The effects of different zilpaterol hydrochloride feed supplements and extended ageing periods on the meat quality of feedlot bulls

By

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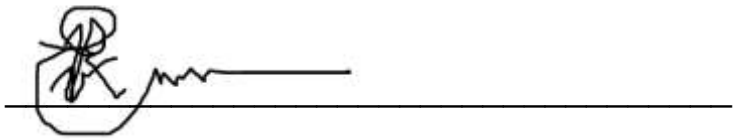
Declaration

I, Rochelle van Emmenis hereby declare that this dissertation, submitted in partial fulfilment of the requirement for the degree MSc Agric. (Animal Science) Production Physiology and Product Quality, in the Department of Animal Science, Faculty of Natural and Agricultural Sciences, University of Pretoria, is my own work and has not previously been submitted by me for a degree at any other university.



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Lastly, I thank my God for opening and closing doors when I need them most. I prayed for every aspect of this MSc and He provided.

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List of Abbreviations

αGPDH - Alpha-glycerophosphate dehydrogenase

AC - Adenyl cyclase enzyme

ADG - Average daily gain

ADP - Adenosine diphosphate

AMSA - American Meat Science Association

ASF - Animal source food

ATP - Adenosine triphosphate

ATPase - Adenosine triphosphatase

βA - Beta-adrenergic agonist

βAR - Beta-adrenergic agonist specific receptors

cAMP - Cyclic adenosine monophosphate

CCI - Carcass compaction index

CIE - Commission Internationale de l'Elcairage

CL – Cooking loss

COMb – Carboxymyoglobin

CT – Conventional treatment group, excluding βAs (negative control)

DAFF - Department of Agriculture, Forestry and Fisheries

DFD – Dark Firm Dry meat

DL – Drip loss

DMb - Deoxymyoglobin

DNA - Deoxyribonucleic acid

DR% - Dressing percentage

EU - European Union

FAO - Food and Agriculture Organization

FG - Fast glycolytic muscle fibers (MHC type 2b)

FGI – Intermediate MHC fast glycolytic muscle fiber type 2X

FOG – MHC muscle fiber type 2a

FT - Subcutaneous fat thickness measurements

GET - Growth-enhancing technology

GF - Conventional treatment group with Grofactor® as added βA

GHG – Greenhouse gas

GLM - General linear model

HCM - Hot carcass mass

IBR - Infectious Bovine Rhinotracheitis

IHC – Immunohistochemical analysis

IMF - Intramuscular fat

KPH fat - fat covering the kidneys, pelvis and heart

LD - Longissimus dorsi muscle

LMA - Longissimus muscle area

Mb – Myoglobin

MHC - Myosin heavy chain

MMb- Metmyoglobin

MRA – Metmyoglobin Reducing Activity

MRL - Maximum residue level

mRNA – Messenger ribonucleic acid

NADH - Nicotinamide adenine dinucleotide (NAD) + hydrogen (H)

NAT - All-natural treatment group receiving no GET

NC-IUB - Nomenclature Committee of the International Union of Biochemistry

OC – Oxygen consumption

OMb – Oxymyoglobin

OPP – Oxygen Partial Pressure

PKA - Protein kinase A enzyme

PMt – Post-mortem time

PSE – Pale Soft Exudative meat

RH - Ractopamine hydrochloride

RNA - Ribonucleic acid

RSA - South Africa(n)

RT - Ractopamine

SDGs - Sustainable development goals

SDH - Succinate dehydrogenase

SO - Slow oxidative muscle fibers

TBA - Trenbolone acetate

UN - United Nations

USA – United States of America (American)

WBSF – Warner-Bratzler Shear Force

WHC - Water holding capacity

ZH - Zilpaterol hydrochloride

ZM - Conventional treatment group with Zilmax® as added βA

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Chapter 1: Aims, objectives and introduction

Abstract

The aim of this study was to evaluate the possible effects of two different zilpaterol hydrochloride (ZH) beta-agonists - with further focus being placed on the viability of the generic Grofactor® product when compared to industry-standard Zilmax® - on the carcass and meat quality characteristics of typical South African feedlot bulls (*Bos taurus* crosses). Additionally, meat quality attributes over an extended ageing period of up to 120 days were studied.

The first objective was to evaluate the effects of dietary ZH-supplementation during the finishing period of feedlot bulls with either Zilmax® or Grofactor® on carcass characteristics namely, hot carcass mass (HCM), dressing percentage (DR%), carcass classification score, carcass compaction index (CCI), subcutaneous fat thickness (FT), carcass composition (bone: muscle: fat), as well as carcass pH and -temperature profiles from 45min to 24hours post-mortem.

The second objective was to critically evaluate the effects of feed ZH-supplementation of feedlot bulls with either Zilmax® or Grofactor® on specific meat quality characteristics such as tenderness (physically measured with Warner-Bratzler shear force [WBSF] values), colour attributes (L*, a*, and b* obtained with a colorimeter as well as chroma and hue), drip losses and cooking losses over an extended ageing period of up to 120 days.

The completely randomised control study consisted of 3 experimental groups receiving a finisher ration during the 30-day feedlot finishing period (with a subsequent 3-day withdrawal period); the first, a negative control (CT) received no ZH-supplementation added to the basal diet, second; ZH-based Zilmax® feed supplementation (ZM) and lastly ZH-based Grofactor® feed

supplementation (GF). ZH-supplementation was mixed into the basal finishing ration at a recommended concentration of 105g ZH/ton.

Each treatment was then randomly allocated to 3 replicates and fed from the first day of the finishing period (D0) for 30 days. The replicates amounted to each treatment being administered to 3 pens x 50 animals/pen = 150 animals. Due to the high costs of proximate and meat sample analyses, only 38 bull carcasses were randomly sub-selected from each treatment to be evaluated further (3 treatments x 38 bull carcasses = 114 carcasses and subsequent meat samples evaluated).

No significant differences were observed between the two ZH-based molecules (ZM vs. GF) for any measured characteristic. Grofactor® performed similar to Zilmax®, supporting the viability of the generic ZH-supplementation with Grofactor®.

Although ZH-supplementation did not yield significantly heavier live masses than CT over the finishing period, increasing the length of finishing period ZH-supplementation, improved the effect of ZH-supplementation i.e., after two weeks ZH bulls were 3kg heavier than CT ($P = 0.64$) and final slaughter masses differed with ZH weighing 5.4kg heavier than CT ($P = 0.12$).

Compared to CT carcasses, ZH-supplemented bull carcasses were 8kg heavier ($P = 0.001$; $\eta^2 = 0.09$), tended to have 0.9% higher DR% ($P = 0.07$; $\eta^2 = 0.03$) and increased CCI (2.2 vs. 2.1; $P < 0.001$ and $\eta^2 = 0.13$). The ZH energy repartitioning mechanism successfully increased carcass muscle% composition with 2.0% ($P = 0.02$; $\eta^2 = 0.05$) and decreased the fat% composition with 1.8% ($P = 0.05$; $\eta^2 = 0.03$) when compared to CT carcasses.

ZH-supplementation did significantly decrease meat tenderness measurements of supplemented bulls when compared to CT on all ageing days ($P < 0.05$; $\eta^2 = 0.24$). The WBSF values between CT and ZH differed with ZH being 0.5-0.8kg

less tender for all ageing days. Post-mortem ageing did however significantly improve the decreased meat tenderness ($P < 0.001$; $\eta^2 = 0.38$). ZH-supplementation had no significant effects on any other meat quality characteristics.

Post-mortem ageing of meat samples had significant effects on all meat quality characteristics investigated ($P < 0.001$; $\eta^2 > 0.30$). And all meat quality characteristics had a trend to sharply decrease or increase in values measured from day 56 to 120.

Meat colour lightness (L*) values fluctuated between increasing and decreasing from day 3 to 56, but ultimately increased from 39.7 on day 3 to 43.2 on day 56, only to have a drastic decrease to 36.0 on day 120 ($P < 0.001$; $\eta^2 = 032$). The chroma values also fluctuated between increasing and decreasing from day 3 to 56, but ultimately decreased from 22.3 on day 3 to 17.1 on day 56, to then have a drastic decrease to 13.1 on day 120 ($P < 0.001$; $\eta^2 = 0.50$). Hue angles drastically decreased from 0.4 on day 3 to 0.1 on day 7, then increased to 0.3 and remained constant up until day 120 ($P < 0.001$; $\eta^2 = 0.40$). Interestingly ZH meat samples insignificantly had a slightly redder hue angle than CT.

Meat drip loss, cooking loss and tenderness (as indicated by decreasing WBSF values) increased from day 7 (4.1%, 28.2% and 6.8kg, respectively) up to 56 days of ageing (10.8%, 30.1% and 4.3kg, respectively), then decreased on 120 days of ageing (8.9%, 22.1% and 4.6kg, respectively).

Further studies would be recommended on the exact mechanisms which influence the tendency of post-mortem meat sample ageing to decrease in meat quality characteristic values from day 56 to day 120.

Keywords: Beta-adrenergic agonist, Zilmax®, Grofactor®, beef cattle, ageing, meat quality

Introduction

Beta-adrenergic agonists (βAs) have been studied and used in beef production for a little over 20 years (Plascencia, Torrentera, & Zinn, 1999; Anderson, Moody, & Hancock, 2005; Maxwell, *et al*., 2015). Although it has been proven to significantly benefit production efficiencies during the finishing phase (Plascencia, *et al.*, 1999; Anderson, *et al.*, 2005; Brooks, *et al.,* 2008), questions were raised about the possible impact these molecules may have on the meat quality and shelf-life. Zilpaterol hydrochloride (ZH)-based Zilmax® (MSD) has thoroughly been researched in livestock production and has been used as a standard finishing period feed supplement in most major feedlots in South Africa. A generic version of Zilmax® has been manufactured by Virbac, known as Grofactor®.

Little research has been done to compare the efficacy of these two different ZH-based molecules (Grofactor® vs. Zilmax®) and the effects thereof on meat quality characteristics (Avendaño-Reyes, *et al.,* 2016). Many feedlot producers may benefit economically if the cheaper generic version is proven to perform equally well as Zilmax®.

Most previous studies only experimented up to 14 days post-mortem while there is interest in longer ageing periods. One of the important aspects that will be investigated is shelf-life, measured by meat tenderness, drip loss and colour stability. The effect of extending meat ageing is an important factor to investigate as positive results would encourage product exportation (that on average would last 120 days, which includes a short retail display).

Hypotheses

H_{01}: Dietary supplementation with one of two different ZH molecules (Zilmax® vs. Grofactor®) in feedlot diets during the finishing period, does not affect the carcass and meat quality characteristics when compared to a negative control.

H_{A1}: Dietary supplementation with one of two different ZH molecules (Zilmax® vs. Grofactor®) in feedlot diets during the finishing period, does affect the carcass and meat quality characteristics when compared to a negative control.

H_{02}: Dietary supplementation with Grofactor® in feedlot diets during the finishing period, does not affect the carcass and meat quality characteristics differently than when Zilmax® is supplemented, when compared to a negative control.

H_{A2}: Dietary supplementation with Grofactor® in feedlot diets during the finishing period, does affect the carcass and meat quality characteristics differently than when Zilmax® is supplemented, when compared to a negative control.

H_{03}: Extending the ageing period of meat samples obtained from all experimental feedlot bulls, does not affect the meat quality characteristics.

H_{A3}: Extending the ageing period of meat samples obtained from all experimental feedlot bulls, does affect the meat quality characteristics.

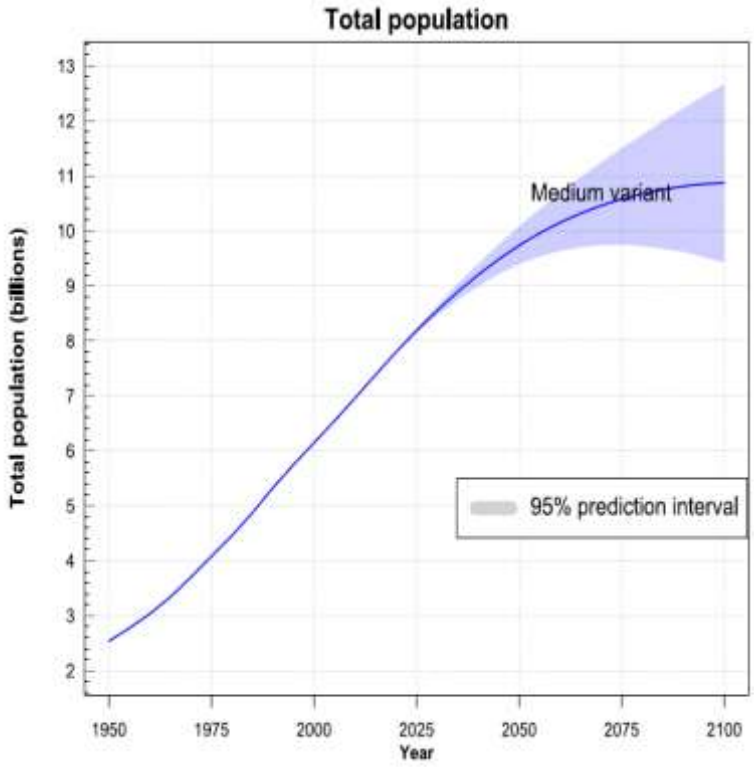
Chapter 2: Literature Review

2.1 The importance of beef production

2.1.1 World population growth

The livestock industry is placed under pressure with the United Nations' (UN) projected population growth to more than 9.5 billion people in 2050, because it is coupled with a projected 70% increase in demand for animal source foods (ASFs) (United Nations [UN], 2011; Capper & Hayes, 2012; Food and Agriculture Organization [FAO], 2018). The UN world population prospects 2019 report still supports this predicted population growth as seen in **Figure 2.1.1,** illustrating the total predicted population growth.

Figure 2.1.1 Total population growth prediction (UN, 2019)



There is a growing concern accompanying the projected population growth regarding the sources, ethical and environmental impact which food production may have (Capper & Hayes, 2012; FAO, 2018; UN, 2019; Paul, Butterbach-

Bahl, Notenbaert, Nderi, & Ericksen, 2021). This is because, despite the obvious increase in the demand for food, many additional factors such as employment rates, creating job opportunities, cost of living and the socioeconomic welfare of citizens need to be considered when the population grows (Department of Agriculture, Forestry and Fisheries [DAFF], 2019; FAO, 2018; Paul, *et al.*, 2021). In this regard, the agricultural industry has been proven to contribute to the sustainable development goals (SDGs) by promoting economic growth, reducing poverty and minimising malnutrition (FAO, 2018).

Developed countries' demand for animal-based proteins will roughly stay the same whilst people living in economically developing countries will see a rise in demand as the purchasing power and coupled expendable income increases (FAO, 2018; Paul, *et al.,* 2021).

To meet the animal protein needs of this growing population, will prove a challenge as the public generally has negative misconceptions of the agricultural sector. There are 5 main areas of negative critique, namely; greenhouse gas (GHG) emissions, land use and the impacts it may have, soil degradation and erosion, water usage and the potential loss of biodiversity (Paul, *et al.*, 2021). The combined effects of the aforementioned areas can contribute to climate change and human health crisises.

To try and minimize the environmental impacts and public criticisms whilst still increasing production, the agricultural sector has become vertically integrated over the years with the aid of modern technology and easier access to educational resources (DAFF, 2019).

2.1.2 South African beef industry and economic implications

The elevation in living standards of people living in economically developing countries, means that more people can afford to include more meat in their diets instead of cheaper field crops, this in turn drives the demand for ASFs higher (Ramanathan, Hunt, Price, & Mafi, 2021). This is supported by the South African (RSA) beef production gross value which raised with R23.4 billion during the past decade (DAFF, 2019). The increase in the gross production value is indicative of the increased demand for beef products as consumers experienced a rise in living standards as a result of income growth.

Subsequently, income growth in RSA drove the beef industry to become the second fastest growing agricultural commodity, following the broiler industry (DAFF, 2019). The RSA beef industry has integrated vertically by making the feedlot-industry a main component with 12 788 million cattle heads vs. the informal beef sector with only 5.69 million cattle heads (DAFF, 2019). This minimizes land-use and improves the efficiency of various aspects in beef production (FAO, 2018).

Accordingly, as cited by DAFF (2019), RSA produced roughly 1 million tons of beef in 2018 of which only 30 000 tons (3%) was exported. RSA further imported almost half as much beef exported, at 14 000 tons in the same year. Indicating that RSA focuses beef production on local consumption. Once the local population demand has been adequately met through increased production, exports can flourish and generate better income for RSA. South Africa currently mainly exports to other African countries and China (DAFF, 2019). The United States of America (USA) and Australia are also mass producers of beef, thus other exporting opportunities would have to be sought from Eurasia (Fields, Therrien, Halstrom, Haggard, & Clayton, 2018).

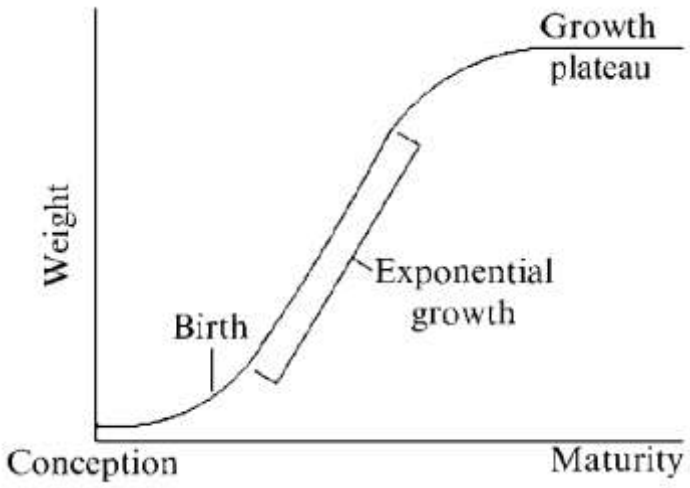
2.2 An overview of feedlot cattle growth

Hossner (2005) states that quantitative muscle (mass) growth is of utmost importance to beef producers to determine whether they achieve growth or efficacy targets, whilst qualitative muscle growth can cascade down to a consumer level and price determination e.g., intramuscular fat (IMF).

Due to increased focus of production being shifted toward leaner carcasses, the meat: fat ratio should be maximised to obtain optimal efficiency and customer satisfaction (Webb & O'Neill, 2008).

Cattle being fed during the feedlot phase of production will initially see exponential growth figures as the animal rapidly grows and matures. Once the animal starts to reach maturation this lean exponential growth will decrease (seen as an inflection point on sigmoidal growth curves) then plateau, and more energy will naturally be directed toward fat (adipose tissue) accumulation instead of muscle accretion.

Figure 2.2.1 Typical sigmoidal growth curve (Hossner, 2005)



Fat tissue is considered a tissue of maturity as it is used to store energy that is in excess of the body's requirements for energy utilisation (Hossner, 2005). It is therefore essential to try and limit unnecessary energy surpluses that can be

transferred to fat deposition, which will yield inefficient production and undesirable final products.

2.2.1 Cellular development relating to muscular growth

In meat animal production, skeletal muscle is the primary product produced and most emphasis is therefore placed on maximising muscle yield and efficacy (Hossner, 2005).

Skeletal muscle cells are large, multinucleated, elongated cylindrical shapes, hence commonly interchangeably referred to as muscle fibers (Purslow, 2017). Muscle fibers are formed by means of myogenesis during embryonic development (Purslow, 2017) and accounts for the majority of skeletal muscle development (Hossner, 2005). Hossner (2005) defines growth at a cellular level as an increase in either cell size (hypertrophy) or cell numbers (hyperplasia).

Muscle fibers are incapable of mitosis (Dwyer, Fletcher, & Stickland, 1993); therefore, fiber numbers are fixed at birth, consequently unable to undergo hyperplasia (Hossner, 2005). The outcome of myogenesis is thus crucial to livestock production as the number of muscle fibers are positively related to the final meat content of a carcass (Dwyer, *et al.*, 1993).

Hypertrophy is when muscle cells increase in volume through protein synthesis or in the case of adipocytes, accumulating lipids (Hossner, 2005). The number of muscle fibers are pre-determined by embryonic development, thus muscular hypertrophy would cause growth in length and cross-sectional areas of these muscle fibers, but the fibers themselves do not multiply post-natally (Dwyer, *et al.*, 1993; Purslow, 2017).

The net effect of muscle fiber hypertrophy is a result of the difference between both dynamic protein synthesis and -degradation processes, known as protein turnover (Purslow, 2017). Protein turnover is described in terms of the amount of protein synthesised or degraded per day, relative to the total amount of muscle

protein (Purslow, 2017). Purslow stated that protein synthesis is dependent upon the available adenosine triphosphate (ATP) content for energy, the amount of deoxyribonucleic acid (DNA) and amino acids, as well as the activity of protein synthesising enzymes. Muscle protein degradation is the proteolytic process which breaks intact proteins down to amino acids by using intracellular proteases. A prominent proteolytic system present in the muscle fibre cytocol, is known as the calpain system that acts on myofibrillar proteins – the "building blocks" of muscle fibres – and will be discussed further later on (Bardsley, *et al.*, 1992).

Additionally, satellite cells (mononucleated cells) are located just outside the muscle fibre cell membrane to fuse with each other and the muscle fiber upon activation (Koohmaraie, Kent, Shackelford, Veiseth, & Wheeler, 2002). This fusion will add more nuclei to the total muscle fibre, increasing DNA content which can subsequently support increased protein synthesis. Ergo, satellite cells provide non-mitotic muscle fibers to increase DNA content, without nuclear replication (Hossner, 2005; Purslow, 2017).

2.2.2 Muscle fiber types

To simplify the different muscle types, they can be classified as either red or white fibre types. Dark meat consists of a larger portion of red muscle fibers which is characterised by a slow twitch contraction velocity. Due to slower contraction velocities, red muscle fibres are located in muscles that are involved in sustained, long-term activities i.e., standing and posture maintenance.

Conversely, white muscle fibers have faster contraction velocities, capable of quick reactions and utilises glycolysis for energy production, making them more energy efficient. Fast twitch muscles are used for locomotion, fine motor control and reflexive actions

Histochemical analyses with three prominent muscle enzymes provide further physiological insights within each muscle fibre type. The myosin adenosine triphosphatase (ATPase) activity of myofibrils is indicative of contraction velocities and can distinguish between fast (red) and slow (white) twitch fibres. Succinate dehydrogenase (SDH) is a mitochondrial enzyme and indicative of oxidative metabolism and mitochondrial concentrations. To measure glycolytic (anaerobic) metabolism α-glycerophosphate dehydrogenase (αGPDH) – located in the cytoplasm -is used. Following the results of the histochemical analyses (summarised in **Table 2.2.1**) red fibers are classified as slow oxidative (SO) fibers whilst white fibers are fast glycolytic (FG) fibers.

Table 2.2.1 Physiological differences between red- and white muscle fiber types as determined by histochemical analyses (Hossner, 2005)

Analysed characteristic (enzyme)	**Contraction velocity** (ATPase)	**Mitochondrial content** (SDH)	**Metabolism** (αGPDH)
Red fiber	Slow	High	Oxidative
White fiber	Fast	Low	Glycolytic

Muscles which depend on oxidative metabolism for energy (red fibres) have smaller fiber diameters than their glycolytic counterparts - white muscle fibers (Hossner, 2005; Joo, Kim, Hwang, & Ryu, 2013). This correlates with the muscle's anatomical location as more active, faster contracting muscles depend on glycolysis for energy sources and have a larger diameter for locomotive functions (Hiner, Hankins, Sloane, Fellers, & Anderson, 1953; Joo, *et al.*, 2013). FG (white) muscle fibers have lower protein turnover rates and can therefore grow faster than their red fibre counterparts. This is due to the inherently higher ATP content which can support protein synthesis (Purslow, 2017).

Immunohistochemical (IHC) analyses further distinguishes between different isoforms of the myosin heavy chain (MHC) of myofibrils when combined with the

results of histochemical analyses. The myosin tail provides an anchor which maintains the position of the MHC (Hossner, 2005). Slow twitch muscles (SO fibers) are now classed as type 1 MHC fibre. Whilst fast twitch muscle fibers are now divided into three MHC fiber sub-types.

MHC muscle fiber type 2X (FGI) displays intermediate properties between 2a (FOG) and 2b (FG) fast twitch muscles, that only represents a small population of skeletal muscle fibers.

Table 2.2.2 Physiological differences between fast- and slow twitch muscle fiber myosin heavy chain (MHC) types as determined with immunohistochemical (IHC) analyses (Hossner, 2005)

Twitch/metabolism	MHC type	Contraction velocity	Mitochondrial content	Metabolism
SO	1	Slow	High	Oxidative
FOG	2A	Fast	High	Glycolytic
FG	2B	Fast	Low	Glycolytic
FGI	2X	Fast	Low	Glycolytic

Using the MHC fiber types, muscle fibers are broadly referred to as either red, type 1 or white, type 2b in literature. Muscle fibers are dynamic with high elasticity expression (Joo, *et al.*, 2013). Plasticity allows the fibers to shift between types following the pathway: 1 ↔ 2a ↔ 2X ↔ 2b (Schiaffino & Reggiani, 1996). Specific transitions between fiber types can be induced by either exercise or ambient temperatures (Rinaldo & Le Dividich, 1991; Herpin & Lefaucheur, 1992; Joo, *et al.*, 2013). E.g., prolonged endurance exercises would induce a shift from type 2b → 2X → 2a → 1, from fast contracting fiber types to slower contacting which now exhibit a slower growth rate. Which additionally motivates beef cattle feedlotting instead of extensive farming.

2.3 The importance of growth-enhancing technologies

With the increased beef demand and awareness of environmental impacts, the beef industry necessitates the research and development of technologies, to enhance efficiency and concurrently limit resource usage. Growth promoters are commonly used to utilize less feed whilst still promoting muscle gain. This improved growth efficiency will lower the beef producer's expenses (Strydom, 2016) and minimize the amount of natural resources used, therefore promoting more sustainable use of natural resources and smaller environmental impact (Capper & Hayes, 2012; Paul, *et al.*, 2021).

For almost 60 years, growth promotants have safely been used in beef cattle production (Capper & Hayes, 2012). Notable effects include improved average daily gains (ADG), feed efficiency and enhanced muscle leanness (Moody, *et al.,* 2000; Brooks, *et al.,* 2008; Montgomery, *et al.,* 2009; Johnson, Ribeiro, & Beckett, 2013; Webb & Erasmus, 2013; Maxwell, *et al.,* 2015).

Examples of growth-enhancing technologies (GET) include dietary additives such as ionophores, anabolic steroids and beta-adrenergic agonists. According to Strydom (2016) genetic manipulation can additionally be considered as a GET. The implementation of all the above-mentioned technologies is considered standard procedure in South African feedlots (DAFF, 2019; Webb & Erasmus, 2013).

These enhancements in performance cascade down in economic benefits to both beef producers and consumers (Capper & Hayes, 2012).

2.3.1 Economic and environmental impacts of exogenous growth enhancing technologies (GET)

Capper and Hayes (2012) quantified the effects which withdrawal of GET from the USA beef production system would have on their environment and economic dynamics. The GET system in their study was similar to the current standard feedlot practice in RSA i.e., combining steroidal implants with in-feed supplements of ionophores and a βA. The alternative system did not adopt any GET. Feed savings were used to compare and calculate the economic impact, global trade and carbon implications that withdrawal of GET can ensue. A summation of their findings is given below.

Table 2.3.1 Effects of the withdrawal of growth-enhancing technology (GET) from the USA beef production system (Capper & Hayes, 2012)

Factor measured	Observed effect
Productivity	↓ based on growth- and slaughter mass
Population size	↑ by 385 $x10^3$ animals to produce 454 $x10^6$ kg beef
Feedstuff use	↑ by 2 830 $x10^3$ tons
Land use	↑ by 265 $x10^3$ ha
Water use	↑ by 20 139 $x10^6$ litres
Manure output	↑ by 1 799 $x10^3$ tons
Carbon emissions	↑ by 714 515 tons to produce 454 $x10^6$ kg beef

↑ increased effects were observed for the measured factor.
↓ decreased effects were observed for the measured factor.

As mentioned earlier, Paul *et al.* (2021) stated 5 main areas of importance to determine environmental sustainability, namely; GHG or carbon emissions, land use and the impacts it might have, soil degradation and erosion, water usage and the potential loss of biodiversity. The Capper and Hayes (2012) study comparatively, shows the positive effect on climate change and environmental impacts that GET inclusion can beget. GHG emissions would be lowered (by minimizing manure and carbon output), water and land would be utiized more

sparingly and soil erosion by crop production can be limited with a decreased feedstuff use.

Anderson *et al.* (2005) as well as Puslow (2017) state that increased lean tissue growth would lead to greater nitrogen retention within the animal, resulting in less nitrogen being lost as waste to the environment.

This proves that 4 out of 5 climate change factors impacted by livestock agriculture, can be improved by implementing GET. Capper and Hayes (2012) therefore illustrated the importance of GET inclusion in beef production for both economic and environmental sustainability.

2.3.2 β-adrenergic agonists as exogenous growth-enhancing technology

β-adrenergic agonists - referred to as β-agonists (βAs) - have repetitively been proven to promote effective growth during the feedlot fattening phase, observed during the last few weeks prior to slaughter, thereby improving feedlot performance and cascades in carcass qualities being more desirable (Mersmann, 1998; Moody, *et al.*, 2000; Montgomery, *et al.,* 2009; Maxwell, *et al.,* 2015; Fields, *et al.*, 2018).

According to the 2005 Encyclopedia of Animal Science, commonly used βAs are from a class of compounds known as phenethanolamines (Mersmann, 1998; Anderson, *et al.*, 2005). Phenethanolamines have successfully been used in human medicine since the 1970s to treat pulmonary distress (Johnson, 1993). The first published demonstration of a βA such as zilpaterol hydrochloride's (ZH) benefits when used in cattle, dates to 1998 (Plascencia, Torrentera, & Zinn, 1999; Anderson *et al.,* 2005).

β-agonists (βAs) are analogues and mimic the actions of catecholamines - such as epinephrine (adrenaline) and norepinephrine (noradrenaline) - that are naturally produced by the adrenal glands in the mammalian body (Mersmann,

1998; Hossner, 2005; Purslow, 2017). Thereby βAs are synthetic non-hormonal compounds which are administered as feed additives (Anderson, *et al.*, 2005).

These compounds are orally active and fed at parts per million (ppm) concentrations during the feedlot finishing period, typically for 30 days, with a 3 day withdrawal period before slaughter (Mersmann, 1998; Johnson, *et al.*, 2013).

The importance and effects of βA-supplementation were investigated by Maxwell *et al.* (2015) who compared an all-natural treatment (NAT), a conventional treatment (same as CT) and their conventional treatment with Zilmax® as added βA (same as ZM) on *B. taurus* crossbred feedlot cattle. The basal diets of all 3 treatments were the same and the NAT cattle received no GET. Both CT and ZM treatments consisted of 40mg estradiol and 200mg trenbolone acetate (TBA) implants upon receival. These cattle were fed monensin and tylosin ionophores daily. The ZM cattle additionally received ZH, in the form of ZM, during the last 20 days on feed which was withdrawn 3-5 days before slaughter.

Both CT and ZM clearly yielded better results in all investigated categories than NAT. ZM had a further few categories where it significantly outperformed CT. Faster gain, better efficiency, heavier HCM, greater longissimus muscle area (LMA) and lower marbling scores were all seen in ZM steers compared to CT, resulting in better USA carcass gradings. Similar effects can be expected in terms of carcass classification in RSA.

Maxell *et al.* (2015) concluded that growth implants, ionophores and βA-supplementation are valuable GETs that greatly improves gain and efficiency in feedlot cattle.

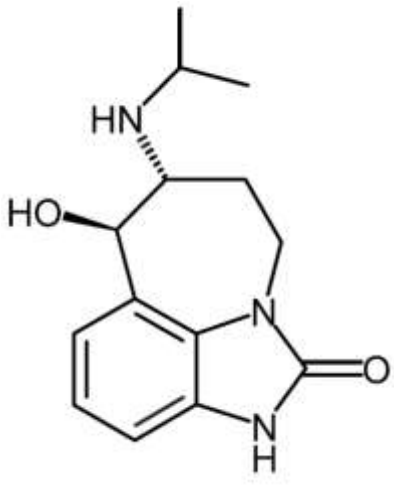
These findings are of utmost importance to the current study as the ZM treatment (additional inclusion of Zilmax® as βA) is seen as standard practice in RSA feedlots today.

2.3.3 Effects of exogenous zilpaterol hydrochloride to improve growth efficiency

The βA compound under current study is ZH which is classified as a β2-agonist because it selectively acts on the β2-adrenergic receptor found on mammalian cell membranes (Mersmann, 1998). Lawrie (2006) states that β2 receptors are associated with striated muscle contractions and vascular smooth muscle relaxations. Further relaxations of the bronchi, trachea and uterus can also be found with β2 receptors. These β2 receptors are associated with muscle metabolism and are consequently an important physiological mechanism to understand when trying to improve the efficiency of livestock growth.

β2-agonists such as ZH and ractopamine hydrochloride (RH) are rapidly metabolized and effectively cleared from tissues, leaving no significant residues (Avendaño-Reyes, *et al.*, 2006). In addition, β2A generally have greater growth effects than β1As even when considering that different studies and βA will naturally vary slightly (Montgomery, *et al*., 2009).

Figure 2.3.1 Chemical structure of zilpaterol hydrochloride (Moody, *et al.*, 2000)



Approval for the usage of ZH in cattle was obtained in 1998 in RSA and Mexico and only later for the USA in 2006 (Brooks, *et al.*, 2008). The ZH compound is now available in the form of two products i.e., Zilmax® and Grofactor®. Since the first Plascencia *et al.* (1999) study on ZH, many have followed suit and Zilmax® is now accepted as a feed supplement in many of the high-throughput beef producing countries. Limited research has been done on the generic Grofactor® product as it is still a relatively new product. The only available published study on Grofactor® is Avendaño-Reyes *et al.* (2016).

Avendaño-Reyes *et al.* (2016) state that the main difference between ZH-based ZM and GF is the location of the ZH molecule. While ZM contains the molecule in the border of feed granules, GF has the molecule dispersed throughout the feed mix.

2.3.3.1 Mechanism of zilpaterol hydrochloride to increase lean growth

In younger animals more energy is naturally directed towards lean tissue deposits instead of fat to promote growth and development. Following the well-known sigmoidal growth curve **(Figure 2.2.1)**, the rate of lean tissue deposition decreases as an animal matures. More mature, heavier animals would therefor direct a larger proportion of energy toward fat deposition instead. This is why βAs are fed during the last 30 days on feed in feedlots (the feedlot finishing period), when animals' rapid growth has been reached and fat deposition normally starts.

βAs are known as repartitioning agents because nutrients are being redirected from fat deposition to muscle accretion instead (Mersmann, 1998). The maturity status of an animal does not influence the degree of ZH repartitioning (Rathmann, *et al.*, 2009). Mersmann (1998) explains that the decreased deposition of fat is brought on by two metabolic pathways; lipogenesis (fat sythesis) that is being reduced and/or lipolysis (fat breakdown) that is being

increased. This results in a slower rate of fat deposition. The action of ZH is reflected particularly at the level of muscle metabolism through increases of skeletal muscle hypertrophy (seen as muscle mass) (Johnson, *et al.*, 2013). This is achieved by increased protein synthesis and decreased protein degradation i.e., decreased muscle protein turnover (Mersmann, 1998; Moody, *et al.,* 2000; Purslow, 2017).

Redirection is brought on by the modification of metabolic signals when βAs bind with specific receptors located on top of muscle and adipose tissue-cells (i.e. myocytes and adipocytes). Biochemical signals are produced within these cells that stimulate or inhibit fat degradation or synthesis, respectively (Anderson, *et al.*, 2005).

The mechanism of growth via satellite cell fusion proposed by Purslow (2017) is still a relatively new concept, so many researchers rather focus on the direct binding of βAs to their specific receptors (Johnson, *et al.*, 2013).

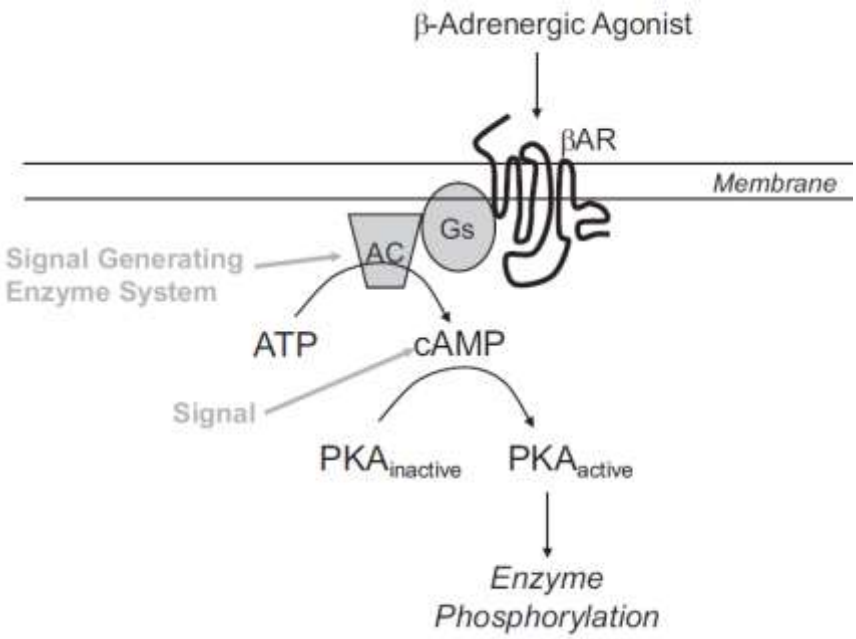
2.3.3.2 βA receptor pathway for energy repartitioning

β-adrenergic receptors are naturally stimulated by neurotransmitters such as the adrenal medullary hormone, adrenaline, and noradrenaline (Mersmann, 1998). Therefore, synthetic analogues such as βAs will compass the same response.

The β-adrenergic receptor (βAR) of a cell membrane is activated when binding to the specific agonist that has a high affinity for the receptor-type e.g. when ZH binds to a β2-receptor on an adipocyte. The binding of ZH to a receptor cascades through signalling events to penultimately either up- or downregulate genes that cause protein accretion and lipogenesis, respectively (Mersmann, 1998). This agonist-receptor complex activates the α-subunit of G_s proteins that in turn activates the enzyme adenyl cyclase (AC), which converts ATP to cyclic adenosine monophosphate (cAMP). cAMP is known to be an intracellular signaling molecule, because high concentrations cAMP would bind to the

regulatory subunit of protein kinase A (PKA), to then release the active catalytic subunit. PKA phosphorylates many intracellular enzymes and regulatory factors that are important for metabolic regulation e.g. phosphorylation can inactivate lipogenic enzymes that regulate fat metabolism within the fat cells.

Figure 2.3.2 Mechanism of signalling cascade from initial binding of a β-adrenergic agonist (βA) with βA specific receptors (βAR) (Anderson, *et al.*, 2005)



In muscle cells the intracellular signalling by cAMP would cascade until the relevant enzymes create an abundance of total ribonucleic acid (RNA) and messenger RNA (mRNA) for myofibrillar proteins to be synthesized. This would result in muscle-cell growth (hypertrophy) and increased lean mass.

β2-adrenergic receptors are affiliated with vascular smooth muscle relaxations which instigated the theory that hypertrophy of muscle can be brought on with increased blood flow. By transporting more substrates and energy sources, needed for protein synthesis, to muscle cells (Purslow, 2017, p. 617).

With the nutrients now being directed toward muscle growth instead, leanness and dressing percentage will increase, feed would be better utilised, and rate and efficiency of gain would be improved.

2.3.4 Global importance of the implementation of β-adrenergic agonists

European Union (EU) member states and Russia have prohibited the use of certain drugs and hormones in livestock production since 1996, under a strong Council legislation; 96/22/EC.

Despite the wide use and acceptance of growth promotants, many countries such as European Union members and Russia ban or restrict the use of βAs (Niño, Granja, Wanschel, & Salerno, 2017). This can negatively influence exporting countries that use these compounds, as a “split system” can be enforced in production. Split systems ensure the eligibility of products from animals not treated in any stage of production with the banned substances (Niño, *et al.,* 2017).

The first maximum residue levels (MRLs) of 10ppb were adopted on July 5, 2012, by the Codex Alimentarius Commission for ractopamine hydrochloride (RH) in beef and pork (Niño, *et al.*, 2017). Niño *et al.* (2017) explain that if residues of banned substances are found to be above MRL concentrations, products can be rejected, recalled or destroyed. Purslow (2017) states that ZH however, has no established international MRLs, rationalizing the bans on meat products containing this drug.

This can negatively affect the economics of exporting countries using the compound, even if withdrawal periods are applied to limit residues. Protective measures can be implemented such as intensive testing or even delisting countries from importation .

2.4 Carcass characteristics related to the beef production efficiency and meat quality

A slaughtered animal is converted into a carcass component and non-carcass component. The non-carcass component is then subdivided into inedible, and edible (variety meat) portions known collectively known as offal. Meat animal carcass weights generally constitute of 50-70% muscle tissue which is then converted to meat post-mortem (Hui, 2012). When food products are derived from either the carcass component or edible offal portion, they are referred to as meat (Seman, *et al.,* 2018).

To ensure that consumers receive a safe and wholesome product, the Meat Safety Act (Act 40 of 2000) serves as a guideline for producers and retailers for the conversion of livestock to meat (Webb, 2015). Carcass quality subsequently affects meat quality which is why it is important to understand the easily measurable characteristics which can directly be related to prominent meat quality characteristics (Webb, 2015).

Many countries have focused their research on producing heavier carcass weights from less resources by improving growth efficiency and muscle growth (Mekonnen & Hoekstra, 2010; Webb & Agbeniga, 2020). Heavier carcasses have increased value per unit of protein produced as no feed, water or labour efficiencies are negatively affected in the production thereof (Mekonnen & Hoekstra, 2010; Webb & Agbeniga, 2020; Ramanathan, *et al.*, 2021). This is currently the practice in South Africa to counter the high grain prices by increasing carcass incomes from animals with heavier carcasses (Webb & Agbeniga, 2020). The most cost-effective method of obtaining ensured heavier carcass weights for all maturity-type cattle, at lower expenses, is to implicate GET such as ZH-based feed additive products (Mekonnen & Hoekstra, 2010; Capper & Hayes, 2012).

To clearly demonstrate the carcass characteristic effects that ZH might ensue, the results of a few different studies will be tabulated in **Tables 2.4.1** and **2.4.2**. Only characteristics relevant to the current study will be fully discussed.

Maxwell *et al.* (2015) treatments are already defined in **Chapter 2.2.3**. The NAT treatment will be considered only to illustrate the additive effects of GET. ZM is considered the standard South African feedlot practice and therefore studies who utilised *B. taurus* (purebred or crossed) cattle with ZM supplementation will be compared, unless stated otherwise. To determine the synergistic effects that ZH brings about, ZM will be compared to the conventional treatment which excludes βA additives (CT).

It is important to note that comparative experiments (unless stated otherwise) tested on feedlot steers instead of bulls. Furthermore, ZM values will be observed at 30 days of ZM supplementation as that is the average recommended supplementation period.

Table 2.4.1 Summary of previous findings about the effects of ZH-supplementation on carcass characteristics of feedlot steers

Study	Montgomery *et al.* (2009)	Hilton *et al.* (2009)	Scramlin *et al.* (2010)	Arp *et al.* (2014)
Carcass characteristics	**Additive effect when ZM is added (ZM vs. CT)**			
HCM (kg)	↑ with 16.4kg (P < 0.001)	No effect (370 vs. 365.5kg; P = 0.38)	↑ with 12.89kg (370.7 vs.357.8kg; P < 0.05)	↑ with 11.1kg (387.7 vs. 376.6kg; P < 0.05)
DR%	↑ with 1.5% (P < 0.001)	N/A	↑ with 1.93% (P < 0.05)	↑ with 1.4% (65.2 vs. 63.8%; P < 0.05)
LMA (cm^2)	↑ with 8.23cm^2 (P < 0.001)	↑ with 9.6cm^2 (88.9 vs. 98.5cm^2; P < 0.001)	↑ with 3.76cm^2 (38.0 vs. 34.2cm^2; P < 0.001)	↑ with 6.7cm^2 (91.4 vs. 84.7cm^2; P < 0.05)
FT (cm)	No effect (P = 0.12)	↓ with 0.23cm (1.2 vs. 1.0cm; P = 0.001)	↓ with 0.17cm (1.1 vs. 1.2cm; P < 0.05)	No effect (P > 0.05)
Abscessed livers	No effect (P = 0.31)	N/A	N/A	N/A
KPH fat	No effect (P= 0.89)	↓ with 0.22% (1.9 vs. 1.7%; P < 0.001)	↓ with 0.95kg (10.1 vs. 11.1kg; P < 0.05)	N/A

HCW = Hot carcass mass (kg).
DR% = Dressing percentage (%).
LMA = Longissimus muscle area (cm^2).
FT = 12th rib fat thickness measured by a P2 calliper (cm).
KPH fat = fat covering the kidneys, pelvis and heart

Table 2.4.2 Continued summary of previous findings about the effects of ZH-supplementation on carcass characteristics of feedlot steers

Study	Maxwell *et al.* (2015)		Avendaño-Reyes *et al*. (2016)
Carcass characteristics	**Effects when GET is added (NAT vs ZM)**	**Additive effect when ZM is added (ZM vs CT)**	**Additive effect when GF is added (CT vs. GF)**
HCM (kg)	↑ (348 vs. 394kg; P < 0.01)	↑ (394 vs. 386kg; P = 0.05)	↑ (301.2 vs. 321kg; P < 0.001)
DR%	NAT steers had the lowest % (63.0 vs. 64.7%; P < 0.01)	↑ with 1.6% (64.7 vs. 63.0%; P < 0.01)	↑ with 2.96% (58.0 vs. 61.0%; P = 0.02)
LMA (cm^2)	↑ by 12.1cm^2 (92.3 vs. 80.2cm^2; P < 0.01)	↑ with 3.6 cm^2 (92.3 vs. 88.7cm^2; P = 0.02)	No effect (60.2 vs. 60.5cm^2; P = 0.97)
FT	No effect (1.1 vs. 1.2cm; P = 0.81)	↓ with 0.12 cm (1.2 vs. 1.1cm; P = 0.03)	No effect (0.7 vs. 0.7cm^2; P = 0.83)
Abscessed livers	No effect of any treatment (P = 0.74)		N/A
KPH fat	N/A		↓ with 0.5% (2.7 vs. 2.2%; P = 0.007)

HCW = Hot carcass mass (kg).
DR% = Dressing percentage (%).
LMA = Longissimus muscle area (cm^2).
FT = 12th rib fat thickness measured by a P2 calliper (cm).
KPH fat = fat covering the kidneys, pelvis and heart

The improvement in carcass gains for all ZH-supplemented animals when compared to CT, is attributed to live performance improvements in both ADG and efficiency during the finishing phase.

Although Avendaño-Reyes *et al.* (2016) used zebu bulls (75% *B. indicus*, 25% *B. taurus*), the authors compared the generic ZH supplement - GF with both a CT treatment and ZM. The authors found no significant effects when comparing the different carcass characteristics for GF supplementation with ZM ($P > 0.05$).

2.4.1 Effects of carcass mass (HCM) on the economics of beef production and carcass quality

The relationship between the live slaughter weight and the weight of the marketable carcass is very important as many cattle are sold on a “dead weight basis” (Lawrence & Fowler, 2002). After being dressed, during which the hide, head, intestines, and feet are removed from the carcass before chilling, the carcass left over is referred to as HCM. This characteristic is a practical indicator of the live growth responses and overall tissue depositions (Lawrence & Fowler, 2002). How these tissues have been deposited will further be determined by the weight, shape and confirmation of the carcass.

Faster growing animals not only have greater deposits of fat subcutaneously, but internally as well (seen by intramuscular fat and fat covering the kidneys, pelvis, and heart [KPH]), whereby these internal depots restrict gut fill capacity (Lawrence & Fowler, 2002). Slower growing or later maturity-type animals would therefore have a greater gut fill capacity which will result in lower carcass mass once the intestines are removed. Beef cattle HCM is on average expected to be 76.5% of the live body mass (Lawrence & Fowler, 2002).

The additive effects of GET can clearly be illustrated by HCM weights as NAT steers were the lightest (348kg; $P \leq 0.05$), CT steers intermediate (386kg) and ZM steers the heaviest (394kg) (Maxwell, *et al.*, 2015). Both Plascencia *et al.*

(1999) and Brooks *et al.* (2008) stated that ZH supplementation consistently, significantly increases HCM ($P < 0.05$). This is supported when comparing previous studies (**Tables 2.4.1 and 2.4.2**) as well as Avendaño-Reyes *et al.* (2006).

Scramlin *et al.* (2010) compared both ZM and ractopamine (RT) supplementation with CT steers and found favourable, significant increases in HCM from both RT and ZM. ZM did however yield greater HCMs than RT ($P < 0.05$).

The HCM component consists of muscle, fat and bone which can subsequently be related to other important carcass characteristics i.e., dressing percentage, fat content and relative muscling determined by composition proportions (Hui, 2012).

2.4.2 Effects of dressing percentage (DR%) on carcass and meat quality

An important economic indicator for producers is DR% as how much meat a carcass will yield and is calculated by $\frac{HCM}{slaughter\ mass}\ x100$ (Schweihofer, 2011). The average DR% for beef cattle is 62% (Hui, 2012) and ranges from 57 – 64% (Schweihofer, 2011). In the past, producers attempted to increase the DR% by focusing on increasing the relative HCM. This was achieved by producing fatter carcasses which would increase the mass yields (Webb & Erasmus, 2013). Webb (2015) explains that in addition to consumers now preferring leaner carcasses, modern GETs now cost-effectively promotes the reduction in carcass fatness. DR% can be lower than expected for carcasses with excessive mud/manure on the hide, gut fill, horns and bruises which increase the slaughter mass but are removed during dressing (Schweihofer, 2011; Hui, 2012).

ZH treatment brings about a significant ($P < 0.05$) improvement in DR% as these animals consistently have a greater percentage yield with 1% up to 5% more than their untreated counterparts, the average report being 1.5%

(Plascencia, *et al.*, 1999; Brooks*, et al.*, 2008). This is supported when comparing previous studies (**Tables 2.4.1** and **2.4.2**).

2.4.3 Effects of subcutaneous fat thickness (FT) on carcass and meat quality

Measurements of FT over muscle, are performed directly with callipers at the 12th rib and is known as a P2 measurement. These measurements are valuable to visually predict the relative carcass composition and in cases where producers are paid on a carcass quality basis (Lawrence & Fowler, 2002).

Although consumer health awareness drives the market toward leaner carcass production, Webb and O'Neill (2008) illustrated that carcass fat significantly contributes to the eating quality of beef. The authors continue to explain that as a result of producers striving toward improved meat quality, beef carcasses will now be produced within a specific fat (classification) code range of 2 and 3.

When comparing additive GET effects, superficial fat deposition (measured as FT) was lower in ZM steers than in CT (1.1 vs. 1.2cm; P = 0.03), this illustrates how nutrients are efficiently being redirected to muscle accretion with βA (Maxwell, *et al.*, 2015). There was however no significant difference between NAT and ZM steers in the same study (1.1cm for both; P = 0.81), suggesting that hormonal treatments (CT) may increase subcutaneous fat deposition without the countering effects of βA.

This is a controversial measurement as the results often vary between different studies. Some report a decrease in FT (Hilton, *et al.,* 2009; Rathmann, *et al.,* 2009; Maxwell, *et al.*, 2015), while others report no significant changes with ZH supplementation (Avendaño-Reyes, *et al.,* 2006; Montgomery, *et al.,* 2009; Arp, *et al.,* 2014; Avendaño-Reyes, *et al.,* 2016).

Cônsolo, Ferrari, Mesquita, Goulart and eSilva (2016) similarly investigated the effects of ZM-supplementation in Nellore (*B. Indicus*) heifers and found that ZM

supplementation had no effect on FT measurements (P = 0.96). Avendaño-Reyes *et al.* (2016) determined that FT values remained the same when CT was compared to GF (0.7cm for both; P = 0.83) but decreased when GF supplementation was compared with ZM (0.7 vs. 0.6cm; P = 0.64) in zebu bulls.

2.4.4 Effects of carcass composition on carcass and meat quality

To determine the repartitioning effect that βAs are known for, carcass composition should be evaluated to determine the relative proportions of meat and fat.

Table 2.4.3 Summary of previous findings on the effects of ZH-supplementation on carcass composition

	Leheska *et al.* (2009)	**Hilton *et al.* (2009)**
Item	**Effect of ZH supplementation**	
Carcass fat%	↓ P = 0.25	↓ P < 0.001
Carcass protein%	↑ P < 0.001	↑ P = 0.002
Carcass bone%	↓ P = 0.14	No effect (P ≥ 0.12)
Protein:fat ratio	↑ P = 0.23	N/A

As mentioned previously; contrasting results have been reported for carcass fat content. This includes Avendaño-Reyes *et al.* (2006) (P > 0.10) as well as Leheska *et al.* (2009) finding no effect (P = 0.25) of ZM supplementation on carcass fat % when evaluating carcass composition for the Montgomery *et al.* (2009) study. However, Hilton *et al.* (2009) reported a significant (P < 0.001) decrease in the carcass fat%.

ZM supplementation resulted in significant increases in carcass protein% for both studies. This is due to the increased muscle protein deposition as well as protein accretion expected with βA supplementation (Mersmann, 1998; Leheska, *et al.,* 2009). Due to the mixed reports of ZM's effect on FT, we can

infer that the effects on carcass fat% are minimal along with those of carcass ash and bone.

2.4.5 Carcass classification as a summation of carcass quality

Carcass quality is defined by using classification (or grading) systems which depict the overall muscular and fat confirmation of a carcass (Lawrence & Fowler, 2002).

To control and specify product standards for both local as well as export purposes, the Agricultural Product Standards Act (Act 119 of 1990) was established. Carcass inspecition, classification or grading and sampling are ascribed witthin this act to ensure product safety and consistency (Webb, 2015).

When considering that consumers differ in their preferences to the same product, both between different and within the same geographic regions (Seman, *et al.*, 2018), it is important to provide a choice of products differing in physical and sensorial characteristics (Webb, 2015). Webb (2015) states that replacing the grading system with a carcass classification system in South Africa in 1992 (Strydom, 2002), served as an end to this specific mean.

Carcass grading systems provide a subjective indication of perceived meat quality and value (Strydom, 2002) by grading carcasses as either of standard, prime or superior carcass grade (Webb, 2015). Whereas carcass classification is based upon the concept that different members of the supply chain, as well as consumers, have different expectations for qualities cascading down from a carcass to meat, to consumption level (Webb, 2015).

Carcass classification can therefore be defined as the combined description of different, clearly defined, carcass characteristics – without referring to their relative economic importance (Kempster, Cuthbertson, & Harrington, 1982; Strydom, 2002; Webb, 2015).

Strydom (2002) illustrated that because the classification system allows prices to be determined by the real value of carcasses, greater pressure is generated towards the producer to better the carcass value and composition, in order to achieve better prices amongst competitors. Improved economic efficacy can be achieved by considering marketing aspects when aligning the biological and economical targets of livestock production, to meet the changing consumer demands (Kempster, *et al.,* 1982; Webb & Erasmus, 2013; Webb, 2015).

Owing to intrinsic factors such as cattle breed, maturity type, age, sex and interactions with extrinsic factors - such as production systems - carcass composition and quality varies significantly (Webb & Erasmus, 2013; Purslow, 2017). Classing similar carcasses within the same classifiaction category will therefore reduce the variation between carcasses and ensure consistent carcass quality (Webb, 2015).

The South African carcass classing categories are clearly defined by physical and compositional characteristics, i.e., physiological age (determined by the number of permanent incisors), fat codes, overall confirmation, carcass damage and sex. Utilising these characteristics ensures that products have specific compositional and quality attributes within the same category (Webb, 2015).

Table 2.4.4 The South African red meat carcass classification system owing to important marketing characteristics (Strydom, 2002; Webb, 2015)

Carcass characteristic	Different classes of each characteristic			
Physiological age category	A	AB	B	C
Number of permanent incisors	0	1-2	3-6	>6
Carcass fatness codes	Ranges from 0 – no fat to 6 – excessively overfat			
Conformation scores	Ranges from 1 – very flat to 5 – very round			
Damage classes	Ranges from 0 – undamaged to 3 – severe damage			

Sex is important to carcass classification because although physiologically young animals (class A) are equally tender as other animals in the same age group, bulls from classes B and C are less tender than their female and castrated male counterparts (Strydom, 2002). Bull carcasses in older classes are thereby distinguished by a “M/D” stamp mark.

Table 2.4.5 Different South African red meat carcass indicative marks according to the physiological age category (Webb, 2015)

Physiological age class	Rollermark code	Rollermark colour
A	AAA	Purple
AB	ABAB	Green
B	BBB	Brown
C	CCC	Red

These classification determination characteristics have significantly been improved since the implementation of the carcass classification system in South Africa in 1992 by means of bettering knowledge on livestock breeds and types, utilising different production systems, improving feedstuffs, and using GETs (Webb & Erasmus, 2013).

Currently the Red Meat Abattoir Association of South Africa estimates that “only 5% - 10% of the carcass classification system is used, notably classes A2, A3,

AB2, AB3" (Webb, 2015). This corroborates the fact that feedlot animals are slaughtered at a similar, relatively young age because of better feeding and management practices to maximise growth, along with the shift in focus to produce more energy effective, leaner carcasses (i.e., lower fat content).

The implementation of certain production enhancing technologies and abattoir practices (e.g., injecting lactate-solutions, electrical stimulation and ageing of carcasses or products) can contribute to additional variation among carcasses (Hope-Jones, Strydom, Frylinck, & Webb, 2010) despite correct classification. Webb (2015) argues that although carcass inspection is compulsory in all South African abattoirs as to (partially) condemn diseased or defected carcasses, carcass classification is not mandatory. This might be in part because in order to be allowed to classify carcasses, an abattoir must register with the Department of Agriculture (Strydom, 2002).

To further exacerbate the matter, the author concludes that the current classification system still creates a bias among carcasses (similar to a grading system), thereby defeating its' original purpose. Age and fatness are the two most important carcass classification criteria therefore, A2/A3 carcasses are of highest marketing and economic value (Strydom, 2002).

2.4.6 Effects of the duration of zilpaterol supplementation on carcass characteristics

Montgomery *et al.* (2009) not only investigated ZM supplemental effects, but additionally whether the duration of supplementation held any merit. Supplementation periods of 20 and 40 days were investigated.

When evaluating the results obtained from Montgomery *et al.* (2009), extending the duration of ZH supplementation from 20 to 40 days had significant increasing effects on HCW, DR% and LMA but not FT of feedlot steers. Strydom *et al.* (1998) also reported a significant increasing trend when increasing ZM

supplementation periods from 15 to 30, to 45 days for carcass mass and DR% ($P < 0.05$).

Table 2.4.6 Literature findings of the effects of different ZH-supplementation durations on feedlot steers' carcass characteristics (Montgomery, *et al.*, 2009)

Carcass characteristic	Effects of extended supplementation duration (40 vs 20 days)	ZH x duration of ZH feeding interaction (P-value)
HCW (kg)	Tend to ↑ (P = 0.06)	0.13
Dressing %	↑ (P = 0.04)	0.06
LMA (cm^2)	↑ (P = 0.02)	0.65
FT (cm)	No effect (P= 0.59)	0.61
Abscessed livers	No effect (P = 0.51)	0.98
KPH fat	No effect (P = 0.45)	0.38
Masculinity	↑ (P = 0.008)	0.01

HCW = Hot Carcass Weight (kg).
DR% = Dressing percentage (%).
LMA = Longissimus muscle area (cm^2).
FT = 12th rib fat thickness measured by a P2 calliper (cm).
KPH fat = fat covering the kidneys, pelvis and heart

To maximize these favourable effects on carcass mass and DR%, it would be recommended to supplement ZM for more than the recommended minimal 20-day period. This is due to the additive nature of performance improvement.

2.5 Meat quality characteristics and factors that influence it

Coupled with the rise in expendable income in developing countries - as discussed earlier - more consumers can afford higher quality and value-added products (Carpenter, Cornforth, & Whittier, 2001; Paul, *et al.,* 2021). This in turn leads to greater scrutiny of meat products.

Meat can be defined as “the flesh of animals used as food” (Lawrie, 2006). A more intricate description would be that meat is the skeletal muscle along with its associated tissues that are derived from any animal species, harvested for human consumption (Seman, *et al.*, 2018).

Savell *et al*. (1987) was the first to postulate that consumers between different geographical regions had different responses to the same kinds of beef. This is because customer satisfaction is driven by the palatability of meat. Meat palatability is mostly determined by a sensory panel instead of being measured instrumentally, and is thus highly subjective to individual preference.

To satisfy consumer eating expectations, focus should be shifted toward the quality of meat which is being produced. The most important factor is meat tenderness. But because consumers first have to purchase the meat, visual factors such as meat colour and drip loss should be emphasized as well (Carpenter, *et al.*, 2001).

2.5.1 Conversion of muscle to meat in the carcass

When slaughtering an animal, different phases of death can be associated with typical physiological changes (Webb, 2020, sl. 10). Understanding this basic concept is important to explain certain meat quality phenomenon and to extrapolate how abnormal physiological changes can result in decreased meat or product quality.

The first stage is *palor mortis,* where the face and body appears pale due to exsanguination (removal of blood from body). The normal blood circulation is

now disturbed, and oxygen can no longer be transported to the relevant muscle cells. Once the muscle depletes its' oxygen supply to generate ATP, the internal carcass environment will attempt to achieve homeostasis through anaerobic glycolysis. As a result of the oxidation-reduction potential being altered, resynthesis of ATP cannot take place through the cytochrome enzyme systems (Lawrie, 2006). During the ensuing breakdown of glycogen, lactic acid is released within the cytosol and lowers the muscle's overall pH because as it cannot be removed by means of an active circulation system (Lawrence & Fowler, 2002). Lawrence and Fowler (2002) state that the normal cattle pH will drop from roughly 7.0 to an ultimate pH of 5.5 within 24-28 hours post-mortem. The authors continue to explain that the rate and extent of the pH decline has a crucial impact on muscle proteins' denaturation. The consequences thereof will be discussed within each meat quality characteristic's section.

Enzymes in the conversion pathway from glycogen to lactic acid are more active in white muscle fibers (Lawrie, 2006) due to their inherent glycolytic metabolism and will have a faster pH drop than their red oxidative counterparts.

Meanwhile, Webb (2020) emphasizes the fact that meat abnormalities, such as pale soft and exudative (PSE) is not a result of only a low ultimate pH but rather that of the rate of pH decline combined with the carcass temperature. During the second phase of death the carcass temperature decreases due to heat dissipation to the external environment, it is ascribed as *algor mortis.* The pH decline is exaggerated at greater carcass temperatures (Purslow, 2017). Purslow (2017) states that the elevated carcass temperature may activate protein kinases which drop the pH lower and at a greater rate of decline. As discussed in **Chapter 2.3.3**, the activation of PKA can additionally be stimulated by ZH interacting with its' specific βAR (**Figure 2.3.2**).

The third phase – *rigor mortis* - is most prominent as this is when muscles begin to stiffen. ATP reserves become depleted as post-mortem biochemistry takes

place consequently, no “new” ATP would be able to bind to the myosin binding site, resulting in the cross bridges not being detached. This will leave muscle filaments immobilised in a contraction, yielding stiff muscles. *Rigor mortis* starts at ca. 2hours post-mortem until all energy reserves are depleted at roughly 8hours post-mortem. The final stage of death is *livor mortis*, where blood starts to collect in certain parts (at the base) of the body due to gravitational pull.

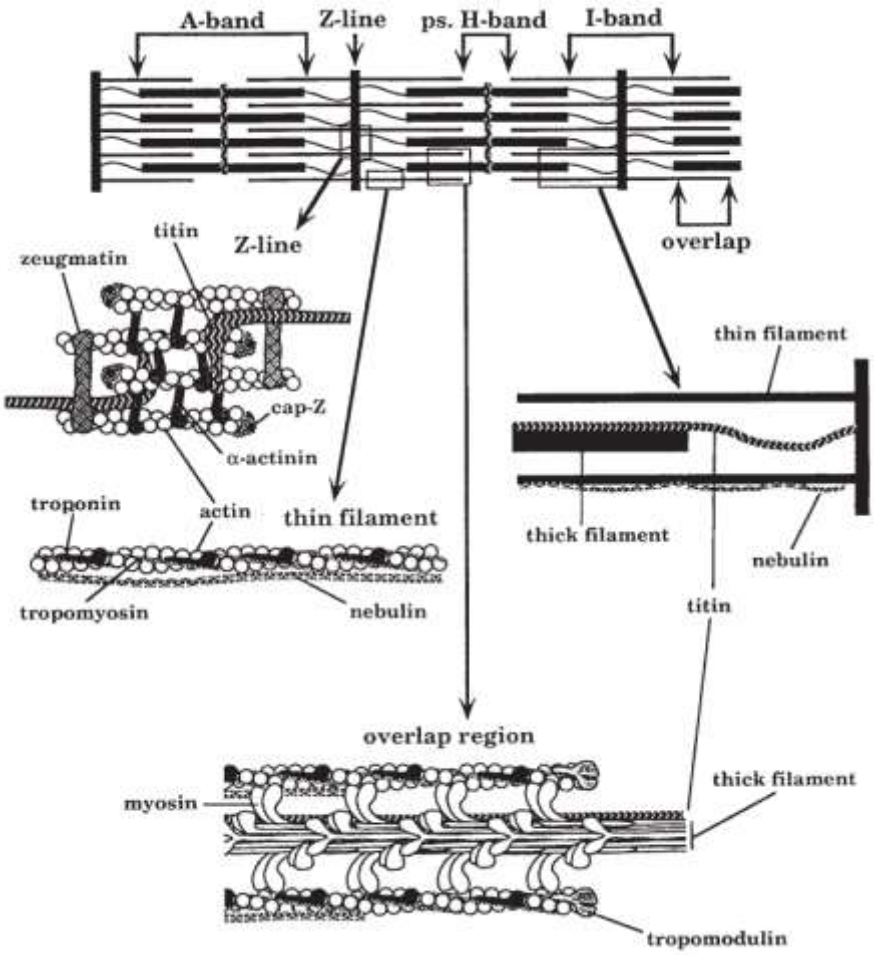
2.5.2 Microscopic muscle fiber structure relating to meat quality aspects

Two main components in muscle fibers that are of biological importance to meat quality are mitochondria and myofibrils.

Myofibrils produce the force of skeletal muscle contractions, to support this function, cross-sectioned skeletal muscle fibers typically contain 500-1000 myofibrils each (Lawrie, 2006; Purslow, 2017). Myofibrils consist of repeated sacromeres, connected end-to-end (Hossner, 2005). Sacromeres represent the smallest contractile unit of a muscle cell. Sacromeres and their contractile nature are most important for meat tenderness and drip or cooking losses.

One sarcomere unit is located between two Z-lines and have two primary components; a thin filament - mainly consisting of the protein actin - which is attached to the Z-lines, and a thick filament - consisting of the protein myosin (Lawrie, 2006). Two other regulatory proteins form part of the thin filament i.e., tropomyosin and troponin which work in unison with ATP to regulate muscle contraction (Sherwood, 2016). Myosin is a large protein consisting of two globular heads attached to one end of a long α-helical tail. These globular heads form the cross bridges between thin and thick filaments by providing a binding site for actin filaments during contraction (seen as the overlapping region in **Figure 2.5.1**).

Figure 2.5.1 Schematic diagram illustrating the relationship between thick (myosin) and thin (actin) filaments, and how thin filaments interact with other proteins at the Z-line (Lawrie, 2006, p. 58)



2.5.3 Effects of meat colour on meat quality

Even though there are many possible factors that can incur meat wastage, the Ramanathan *et al.* (2021) states that the total global meat losses that are solely due to meat discolouration, was over 5.8 tons for 2020. Meat discolouration can superficially be described as a brown-coloured surface appearance of raw (or interchangeably referred to as fresh) meat. This discolouration is seen by consumers as meat quality deterioration (Livingston & Brown, 1981; Faustman & Cassens, 1990b).

This is due to the fact that consumers cannot evaluate any meat quality characteristics (i.e. texture and odour) other than meat colour, in packaged meat at the point of sale (Suman & Joseph, 2013). Making a bright cherry-red colour the most important consumer demand.

Post-mortem muscle has both chromatic (contributing to colour pigment) and achromatic components which can influence visual perception (Ramanathan, *et al.,* 2021). A few examples include, heme proteins (chromatic), mitochondria and structural components such as fat, connective tissue and myofibrillar proteins. Post-mortem muscle fibres allow light to be absorbed, reflected or scattered, owning to their unique matrices (Purslow, Warner, Clarke, & Hughes, 2020b). Ramanathan *et al.* (2021) state that the visual perception of chromatic components depends on these light properties which can be further influenced by either chromatic or achromatic components. One such instance is when a decreased post-mortem pH results in the degradation of myofibrillar proteins which disturbs the inherent structural light scattering properties of muscle fibers (Lawrence & Fowler, 2002).

2.5.3.1 Economic implications of meat discolouration

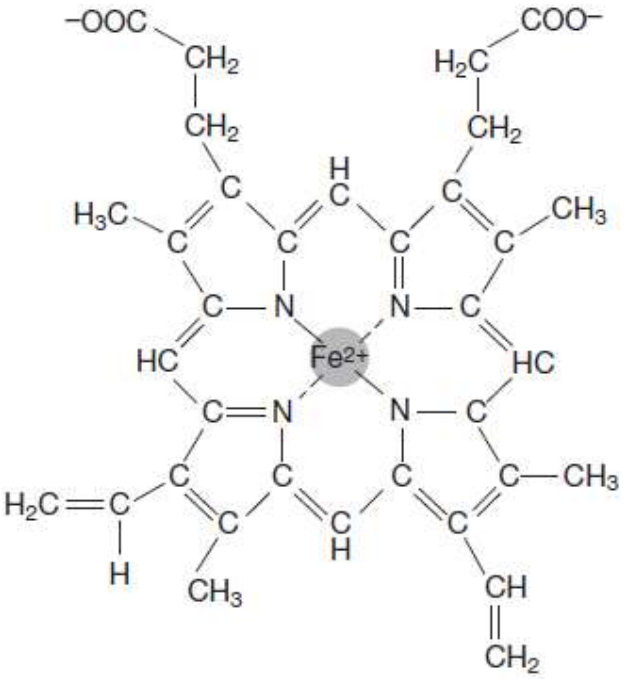
Ramanathan *et al.* (2021) admits that it is difficult to determine the precise economic losses that are solely due meat discolouration. This is due to the complex variety of global meat merchandising systems such as farm-to-fork. Other examples include in-store meat processing and displaying as well as case-ready products being readily shipped to distributers.

Ramanathan *et al.* (2021) reported figures of $3 billion in the USA and $14.2 billion globally that are being lost in the retail meat industry as a result of meat colour not being acceptable for consumer consumption.

2.5.3.2 Effects of heme proteins on meat colour

According to the American Meat Science Association [AMSA] (2012) guidelines to meat colour measurement, heme proteins impart colour because of the presence of a transition metal (Fe^{2+}) and inherent porphyrin structure. One view is that “hemes, or iron–porphyrin complexes, are the versatile and ubiquitous active centers of these proteins” (Paoli, Marles-Wright, & Smith, 2002, p. 271). The concentration and chemical state of these proteins determine the colour in red meat especially. Prominent chromatic heme proteins in meat science are haemoglobin, cytochrome c and myoglobin (Mb) (Suman & Joseph, 2013). All three are associated with the transport of oxygen and have critical functions in cellular metabolism. The inherent heme proteins’ structure has both iron and conjugated double bonds of protoporphyrin which allows the absorption and reflection of light (Purslow, 2020b; Ramanathan, *et. al,* 2020a).

Figure 2.5.2 Basic chemical structure of a heme protein (Everse, 2013)



Haemoglobin

A popular belief exists that meat is inherent of its' red colour due to blood content. Upon slaughter of an animal, blood is removed from the body through exsanguination. This will induce *palor mortis* – as discussed earlier – resulting in a pale appearance of the body.

To evaluate the average residual blood content, the effectiveness of bleeding was evaluated by Warriss and Rhodes (1977), who determined that small capillaries in the post-mortem muscle of a carcass can retain 1-4% of the total blood volume of a normal steer. Normal butcher's meat was found to only contain 0.3% residual blood content. These findings led to the conclusion that in commercial slaughtering, no "bad bleeding" occurred and that neither carcass nor meat contain significant amounts of blood to contribute to the red colour. It follows that with slaughtering techniques being researched and improved over time, the same holds true for modern bleeding practices.

The belief physiologically stems from the fact that haemoglobin is a main component of blood and contains a heme prosthetic group, making it a heme protein. The oxygenated form of this heme prosthetic group is responsible for the red appearance of blood when it is exposed to oxygen.

It follows that since most of the haemoglobin is removed during exsanguination in cattle (Warriss & Rhodes, 1977), Mb and cytochromes would subsequently contribute to meat colour instead of haemoglobin.

Cytochromes

Cytochromes are defined as intracellular heme proteins that transfer electrons by undergoing oxidation-reduction (Nomenclature Committee of the International Union of Biochemistry [NC-IUB], 1992). This function is inherent due to the structural nature and abundance of cytochromes throughout the mammalian

body. Cytochromes can be found as membrane proteins or globular proteins depending on their function and are closely associated with mitochondria.

Cytochromes are found in mitochondria which are abundantly spread throughout the muscle cells, coupled with the main function of electron transfer, it suggests that the concentration and different forms of cytochrome play a more vital role in post-mortem pH changes as mitochondria remain active. These active mitochondria both comsumes oxygen and contribute to post-mortem pH changes when undergoing oxidation-reduction. Both actions negatively influence biochemical processes that not only incluence meat colour, but other meat quality attributes as well.

Myoglobin

Due to the negligible direct contribution to meat colour by haemoglobin and cytochromes, Mb ultimately determines meat colour (Livingston & Brown, 1981). Haemoglobin and Mb are differently classed from cytochromes due to their specific associations with oxygen transport and storage, whereas cytochromes have a more focused function of electron transfer (NC-IUB, 1992; Paoli, *et al.*, 2002). These two molecules differ further in their quaternary structure allowing Mb to bind oxygen more tightly than haemoglobin. This difference in binding energy allows haemoglobin to transport oxygen from the bloodstream to the muscle cells that contain Mb.

Bechtold, Mafi, VanOverbeke and Ramanathan (2018) compared the relative redox stabilities of all three heme proteins, at two different temperatures (4°C vs. 25°C). The ratio of oxidation: reduction was used to compare oxidation between the different heme proteins, as all three have differing oxidation–reduction peaks. The authors found that Mb had the greatest oxidation, followed by haemoglobin and then cytochrome c ($P < 0.05$) for the two different temperatures. Cytochrome c remained stable and underwent minimal oxidation,

compared to the other two proteins. This supports the theory that Mb determines meat colour since it is the most susceptible to oxidation-redox reactions as intrinsic circumstances change post-mortem.

Myoglobin (Mb) is a water-soluble, sarcoplasmic heme protein that consists of the chromatic heme portion and achromatic globin protein portion (Suman & Joseph, 2013). This globin portion contains amino acid residues. Both the number of these residues as well as the heme are structurally the same in beef animals and poultry. The distinguishing factor between species lies in the actual amino acid composition (AMSA, 2012).

Due to the inherent heme structure the Mb ligand can be interchanged according to oxidation-reduction reactions; the ligand - along with the ensuing valence state of the iron atom - is therefore a key contributing factor in determining the different forms of Mb (NC-IUB, 1992). These different forms of Mb dictate meat colour. Fresh meat can naturally contain deoxy- (DMb), oxy- (OMb), carboxy- (COMb) and metmyoglobin (MMb). These different forms along with their structural differences and associated meat colour are tabulated below.

Table 2.5.1 Myoglobin forms' valence states and ligands in fresh meat along with their associated meat colours

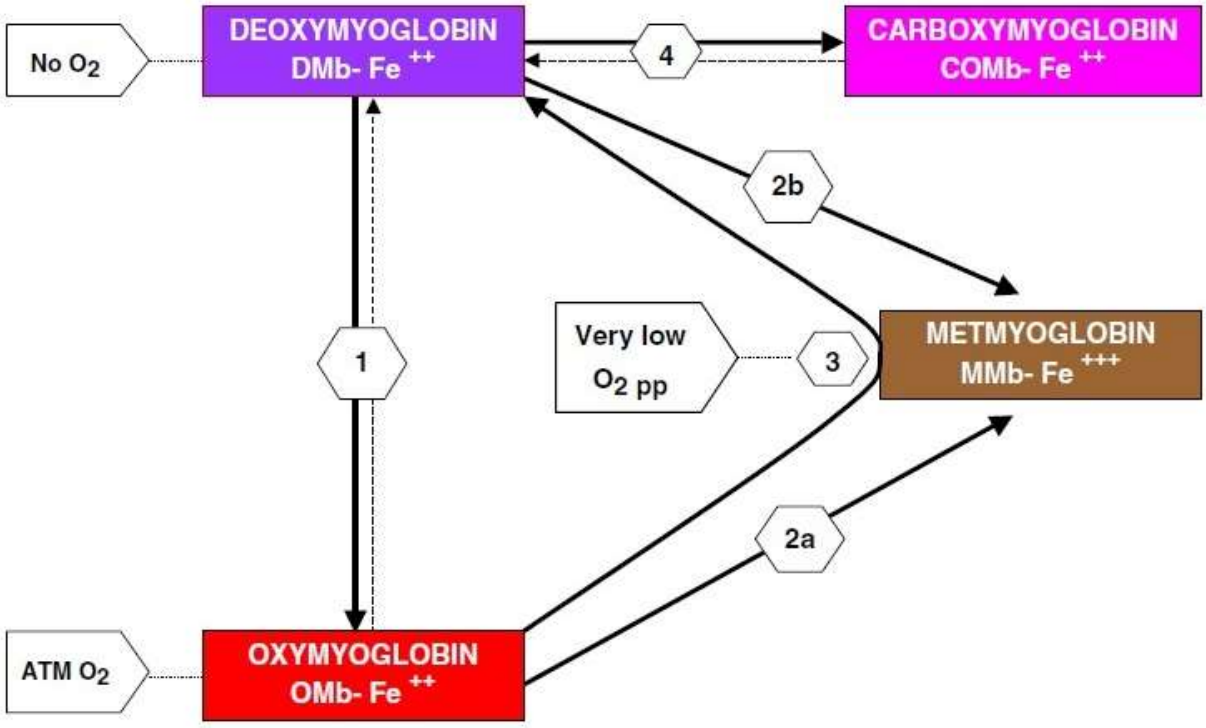
Myoglobin form	Ligand	Valence state	Meat colour
DMb	None	Fe^{2+}	purplish-red
COMb	CO	Fe^{2+}	bright red
OMb	O_2	Fe^{2+}	bright red
MMb	H_2O	Fe^{3+}	brown or yellowish

2.5.3.3 Reactions between myoglobin derivatives in fresh meat

In live muscle Mb binds to oxygen and then delivers it to the mitochondria in muscle cells, allowing physiological maintenance processes to take place (Livingston & Brown, 1981; Renerre, 1990). The conversions between the

different derivatives of Mb are therefore governed by the oxygen affinity of the heme protein (Suman & Joseph, 2013).

Figure 2.5.3 Conversions between myoglobin derivatives that are visible on the surface of meat (Mancini & Hunt, 2005)



DMb is typically associated with the purplish-red colour of muscle immediately after cutting the carcass. This is due to the absence of a ligand at the 6th coordination site of the heme iron, resulting in its' ferrous state (Fe^{2+}). Mb has a very low oxygen tension threshold (<1.4 mm Hg) that maintains it in a deoxygenated state (Brooks, 1935).

Oxygenation

Upon post-mortem exposure of muscle to atmospheric oxygen above 1.4 mm Hg, reaction 1 occurs where DMb is oxygenated to OMb. A diatomic oxygen now occupies the 6th coordination site as ligand. This reaction is known as oxygenation instead of oxidation because the ferrous state remains the same (Fe^{2+}). As with haemoglobin, oxygenation of myoglobin to OMb changes the

meat colour to the preferred bright cherry red. The process of changing colour in meat is known as blooming. OMb will continue to penetrate further below the meat's surface as the meat's exposure to oxygen increases. The depth and thickness of this OMb layer thereby relies on the meat's oxygen partial pressure (OPP) and competition for oxygen by other molecules (such as mitochondria) and respiratory processes (Mancini & Hunt, 2005). Other factors that influence the oxygenation capacity of meat include meat temperature and pH post-mortem as this influences the conversion of muscle to meat as well as intramuscular biochemistry.

The superficial DMb is converted to OMb and continues to penetrate the meat in depth, resulting in a "top red" layer of OMb and "bottom purplish" layer of DMb which could be expected.

Oxidation

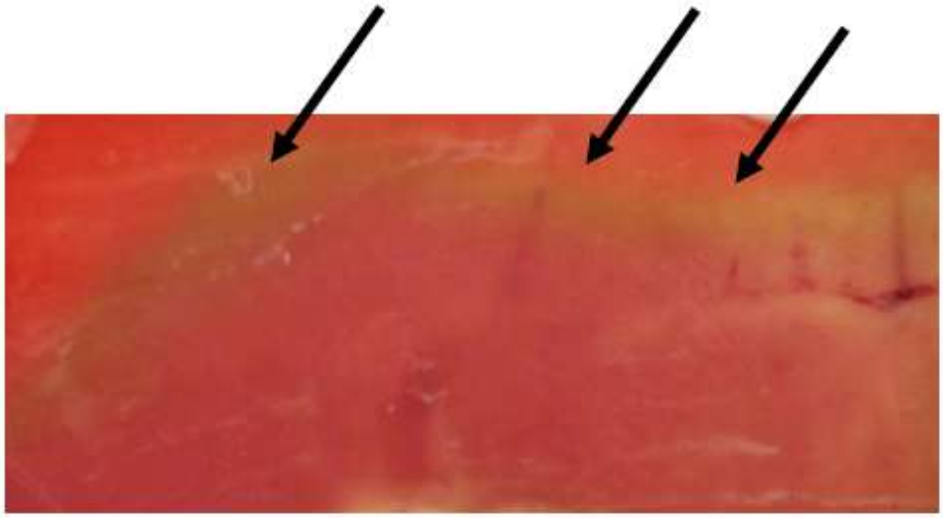
Discolouration of meat occurs when both ferrous forms of myoglobin (OMb and DMb) oxidize to ferric iron (Fe^{3+}). These reactions are indicated as 2a and 2b on **Figure 2.5.3** and the process is known as autooxidation. This resulting derivative is known as MMb and has a water molecule as ligand (Wallace, Houtchens, Maxwell, & Caughey, 1982).

Discolouration is defined by Mancini and Hunt (2005) as the amount of meat surface area which is covered by MMb. The authors explain that the subsurface myoglobin derivatives also contribute to the overall appearance as an additional yellowish-brown layer will form between the OMb and DMb layers when both derivatives oxidize to MMb. The MMb layer will thicken and migrate towards the surface if discolouration continues. This phenomenon is illustrated in **Figure 2.5.4**, obtained and enhanced from Ramanathan *et al.* (2020a).

MMb formation depends on a few of the same factors as OMb formation (i.e. OPP, temperature and pH) with an addition of meat's reducing activity (Wallace,

et al., 1982; Mancini & Hunt, 2005). The reducing activity plays a vital role which will be elucidated shortly. In some cases microbial growth can influence MMb formation (Mancini & Hunt, 2005).

Figure 2.5.4 Interior metmyoglobin layer formation between oxymyoglobin (top) and deoxymyoglobin (bottom) as indicated by arrows (Ramanathan, *et al.*, 2020a)



Since Rousseaux, Dautrevaux and Han (1976) established that ruminant Mb (beef, lamb and deer) is phylogenetically distant from pork Mb, Gutzke and Trout (2002) compared and determined that ruminant Mb was more susceptible to autoxidation than pork Mb i.e., pork meat was less susceptible to meat discolouration.

This decreased susceptibility to meat discolouration in pork meat can further be explained by differing muscle fiber types. Beef is classified as a red meat due to the majority proportion of meat containing oxidative, red muscle fibers (type 1). Whereas pork has a mixture of both red and white, with a larger proportion of glycolytic white fibers (type 2). Oxidative red muscle fibers inherently have greater myoglobin and mitochondria concentrations, making them more susceptible to MMb formation (through oxidation) to MMb, resulting in meat

discolouration (Ramanathan, *et al.*, 2021). Following this reasoning, glycolytic muscle would be more colour stable with a redder appearance than their oxidative counterparts.

Reduction

OMb is not directly reduced back to DMb, but first oxidizes to MMb at a very low OPP (reaction 3 in **Figure 2.5.3**). This (very) low OPP is achieved through oxygen consumption (OC) which is defined as the endogenous removal of oxygen e.g., by mitochondria.

MMb can then be reduced back to DMb which is crucial to meat colour life and stability. In post-mortem systems the reduction depends on the muscle's reducing enzyme systems, nicotinamide adenine dinucleotide (NAD) + hydrogen (H) [NADH] pool and of course oxygen scavenging enzymes, due to the vital role oxygen plays in myoglobin derivative conversions (Wallace, *et al.*, 1982). As post-mortem time progresses both the muscle's reducing enzyme- and NADH pools deplete as the muscle is naturally converted to meat. DMb formation therefore relies on the muscle's reduction capacity and further reductions in OPP through OC.

Carboxymyoglobin (COMb)

The COMb derivative is considered as a relevant redox state of myoglobin because of the growing interest in specialized packaging methods that include carbon monoxide. This is a result of the consumer preference for bright-red meat that can alternatively be achieved with COMb formation instead of OMb. The US and a few other countries have approved carbon monoxide inclusion levels of 0.4% in packaging, but not the EU member countries (Ramanathan, *et al.*, 2021a).

Although the exact mechanism which forms COMb is still unclear, it is accepted that COMb is more readily converted from DMb (reaction 4) than the other

derivatives (Mancini & Hunt, 2005; Suman, Mancini, & Faustman, 2006). Carbon monoxide will now be bound to the 6th coordination site of myoglobin and remain in its' ferrous state (Fe^{2+}). COMb can be used as an alternative to OMb due to greater stability which is achieved through the structural configuration between carbon monoxide and the iron-porphyrin (Wallace, *et al.*, 1982; Suman, *et al.*, 2006; Suman & Joseph, 2013). Despite this improved stability, it still discolours similar to OMb (Hunt, *et al.,* 2004; Suman, *et al.*, 2006). Additionally, it poses no risk to consumer welfare as the carbon monoxide will simply dissociate from myoglobin, upon exposure to atmospheres free of carbon monoxide (Mancini & Hunt, 2005; Suman, *et al.*, 2006). Regardless of how COMb is formed, it has the potential to become an important derivative due to its' sought-after colour implications.

2.5.3.4 Muscle biochemistry relating to meat colour

To intricately understand meat discolouration, it is important understand the biochemical processes which may influence the state of myoglobin and internal post-mortem muscle environment (Livingston & Brown, 1981).

Darker, redder meat has positively been correlated with higher Mb concentrations (Purslow, 2017). Protein degradation decreases as an animal ages and consequently Mb content will increase (Warriss & Rhodes, 1977; Morgan, *et al.*, 1993; Jeong, *et al*., 2009; Joo, *et al.*, 2013). Muscles rich in slow oxidative fibres (type 1) have greater Mb concentrations than their fast glycolytic (type 2) counterparts and will appear redder (Hossner, 2005).

Any changes in post-mortem energy metabolism can affect meat colour (Purslow, 2017). The previously given example of PSE is when carcass pH declines too low (pH < 5.4) a paler meat is observed, while a conversely higher ultimate pH (pH > 6.0) will result in darker meat cuts known as dark firm dry (DFD) meat.

Cutting carcasses immediately after slaughter will result in the visually observed blooming of meat colour from purplish to red, as DMb on the meat's surface is exposed to oxygen in the atmosphere. This is linked to the skeletal muscle metabolism now changing from an aerobic state to anaerobic as oxygen reserves are depleted within the muscle cells. Because anoxic post-mortem muscle is still biochemically active (Ramanathan, *et al.*, 2020b) several biochemical factors have been identified by Mancini and Hunt (2005) that influence meat discolouration namely, OPP, MMb reducing activity (MRA), OC, lipid oxidation and microbial growth (Ramanathan, *et al.*, 2021).

Ramanathan and Mancini (2018) conclude that the most important processes which impact meat colour are MRA and OC. Both processes are interrelated during the reduction of OMb and MMb to DMb through mitochondria, NADH and succinate (Bekhit, *et al.*, 2003; Mancini & Hunt, 2005; Ramanathan, *et al.*, 2021). Both MRA and OC should therefore be considered in tandem when trying to limit meat discolouration.

2.5.3.5 Effects of ZH-supplementation and ageing on meat colour

Standard practice is to use a calibrated colorimeter to obtain the three Commission Internationale de l'Elcairage (CIE) colour model, colour attribute values of which chroma and hue can be derived. Other studies have an overall colour score which ranges from 1 = extremely dark red to 8 = extremely bright cherry red (which is preferred). A few studies utilize dark cutters which is determined by the percentage of cattle that cut ≥ 34% DFD meat.

Both Montgomery *et al.* (2009) and Hilton *et al.* (2009) determined that ZM supplementation had no effect on colour scores ($P = 0.12$ and 0.14, respectively). Additionally, Montgomery *et al.* (2009) determined that ZM supplementation did not increase the % dark cuttters ($P = 0.37$).

Avendaño-Reyes *et al*. (2016) determined that at 2days post-mortem, GF chroma was significantly higher than ZM (P = 0.02; 22.1 vs. 18.6) but remained within the acceptable colour range, whilst GF only tended to have a greater chroma than CT (P = 0.06; 22.1 vs. 19.7). Additionally the hue angle tended to be greater (yellower) for GF when compared to ZM (P = 0.08; 39.7 vs. 37.1°) but GF hue angle did not differ significantly from CT (P = 0.22; 39.7 vs. 38.0°). Noticably ZM yielded numerically similar results to CT (as opposed to GF) for all colour attributes.

At 14days post-mortem no significant differences were observed between either CT vs. GF nor GF vs. ZM when considering all colour attributes measured for the Avendaño-Reyes *et al*. (2016) study.

Avendaño-Reyes *et al*. (2006) found that ZM had no effect on meat colour attributes. But the authors determined that ageing tended to yield paler meat, while there was still more red pigment than yellow.

These findings along with those investigasted by Brooks *et al.* (2008), indicate that ZH-suppplementation has no detrimental effect on meat colour and can even marginally enhance colour scores (Brooks, *et al.,* 2008).

2.5.4 Water holding capacity (WHC)

Rigor muscle can consist of up to 75% water and 22% protein (Honikel, 2004). Huff-Lonergan and Lonergan (2005) established that muscle cell volume comprises of 82-87% myofibrils and those myofibrils further contain up to 85% water. The water found within myofibrils is known as immobilised water due to the interaction it has with the protein structures and, as a result, water is inhibited in molecular movement (Honikel, 2004).

The ability of fresh meat to retain water is known as water-holding capacity (WHC) (Huff-Lonergan & Lonergan, 2005) and is an important intrinsic factor which can affect meat appearance, juiciness and tenderness (Destefanis, Barge,

& Brugiapaglia, 1994). Water released from meat is often referred to as drip (loss), purge, exudate or even cooking loss (Hughes, Oiseth, Purslow, & Warner, 2014). Consequently processes which induce these water releases can be ascribed to evaporation, drip loss or cooking loss (den Hertog-Meischke, van Laack, & Smulders, 1997).

den Hertog-Meischke, van Laack and Smulders (1997) state that evaporation losses occur during carcass chilling as a result of vapor pressure differing between the hot carcass surface and the cold air of the chiller. Evaporation losses can amount to 2% of the total carcass mass. The total amount of water lost due to evaporation depends on carcass temperature as well as chilling room air attributes (temperature, humidity and velocity).

Drip (loss) emerges from the cut surface of meat. This drip has a red appearance and is a concentrated solution of intracellular proteins – notably myoglobin and glycolytic enzymes (den Hertog-Meischke, *et al,* 1997). Drip contains intracellular proteins - up to two-thirds of the whole meat protein concentration – thereby loss can affect the nutritive value of meat products (Huff-Lonergan & Lonergan, 2005).

During the cooking process meat inherently heats up (increases in temperature). Consequently, protein structural changes are incurred and result in contained fluid being expelled, due to the protein denaturation process which occurs at higher temperatures (den Hertog-Meischke, *et al.*, 1997).

Additionally, because myofibrillar proteins reach their isoelectric points faster, decreasing pH values will result in myosin and actin to have no electrical charge to maintain their bond with the intracellular water, resulting in a rapid loss of water from within the meat (Lawrence & Fowler, 2002).

Destefanis *et al.* (1994) established that muscular development is negatively related to the WHC of meat. As muscular development increases, less water is

preserved within the muscle (Lawrie, 2006). ZH-supplementation encourages muscular development which could lead to an expected decrease in WHC (increased drip- and cooking loss).

Avendaño-Reyes *et al*. (2016) compared the WHC of meat samples obtained from either control or ZM-treated meat samples at 48hours post-mortem and again after 14 days of ageing. Initially at 48hours post-mortem, ZM meat samples had significantly lower WHC than CT (P = 0.01; 87.3 vs. 90.9%). With no difference between the two ZH-based products (P = 0.43; ZM = 87.3% and GF = 88.3%). After 14days of ageing, no significant difference was observed between any treatment group and CT (P > 0.20; 88.4 vs. 88.4 vs. 88.7%).

Contrastingly, Avendaño-Reyes *et al*. (2006) found no significant difference between CT and ZM when measuring WHC at 14days post-mortem (P = 0.75; 60.7 vs. 61.3%). But that the ZM drip loss was significantly higher than that of CT (P > 0.001; 4.1 vs. 6.2%).

Leheska *et al.* (2009) reported no significant difference between ZM and CT for cooking losses (P = 0.74). This is supported by Hilton *et al.* (2009) with (15.1 vs. 16.0% values) P = 0.13.

Additionally, Cônsolo *et al.* (2016) found that both ZM-supplementation and post-mortem ageing up to 14days post-mortem had no effect on cooking losses (P > 0.5) in Nellore (*B. Indicus*) heifers.

2.5.5 Meat tenderness

As discussed previously, each globular myosin head serves as a binding site, crucial to the contraction process. One for actin binding and another for ATP-splitting (known as the myosin ATPase site) (Sherwood, 2016).

With *rigor mortis* inactive muscle cell membranes would witness an influx of Ca^{2+} molecules into the cytosol. The binding of Ca^{2+} to troponin allows contraction as myosin cross bridges were already charged with ATP before death (Sherwood,

2016). With no new ATP being regenerated post-mortem, no ATP molecules would be available to displace the adenosine diphosphate (ADP) at the myosin ATPase site, resulting in a permanent cross-bridge being formed, known as actinomyosin.

A Warner-Bratzler instron is used to measure the (shear) force which it would take to cut the meat perpendicularly to the muscle fibres (Honikel, 1998). This measurement is used as an indicator of meat tenderness as consumers can detect slight changes in shear force values when evaluating meat tenderness (Shackelford, Morgan, Cross, & Savell, 1991; Miller, *et al.,* 1995).

Avendaño-Reyes *et al.* (2016) determined that Warner-Bratzler Shear Force (WBSF) values measured at both 2- and 14 days post-mortem, did not significantly differ between either control and GF, nor GF and ZM ($P > 0.05$) for bulls.

Leheska *et al.* (2009) found that ZH supplementation increased WBSF values by 22% ($P < 0.001$) in steers. Hilton *et al.* (2009) measured WBSF over 21 days to additionally test post-mortem ageing effects and the interaction it might have with ZM supplementation effects.

Table 2.5.2 Summary of previous findings about the effects of ZM-supplementation on *Longissimus* muscle WBSF values measured up to 21 days post-mortem

	Hilton *et al.* (2009)	Scramlin *et al.* (2010)
Post-mortem ageing period	**ZM effect on WBSF values (CT vs. ZM)**	
3 days	N/A	↑ with 2.2kg (4.7 vs. 6.9; P < 0.05)
7 days	↑ with 0.8kg (3.7 vs. 4.5; P < 0.001)	↑ with 2.2kg (4.2 vs. 6.3; P < 0.05)
14 days	↑ with 0.4kg (3.6 vs. 4.0; P < 0.001)	↑ with 1.7kg (3.6 vs. 5.3; P < 0.05)
21 days	↑ with 0.3kg (3.1 vs. 3.4; P = 0.001)	↑ with 1.2kg (3.1 vs. 4.3; P < 0.05)

Longissimus muscle WBSF increased significantly (P = 0.001) with ZM supplementation (Hilton, *et al.*, 2009). The effects of this increase in WBSF were drastically mitigated with post-mortem ageing (P < 0.001).

Strydom and Nel (1996) who aged ZM-treated steaks up to 14 days post-mortem and found that ageing had favourable decreasing effects on increased WBSF values that are due to ZM supplementation. Brooks *et al.* (2008) gathered that post-mortem ageing up to 21 days can improve WBSF in ZM treated steaks by 10-11.9%.

Similarly Cônsolo *et al.* (2016) established that ZM supplementation significantly ($P > 0.05$) increased WBSF values of *Longissimus* muscles from *B. Indicus* heifers with 18% and 29%, for 7- and 14 days post-mortem, respectively. At 21days post-mortem no significant difference was observed between control or ZM-treated steaks. This lack of effect at 21 days indicates a significant interaction between ZM supplementation and ageing ($P = 0.03$) for WBSF values.

Scramlin *et al.* (2010) compared both ZM and ractopamine (RT) supplementation with CT steers. The authors' results indicated that during all post-mortem ageing periods (3-21 days), ZM steaks yielded higher WBSF values than CT steaks ($P < 0.05$). At days 3 and 7 days post-mortem RT steaks had higher WBSF values than CT ($P < 0.05$), but at 14 and 21 days no significant difference was observed between the two treatments (CT vs. RT), while ZM was still sighnificantly higher ($P < 0.05$). These findings indicate a greater tendency towards post-mortem tenderization with RT treatment instead of ZM. Although ZM values did decrease marginally with ageing. The aforementioned findings for ZM supplementation were confirmed with Rathmann *et al.* (2009) who observed greater WBSF values for ZM steaks than CT at 7, 14 and 21 days post-mortem ($P < 0.01$) even as they decreased with ageing.

These results support post-mortem ageing as a tenderisation method to improve meat toughness of animals supplemented ZH.

2.5.5.1 Consumer acceptability of meat from ZH-supplemented cattle

Although ZH supplementation does affect meat tenderness, WBSF values still remain below consumer rejection limits (Miller, Carr, Ramsey, Crockett, & Hoover, 2001). This is supported by another follow-up of the study performed by Montgomery, Hilton *et al.* (2009), which found that ZM treated steaks were still deemed acceptable (92.8%) by consumers when compared to control steaks

(89.1%), when both were aged for 14 days post-mortem. Brooks *et al.* (2008) inferred overall combined results from previous research supports this finding with an average consumer acceptability of 92.4 and 94.4%, respectively for control steaks and ZM supplemented steaks.

Shackelford *et al*. (1991) identified critical threshold values of 3.86 and 4.59kg for both rib and loin cuts as well as round and chuck cuts, respectively, when WBSF is used as a tenderness benchmark measurement. To achieve maximum levels of consumer acceptability (98%) a WBSF threshold value of ≤ 4.1kg would suffice on any meat cut (Huffman, *et al.*, 1996).

2.5.5.2 Factors which may influence meat tenderness

A summation of various previous studies establishes that βA supplementation increases WBSF values with a moderate effect. Proposed contributing factors include a change in muscle fiber type, increases in muscle fiber diameters as well as the amount of heat-stable collagen present in muscle (Mersmann, 1998; Brooks, *et al.,* 2008). The age of an animal also influences meat tenderness through increaed heat-stable collagen (Webb, 2015) which is why it is included in the South African carcass classification (**Table 2.4.4**).

Purslow (2017) states that a muscle pH above 6.0 combined with a muscle temperature below 10-15°C causes cold shortening (meat toughening), as energy reserves are depleted prematurely to generate heat in an attempt to aschieve homeostasis.

Muscle comprises of three protein fractions namely, myofibrillar, connective tissue and sarcoplasmic proteins (Koohmaraie, *et al.,* 2002). The authors continue to explain that connective tissue contributes to meat tenderness through background toughness. And that because sarcoplasmic proteins are not structural proteins, they can not directly contribute to meat tenderness.

Myofibrils are both the most abundant in muscle fibers and known as the contractile organelles, therefore the post-mortem degradation of these proteins are associated with meat tenderness (Huff-Lonergan, *et al.*, 1996; Purslow, 2017).

Protein degradation is seldomly measured directly and protease activities within muscle samples are measured instead (Mersmann, 1998). Mersmann further explains that βA treatment often elicits either a reduction in protease activities or an increase in the concentration of protease inhibitors. The primary protease activity in question is the endogenous, sarcoplasmic proteolytic enzyme system known as the calpain system.

The calpain system is important for post-mortem proteolysis which tenderizes meat (Koohmaraie, *et al.*, 2002; Purslow, 2017). This system amounts to the interaction between four proteins in muscle; μ-calpain (calpain 1), m-calpain (calpain 2), calpastatin and to a lesser extent p94 calpain (calpain 3). Calpains 1 and 2 are ubiquitous in all cell types and both depend on calcium concentrations for protein degradation activity (Purslow, 2017). Calpastatin is the inhibitor of caplains 1 and 2 and requires similar (or lower) calcium concentrations to those required to activate calpain (Cônsolo, *et al.*, 2016).

Purslow (2017) elucidated that calpains with high levels of activity results in high cleavage levels of specific myofribrillar proteins (e.g. titin and desmin), the degradation of these proteins will result in improved meat tenderness. Conversely, high concentrations of calpastatin will decrease protein degradation, thereby resulting in less tender (tougher) meat.

Bardsley *et al.* (1992) proposes that βA-supplementation may influence the cellular mebrane's electrochemical gradient and prevent ionized calcium from actively being transported into the muscle cells. This can lead to excessive calpastatin expression that can block the beneficial effects of calpain activity

(Cônsolo, *et al.,* 2016). βA-supplementation has been correlated with increases in both meat toughness as well as calpastatin activity (Bardsley, *et al.*, 1992; Brooks, *et al.,* 2008).

The calpain system and its' activity relies on many factors besides calcium concentrations. As with most biochemical porcesses post-mortem pH and temperature have principal roles in regulating the metabolic tenderisation process (Purslow, 2017). During post-mortem storage, the temperature and pH gradually declines to aid the conversion of muscle to meat. This in turn can compromise the calpain system's activity and should therefore be managed accordinglky to avoid occurences of DFD or PSE meat (Huff-Lonergan, *et al*., 1996).

Strydom *et al.* (1998) state that ZH is a moderately active repartitioning agent as determined by the weak affinity it has for specific receptors. Following this reasoning, ZH-treated animals will have a less pronounced effect on muscle tenderness when compared to other molecules - such as Cimaterol – that have a higher repartitioning activity due to stronger receptor affinity.

Chapter 3: Materials and methods

3.1 Hypotheses

H_{01}: Dietary supplementation with one of two different ZH molecules (Zilmax® vs. Grofactor®) in feedlot diets during the finishing period, does not affect the carcass and meat quality characteristics when compared to a negative control.

H_{A1}: Dietary supplementation with one of two different ZH molecules (Zilmax® vs. Grofactor®) in feedlot diets during the finishing period, does affect the carcass and meat quality characteristics when compared to a negative control.

H_{02}: Dietary supplementation with Grofactor® in feedlot diets during the finishing period, does not affect the carcass and meat quality characteristics differently than when Zilmax® is supplemented, when compared to a negative control.

H_{A2}: Dietary supplementation with Grofactor® in feedlot diets during the finishing period, does affect the carcass and meat quality characteristics differently than when Zilmax® is supplemented, when compared to a negative control.

H_{03}: Extending the ageing period of meat samples obtained from all experimental feedlot bulls, does not affect the meat quality characteristics.

H_{A3}: Extending the ageing period of meat samples obtained from all experimental feedlot bulls, does affect the meat quality characteristics.

3.2 Experimental animals and treatment allocation

The current research formed part of a larger research study on the effects of beta-adrenergic agonist feed additives on the growth performance, carcass and meat quality of typical South African feedlot cattle. The present experiment focused specifically on the carcass and meat quality attributes of ZH-supplemented bulls, including shelf-life of meat up to 120 days post-mortem. The original ethics approval was obtained for this research with approval number; NAS390/2019, with an amendment to conduct shelf-life and meat colour research, approved on 12 April 2021.

The research study was conducted at the Beefcor feedlot, located in Boschkop, Bronkhorstspruit, 1020, Gauteng, South Africa. A total of 2 000 typical intact feedlot bulls were made available for the random selection 450 homogenous bulls for our study and the cattle remained the property of the feedlot owner. To ensure that the selected population (identified with ear tags) closely represents the majority of South African feedlot cattle, they had to have a medium sized maturity type (e.g., Bonsmara crosses), not have been castrated at weaning and have an average initial mass of ca. 400kg when the experiment commenced (D0).

All cattle were received and subjected to the same routine processing and management procedures according to Beefcor standards to limit variation. This included preventative treatments and vaccinations for internal and external parasites, injecting vitamin supplementation and immunisation against Clostridial and viral diseases – for respiratory diseases such as infectious bovine rhinotracheitis (IBR). During processing all cattle received an initial hormonal growth ear implant i.e., Ralgro and were then re-implanted with Revalor H after 45 days. Revalor H was then effective for another 60 – 70 days.

All bulls were housed in large soil-surfaced floor pens and fed the same starter and grower diet after an adaption period of 14 days. Each pen housed 50 bulls and 9 pens were used (50 bulls/pen x 3 replicates (pens)/treatment x 3 treatments = 450 bulls). 14 days prior to the normal finishing period (D-14), cattle were weighed and randomly allocated to different treatments to commence (D0) the finishing diet with. These pens were treated as replicates of the treatment groups.

All treatments were then administered for 30 days followed by a 3-day withdrawal period before slaughter (D33). The completely randomised control study consisted of 3 experimental groups during the feedlot finishing period (i.e., from D0 to D33); the first, a negative control (CT) received no supplementation, second; Zilmax® feed supplementation (ZM) and lastly Grofactor® feed supplementation (GF).

Each treatment was then randomly allocated to 3 replicates. The replicates then amounted to each treatment being administered to 3 pens x 50 animals/pen = 150 animals. Thus, accounting for all 450 cattle allocated to the trial. But due to the high costs of proximate and meat sample analyses, 38 bull carcasses were randomly sub-selected from each treatment to be evaluated further (3 treatments x 38 bulls = 114 carcasses and subsequent meat samples evaluated). Unfortunately, one bull from CT jumped the dividing fence between the two pens, therefore yielding the following replicates per treatment:

Table 3.1.1 Treatment allocation to experimental feedlot bull carcasses that were randomly selected for further analyses

Treatment	Replicates
Control (CT)	37
Grofactor® (GF)	38
Zilmax® (ZM)	39
Total	114

3.3 Feed

Following standard feedlot operating procedures, animals were fed in bulk feed troughs that ensured adequate feeding space per animal. Animals received a standard balanced, dry feedlot concentrate ration ad libitum, that provided 10.5 MJ ME/kg DM energy during the finishing phase of feeding. Daily allotment of feed for each pen was estimated and recorded by bunk-reading the feed consumed from that provided. Expected or predicted feed intake was calculated using an estimate of roughly 3% of the live weight (kg), being consumed per day for live performance analyses. Animals had free access to good quality water.

The trial commenced during the feedlot finishing period (last 30 days on feed) when treatment groups received Zilmax® or Grofactor® feed supplementations as part of the finisher ration. Supplementations were mixed into the 75% DM content feed rations at a standard recommended concentration of 105g of ZH/ton.

Every batch finishing ration mixed was sampled and bio-assayed to confirm β-agonist concentrations. An additional ZH withdrawal period of 3 days was observed.

3.4 Slaughter, meat sample collection and storage

On 10 December 2019, slaughter mass (D33 mass) of bulls were obtained and then transported to the commercial abattoir Chamdor Meat Packers in Krugersdorp, 1740, Gauteng, South Africa. After an 24hour lairage animals were mechanically stunned by means of a pneumatic stunner (captive bolt), then hung from their hocks during exsanguination.

Carcass classifications were performed by an external, non-biased South African government grader. From the 450 experimental animals, 38 bulls per treatment were sampled for carcass analyses (38 x 3 treatments = 114

carcasses). Carcasses were placed in a chiller (2-4°C) at approximately 45min post-mortem. Initial carcass pH and temperature readings were performed before chilling the carcasses with a portable pH/temperature meter (Hanna Instruments, code- HI99163). The measurements were performed on the *Longissimus dorsi* muscle (LD), between the 10th and 11th ribs at 45min, 3hours, 6hours, 12hours and 24hours post-mortem.

Muscle samples were then only removed from the carcasses after being chilled for 24hours. Samples were excided from the LD, between the 9th and 12th ribs on the left side of each carcass. Muscle samples were labelled, vacuum packed and frozen until meat quality analyses were performed. Prior to storage, the fresh meat samples were placed at 25°C (room temperature) for 20min to bloom (i.e., turning bright red due to oxygen exposure).

3.5 Carcass and meat quality evaluations performed

3.5.1 Carcasses quality analyses

- DR% was determined by $\frac{HCM}{D33\ mass} x100\%$. Where:

D33 mass was the final mass (kg) of a bull before it was slaughtered.

- HCM was the mass yielded (kg) after a bull had been stunned, exsanguinated, skinned, eviscerated, and beheaded.

- Carcass CCI was derived from HCM by ($\frac{HCM}{carcass\ length}$). Where:

Carcass length was measured from the 1st rib up to the symphysis of the pelvis (cm).

- Carcass composition (bone: meat: fat) estimated with an approximate analysis.

- Carcass FT obtained by measuring the subcutaneous fat over the 13th rib, 50mm lateral from the vertebrae.

- Carcass classification performed by the previously mentioned carcass grader.

3.5.2 Meat quality analyses

The following meat quality evaluations were performed after beef samples were thawed at 4°C for 36hours on days 7, 14, 28, 56 and 120:

- WHC by determining the amount of water pressed out of the meat, under standardised conditions set out by Babiker and Lawrie (1983). Whatman filter paper (No. 4) was stored over a saturated potassium chloride solution, in a desiccator. 0.5g minced meat sample was then placed on a humidified filter paper and pressed at 25kg between two plexiglass plates for 2min. A ballpoint pen was used to define the area of meat impression made on the filter pen and the paper was then dried. The WHC ratio was calculated as $\frac{(loose\ water\ area - meat\ film\ area)}{meat\ fil\ area}$. The meat and loose water areas (cm^2) were

determined using a planimeter. A large WHC ratio can be indicative of either watery conditions of the meat or the decline in WHC of muscle (Biraima, Mohammed, & Webb, 2019).

- Drip loss was the percentage mass of (high protein) fluid that was lost from fresh meat during storage. This was calculated as $\frac{(original\ mass - mass\ pre{-}cook)}{original\ mass} x100\%$.

- Cooking losses were determined using the Honikel (1998) method. Meat samples were weighed then placed in a plastic bag to be cooked in a water bath at 80°C until the internal temperature of meat samples was 70°C. The cooked samples were then chilled overnight at 4°C. Later the samples were blotted dry and weighed to calculate the weight loss after cooking. Cooking loss was calculated as a percentage of the initial mass by $\frac{mass\ loss\ after\ cooking}{initial\ sample\ mass} x100\%$.

- Meat tenderness was tested on the same samples used for cooking loss measurements by a shear force (SF) test performed with a Warner-Bratzler instrument (G-R Elec. Mfg. Co. Manhattan, Kansas 606502). The WBSF measurement was used as a physical indicator of meat tenderness instead of a perceived sensory panel score. Rectangular meat samples (1 x 1.5 x 10 cm) were excised from the cooked meat samples. A mean peak force value (kg) was obtained and is defined as the force required to cut the meat perpendicularly to the muscle fibres. The mean was derived from two peak force recordings.

- Meat colour was evaluated using a colorimeter - Hunter Lab ColorFlex EZ (Model 45/0 LAV, Hunter Laboratory Associates, Inc., Reston, Virginia, USA). According to manufacturer guidelines, the ColorFlex EZ was calibrated immediately before readings against black and white tiles. L* (lightness), a* (redness), and b* (yellowness) values were determined utilising a standard observation made at 10° using illuminant D65. Three random readings were made to obtain a mean value recorded. Chroma (red colour intensity) and hue

(meat discolouration) values were calculated using the following formulas as determined by Hunt *et al*. (1991): chroma = $(a^{*2} + b^{*2})^{1/2}$ and hue angle = $\tan^{-1}(\frac{b*}{a*})$. An additional recording was made on day 3 post-mortem, for colour only.

3.6 Statistical analyses of data

Recorded data was imported from an excel spreadsheet to the IMB SPPS (version 28) package of 2022. Any and all statistical analyses were performed using only this program.

The number of bulls supplemented with each treatment varied (because one jumped the dividing fence) and some manual errors occurred during measurements, Bonferroni's multiple range test was implemented as a result to accurately analyse the differences between least squares means in an unbalanced experimental design for both pooled and pairwise comparisons.

ZH-supplementation and differences between the two ZH-molecules were investigated by general linear model (GLM) analyses. Live mass GLM analyses included D0 as covariate because small differences existed among the experimental groups before the treatments commenced, despite the random allocation of animals to experimental groups.

Effects investigating time (i.e., days of finishing period, post-mortem time and ageing period) were analysed with univariate GLMs to include the time variable as an additional fixed factor with the ZH-supplementation or treatment effect group.

Chapter 4: Results and Discussion

4.1 Live- and hot carcass mass of feedlot bulls as influenced by dietary supplementation of zilpaterol hydrochloride either as Zilmax® or Grofactor®

The pooled average initial mass (D0) was 398.0kg for the 114 experimental feedlot bulls upon starting the finishing period. As seen in **Table 4.1.1**, average D0 mass varied marginally - but was proven to not be statistically significantly different (P = 0.56) among experimental groups.

Table 4.1.1 Differences in average initial (D0) mass (kg) between the three different experimental groups of feedlot bulls before ZH-supplementation commenced

Experimental group	Average D0 mass (kg)	Difference from pooled average D0 mass (kg)	Std. deviation	Std. error	N
Control	398.0	0.01	24.18	2.07	37
Grofactor®	400.0	2.00	24.00	2.04	38
Zilmax®	396.0	-1.75	27.24	2.02	39

(Negative difference indicates that the mean treatment group mass weighed less than the total estimated mean)

As seen in **Table 4.1.1**, average D0 mass varied marginally among experimental groups with the GF group weighing only 2.0kg more than the pooled average of 398.0kg. The ZM group weighed only 2.0kg less than the pooled average. The CT group weighed the closest to the pooled average by only differing with a negligible 0.01kg. These initial weights indicated that the random allocation of experimental animals was very successful. Nevertheless, D0 mass was included as a covariant for subsequent statistical analyses.

D0 had significant (P < 0.001) between-subject effects on average mass when measured on subsequent weighing days (i.e., D15 mass, D33 mass and HCM).

The inclusion of D0 as covariant had a large effect size of 0.83, 0.65 and 0.67 on respectively day 15 mass (D15), day 33 mass (D33) and HCM, respectively. D0 also had a significant (P = 0.01) effect on DR%, but with a small effect size of 0.06.

When performing a regression model for D0 between D15, D33, HCM and DR%, respectively the following results were obtained:

Table 4.1.2 Regression model summaries utilizing initial (D0) mass (kg) as independent variable with day 15 and 33 mass (D15 and D33; kg), hot carcass mass (HCM; kg), dressing percentage (DR%) and carcass compactness index (CCI) as dependent variables

Dependent variable	R	R^2	Sig.
D15 mass (kg)	0.91	0.83	< 0.001
D33 mass (kg)	0.81	0.65	< 0.001
HCM (kg)	0.81	0.65	< 0.001
DR%	0.24	0.06	0.01
CCI	0.75	0.56	< 0.001

From these results it was concluded that a strong correlation exists between D15 and D0 mass (R = 0.91). For this regression model, 83% of the variation in D15 mass was due to D0 mass.

Both D33 mass and HCM also had a strong correlation (albeit weaker than D15 mass) with D0 (R = 0.81). For both these regression models, 65% of the variation in both D33 mass and HCM were due to D0 mass. The contribution of D0 mass to D33 mass and HCM variation was expected to be smaller than for D15, due to anticipated improvements in growth efficiency. These improvements would yield a greater contribution to variation in HCM and D33 mass than D15, thereby lowering the contribution of D0 mass to variation.

The correlation between D0 and DR% was 0.24. For this regression model only 6% of the variation in DR% was due to D0 mass**.** DR% was included in this section because it was derived from both slaughter mass (D33 mass) and HCM ($\frac{HCW}{D33\ mass} x100\%$) which both utilized D0 as covariate.

CCI had a strong correlation with D0 of 0.75. For this regression model 56% of the variation in CCI was due to D0 mass**.** CCI was included in this section because it was derived from HCM ($\frac{HCW}{CCI}$) which utilized D0 as covariate.

4.1.1 Live mass data

Overall ZH-supplementation effect

The mean observed mass of the CT group and ZH-supplemented group (ZH) from commencement of the supplementation period are visualized in **Figure 4.1.1** and given in **Table 4.1.1** (initial mass) as well as **Table 4.1.3** (D15 and D33 mass).

The pooled mean D15 mass was 426.9kg for the 114 experimental feedlot bulls. ZH weighed 3.0kg more than CT when comparing the mean D15 mass of each group (424.9 vs. 427.9kg).

The pooled mean D33 mass was 424.7kg for the 114 experimental feedlot bulls. ZH weighed 5.4kg more than CT when comparing the mean D33 mass of each group (421.1 vs. 426.5kg).

A tendency to decrease in mean mass was observed for both groups from D15 to D33. This decrease was due to losses associated with transportation to the abattoir and lairage time (Hui, 2012).

Figure 4.1.1 Observed mean control and ZH-supplemented treatment groups live mass (kg) over the finishing period

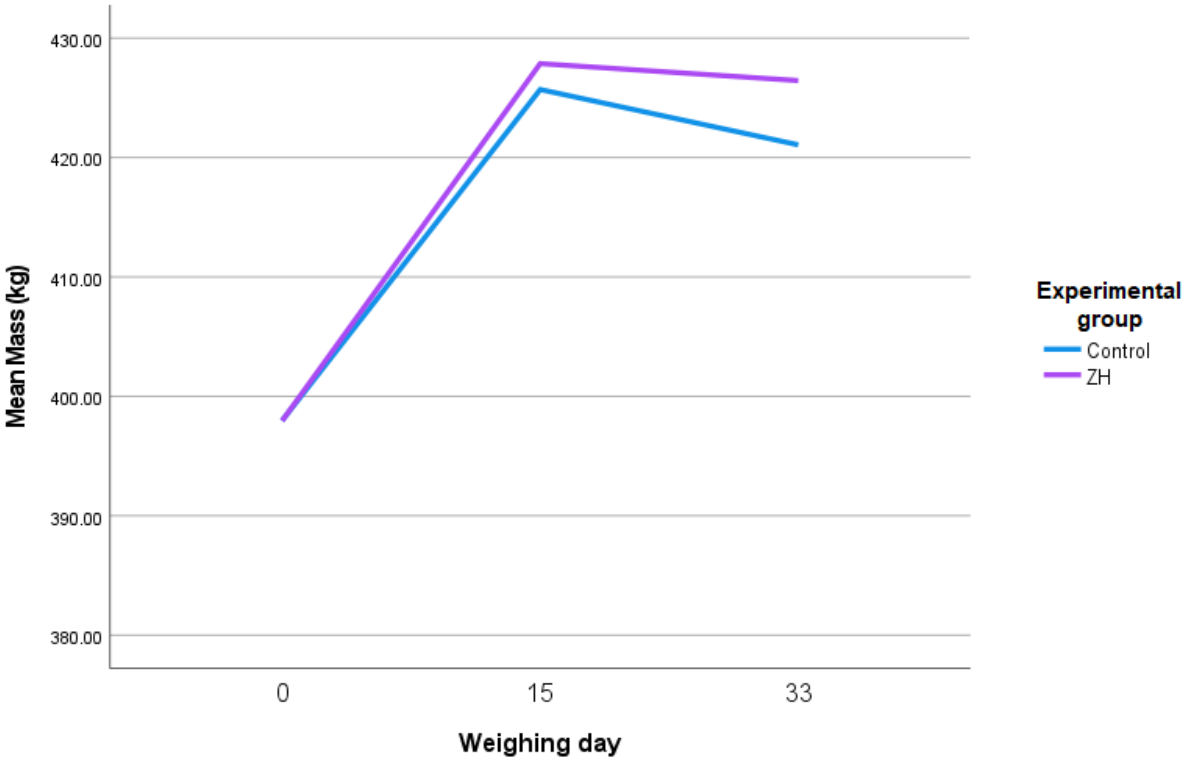


Table 4.1.3 Observed mean mass (kg) measured on different weighing days over the finishing period for control and ZH-supplemented groups

	Experimental group	Mean mass (kg)	Std. deviation	N
D15	Control	424.9	29.58	37
	ZH	427.9	29.80	77
D33	Control	421.1	26.31	37
	ZH	426.5	30.53	77

From **Table 4.1.4** it is evident that ZH-supplementation unfortunately had no significant effect on either weighing day (P > 0.05) live mass as expected. The effect of ZH-supplementation on live mass did however increase from D15 to D33 but still remained insignificant (P > 0.05). This increase in ZH-supplementation effect is seen by decreasing P-values coupled with a slight increase in effect size.

Table 4.1.4 ZH-supplementation effects and effect sizes for individual weighing days over the finishing period

Weighing day	Sig.	η^2
D15	0.23	0.01
D33	0.12	0.02

An increase in difference between the ZH-supplemented and control group mean mass were observed when comparing D15 to D33 (**Table 4.1.3**). With the difference being 2.4kg heavier at D33 than D15 (5.4 – 3.0 = 2.4kg). When coupled with the increase in ZH-supplementation effect from D15 to D33 (**Table 4.1.4**) it suggests that increasing the ZH-supplementation period would increase live mass.

Treatment effect comparisons

The mean observed mass of the different treatment groups from commencement of the supplementation period are visualized in **Figure 4.1.2** and given in **Table 4.1.1** (initial mass) as well as **Table 4.1.5** (D15 and D33 mass).

As explained previously, trial bulls were selected (and randomly allocated to treatments) to represent a homogenous group so that treatment differences can have been accurately calculated. As supplementation has not yet commenced on D0, virtually no differences in mean live mass were observed at D0 among treatment groups.

Figure 4.1.2 Observed mean experimental group live mass (kg) over the finishing period

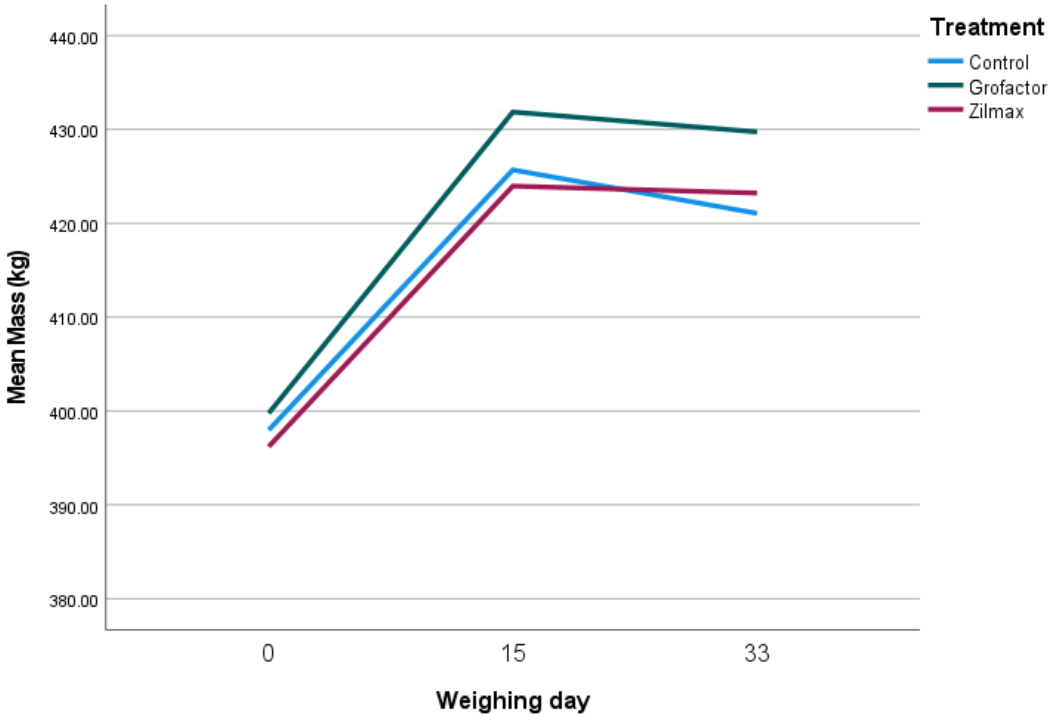


Table 4.1.5 Observed mean live mass (kg) measured on different weighing days for the three different experimental groups over the finishing period

	Experimental group	Mean mass (kg)	Std. deviation	N
D15	Control	424.9	29.58	37
	Grofactor®	431.9	27.58	38
	Zilmax®	424.0	31.69	39
	Total	426.9	29.63	114
D33	Control	421.1	29.63	37
	Grofactor®	429.8	26.31	38
	Zilmax®	423.2	30.01	39
	Total	424.7	29.22	114

The pooled average D15 mass was 426.9kg for the 114 experimental feedlot bulls. Bulls from GF weighed the heaviest with a D15 mean mass of 431.9kg (5.0kg heavier than the pooled D15 mean mass of 426.9kg). Bulls from ZM

weighed the lightest with a D15 mean mass of 424.0kg (2.9kg lighter than the pooled D15 mean mass). Bulls from CT weighed in as an intermediate with a D15 mean mass of 424.9kg (2.0kg lighter than the pooled D15 mean mass). CT only weighed 0.92kg heavier than ZM at D15.

The pooled average D33 mass was 424.7kg for the 114 experimental feedlot bulls. GF still weighed the heaviest with a D33 mean mass of 429.8kg (5.1kg heavier than the pooled D33 mean mass of 424.7kg). CT now weighed the lightest with a D33 mean mass of 421.1kg (3.6kg lighter than the pooled D33 mean mass). With the intermediate group now being ZM with a D33 mean mass of 423.2kg (only 1.5kg lighter than the pooled D33 mean mass). ZM weighed 2.2kg more than CT at D33.

The previously described tendency to decrease in D15 mass to D33 mass - due to losses associated with transportation to the abattoir and lairage time (Hui, 2012) – is still observed. But ZM now has a pronounced effect which was not apparent in **Figure 4.1.1**. The overall differences among experimental groups, both within and between weighing days, were analyzed in **Table 4.1.6**.

Table 4.1.6 Overall treatment effects and effect sizes for different weighing days over the finishing period

Weighing day	Sig.	η^2
D15	0.17	0.03
D33	0.22	0.03

Increases in the different treatment D0 mean mass were observed from mean D15 mass (**Figure 4.1.1**). However, these increased D15 mean mass did not differ significantly between the different treatment groups (P = 0.17 with a small η^2 = 0.03). The same holds true for increases in the different treatment D0 mean mass were observed from mean D33 mass (P = 0.22 with a small η^2 = 0.03).

Therefore, a pooled treatment effect did not yield significant differences amongst treatment groups on either D15 or D33. Unlike **Table 4.1.4** which evaluated the broad ZH-supplementation effects, the overall (individual) treatment effect did not increase over time (P-value did not decrease but increased in **Table 4.1.6**). The observed decrease in treatment effect from D15 to D33 is due to GF and ZM now being analyzed as separate treatments and not as a pooled ZH-supplementation effect. A pairwise comparison was created **(Table 4.1.7)** to analyze these differences between GF and ZH, as well as each treatment with CT. But first, means had to be adjusted (Bonferroni's multiple range test) (**Table 4.1.7**) because experimental groups had uneven numbers of observations (seen as N in **Table 4.1.5**).

Table 4.1.7 Estimated marginal live mass means (kg) for the different experimental groups over the finishing period

	Treatment	Mean	Std. Error
D15 mass (kg)	Control	424.9[a]	2.013
	Grofactor®	429.9[a]	1.988
	Zilmax®	425.9[a]	1.963
D33 mass (kg)	Control	421.1[a]	2.847
	Grofactor®	428.1[a]	2.812
	Zilmax®	424.9[a]	2.776

a. Covariates appearing in the model are evaluated at the following values: D0 mass = 398.0kg.

Adjusting the mean mass (**Table 4.1.7**) resulted in **Figure 4.1.3** which fit expected effects of ZH-supplementation better – i.e., increased mass for both groups treated with ZH, above that of control. ZM now yields heavier mean live mass than CT on both D15 and D33 weighing days.

When comparing the adjusted live mass means in a pairwise manner (**Table 4.1.8**) the following was found:

No statistically significant differences for live D15 mass were observed for the ZM treatment when compared to either CT or GF (P = 1.00). Meaning that ZM had no significant treatment effect on D15 mean live mass.

GF treatment indicated a greater treatment effect on D15 mean live mass when compared to CT (429.94 vs. 424.9kg; P = 0.23) than ZM (P = 1.00) but remained insignificant. Regardless of the greater numerical GF treatment effect, GF D15 mean live mass did not significantly differ from ZM (429.9 vs. 425.9kg; P = 0.44). GF had a greater treatment effect on D15 mean live mass when compared to CT (P = 0.23) than ZM (P = 0.44), meaning that although insignificant, ZM did yield a numerically heavier mean D15 live mass than CT.

The same holds true for D33 mean live mass comparisons. D33 mean live mass for ZM did not differ from either CT or GF (P = 1.00). Whereas GF had a greater insignificant treatment effect when compared to CT D33 mean live mass (P = 0.25).

Figure 4.1.3 Estimated marginal live mass means (kg) of the three different experimental groups over the finishing period

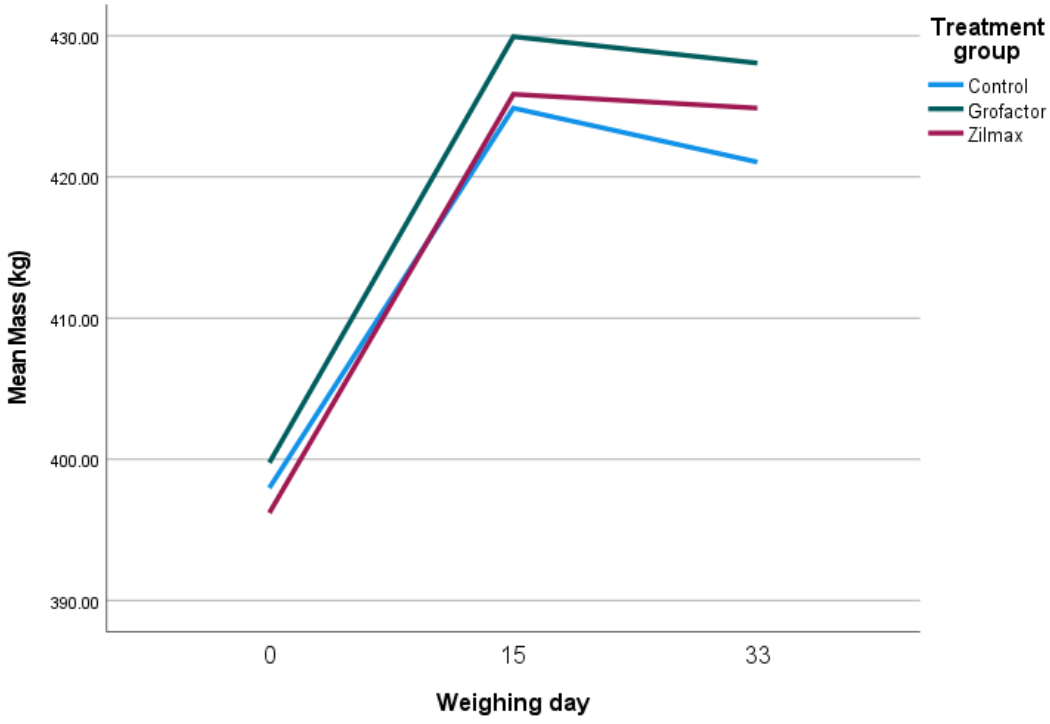


Table 4.1.8 Pairwise comparisons of the different treatment effects on live mass (kg) over the finishing period

	(I) Treatment	(J) Treatment	Mean difference (I-J)	Sig.	Std. Error
D15 mass (kg)	Control	Grofactor®	-5.06	0.23	2.83
		Zilmax®	-0.98	1.00	2.81
	Grofactor®	Control	5.06	0.23	2.83
		Zilmax®	4.08	0.44	2.80
	Zilmax®	Control	0.98	1.00	2.81
		Grofactor®	-4.08	0.44	2.80
D33 mass (kg)	Control	Grofactor®	-7.01	0.25	4.00
		Zilmax®	-3.82	1.00	3.98
	Grofactor®	Control	7.01	0.25	4.00
		Zilmax®	3.19	1.00	3.96
	Zilmax®	Control	3.82	1.00	3.98
		Grofactor®	-3.19	1.00	3.96

Based on estimated marginal live mass means for each experimental group (**Table 4.1.7**).

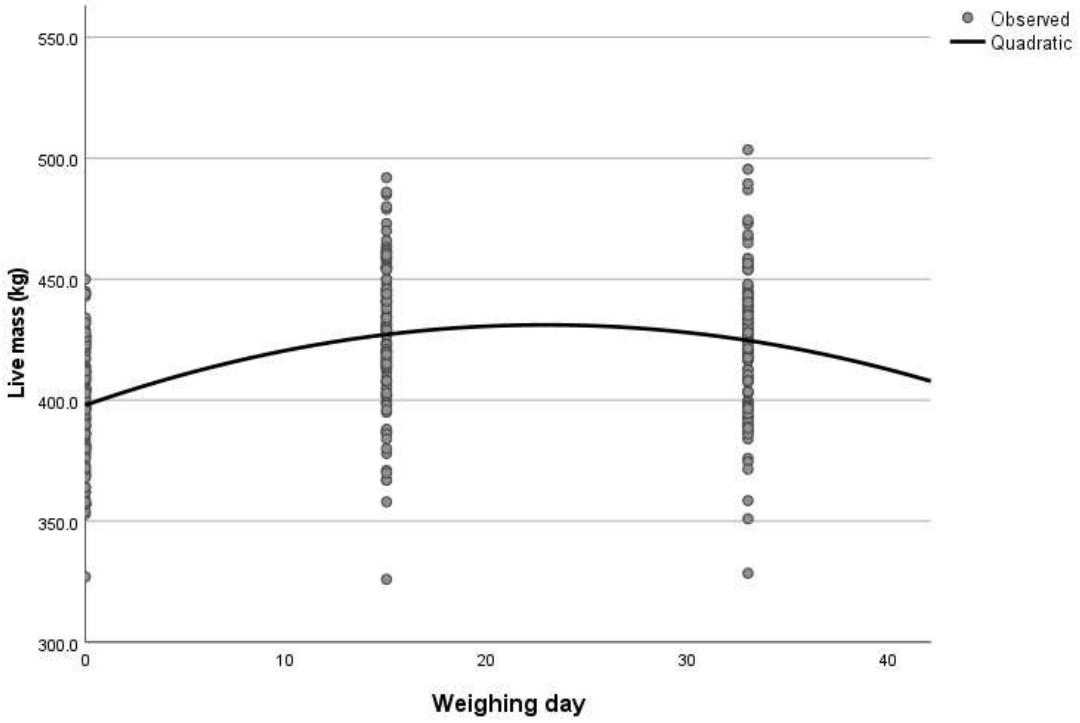
From these results we could derive that despite no significant treatment effects were observed for the ZH-supplementation treatments, GF had a larger (yet insignificant; $P > 0.05$) numerical treatment effect than ZM on both D15 and D33 mean live mass yields.

The insignificant effects ZH-supplementation of individual treatments had on both D15 and D33 mean live mass, suggest that the length of finishing period had a contributing effect to differences in mass. This was analyzed in the following sub-section.

Length of finishing period effect

When analyzing live mass as the dependent variable in a regression model with weighing day as independent variable, the relationship between these two variables was significant with P < 0.001. Live mass had a large correlation of 0.43 with ageing day. Based on this regression model, 18% of the variation in live mass was due to the ageing length of finishing period effect. This relationship is plotted against observed data below.

Figure 4.1.4 Relationship between live mass (kg) and weighing day



Quadratic fit line formula: $y = 2.89x - 0.06x^2 + 397.97$
y = live mass(kg).
x = weighing day.

As seen in **Figure 4.1.4** live mass are expected to increase as weighing day (length of the finishing period) increases.

To analyse the possible effect which the length of the finishing period might have had on live mass, weighing day (WD) was analyzed as one of two fixed factors in a univariate GLM. The other fixed factor was the overall experimental group (OG) to evaluate ZH-supplementation. Another univariate GLM was not

performed for the experimental groups, since it was well established that no live mass difference was present among the two ZH-based products ($P > 0.05$) on either D15 or D33. D0 was included as covariant in both GLMs.

D0 mass was a significant ($P < 0.001$) covariant with a large effect size of 0.80.

ZH-supplementation had a tendency ($P = 0.08$) to influence live mass improvements over the entire supplementation period, but remained insignificant, with a small effect size of 0.01. Weighing day between-subject effects were significant ($P < 0.001$) with a large effect size (0.49). The subsequent ZH-supplementation x weighing day interaction was insignificant ($P = 0.31$) with a small effect size ($\eta^2 = 0.01$)

To evaluate the effect weighing day had on overall live mass, live mass was pooled together for individual weighing days and included D0 as covariate, through which estimated means in **Table 4.1.9** were obtained. This would allow us to analyze the effect of only weighing day, without considering the ZH-supplementation effect.

Table 4.1.9 Estimated pooled live mass means (kg) of the experimental groups over the finishing period, adjusted for initial (D0) mass inclusion as covariate

Weighing day	Pooled mean mass (kg)	Std. Error
0	398.0[a]	1.25
15	426.8[a]	1.25
33	423.8[a]	1.25

a. Covariates appearing in the model are evaluated at the following values: D0 mass = 398.0kg.

The statistical pairwise comparisons of pooled live mass between weighing days, yielded the following results:

Table 4.1.10 Pairwise pooled live mass (kg) comparisons between different weighing days over the finishing period (i.e., not testing ZH-supplementation effect)

(I) Weighing day	(J) Weighing day	Mean Difference (I-J)	Sig.[b]	Std. Error
0	15	-28.82*	<0.001	1.77
	33	-25.79*	<0.001	1.77
15	0	28.82*	<0.001	1.77
	33	3.03	0.26	1.77
33	0	25.79*	<0.001	1.77
	15	-3.03	0.26	1.77

Based on estimated pooled means for different weighing days (**Table 4.1.9**).
* The mean difference is significant at the 0.05 level.
b. Adjustment for multiple comparisons: Bonferroni.

Both D15 and D33 pooled mean mass respectively differed significantly from D0 ($P < 0.001$). The heavier D33 pooled mean mass did however not differ significantly from D15 ($P > 0.20$). Indicating that length of finishing period (or ZH-supplementation period) had significant individual effects on pooled live mass when compared to D0, but that no difference exists between D15 and D33 pooled live mass.

Table 4.1.11 Comparisons of ZH-supplementation effect within each weighing day over the finishing period

Weighing day	Mean mass difference (ZH-CT)	Sig.	η^2	Std. Error
D0	2.32×10^{-5}	1.00	0.00	2.50
D15	2.18	0.23	0.01	2.50
D33	5.39	0.12	0.02	2.50

Although the ZH-supplementation effect remained insignificant ($P > 0.05$) for the difference between experimental groups, an increase in effect on live mass was

seen as days of supplementation increased (decreasing P-values and increasing η^2).

As expected ZH-supplementation had virtually no effect on D0 live mass (P = 1.00 and η^2 = 0.00) as supplementation has not yet started and experimental groups were homogenously randomly allocated beforehand. As duration of ZH-supplementation increased (to 15 then 33 days), a greater ZH-supplementation effect on live is observed between the experimental groups, although still insignificant (**Table 4.1.6** and **Table 4.1.11**).

In conclusion, ZH-supplementation does not yield significant effects on live mass as expected for different weighing days, but the effects thereof do increase over the length of the finishing period. Increasing the finishing period from 15 to 33 days yielded significant increasing effects on live mass, as suggested by Montgomery *et al.* (2009). However, the difference between day 15 and 33 live mass were not significant due to losses from transport and lairage (Hui, 2012).

4.1.2 Hot carcass mass (HCM)

As established earlier; D0 as covariant had a significant (P < 0.001) effect on HCM with a large effect size of 0.67. There was a strong correlation of R = 0.81 between D0 mass and HCM (**Table 4.1.2**). Subsequently, because R^2 = 0.65, 65% of the variation in HCM was due to D0 mass. Therefore, D0 was included as covariant in all HCM statistical analysis.

Overall ZH-supplementation effect

The pooled mean HCM was 261.1kg for the 114 experimental feedlot bulls. The ZH group HCM weighed 8kg more than CT when comparing the mean HCM of each group (263.7 vs. 255.7kg).

Table 4.1.12 Observed and estimated mean hot carcass mass (HCM; kg) for the control and ZH-supplemented treatment groups

Group	Mean HCM (kg)	Std. Deviation	Estimated marginal mean	Std. error	N
Control	255.7	20.21	255.7[a]	1.18	37
ZH	263.7	20.65	263.7[a]	1.95	77
Total	261.1	20.76	259.7[a]	1.35	114

a. Covariates appearing in the model are evaluated at the following values: D0 mass = 398.0kg.

As expected, there was a trend for HCM to increase with ZH-supplementation. Statistical analysis through a GLM, support this observation by yielding a significant ZH-supplementation effect on HCM (P = 0.001) with an effect size of 0.09. This was in agreeance with Plascencia *et al. (*1999), Avendaño-Reyes *et al.* (2006), Brooks *et al.* (2008), Montgomery *et al.* (2009), Scramlin *et al.* (2010), Maxwell *et al.* (2015) and Avendaño-Reyes *et al.* (2016).

Treatment effect comparisons

GF numerically yielded a heavier mean HCM (265.82kg) than both ZM and CT. With CT yielding the lightest mean HCM (255.68kg).

Table 4.1.13 Observed and estimated mean hot carcass mass (HCM; kg) for the three different experimental groups

Group	Mean HCM (kg)	Difference from pooled mean (kg)	Std. Deviation	N
Control	255.7	-5.40	20.21	37
Grofactor®	265.8	4.74	19.68	38
Zilmax®	261.6	0.51	21.59	39
Total	261.1	N/A	20.76	114

Statistical analysis through a GLM confirmed that overall treatments yielded a significant (P = 0.004) effect (η^2 = 0.10). To critically evaluate this effect, a

pairwise analysis was performed by adjusting means with the Bonferroni's multiple range test for uneven number of observations.

Table 4.1.14 Pairwise comparisons of estimated marginal hot carcass mass (HCM; kg) means for the three different experimental groups

(I) Treatment	(J) Treatment	Estimated marginal HCM means (kg)	Mean Difference (I-J)	Sig.[b]	Std. Error
Control	Grofactor®	264.6[a]	-8.95*	0.004	2.74
	Zilmax®	262.8[a]	-7.08*	0.03	2.72
Grofactor®	Control	255.7[a]	8.95*	0.004	2.74
	Zilmax®	262.8[a]	1.87	1.00	2.71
Zilmax®	Control	255.7[a]	7.08*	0.03	2.72
	Grofactor®	264.6[a]	-1.87	1.00	2.71

Based on estimated marginal means
*. The mean difference is significant at the 0.05 level.
a. Covariates appearing in the model are evaluated at the following values: D0 mass = 398.0kg.
b. Adjustment for multiple comparisons: Bonferroni.

Both GF and ZM treatments had significant effects on HCM when compared to CT (P = 0.004 and 0.03, respectively). GF had a larger effect than ZM (seen by a smaller P-value). GF and ZM did however not differ statistically (P = 1.00). This finding supports that of Avendaño-Reyes *et al.* (2016) who found no HCM difference between that of GF and ZM (P = 0.78).

This leads to the conclusion that ZH-supplementation had a significant increasing effect on HCM (P = 0.004) above CT, but that the ZH-based products did not differ statistically (P = 1.00). GF did yield numerically heavier HCWs than ZM.

4.1.3 Dressing percentage (DR%)

DR% was derived from both D33 mass and HCM ($\frac{HCW}{D33\ mass} x100\%$) which utilized D0 as covariate, therefore D0 was included as covariate in all statistical analyses pertaining to DR% as well. D0 had a significant (P = 0.01) effect on DR%, with a small effect size of 0.06.

The regression analysis obtained (**Table 4.1.2**) indicated that the correlation between D0 and DR% is 0.24. For this regression model only 6% of the variation in DR% was due to D0 mass**.**

Overall ZH-supplementation effect

The pooled mean DR% was 59.9% for the 114 experimental feedlot bulls. This is lower than the average 62% proposed by Hui (2012), but still falls within the range of 57 – 64% proposed by Schweihofer (2011).

A clear ZH-supplementation numerical difference was evident from the observed data, with the ZH-supplemented groups having a 0.9% higher DR% than the CT group. When investigating the statistical difference of this small increase with estimated marginal means, ZH-supplementation only tended to significantly increase the DR% (P = 0.07) with a small effect size (η^2 = 0.03).

From the literature it was determined that ZH-supplementation on average, significantly improves DR% with 1 - 5% more than their untreated counterparts, with and average value of about 1.5% (Plascencia, Torrentera, & Zinn, 1999; Brooks*, et al.*, 2008).

Table 4.1.15 Observed mean dressing percentage (DR%) for the control and ZH-supplemented treatment groups

Treatment group	Observed mean	Std. Deviation	N
Control	59.2	2.73	37
ZH	60.1	2.43	77
Total	59.9	2.55	114

Treatment effect comparisons

From the observed data it follows that no numerical difference exists between GF and ZM treatment group in terms of DR%, but that both these treatments differed numerically from the CT group. When investigating the statistical relevance of this observation with estimated marginal means, no significant overall difference existed amongst the experimental groups (P = 0.19 with η^2 = 0.03).

Table 4.1.16 Observed mean dressing percentages (DR%) for the three different experimental groups

Experimental group	Observed mean DR%	Std. Deviation	N
Control	59.2	2.73	37
Grofactor®	60.2	2.55	38
Zilmax®	60.1	2.34	39
Total	59.9	2.55	114

Pairwise comparisons of the different treatment groups once again indicated that there is no statistical difference between GF and ZM treatment results (P = 1.00; 60.2 vs. 60.1%) for DR%. This finding was in agreement with that of Avendaño-Reyes *et al.* (2016) who found no DR% difference between the GF and ZM treatments (P = 0.99; with 61.0% for both ZM and GF). Although no significant treatment effects were observed between either ZM or GF when compared to CT on DR%, ZM had a greater treatment effect than the GF treatment (P = 0.30 vs. 0.40, respectively). However, GF yielded greater effects than ZM for both live mass and HCM.

Table 4.1.17 Pairwise comparisons of estimated marginal dressing percentage (DR%) means for the three different experimental groups

(I) Treatment	(J) Treatment	Estimated marginal DR% means (kg)	Mean Difference (I-J)	Sig.[b]	Std. Error
Control	Grofactor®	60.1[a]	-0.86	0.40	0.57
	Zilmax®	60.2[a]	-0.95	0.30	0.57
Grofactor®	Control	59.2[a]	0.86	0.40	0.57
	Zilmax®	60.2[a]	-0.08	1.00	0.57
Zilmax®	Control	59.2[a]	0.95	0.30	0.57
	Grofactor®	60.1[a]	0.08	1.00	0.57

Based on estimated marginal means

a. Covariates appearing in the model are evaluated at the following values: D0 mass = 398.0kg.

b. Adjustment for multiple comparisons: Bonferroni.

4.2 Carcass characteristics, -composition and - classification of feedlot bulls as influenced by dietary supplementation of zilpaterol hydrochloride either as Zilmax® or Grofactor®

4.2.1 Carcass compactness index (CCI)

Following that CCI was derived from HCM and the carcass length (cm) ($\frac{HCM}{carcass\ length}$), where HCM utilized D0 as covariate, D0 was included as covariate in all statistical analyses pertaining to CCI as well. D0 had a significant ($P < 0.001$) effect on CCI, with an effect size of 0.59.

The regression analysis obtained (**Table 4.1.2**) indicated that the correlation between D0 and CCI was 0.75. For this regression model 56% of the variation in ICC was due to D0 mass**.**

Overall ZH-supplementation effect

The pooled mean CCI was 2.2 for the 114 experimental feedlot bulls. A small ZH-supplementation numerical increase above CT is evident from the observed data (2.2 vs. 2.1).

Table 4.2.1 Observed and estimated mean carcass compactness index (CCI) for the control and ZH-supplemented treatment groups

Group	Mean CCI	Std. Deviation	Estimated marginal mean	Std. error	N
Control	2.1[x]	0.15	2.1[a]	0.02	37
ZH	2.2[y]	0.15	2.2[a]	0.01	77
Total	2.2[y]	0.15	2.2[a]	0.01	114

[x, y] Different superscript letters in the same column indicates significant differences ($P < 0.05$)

[a.] Covariates appearing in the model are evaluated at the following values: D0 mass = 398.0kg.

When investigating the statistical difference of this small increase with estimated marginal means, ZH-supplementation significantly increased the CCI ($P < 0.001$) with an effect size of 0.13.

Treatment effect comparisons

Both GF and ZM numerically yielded a greater mean CCI (2.2) than CT. With CT yielding the smallest mean CCI (2.1).

Table 4.2.2 Observed and estimated mean carcass compactness index (CCI) for the three different experimental groups

Group	Mean CCI	Std. Deviation	Estimated marginal mean	Std. error	N
Control	2.1[x]	0.15	2.1[a]	0.02	37
Grofactor®	2.2[y]	0.14	2.2[a]	0.02	38
Zilmax®	2.2[y]	0.16	2.2[a]	0.02	39
Total	2.2	0.15	2.2[a]	0.01	114

[x, y] Different superscript letters in the same column indicates significant differences (P < 0.05)

a. Covariates appearing in the model are evaluated at the following values: D0 mass = 398.0kg.

Statistical analysis through a GLM confirmed that overall treatments yielded a significant (P < 0.001) effect (η^2 = 0.13). To critically evaluate this effect, a pairwise analysis was performed with the adjusted (estimated) marginal means.

Table 4.2.3 Pairwise comparisons of estimated marginal carcass compactness index (CCI) means for the three different experimental groups

(I) Treatment	(J) Treatment	Mean difference (I-J)	Sig.[b]	Std. Error
Control	Grofactor®	-0.08*	<0.001	0.02
	Zilmax®	-0.08*	0.003	0.02
Grofactor®	Control	0.08*	<0.001	0.02
	Zilmax®	0.01	1.00	0.02
Zilmax®	Control	0.08*	0.003	0.02
	Grofactor®	-0.01	1.00	0.02

Based on estimated marginal means (**Table 4.2.2)**

*. The mean difference is significant at the 0.05 level.

b. Adjustment for multiple comparisons: Bonferroni.

Both GF and ZM treatments had significant effects on CCI when compared to CT ($P < 0.001$ and 0.003, respectively). GF had a larger effect than ZM (seen by a smaller P-value). GF and ZM did however not differ statistically ($P = 1.00$).

This leads to the conclusion that ZH-supplementation had a significant increasing effect on CCI ($P < 0.001$), and that both ZH-treatments were equally efficient in improving CCI.

4.2.2 Carcass subcutaneous fat

Overall ZH-supplementation effect

The pooled mean subcutaneous fat was 5.5mm for the 114 experimental feedlot bulls. The ZH-supplemented group had a lower mean SC fat measurement than the control group (5.3 vs. 5.8mm). Performing a GLM established that this difference was not due to a significant between-subject ZH-supplementation effect ($P = 0.29$ with $\eta^2 = 0.01$).

Table 4.2.4 Observed mean subcutaneous fat thickness (FT; mm) for the control and ZH-supplemented treatment groups

	Observed mean		
Group	**FT (mm)**	**Std. Deviation**	**N**
Control	5.8	2.25	37
ZH	5.3	2.19	74
Total	5.5	2.21	111

Although FT results are controversial in literature, our findings support that of Avendaño-Reyes *et al.* (2006), Montgomery *et al.* (2009), Arp *et al.* (2014) and Avendaño-Reyes *et al.* (2016) who found that ZH-supplementation had no significant influence on the SC fat measurement.

Treatment effect comparisons

From the observed data it seems that a Zilmax®-supplementation yielded the smallest mean FT measurements (4.9mm), whilst the control and Grofactor®-

supplemented groups yielded numerically similar measurements (5.8 and 5.7mm, respectively). When investigating the statistical relevance of these observations with estimated marginal means, no significant overall difference existed amongst the experimental groups (P = 0.19 with η^2 = 0.03).

Table 4.2.5 Observed and estimated mean subcutaneous fat thickness (FT; mm) for the three different experimental groups

Experimental group	Mean FT (mm)	Std. Deviation	N
Control	5.8	2.25	37
Grofactor®	5.7	2.40	37
Zilmax®	4.9	1.91	37
Total	5.5	2.21	111

Pairwise comparisons eluded that no significant differences were present amongst the different experimental groups (P > 0.05). There was no GF-supplementation effect when compared to CT (P = 1.00), whereas ZM numerically yielded more (insignificant) favourable mean FT results when compared to CT (P = 0.29 vs. 1.00). There was an insignificant numerical difference between FT means for ZM and GF (P = 0.42). This finding mirrors that of Avendaño-Reyes *et al.* (2016) where ZM yielded numerically lower FT measurements than GF, but that the two products did not differ on a statistical level (P = 0.64; 6.1 vs. 6.9mm).

Although insignificant, ZM numerically yielded leaner carcasses than CT, which represented the theoretical energy repartitioning mechanism of ZH-supplementation. GF did not repartition the energy as effective since the mean FT did not differ at all from CT. ZM had greater (insignificant) effects on both DR% and SC fat than GF, whereas GF yielded greater effects on live mass and HCM. The effects of GF on HCM were significant.

Table 4.2.6 Pairwise comparisons of estimated marginal subcutaneous fat thickness (FT; mm) means for the three different experimental groups

(I) Treatment	(J) Treatment	Estimated marginal SC fat means (mm)	Mean Difference (I-J)	Sig.[b]	Std. Error
Control	Grofactor®	5.7	0.09	1.00	0.51
	Zilmax®	4.9	0.85	0.29	0.51
Grofactor®	Control	5.8	-0.09	1.00	0.51
	Zilmax®	4.9	0.76	0.42	0.51
Zilmax®	Control	5.8	-0.85	0.29	0.51
	Grofactor®	5.7	-0.76	0.42	0.51

Based on estimated marginal means
b. Adjustment for multiple comparisons: Bonferroni.

4.2.3 Carcass composition

Overall ZH-supplementation effect

The pooled mean muscle% was 61.7% for the 114 experimental feedlot bulls. ZH numerically yielded about 2.0% more muscle than CT.

The pooled mean fat% was 22.3% for the 114 experimental feedlot bulls. ZH numerically yielded about 1.8% less fat than CT.

The pooled mean bone% was 13.5% for the 114 experimental feedlot bulls. Both ZH and CT had a 13.5% bone composition as expected. The experimental feedlot bulls were selected from a homogenous *B. Taurus* crossbred population; therefore, the inherent bone compositions were expected to be nearly identical in carcasses of all the experimental animals.

Figure 4.2.1 Observed mean carcass composition percentages for the control and ZH-supplemented treatment groups

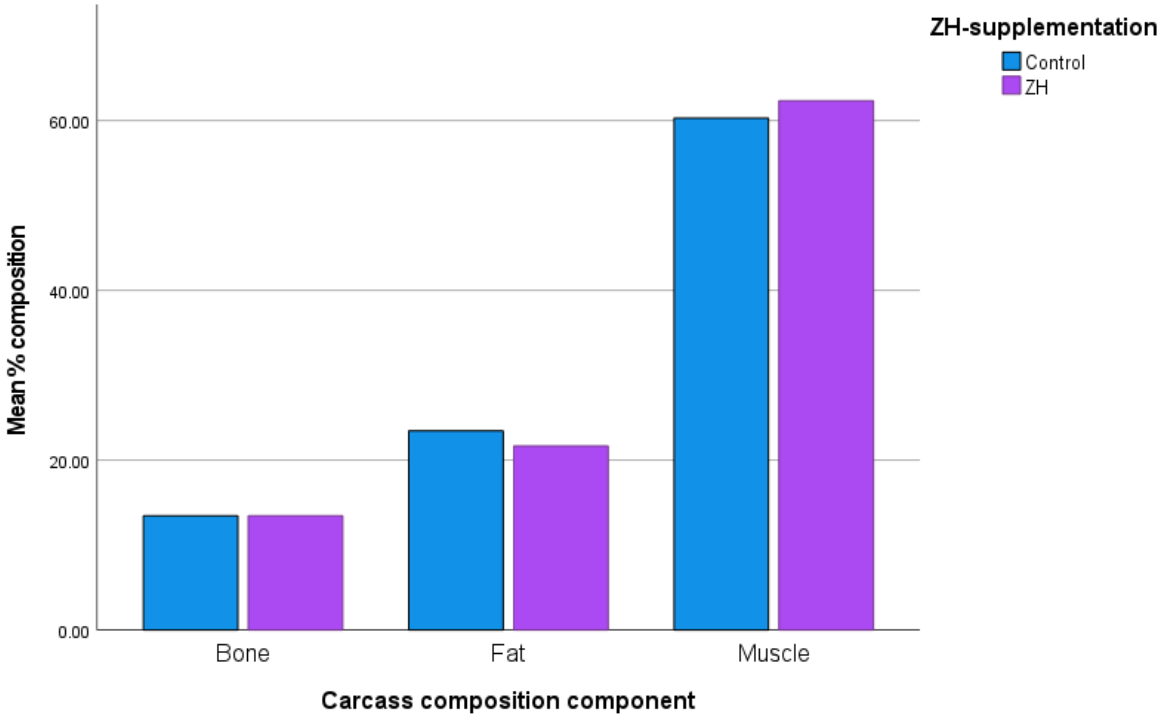


Table 4.2.7 Observed mean carcass composition percentages for the control and ZH-supplemented treatment groups

Component		Mean %	Std. Deviation	N
Muscle	Control	60.3	3.65	37
	ZH	62.3	4.53	75
	Total	61.7	4.35	112
Fat	Control	23.5	4.17	37
	ZH	21.7	4.65	75
	Total	22.3	4.56	112
Bone	Control	13.5	1.64	37
	ZH	13.5	1.97	75
	Total	13.5	1.86	112

When evaluating the overall ZH-supplementation effect, there was an insignificant supplementation effect (P = 0.10 with η^2 = 0.06) across all three composition components. When evaluating the ZH-supplementation effect on

each individual carcass composition component, the following results were found:

Table 4.2.8 ZH-supplementation effects and effect sizes for the different carcass composition components

Composition component	Sig.	η^2
Muscle%	0.02	0.05
Fat%	0.05	0.03
Bone%	0.99	0.00

As expected, no statistical difference was observed in the bone% with ZH-supplementation (P = 0.99). This is due to the physiological mechanism of ZH focusing on repartitioning energy from fat deposition to muscle synthesis instead (Mersmann, 1998).

Since both muscle% and fat% significantly ($P \leq 0.05$) increased and decreased, respectively, the repartitioning mechanism of ZH is supported. The results from this study confirm the larger effect size of ZH-supplementation on muscle% than fat% ($P = 0.02 < 0.05$; $\eta^2 = 0.05$ vs. 0.03, respectively), which supports the findings of Johnson *et al.* (2013). This increased the muscle:fat ratio favourably from 2.6 for the control group to 2.9 for the ZH-supplemented group.

The significant decreased fat% observed in this study, supports that of Hilton *et al.* (2009). Additionally, Avendaño-Reyes *et al.* (2006), Leheska *et al.* (2009) and Hilton *et al.* (2009) also found that ZH-supplementation significantly increases the muscle%.

Treatment effectiveness comparisons

CT had the smallest muscle% composition (60.3%), with GF and ZH having larger, similar muscle % compositions (62.2 and 62.5%, respectively).

The smallest fat% composition was yielded by ZM (21.3%), with GF yielding a marginally larger fat% than ZM (22.0%) and CT yielding the overall largest fat% (23.5%).

Although small numerical differences exist amongst the different experimental groups, it could be expected not to differ significantly on a statistical level given the abovementioned results between CT and the ZH-supplemented treatment groups.

Figure 4.2.2 Observed mean carcass composition percentages for the three different experimental groups

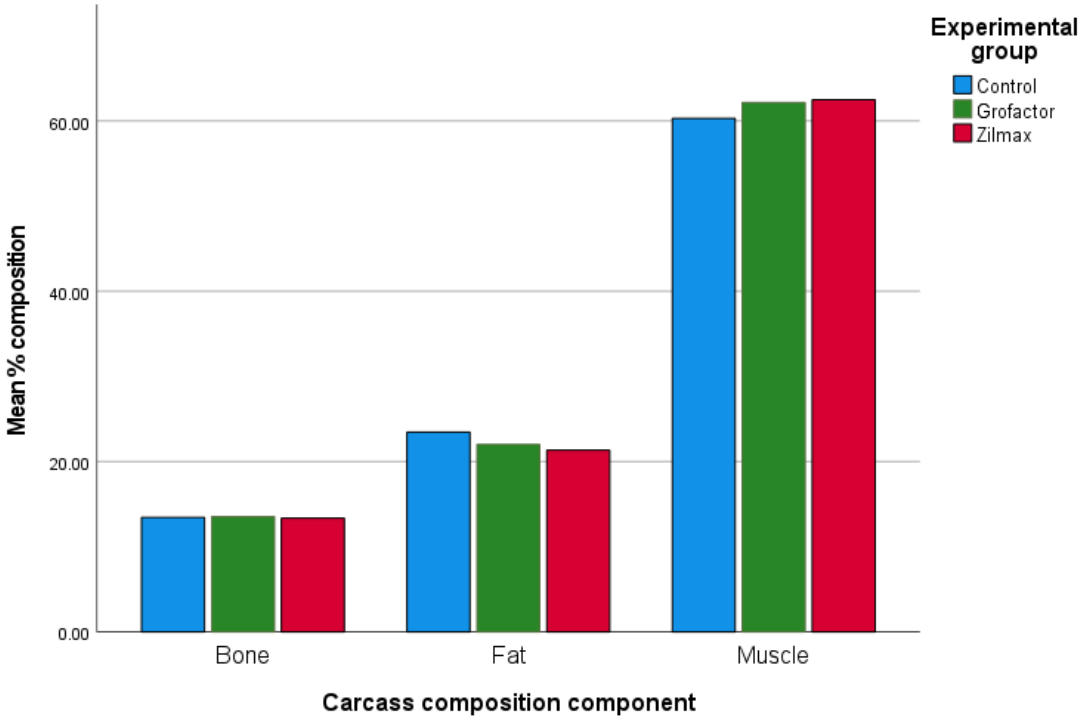


Table 4.2.9 Observed mean carcass composition percentages for the three different experimental groups

Component	Treatment	Mean %	Std. Deviation	N
Muscle	Control	60.3	3.65	37
	Grofactor®	62.2	5.00	37
	Zilmax®	62.5	4.08	38
	Total	61.7	4.35	112
Fat	Control	23.5	4.17	37
	Grofactor®	22.0	4.87	37
	Zilmax®	21.3	4.47	38
	Total	22.3	4.56	112
Bone	Control	13.5	1.64	37
	Grofactor®	13.5	1.97	37
	Zilmax®	13.4	1.99	38
	Total	13.5	1.86	112

Because no covariate was included in the GLM, only Bonferroni's multiple range test was performed to compare the three uneven different experimental groups.

Table 4.2.10 Pairwise comparisons of carcass composition component (%) means for the three different experimental groups

	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.[b]
Muscle	Control	Grofactor®	-1.86	1.00	0.19
		Zilmax®	-2.19	1.00	0.09
	Grofactor®	Control	1.86	1.00	0.19
		Zilmax®	-0.33	0.99	1.00
	Zilmax®	Control	2.19	0.99	0.09
		Grofactor®	0.33	0.99	1.00
Fat	Control	Grofactor®	1.44	1.05	0.52
		Zilmax®	2.12	1.04	0.13

	Grofactor®	Control	-1.44	1.05	0.52
		Zilmax®	0.68	1.04	1.00
	Zilmax®	Control	-2.12	1.04	0.13
		Grofactor®	-0.68	1.04	1.00
Bone	Control	Grofactor®	-0.09	0.44	1.00
		Zilmax®	0.09	0.43	1.00
	Grofactor®	Control	0.09	0.44	1.00
		Zilmax®	0.18	0.43	1.00
	Zilmax®	Control	-0.09	0.43	1.00
		Grofactor®	-0.18	0.43	1.00

*. The mean difference is significant at the 0.05 level.
b. Adjustment for multiple comparisons: Bonferroni.

For muscle composition, no significant differences were observed among the different experimental groups. When compared to the mean muscle% of CT, GF had no significant increasing effect (P = 0.19), whereas ZM tended to increase muscle% (P = 0.09). Although ZM did have a greater (insignificant effect) than GF when compared to CT (P = 0.09 < 0.19), no difference was observed between ZM and GF treatment effects (P = 1.00).

Similar to muscle% results, fat% yielded no significant treatment effects when compared to CT (P = 0.52 and 0.13 for GF and ZM, respectively). ZM still had a greater (insignificant effect) than GF when compared to CT (P = 0.13 < 0.52). As with muscle%, no difference was observed between ZM and GF treatment effects (P = 1.00).

As expected, no treatment effects were present for bone% (P = 1.00). The small insignificant effects which were present existed for muscle and fat percentages, since ZH and GF are both ZH-supplementations which repartition energy from fat to muscle accretion. Whilst no difference was present between the two ZH-supplementations, ZM did have greater (insignificant) treatment effects when increasing muscle% and decreasing fat%, better than GF. Thereby (insignificantly) altering the muscle:fat ratio favourably.

4.2.4 Carcass classification

Due to the categorical nature of classification measurements the carcass classification results would not be as intricately explored as the previous numerical results.

As seen in **Table 4.2.11** and **Figure 4.2.3**, the observed counts in different categories (A2 specifically) for GF and ZM were numerically higher than CT. To determine whether these differences in classification category counts were significantly associated with the treatments, a chi-square test was performed.

Figure 4.2.3 Carcass classification counts for the three different experimental groups

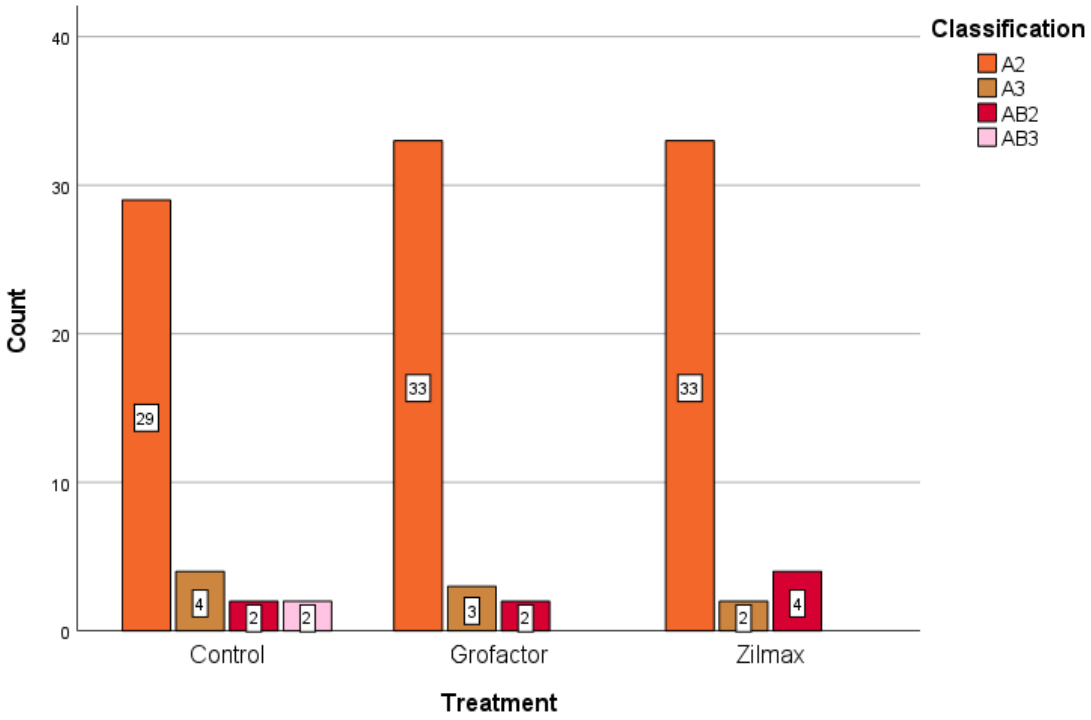


Table 4.2.11 Crosstabulation of the observed and expected carcass numbers of each experimental group in their respective classification categories

			Classification				Total
			A2	A3	AB2	AB3	
Treatment	Control	Count	29	4	2	2	**37**
		Expected	30.8	2.9	2.6	0.6	**37.0**
	Grofactor®	Count	33	3	2	0	**38**
		Expected	31.7	3.0	2.7	0.7	**38.0**
	Zilmax®	Count	33	2	4	0	**39**
		Expected	32.5	3.1	2.7	0.7	**39.0**
Total		Count	**95**	**9**	**8**	**2**	**114**
		Expected	**95.0**	**9.0**	**8.0**	**2.0**	**114.0**

Table 4.2.12 Chi-square test results for carcass classification and treatment association

	Value	df	Asymptotic Significance (2-sided)
Pearson Chi-Square	6.00[a]	6	0.42
Likelihood Ratio	6.31	6	0.39
N of Valid Cases	114		

a. 9 cells (75.0%) have expected count less than 5. The minimum expected count is 0.65.

Because more than 20% of the cells had an expected count less than 5, the likelihood ratio is utilised instead of the Pearson chi-square value. No significant association was present between the treatment and carcass classification (P > 0.05). It can thus be concluded that no significant statistical difference exists between treatments, among the different category counts.

It is important to note, that although only numerical, ZH-supplementation yielded more carcasses of A2 classification than CT. This is due to the production focus being placed on carcasses being leaner (Webb, 2015).

4.3 Carcass temperatures and pHs of feedlot bulls as influenced by dietary supplementation of zilpaterol hydrochloride either as Zilmax® or Grofactor®, over 48hours

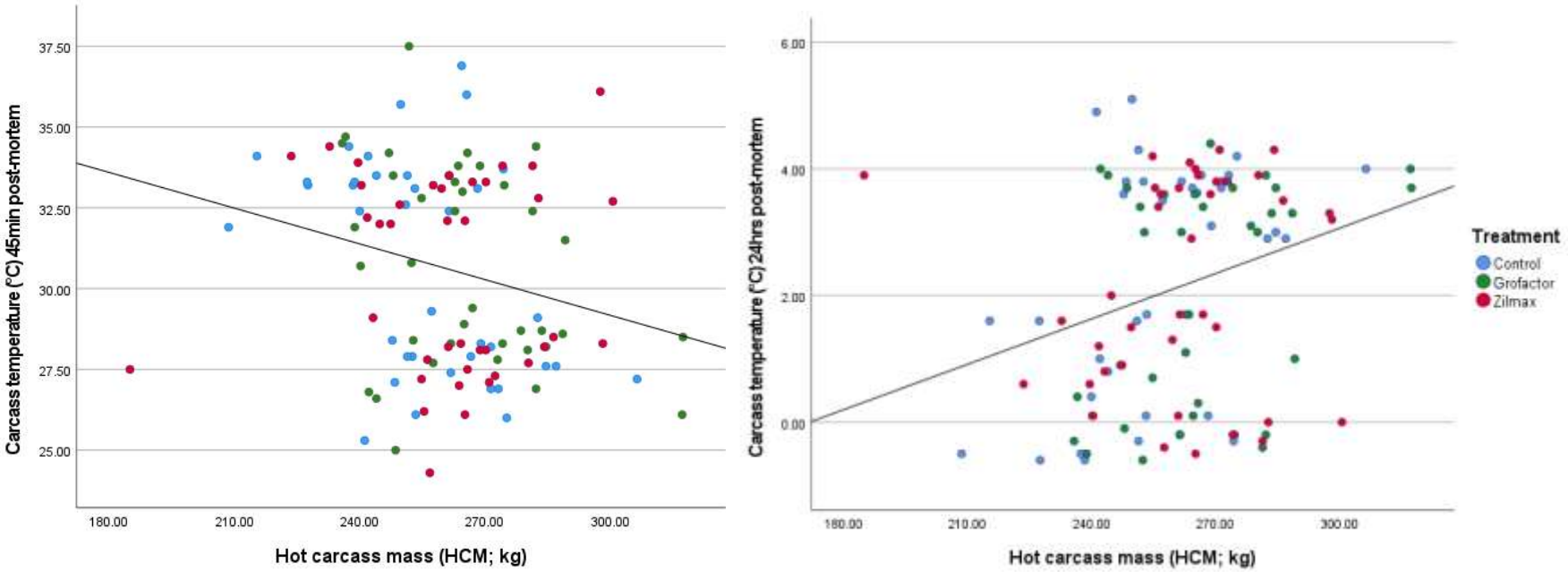
Regression models were performed for HCM and the carcass temperatures. Initial carcass temperatures measured at 45min post-mortem, indicated that a negative relationship existed between the carcass temperatures and HCM i.e., heavier HCM had colder carcass temperatures at 0.75hrs post-mortem. Carcass temperatures measured at 24hrs post-mortem had a positive relationship with HCM i.e., heavier carcasses now had hotter carcass temperatures at 24hrs post-mortem, as expected (Webb & Agbeniga, 2020).

HCM had a significant effect ($P = 0.01$) on the carcass temperature at 45min post-mortem. The carcass temperature at 45min post-mortem had a correlation of 0.24 with HCM. Based on this regression model 5.6% of the variation in carcass temperature at 45min post-mortem was due to HCM. The coefficient in this relationship was -0.04, meaning that for every 1kg increase in HCM, the estimated carcass temperature at 45min post-mortem decreased by 0.4°C.

HCM had a significant effect ($P < 0.05$) on the carcass temperature at 24hrs post-mortem. Carcass temperature at 24hrs post-mortem had a correlation of 0.28 with HCM. This is 0.04 units higher than the HCM and 45min post-mortem carcass temperature relationship. Based on this regression model 7.8% of the variation in carcass temperature at 24hrs post-mortem was due to the HCM. The coefficient in this relationship was 0.02, meaning that for every 1kg increase in HCM, the estimated carcass temperature at 24hrs post-mortem increased with 0.2°C.

The regression models for carcass temperature and HCM - with formulas - are given on the following page:

Figure 4.3.1a & b Relationships for hot carcass mass (HCM; kg) and carcass temperatures (°C) measured at 45min (a) and 24hrs (b) post-mortem, plotted for experimental group differences



(a) Linear fit line formula: y = 40.2 - 0.04x

(b) Linear fit line formula: y = -4.1 + 0.02x

y = carcass temperature (°C) at 45min or 24hrs post-mortem.

x = hot carcass mass (HCM; kg).

Table 4.3.1 Summarised regression model results for hot carcass mass (HCM; kg) on carcass temperatures (°C) measured at 45min and 24hrs post-mortem

Carcass temperature (°C)	45min	24hrs
Sig.	0.01	0.003
Relationship	Negative	Positive
Coefficient	-0.04	0.02
R (correlation)	0.24	0.28
R^2 (% of variation)	0.06	0.08

The independent variable is HCM (kg).
The dependent variable is carcass temperature(s) measured at either 45min or 24hrs post-mortem.

This coincides with the conclusion drawn by Webb and Agbeniga (2020, p. 968) that heavier carcasses will have higher rigor temperatures than lighter counterparts.

Additionally, regression models were performed for HCM and the carcass pHs. Both carcass pHs measured at 45min and 24hrs post-mortem, indicated that a negative relationship existed between the carcass pHs and HCM i.e., heavier HCM had lower carcass pHs than lighter carcasses.

HCM had a significant effect (P = < 0.001) on the carcass pH at 45min post-mortem. The carcass pH at 45min post-mortem had a correlation of 0.26 with HCM. Based on this regression model 6.7% of the variation in carcass pH at 45min post-mortem is due to HCM. The coefficient was -0.005 in this relationship, meaning that for every 1kg increase in HCM, the estimated carcass pH at 45min post-mortem will decrease with 0.005 units.

HCM had a significant effect (P = 0.002) on the carcass pH at 24hrs post-mortem. The carcass pH at 24hrs post-mortem had a correlation of 0.29 with HCM. This is 0.03 units stronger than the HCM and 45min post-mortem carcass pH relationship, as seen with the individual carcass temperature regressions. Based on this regression model 8.2% of the variation in carcass pH at 24hrs post-mortem is due to the HCM. The coefficient in this relationship was -0.002,

meaning that for every 1kg increase in HCM, the estimated carcass pH at 24hrs post-mortem increased with 0.002 units.

Although HCM had significant ($P < 0.05$) effects on carcass pHs, the effect size was miniscule in practice (seen by coefficient explanations).

Table 4.3.2 Summarised regression model results for hot carcass mass (HCM; kg) on carcass pHs measured at 45min and 24hrs post-mortem

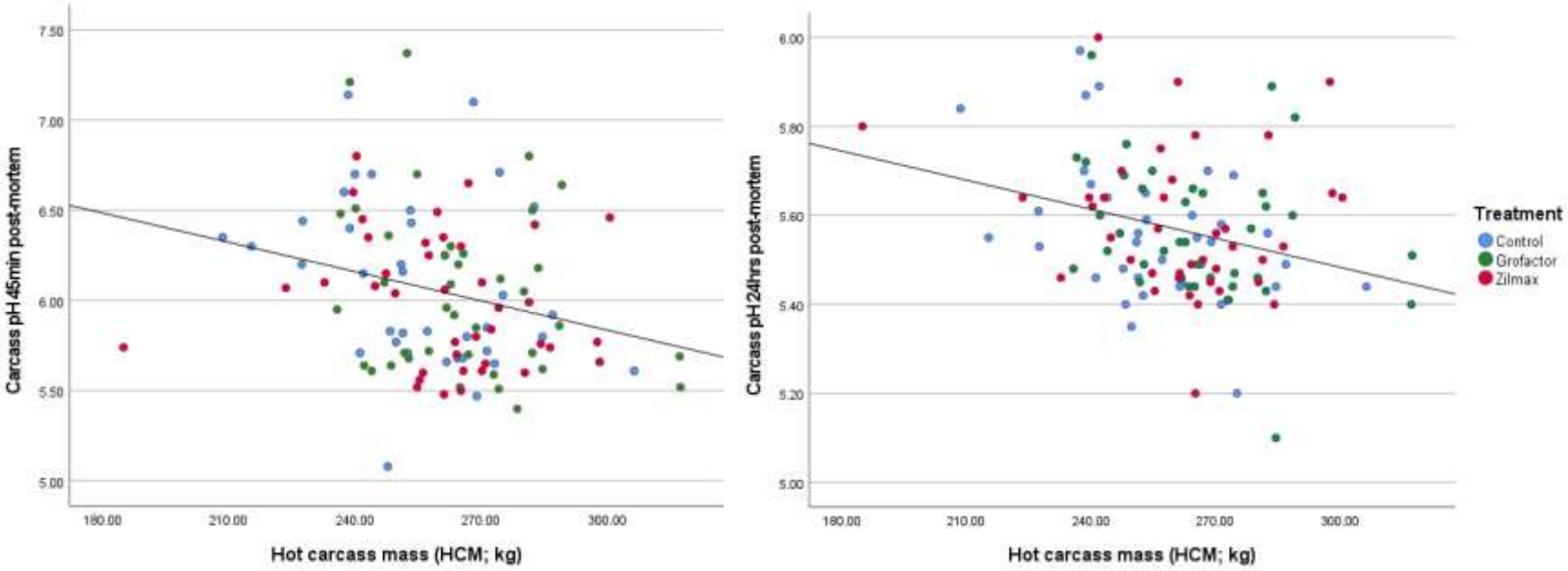
Carcass pH	45min	24hrs
Sig.	< 0.001	0.002
Relationship	Negative	Negative
Coefficient	-0.005	-0.002
R (correlation)	0.26	0.29
R^2 (% of variation)	0.07	0.08

The independent variable is HCM (kg).
The dependent variable is carcass pH(s) measured at either 45min or 24hrs post-mortem.

The regression models for carcass pH and HCM with formulas are given on the following page(s).

In conclusion, HCW was not included in the statistical analyses for either carcass temperature or pH as focus was placed on the possible effects caused by ZH-supplementation for the two different ZH-based products. Additionally, because HCM was analysed as a dependent variable to achieve the ZH-supplementation affects, it cannot be utilised as a covariant for a different dependent variable.

Figure 4.3.2a & b Relationships for hot carcass mass (HCM; kg) and carcass pHs measured at 45min (a) and 24hrs (b) post-mortem, plotted for experimental group differences



(a) Linear fit line formula: $y = 7.5 - 0.005x$ (b) Linear fit line formula: $y = 6.1 - 0.002x$

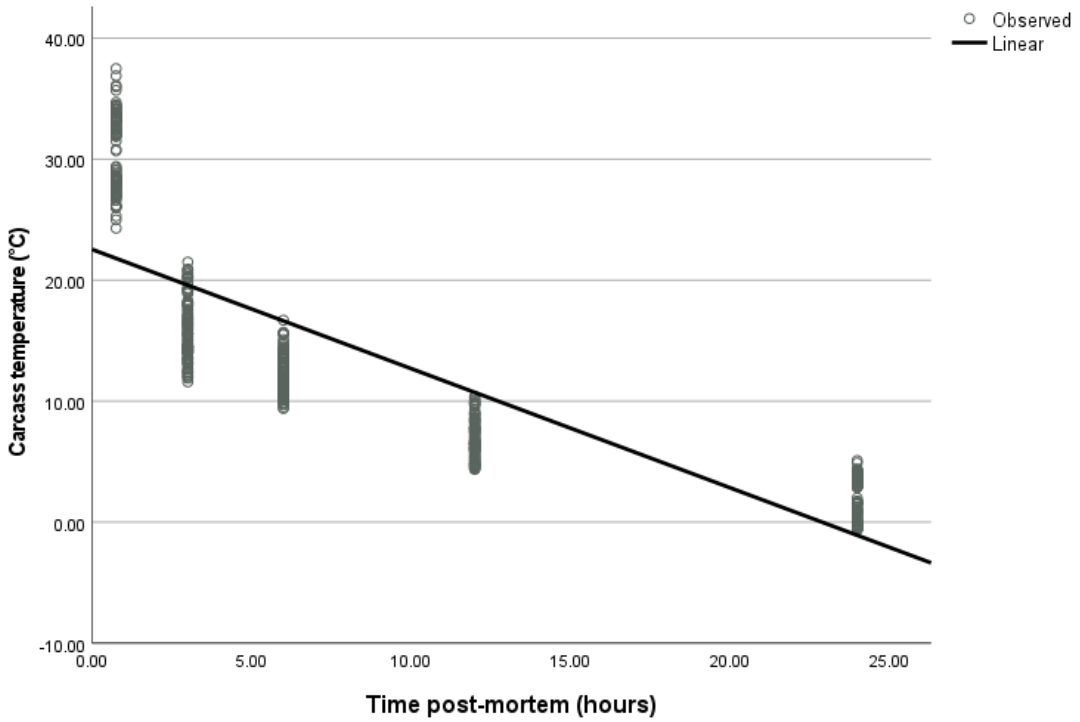
y = carcass pH at 45min or 24hrs post-mortem.

x = hot carcass mass (HCM; kg).

4.3.1 Carcass temperature

Carcass temperature as dependent variable had a negative, significant (P < 0.001) relationship with time post-mortem. The carcass temperature had a large correlation of 0.82 with time post-mortem. Based on this regression model 67.7% of the variation in carcass temperature was due to post-mortem time. The coefficient in this relationship was -0.98, meaning that for every 1hr increase in post-mortem time, the estimated carcass temperature decreased with 1.0°C. This linear relationship is plotted against observed data in **Figure 4.3.3.** We would therefore expect all carcass temperatures to decrease over time with ZH-supplemented carcasses to additionally be hotter than the control group carcasses, due to expected heavier carcass yields (Webb & Agbeniga, 2020).

Figure 4.3.3 Relationship between carcass temperature (°C) and time post-mortem (hours)



Linear fit line formula: y = -0.98x + 22.55
y = carcass temperature (°C).
x = time post-mortem (hrs).

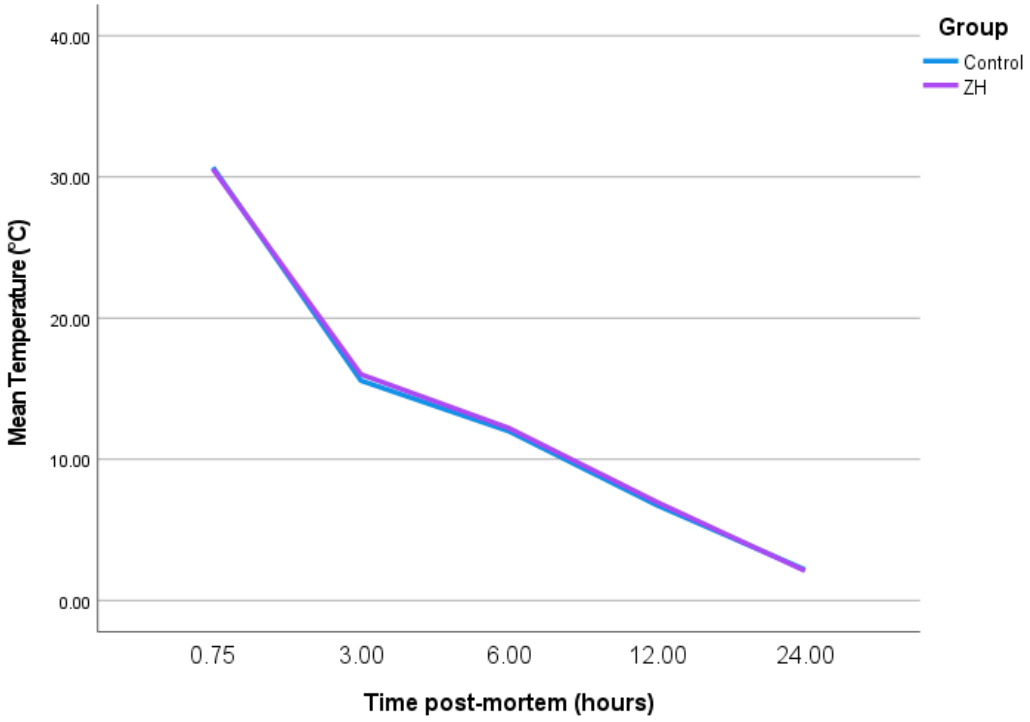
Overall ZH-supplementation effect

ZH-supplemented cattle yielded carcasses that had numerically colder carcass temperatures at 0.75 and 24hrs post-mortem. These are the initial and final carcass temperatures, respectively. As seen in **Figure 4.3.4,** there was a gradual decrease in carcass temperatures over time post-mortem, both CT and ZH had similar numerical values.

Table 4.3.3 Observed mean carcass temperatures (°C) over 24hrs post-mortem for the control and ZH-supplemented treatment groups

Time post-mortem	Group	Mean carcass temperatures (°C)	Std. Deviation	N
0.75	Control	30.8	3.35	36
	ZH	30.6	3.05	77
	Total	30.6	3.14	113
3	Control	15.6	2.27	36
	ZH	16.0	2.59	77
	Total	15.9	2.49	113
6	Control	12.0	1.54	36
	ZH	12.2	1.55	77
	Total	12.2	1.54	113
12	Control	6.8	1.68	36
	ZH	7.0	1.54	77
	Total	6.9	1.58	113
24	Control	2.2	1.91	36
	ZH	2.1	1.69	77
	Total	2.1	1.76	113

Figure 4.3.4 Observed mean carcass temperatures (°C) over 24hrs post-mortem for the control and ZH-supplemented treatment groups



Overall, between-subject effect analysis found that ZH-supplementation had no significant effect on carcass temperatures over the entire post-mortem time period (P = 0.93; η^2 = 0.01). To investigate the possible effects which ZH-supplementation might have had on carcass temperatures on individual measuring time periods, Bonferroni's multiple range test was performed:

Table 4.3.4 ZH-supplementation effects and effect sizes for carcass temperatures (°C) measured at different times post-mortem (hours)

Time post-mortem (hrs)	Sig.	η^2
0.75	0.75	0.001
3	0.43	0.006
6	0.55	0.003
12	0.48	0.004
24	0.87	0.001

Adjustment for multiple comparisons: Bonferroni.

From the table above it is evident that ZH-supplementation had no significant effect on carcass temperatures at any measuring times (P > 0.05).

Treatment effect comparisons

No significant between-subject treatment effects were observed on any post-mortem times (P > 0.05) for carcass temperatures.

Carcass temperatures were expected to decrease over time due to the negative, significant (P < 0.001) relationship with time post-mortem (**Figure 4.3.4)**. This overall trend holds true when evaluating individual experimental group carcass temperatures over time (**Figure 4.3.5**).

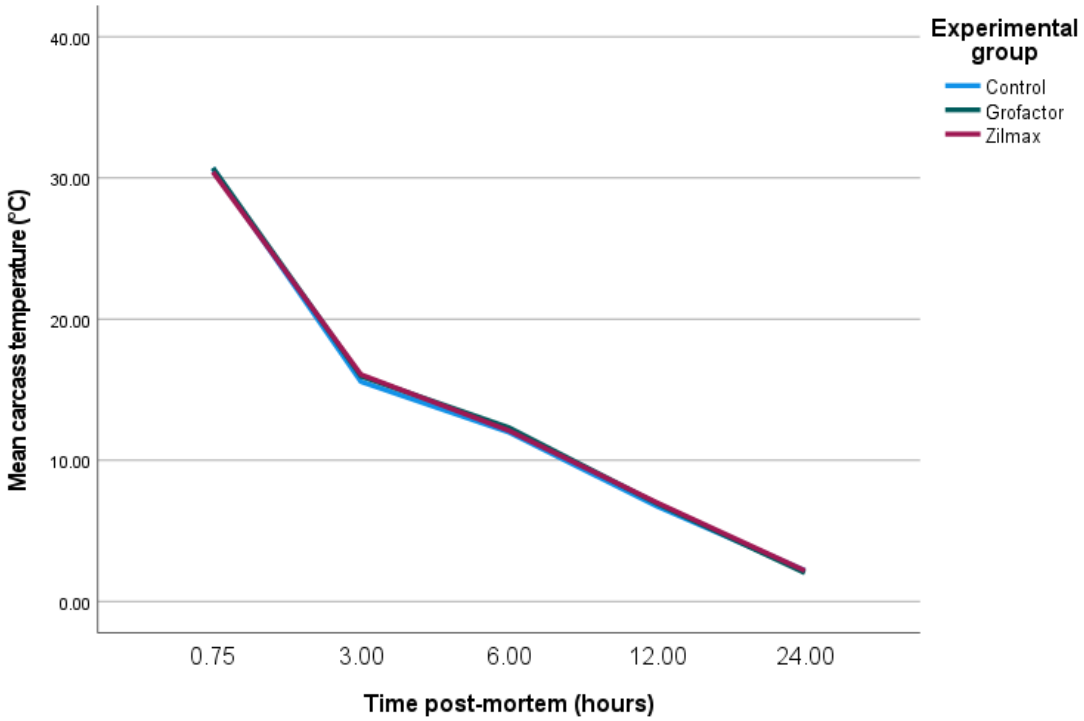
Following the previously stated assumption that ZH-supplemented carcasses would be colder than the control group carcasses, we can now expect CT carcasses to be hotter numerically than both GF and ZM, because GF and ZM form the broad ZH-supplementation group.

Table 4.3.5 Observed mean carcass temperatures (°C) over 24hrs post-mortem for the three different experimental groups

Time post-mortem (hrs)	Experimental group	Mean carcass temperature (°C)	Std. Deviation	N
0.75	Control	30.8	3.35	36
	Grofactor®	30.7	3.08	38
	Zilmax®	30.4	3.07	39
	Total	30.6	3.14	113
3	Control	15.6	2.27	36
	Grofactor®	16.0	2.56	38
	Zilmax®	16.1	2.65	39
	Total	15.9	2.49	113
6	Control	12.0	1.54	36
	Grofactor®	12.3	1.4	38
	Zilmax®	12.1	1.68	39
	Total	12.2	1.54	113
12	Control	6.8	1.68	36
	Grofactor®	6.9	1.41	38
	Zilmax®	7.0	1.68	39
	Total	6.9	1.58	113

24	Control	2.16	1.91	36
	Grofactor®	2.03	1.75	38
	Zilmax®	2.19	1.66	39
	Total	2.13	1.76	113

Figure 4.3.5 Mean carcass temperatures (°C) over 24hrs post-mortem for the three different experimental groups



Although experimental group mean carcass temperatures did marginally differ numerically, no clear trend was upheld, i.e., neither ZH-supplementation group (GF or ZM) consistently yielded colder carcasses than CT (**Table 4.3.5**).

To investigate whether these small numerical differences amongst experimental groups might be statistically significant, Bonferroni's multiple range test was performed to accurately analyse the GLM.

The small numerical differences among experimental group carcass temperatures were insignificant (P = 1.00) when evaluated within each specific post-mortem time (e.g., 45min). This finding is similar to the results obtained with live mass evaluations (that experimental groups did not differ from each other on the same weighing day). Therefore, time post-mortem should be investigated as a possible factor which influences the carcass temperatures.

Post-mortem time (PMt) effect

To analyse the possible effect which the post-mortem time (PMt) might have had on carcass temperatures (°C), PMt was analyzed as one of two fixed factors in two univariate GLMs. The other fixed factor was the overall experimental group (OG) to evaluate ZH-supplementation. Another univariate GLM was not performed for the experimental groups, since it was well established that no carcass temperature differences were present among the two ZH-based products ($P > 0.05$) on any post-mortem time.

Table 4.3.6 Between-subject overall effects on carcass temperatures (°C) with both post-mortem time (PMt; hours) and ZH-supplementation (ZH) as fixed factors

Fixed factors	Sig.	η2
ZH	0.48	0.001
PMt	< 0.001	0.95
ZH x time interaction	0.89	0.002

The overall ZH-supplementation effect reiterates that ZH had no significant on the carcass temperatures. The ZH x time interaction was insignificant (0.89) with a negligible effect size of 0.002. PMt had significant ($P < 0.001$) overall effects on the carcass temperatures with a large effect size ($\eta^2 = 0.95$). Indicating that the post-mortem period had a greater (significant) effect on the carcass temperature decline instead of ZH-supplementation.

Table 4.3.7 Pooled mean carcass temperatures (°C) for the three different experimental groups at different post-mortem times (hours)

Time post-mortem (hours)	Pooled mean	Std. Error
0.75	30.63	0.22
3.00	15.80	0.22
6.00	12.10	0.22

12.00	6.86	0.22
24.00	2.15	0.22
Total	13.51	0.10

To evaluate the PMt effect on carcass temperatures the pooled mean temperatures of the different time periods were analysed in a pairwise comparison. Because the carcass temperatures are pooled, ZH-supplementation cannot be tested and only the effects of PMt will be obtained.

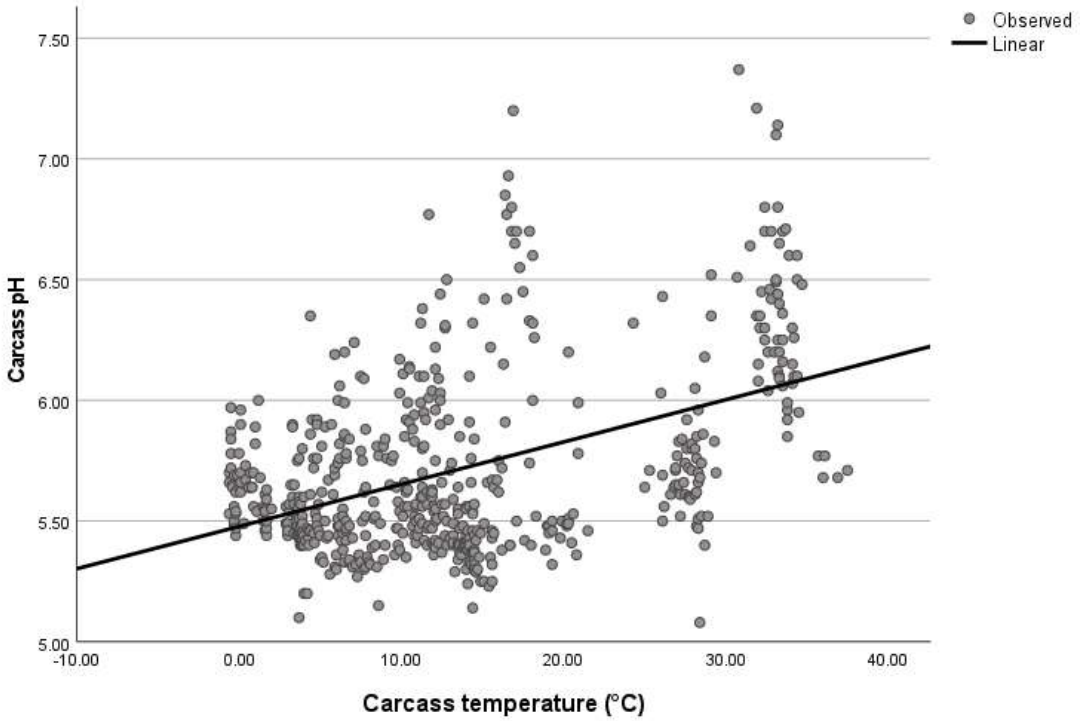
All time periods significantly differed from each other ($P < 0.001$), indicating that the decreasing carcass temperatures over the post-mortem time period, decreased significantly as a result of lengthening post-mortem time. This coincides with the overall between subject-effects given in **Table 4.3.6**.

When executing pairwise comparisons (not pooled) for the different ZH-supplementation groups (OG and TG) within a specific time period, the different groups did not differ statistically from each other ($P = 1.00$). But when executing pairwise comparisons for the different PMt within a specific ZH-supplementation group (OG and TG), all values differed significantly from each other ($P < 0.001$). Reiterating that the ZH-supplementation had no effect on carcass temperature decreases, but rather the PMt.

4.3.2 Carcass pH

Carcass pH as dependent variable had a positive, significant ($P < 0.001$) relationship with carcass temperature. The carcass pH had a correlation of 0.46 with carcass temperature. Based on this regression model 21% of the variation in carcass pH will be due to carcass temperature. The coefficient in this linear relationship was 0.02, meaning that for every 1°C decrease in carcass temperature, the estimated carcass pH decreased with 0.02units. This linear relationship is plotted against observed data in **Figure 4.3.6**, unfortunately IBM SPSS would not allow the carcass temperature axis to decrease instead of increase.

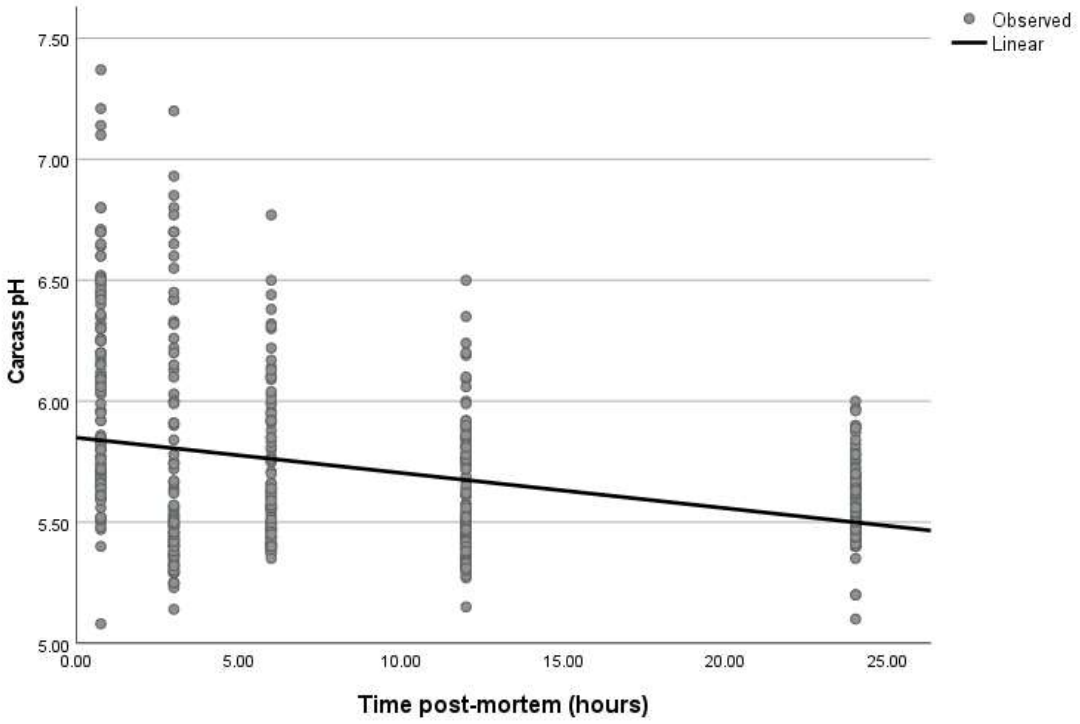
Figure 4.3.6 Relationship between carcass pH and -temperature (°C)



Linear fit line formula: $y = 0.02x + 5.48$
y = carcass pH.
x = carcass temperature (°C).

Carcass pH as dependent variable had a significant (P < 0.001) relationship with time post-mortem. The carcass pH had a correlation of 0.32 with time post-mortem. Based on this regression model 10% of the variation in carcass temperature was due to time post-mortem. The coefficient in this relationship was -0.02, meaning that for every 1hr increase in time post-mortem, the estimated carcass pH decreased with 0.02units. This relationship is plotted against the observed data in **Figure 4.3.7.** We would therefore expect all carcass pH to decrease over time.

Figure 4.3.7 Relationship between carcass pH and time post-mortem (hours)

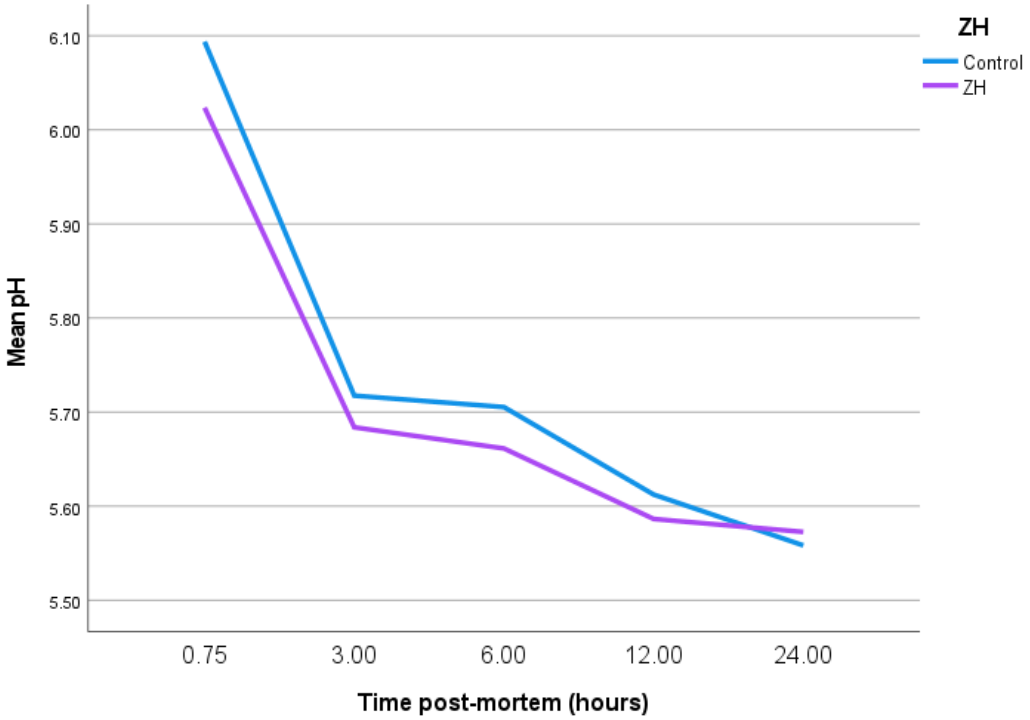


Linear fit line formula: y = -0.02x + 5.85
y = carcass pH.
x = time post-mortem (hrs).

Overall ZH-supplementation effect

Carcasses from ZH-supplemented cattle yielded numerically lower pHs at all time periods except at 24hrs post-mortem where ZH-supplemented carcasses yielded numerically similar pHs (5.6 for both) to the control group. As seen in **Figure 4.3.8,** there was a tendency to decrease carcass pHs over the post-mortem period.

Figure 4.3.8 Mean carcass pH over 24hrs post-mortem for the control and ZH-supplemented treatment groups



ZH-supplementation yielded no significant between-subject effects on carcass pHs on any of the times post-mortem (P = 0.66; η^2 = 0.03). To investigate the possible effects which ZH-supplementation might have had on carcass pH on individual measuring time periods, Bonferroni's multiple range test was performed to obtain the results in **Table 4.3.9**. It is evident from these results that ZH-supplementation had no significant effect on carcass pHs at any measuring times (P > 0.05).

Table 4.3.8 Observed mean carcass pH values over 24hrs post-mortem for the control and ZH-supplemented treatment groups

Time post-mortem	Group	Mean carcass pH values	Std. Deviation	N
0.75	Control	6.1	0.46	36
	ZH	6.0	0.42	77
	Total	6.1	0.43	113

3	Control	5.7	0.45	36
	ZH	5.7	0.48	77
	Total	5.7	0.47	113
6	Control	5.7	0.28	36
	ZH	5.7	0.31	77
	Total	5.7	0.30	113
12	Control	5.6	0.28	36
	ZH	5.6	0.26	77
	Total	5.6	0.26	113
24	Control	5.6	0.16	36
	ZH	5.6	0.16	77
	Total	5.6	0.16	113

Table 4.3.9 ZH-supplementation effects and effect sizes for carcass pH values measured at different times post-mortem (hours)

Time post-mortem (hrs)	Sig.	η^2
0.75	0.42	0.006
3	0.72	0.001
6	0.47	0.005
12	0.62	0.002
24	0.65	0.002

Adjustment for multiple comparisons: Bonferroni.

It can therefore be concluded that ZH-supplementation had no significant effect on carcass pHs at any of the post-mortem times.

Treatment effect comparisons

No significant between-subject treatment effects were observed on any individual times post-mortem ($P > 0.05$) for carcass pH.

As indicated by **Figure 4.3.7**, carcass pH was expected to decrease over time due to the negative, significant ($P < 0.001$) relationship with time post-mortem. This is supported by **Figure 4.3.8** and **Table 4.3.8**. Following our previous

assumption that ZH-supplemented carcasses would have lower pH values than the control group carcasses, we can now expect CT carcasses to have numerically higher pH values than both GF and ZM, because GF and ZM form the broad ZH-supplementation group.

Figure 4.3.9 Mean carcass pH values over 24hrs post-mortem for the three different experimental groups

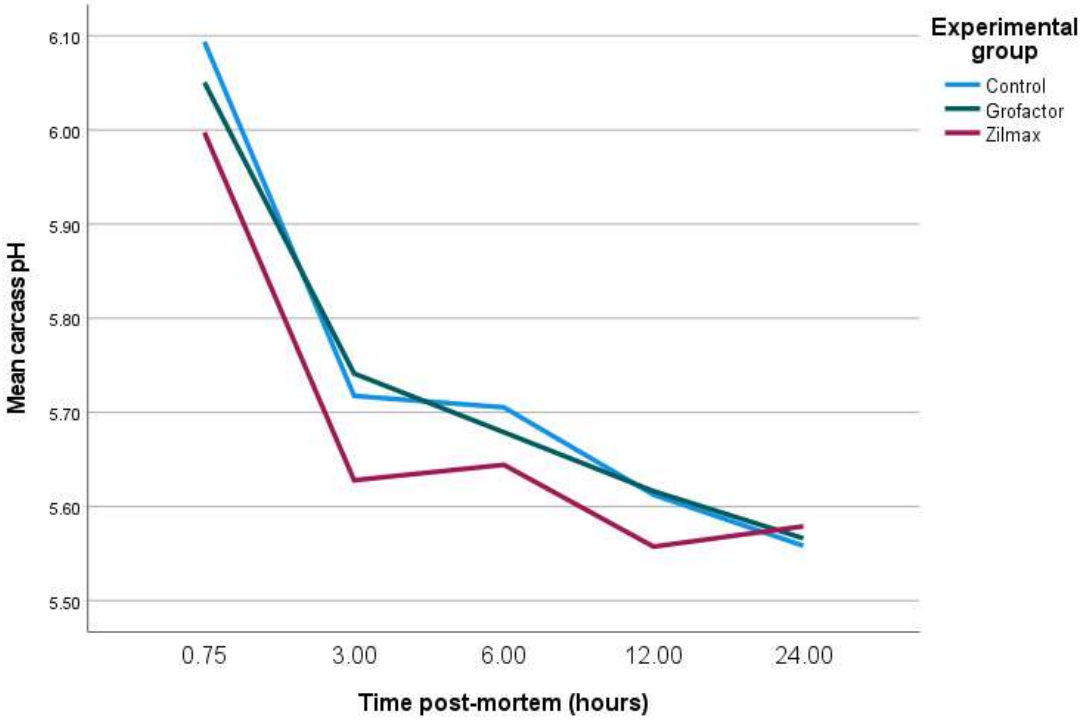


Table 4.3.10 Observed mean carcass pH values over 24hrs post-mortem for the three different experimental groups

Time post-mortem (hrs)	Experimental group	Mean carcass pH value	Std. Deviation	N
0.75	Control	6.1	0.46	37
	Grofactor®	6.1	0.48	38
	Zilmax®	6.0	0.37	39
	Total	6.1	0.43	114
3	Control	5.7	0.45	37
	Grofactor®	5.7	0.53	38
	Zilmax®	5.6	0.41	39
	Total	5.7	0.47	114

6	Control	5.7	0.28	37
	Grofactor®	5.7	0.34	38
	Zilmax®	5.6	0.28	39
	Total	5.7	0.30	114
12	Control	5.6	0.28	37
	Grofactor®	5.6	0.28	38
	Zilmax®	5.6	0.23	39
	Total	5.6	0.26	114
24	Control	5.6	0.16	37
	Grofactor®	5.6	0.16	38
	Zilmax®	5.6	0.16	39
	Total	5.6	0.16	114

To investigate whether the numerical differences amongst experimental groups might have been statistically significant, Bonferroni adjustments were performed to accurately analyse the GLM.

The small numerical differences among experimental group carcass pHs were insignificant (P = 1.00) when evaluated at 0.75, 6 and 24hrs post-mortem. At 3 and 12hrs post-mortem only the comparison between GF and ZH did not yield a P-value of 1.00 but rather 0.87 and 0.98, respectively. Indicating small differences between the two ZH-based products at those specific times. Nonetheless, no significant differences existed among the different experimental groups for all the post-mortem times.

This finding is similar to the results obtained with both carcass temperature as well as live mass evaluations (that experimental groups did not differ from each other within the same time period). Therefore, post-mortem time should be investigated as a possible factor which influences the carcass pH.

Post-mortem time (PMt) effect

To analyse the possible effect which the ZH-supplementation period might have had on carcass pH values, PMt was analyzed as one of two fixed factors in two univariate GLMs. The other fixed factor the ZH-supplementation effect. Another univariate GLM was not performed for the experimental groups, since it was well

established that no carcass pH differences were present among the two ZH-based products (P > 0.05) on any post-mortem time.

Table 4.3.11 Between-subject overall effects on carcass pHs with both post-mortem time (PMt; hours) and ZH-supplementation (ZH) as fixed factors

Fixed factors	Sig.	η2
ZH	0.30	0.002
PMt	<0.001	0.19
ZH x time interaction	0.94	0.001

PMt had significant (P < 0.001) overall effects on the carcass pHs. Whereas carcass temperatures were affected by PMt with a large effect size (η^2 = 0.95), carcass pHs had a moderate effect size (η^2 = 0.19). Indicating that the post-mortem period had a greater (significant) effect on the carcass pH decline instead of ZH-supplementation.

Table 4.3.12 Pooled mean carcass pH values for the experimental groups at different post-mortem time periods (hours)

Time post-mortem (hours)	Pooled mean pH	Std. Error
0.75	6.1	0.03
3.00	5.7	0.03
6.00	5.7	0.03
12.00	5.6	0.03
24.00	5.6	0.03
Total	5.7	0.02

To evaluate the PMt effect on carcass pHs the pooled mean pH values (**Table 4.3.12**) of the different time periods were analysed in a pairwise comparison. Because the carcass pHs were pooled, ZH-supplementation was not tested and only the effects of PMt were obtained.

Unlike carcass temperatures, not all time periods differed significantly from each other for pooled carcass pHs. Initial pHs differed significantly ($P < 0.001$) from all subsequent PMts, indicating that time post-mortem for any period would significantly decrease the carcass pHs. However, all subsequent PMts did not differ significantly ($P > 0.05$) from each other. Only pooled carcass pH measured at 3hrs post-mortem had a tendency ($P = 0.06$) to differ from the final carcass pHs, measured at 24hrs post-mortem. This can be illustrated by **Figure 4.3.8** through the stark difference (or incline) from carcass pHs at 0.75hrs post-mortem compared to the other times. While only looking at post-mortem times from 3hrs post-mortem onwards, no “severe” differences can be observed for the carcass pHs.

But when executing pairwise comparisons for the different PMt within a specific ZH-supplementation group (OG and TG), all values differed significantly from 0.75hrs post-mortem ($P < 0.001$), but not amongst the subsequent times. Reiterating that the ZH-supplementation had no effect on carcass pH decreases.

When executing pairwise comparisons (not pooled) for carcass pH between the ZH-supplemented group and CT within a specific post-mortem time, no significant differences were obtained. But as the post-mortem time elapsed, the insignificant ZH-supplementation effect decreased even further (seen by increasing P-values). Indicating that changes in pH are now due to inherent physiological post-mortem changes (e.g., OC and oxidative-reduction reactions).

Comparing the different experimental group carcass pHs within a specific post-mortem time indicated no significant differences among the different groups. ZM did however (insignificantly) have a marginally lower pH at 0.75- and 3hrs post-mortem than CT ($P = 0.67$ and 0.77, respectively). No differences were present between ZM and GF treatments ($P = 1.00$) for majority of the post-mortem times. Only 3hrs post-mortem indicated that ZM carcass pHs was insignificantly lower than GF with $P = 0.45$.

In conclusion, ZH-supplementation had no significant effects on carcass pHs. PMt did have a significant overall effect on carcass pHs. Increasing the PMt, decreased the already insignificant effect which ZH-supplementation might have had on carcass pHs.

4.4 Meat quality characteristics of feedlot bulls as influenced by dietary supplementation of zilpaterol hydrochloride either as Zilmax® or Grofactor®

4.4.1 Meat colour attributes

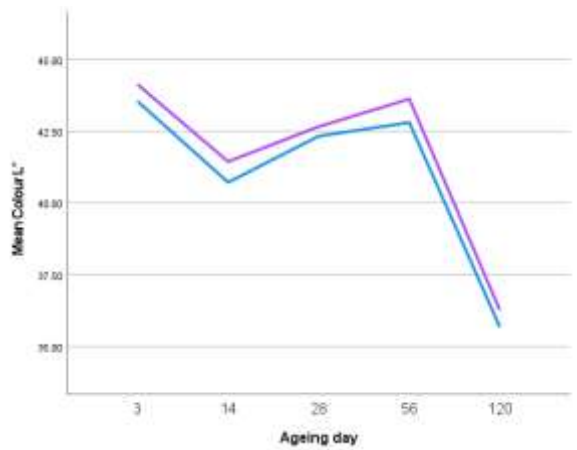
The three CIE colour attribute values (L*, a* and b*) were obtained to evaluate the meat colour lightness (L*), chroma ($(a^2 + b^2)^{\frac{1}{2}}$) and hue angle ($\tan^{-1}(\frac{b}{a})$). The figures illustrating the observed mean values for the meat colour attributes excluded day 7 as only half of the meat samples were measured that day, but statistical analyses included the day 7 values as adjustments were made for the uneven number of observations by employing Bonferoni's multiple comparisons methodology. Additionally, all three meat colour attribute figures indicating the same groups were placed together on the same page, as to ease formatting.

Overall ZH-supplementation effect

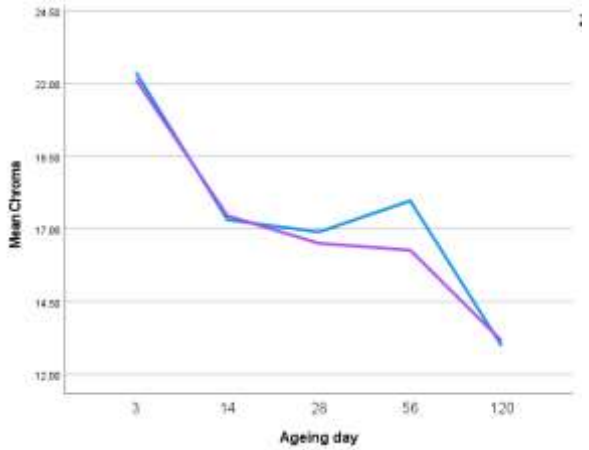
Both control and ZH-supplemented groups had comparable colour attribute values (difference did not exceed 1 unit). No large numerical differences were observed between ZH and CT meat colour lightness (L*) throughout the entire ageing period (see **Figure 4.4.1**). The meat sample chroma values were numerically similar for all ageing days, except day 56 where ZH was numerically lower than CT, but not statistically significant. Hue angles for both ZH and CT were similar on ageing days 7, 28 and 120 with numerical differences on days 14 and 56.

Performing a GLM confirmed that no significant between-subject ZH-supplementation effects were present for any of the colour attributes for the combined ageing period (P = 0.64 with η^2 = 0.31). This insignificant ZH-supplementation effect was then evaluated for individually measured ageing days.

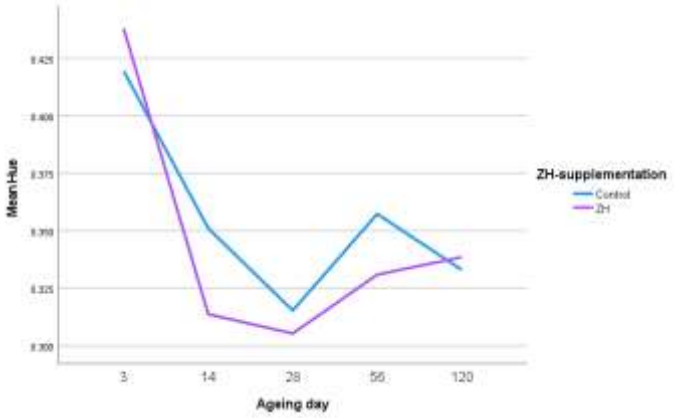
Figure 4.4.1a, b & c Mean L* (a), chroma (b) and hue angle (c) colour attribute values of meat samples over 120 days of ageing for the control and ZH-supplemented treatment groups



(a)



(b)



(c)

To investigate the possible ZH-supplementation effects on individually measured ageing days, Bonferroni's multiple comparisons were performed (**Table 4.4.1**).

Table 4.4.1 ZH-supplementation effects and effect sizes for the three meat colour attributes measured over 120 days of ageing

Meat colour attribute	Ageing day	Sig.	η^2
Lightness (L*)	3	0.90	0.000
	7	0.55	0.007
	14	0.89	< 0.001
	28	0.68	0.003
	56	0.72	0.002
	120	0.71	0.003
Chroma	3	0.81	0.001
	7	0.78	0.002
	14	0.42	0.01
	28	0.95	< 0.001
	56	0.20	0.03
	120	0.87	0.001
Hue angle (°)	3	0.68	0.003
	7	0.54	0.007
	14	0.47	0.01
	28	0.81	0.001
	56	0.53	0.008
	120	0.50	0.009

Adjustment for multiple comparisons: Bonferroni.

From **Table 4.4.1** it is evident that ZH-supplementation had no significant effect on any meat colour attribute on any of the individual ageing days ($P > 0.05$). This supports the conclusion drawn by Montgomery, *et al.* (2009), Hilton, *et al.* (2009) and Avendaño-Reyes, *et al*. (2016) that ZH does not affect meat colour. However, these results contradict reports from Avendaño-Reyes, *et al*. (2006) that ZH-supplementation will yield paler (increased L*) meat.

Treatment effect comparisons

The observed mean lightness values for each experimental group are illustrated in **Figure 4.4.2** for each individual ageing day. To investigate whether the numerical differences amongst experimental groups differed significantly on each individual ageing day, Bonferroni's multiple range test was performed to accurately analyse the GLM in pairwise comparisons.

No significant differences in colour lightness were observed among the three experimental groups for any individual ageing day (P > 0.05). Only colour lightness measured on day 28 had small insignificant differences among the experimental groups. Insignificantly, the day 28 colour lightness for GF was marginally lighter than CT (42.8 vs. 41.3; P = 0.83) with GF having a greater lightness difference from ZM (42.8 vs. 40.9; P = 0.43). ZM colour lightness did however not differ from CT (40.9 vs. 41.3; P = 1.00).

No significant differences in colour chroma values were observed among the three experimental groups for any individual ageing day (P > 0.05). No differences were observed in colour chroma values among the experimental groups for ageing days 3, 7 and 120 (P = 1.00). On day 14 no differences were detected between GF and ZM saturation (17.3 vs. 16.5; P = 1.00), but GF was insignificantly more saturated than CT (17.3 vs. 16.3; P = 0.76), whilst ZM did not differ from CT (16.5 vs. 16.3; P = 1.00). On day 28 no differences were present between CT saturation when compared to either GF or ZM (P = 1.00), but GF was insignificantly more saturated than ZM (15.8 vs. 15.0; P = 0.96). On day 56 no differences were present between GF and ZM saturation (15.8 for both; P = 1.00), but both GF and ZM were insignificantly more saturated than CT (15.8 vs. 16.6; P = 0.81).

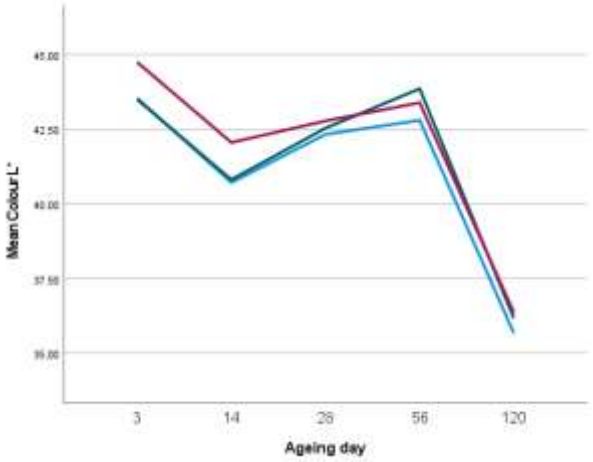
No significant differences in colour hue angles were observed among the three experimental groups for any individual ageing day (P > 0.05). No differences

were observed in colour hue angles among the experimental groups for ageing days 3, 14 and 120 (P = 1.00). The ZM day 7 colour hue angle did not differ from CT (0.2 for both; P = 1.00). The difference between day 7 hue angles for GF and CT was marginally lower than the difference in hue angles for GF and ZM (P = 0.75 vs. 0.62) i.e., GF was insignificantly more yellow than ZM and CT.

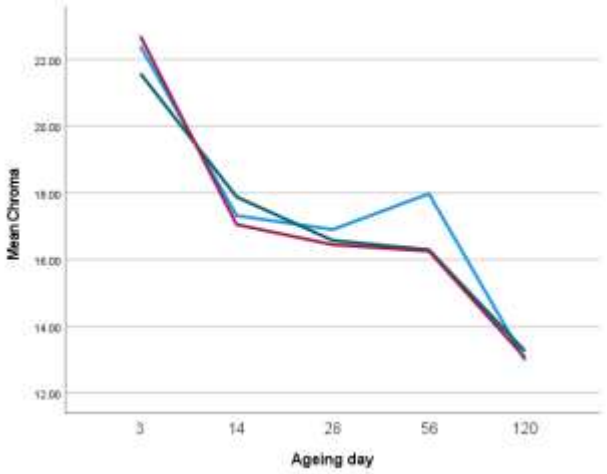
On day 28 neither GF nor ZM hue angles differed from CT (P = 1.00), but GF was insignificantly more yellow than ZM (0.3 vs. 0.2; P = 0.59). GF colour hue angles on day 56 did not differ from CT (0.3 for both; P = 1.00). The difference between day 56 hues for ZM and CT was marginally lower than the difference in hue for ZM and GF (P = 0.9 vs. 0.8) i.e., ZM was insignificantly marginally more yellow than GF than CT.

In conclusion, ZH-supplementation had no significant effect on any colour attribute on any of the individual ageing days. The two ZH-based products did not significantly differ from each other or CT in terms of colour attributes.

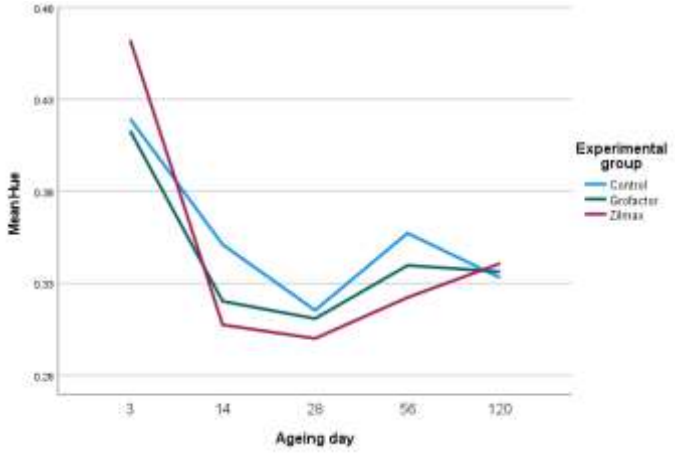
Figure 4.4.2a, b & c Mean colour L* (a), chroma (b) and hue angle (c) colour attribute values of meat samples over 120 days of ageing for the three different experimental groups



(a)



(b)



(c)

Ageing effects

Each colour attribute was analyzed as the dependent variable in a regression model with ageing day as independent variable. These relationships are plotted against observed data in **Figure 4.4.3.**

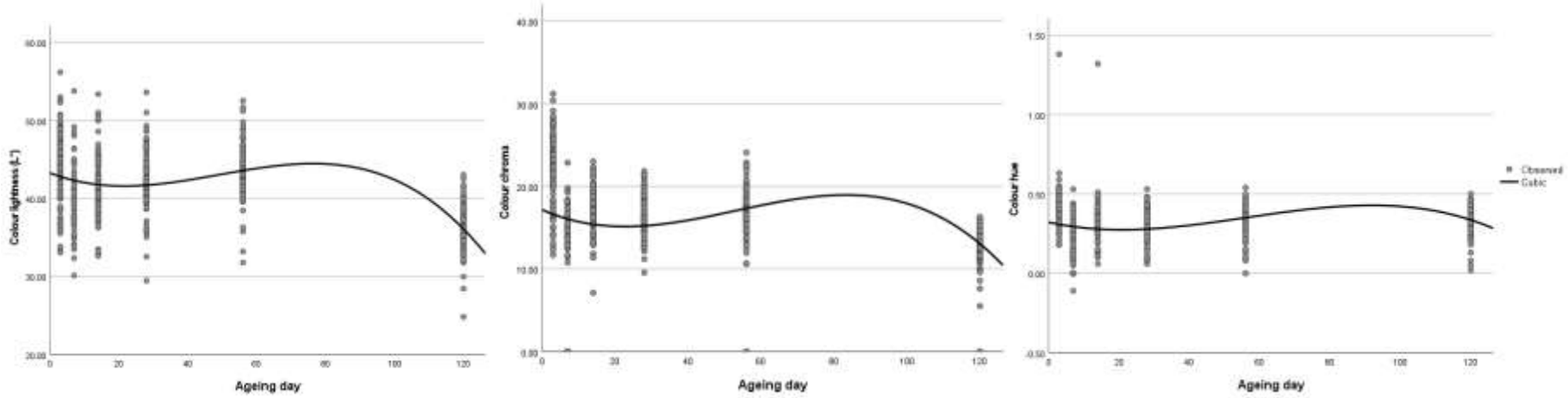
When analyzing colour lightness as the dependent variable in a regression model with ageing day as independent variable, the relationship between these two variables was significant with $P < 0.001$. Colour lightness correlation ($R = 0.55$) with ageing day. Based on this regression model, 30% of the variation in colour lightness was due to the ageing effect. The regression equation for this cubic relationship was $y = -0.17x + 0.005\,x^2 - 3.49\text{x}10^{-5}\,x^3 + 43.27$.

When analyzing colour chroma (saturation) as the dependent variable in a regression model with ageing day as independent variable, the relationship between these two variables was significant with $P < 0.001$. Colour hue had a correlation of 0.22 with ageing day. Based on this regression model, only 5% of the variation in colour hue was due to the ageing effect. The regression equation for this cubic relationship was $y = -0.20x + 0.005\,x^2 - 3.44\text{x}10^{-5}\,x^3$.

When analyzing colour hue angle as the dependent variable in a regression model with ageing day as independent variable, the relationship between these two variables was significant with $P < 0.001$. Colour hue angle had a correlation of 0.18 with ageing day. Based on this regression model, only 3% of the variation in colour hue angle was due to the ageing effect. The regression equation for this cubic relationship was $y = -0.005x + x^2 - 8.59\text{x}10^{-7}\,x^3 + 0.32$.

Ageing the meat samples up to 56 days did show an overall slight increasing trend for the colour attributes when compared to day 7 values, on different ageing days. After 56 days of ageing all the colour attributes tended to decrease in values for day 120. The significance of these increases and decrease were investigated.

Figure 4.4.3a, b & c Colour lightness (a), chroma (b) and hue angle (c) in relationship with ageing day



(a) Cubic fit line formula:
$y = -0.17x + 0.005x^2 - 3.49\text{x}10^{-5}x^3 + 43.27$

y = colour lightness (L*).
x = ageing day.

(b) Cubic fit line formula:
$y = -0.20x + 0.005x^2 - 3.44\text{x}10^{-5}x^3$

y = colour chroma.
x = ageing day.

(c) Cubic fit line formula:
$y = -0.005x + x^2 - 8.59\text{x}10^{-7}x^3 + 0.32$

y = colour hue angle.
x = ageing day.

Both ZH and CT meat colour lightness (L*) decreased from day 3 to 14, then increased from day 14 to day 56, only to have a sharp numerical decrease to day 120 (see **Figure 4.4.1**). The chroma values decreased from day 3 to 28, then increased on day 56, only to have yet another sharp numerical decrease to day 120. Hue angles for both ZH and CT sharp numerical decreased from day 3 to day 7, then increased and remained constant, with ZH meat samples having a lower hue angle than CT.

To analyse the possible effect which the ageing period might have had on each individual colour attribute, ageing day was analyzed as one of two fixed factors in a univariate GLM. The other fixed factor was ZH-supplementation. Another univariate GLM was not performed for the experimental groups, since it was well established that no difference was present among the two ZH-based products ($P > 0.05$) on any ageing day for any of the three colour attributes.

To statistically evaluate the ageing effect on each colour attribute, the pooled mean values of the different ageing days were analysed in pairwise comparisons. Bonferroni's multiple range test was performed to compensate for the uneven number of observations between the experimental groups. Because the values are pooled, ZH-supplementation cannot be tested and only the storage effects would be obtained.

Colour lightness (L)*

Ageing the meat samples had a significant effect on colour lightness ($P < 0.001$) with a large effect size of $\eta^2 = 0.32$. It follows that the interaction between ageing day and ZH-supplementation was insignificant ($P = 1.00$) with no effect size ($\eta^2 = 0.00$).

Table 4.4.2 Pooled mean colour lightness (L*) values for the three different experimental groups measured over 120 days of ageing

Ageing day	Mean pooled colour L*	Std. Error
3	43.8	0.38
7	39.7	0.55
14	41.1	0.38
28	42.5	0.38
56	43.2	0.38
120	36.0	0.38

The pooled mean lightness values significantly decreased from day 3 to 7 so that the pooled mean lightness on day 7 was significantly lower than day 3 (43.8 vs. 39.7; $P < 0.001$).

The pooled lightness values increased marginally from day 7 to 14 so that the pooled mean lightness on day 14 was only numerically lighter than day 7 (41.08 vs. 39.67; $P = 0.55$), yet still significantly darker than day 3 (41.1 vs. 43.8; $P < 0.001$).

The pooled lightness values increased marginally from day 14 to 28 so that the pooled mean lightness on day 28 was lighter than day 14 (42.5 vs. 41.1; $P = 0.13$). Because the increase in pooled lightness from day 14 to 28 was only slight, day 28 was still significantly lighter than day 7 (42.5 vs. 39.7; $P < 0.001$) but on the other hand, the increase was to such extent that day 28 pooled lightness now resembled that of day 3 (42.5 vs. 43.8; $P = 0.19$).

Similarly, the pooled mean lightness increased marginally from day 28 to 56 so that the day 56 pooled lightness value now resembled that of both day 3 and 28 (42.5 vs. 43.2 vs. 43.8; $P = 1.00$). The day 56 pooled lightness value was still significantly higher than both days 7 and 14 (43.2 vs. 39.7 vs. 41.1; $P < 0.001$).

The decrease in lightness from day 56 to 120 was significant (43.2 vs. 36.0; $P < 0.001$) and the subsequent lightness for day 120 was significantly lower than all the previous pooled lightness values ($P < 0.001$).

When comparing the specific lightness values for each individual ageing day, within either ZH or CT, ageing time had significant effects on meat sample lightness for both groups ($P < 0.001$). Even though the lightness means did not differ between CT and ZH, implementing ageing had a greater numerical increasing effect on ZH-supplemented meat samples' lightness values than those of control samples ($\eta^2 = 0.26$ vs. 0.15).

Colour chroma (saturation)

Ageing the meat samples had a significant effect on colour chroma values ($P < 0.001$) with a large effect size of $\eta^2 = 0.50$. It follows that the interaction between ageing day and ZH-supplementation was insignificant ($P = 0.49$) with a negligible effect size ($\eta^2 = 0.007$).

Table 4.4.3 Pooled mean colour chroma values for the three different experimental groups measured over 120 days of ageing

Ageing day	Mean pooled colour chroma	Std. Error
3	22.3	0.43
7	7.4	0.43
14	17.4	0.43
28	16.7	0.43
56	17.1	0.43
120	13.1	0.43

In **Figure 4.4.2** an overall decreasing trend can be seen for chroma over the ageing period (when day 7 is excluded).

The pooled mean chroma values severely decreased from day 3 to 7 so that the pooled mean chroma on day 7 was significantly lower than day 3 (7.4 vs. 22.3; P

< 0.001). The day 7 pooled mean chroma was significantly lower than all other ageing day pooled mean chromas (P < 0.001). In contrast, the day 3 pooled mean chroma remained significantly higher (more saturated) than all subsequent ageing days pooled mean chroma (P < 0.001). And following discussions will thus only focus on comparing days 14 to 120 with each other.

The pooled chroma values increased just as sharply from day 7 to 14 so that the pooled mean chroma on day 14 was significantly higher than day 7 (17.4 vs. 7.4; P < 0.001).

The pooled chroma values decreased marginally from day 14 to 28 so that the pooled mean saturation on day 14 was higher than day 28 (17.4 vs. 16.7; P = 0.13). Similarly, the pooled mean chroma only increased marginally from day 28 to 56 so that the day 56 saturation now resembled that of both day 14 and 28 (17.1 vs. 17.4 and 16.7; P = 1.00).

The decrease in chroma from day 56 to 120 was significant (17.1 vs.13.1; P < 0.001) and the subsequent saturation for day 120 was significantly lower than all the previous pooled chroma values (P < 0.001) – except day 7, where day 120 remained significantly more saturated than day 7 pooled mean (P < 0.001).

When comparing the specific chroma values for each individual ageing day, within either ZH or CT, ageing time had significant effects for both groups (P < 0.001). Even though the chroma means did not differ between CT and ZH, implementing ageing had a greater numerical decreasing effect on ZH-supplemented meat samples' chroma values than those of control samples (η^2 = 0.41 vs. 0.29).

Colour hue angle

Ageing the meat samples had a significant effect on colour hue angles (P < 0.001) with a large effect size of η^2 = 0.40. It follows that the interaction between

ageing day and ZH-supplementation was insignificant (P = 0.39) with a negligible effect size (η^2 = 0.008).

Table 4.4.4 Pooled mean colour hue angles (°) for the three different experimental groups measured over 120 days of ageing

Ageing day	Mean pooled hue angles (°)	Std. Error
3	0.4	0.01
7	0.1	0.01
14	0.3	0.01
28	0.3	0.01
56	0.3	0.01
120	0.3	0.01

The pooled mean hue angle significantly decreased from day 3 to 7 so that the pooled mean hue angle on day 7 was statistically significantly lower (redder) than day 3 (0.1 vs. 0.4°; P < 0.001). The day 7 pooled mean hue angle was significantly lower than all other ageing day pooled mean hue angles (P < 0.001). In contrast, the day 3 pooled mean hue angle remained significantly higher (more yellow) than all subsequent ageing days' pooled mean hue angles (P < 0.001).

The pooled hue angle increased significantly (become more yellow) from day 7 to 14 and remained the same for all subsequent ageing days (0.1 vs. 0.3°; P < 0.001).

Even though the hue angle means did not significantly differ between CT and ZH, sample ageing had a greater decreasing effect on ZH-supplemented meat samples' hue angles than those of CT samples (η^2 = 0.33 vs. 0.21) i.e., ZH meat samples were insignificantly redder than CT even when aged.

4.4.2 Drip losses (%)

Overall ZH-supplementation effect

Both control and ZH-supplemented treatment groups had drip loss percentages (%) close in numerical values, which observed a trend for mean drip loss to increase from day 7 to 56, where a maximum drip loss was obtained at day 56. A sharp numerical decrease in drip loss was then observed from day 56 to day 120 (**Figure 4.4.4** and **Table 4.4.5**).

Performing a GLM established that no significant between-subject ZH-supplementation effects were present for drip loss for the combined ageing period (P = 0.38 with η^2 = 0.05). This insignificant ZH-supplementation effect was then evaluated for individually measured ageing days.

Figure 4.4.4 Observed mean drip losses (%) over 120 days of ageing for the control and ZH-supplemented treatment groups

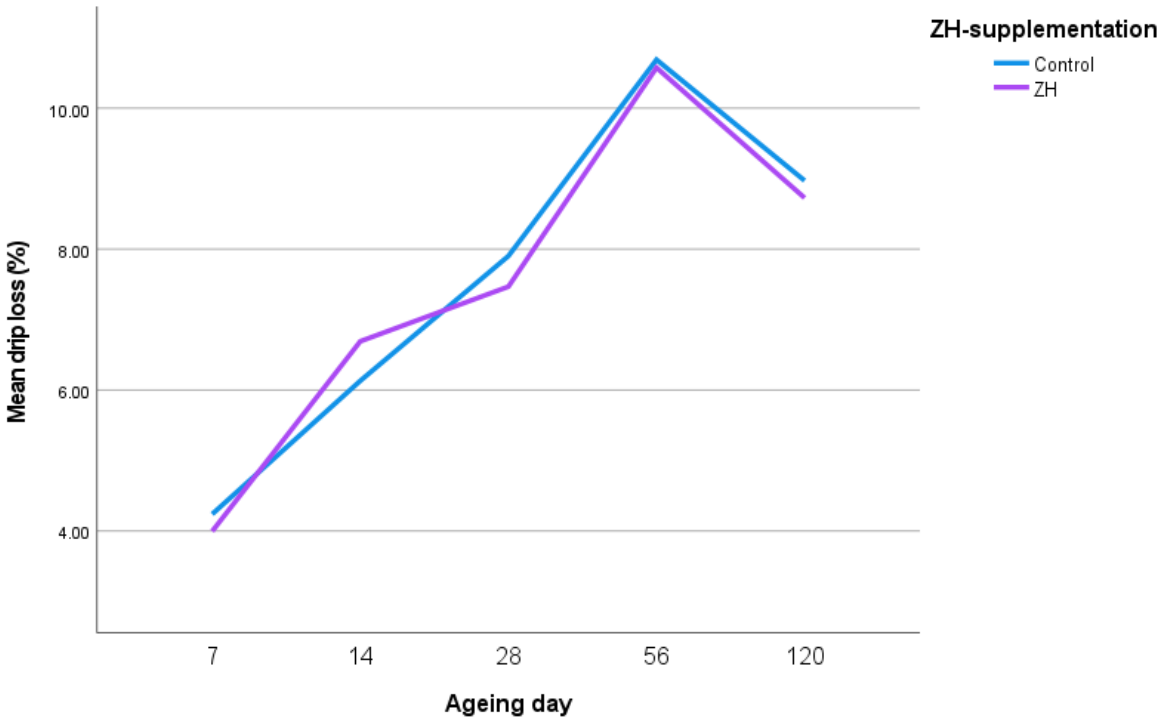


Table 4.4.5 Observed mean drip losses (%) over 120 days of ageing for the control and ZH-supplemented treatment groups

Ageing day	ZH-supplementation	Mean drip loss (%)	Std. Deviation	N
7	Control	4.2	1.44	36
	ZH	4.0	1.56	74
	Total	4.1	1.52	110
14	Control	6.1	2.59	36
	ZH	6.9	2.27	74
	Total	6.6	2.40	110
28	Control	7.9	5.87	36
	ZH	7.5	2.26	74
	Total	7.6	3.81	110
56	Control	10.7	3.14	36
	ZH	10.8	2.95	74
	Total	10.8	3.00	110
120	Control	9.2	2.27	36
	ZH	8.8	4.78	74
	Total	8.9	4.12	110

To investigate the possible ZH-supplementation effects on individually measured ageing days, Bonferroni's multiple range test was performed to obtain the following:

Table 4.4.6 ZH-supplementation effects and effect sizes for drip loss (%) measured over 120 days of ageing

Ageing day	Sig.	η^2
7	0.56	0.003
14	0.12	0.02
28	0.58	0.003
56	0.85	< 0.001
120	0.59	0.003

Adjustment for multiple comparisons: Bonferroni.

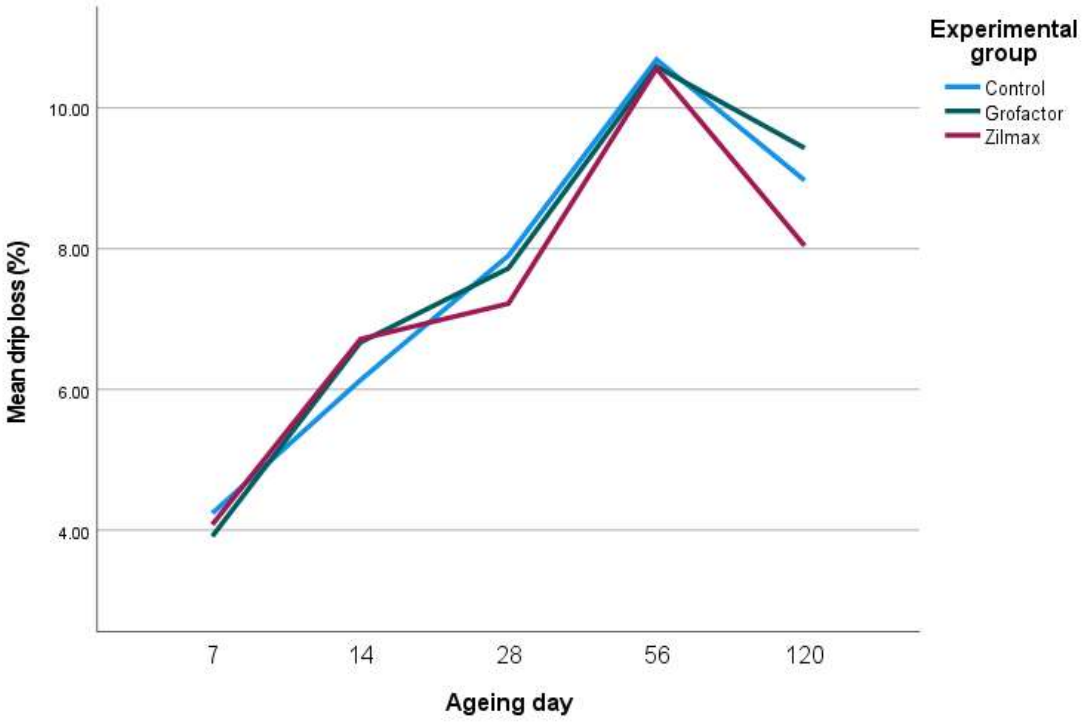
From the table above it was evident that ZH-supplementation had no significant effect on drip loss on any ageing days (P > 0.05). This agrees with results by Avendaño-Reyes *et al*. (2016). No significant trend was observed for ZH-supplementation effect on drip losses over the ageing period.

Treatment effect comparisons

Table 4.4.7 Observed mean drip losses (%) over 120 days of ageing for the three different experimental groups

Ageing day	Experimental group	Mean drip loss (%)	Std. Deviation	N
7	Control	4.2	1.44	36
	Grofactor®	4.0	1.70	37
	Zilmax®	4.1	1.44	37
	Total	4.1	1.52	110
14	Control	6.1	2.59	36
	Grofactor®	6.7	1.96	37
	Zilmax®	7.0	2.57	37
	Total	6.6	2.40	110
28	Control	7.9	5.87	36
	Grofactor®	7.7	2.38	37
	Zilmax®	7.2	2.13	37
	Total	7.6	3.81	110
56	Control	10.7	3.14	36
	Grofactor®	10.9	3.07	37
	Zilmax®	10.7	2.87	37
	Total	10.8	3.00	110
120	Control	9.2	2.27	36
	Grofactor®	9.4	2.47	37
	Zilmax®	8.2	6.28	37
	Total	8.9	4.12	110

Figure 4.4.5 Observed mean drip losses (%) over 120 days of ageing for the three different experimental groups



To investigate whether these small numerical differences amongst experimental groups were statistically significant, by employing Bonferroni's multiple range test to analyse differences between means.

The small numerical differences among experimental group drip losses were insignificant (P > 0.05). In addition to day 7 and 56 which visually did not differ for drip losses amongst groups, day 28 also did not exhibit any differences among experimental groups (P = 1.00). Although there were small differences among the experimental groups on day 14, they proved insignificant with ZM having a greater insignificant difference from CT (7.0 vs. 6.1%; P = 0.32) than the difference between GF and CT (6.7 vs. 6.1%; P = 0.83). ZM and GF did not differ for drip loss means on day 14 (7.0 vs. 6.7; P = 1.00).

The insignificant differences on day 120 were of such nature that CT and GF had no difference for drip loss (P = 1.00) while ZM had a lower drip loss when compared to GF (P = 0.61) than compared to CT (P = 0.82).

In conclusion, ZH-supplementation had no significant effect on drip losses on any of the individual ageing days. The two ZH-based products did not differ from each other for drip loss. Avendaño-Reyes *et al*. (2016) also determined that there was no difference between the two ZH-based products (P = 0.43; ZM = 87.0% and GF = 88.3%).

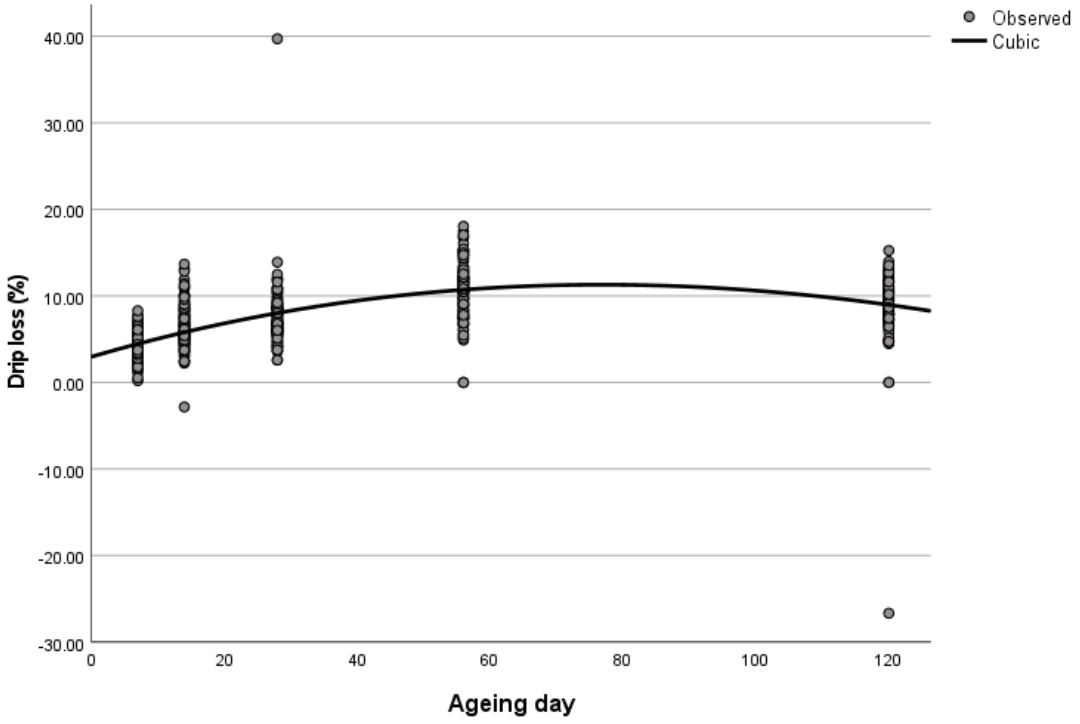
Ageing effects

When analyzing drip losses as the dependent variable in a regression model with ageing day as independent variable, the relationship between these two variables was significant with P < 0.001. Drip losses had a large correlation of 0.56 with ageing day. Based on this regression model, 31% of the variation in drip losses were due to the ageing effect. The coefficients in this quadratic relationship were 0.21 and -0.001. This relationship is plotted against observed data in **Figure 4.4.6**.

Ageing the meat samples up to 56 days did numerically increase the drip losses when compared to drip losses at day 7. After 56 days of ageing the drip losses decreased on day 120, which is favourable.

To analyse the possible effect which the ageing period may have had on drip losses, ageing day was analyzed as one of two fixed factors in a univariate GLM. The other fixed factor was ZH-supplementation. Another univariate GLM was not performed for the experimental groups, since it was well established that no drip loss difference was present among the two ZH-based products (P > 0.05) on any ageing day. These results are tabulated in **Table 4.4.7**.

Figure 4.4.6 Relationship between drip losses (%) and ageing day



Cubic fit line formula: $y = 0.21x - 0.001\,x^2 + 3.12$
y = drip loss (%).
x = ageing day.

Table 4.4.8 Between-subject overall effects on drip loss (%) with both ageing day and ZH-supplementation (ZH) as fixed factors

Fixed factors	Sig.	η2
Ageing day	<0.001	0.30
ZH	0.74	< 0.001
Ageing day x ZH interaction	0.84	0.003

Once again it was reiterated that ZH-supplementation had no significant effect on drip losses with P = 0.74 and a negligible effect size of $\eta^2 < 0.001$. It follows that the interaction between ageing day and ZH-supplementation was insignificant (P = 0.84) with an equally small effect size ($\eta^2 = 0.003$).

Ageing the meat samples had a significant effect on drip losses (P < 0.001) with a large effect size of $\eta^2 = 0.30$. To evaluate the ageing effect on drip losses, the pooled mean drip loss for the different ageing days was analysed in a pairwise

comparison. Bonferroni's multiple range test was employed to compensate for differences in the number of observations per group. Because the drip loss values were pooled, ZH-supplementation could not be tested and only the effect of ageing was determined.

Table 4.4.9 Pooled mean drip losses (%) for the three different experimental groups measured over 120 days of ageing

Ageing day	Mean pooled drip losses (%)	Std. Error
7	4.1	0.32
14	6.4	0.32
28	7.7	0.32
56	10.6	0.32
120	8.9	0.32

For almost all the ageing days, the pooled drip loss means for each individual ageing day differed significantly from each other ($P \leq 0.001$). Only the day 28 and 120 pooled drip loss means did not differ significantly from each other ($P = 0.11$). This is because the pooled drip loss mean decreased from day 56 to day 120, instead of increasing. But overall, a significant storage effect was observed.

The pooled mean drip loss for day 7 was significantly lower than all subsequent ageing days ($P < 0.001$).

The pooled mean drip loss for day 14 was significantly higher than day 7, and significantly lower than all subsequent ageing days ($P < 0.05$). As seen in **Figure 4.4.4**, the increase in drip loss from day 14 to day 28 of ageing was small ($P = 0.05$) whereas all subsequent days after day 28 had a greater significance ($P < 0.001$) in differences for drip loss from day 14.

The day 28 pooled mean drip loss was significantly higher than both day 7 and 14 drip losses ($P < 0.001$ and $P = 0.05$, respectively) and significantly lower than

the day 56 pooled mean drip loss (P < 0.001). However, because the drip losses significantly decreased (P = 0.001) from day 56 to 120, the day 120 drip loss now resembles that of day 28, thereby yielding no significant difference (P = 0.11).

The day 56 pooled mean drip loss was significantly higher than all the previous days' mean drip losses (P < 0.001), as well as additionally being significantly higher than the decreased day 120 pooled mean drip loss (P = 0.001).

Therefore, the day 120 mean drip loss was significantly higher than days 7 and 14 pooled mean drip losses and lower than day 56 (P <= 0.001). No significant difference was observed between day 120 pooled mean drip losses and that of day 28 (P = 0.11) because day 120 deviated from the trend to increase drip loss over time and decreased instead.

In conclusion, ageing the meat samples up to 56 days significantly increased in drip loss for both CT and ZH (P < 0.001). The ageing effect size on CT drip loss means was smaller than the effect size on ZH drip loss means (η^2 = 0.14 vs. 0.24). Indicating that even though the drip loss means did not differ between CT and ZH, implementing ageing had a greater effect on ZH-supplemented meat samples' drip loss than those of control samples.

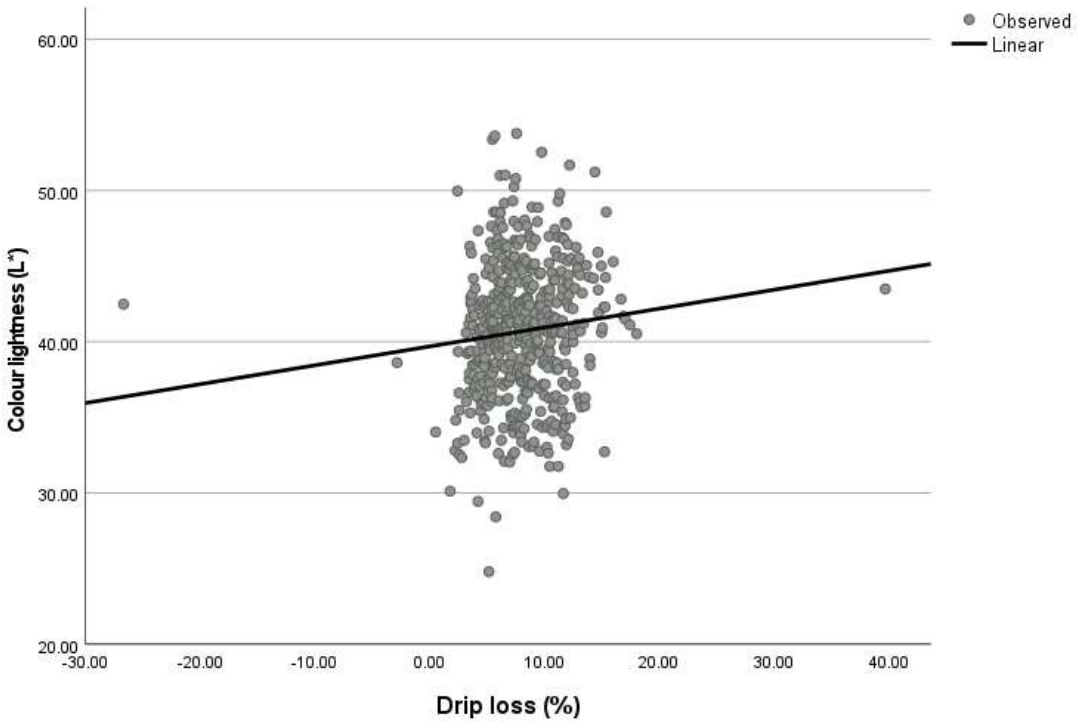
Additional effects analyzed

Because Kim *et al.* (2010) determined that lighter and less red pork had higher drip loss values, the relationship between the two variables was investigated.

When analyzing drip losses as the independent variable in a regression model with colour lightness (L*) as dependent variable, the relationship between these two variables was positive and significant with P = 0.02. Drip losses had a correlation of 0.11 with colour lightness. Based on this regression model, only 1% of the variation in colour lightness was due to the relationship with drip loss. The coefficient in this linear relationship was 0.13, meaning that for every 1%

increase in drip loss, the estimated lightness increased with 0.13%. This relationship is plotted against observed data in **Figure 4.4.7.**

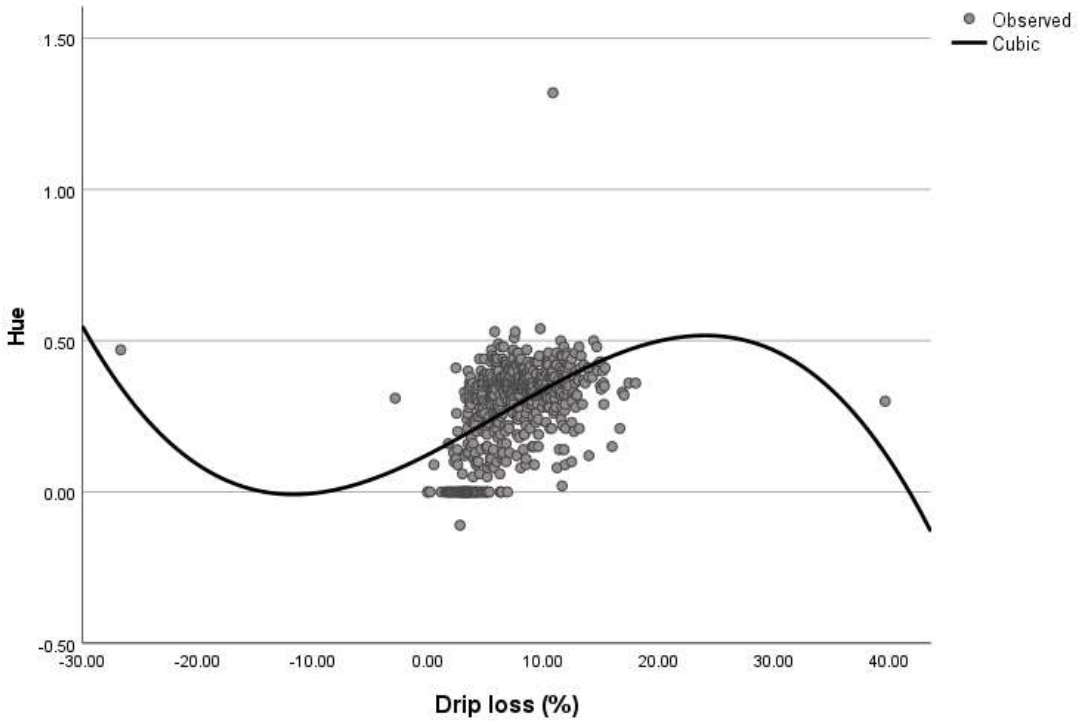
Figure 4.4.7 Relationship between colour lightness (L*) and drip loss (%)



Linear fit line formula: $y = 0.13x + 39.69$
y = colour lightness (L*).
x = drip loss (%).

When analyzing drip losses as the independent variable in a regression model with colour hue angle as dependent variable, the positive relationship between these two variables was significant with $P < 0.001$. Indicating that when drip loss increased, the hue angle significantly increased as well i.e., the meat colour became more yellow. Drip losses had a correlation of 0.29 with hue angle. Based on this regression model, 8% of the variation in hue angles were due to the relationship with drip loss. This relationship is plotted against observed data in **Figure 4.4.8**.

Figure 4.4.8 Relationship between hue angle (relative yellowness; °) and drip loss (%)



Cubic fit line formula: $y = 0.02x + x^2 - 2.3x10^{-5}x^3 + 0.12$
y = hue angle (relative yellowness).
x = drip loss (%).

The majority of the drip loss observations (excluding outliers) fell within the range of 0 – 20% drip loss. In this range a clear increasing trend was evident between the hue angle and drip loss i.e., as drip loss increased, the meat samples had a more yellow appearance.

The increases in both colour lightness and relative yellowness because of increased drip losses, are a combined result of both the muscle fibre structure being damaged (through protein degradation) which releases intracellular fluids (Lawrence & Fowler, 2002; Huff-Lonergan & Lonergan, 2005) and the chromatic pigments (mainly myoglobin) being excreted in the drip (den Hertog-Meischke, van Laack, & Smulders, 1997).

4.4.3 Cooking losses (%)

Overall ZH-supplementation effect

Both control and ZH-supplemented groups illustrated an increasing trend for cooking losses from day 7 to a maximum cooking loss at day 56. A sharp numerical decrease in cooking loss was then observed for both groups on day 120 (see **Figure 4.4.9** and **Table 4.4.10**). ZH consistently yielded lower numerical cooking loss values than CT.

Performing a GLM established that the between-subject ZH-supplementation decreasing effects on cooking losses were insignificant (P = 0.47 with η^2 = 0.04) for the combined ageing days (i.e., pooled the cooking losses for each individual ageing day together for CT and ZH).

Figure 4.4.9 Observed mean cooking losses (%) over 120 days of ageing for the control and ZH-supplemented treatment groups

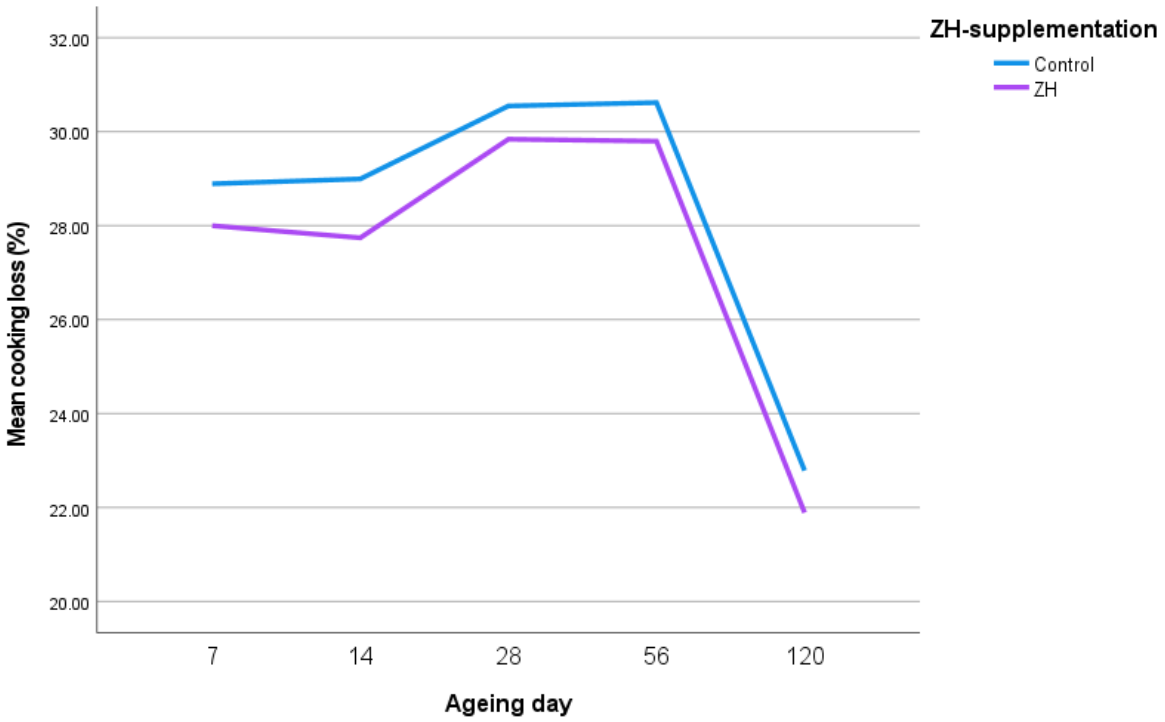


Table 4.4.10 Observed mean cooking losses (%) over 120 days of ageing for the control and ZH-supplemented treatment groups

Ageing day	ZH-supplementation	Mean cooking loss (%)	Std. Deviation	N
7	Control	28.9	3.86	36
	ZH	27.8	4.053	73
	Total	28.2	4.01	109
14	Control	28.9	2.54	36
	ZH	27.7	3.08	73
	Total	28.1	2.95	109
28	Control	30.6	3.14	36
	ZH	29.8	3.33	73
	Total	30.0	3.28	109
56	Control	30.6	3.17	36
	ZH	29.8	3.76	73
	Total	30.1	3.58	109
120	Control	22.8	2.81	36
	ZH	21.8	4.48	73
	Total	22.1	4.02	109

To investigate the possible ZH-supplementation effects on individually measured ageing days, Bonferroni multiple range test was performed (**Table 4.4.11**).

Table 4.4.11 ZH-supplementation effects and effect sizes on cooking loss (%) measured over 120 days of ageing

Ageing day	Sig.	η^2
7	0.18	0.02
14	0.05	0.04
28	0.20	0.02
56	0.24	0.01
120	0.24	0.01

Adjustment for multiple comparisons: Bonferroni.

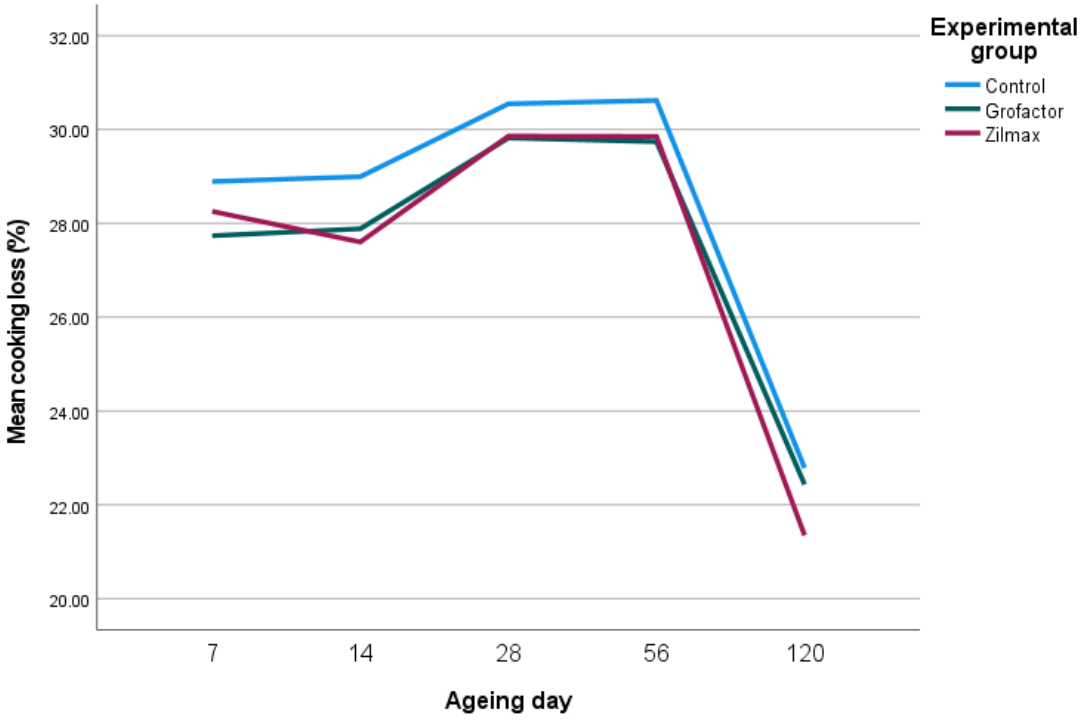
From the table above it is evident that ZH-supplementation had no significant decreasing effect on the individual ageing days for cooking losses, except on day 14 (P = 0.05) where ZH yielded significantly lower cooking losses than CT (27.7 vs. 28.9%). Leheska *et al.* (2009) and Hilton *et al*. (2009) also found no significant ZH-supplementration effects on cooking losses.

Treatment effect comparisons

Table 4.4.12 Observed mean cooking losses (%) over 120 days of ageing for the three different experimental groups

Ageing day	Experimental group	Mean cooking loss (%)	Std. Deviation	N
7	Control	28.9	3.86	36
	Grofactor®	27.6	3.49	36
	Zilmax®	28.1	4.57	37
	Total	28.2	4.01	109
14	Control	28.9	2.54	36
	Grofactor®	27.9	2.99	36
	Zilmax®	27.5	3.20	37
	Total	28.1	2.95	109
28	Control	30.6	3.14	36
	Grofactor®	29.8	3.52	36
	Zilmax®	29.7	3.18	37
	Total	30.0	3.28	109
56	Control	30.6	3.17	36
	Grofactor®	29.7	3.45	36
	Zilmax®	29.9	4.08	37
	Total	30.1	3.58	109
120	Control	22.8	2.81	36
	Grofactor®	22.4	5.10	36
	Zilmax®	21.3	3.77	37
	Total	22.1	4.02	109

Figure 4.4.10 Observed mean cooking losses (%) over 120 days of ageing for the three different experimental groups



To investigate whether these small numerical differences amongst experimental groups were statistically significant, Bonferroni's multiple range test was performed to accurately analyse the GLM.

Although there were small differences among the experimental groups' cooking losses, these differences proved insignificant ($P > 0.05$) for each individual ageing day.

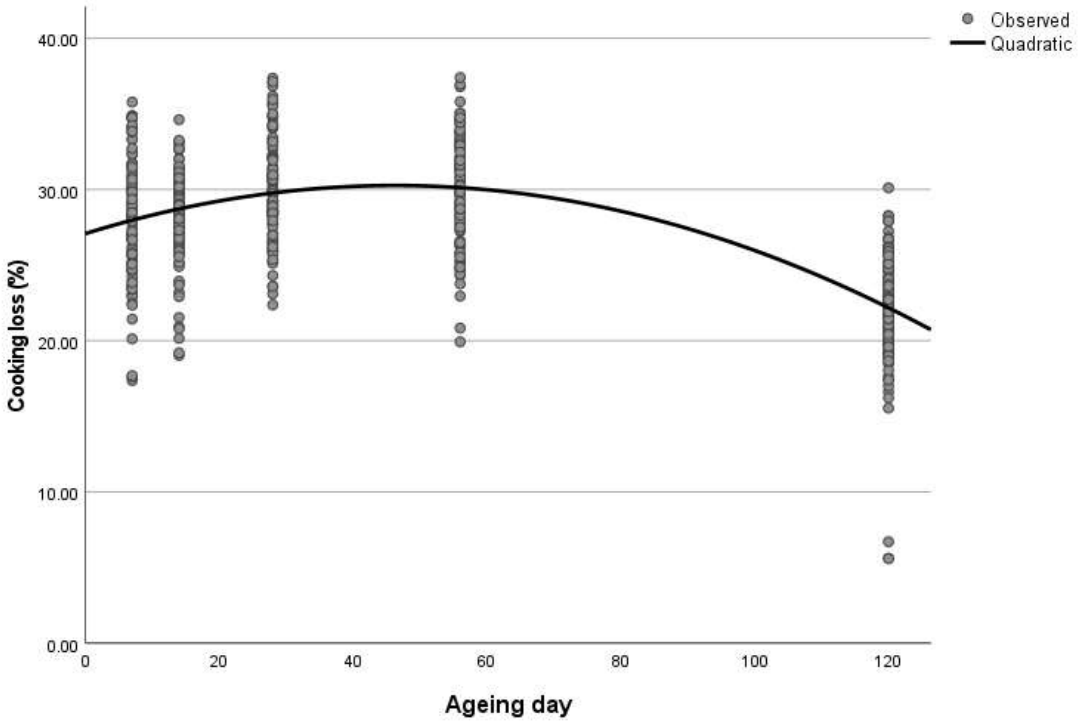
The insignificant differences on day 120 were of such nature that CT and GF had no difference for drip loss ($P = 1.00$) while ZM had a smaller drip loss when compared to CT ($P = 0.55$) than compared to GF ($P = 0.32$).

In conclusion, ZH-supplementation had no significant effect on drip losses on any of the individual ageing days. The two ZH-based products did not differ from each other ($P = 1.00$) for days 7 to 56, and only differed marginally on day 120 ($P = 0.94$).

Ageing effects

When analyzing cooking loss as the dependent variable in a regression model with ageing day as independent variable, the relationship between these two variables was significant with P < 0.001. Cooking loss had a large correlation of 0.63 with ageing day. Based on this regression model, 39% of the variation in cooking losses were due to the ageing effect. The regression equation for this quadratic relationship was $y = 0.14x - 0.001\ x^2 + 27.08$. This relationship is plotted against observed data below.

Figure 4.4.11 Relationship between cooking loss (%) and ageing day



Quadratic fit line formula: $y = 0.14x - 0.001\ x^2 + 27.08$
y = cooking losses (%).
x = ageing day.

Ageing the meat samples up to 56 days did numerically increase the cooking losses when compared to initial day 7 losses. After 56 days of ageing, the cooking losses dropped on day 120, which is favourable. The significance of these increases and decrease was investigated below.

To analyse the effect which the ageing period had on cooking loss, ageing day was analyzed as one of two fixed factors in a univariate GLM. The other fixed factor was ZH-supplementation. Another univariate GLM was not performed for the experimental groups, since it was well established that no cooking loss difference was present among the two ZH-based products ($P > 0.05$) on any ageing day, while ZH-supplementation differed significantly from CT on day 14.

Ageing the meat samples had a significant effect on cooking losses ($P < 0.001$) with a large effect size of $\eta^2 = 0.37$. It follows that the interaction between ageing day and ZH-supplementation was insignificant ($P = 0.84$) with an equally small effect size ($\eta^2 = 0.003$).

To statistically evaluate the ageing effect on cooking loss, the pooled mean cooking losses of the different ageing days were analysed in a pairwise comparison. Bonferroni's multiple range test was performed to compensate for differing group sizes. Because the cooking loss values are pooled, ZH-supplementation cannot be tested and only the ageing effects would be obtained.

Table 4.4.13 Pooled mean cooking loss (%) for the three different experimental groups measured over 120 days of ageing

Ageing day	Mean pooled cooking loss (%)	Std. Error
7	28.5	0.36
14	28.4	0.36
28	30.2	0.36
56	30.2	0.36
120	22.3	0.36

The pooled mean cooking loss decreased insignificantly from day 7 to 14 (28.5 vs. 28.4%; $P = 1.00$). But the day 7 pooled cooking loss still significantly differed

($P < 0.05$) from all other subsequent ageing days. The increase in cooking loss from day 14 to 28 was significant (28.4 vs. 30.2%; $P = 0.003$) and day 14 pooled mean cooking losses remained significantly lower than day 56 ($P = 0.003$), yet significantly higher than day 120 ($P < 0.001$).

The increase in pooled cooking losses from day 28 to 56 was insignificant (30.2 vs. 30.2; $P = 1.00$), whilst both pooled cooking losses on days 28 and 56 were significantly higher than those of days 7, 14 and 120 ($P < 0.05$).

The decrease in cooking loss from day 56 to 120 was significant (30.2 vs. 22.3; $P < 0.001$) and the pooled cooking loss was significantly lower than all the previous pooled cooking losses ($P < 0.001$).

When comparing the specific cooking losses for each individual ageing day, within either ZH or CT, ageing time had significant effects for both groups ($P < 0.001$). Even though the cooking loss means did not differ between CT and ZH, implementing ageing had a greater numerical effect on ZH-supplemented meat samples' drip loss than those of control samples ($\eta^2 = 0.31$ vs. 0.18).

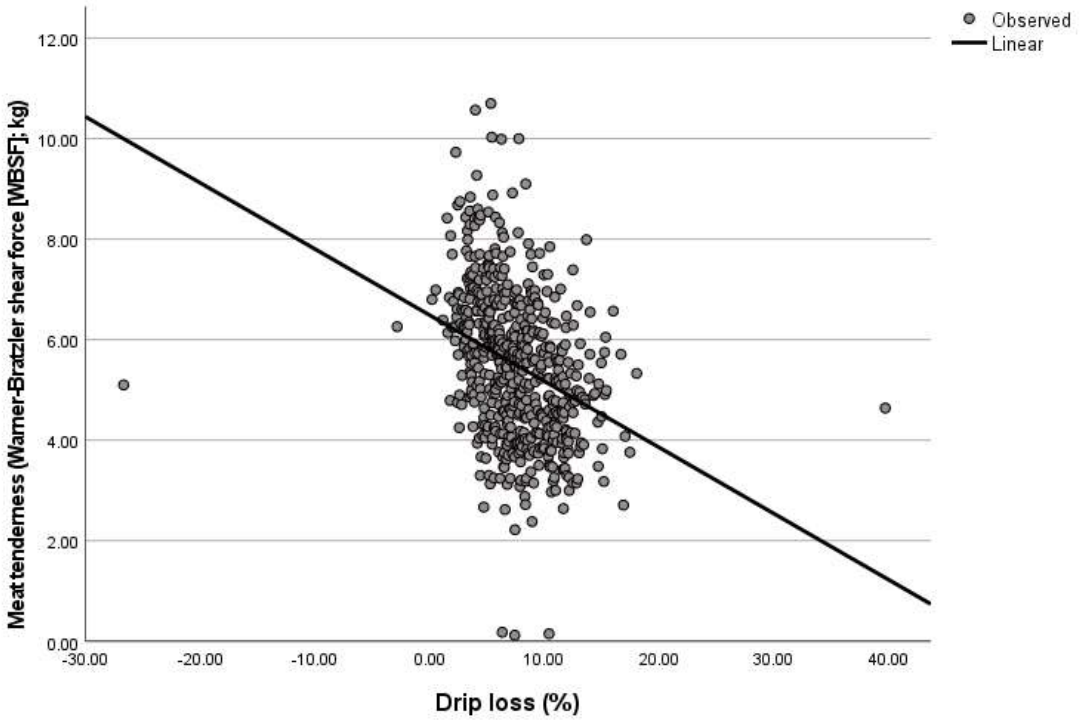
4.4.4 Meat tenderness

To ensure an accurate, instrumental and easily replicable finding with regards to meat tenderness WBSF values were obtained instead of a sensory panel, as sensory scores are the perceived tenderness by a trained panel

Because structural damages influenced drip and cooking losses, the relationships between the two WH) variables and meat tenderness were investigated.

When analyzing drip loss (%) as the independent variable in a regression model with Warner-Bratzler shear force (WBSF; kg) as dependent variable, the relationship between these two variables was significant with $P < 0.001$. WBSF had a correlation of 0.33 with drip loss. Based on this regression model, 11% of the variation in WBSF was due to the relationship with drip loss. The coefficient in this relationship was -0.13, meaning that for every 1% increase in drip loss, the estimated WBSF value decreased with 0.13kg. This relationship is plotted against observed data in **Figure 4.4.12**.

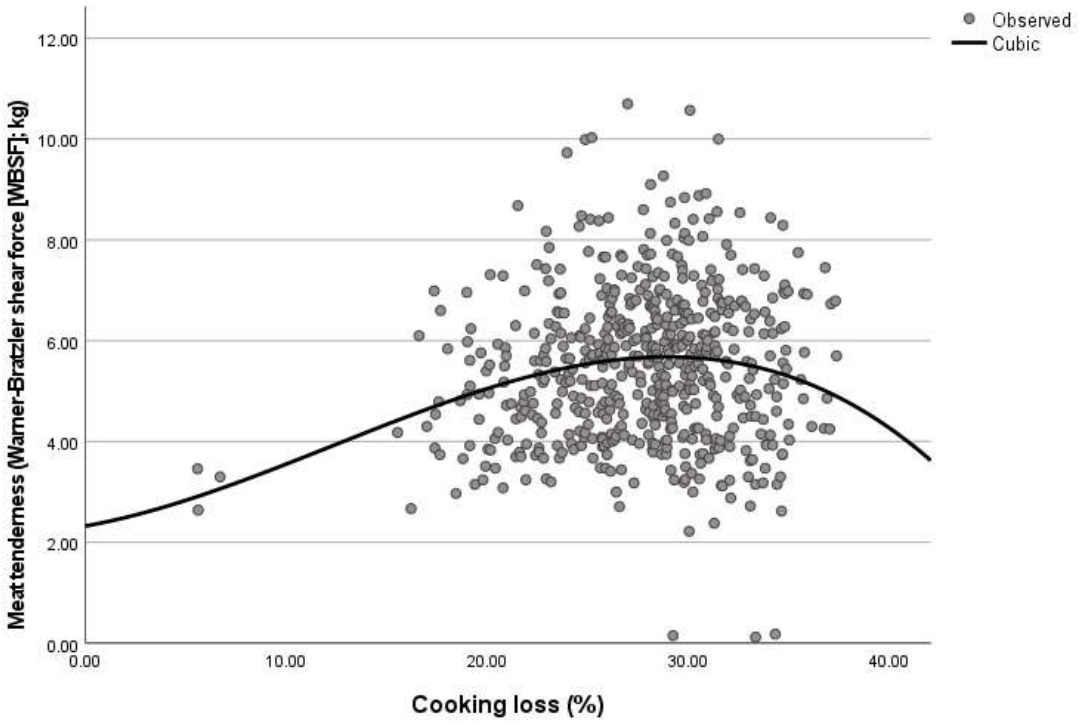
Figure 4.4.12 Relationship between meat tenderness (Warner-Bratzler shear force [WBSF]; kg) and drip loss (%)



Linear fit line formula: y = - 0.13 x + 6.49
y = WBSF (kg).
x = drip loss (%).

When analyzing cooking loss as the independent variable in a regression model with WBSF as dependent variable, the relationship between these two variables was significant with P < 0.001. WBSF had a correlation of 0.19 with cooking loss. Based on this regression model, 4% of the variation in WBSF was due to the relationship with cooking loss. This relationship is plotted against observed data in **Figure 4.4.13**.

Figure 4.4.13 Relationship between meat tenderness (Warner-Bratzler shear force [WBSF]; kg) and cooking loss (%)



Cubic fit line formula: $y = 0.07\ x + 0.007\ x^2 + x^3 + 2.32$
y = WBSF (kg).
x = cooking loss (%).

Both drip and cooking losses had significant relationships with meat tenderness ($P < 0.001$). Drip loss had a greater correlation with WBSF than cooking loss and subsequently drip loss accounted for a larger portion of the variation in WBSF than cooking loss ($R = 0.39$ vs. 0.19; $R^2 = 0.15$ vs. 0.04). The decrease in relationship between WBSF is due to external factors (such as heating) influencing the cooking loss instead of only inherent factors as with drip loss.

As seen in **Figure 4.4.12**, majority of the observations (excluding outliers) were present within the 0 – 20% drip loss rage. Within this range WBSF had a negative relationship with drip loss i.e., as drip loss increased, WBSF decreased which yielded more tender results. This is due to the protein degradations associated with drip losses (Huff-Lonergan & Lonergan, 2005).

Overall ZH-supplementation effect

Figure 4.4.14 clearly illustrates how CT mean WBSF decreased from 6.4kg on day 7, to 3.8kg on day 56, as one instance, then increased to 4.3kg on day 120. ZH had a stepwise decrease from 7.0kg on day 7, to 6.4kg on day 14, to 6.1kg on day 28, with a final stark decrease to 4.5kg on day 56, before increasing to 4.8kg on day 120. ZH-supplementation did numerically decrease meat tenderness – seen by increased WBSF values. The ZH values remained well above the critical threshold value of 4.59kg identified by Shackelford *et al*. (1991) for consumer acceptability. Performing a GLM established that this difference was due to significant between-subject ZH-supplementation effects on WBSF throughout the entire storage period ($P < 0.001$ with $\eta^2 = 0.24$).

Figure 4.4.14 Observed mean Warner-Bratzler shear force values (WBSF; kg) of meat samples over 120 days of ageing for the control and ZH-supplemented treatment groups

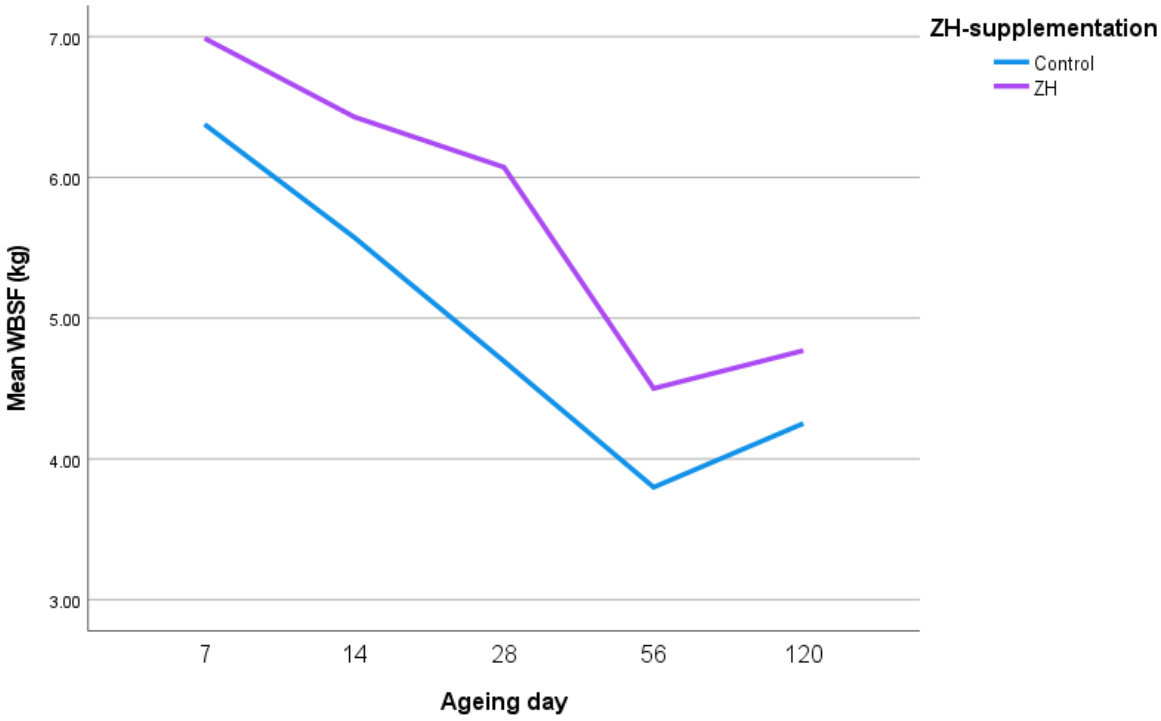


Table 4.4.14 Observed mean Warner-Bratzler shear force values (WBSF; kg) over 120 days of ageing for the control and ZH-supplemented treatment groups

Ageing day	Group	Observed mean WBSF	Std. Deviation	N
7	Control	6.4	1.17	36
	ZH	7.0	1.14	73
	Total	6.8	1.18	109
14	Control	5.6	1.43	36
	ZH	6.4	1.32	73
	Total	6.2	1.41	109
28	Control	4.7	1.11	36
	ZH	6.1	1.26	73
	Total	5.6	1.37	109
56	Control	3.8	0.77	36
	ZH	4.5	1.05	73
	Total	4.3	1.02	109
120	Control	4.3	0.95	36
	ZH	4.8	0.95	73
	Total	4.6	0.98	109

As expected, meat tenderness significantly ($P < 0.05$) decreased (increased WBSF values) with ZH-supplementation on each individual ageing day.

Table 4.4.15 ZH-supplementation effects and effect sizes on Warner-Bratzler shear force values (WBSF; kg) measured over 120 days of ageing

Ageing day	Sig.	η^2
7	0.01	0.06
14	0.002	0.08
28	<0.001	0.23
56	<0.001	0.10
120	0.01	0.06

Adjustment for multiple comparisons: Bonferroni.

Treatment effect comparisons

Now that we have established that ZH-supplementation did indeed decrease meat tenderness on each individual ageing day, we can evaluate the efficacy of the two ZH-based products in comparison with each other and the control group. Observed values (**Figure 4.4.15** and **Table 4.4.16**) indicate that ZH and GF have similar values for WBSF measured up until day 56, after which both values increase on day 120, with ZM numerically having more tender meat than GF.

Figure 4.4.15 Observed mean Warner-Bratzler shear force values (WBSF; kg) of meat samples over 120 days of ageing for the three different experimental groups

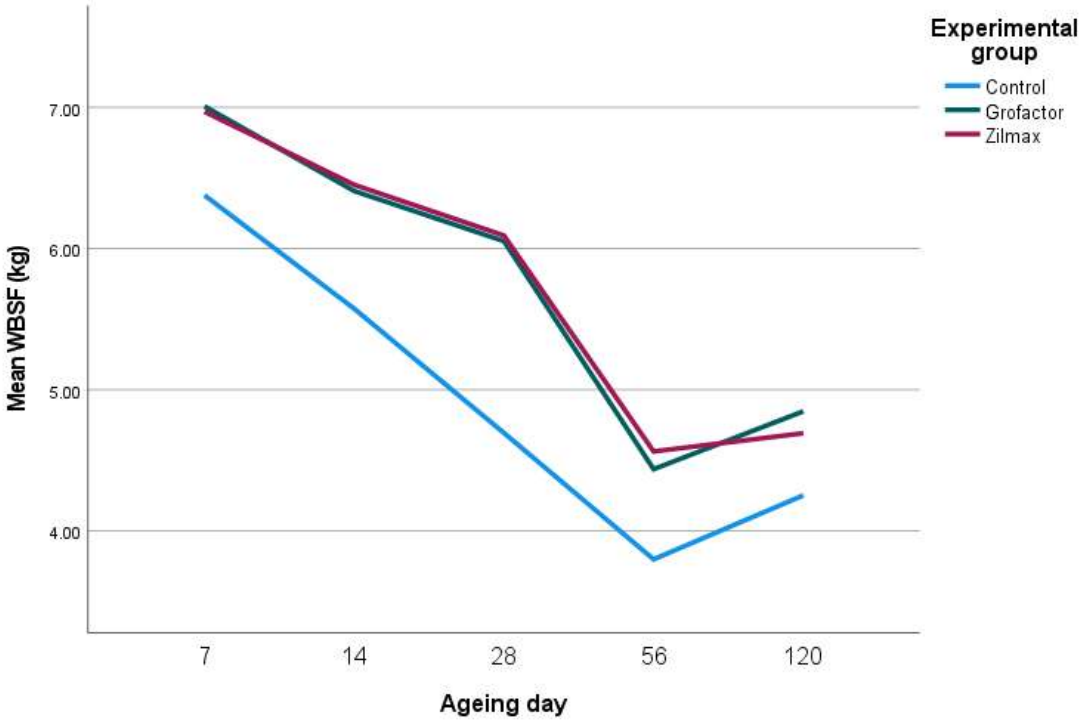


Table 4.4.16 Observed mean Warner-Bratzler shear force values (WBSF; kg) over 120 days of ageing for the three different experimental groups

Ageing day	Treatment	Mean	Std. Deviation	N
7	Control	6.4	1.17	36
	Grofactor®	7.0	1.20	37
	Zilmax®	7.0	1.09	36
	Total	6.8	1.18	109
14	Control	5.6	1.43	36
	Grofactor®	6.4	1.39	37
	Zilmax®	6.5	1.27	36
	Total	6.2	1.41	109
28	Control	4.7	1.11	36
	Grofactor®	6.0	1.51	37
	Zilmax®	6.1	0.95	36
	Total	5.6	1.37	109
56	Control	3.8	0.77	36
	Grofactor®	4.4	1.13	37
	Zilmax®	4.6	0.98	36
	Total	4.3	1.02	109
120	Control	4.3	0.95	36
	Grofactor®	4.8	1.09	37
	Zilmax®	4.7	0.79	36
	Total	4.6	0.98	109

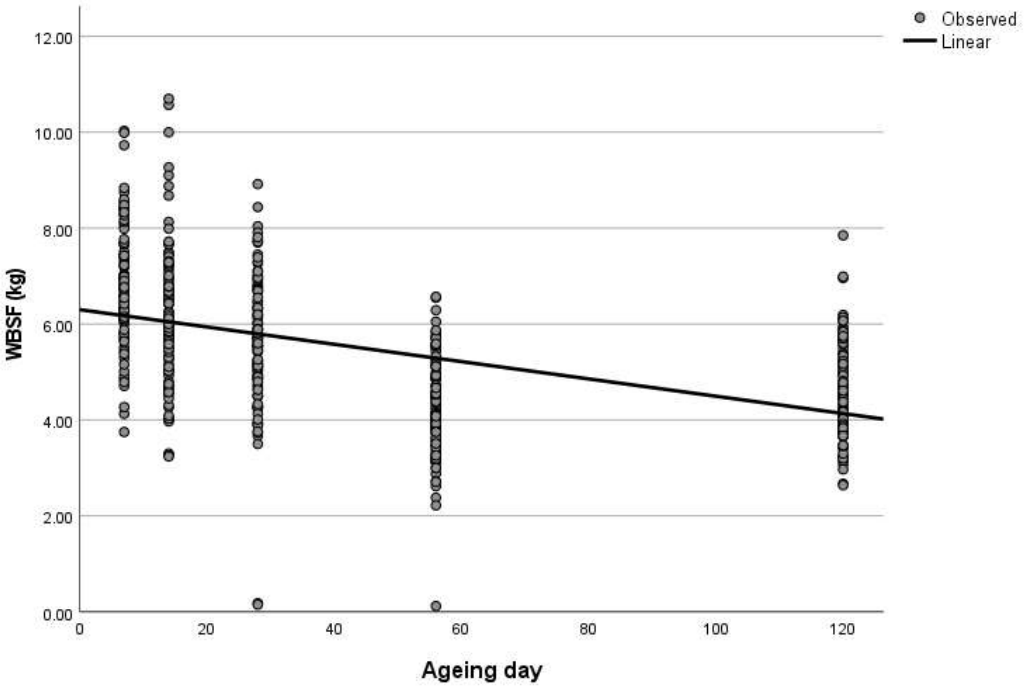
Statistical GLM analysis with Bonferroni's multiple range test was used to compare the means of different experimental groups, yielded results easily discernible in **Figure 4.4.15**. Throughout the entire ageing period ZM and GF WBSF means did not differ from each other (P = 1.00) on individual ageing days. Regardless of the numerical difference between the two ZH-based treatments and the control group, no significant difference existed between the three experimental groups on day 7. From day 14 to 56, both ZM and GF mean WBSF values were significantly less tender than CT (P < 0.05), with ZM

insignificantly yielding the least tender WBSF values. On day 120 of ageing, GF yielded the least tender WBSF values, instead of ZM, and both significantly differed from CT (P < 0.05), but not each other (P = 1.00). Avendaño-Reyes *et al.* (2016) also determined that GF and ZM WBSF values measured at both 2- and 14 days post-mortem, did not significantly differ (P > 0.05) for bulls.

Ageing effects

When analyzing meat tenderness (WBSF) as the dependent variable in a regression model with ageing day as independent variable, the relationship between these two variables was significant with P < 0.001. Meat tenderness had a large correlation of 0.49 with ageing day. Based on this regression model, 24% of the variation in WBSF values were due to the ageing effect. The coefficient in this relationship was -0.02, meaning that for every 1 day increase in ageing time, the estimated WBSF value decreased with 0.02kg.

Figure 4.4.16 Relationship between Warner-Bratzler shear force values (WBSF; kg) and ageing day



Linear fit line formula: y = -0.02x + 6.30
y = WBSF (kg).
x = ageing day.

Ageing the meat samples up to 56 days did numerically increase the meat tenderness (by decreasing WBSF) when compared to day 7 losses. After 56 days of ageing the meat tenderness dropped on day 120. The significance of these increases and decrease was investigated below.

To analyse the possible effect which the ageing period may have had on meat tenderness, ageing day was analyzed as one of two fixed factors in a univariate GLM. The other fixed factor was the overall experimental group (OG) to evaluate ZH-supplementation. Another univariate GLM was not performed for the experimental groups, since it was well established that no WBSF difference was present among the two ZH-based products (P = 1.00) on any ageing day.

Table 4.4.17 Between-subject overall effects on meat tenderness with both ageing day and ZH-supplementation (ZH) as fixed factors

Fixed factors	Sig.	η2
Ageing day	<0.001	0.38
ZH	<0.001	0.10
Ageing day x ZH interaction	0.06	0.02

Once again it was reiterated that ZH-supplementation had significant effects on meat tenderness (i.e., decreased tenderness) with $P < 0.001$ and a large effect size of $\eta^2 = 0.38$.

The interaction between ageing day and ZH-supplementation tended to be significant ($P = 0.06$) but with a small effect size ($\eta^2 = 0.02$). To evaluate the ageing effect on meat tenderness, the pooled mean WBSF values of the different ageing days were analysed in a pairwise comparison. Bonferroni's multiple range test was implemented to compensate for differing group sizes. Because the WBSF values were pooled, ZH-supplementation could not be tested and only the ageing effects were assessed.

For almost all the ageing days, the pooled WBSF means for each individual day differed significantly from each other ($P \leq 0.001$). Only day 56 and 120 pooled WBSF means did not differ significantly from each other ($P = 0.25$). This is because the pooled WBSF mean increased from day 56 to day 120. But overall, a significant ageing effect is observed.

Table 4.4.18 Pooled mean Warner-Bratzler shear force values (WBSF; kg) for the three different experimental groups measured over 120 days of ageing

Ageing day	Mean pooled WBSF (kg)	Std. Error
7	6.7	0.11
14	6.0	0.11
28	5.4	0.11
56	4.2	0.11
120	4.5	0.11

When comparing the ageing day effects on WBSF values within either CT or ZH, the results are once again easily discernible on **Figure 4.4.14**.

For CT all subsequent days up to day 56, WBSF means decreased to differ significantly from the previous days' mean WBSF ($P < 0.05$) whilst still being significantly higher than those of subsequent days. i.e., the WBSF mean for CT measured on day 14 was significantly lower than day 7, but still higher than all subsequent days including day 56. Whilst day 28's WBSF was significantly lower than both day 7 and 14, but significantly higher than day 56. Additionally on day 28, because of the increase in mean WBSF on day 120 from day 56, day 120 WBSF mean did not significantly differ from day 28 ($P = 0.94$), as the increase for day 120 now yielded a WBSF mean only 0.5kg lighter than day 28. Similarly, all previous storage day WBSF means were significantly higher than

that of day 56 ($P < 0.05$), but the day 120 WBSF mean was insignificantly only 0.45kg higher than day 56 ($P = 0.86$). A greater difference was observed between days 120 and 56 than 120 and 28 because the day 120 WBSF mean increased from day 56 to a value similar to that of day 28.

WBSF means for ZH-supplementation decreased in a stepwise manner (3 steps) throughout the ageing period, then increased from day 56 to day 120. Day 7 ZH WBSF values were significantly higher than values for all subsequent storage days ($P < 0.05$). Following **Figure 4.4.14**, the ZH WBSF mean only decreased marginally from day 14 to day 28, this was deemed insignificant ($P = 0.51$). Day 14 mean ZH WBSF was still significantly lower than day 7 and higher than all subsequent days after day 28 ($P < 0.05$). Following this finding the WBSF mean on day 28 was significantly lower than day 7 ($P < 0.001$), marginally (insignificantly) lower than day 14, but still higher than all subsequent storage day WBSF means ($P < 0.001$) for ZH. Unlike the CT WBSF means, day 120 ZH WBSF mean did not increase to such extent that it was similar to the day 28 CT WBSF mean, but rather that ZH WBSF remained significantly lower on day 120 than day 28 ($P < 0.001$). As expected, all previous storage day WBSF means were significantly higher than that of day 56 ($P < 0.001$), whereas the increase in WBSF for day 120 resulted in a similar value to that of day 56 ($P = 1.00$). Ultimately, the mean WBSF on day 120 still remained significantly lower than all previous days before day 56 ($P < 0.001$).

In conclusion, storing the meat samples significantly decreased WBSF values for both CT and ZH. The ageing effect size for CT WBSF means was smaller than the effect size for ZH WBSF means ($\eta^2 = 0.18$ vs. 33). From these results it was found that utilising storage as a tenderization method was more effective for ZH-supplemented meat samples than those of control samples. The ZH values remained well above the critical consumer threshold value of 4.59kg identified by Shackelford *et al*. (1991) regardless of tenderization due to ageing.

4.5 Table summaries of results

Table 4.5.1 Summation of live mass and carcass characteristic results for overall zilpaterol hydrochloride (ZH) supplementation effects, the differences in Zilmax® (ZM) vs. Grofactor® (GF) forms and length of finishing period (if applicable)

Characteristic	Effect of ZH-supplementation	ZM vs. GF	Length of finishing period effect
Live mass (kg)	No overall effect ($P = 0.64$; $\eta^2 = 0.31$) No effect on either D15 or D33 ($P = 0.23$ and 0.12, respectively)	No significant differences. D15 GF yielded a numerically heavier live mass ($P = 0.44$). No difference on D33 ($P = 1.00$).	↑ live mass from D0 ($P < 0.001$; $\eta^2 = 0.49$)
Hot carcass mass (HCM; kg)	↑ ($P = 0.001$; $\eta^2 = 0.09$)	No difference ($P = 1.00$). GF yielded a numerically heavier mass than ZM	N/A
Dressing percentage (DR%)	Tended to ↑ ($P = 0.07$; $\eta^2 = 0.03$)	No difference ($P = 1.00$). ZM had a marginally greater ↑ effect compared to CT than GF ($P = 0.30$ vs. 0.40)	N/A
Carcass classification	No significant effect ($P > 0.05$)	No difference ($P > 0.05$)	N/A
Subcutaneous fat thickness (mm)	No significant effect ($P = 0.29$; $\eta^2 = 0.01$)	No difference ($P = 0.42$). ZM had a marginally greater ↓ effect compared to CT than GF ($P = 0.29$ vs. 1.00)	N/A

Table 4.5.2 Summation of carcass composition, temperature, and pH results for overall zilpaterol hydrochloride (ZH) supplementation effects, the differences in Zilmax® (ZM) vs. Grofactor® (GF) forms and post-mortem time (PMt) effects (if applicable)

Characteristic	Effect of ZH-supplementation	ZM vs. GF	PMt effect
Carcass compactness index (CCI)	↑ ($P < 0.001$; $\eta^2 = 0.13$)	No difference ($P = 1.00$)	N/A
Carcass composition	↑ muscle composition % and ↓ fat% ($P = 0.02$ and 0.05; $\eta^2 = 0.05$ and 0.03)	No difference ($P = 1.00$). ZM had a marginally greater ↑ effect on muscle% compared to CT than GF ($P = 0.09$ vs. 0.19) and ↓ effect on fat% ($P = 0.13$ vs. 0.52).	N/A
Carcass temperature (°C)	No significant effect ($P = 0.93$; $\eta^2 = 0.01$)	No difference ($P = 1.00$).	↓ carcass temperatures over post-mortem time ($P < 0.001$; $\eta^2 = 0.95$)
Carcass pH	No significant effect ($P = 0.66$; $\eta^2 = 0.03$)	No significant differences for hours 0.75, 6 or 24 ($P = 1.00$). ZM yielded a numerically colder carcasses than GF on hours 3 and 12 ($P = 0.87$ and 0.98)	↓ carcass pHs over post-mortem time ($P < 0.001$; $\eta^2 = 0.19$)

Table 4.5.3 Summation of meat quality characteristic results for of overall zilpaterol hydrochloride (ZH) supplementation effects, the differences in Zilmax® (ZM) vs. Grofactor® (GF) forms and ageing effects

Characteristic	Effect of ZH-supplementation	ZM vs. GF	Ageing effect
All 3 meat colour attributes	No effect ($P = 0.64$; $\eta^2 = 0.31$)	No difference for either colour attribute ($P > 0.05$)	($P < 0.001$)
Meat colour lightness (L*)	No effect ($P = 0.64$; $\eta^2 = 0.31$)	No difference ($P > 0.05$)	↑ L* for 14-56 DOA, then ↓ on day 120 ($P < 0.001$; $\eta^2 = 0.32$)
Meat colour chroma	No effect ($P = 0.64$; $\eta^2 = 0.31$)	No difference ($P > 0.05$)	↓ saturation ($P < 0.001$; $\eta^2 = 0.50$)
Meat colour hue angle (°)	No effect ($P = 0.64$; $\eta^2 = 0.31$)	No difference ($P > 0.05$)	↓ yellow for 3-7 DOA then ↑ from 7-14 DOA remained constant ($P < 0.001$; $\eta^2 = 0.40$)
Meat drip loss (DL; %)	No effect ($P = 0.38$; $\eta^2 = 0.05$)	No difference ($P = 1.00$ for days 7-56; $P = 0.61$ for day 120)	↑ DL up to 56 DOA, then ↓ on day 120 ($P < 0.001$; $\eta^2 = 0.30$)
Meat cooking loss (CL; %)	No effect ($P = 0.47$; $\eta^2 = 0.04$)	No difference ($P = 1.00$ for days 7-56; $P = 0.67$ for day 120)	↑ CL up to 56 DOA, then ↓ on day 120 ($P < 0.001$; $\eta^2 = 0.37$)
Meat tenderness	↓ tenderness ($P < 0.05$; $\eta^2 = 0.24$)	No difference ($P = 1.00$)	↑ tenderness up to 56 DOA, then ↓ on day 120 ($P < 0.001$; $\eta^2 = 0.38$)

DOA = Days of ageing

Chapter 5: Conclusion

It was demonstrated that bulls fed the β-adrenergic agonist, zilpaterol hydrochloride (ZH) for 30 days (either in the form of Zilmax® or Grofactor® supplementation), significantly produced heavier and more compact carcasses, significantly increased the carcass muscle:fat ratio and tended to yield higher dressing percentages, than the negative control group. ZH-supplementation had a significantly negative effect on meat tenderness. Thereby the H_{01} is rejected since ZH-supplementation did significantly affect carcass and meat quality characteristics when compared to a negative control.

The H_{02} is accepted since dietary supplementation with Zilmax® in feedlot diets during the finishing period, did not significantly affect the carcass and meat quality characteristics differently than when Grofactor® is supplemented, when compared to a negative control. Thereby supporting the viability of the generic ZH-supplementation with Grofactor®.

Post-mortem ageing of meat samples did significantly affect meat quality characteristics. Specifically, meat tenderness - which was negatively affected by ZH-supplementation - significantly improved with post-mortem ageing. Thereby the H_{03} is rejected.

Further studies would be recommended on the exact mechanisms which influence the tendency of post-mortem meat sample ageing to suddenly decrease or increase in meat quality characteristic values from day 56 to day 120.

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