se	139.4	328.3	548.7	723.6	324.7	2081
Org Zn + Org Se	138.7	328.4	542.6	735.3	326.4	2090
Standard error			5.387			11.527
P-value			0.1839			0.1956
Duration[5]						
0–32 d	135.3	328.3	544.8	735.5	335.7	2086
21–32 d	139.1	330.7	546.3	733.7	326.2	2101
Standard error			4.398			9.412
P-value			0.5703			0.2600

[1]Two-way ANOVA to determine the main effects of mineral source and duration of supplementation
[2]Cumulative body weight gain
[3]Inorganic Zn = zinc sulphate; inorganic Se = sodium selenite; organic Zn and Se = Availa®
[4]InO = inorganic; Org = organic
[5]Supplementation throughout trial = 0-32 d; supplementation only during finisher phase = 21-32 d

4.2.2 Feed intake

4.2.2.1 Weekly and cumulative feed intake

As shown in Table 4.9, there were no significant (P>0.05) treatment differences in weekly or cumulative broiler feed intake measured throughout the entire feeding period. Mineral source and duration of organic mineral supplementation had no significant (P<0.05) effect on weekly feed intake (Table 4.10). Also, cumulative feed intake was affected (P>0.05) neither by source nor duration of supplementation. No significant (P>0.05) interactive effects were observed for feed intake.

4.2.2.2 Feed intake during the starter, grower, and finisher periods

The treatment effects on feed intake of broilers for the starter, grower, and finisher growth periods are shown in Table 4.11. There were no significant (P>0.05) treatment differences observed on feed intake during the starter, grower, or finisher period. However, birds fed the inorganic control diet (T1; 0–32 d) showed a numerically higher feed intake during the finisher period compared to the other treatments.

The effect of different Zn and Se sources and duration of supplementation on feed intake of broilers for the starter, grower, and finisher periods are presented in Table 4.12. The source of Zn and Se had no significant (P>0.05) effect on feed intake during the starter, grower, or finisher periods. Also, the supplementation duration showed no significant effect on broiler feed intake during the production periods. No significant (P>0.05) interactive effects were observed for feed intake during any of the feeding periods.

Table 4.9 The treatment effects on weekly and cumulative feed intake (g) of broilers from 0–32 days of age[1]

Treatments (T)	Weekly					cum.[2]
	0–7	7–14	14–21	21–28	28–32	0–32
T1 (Control)	161.1	427.3	736.0	1076	591.1	3028
T2	170.0	424.0	731.1	1073	563.0	2955
T3	164.8	423.9	730.9	1047	552.6	2930
T4	168.0	426.9	727.0	1065	567.8	2967
T5	164.2	421.3	734.9	1076	554.2	2967
T6	166.7	433.3	740.3	1086	563.9	3025
T7	170.8	424.0	727.0	1085	511.9	3000
Standard error			14.440			44.646
P-value			0.5448			0.6619

T1 (100% $ZnSO_4$, 100% SS; 0–32 d); T2 (40% Availa®Zn, 100% SS; 0–32 d); T3 (100% $ZnSO_4$, 50% Availa®Se; 0–32 d); T4 (40% Availa®Zn, 50% Availa®Se; 0–32 d); T5 (40% Availa®Zn, 100% SS; 21–32 d); T6 (100% $ZnSO_4$, 50% Availa®Se; 21–32 d); T7 (40% Availa®Zn, 50% Availa®Se; 21–32 d)
[1]One-way ANOVA to determine differences between dietary treatments
[2]Cumulative feed intake

Table 4.10 The effect of different Zn and Se sources and duration of supplementation on weekly and cumulative feed intake (g) of broilers from 0–32 days of age[1]

Effects	Weekly					cum.[2]
	0–7	7–14	14–21	21–28	28–32	0–32
Source[3, 4]						
Org Zn + InO Se	167.1	422.7	733.0	1074	558.6	2961
InO Zn + Org Se	165.7	428.6	735.6	1067	558.3	2977
Org Zn + Org Se	169.4	425.5	727.0	1075	539.9	2984
Standard error			10.056			28.839
P-value			0.9435			0.8532
Duration[5]						
0–32 d	167.6	425.0	729.6	1062	561.2	2951
21–32 d	167.2	426.2	734.1	1082	543.4	2997
Standard error			8.210			23.547
P-value			0.2297			0.1679

[1]Two-way ANOVA to determine the main effects of mineral source and duration of supplementation
[2]Cumulative feed intake
[3]Inorganic Zn = zinc sulphate; inorganic Se = sodium selenite; organic Zn and Se = Availa®
[4]InO = inorganic; Org = organic
[5]Supplementation throughout trial = 0-32 d; supplementation only during finisher phase = 21-32 d

Table 4.11 The treatment effects on feed intake (g) of broilers for the starter, grower, and finisher period[1]

Treatments (T)	Starter (0–10 d)	Grower (10–21 d)	Finisher (21–32 d)
T1 (Control)	287.7	1039	1674
T2	297.4	1028	1631
T3	292.2	1029	1600
T4	295.6	1027	1633
T5	292.1	1033	1628
T6	294.9	1045	1661
T7	299.8	1024	1635
Standard error		19.669	
P-value		0.9262	

T1 (100% ZnSO4, 100% SS; 0–32 d); T2 (40% Availa®Zn, 100% SS; 0–32 d); T3 (100% ZnSO4, 50% Availa®Se; 0–32 d); T4 (40% Availa®Zn, 50% Availa®Se; 0–32 d); T5 (40% Availa®Zn, 100% SS; 21–32 d); T6 (100% ZnSO4, 50% Availa®Se; 21–32 d); T7 (40% Availa®Zn, 50% Availa®Se; 21–32 d)
[1]One-way ANOVA to determine differences between dietary treatments

Table 4.12 The effect of different Zn and Se sources and duration of supplementation on feed intake (g) of broilers for the starter, grower, and finisher period[1]

Effects	Starter (0–10 d)	Grower (10–21 d)	Finisher (21–32 d)
Source[2, 3]			
Org Zn + InO Se	294.7	1030	1630
InO Zn + Org Se	293.5	1037	1631
Org Zn + Org Se	297.7	1025	1634
Standard error		12.808	
P-value		0.9733	
Duration[4]			
0–32 d	295.1	1028	1622
21–32 d	295.6	1034	1641
Standard error		10.458	
P-value		0.6347	

[1]Two-way ANOVA to determine the main effects of mineral source and duration of supplementation
[2]Inorganic Zn = zinc sulphate; inorganic Se = sodium selenite; organic Zn and Se = Availa®
[3]InO = inorganic; Org = organic
[4]Supplementation throughout trial = 0-32 d; supplementation only during finisher phase = 21-32 d

4.2.3 Feed conversion ratio

4.2.3.1 Weekly and cumulative feed conversion ratio

The treatment effects on the weekly and cumulative feed conversion ratio of broilers from 0–32 days of age are shown in Table 4.13. There was no significant difference (P>0.05) observed in weekly FCR between the seven dietary treatments. Also, there were no significant differences (P>0.05) observed in FCR over the entire feeding period between birds fed different treatment diets. Birds had a numerically lower (P>0.05) cumulative FCR when diets were partially substituted with organic Zn (T5) during the finisher period (21–32 d).

The effect of different Zn and Se sources and duration of supplementation on weekly and cumulative feed conversion ratio of broilers from 0–32 days of age is represented in Table 4.14. In the current study, no significant (P>0.05) difference was observed among different Zn and Se sources for weekly and cumulative FCR. The duration of organic source supplementation showed a significant (P=0.0213) difference in FCR during the last week of the study. Partial organic source supplementation during the last two weeks of the study improved weekly FCR at 28–32 d of age compared to inorganic supplementation throughout the trial with 1.54 and 1.65, respectively. The duration of supplementation had no significant (P>0.05) effect on cumulative FCR, and no significant (P>0.05) interactive effects were observed for FCR.

4.2.3.2 The feed conversion ratio for the starter, grower, and finisher periods

The treatment effects on feed conversion ratio of broilers for the starter, grower, and finisher period are presented in Table 4.15. Birds receiving inorganic treatment diets (T1 and T6) during the starter period (0–10 d) obtained the lowest (1.16; P=0.0024) FCR compared to birds fed organic diets (T2 and T4); however, these treatments were non-significantly different from T3, T5, and T7. Birds fed T5 (1.46) and T7 (1.48) during the finisher period (21–32 d) had a significantly (P=0.0024) lower FCR compared to the other treatments.

As shown in Table 4.16, the source had a significant effect on FCR (P=0.0020) during the starter and grower period. In the starter period, the FCR among birds receiving organic Zn and Se (1.196) was non-significantly different (P>0.05) from birds fed organic Zn (1.201) or organic Se (1.173). However, birds fed organic Se with inorganic Zn had the lowest (P<0.05) FCR compared to birds fed organic Zn with inorganic Se. In the grower period, birds fed organic Se (1.348) had the highest (P<0.05) FCR compared to birds receiving organic Zn (1.331) or a combination of organic Zn and Se (1.339). Duration of organic mineral supplementation had no significant (P>0.05) effect on FCR during any of the feeding periods. No significant (P>0.05) interactive effect was observed in feed conversion ratio during any of the feeding periods

Table 4.13 The treatment effects on weekly and cumulative feed conversion ratio[1] (g:g) of broilers from 0–32 days of age[2]

Treatments (T)	Weekly					cum.[3]
	0–7	7–14	14–21	21–28	28–32	0–32
T1 (Control)	1.17	1.28	1.33	1.44	1.69	1.42
T2	1.27	1.28	1.33	1.44	1.65	1.41
T3	1.20	1.30	1.33	1.44	1.62	1.41
T4	1.25	1.29	1.34	1.41	1.67	1.41
T5	1.23	1.26	1.33	1.41	1.51	1.39
T6	1.17	1.30	1.34	1.47	1.66	1.43
T7	1.20	1.29	1.32	1.47	1.46	1.40
Standard error			0.039			0.011
P-value			0.2885			0.1730

T1 (100% ZnSO4, 100% SS; 0–32 d); T2 (40% Availa®Zn, 100% SS; 0–32 d); T3 (100% ZnSO4, 50% Availa®Se; 0–32 d); T4 (40% Availa®Zn, 50% Availa®Se; 0–32 d); T5 (40% Availa®Zn, 100% SS; 21–32 d); T6 (100% ZnSO4, 50% Availa®Se; 21–32 d); T7 (40% Availa®Zn, 50% Availa®Se; 21–32 d)
[1]Mortality corrected
[2]One-way ANOVA to determine differences between dietary treatments
[3]Cumulative feed conversion ratio

Table 4.14 The effect of different Zn and Se sources and duration of supplementation on mortality corrected weekly and cumulative feed conversion ratio (g:g) of broilers from 0–32 days of age[1]

Effects	Weekly					cum.[2]
	0–7	7–14	14–21	21–28	28–32	0–32
Source[3, 4]						
Org Zn + InO Se	1.25	1.27	1.33	1.42	1.58	1.40
InO Zn + Org Se	1.19	1.30	1.34	1.45	1.64	1.42
Org Zn + Org Se	1.22	1.29	1.33	1.44	1.56	1.40
Standard error			0. 024			0.007
P-value			0.2368			0.1313
Duration[5]						
0–32 d	1.24	1.29	1.33	1.43	1.65[a]	1.41
21–32 d	1.20	1.28	1.33	1.45	1.54[b]	1.40
Standard error			0.020			0.006
P-value			0.0213			0.3232

[a, b]Column means with the same superscript do not differ significantly ($P<0.05$)
[1]Two-way ANOVA to determine the main effects of mineral source and duration of supplementation
[2]Cumulative feed conversion ratio
[3]Inorganic Zn = zinc sulphate; inorganic Se = sodium selenite; organic Zn and Se = Availa®
[4]InO = inorganic; Org = organic
[5]Supplementation throughout trial = 0-32 d; supplementation only during finisher phase = 21-32 d

Table 4.15 The treatment effects on feed conversion ratio[1] (g:g) of broilers for the starter, grower, and finisher period[2]

Treatments (T)	Starter (0–10 d)	Grower (10–21 d)	Finisher (21–32 d)
T1 (Control)	1.16[b]	1.33	1.54[a]
T2	1.22[a]	1.33	1.51[a]
T3	1.18[ab]	1.35	1.51[a]
T4	1.21[a]	1.34	1.50[a]
T5	1.19[ab]	1.33	1.46[b]
T6	1.16[b]	1.35	1.54[a]
T7	1.18[ab]	1.34	1.48[b]
Standard error		0.014	
P-value		0.0024	

T1 (100% ZnSO4, 100% SS; 0–32 d); T2 (40% Availa®Zn, 100% SS; 0–32 d); T3 (100% ZnSO4, 50% Availa®Se; 0–32 d); T4 (40% Availa®Zn, 50% Availa®Se; 0–32 d); T5 (40% Availa®Zn, 100% SS; 21–32 d); T6 (100% ZnSO4, 50% Availa®Se; 21–32 d); T7 (40% Availa®Zn, 50% Availa®Se; 21–32 d)
[a, b]Column means with the same superscript do not differ significantly ($P<0.05$)
[1]Mortality corrected
[2]One-way ANOVA to determine differences between dietary treatments

Table 4.16 The effect of different Zn and Se sources and duration of supplementation on feed conversion ratio[1] (g:g) of broilers for the starter, grower, and finisher period[2]

Effects	Starter (0–10 d)	Grower (10–21 d)	Finisher (21–32 d)
Source[3, 4]			
Org Zn + InO Se	1.201[a]	1.331[b]	1.483
InO Zn + Org Se	1.173[b]	1.348[a]	1.523
Org Zn + Org Se	1.196[ab]	1.339[b]	1.493
Standard error		0.009	
P-value		0.0020	
Duration[5]			
0–32 d	1.203	1.341	1.505
21–32 d	1.177	1.338	1.494
Standard error		0.007	
P-value		0.2581	

[a, b]Column means with the same superscript do not differ significantly ($P<0.05$)
[1]Mortality corrected
[2]Two-way ANOVA to determine the main effects of mineral source and duration of supplementation
[3]Inorganic Zn = zinc sulphate; inorganic Se = sodium selenite; organic Zn and Se = Availa®
[4]InO = inorganic; Org = organic
[5] Supplementation throughout trial = 0-32 d; supplementation only during finisher phase = 21-32 d

4.2.4 Mortality rate

The effect of different Zn and Se sources and duration of supplementation had no significant (*P*>0.05) effect on mortality rate. Over the entire trial period, there were a total of 40 mortalities totalling to a rate of 2.08% (data not shown). There was no significant (*P*>0.05) main effect in terms of mineral source or duration of supplementation on mortality.

4.3 Carcass characteristics

The treatment effects on carcass characteristics measured in this study are presented in Table 4.17. Live weight, hot carcass weight, cold carcass weight, breast weight, carcass yield, and breast yield were non-significantly (*P*>0.05) different between treatments. Thus, none of the carcass characteristics measured in this study performed significantly different from the control. Overall, birds fed T5 numerically showed the most benefit from receiving organic mineral sources during the final growth stage.

The effect of different Zn and Se sources and duration of supplementation on carcass characteristics are presented in Table 4.18. The source of Zn and Se had no significant effect (*P*>0.05) on any of the carcass characteristics measured in this study. However, diets with a combination of organic Zn and Se (78.87 %) tended (*P*=0.0865) to obtain a higher carcass yield when compared to organic Zn (78.42 %), or organic Se (78.43 %). The duration of supplementation had a significant (*P*=0.0300) effect on broiler body weight before slaughter, signifying the partial replacement of inorganic with organic sources during the last growth stage (21–32 d) obtained a higher final body weight (2218 g and 2198 g) when compared to birds fed organic sources throughout the trial (0–32 d). Similarly, supplementation of organic minerals during the finisher period (21–32 d) showed a tendency (*P*=0.0897) to obtain a higher hot carcass weight when compared to organic source supplementation throughout the study (0–32 d) with 1742 g and 1728 g, respectively. Furthermore, the duration of organic mineral supplementation did not affect (*P*>0.05) cold carcass weight, breast weight, carcass, or breast yield. No significant (*P*>0.05) interactive effects were observed for carcass characteristics.

Table 4.17 The treatment effects on live weight, hot and cold carcass weight, carcass yield, breast weight, and breast yield[1]

Treatment (T)	Live weight (g)	Hot carcass weight (g)	Cold carcass weight (g)	Carcass yield (%)	Breast weight (g)	Breast yield (%)
T1 (Control)	2198	1727	1670	78.61	450.0	21.10
T2	2213	1734	1678	78.37	455.9	21.92
T3	2190	1721	1666	78.64	447.5	21.39
T4	2192	1729	1672	78.94	446.9	21.21
T5	2221	1747	1684	78.46	448.7	21.52
T6	2223	1738	1684	78.21	448.2	21.12
T7	2210	1742	1682	78.80	448.0	21.37
Standard error	10.630	9.437	9.442	0.238	4.294	0.268
P-value	0.1560	0.5179	0.7196	0.3672	0.8003	0.3522

T1 (100% ZnSO4, 100% SS; 0–32 d); T2 (40% Availa®Zn, 100% SS; 0–32 d); T3 (100% ZnSO4, 50% Availa®Se; 0–32 d); T4 (40% Availa®Zn, 50% Availa®Se; 0–32 d); T5 (40% Availa®Zn, 100% SS; 21–32 d); T6 (100% ZnSO4, 50% Availa®Se; 21–32 d); T7 (40% Availa®Zn, 50% Availa®Se; 21–32 d)
[1]One-way ANOVA to determine differences between dietary treatments

Table 4.18 The effect of different Zn and Se sources and duration of supplementation on carcass characteristics[1]

Effects	Live weight (g)	Hot carcass weight (g)	Cold carcass weight (g)	Carcass yield (%)	Breast weight (g)	Breast yield (%)
Source[2, 3]						
Org Zn + InO Se	2217	1740	1681	78.42^{y}	452.3	21.72
InO Zn + Org Se	2206	1730	1675	78.43^{xy}	447.9	21.26
Org Zn + Org Se	2201	1735	1677	78.87^{x}	447.5	21.29
Standard error	7.711	7.022	7.027	0.163	4.465	0.193
P-value	0.3461	0.5863	0.8258	0.0865	0.4874	0.1750
Duration[4]						
0–32 d	2198^{b}	1728^{y}	1672	78.65	450.1	21.51
21–32 d	2218^{a}	1742^{x}	1684	78.49	448.3	21.34
Standard error	6.296	5.734	5.738	0.133	3.646	0.158
P-value	0.0300	0.0897	0.1533	0.4142	0.6306	0.4526

$^{a, b}$Column means with the same superscript do not differ significantly ($P<0.05$)
$^{x, y}$Column means with different superscript indicate a tendency to differ ($P<0.10$)
[1]Two-way ANOVA to determine the main effects of mineral source and duration of supplementation
[2]Inorganic Zn = zinc sulphate; inorganic Se = sodium selenite; organic Zn and Se = Availa®
[3]InO = inorganic; Org = organic
[4]Supplementation throughout trial = 0-32 d; supplementation only during finisher phase = 21-32 d

4.4 Meat quality characteristics

The treatment effects on meat pH, colour, drip, and cook loss are represented in Table 4.19. Meat pH, colour, drip, and cook loss measured in this study did not perform significantly (P>0.05) differently from the control. However, the meat pH_{24} tended (P=0.0949) to be lower in the T2 (6.03) group compared to the control (6.08), T4 (6.09), and T7 (6.09). In addition, T2 was non-significantly different from T3 (6.07), T5 (6.06), and T6 (6.07). Overall, T6 presented overall better meat quality compared with the other dietary treatments.

The source of Zn and Se had no significant effect on pH_{15}, colour attributes, drip, or cook loss as seen in Table 4.20. The meat pH_{24} from the groups fed organic Se (6.07) was non-significantly (P>0.05) different from the birds fed organic Zn (6.05) or a combination of organic Zn and Se (6.09), but birds fed a combination of organic Zn and Se had a significantly (P=0.0152) higher ultimate meat pH compared to birds fed organic Zn. The duration of organic mineral supplementation showed no improvement (P>0.05) on any of the meat quality parameters measured in this study. No significant (P>0.05) interactive effects were observed for meat quality characteristics.

Table 4.19 The treatment effects on meat pH, colour, drip, and cook loss[1]

Treatments (T)	pH[2]		Colour[3]			Drip loss (%)			Cook loss (%)
	pH_{15}	pH_{24}	L*	a*	b*	24 h	48 h	72 h	
T1 (Control)	6.70	6.08^{x}	49.919	2.095	6.799	0.89	1.57	2.61	13.82
T2	6.66	6.03^{y}	49.923	2.241	7.475	1.05	1.75	2.89	13.36
T3	6.69	6.07^{xy}	50.812	2.222	7.083	0.94	1.78	2.67	13.96
T4	6.65	6.09^{x}	50.128	2.256	6.780	0.94	1.65	2.72	13.84
T5	6.67	6.06^{xy}	49.987	2.304	7.040	1.04	1.66	2.71	13.74
T6	6.53	6.07^{xy}	49.694	2.107	7.011	0.95	1.58	2.58	13.45
T7	6.64	6.09^{x}	49.860	2.441	6.999	0.95	1.60	2.69	14.08
Standard error	0.053	0.014	0.478	0.140	0.296	0.073	0.093	0.090	0.284
P-value	0.3356	0.0949	0.7501	0.6406	0.7262	0.7123	0.5771	0.3226	0.5498

T1 (100% ZnSO4, 100% SS; 0–32 d); T2 (40% Availa®Zn, 100% SS; 0–32 d); T3 (100% ZnSO4, 50% Availa®Se; 0–32 d); T4 (40% Availa®Zn, 50% Availa®Se; 0–32 d); T5 (40% Availa®Zn, 100% SS; 21–32 d); T6 (100% ZnSO4, 50% Availa®Se; 21–32 d); T7 (40% Availa®Zn, 50% Availa®Se; 21–32 d)

$^{x, y}$Column means with different superscript indicate a tendency of treatments to differ (P<0.10)

[1]One-way ANOVA to determine differences between dietary treatments

[2]pH_{15} = 15 minutes post-slaughter; pH_{24} = ultimate pH 24-hours post-slaughter

[3]L* = lightness; a* = redness; b* = yellowness

Table 4.20 The effect of different Zn and Se sources and duration of supplementation on meat quality characteristics[1]

Effects	pH[2]		Colour[3]			Drip loss (%)			Cook loss (%)
	pH_{15}	pH_{24}	L*	a*	b*	24 h	48 h	72 h	
Source[4, 5]									
Org Zn + InO Se	6.67	6.05[b]	49.955	2.273	7.258	1.05	1.71	2.80	13.55
InO Zn + Org Se	6.61	6.07[ab]	50.253	2.164	7.047	0.94	1.68	2.63	13.70
Org Zn + Org Se	6.64	6.09[a]	49.994	2.348	6.890	0.95	1.63	2.71	13.96
Standard error	0.040	0.010	0.337	0.092	0.208	0.055	0.070	0.067	0.191
P-value	0.6279	0.0152	0.7943	0.3679	0.4599	0.3286	0.7254	0.2059	0.3216
Duration[6]									
0–32 d	6.67	6.06	50.288	2.240	7.113	0.98	1.73	2.76	13.72
21–32 d	6.61	6.07	49.847	2.284	7.017	0.98	1.61	2.66	13.75
Standard error	0.033	0.008	0.275	0.075	0.170	0.045	0.057	0.054	0.156
P-value	0.2454	0.4900	0.2626	0.6769	0.6909	0.9898	0.1581	0.2009	0.8659

[a, b]Column means with the same superscript do not differ significantly ($P<0.05$)
[1]Two-way ANOVA to determine the main effects of mineral source and duration of supplementation
[2]pH_{15} = 15 minutes post-slaughter; pH_{24} = ultimate pH 24-hours post-slaughter
[3]L* = lightness; a* = redness; b* = yellowness
[4]Inorganic Zn = zinc sulphate; inorganic Se = sodium selenite; organic Zn and Se = Availa®
[5]InO = inorganic; Org = organic
[6]Supplementation throughout trial = 0-32 d; supplementation only during finisher phase = 21-32 d

Chapter 5
Discussion

5.1 Experimental diets

A feeding programme heavily influences the success of a poultry operation as broiler performance could be improved by way of nutritional manipulation having a direct effect on final product presentation. The growth rate of broilers has increased rapidly due to genetic alteration; therefore, the nutrients supplied by a diet need to match the new requirements of the bird. Feeding programmes are implemented to match specific growth stages of broilers, ensuring that optimal genetic potential is achieved through nutrition.

Feed ingredients used to construct diets make up 70% of broiler production costs, whereby energy makes up approximately 60% of total feed costs (Kleyn & Chrystal, 2020). Protein levels are decreased with broiler age, as high levels of protein during later stages of maturity can result in liver damage and reduced performance (O'Neill, 2021). The conversion of feed protein into animal protein highlights the necessity of amino acid requirements which steadily decrease with maturity due to the productive state of the bird (NRC, 1994; Kleyn & Chrystal, 2020). During the starter period, chicks have the lowest feed intake, however; the uppermost requirements for nutrients as early broiler performance has been shown to drastically influence final body weight (Mendes *et al.*, 2011; Abousekken *et al.*, 2017).

The main protein source that constructed the base of the experimental diet was soybean meal and full-fat soya, which is known for its high protein content and amino acid profile, except for methionine (Dei, 2011). This underlines the inclusion of synthetic methionine into the diet as seen in Table 3.1. Sunflower meal also served as a protein source for the same qualities as soybean meal, yet its use was restricted due to the impact on the crude fibre content within the diet (Senkoylu & Dale, 1999). Although the inclusion rate of sunflower meal was low, it could explain the higher crude fibre content analysed in the diets. Sunflower does not contain antinutritional factors like soybean meal; however, the addition of synthetic lysine is required (Senkoylu & Dale, 1999).

The mineral analyses of the treatment diets are presented in Table 4.2. The Zn and Se used in the treatment diets was mixed with ground yellow maize which served as a carrier. The final diet inclusion of trace minerals in relation to other dietary additives are extremely low, therefore some degree of premixing is needed prior to their incorporation into diets or premixes (Leeson & Summer, 2005). The maize carrier-premix was thereafter added on top of each basal batch and blended, constructing each dietary treatment. Research has shown that

maize–soybean or wheat–corn–soybean basal diets contain approximately 29 to 34 mg/kg of background Zn (Sunder *et al.*, 2008; Star *et al.*, 2012; Zhang *et al.*, 2018). Hygroscopicity, along with background Zn present within the feedstuff used to construct the diet, could explain the varying levels of Zn (125.34 to 145.44 mg/kg) obtained within the treatment diets. Maize grain in South Africa is a poor source of Se which constructs the majority of broiler diets highlighting the necessity of supplementation (Courtman *et al.*, 2012). The analysed levels of Se in the diet ranged from 0.50 to 0.95 mg/kg which is higher than the formulated inclusion of 0.30 mg/kg, but none the less acceptable. Selenium doses lower than 3–5 mg/kg are not associated with toxicity, and only when the dose exceeds the physiological requirement, at least 10-fold, is it classified as toxic (Surai, 2002).

5.2 Broiler performance

The results from the study suggest the nutrient requirements of the broilers were met as reported in the performance results. No additional benefit was observed when incorporating organic Zn and Se on an *iso*-basis into inorganic broiler diets for the total duration of the trial nor during the last growth stage. The supplementation of Zn and Se was sufficient for maximum broiler performance, however; a higher mineral bioavailability could not attribute towards higher performance. Organic forms of trace mineral supplementation have been known to improve broiler performance due to a higher bioavailability (Wedekind *et al.*, 1992; Nollet *et al.*, 2007; Yoon *et al.*, 2007; Star *et al.*, 2012), and less absorptive antagonistic reactions in the intestinal tract (Oliveira *et al.*, 2014). Previous research has shown birds reared on diets including organic forms of Zn or Se trace minerals improved broiler growth performance (Ao *et al.*, 2009; Saenmahayak *et al.*, 2010; Das *et al.*, 2014), and feeding efficiency (Saenmahayak *et al.*, 2012; Marković *et al.*, 2018a; Ibrahim *et al.*, 2019).

The results from this study established that partial replacement of inorganic Zn and Se by organic sources during the final production stage (21–32 d) attained similar broiler performance than feeding inorganic or organic sources for the cumulative experimental period (0–32 d). This statement is evident by evaluating final broiler BW, BWG, FI, and FCR results by which no significant treatment or main effect responses were obtained. These results are consistent with Silva *et al.* (2019), who found no difference in BWG, FI, or FCR when inorganic SS was replaced with organic Se-yeast at different ages in broilers. Thus, regardless of the form or age of mineral supplementation, cumulative broiler performance was not influenced. Zakaria *et al.* (2017) also found no significant effect on cumulative broiler BW, BW gain, FI, and FCR when birds were fed inorganic Zn-oxide (ZnO) or a combination of ZnO and organic Zn (Availa®Zn). Similarly, several authors found that the source in which Zn or Se had been included in a broiler diet had no significant effect on growth performance during the entire

measured period (Downs *et al.*, 2000a; De Grande *et al.*, 2020). Overall, the final growth performance of the broilers was comparable to that of the Ross standards.

In the starter (0–10 d) period, birds fed inorganically supplemented diets obtained the lowest FCR compared to birds receiving *iso*-based organic diets, which seemed to be less efficient. Contradictory results were found by Nollet *et al.* (2007) where the FCR of broilers fed an organic mineral diet (Mn, Fe, Zn, Cu) tended to be more efficient during the starter phase (0–14 d) compared to the inorganic counterpart diet. Similarly, De Grande *et al.* (2020) reported a decrease in FCR during the starter period (0–10 d) when broilers were fed organic Availa®Zn as compared to birds receiving inorganic $ZnSO_4$ owing to an increase in BW gain. The first seven days of a broiler's life corresponds to 16–20% of its entire productive life, making it a rather important task to supply the correct nutrients to a chick to obtain superior final broiler performance (Yerpes *et al.*, 2020).

The decrease in FCR when birds received inorganic minerals could be explained by the observations in BW during the first week of the trial. Birds tended to yield better 7-day BW results when inorganic forms of Zn and Se were supplemented from the start of the trial as opposed to later stages. Also, the tendency of birds to be heavier when fed inorganic mineral supplemented diets during the starter period (0–10 d) could further explain the improvement in FCR. Inorganic mineral supplementation in broiler diets is based on higher inclusion levels allowing a large safety margin to accommodate for low and variable mineral availability, owing to the relatively low cost of its use (Zhao *et al.*, 2010). The use of organic counterparts has become quite common due to its alternative absorption pathway, longer retention time in the body and being more bioavailable to animals, improving overall performance (Zhao *et al.*, 2010; Wei *et al.*, 2021). The inconsistent effects of dietary Zn and Se source supplementation on broiler growth performance from this study could be attributed by various factors; the presence of ligands which hinder mineral absorption due to antagonistic effects (Ao *et al.*, 2009; Hidayat *et al.*, 2020), selective metabolism of Zn by numerous metalloenzymes influencing enzymes potentially involved in broiler performance (Hudson *et al.*, 2005), and varying levels of background mineral levels in feed stuff (Saenmahayak *et al.*, 2010).

The results of the present research agrees with those of several other studies in which the mortality rate remained unaffected, irrespective of the source of Zn (Saenmahayak *et al.*, 2010; Yogesh *et al.*, 2013; De Grande *et al.*, 2020) or Se (Choct *et al.*, 2004; Deniz *et al.*, 2005; Perić *et al.*, 2009). Thus, Zn and Se trace mineral supplementation was sufficient in producing healthier birds. This signifies the importance of trace mineral supplementation in preventing extreme mortality rates during the experimental period.

5.3 Carcass characteristics

The indication of overall good broiler performance is not only related to live production measures, but post-slaughter carcass characteristics as well. Numerous studies have reported Zn supplementation to increase the fibrous protein (collagen) content within the muscles' connective tissues, relating to greater skin strength and preventing skin-associated carcass downgrading (Saenmahayak *et al.*, 2010), and improving carcass quality (Downs *et al.*, 2000b; Rossi *et al.*, 2007; Salim *et al.*, 2008). In addition, Se supplementation forms an integral part of selenoproteins and triiodothyronine (T3), a growth regulator, controlling protein deposition and energy utilisation within the body (Wang *et al.*, 2021).

The supplementation of Zn and Se had an overall positive effect on the measures of carcass characteristics measured in this study, although none of the carcass parameters measured in the current study performed significantly different from the control. These findings are consistent with those from Zakaria *et al.* (2017) who observed no effect on carcass yield parameters when inorganic zinc oxide (ZnO) and organic Zn-Met (Availa®Zn) were combined to total a higher concentration of 122 mg/kg compared to the 80 mg/kg ZnO control. Similarly, other studies have shown no significant effect on carcass yield or breast meat yield when broilers were fed inorganic or organic Se (Deniz *et al.*, 2005; Bakhshalinejad *et al.*, 2019). Contrary to the above-mentioned results, Marković *et al.* (2018) found significantly higher hot and cold carcass weights when broilers were fed 0.90 mg/kg of Se throughout the entire experimental period (1–42 d) and during the finisher period (22–42 d) when the level of Se was increased from 0.30 to 0.90 mg/kg of Se. Based on the carcass characteristics measured in the current study, some parameters had numerically higher results when organic Zn partially replaced inorganic $ZnSO_4$ during the finisher phase (T5) such as live weight and hot and cold carcass weight. Also, T5 obtained the second heaviest breast yield compared to T2 which birds received organic Zn (*iso*-basis) from day 1, this potentially signifying the value of duration supplementation of organic Zn on broiler carcass quality.

The main effect of mineral source tended to yield heavier carcasses when birds received a combination of organic Zn and Se, compared to organic Zn or organic Se alone. Jahanian & Rasouli (2015) reported a numerical improvement in carcass yield when inorganic ZnO was partially substituted with organic Zn-Met. Complete substitution of inorganic ZnO or $ZnSO_4$ by organic Zn-Met, however, significantly improved carcass and breast yields. The increase in carcass and breast meat yield may be explained by the role of Zn in protein and DNA biosynthesis, whilst deposition occurs throughout the body. The assumptions concerning the mechanism of organic Zn absorption and bioavailability highlights the importance of an organic ligand (amino acid, protein, carbohydrate, or lipid) acting as a protective structure against

antagonistic compounds that hinder the absorption of the mineral (Yu *et al.*, 2017; Zafar & Fatima, 2018; De Grande *et al.*, 2020). The enhancement of protein deposition with Se supplementation supports other authors who found an increase in carcass weight and dressing percentage (Choct *et al.*, 2004; Marković *et al.*, 2018a). Inorganic minerals are absorbed by simple diffusion across the gastrointestinal wall (Woods *et al.*, 2020). Organic selenomethionine (Se-Met) is the only form of Se that allows the body to build Se reserves in the muscle (Surai & Fisinin, 2014). This form of Se is not used for selenoprotein synthesis, unlike inorganic selenium selenite; it replaces methionine which enables tissue protein deposition or conversion into Se-Cys enabling active transport (Choct *et al.*, 2004; Surai & Fisinin, 2014; Surai *et al.*, 2018). Therefore, a synergistic effect could explain the addition of organic Zn and Se in combination owing to an increased protein build-up in the muscle.

Several studies have shown the benefits of pre-slaughter supplementation of additives or minerals in feed or drinking water in pigs and broilers (Satterlee *et al.*, 1989; Rey *et al.*, 2020). The results of the study might suggest that the physiological requirements of broilers were met by the provision of more bioavailable trace mineral sources during the last stage of the growth cycle. Overall, birds fed inorganic treatment diets during the starter and grower periods, after which organic sources partially substituted inorganic sources on an *iso* basis during the finisher period, obtained better carcass characteristics.

5.4 Meat quality characteristics

Meat quality in recent years has become the validation of product appearance and acceptability as consumer standards and procurement have altered the means of final product presentation. The meat quality characteristics measured in the current study were comparable between birds fed different sources of Zn and Se throughout (0–32 d) the trial period or during the final feeding stage (21–32 d) when organic sources partially replaced inorganic sources. The results from this study indicate the meat to be of normal quality concerning the pH and lightness (L*) of the breast fillets ($5.9 < pH < 6.1$ and $46 < L^* < 53$; Zhang & Barbut, 2005).

Birds fed diets with organic Zn (T2) supplementation from the start of the trial tended to have a lower ultimate pH than the other treatment groups. A more rapid decline in meat pH 24 hours after slaughter could explain the numerical, but not significantly higher drip loss observed when birds received diets partially supplemented with organic Zn (T2). However, when diets were partially substituted with organic Zn in T5 during later stages (21–32 d) of the trial, the ultimate pH was similar to the other treatments.

The breast muscle comprises white, fast-twitching fibres known to have a greater pH drop after slaughter, attributable to higher glycogen stores which have been reported to

increase drip losses (Listrat *et al.*, 2016). The effect of a higher ultimate meat pH after slaughter on the improvement of WHC has been well established by numerous studies (Berri *et al.*, 2008; Liu *et al.*, 2011; Albrecht *et al.*, 2019). The release of muscle water post-harvest can be explained by pre-*rigor* sarcomere shortening induced by the rate of pH fall (Honikel *et al.*, 1986; Ertbjerg & Puolanne, 2017; Kralik *et al.*, 2018). No significant differences were observed in meat quality or carcass parameters. Agbeniga & Webb (2018) stated the reduction in WHC due to electrical stimulation in beef carcasses. The carcasses were not electrically stimulated pre-scalding due to equipment limitations which could have resulted in the lack of differences observed. Dietary groups partially supplemented with organic Se (T6) at the start of the finisher phase (21–32 d) obtained numerically lower drip loss results at 48 and 72 hours, stressing the potential improvement in product yield as a result of more bioavailable sources supplied during later stages of the growth cycle, which could explain the prolonged rate of pH decline as the muscle approaches the state of permanent sarcomere shortening.

The source of Zn and Se had a significant effect on the ultimate meat pH, indicating a higher pH at 24 hours postmortem when birds received organic forms of Zn and Se. Although, this minute difference in breast meat pH has no physiological importance on meat quality. Ibrahim *et al.* (2019) reported the pH of breast meat from broiler groups receiving Se-Met presented an increased meat pH at two intervals postmortem in comparison to the inorganic selenite groups. On the contrary, Bakhshalinejad *et al.* (2019) reported no effect of source or level of Se on 24 h postmortem pH in the breast and thigh of broilers, which was in accordance with results by Perić *et al.* (2009). In addition, the author also found no correlation between drip loss and the pH of the breast muscle. Liu *et al.* (2011) reported a higher ultimate meat pH measured in the thigh of broilers fed diets containing Zn, irrespective of the source, compared to broilers fed the control diet without additional Zn supplementation.

Haem proteins, namely myoglobin and haemoglobin, can bind oxygen in live muscles, aiding in the presentation of meat with its distinctive reddish colour (Kranen *et al.*, 1999; Suman & Joseph, 2013). The oxygenation of meat enables four different redox states to change the colour of meat from cherry-red to brown which heavily influences consumer acceptability. It has been reported that Zn can bind myoglobin, increasing the redness and maintaining the fresh-looking colour of broiler meat (Saenmahayak *et al.*, 2010; Zakaria *et al.*, 2017). Sałek *et al.* (2020) failed to improve the redness (a*) of broiler meat with organic Zn-Met supplementation compared to inorganic ZnO. The results from the current study showed groups receiving organic Zn (T6) during the finisher phase (21–32 d) numerically obtained the lowest L* values, which could permit the breast muscle to appear less light to customers. Results by other authors showed an increase in the redness (a*) of meat with supplementary Zn (Saenmahayak *et al.*, 2010). The results of the current study are in agreement with Zakaria

et al. (2017) and Silva *et al.* (2019), who found no significant effect on broiler meat colour with Zn or Se supplementation.

Groups where organic Zn partially replaced inorganic $ZnSO_4$ (T6) during the finisher phase (21–32 d) numerically obtained one of the lowest cooking loss values in comparison to birds receiving organic Zn (T2) from the first day of the feeding period. Replacing inorganic with organic trace minerals relative to different times of supplementation has been shown to reduce cooking losses and oxidative damage to breast fillets. Silva *et al.* (2019) supplemented organic Se into broiler diets in whole or part at different ages and concluded that broilers took better advantage of the micromineral in organic form when fed in the final phase of their growth cycle, as indicated by improved oxidative stability and reduced cellular water loss during the cooking process. This finding was supported by the author's conclusions that broilers that received Se yeast throughout the experimental period or only during the last week before slaughter performed the same. However, meat samples from treatment groups fed organic Se throughout the experimental period or only during the last week showed less weight loss after cooking when compared to inorganic SS fed throughout the experimental period. Numerous authors obtained similar results to the current study whereby no significant differences between treatments were observed in cooking losses (Downs *et al.*, 2000b; Sunder *et al.*, 2008; Saenmahayak *et al.*, 2010; Zakaria *et al.*, 2017).

Chapter 6
Conclusion

The production of broiler meat has intensified on a large scale to meet the global demand for affordable animal protein. Consumers demand sustainable and affordable meat products that are health-promoting, responsibly produced, and are of optimum quality. Increasing selection towards heavier carcasses containing less fat has led to an upsurge of modernised issues in poultry meat production. Drip and cook loss are quality-related challenges in the poultry meat industry characterised by liquid seeping from the tissue – known to impair product appearance and performance. Inconsistent carcass composition further limits the processing of secondary products and, therefore, emphasis should be placed on the quantifiable properties of broiler meat as most economic relevance lies within these parameters.

Numerous studies have reported the lack of additional Zn or Se supplementation in broiler diets to cause depressed growth and impair immune function. In the present study, the nutrient requirements of the broilers were met as reported in the performance results. The results of the study did not find any significant treatment effect on growth performance. However, based on broiler BW and FCR results, there were positive mineral source and duration of supplementation effects during certain phases of production. The FCR was significantly lower when birds were fed treatments with inorganic Zn and Se in the starter phase. This is supported by the effect of supplementation duration, which tended to yield heavier body weights with inorganic treatments during the first week of the trial. Also, on day 7, BW tended to be heavier when birds received inorganic mineral sources. However, during the finisher phase, the FCR was significantly lower when birds received diets partially substituted with organic sources of Zn and Se.

In addition, the effect of supplementation duration was shown to significantly lower the FCR when organic sources partially replaced inorganic sources during the last week of the trial. Mineral source significantly lowered the FCR in the starter and grower phase when birds received organic Se and a combination of organic Zn and Se, respectively. The mortality rate remained unaffected during the current research. Broilers obtained overall better results as they were more efficient when fed dietary treatments containing inorganic forms of Zn and Se in the early growth stages, followed by the partial substitution of inorganic Zn and Se with organic forms during the start of the finisher phase (21–32 d).

None of the carcass parameters measured in the current study performed significantly different from the control. A synergistic source effect tended to yield heavier carcasses when

birds received a combination of organic Zn and Se. Supplementing organic Zn and Se during the finisher phase produced significantly heavier birds at slaughter. A similar trend was observed in HCW by which carcasses tended to be heavier when organic sources of Zn and Se were included in the diet during later stages of the feeding period. No treatment significance was found in the meat quality attributes measured in the current study. A tendency for a lower ultimate breast meat pH was observed when birds received organic Zn supplementation from the start of the trial. Furthermore, mineral source effect significantly lowered the ultimate meat pH when diets partially contained organic Zn.

In conclusion, early broiler growth performance was achieved with inorganic Zn and Se supplementation, whereafter partial substitution of more available forms of Zn and Se during the final stage of production presented overall better performance, carcass, and meat quality results. Considering the general outcomes of the current research, partial substitution of inorganic Zn and Se with organic sources at later stages could be implemented without affecting broiler performance as a cost-effective strategy for more sustainable broiler production.

Chapter 7

Critical review and recommendations

The current study did not take account of the different inclusion levels of Zn and Se. The inclusion level was kept constant whereby minerals were added into the diet on an *iso* basis, each in constant ratios to construct a combined total of 100 mg/kg of Zn and 0.30 mg/kg of Se, irrespective of source. Studies have reported differences in broiler performance when different inclusion levels of Zn or Se were incorporated as the main effect (Sunder *et al.*, 2008; Jahanian & Rasouli, 2015; Ibrahim *et al.*, 2019). Perhaps altering the mineral levels on an *iso* basis or full replacement of inorganic sources could have shown more statistically significant outcomes on performance, meat quality, or carcass characteristics. Lastly, due to the limitations of the number of personnel assisting on sampling days the measurements were not carried out by the same person, which could have resulted in some inconsistencies. Thus, there was room for human error on sampling days.

Broiler production is expected to reach greater heights than predicted in the future, thus the importance of research aimed at animal nutrition is irrefutable. Prospective research should explore relative supplementation levels and different durations at which organic sources replace inorganic sources, either partially (*iso*-basis) or fully, to improve the foundation of responsible and sustainable broiler production on a more profitable basis. This could provide a better insight into the mode of which minerals and sources equip modern broilers to perform at an optimal level, as well as the age at which these minerals are in a high demand by the animal.

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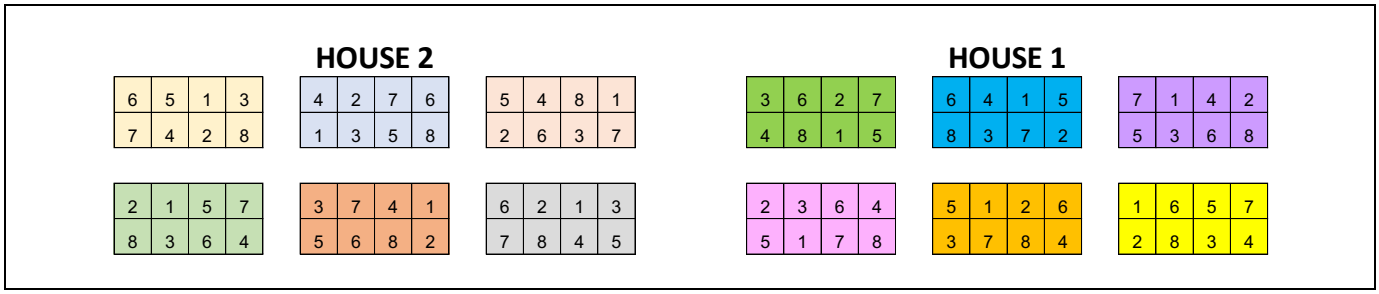
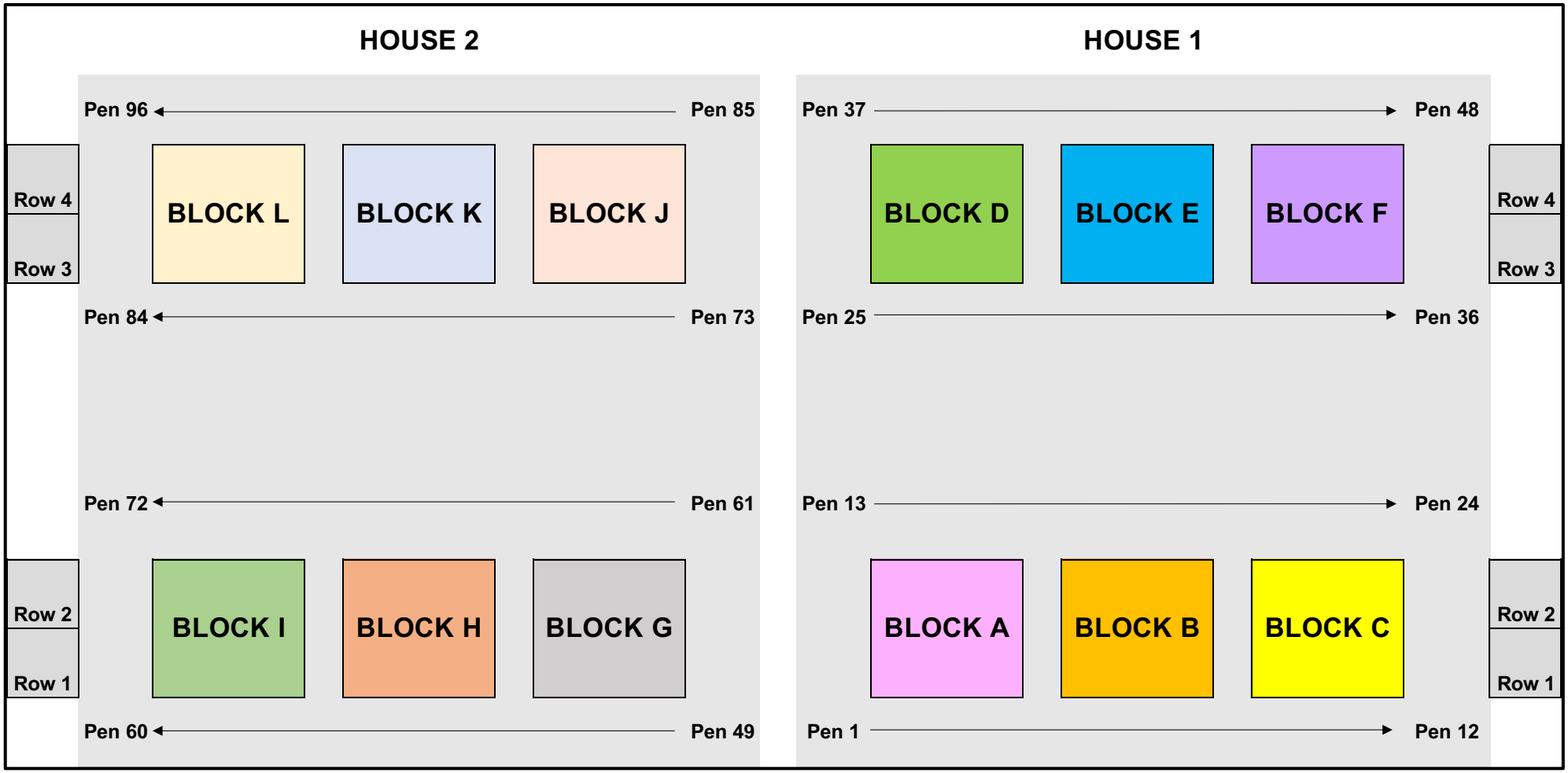
Appendix 1



Randomisation of treatments within the 96-pen research facility separated into house 1 and house 2

Appendix 2



Blocking design used to allocate treatments to the 96-pen research facility separated into house 1 and house 2

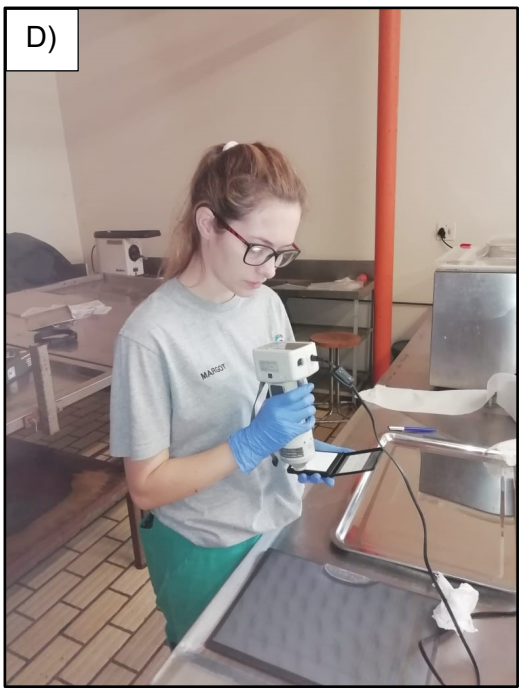
Appendix 3

Images of trial setup and apparatus

A) Chicks on placement day

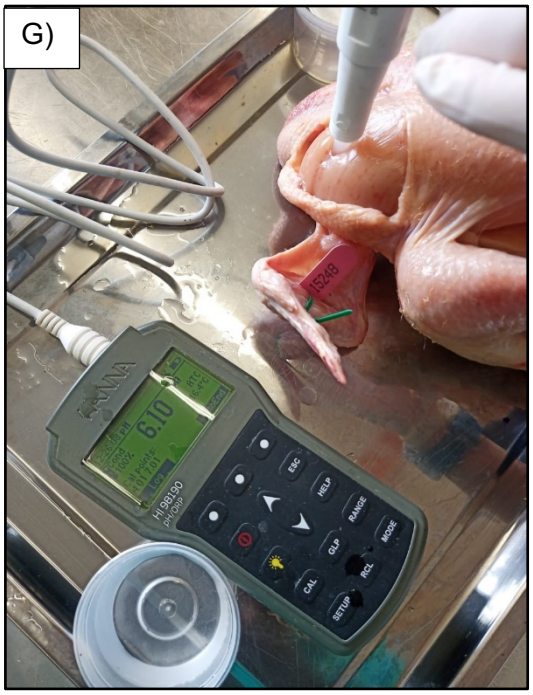
B) Sampling of fillets from carcass

C) Neck tag and treatment specific band accompanying left and right fillets for further sampling



C) Student calibrating CR-400 Konica Minolta chroma meter

E) Minolta calibration plate

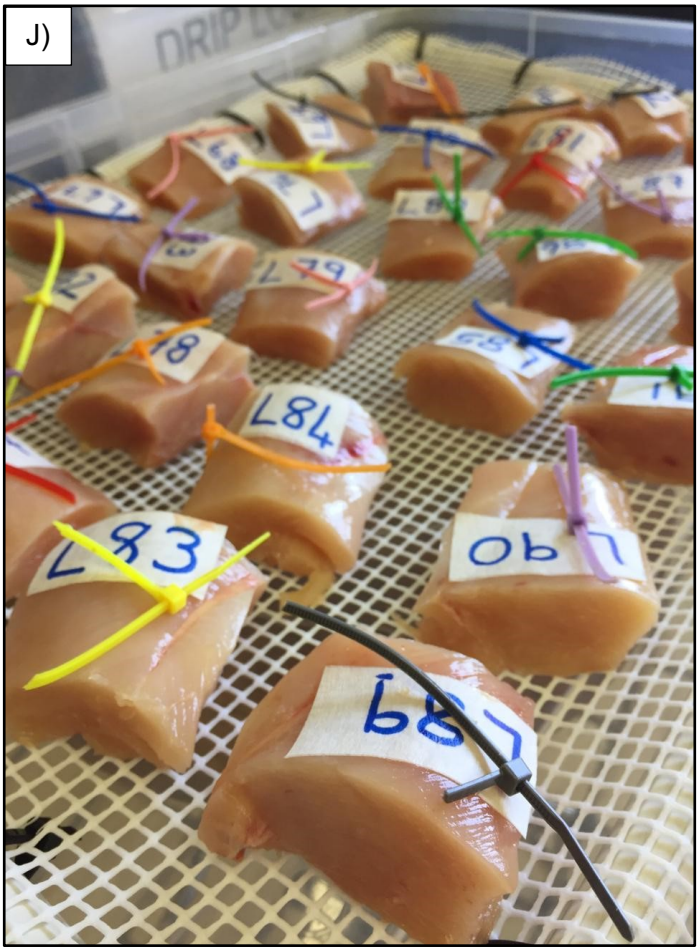


F) Student measuring pH on breast fillet 15 min after slaughter

G) Hanna portable pH meter and probe

H) Carcass hanging on rack in cooler with neck tag and treatment band

I) Carcass hanging system



J) Drip loss samples placed on mesh plastic wire inside plastic boxes