Comparison between the effects of zilpaterol hydrochloride and R-salbutamol during the finishing period on the growth performance and carcass characteristics of feedlot cattle

By

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Abstract

This study was conducted on 228 typical South African feedlot steers, of which 114 steers were fed 60 mg of zilpaterol HCl per steer per day for the final 30 days of the feedlot finishing period and the other 114 steers were fed 120 mg of R-salbutamol per steer per day for the last 30 days of the feedlot finishing period. The animals were slaughtered at the same abattoir after waiting the 3-day withdrawal period of zilpaterol HCl and R-salbutamol. The recorded growth and feedlot parameters included starting weight, slaughter weight, average daily gain, live weight gain and lean gain. The recorded carcass and meat characteristics parameters included warm and cold carcass weights, carcass length, carcass fat thickness measured over the 13th rib, carcass classification score, age code as well as fat code using the current South African classification system, dressing percentage, and carcass compactness.

Declaration

I, Candice Stock, declare that this dissertation which I hereby submit for the degree MSc (Agric) Animal Science with specialisation in Production Physiology and Product Quality in the Department of Animal Science at the University of Pretoria, is the work of my own and has not yet been submitted by myself or any other person for a degree at this or any other tertiary institution.

Signed: ……..

Date: …….

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Abbreviations

AC	Adenylyl cyclase
ADG	Average daily gain
ATP	Adenosine triphosphate
MANOVA	Multivariate analysis of variance
cAMP	Cyclic adenosine monophosphate
C-C	Carbon-carbon
CCW	Cold carcass weight
CFI	Cumulative feed intake
Dress%	Dressing percentage
FA	Fatty acid
FCR	Feed conversion ratio
HCl	Hydrochloride
LSMeans	Least square mean
PKA	Protein kinase A
s.e.	Standard error
SDGs	Sustainable development goals
WCW	Warm carcass weight

Chapter 1. Introduction

1.1 Project theme

Growth and physiology of feedlot cattle

1.2 Project title

Comparison between the effects of zilpaterol hydrochloride and R-salbutamol during the finishing period on the growth performance and carcass characteristics of feedlot cattle

1.3 Aim

The aim of this project was to compare the effects of R-salbutamol (Salbutamate® 10%) at 120 mg per head cattle per day in the feed on feedlot cattle during the last 30 days of the finishing phase preceding slaughter, versus zilpaterol hydrochloride (Zilmax®), which will be referred to as zilpaterol HCl from now on, at 60 mg per head per day in the feed for the last 30 days preceding slaughter on the parameters below:

1) Growth and feedlot performance:
   - Starting weight
   - Slaughter weight
   - Live weight gain
   - Average daily gain (ADG)
   - Lean gain

2) Carcass characteristics
   - Carcass weights (WCW & CCW)
   - Dressing percentage (Dress%)
   - Carcass classification score (carcass conformation score)
   - Age code (A-code)
   - Fat code
   - Fat thickness
   - Carcass length
   - Carcass compactness (calculated amount)

3) Possible diseases
   - Lung lesions and adhesions (Although these diseases were monitored by the consulting veterinarian, they did not form a part of the study).

1.4 Motivation

Because of the ever-growing population of humans, changes in wealth, health and life expectancy there have been significant increases in the demands for products of animal origin (Webb & Erasmus, 2013). Global livestock production is projected to double by the year 2050 to satisfy the demands of the estimated global population, suggesting a faster than expected growth when compared to all agricultural sectors. In the next 8 years, the demands for milk, meat and eggs are anticipated to increase by approximately 30% (Webb, 2013).

With increasing consumer concern surrounding dietary animal fats associated with heart disease and atherosclerosis in certain individuals. The demand of Western consumers is to have less fat in their meat (Higgans, 2004). Fat is an expensive commodity to produce and because of the world's already limited feed resources, having to cut off 5-6% of carcass fat off at the abattoir and a further 5-6% is trimmed at the butcher the production of lean meat is imperative. The majority of consumers look for a larger cut consisting of less fat which means more value for money. Therefore, there has been a considerable amount of interest and investment in attempting to find pharmaceutical, genetic, economically and biologically practical ways of producing the target product. The application of certain growth promoters has been studied and used in production of livestock for many decades.

If the South African feedlot industry did use of β-adrenergic agonists, the industry would be unable to produce enough meat in an efficient manner. Red meat is an exceptionally good source of protein and using β-agonists to produce this protein in a manner that limits the environmental impacts of beef production.

It is of great economic importance that growth in animal production is efficient, especially when intensive animal production is practiced such as in feedlots. Animal growth can be studied at various levels, from cellular and molecular to the entire animal (Hossner, 2005). Growth in livestock can be routinely measured as the change in live weight (Owens *et al*., 1995). The body weight for cattle obtained 24 hours post feed, but with access to water, provides a shrink body weight, which is highly correlated with the empty body weight, the body weight minus the gut contents (Hossner, 2005).

Due to males having leaner carcasses compared to females among cattle, the sex of the animals is an important factor that affects the body composition and growth (Trenkle & Marple, 1983; Monteiro *et al*., 2006). In the 2nd half of the 20th century already (Berg & Butterfield, 1976) it was established that the androgenic effect of male hormones, e.g., in bulls and not in steers or heifers, are required to achieve the full pattern of muscle development. It is necessary to increase livestock profits, which may be achieved by increasing average daily gains and feed conversion efficiency, while maintaining meat composition, palatability and quality. If the

specific market requirements are met the feedlot operator is paid a premium (Block *et al*., 2001). Feed cost determines profit made by the feedlot, therefore it is of utmost importance to improve feed conversion (Silence, 2004).

Anabolic agents decrease the amount of fat while increasing the rate of both muscle protein synthesis and deposition as well as decreasing protein degradation at a particular live weight (Dunshea *et al*., 2005). Anabolic agents such as implants cause an increase in feed intake of approximately 5 to 10%, decreasing the total energy required for maintenance, meaning this energy is now available for growth and thereby the feed efficiency is improved between 5 to 15%. Cattle that are fed high concentrate diets in conjunction with an aggressive implant strategy result in an improved daily gain of up to 25% (Bartle *et al*., 1992; Johnson *et al*., 1996).

Beta-agonists are synthetic organic compounds that are naturally occurring and have a common chemical structure of compounds that fall under the classification of phenethanolamines (Beerman & Dunshea, 2005). It is also stated that beta-agonists act as repartitioning agents, which are chemical or hormonal agents that direct substrates (i.e. consumed nutrients) from adipose tissue and towards muscle accretion in growing cattle, sheep, swine, turkeys and broilers (Unruh, 1986; NRC, 1994; Moody *et al*., 2002). Beta-agonists are active orally and are effective at 5-30 parts per million (ppm) of feed for small periods of time (28-42 days) near the finishing period (Beerman & Dunshea, 2005). The response diminishes with time, which indicates that careful planning should be done to determine the optimal period of use. They do not improve lactation nor have they been approved for use in animals that are breeding (Moody *et al*., 2002). Beta-adrenergic agonists have similar characteristics to natural catecholamines such as dopamine, epinephrine and norepinephrine (NRC, 1994; Bell *et al*., 1998).

Examples of these beta-adrenergic agonists are clenbuterol, ractopamine hydrochloride, R-salbutamol and zilpaterol hydrochloride. The beta-adrenergic agonists are taken orally and bind to specific beta-adrenergic receptors in fat and muscle tissue, thus changing the biochemical growth processes that occur within these tissue cells. This leads to increased protein accretion, decreased protein degradation, increased lipolysis, decreased lipogenesis or combinations of these (Wheeler & Koohmaraie, 1992; Mersmann, 1998). This repartitioning of nutrients consumed by the animal in the direction of the synthesis of protein and muscle growth instead of for the deposition of fat, increases profitability by lowering feed costs, improved dressing percentage and improve carcass leanness (Brooks *et al*., 2009).

The three main beta-adrenergic receptor subtypes that occur in mammal tissues are $beta_1$-adrenergic receptors, $beta_2$-adrenergic receptors, and $beta_3$-adrenergic receptors (Mersmann, 1998; 2002). Different subtypes are accommodated by different tissues and metabolic

pathways are addressed differently by cells and tissues. Therefore, the actions or effects of the different beta-agonists will not be the same depending on the type of beta-adrenergic subtype. Many factors influence the results such as treatment period, type of beta-adrenergic agonist treatment and the concentration of that beta-adrenergic agonist, as well as the type of species that was treated (Dunshea *et al*., 2005). Observations were made in cattle that were treated with zilpaterol HCl and clenbuterol, showing a significant advantage of beta-agonist treated cattle over cattle that were not fed any beta-agonist. Cattle that were given ractopamine HCl for adjusted feed conversion ratios had higher dressing percentages (Strydom *et al*., 2009). Because of clenbuterol's strong receptor affinity, it is illegal to use as a beta-agonist due to adverse effects such as an increase heart rate and depression of appetites throughout the initial phase of the treatment period (Spurlock *et al*., 1993; Ricks *et al*., 1984).

Zilpaterol hydrochloride, is a beta-agonist with the formula $C_{14}H_{19}N_3O_2$.HCl and a molecular mass of 297,78 g/mol, that has been legal for use in South Africa and Mexico for an excess of 15 years and it was approved by the FDH to be used in USA feedlots since 2006 (Avendaño-Reyes *et al*., 2006; Brooks *et al*., 2009; Shook *et al*., 2009). Zilpaterol hydrochloride is observed to have negative effects on the tenderness of meat (Rathmann *et al*., 2009; Strydom *et al*., 2011; Hope-Jones *et al*., 2012).

R-salbutamol is a beta-adrenergic agonist with the chemical formula $C_{13}H_{21}NO_3$ and molecular mass of 239,311 g/mol and is one of the new products which has been introduced as a growth modifier in production animals. It is a purified derivative of racemic salbutamol (RS) and used in the treatment of human respiratory disorders and is a $beta_2$-adrenergic molecule. In animals, there have been more recent studies focussing on the effects that R-salbutamol has on poultry or swine, where it positively effects carcass growth and composition during a study on finishing pigs (Marchant-Forde *et al*., 2012). There have been various salbutamol versus zilpaterol HCl studies conducted on typical South African feedlot cattle, however these studies did not yield conclusive results (Steenekamp, 2014). The effects of zilpaterol HCl have been reported, however the results of R-salbutamol on feedlot cattle have not been clear. An objective study conducted on a larger scale is therefore required to inform the industry about the newer molecule.

There have been several studies testing the efficacy zilpaterol HCl in feedlot conditions and many of these studies were conducted in South Africa. The use of beta-adrenergic agonists or beta-agonists as they are commonly referred to, is vital for the efficient and safe production of feedlot cattle for meat, ensuring that the demand for red meat in South Africa and its neighbouring countries is met. R-salbutamol has been approved for use in South Africa more recently, and it provides an alternative molecule, which increases the competitiveness of this

industry. A few studies have been conducted at the University of Pretoria on the use of zilpaterol HCl in feedlot cattle (Morris, 1997; Steenekamp, 2014). One study was conducted at the University of Pretoria, and several were conducted at private feedlots, but the results were inconclusive and not published. However, previous studies with Salbutamate® 10% have been inconclusive and due to its aggressive marketing in South Africa there have been numerous requests for an objective evaluation of the effects of R-salbutamol compared to zilpaterol HCl on the growth of feedlot cattle during the finishing phase and subsequent carcass characteristics.

The study was conducted during June, July and August of 2016 at the Taaiboschbult Feedlot in the Free State province, which is now owned and managed by Sparta Taaiboschbult Feedlot (Pty) Ltd. The study simulated typical feedlot conditions to compare the use of the beta-agonists Zilmax® and Salbutamate® 10% when supplemented in the finishing feed for the last 30 days of the fattening phase for typical South African feedlot cattle. Zilpaterol HCl was registered for use as beta-adrenergic agonist, feed additive, more than 25 years ago and has become the industry norm. It is used in the majority of local feedlots as a feed additive to improve feed conversion, growth performance and carcass characteristics. The weaners from which the experimental cattle were selected underwent backgrounding before entering the feedlot, which ensured that they were more uniform in terms of adaptation and weight. This allowed for the selection of cattle in each treatment group to be more similar in terms of starting weight and thus comparable, ensuring a completely randomized control study.

1.5 Hypotheses

$H1_O$: There is no significant difference between the use of R-salbutamol or Zilmax on the growth performance and efficiency of feedlot steers.

$H1_A$: There is a significant difference between the use of R-salbutamol or Zilmax on the growth performance and efficiency of feedlot steers.

$H2_O$: There is no significant difference between the use of R-salbutamol and Zilmax on the carcass characteristics of feedlot steers.

$H2_A$: There is a significant difference between the use of R-salbutamol and Zilmax on the carcass characteristics of feedlot steers.

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Chapter 2. Literature review

2.1 Beef production in South Africa

According to the 2030 Agenda for Sustainable Development (UN) there are 17 SDGs, of which there are several goals in which agriculture plays a vital role. The goal for there to be zero hunger in the world by the year 2030 which is achievable through effect food distribution and minimizing food wastage. Throughout the world there is enough food produced to solve the problem of world hunger however, the food that is produced in developed countries cannot be distributed throughout the world to the developing countries which have a shortage of food (SDGs, 2015). It is estimated that one third of the food produced throughout the world goes to waste, this is equal to 1,3 billion tons of food annually (Ishangylyyev *et al*., 2019). With the current world population estimated to be nearing 8 billion people and by 2050 the expected population size will be greater than 9,5 billion people (UN, 2019).

In order not only to solve this food distribution crisis, but also the exponential growth of the world's population the way people produce food must be streamlined and made more efficient. Another important factor is that the environmental impact of agriculture and more specifically beef production. It is vital to produce meat in a sustainable manner that is ethically acceptable, which can be achieved through vertically integrating production systems, using unconventional species, using technologies that enhance production responsibly and producing cultured meats (Webb, 2021). The unavailability of land for agricultural use is caused by increasing urban development from cities mean that producing meat in an efficient manner, using minimal resources and land is the only way to keep up with the growing demands. This can be done by using growth enhancing molecules such as steroidal implants and beta-adrenergic agonists (Anderson *et al*., 2005).

2.1.1 The SA beef feedlot industry

The South African beef industry makes up approximately 80% of the total heads of cattle in South Africa, which is then split into intensive (feedlot cattle) and extensive farming (DAFF, 2019). About 80% of the beef production in South Africa is from feedlot cattle and depending on the economics and management, it can deliver up to 1,7 million animals per annum, which yielded 531 662 animals on feed during December 2016 (SAFA, 2017). Feedlot animals spend an average of 140 days on feed at full capacity with varying feedlot sizes from less than 200 animals to more than 160 000 animals in one feedlot consisting of farmer feeders, seasonal feeders and commercial feeders (Spies, 2018).

In order for the agricultural sector to overcome the decrease in usable water and raw animals feed materials and the higher worldwide food demand, the agricultural sector is required to

increase the efficiency of production. This can only be done through the utilization of feedlot systems for growing and finishing animals thus increasing the production of land per unit (du Toit, 2017). Through the intensification of production systems, producers are able to increase the stocking density, limit predation and stock theft, protect the pasture health by preventing over grazing and thus protecting the entire production system by maintaining the supply of higher quality feed that are used in the feedlot (Webb, 2013; Du Toit, 2017). Intensive production systems allow the control of problematic factors associated with animal growth and product quality that are normally attributed with an extensive production system. Other advantages of feedlotting are that animals achieve a higher growth rate, improved carcass quality with a higher dressing percentage and improved carcass consistency (Cloete et al., 2012a; Webb & Erasmus, 2013a).

The typical SA feedlot cattle are split into different maturity types; with early maturing breeds, including Hereford, Angus, Sussex and Shorthorn; medium-early maturing breeds such as Afrikaner and Brahman; a medium-maturity breed is Bonsmara; medium-late maturing breeds are Simmentaler, Limousin, Santa Gertrudis, Brown Switzer and South Devon; and late-maturing breeds being Charolais, Chianina, Blonde d' Aquitaine, Pinzgauer and Friesian (Strydom, 2002a). The SA feedlot industry tends to use composite breeds of *Bos taurus* (European breeds) x *Bos indicus* with medium maturity types, producing leaner carcasses at the same slaughter weight and a higher percentage of muscle compared to early maturing breeds (Strydom, 2002a). The importance of producing a product that falls within consumer specifications with regards to meat leanness which has recently become even more important due to growing health concerns.

2.1.2 South African beef feedlot norms

The slaughter weight (kg) of feedlot cattle has increased over the years because it has become more economical to feed cattle for slightly longer, with larger frame sizes and to a heavier slaughter weight, however a leaner carcass is desired by consumers. The average slaughter weight of Bonsmara steers fed zilpaterol HCl for 30 days during the finishing phase was 575,5 kg (Strydom *et al*., 2009). The final mean slaughter weight of two North American studies where zilpaterol HCl was fed were 565,0 kg (Montgomery *et al*., 2008) and 614,0 kg (Baxa *et al*., 2004). Another South African study feedlot study where zilpaterol HCl was fed as a feed additive observed a mean final live weight of 453,0 kg (Steenekamp, 2014). Another study observed a mean slaughter weight of 535 kg for steers fed zilpaterol HCl (Moholisa *et al*., 2018). The average daily gain (kg/day), henceforth referred to as ADG, throughout the feedlot period varies depending on the phase of growth and therefore feeding. During the final phase of finishing the ADG decreases because the animal is no longer depositing predominantly

muscle tissue but instead fat, which is more nutrient demanding (Hossner, 2005). In a North American feedlot study, during the final 50 days of the feedlot the ADG obtained was 1,27 kg/day, compared to day 0 to -50 days before the end which observed an ADG of 1,89 kg/day (Elam *et al*., 2009). In another North American study the mean ADG of feedlot steers fed zilpaterol HCl for the final 30 days observed was 1,59 kg/day (Montgomery *et al*., 2008). The cold carcass weights of two South African feedlot studies measured 311 kg (Moholisa *et al*., 2018) and 353,5 kg (Strydom *et al*., 2009). In the current study the carcasses were also classified by frame size at slaughter by making use of the CCW, namely categorization of carcasses into light/small (200 to 225 kg), medium (226 to 275 kg) and heavy/large (>275 kg) as described by Webb & Agbeniga (2020). The dressing percentage is calculated as the warm carcass weight (WCW) divided by the live weight of the animal at slaughter (excluding gut fill) which is then expressed as a percentage.

2.2 Beta-adrenergic agonists

2.2.1 Introduction to beta-adrenergic agonists

Animals undergo stress due to environmental challenges such as weather stressors, feed and mating competition and predator threats. The responses to these stressors are regulated by the components of the central and autonomic nervous systems as well as the endocrine system. This is known as the ‘fight or flight’ syndrome by W. B. Cannon in 1932 and is accompanied by important physiological adaptations that mobilize energy and provide energy to the organ systems which are involved in this reflex (Cannon & De La Paz, 1911; Hossner, 2005). Changes in the cardiovascular system, the respiratory system and the gastrointestinal tract are influenced by the sympathetic portion of the autonomic nervous system and the adrenal glands. These changes are stimulated by the release of the catecholamines; norepinephrine (a neurotransmitter in the sympathetic nervous system) and epinephrine (the adrenal gland hormone). Dopamine is hydroxylated to produce norepinephrine which is then methylated to produce epinephrine. DOPA is decarboxylated from dopamine and DOPA is the hydroxylation product of tyrosine (Mersmann, 1998; Mersmann, 2002; Hossner, 2005).

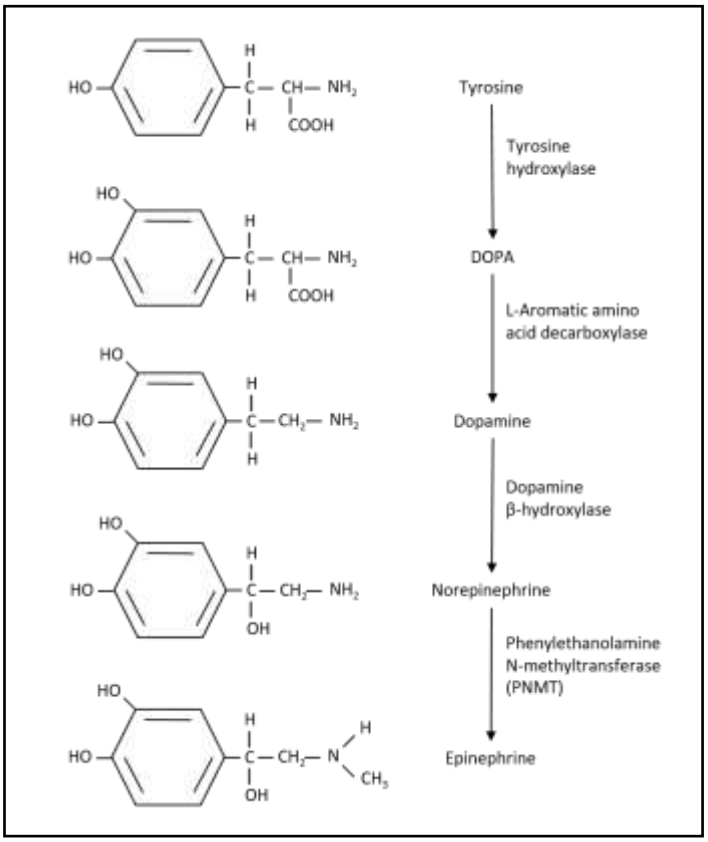

Figure 2.1 The biological synthesis of catecholamines (Adapted from Hossner, 2005)

2.2.2 The physiological mechanism of a beta-adrenergic agonist

The release of dopamine, norepinephrine and epinephrine results in the mobilization of lipids and glycogen, and the catabolism of these molecules by lipolysis and glycogenolysis provides the animal with energy in order to function. Subsequently, the rate and strength of cardiac contractions are increased, and the vasoconstriction of blood vessels decreases blood flow to the gastrointestinal tract. This then causes the vasodilation of blood vessels increasing blood flow to the heart, brain and skeletal muscle. Catecholamine derivatives, specifically β-agonists, are therefore useful as agents to alter food animal body composition and improve production efficiency (Hossner, 2005).

Beta-adrenergic agonists or beta-agonists are synthetic chemicals that fall under the compound class phenethanolamines and bind to specific β-adrenergic receptors, mimicking the effects of catecholamines (Hossner, 2005; Anderson *et al*., 2005). They are orally active, do not have any antibiotic activity, are not anabolic steroids and can be used as feed supplements in animal production (Anderson *et al*., 2005). When administered orally, β-agonists cause the modification of growth by decreasing the amount of fat accretion and repartitioning these nutrients to skeletal muscle accretion (Webb, 2021; Anderson et al., 1991, 2010; Mersmann, 1998). The body is able to do this by increasing gluconeogenesis (the

process of the transformation of specific non-carbohydrate carbon substrates into glucose) and glucogenolysis (the process of the transformation of glycogen in the liver to glucose) which occurs in the liver and increases glycogenolysis that occurs in the skeletal muscle cells of the animal (Cherrington et al., 1984; Chung et al., 2015). The body is able to maintain its high blood glucose levels through the suppression of the release of pancreatic insulin and the induction of the secretion of glucagon into the bloodstream. Additionally, the body causes an increase in the lipolysis of adipose tissue (the degradation of lipids through hydrolysis), ensuring that there is a sufficient amount of glycerol and free fatty acids. A source of readily available energy is provided by these substrates or they can be recycled through the process of gluconeogenesis, producing glucose (Östman *et al*., 1979; Hossner, 2005b). When the body undergoes the stress response induced by the beta-adrenergic agonist it ensures that the involved organs receive enough energy and oxygen to respond. These responses are an increased heart rate, vasoconstriction of gastrointestinal tract blood vessels, which decreases the blood flow and increased blood circulation to the skeletal muscle, including the brain and heart (Mersmann, 2002; Hossner, 2005b).

Beta-agonists are repartitioning agents and this re-direction of nutrients used by skeletal muscle, which were originally meant for adipose tissue, is important for the efficiency and efficacy of their use in livestock. Beta-agonists are more effective in cattle and sheep compared to swine and poultry (Ricks *et al*., 1984; Hossner, 2005; Anderson *et al*., 2005). When beta-agonists are treated in the for the last 20-42 days of the fattening period, a leaner carcass with improved conformation is produced (Webb, 2021; Webb & Casey, 1995; Johnson *et al*., 2013; Strydom *et al.,* 2011). By using these beta-agonists the distribution and proportion of lean meat can be improved (Moloney *et al*., 1990; Webb, 2021). By producing a leaner carcass, the meat industry is able to meet the consumer demands for less fat in their meat and producing meat more efficiently (Higgans, 2004). The use of Zilpaterol HCl shows a decrease fat content of the carcasses and positively influences the composition of FAs in beef (Webb & Casey, 1995; Webb & Casey, 1997; Webb, 2021).

The effects of β-agonists are mediated by the modification of particular metabolic signals within fat and muscle cells, resulting in an increase in nutrients that are directed towards lean muscle growth (Anderson *et al*., 2005). β-adrenergic agonists bind to the β-adrenergic receptors that are found on the fat and muscle cell surface and this produces these metabolic signals. When they bind to receptors on the fat cell surface, the cells produce biochemical signals inside which decrease the synthesis of fat and increase the degradation of fat. This intracellular signalling in muscle cells causes significant increased rates of synthesis and deposition of muscle protein. This results in a slower deposition rate of fat, therefore a slower synthesis of fat and a faster synthesis of muscle. Fat requires double the amount of energy for deposition compared

to lean meat, therefore the leaner animal uses feed more efficiently and produces a more muscular carcass with a higher dressing percentage.

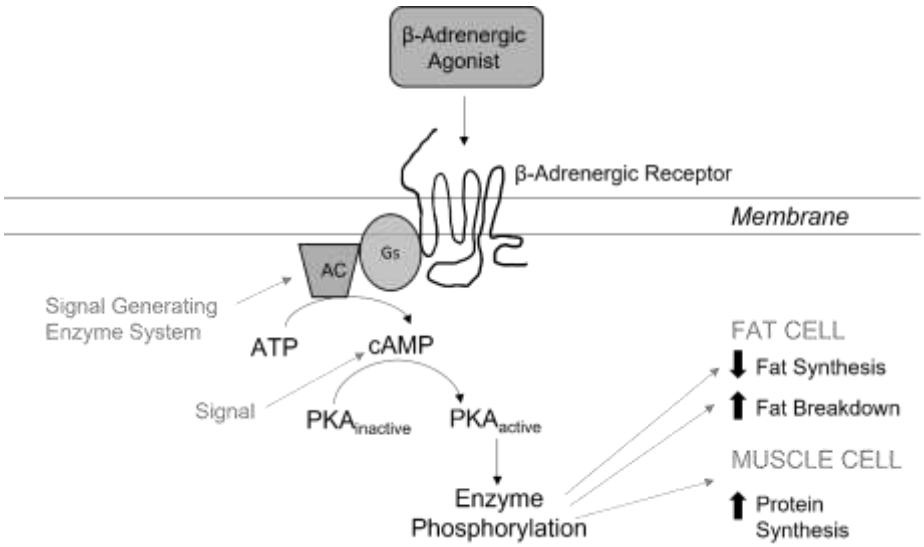

Figure 2.2 Mechanism of the transduction of biochemical signals from β-adrenergic receptors (*Adapted from Farm Animal Metabolism and Nutrition: Critical Reviews*).

In figure 2.2.2, the receptor is activated by a beta-adrenergic agonist, causing an interacting between the receptor and the Gs protein. Adenylyl cyclase (AC) is then stimulated by these Gs proteins to convert adenosine triphosphate (ATP) into cyclic adenosine monophosphate (cAMP), cAMP then mimics an intracellular signalling molecule. This increase in cAMP levels activate the protein kinase A (PKA), releasing its catalytic subunit which phosphorylates numerous enzymes and regulating factors that are vital for the regulation of metabolic processes. β-adrenergic receptors and the cAMP signalling pathway are activated by the β-adrenergic agonists that trigger the rate limiting enzymes, which are activated in lipolysis, and the lipogenic enzymes involved in de novo synthesis of FAs and triglycerides are inactivated (Moody et al., 2000).

2.2.3 β-Adrenergic receptor subtypes

A physiological response occurs due to the binding of a β-adrenergic agonist to a β-adrenergic receptor. Epinephrine and norepinephrine are the physiological β-adrenergic receptor agonists. There are three sub-types of β-adrenergic receptors; namely the β_1-adrenergic receptor, the β_2-adrenergic receptor, and the β_3-adrenergic receptor. These β-adrenergic receptors are found in most mammal tissue cells, with varying distribution and proportion of each subtype between tissues of different species as well as between the tissues in a given species. A variation exists between the amino acid sequences across species for a given β-adrenergic receptor subtype (Mersmann, 1998). At lower

concentrations, norepinephrine is more effective for β_1-adrenergic receptor than for β_2-adrenergic receptor.

Table 2.1 The effects of oral administration of β-adrenergic receptor agonists on farm animals

Animal	Weight gain	Feed intake	Feed efficiency	Muscle accretion	Fat accretion
Cattle	+10	-5	+15	+10	-30
Chickens	+2		+2	+2	-7
Pigs	+4	-5	-5	+4	-8
Sheep	+15	+2	+15	+25	-25

The values in the Table 2.1 above are indicative of the percentage change and these approximate values are adapted from Moloney *et al*. (1991).

It is suggested that in cattle there are predominantly β_2-adrenergic receptors on skeletal muscle, with approximately 75% β_2-adrenergic receptors and 25% β_1-adrenergic receptors on adipocytes (Sillence and Matthews, 1994). A recent study however, found that β_2-adrenergic receptors are predominant in intramuscular and subcutaneous adipose tissue, with β_3-adrenergic receptors being the second most abundant in adipose tissue (Hwang *et al*., 2021) Each β-adrenergic receptor subtype executes a specific part in that beta-adrenergic response (Mersmann, 1998; Hossner, 2005b).

Table 2.2 β-adrenergic receptor subtypes and their specific functioning (Mersmann, 1998; Hossner, 2005b)

β-adrenergic receptor subtype	**Tissue in which β-receptor subtype is present**	**Response to epinephrine vs norepinephrine**	**Function of subtype**	**Abundance**
β_1	80% in adipose 70% of heart 65% of lung 60% of skeletal 50% of liver	Equally to both circulating epinephrine and neural norepinephrine. The primary neural system adrenergic receptor.	1. Mediate lipolysis in adipose tissue 2. Other effects in smooth intestinal muscle and cardiac muscle	Most abundant in most tissues
β_2	Lung tissue, uterine tissue and smooth muscle tissue.	Epinephrine is much more effective in stimulating β_2 receptors compared to norepinephrine, which has a weak interaction.	Mediate glycogenesis in the liver, uterine smooth muscle relaxation, vasodilation and bronchodilation.	In specific tissues, however not as abundant as β_1-adrenergic receptor subtypes.
β_3	In humans and pigs, consist of less than 10% in adipose tissue and 2% in other tissues. In rodents, it is the most abundant receptor subtype in white and brown adipose tissues.	NA	Mediate lipolysis in rodent adipose tissue and thermogenesis.	Least abundant receptor subtype.

These beta-adrenergic receptor subtypes are present in most tissues, and they show that there is a complex ratio that exists between the receptor subtypes and receptor types and could be the explanation to why specific tissues show varying sensitivities to beta-agonists (Minneman et al., 1979; Mersmann, 1989, Dunshea et al., 2005, Du Toit, 2017).

2.2.4 Effects of β-adrenergic agonists on growth and body composition

When administered orally as feed supplements, beta-agonists result in improved feed efficiency, an increase in the rate of weight gain, reduced fatness, and increased carcass protein deposition, thus an increase in muscle weight of animals treated (Miller *et al*., 1988; Anderson *et al*., 1991; Vestergaard *et al*., 1994; Johnson, 2004; Anderson *et al*., 2005; Strydom

et al., 2009; Webb & Allen, 2015). The effects of beta-agonists are dependent on the type and amount of beta-agonist used and the species of animal that is treated (Dunshea *et al*., 2005). These effects are due to the direct actions that the beta-agonists have on the tissues of interest, adipose tissue and skeletal muscle (Anderson *et al*., 2005). Beta-agonists are able to trigger an increase in blood flow to major body parts such as the hindquarters of cattle and sheep. This could be as a result of the interaction between various beta-agonist receptor subtypes that are found in different types of cells in the body (Mersmann, 1998).

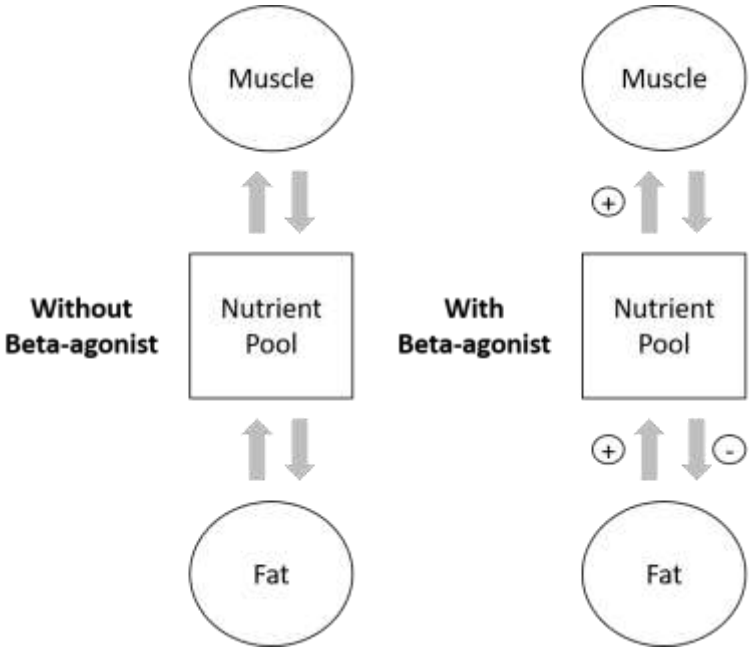

Figure 2.3 Beta-adrenergic agonists modify metabolic signals in muscle and fat cell to achieve enhanced lean muscle growth rate (Adapted from Anderson *et al*., 2005).

Animals treated with beta-agonists do not have any changes in the proportion of circulating hormones in the tissues which are affected by beta-agonists. They are the most potent inducers of skeletal hypertrophy, a condition distinguished by extreme skeletal muscle development, decreased subcutaneous fat depots, thinner skin and lighter bones (Butterfield, 1966; Hossner, 2005). The response to beta-agonists is muscle protein accretion and is primarily due to a decrease in muscle protein degradation. Animals treated with beta-agonists show a reduction in lysosomal protease activity and in non-lysosomal protease activity. Skeletal muscle effects are time-dependent and are characterized by rapid early growth which decreases over time. This decrease could be due to the downregulation of β-receptors in skeletal muscle tissue which can potentially be avoided by intermittent treatments (e.g. treat for 2 days and rest for 2 days) (Hossner, 2005).

The increased demand for lean tissue deposition requires adequate nutrition, specifically balanced amino acids in regard to lysine concentrations, which is a common characteristic of

animals that receive some form of production enhancers. These feed supplements are most effective when given to older, heavier animals and at lower doses (<20mg/kg) otherwise weight gain is reduced. This reduced weight gain is due to lower feed intake caused by a reduced appetite at higher supplement doses (Hossner, 2005).

2.3 Fatty acid composition of meat and human health concerns

Fatty acids (FAs) that consist of single bonds only are 'saturated' and if they have carbon-carbon (C-C) double bonds in a FA chain they are "unsaturated" FAs (Campbell, 1995). Fatty acids containing one C-C double bond are "monounsaturated" and those containing more than one double bond are termed "polyunsaturated". Polyunsaturated FAs are typically found in vegetable oils and the consistency of a lipid is determined by the length of FA chains as well as the presence or absence of double bonds (Webb & O'Neill, 2008; Campbell, 1995; IUPAC, 1978). Fatty acids that are essential, are those that are vital in the diets of humans and other monogastric animals, and they cannot be synthesized by the body. These dietary essential FAs are n-3 and n-6 fatty acids, and they function as transporters of the four fat-soluble vitamins (Vitamins A, D, E & K), playing a vital role in the immune response of both humans and animals (Webb & O'Neill, 2008). The 20-carbon essential FAs that are considered the most important are arachidonic acid (C20:4n-6) and eicosapentaenoic acid (C20:5n-2, EPA), which are produced through the desaturation and elongation of linoleic and α-linoleic acid respectively. The only noteworthy dietary sources of arachidonic acid and docosahexaenoic acid (C22:6n-3) are fish, fish oil and meat, with meat containing lower concentrations of these polyunsaturated FAs compared to oily fish (Seppänen-Lakso *et al*., 2002). Of these n-3 fish oil FAs, eicosapentaenoic acid and docosahexaenoic acid are the most readily found (Smith, 2007).

Non-essential FAs are those that cannot be obtained from the diet but can be synthesized from acetyl Coenzyme A (CoA) with various cofactors. Glycerophospholipids and sphingolipids, the 16- and 18-carbon saturated FAs namely palmitic and stearic acids and n-9 monounsaturated FAs such as oleic acid are the major non-essential FAs. Linoleic acid (C18:2) is the precursor of the n-6 FA essential FA class. Animals are able to incorporate the double bonds at the position n-9 or nearer to the carboxyl group (Webb & O'Neill, 2008; Smith, 2007).

Conjugated linoleic acid (CLA) is an unsaturated fatty acid that contains conjugated double bonds, many of these FAs occur naturally in ruminant animal fats such as beef tallow and milk fat (Christie *et al*., 1997; Webb & O'Neill, 2008). The cis-9, trans-11 CLA isomer is the most important with regards to human health, with between 2,9 and 11,3 mg/g CLA found to be present in body and milk fat of ruminants (Larquè *et al*., 2001; Webb & O'Neill, 2008). Despite there being some endogenous synthesis of CLA through the desaturation of C18:1_11t, CLA

that is detected in human tissues is of dietary origin. In human adipose tissue, there is a good correlation between the amount of milk consumer and the presence of CLA (Webb & O'Neill, 2008).

Table 2.3 Fatty acid composition (w/w%) of subcutaneous adipose tissue and muscle of loin (*Musculus longissimus*) steak in cattle (Enser *et al*., 1996).

Fatty acid	Adipose tissue in cattle	Loin muscle in cattle
C14:0	3,7[b]	2,7[b]
C16:0	26,1[c]	25,0[c]
C16:1*cis*	6,2[b]	4,5[c]
C18:0	12,2[a]	13,4[b]
C18:1*cis*-9	35,3[b]	36,1[b]
C18:2n-6	1,1[a]	2,4[a]
C18:3n-3	0,5[a]	0,70[a]
C20:4n-6	ND	0,63[a]
C20:5n-3	ND	0,28[a]
n-6:n-3	2,3	2,1
P:S	0,5	0,11
Total	86,31	109,52

[a, b, c] Mean that different superscripts are significantly different ($p < 0,05$).

P:S – polyunsaturated: saturated fatty acid ratio.
ND – no data

Taste and nutrition are two of the most important aspects of meat quality, and fat has a significant impact on the meat's eating quality (Wood, 1990; Webb, 2006; Webb, 2021). The general public has the opinion that dietary fat increases the risk of colorectal cancer and is considered unhealthy; however, analysis of various studies found that an association between dietary fat intake and the incidence of colorectal cancer does not exist (Lin *et al*., 2004; Webb & O'Neill, 2008). In both muscle and adipose tissue, fat and particularly long-chain FAs are important contributing factors to meat quality and play a significant role in the nutritional and sensory meat values (Webb & O'Neill, 2008).

2.3.1 How do growth promoters influence fatty acid composition?

Due to consumer health concerns with regards to fat consumption and specifically fatty acid composition, particular interest must be paid to how beta-agonist supplementation alters the FA composition of steers (Webb & Casey, 1995). In the subcutaneous fat of supplemented steers, there was a greater proportion of oleic acid compared to non-supplemented steers (Webb, 2021). In Table 3 (Enser *et al*., 1996) oleic acid consisted of approximately 40% the total amount of FAs in subcutaneous adipose tissue, the highest concentration of all the FAs. This could be explained through the repartitioning of energy by the events that stimulate lipolysis and inhibit lipogenesis in fat tissue. Anabolic steroids stimulate growth via an increase

in growth rate and protein (muscle) synthesis, consequently delaying the active fat deposition phase (Strydom, 2002b). The two molecules, beta agonists and anabolic steroids, are often used simultaneously in feedlot steers (Webb & Casey, 1995). In the study conducted by Webb and Casey (1995) on feedlot steers treated with a beta-agonist compared to those that did not receive any beta-agonist and those treated with trenbolone acetate as well as a combination of the two, the deposits of oleic acid (C18:1) in subcutaneous fat were significantly greater in the steers that received the beta agonist treatment than those that did not or that received the combination (Webb & Casey, 1995; Webb & Casey, 1997; Webb, 2021).

2.3.2 What is the nutritional importance of lipids?

It was originally perceived that dietary fat intake in humans should be limited in order to reduce predisposed medical conditions such as obesity, coronary heart disease and others (Webb & O'Neill, 2008). The current evidence shows that instead of limiting the amount of fat, attention should rather be on the quality of the fat which focusses on the FA composition, saturation and unsaturation instead. An example of this is that monounsaturated and polyunsaturated FAs are considered to be of more importance than the total amount of fat ingested when specifically looking at middle-aged men and how the risk of cardiovascular disease can be reduced (Laaksonen *et al*., 2005). As was found in adults, infants that ingest more polyunsaturated and less saturated FAs could decrease the total and low-density lipoprotein cholesterol (LDL-C) in early life. Again, the importance of fat quality over the fat quantity for humans to have a lower risk of cardiovascular disease (Öhlund *et al*., 2007).

Dietary lipids are required by humans and animals not only for providing metabolic energy but for the synthesis of phospholipids which are vital in the structural integrity of biological membranes (Webb & O'Neill, 2008).

2.4 The effects of beta-adrenergic receptors on meat quality

Beta-adrenergic agonists play a significant role on muscle metabolism, and therefore unfavourable effects are to be expected. Beta-adrenergic agonist treatment resulted in a reduction in intramuscular fat and an increase in both the drip loss and pH (Dunshea *et al*., 2005). Measured by the Warner-Bratzler shear force test it has been determined that beta-agonists have a major significant drawback which is increased meat toughness (Hossner, 2005). In a trial conducted at the University of Pretoria zilpaterol HCl and R-salbutamol were given to feedlot bulls and the shear force of the meat was compared. R-salbutamol had more tender meat when given to feedlot bulls for 40 days prior to slaughter day compared to zilpaterol hydrochloride (Steenekamp, 2014). This meat toughening could be explained by beta-agonists having the ability to affect the activity of certain proteolytic enzymes that are

found in the calpain system. This system is responsible for calpastatin functioning as a calpain inhibitor and the turnover of myofibril proteins (the natural degradation process of protein), which is a predominant collection of proteins in striated muscles (Goll *et al*., 1992). Thus, because beta-agonists increase the activity of calpastatin and therefore resulting in decreased calpain activity, meat toughness can be expected to increase (Strydom *et al*., 2009; 2011).

2.5 Beta-adrenergic agonist molecules

2.5.1 Clenbuterol

Clenbuterol is an illegal beta-adrenergic agonist for the use in meat producing animals because of its strong receptor affinity and its adverse effects on animals during the treatment period such as increased heart rates and reduced appetites and is therefore banned by the FDA and in the EU (Ricks *et al*., 1984; Spurlock *et al*., 1993). When administered orally, clenbuterol initiates increased muscle weight and a reduction in the carcass fat in production animals and increased weight gain and enhanced feed conversion occurred in some species (Ricks *et al*., 1984; Strydom *et al*., 2009). When measuring meat tenderness in Warner-Bratzler Shear Force (WBSF) an increase compared to the control, which was without any beta-adrenergic agonist supplementation, indicated that clenbuterol causes meat toughening (Mersmann, 1998).

2.5.2 Cimaterol

Cimaterol is another beta-adrenergic agonist that is banned for use in production animals because of its adverse effects on the animals as well as the residue levels in the meat that are potentially toxic for the consumer (Dikeman, 2007). The response to cimaterol is similar to that of clenbuterol, producing a heavier and leaner carcass, increasing the protein content and also reducing the time that the animal was required to spend in the feedlot. As was the case with clenbuterol, cimaterol causes meat toughening which is another negative attribute (Fiems *et al*., 1990; Schiavetta *et al*., 1990; Chikhou *et al*., 1993; Vestergaard *et al*., 1994).

2.5.3 Ractopamine hydrochloride

Ractopamine HCl has been registered as a beta-adrenergic agonist feed additive since 2000 in pig rations and 2003 in cattle rations by the FDA (Hossner, 2005a). In a feedlot study testing three beta-adrenergic agonists versus a control group showed ractopamine HCl and zilpaterol HCl to have improved feed conversion rates compared to the control, zilpaterol HCl and clenbuterol (Strydom *et al*., 2009). Ractopamine HCl increases ADG, FCR and produces heavier, yet leaner carcasses with improved dressing percentage over various livestock species (Apple *et al*., 2007; Dunshea *et al*., 2005; Dikeman, 2007). Several sources have noted an increase in meat toughness compared to animals who do not receive beta-

adrenergic agonist supplementation (Aalhus *et al*., 1990; Stites *et al*., 1991; Dunshea *et al*., 1993; Schroeder et al., 2003; Carr *et al*., 2005; Needham & Hoffman, 2015).

2.5.4 Zilpaterol hydrochloride (Zilmax®)

Zilpaterol hydrochloride (Zilpaterol HCl) with the formula $C_{14}H_{19}N_3O_2$.HCL and the molecular mass of 297,8 g/mol is a beta-agonist used in a total of 11 countries including South Africa, Mexico and the USA, (Avendaño-Reyes *et al*., 2006; Brooks *et al*., 2006; Shook *et al*., 2009; Reinhardt *et al*., 2014; NlH, 2021a). There have been a few different studies on using zilpaterol HCl in feedlot cattle that were done at the University of Pretoria (Morris, 1997; Steenekamp, 2014). Zilpaterol HCl has been approved for use for more than 25 years in South Africa (1995) and Mexico (1996) and was approved for use in USA feedlots by the FDA in 2006 (Avendaño-Reyes *et al*., 2006; Brooks *et al*., 2009; Shook *et al*., 2009). In a recent study on the characterization of β-adrenergic receptors in bovine intramuscular and subcutaneous adipose tissue, zilpaterol HCl had higher β_2-adrenergic receptor activity compared to a lower β_3-adrenergic receptor activity and negligible β_1-adrenergic receptor activity (Hwang *et al*., 2021).

During the summer the dry matter intake in feedlot cattle decreases slightly after the start of Zilmax feeding. After this depression in dry matter intake (DMI), some instances showed a return to intake levels and others observed a constant decreased intake rate (Reinhardt *et al*., 2014).

The use of zilpaterol HCl in feedlots improves the final body weight of the slaughtered animal, the carcass weights and lean carcass yield, increase the carcass protein and moisture, decrease carcass fat, improves average daily gain and feed conversion ratio meaning feed efficiency, dressing percentage and increases protein-to-bone ratio (Maritz, 1996; Morris, 1997; Vasconcelos *et al*., 2008; Elam *et al*., 2009; Hilton *et al*., 2009; Montgomery *et al*., 2009a; 2009b; Leheska *et al*., 2009).

Various sources concluded that the supplementation with zilpaterol HCl reduces meat tenderness by measuring an increase in the Warner-Bratzler Shear Force (WBSF) which is indicative of an increase in toughness of meat produced by zilpaterol treated steers (Strydom *et al*., 2002; Brooks *et al*., 2009; Kellermeier *et al*., 2011; Hope-Jones *et al*., 2010; Hope-Jones *et al*., 2012). This increase in meat toughness is caused be increased calpastatin activity and can be reduced by using electrical stimulation on the carcass during early rigor and triggering the calpains, improving the meat's tenderness (Hope-Jones *et al*., 2010). The meat toughening effect of Zilmax was not entirely eliminated by electrical stimulation; however, the effects were better than without electrical stimulation (Hope-Jones *et al*., 2010). According to numerous studies conducted in 2009 the aging of meat can reduce this toughening effect of Zilmax;

however, a study by Rathmann *et al*. (2009) stated that meat aged for 7 to 21 days did not reduce this toughening effect (Brooks *et al*., 2009; Holmer *et al*., 2009; Shook *et al*., 2009).

Zilmax has been shown to cause a reduction in the redness of meat and an increase in drip loss (Hope-Jones *et al*., 2012). Various sources have shown that Zilmax decreases the quality grade of the meat by decreasing the marbling score (Vasconcelos *et* al., 2008; Hilton *et al*., 2009; Montgomery *et al*., 2009b) and another study showed that Zilmax can negatively affect palatability traits of meat (Leheska *et al*., 2009).

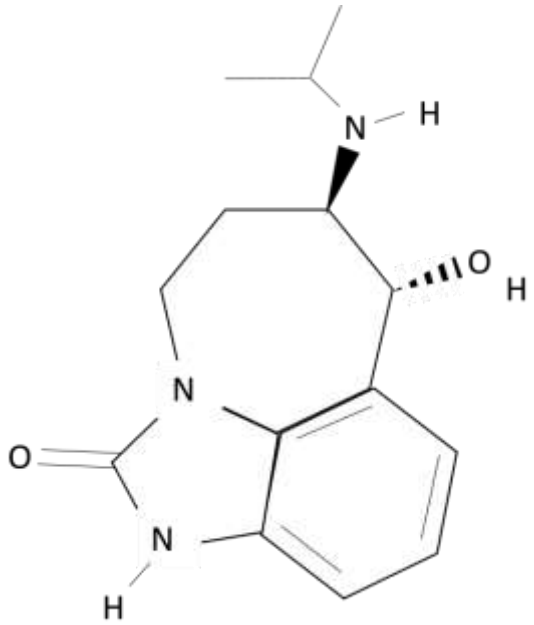

Figure 2.4 The chemical structure of Zilpaterol hydrochloride (Zilmax) adapted from PubChem (NlH, 2021a).

2.5.5 R-Salbutamol

R-Salbutamol is a predominantly β2-adrenergic agonist with the formula $C_{13}H_{21}NO_3$ and a molecular mass of 239,31 g/mol (Boulton & Fawcett, 2001; Anderson *et al*., 2005; NlH, 2021b). R-salbutamol was initially developed and used in the treatment of asthma in humans, acting as a bronchodilator as well as to suspend premature labour by being a uterine relaxant and is metabolised to an inactive metabolite predominantly by sulphotransferase (SULT) 1A3 (Boulton & Fawcett, 2001). R-salbutamol is derived through the purification of racemic (RS-) salbutamol which separates the two enantiomers, removing the unwanted enantiomer which causes unwanted effects (White et al., 1989; Ameredes & Calhoun, 2009; Marchant-Forde *et al*., 2012). The studies that were conducted by Oksbjerg *et al* (1996) were on finishing pigs, monogastric animals, and therefore processing the molecule differently to ruminants, more specifically feedlot cattle. There have not been as many studies conducted testing R-salbutamol on feedlot cattle compared to that in poultry and swine. Following this study by

Oksberg *et al* (1996), R-salbutamol was later used in lamb production (Bastos *et al*., 1997) and poultry production (Fawcett *et al*., 2004).

The study that was conducted by Steenekamp (2014) tested the effects that R-salbutamol had on growth performance and meat characteristics of feedlot cattle. This trial was conducted at the University of Pretoria, and it was reported that the addition of R-salbutamol did not induce apparent differences between growth performance characteristics. Steenekamp's trial tested the influence of R-salbutamol on the meat tenderness and found that R-salbutamol did not adversely affect meat tenderness which is a quality of other beta-agonists such as zilpaterol HCl, resulting in tougher meat (Steenekamp, 2014; Du Toit, 2017). This absence of the toughening effect might be explained by the higher incidence of lipolysis when R-salbutamol was administered compared to that of zilpaterol HCl which decreased fat synthesis and increased protein turnover (Steenekamp, 2014). In Steenekamp's trial however, the use of the electromagnetic gate feeders (Calan feeding gate system) resulted in low feed intake, this can be attributed to cattle being social feeders. Steenekamp's trial was conducted in an unexpectedly cold and rainy season, considering this along with bull variation and the low feed intake throughout the trial, there is a need for the trial to be reproduced in typical feedlot conditions on typical South African feedlot type cattle.

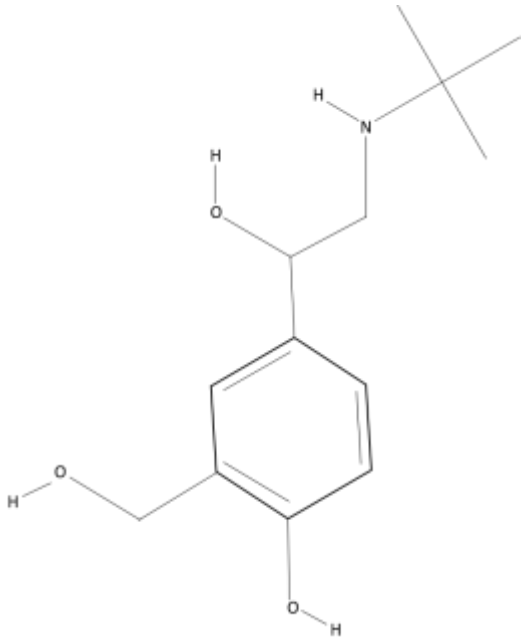

Figure 2.5 The chemical structure of R-salbutamol sulphate adapted from PubChem (NlH, 2021b).

2.6 How beta-adrenergic agonist supplementation differs between species

As mentioned previously, beta-agonist efficacy is dependent on the species in which it is activated (Mersmann, 1998). This could be as a result of multiple reasons including: the coupling of the signal transduction system to the agonist-receptor complex, differences in the β-agonist affinity for the receptor(s), as well as the factors that could influence the transport of the compound to the receptor sites (Mersmann, 1998). A certain species may have less receptors on a specific tissue that is targeted when compared to another species, resulting in a reduced beta-agonist response or that the receptor becomes inactive after a short length of time.

By using ligand binding researchers were able to determine the proportions of beta-receptor subtypes that are found in the tissue cells (Sillence & Mathews, 1994) and adipocytes (Sillence & Mathews, 1994; Van Liefde *et al*., 1994) of bovines as well as ovine adipocytes (Bowen *et al*., 1992) there are predominantly $beta_2$-receptors. In pharmacological and mRNA data for porcine adipocytes the results showed that over 70% of the receptor subtypes were $beta_1$-receptors (Mersmann, 2002). Therefore, due to the different receptor subtypes, the distribution of these receptor subtypes throughout tissue types and the pharmacological variation of the receptor subtypes, different species elicit varying responses in their tissues to beta-agonists.

Cattle, sheep and turkeys all respond well to beta-adrenergic agonists, with pigs having a slightly poorer response and chickens having the poorest response (Mersmann, 1998; Moody *et al*., 2000; Mersmann, 2002; Hossner, 2005). Due to genetic selection for maximum growth over many years, it is hypothesised that broiler chickens respond poorly to beta-adrenergic agonists in terms of growth for they are already growing at their biological peak (Mersmann, 1998; 2002). Pigs have also undergone intensive selection for leaner carcasses, meaning they are already lean and genetically more advanced compared to ruminants, having minimal subcutaneous fat and therefore making it difficult to detect differences in the fat deposition between the control versus beta-agonist-treated animals (Mersmann, 2002).

2.7 References

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Chapter 3. Methods and materials

3.1 Animals

The experimental animals were typical South African feedlot cattle (steers) obtained from Sparta Taaiboschbult Feedlot (North West, South Africa) at an average starting weight of 240 kg before entering the feedlot. The weaners were selected from a larger group, which ensured a uniform group of cattle which were compared, they all underwent backgrounding before entering the feedlot to further ensure uniformity between and within the treatment groups. Experimental animals were adapted and grown for 80 days in the feedlot and then stratified into the two treatment groups (R-salbutamol and zilpaterol hydrochloride). The animals' starting weights were stratified and then allocated into each of the experimental groups by placing animals of similar weights into each group, this was done to ensure that the starting weights of the two groups were similar for a completely impartial comparison of the two molecules. The animals were then randomly allocated into replicate groups. They were then fed the finishing phase diet for 30 days with the beta-agonists given as feed additives for the duration of the finishing period. The beta-agonists were withdrawn after 30 days and a 3-day withdrawal period on finishing feed was ensured before slaughter. The animals slaughter weights were measured before the 3-day withdrawal period.

3.2 Ethical approval

Ethical approval (EC-040/10) was obtained in 2016 when the trial took place and was granted by the Faculty of Natural and Agricultural Sciences and the Animal Ethics Committee from the Faculty of Veterinary Science, University of Pretoria. The procedures for the trial and the general animal husbandry were strictly managed in accordance with the research protocol and was conducted at Taaiboschbult Feedlot, a typical South African feedlot and the general health of the animals were monitored by Dr Shaun Morris (BVSc, MSc Agric). The feedlot manager monitored animal health daily through observations of the animals, identifying any potentially sick animals. During the trial the animals were monitored for possible diseases and after slaughter the lungs were inspected for adhesions and lesions, all of which came back negative.

3.3 Farm & farm conditions

The trial was conducted at Sparta Beef Taaiboschbult feedlot, located in the Northwest, R501, Potchefstroom, 2091 (-26.924506; 27.058669). The trial was conducted in August 2016, with the entire feedlot period taking place from June to August 2016. The weather conditions for June to August average 18°C - 22°C maximum daily temperatures, with between 5°C and 11°C minimum temperatures. During these months Potchefstroom

experienced an average of 1 - 2 rainy days, receiving between 2,12 – 5,12mm of rainfall per month, and humidity ranging between 35 - 47% (WWO, 2021). The trial was conducted during this particular period due to the climatic conditions being moderate and therefore ideal feedlotting conditions.

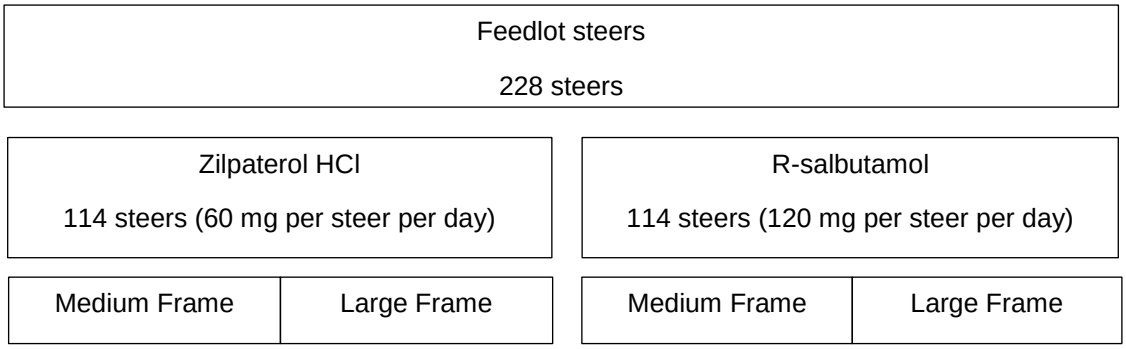
3.4 Experimental design

The trial compared two treatment groups of cattle, the first treatment group was given R-salbutamol (Salbutamate® 10%), the recently registered molecule compared with the beta-agonist that was first registered in South Africa and is the current industry standard, namely zilpaterol hydrochloride (Zilmax®) which will from henceforth be referred to as zilpaterol HCl. Therefore 114 steers were treated with 60 mg of zilpaterol HCL per steer per day and 114 steers were treated with 120 mg of R-salbutamol per steer per day for the last 30 days of the fattening phase and their subsequent growth and feedlot performance were recorded. To reduce the variation between animals, cattle of a similar maturity type were selected and randomly allocated to the experimental groups. 240 animals were acquired for the purpose of the study, however 12 animals were excluded from the study as they were excluded because they were outliers in terms of growth parameters at the end of the study before doing the analysis. The mean final weights were plotted, and a 95% confidence interval was obtained on all of the animals. There were 12 animals that fell above or below the confidence intervals and these were then removed from the study.

Each treatment group was split into smaller pens of 20 animals per pen which was for easy management and recording. The trial is based on a completely randomized control study (after stratifying the starting weights to ensure each of the treatment groups has similar mean starting weights), consisting of a 2 x 2 factorial experimental design, e.g. 2 treatment groups x 2 weight categories. The weight categories were only allocated after slaughter by using the CCW and they were allocated into medium and large carcasses.

Figure 3.1 provides an indication of how the treated animals were randomly allocated to each treatment group. The treatments were as follows:

1. Zilpaterol HCl treatment (60 mg per steer per day for 30 days; e.g. inclusion in feed at 105 g/ton of feed at a DM level of 90%).
2. R-salbutamol treatment (120 mg per steer per day for 30 days; e.g. inclusion in feed at 210 g/ton of feed at a DM level of 90%).



*Cattle were sorted into frame size at slaughter

Figure 3.1 Illustration of the allocation of treatments by each frame size and the number of animals in each group.

3.5 Feed and water

The animals were fed *ad libitum* feed and had constant access to fresh and clean water throughout the feedlot period.

Table 3.1 Trial feed ration for R-salbutamol versus zilpaterol HCl

Feed ingredient	**Kg/Feed wagon**
Sunflower/Oilcake 36% protein	40
Soya Oilcake	50
Eragrostis curvula/Smuts finger grass	80
Feed lime	30
Maize bran	60
Molasses	90
Hominy Chop	1380
Silage	486
Peanut Shell	60
Sunflower Hull	40
Water	53,35
Salt (dry)	10,51
Urea (dry)[1]	18,22
PREMIX T2 9029[2]	0,9
Rumensin20%	0,384
Salbutamate® 10%[3]	0,288
Tylan® Premix (Tylosin)	0,2256
	2400,02

[1] Added as 82,08 kg 22,2% urea and 12,8% salt

[2] Premix T2 9029 (FE6792)

[3] (130 mg/cattle at 10,77 kg feed intake) = (130/10,77) * 10 = 120,7 grams per ton of feed of Zilmax® 0,2592 kg per feed wagon

Table 3.2 Nutrient value of finisher feed ration fed to the steers

Nutrient	Amount
DM (%)	78,44
Daily feed intake (kg/day)	10,77
Dry Matter Intake (kg/day)	8,44
peNDF	13,54
EE (%DM)	6,44
CP (%)	14,84
Monensin (mg/day)	32,74
ME (MJ/kg)	11,30
Minerals and Vitamins	
Ca (g/kg)	5,98
P (g/kg)	3,80
K (g/kg)	8,90
Mg (g/kg)	2,32
S (g/kg)	0,80
Na (g/kg)	2,41
Cl (g/kg)	7,38
Fe (g/kg)	143,07
Zn (g/kg)	15,12
Co (g/kg)	0,09
I (g/kg)	0,004
Mn (g/kg)	19,99
Se (g/kg)	0,033
Cu (g/kg)	12,10
Vit-A (KIU)	0,3
Vit-D (KIU)	0,04
Vit-E (IU)	0,11

3.6 Growth and feedlot performance

The following parameters were recorded and will be statistically analysed:

Starting weight (kg) is measured at the end of the growing period and the start of the finishing period in the feedlot. This weight was also used to allocate animals into treatment groups, selecting animals of similar weight to try and eliminate variation caused by differing starting weights.

Slaughter weight (kg) is the weight of the feedlot steer measured after 30 days after the finishing feed and beta-adrenergic agonists were fed. The average final weight of Bonsmara

steers fed zilpaterol HCl during the finishing phase was 575,5 kg (Strydom *et al*., 2009) In another study, conducted in North America, the finishing steers were slaughtered at a final body weight of 614,0 kg (Baxa *et al*., 2004). It is important to note that once the slaughter weight is measured, the animals then still undergo 3 days of finisher feeding without any beta-agonist supplementation as a withdrawal period, the actual weight at slaughter is therefore not measured.

Live weight gain (kg) is measured by subtracting the starting weight from the slaughter weight of the live animal. This is the total weight that the animal gained during the finishing phase and is required in order to calculate the ADG during this period.

Average daily gain, ADG (kg/day) is calculated by dividing the total weight gain by the number of days (30 days). Feedlot records of ADG in the finishing phase only of beef feedlot have not been observed in South African feedlot studies, however North American studies observed AGDs of 1,27 kg/day when fed zilpaterol HCl for 30 days during the finishing phase (Elam *et al*., 2009). During the entire feedlot period of the Elam *et al*. (2009) study the ADG ranges from 1,27 kg/(steer/day) in the finishing phase to 1,89 kg/(steer/day) in the starter and grower phase, with an overall ADG of 1,66 kg/(steer/day).

The range for ADG should be approximately 2 kg/day during the growing phase and decreases to approximately 1,3 kg/day during the finishing phase.

Lean gain (kg) is a calculated amount that measures the amount of weight gain (kg) that is muscle weight (weight gained minus the gut fill). The calculation was done as follows: Lean gain (kg) = [Percentage muscle (%) x Live weight gain (kg)] / 100. Lean gain is an estimation of the muscle added onto the carcass during the finishing period and is a notable factor for the overall income for the feedlot.

3.7 Carcass characteristics measured at the abattoir

Warm carcass weight (WCW) is measured by weighing the carcass directly after slaughter. Cold carcass weight (CCW) is measured after evaporation loss occurs while the carcass is chilled in cold storage. The carcasses can be categorised into light (200 - 225 kg), medium (226 - 275 kg) and heavy (275 - 350 kg) carcasses in South African abattoirs (Webb & Agbeniga, 2020). The carcasses were then grouped according to the above unofficial categories for the purpose of investigating the effects of the beta-agonist treatments on medium and heavy carcasses. The average ‘final carcass weight (kg)’ for zilpaterol HCl fed Bonsmara feedlot steers was measured as 353,5 kg (Strydom et al., 2009). In the North American study the Hot Carcass Weight (HCW) after 30 days of feeding zilpaterol HCl was measured to be 405,6 kg (Elam *et al*., 2009). In a more recent South African study on

Bonsmara steers, those fed zilpaterol HCl obtained average carcass weights of 311 kg (Moholisa *et al*., 2018).

Carcass length (cm) is measured from the caudal edge of the final sacral vertebra to the dorso-cranial edge of the atlas (Webb, 1992). The carcass length can be categorised into three groups short (106 - 114 cm), intermediate (114 - 122 cm) and long (122 - 130 cm) which is indicative of the cattle frame size and therefore maturity type (Berry *et al*., 1973).

Fat thickness (mm) is a measure of the subcutaneous fat thickness is measured at the 13th rib. Carcass fattening is the most vital factor in the production of meat yield for sale. If carcass weight is kept constant and there in an increase in fat thickness the result is that there is a decrease in percentage edible meat and the percentage of expensive meat in the carcass. In the study on Bonsmara steers, conducted by Strydom *et al*. (2009), the adjusted fat thickness (cm) for zilpaterol HCl fed steers was measured as 0,91 cm.

Dressing percentage was calculated as the warm carcass weight (WCW) divided by the live weight of the animal at slaughter (excluding gut fill) and expressing this value as a percentage. Cold dressing % is a measured value.In the study by Strydom et al. (2009) the Bonsmara feedlot steers that were fed zilpaterol HCl during the finishing phase produced a dressing percentage of 61,4%. In a North American study the dressing percentage of feedlot cattle fed zilpaterol HCl for 30 days was 65,03% (Elam *et al*., 2009).

Age code is one aspect of the South African Beef Classification System that sorts carcasses into four age groups as follows: Age Class A (no permanent incisors), Age Class AB (1 – 2 permanent incisors), Age Class B (3 – 6 permanent incisors) and Age Class C (more than 6 permanent incisors) (Meat Classification Regulation No. 863 in Government Gazzette, September 2006).

Fat code is another aspect of the South African Beef Classification System that sorts carcasses into seven fat classes which are grouped as follows: 0 (no visible fat), 1 (very lean; 0 – 1 mm fat thickness measured between the 10th and 11th ribs. 50 mm from the median line of the cold, non-quartered carcasses), 2 (lean; 1 – 3 mm), 3 (medium; 3,1 – 5 mm), 4 (fat; 5,1 – 7 mm), 5 (moderately over fat; 7,1 – 10mm) and 6 (excessively over fat; >10mm) (Meat Classification Regulation No. 863 in Government Gazzette, September 2006).

Carcass conformation score is determined by the visual assessment of the soft tissue component of the carcasses with similar fatness score. Carcass conformation score is classified according to the South African Beef Classification System into the classes 1 (very flat), 2 (flat), 3 (medium), 4 (round) or 5 (very round) (Meat Classification Regulation No. 863 in Government Gazzette, September 2006). It also gives a good indication of muscle thickness

within carcasses that have the same fatness score. It was traditionally accepted that conformation score was a good indication of saleable meat yield (percentage expensive cuts) however fat code is the most important factor to determine this.

Percentage muscle was calculated using a regression equation that was formulated in a previous study (Steenekamp, 2014). The equation is as follows: Percentage muscle = [55,515 – (0,897 x Subcutaneous fat thickness) – (0,022 x Slaughter weight) + (0,332 x Cold Dressing %)].

Carcass compactness is a calculated amount that is obtained by dividing the CCW by the carcass length (Webb, 1992).

3.8 Statistical analysis

The data was stored in excel and transferred to IBM SPSS Statistics 27. The data was then tested for normal distribution and frequency distributions were conducted. Thereafter the effect of treatment on the measured variables, with starting weight included as a covariate, was analysed through a General Linear Model procedure using IBM SPSS Statistics 27. This data was analysed using $P < 0,05$ and $P < 0,01$ significance levels.

The results were analysed by means of multivariate analysis of variance (MANOVA) and the least square mean values (LSMeans) ± standard deviation (SD) are depicted in Table 4.1 below. Although animals were stratified according to initial weights before the trial commenced and then randomized into experimental groups for the duration of the trial. Starting weight was included as a covariate to further increase the accuracy of the analysis and therefore the levels of significance. By including starting weight as a covariate the significance levels were increased to $P < 0,01$ compared to $P < 0,05$ when starting weight was not included as a covariate in the analysis. Even though animals are allocated randomly to the experimental groups there were still small numerical differences between the average starting weights. In order to correct these very small differences from a statistical point of view a covariant was included. The starting weight was recorded after steers spent 80 days on the feedlot diet and before feed additives were given during the finisher phase, i.e. this specific trial only reports on the finishing phase of the feedlot. Slaughter weight is the weight of the steers after 30 days of treatment with R-salbutamol or zilpaterol HCl and after the 3-day withdrawal period.

The fat code and carcass classification were evaluated by conducting a Chi-square analysis using a $P < 0,05$ level of significance.

The means of carcasses were separated into medium (240 - 320 kg) and large (321 - 410 kg) weight categories by CCW. This was done to determine the influence of carcass weight or size on the R-salbutamol and zilpaterol HCl experimental groups for each factor. The comparisons

between means were done by means of Bonferroni multiple range tests because the number of observations (n) differed between experimental groups, hence the necessity for this statistical method of multiple comparisons.

3.9 References

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Chapter 4. Results and discussion

4.1 Effects of zilpaterol HCl and R-salbutamol on growth and feedlot performance

4.1.1 Results

Starting weight is measured at the end of the growing phase and at the beginning of the finishing phase, where slaughter weight is measured at the end of the finishing period, before the 3-day beta-agonist withdrawal period. Live weight gain is the difference between the slaughter weight and starting weight, e.g. the weight gained during the trial duration. ADG (meaning average daily gain, will be from henceforth referred to as ADG) is the live weight gain divided by the number of days (30 days). Lean gain (kg) is a calculated amount that measures the amount of weight gain (kg) that is muscle weight (weight gained minus the gut fill).

Table 4.1 Growth and feedlot performance (LS Means ± s.e.) of feedlot steers fed 60 mg zilpaterol HCl per steer per day versus fed 120 mg R-salbutamol per steer per day

	R-salbutamol (n = 114)	Zilpaterol HCl (n = 114)	Pooled average (n = 228)
Starting weight (kg)	460,2 ± 44,15	467,6 ± 47,37	463,9 ± 45,84
Slaughter weight (kg)	494,1 ± 47,67^{A}	506,6 ± 48,94^{B}	500,4 ± 48,61
Live weight gain (kg)	33,9 ± 15,11^{A}	39,0 ± 14,16^{B}	36,5 ± 14,83
ADG (kg/day)	1,1 ± 0,50^{A}	1,3 ± 0,47^{B}	1,2 ± 0,49
Lean gain (kg)	19,7 ± 8,84^{A}	22,7 ± 8,43^{B}	21,2 ± 8,75

$^{a, b}$ Each superscript is significantly different at a 95% level of significance (P <0,05)
$^{A, B}$ Each superscript is significantly different at a 99% level of significance (P <0,05)

The data in Table 4.1 was analysed statistically by doing a MANOVA with P <0,05 and P <0,01 for significant differences. There were differences found between the means for the experimental groups for slaughter weight, live weight gain, ADG and lean gain, which will be discussed individually in the section below.

4.1.2 Discussion

a) The effect of the beta-agonist treatments on starting weight

The starting weight (kg) of steers fed R-salbutamol (460,2 kg) was not significantly different compared to that of the experimental group fed zilaterol HCl (467,6 kg), however because there was a numerical difference of 7,4 kg between the experimental groups, starting weight was included as a covariate in the analysis. The beta-agonist treatments were only

administered after the starting weight was measured; thus they had no influence on the starting weights. By comparing the pooled average (463,9 kg) of the mean starting weights with that of a South African feedlot study, feedlot steers enter the finishing phase at approximately 500 kg (Strydom *et al*., 2009).

b) The effect of the beta-agonist treatments on slaughter weight

The mean slaughter weight of R-salbutamol fed steers (494,1 kg; *P* <0,01) is significantly lower than zilpaterol HCl fed steers (506,6 kg). The trial had a mean slaughter weight of 500,4 kg. The final body weight of steers two North American studies were slaughtered at mean slaughter weights of 565,0 kg (Montgomery *et al*., 2008) and 614,0 kg (Baxa *et al*., 2004). These studies produced much higher slaughter weights compared to the presented study. In a South African study, the final weight of zilpaterol HCl treated steers was 575,5 kg (Strydom *et al*., 2009). The lower slaughter weights in the presented trial are around 60 kg less compared to the trials conducted between 2004 and 2009, which could be due to the demand for leaner carcasses and therefore less fat on the carcass (Webb & Erasmus, 2013). The mean slaughter weight of another South African study of steers fed zilpaterol HCl 535 kg which is slightly higher compared to the presented study (Moholisa *et al*., 2018).

c) The effect of the beta-agonist treatments on live weight gain

Measured by subtracting starting weight from slaughter weight. The live weight gain of R-salbutamol fed steers (33,9 kg; *P* <0,01) was significantly lower than the live weight gain of zilpaterol HCl fed steers (39,0 kg). The zilpaterol HCl experimental group gained on average 5,1 kg more than the R-salbutamol treatment group during the final 30 days of the finishing period. Therefore zilpaterol HCl has more of abeta-agonist effect in terms of increasing live weight gain during the finishing phase. Most feedlot studies measure live weight gain from the beginning of the feedlot period after adaptation from backgrounding. In the North American study by Elam *et al*. (2009), during the final 50 days on feed before slaughter, including 30 days of zilpaterol HCl feeding, the live weight gain was 57,7 kg (624,1 – 566,4 kg).

d) The effect of the beta-agonist treatments on ADG

Zilpaterol HCl treated steers (1,3 kg/day; *P* <0,05) had a significantly higher mean ADG (kg/day) during the finishing phase compared to R-salbutamol fed steers (1,1 kg/day). In two North American feedlot studies, the ADGs measured feeding zilpaterol HCl for 30 days and 50 days respectively as well as a 5 days withdrawal were 1,59 kg/day (Montgomery *et al*., 2008) and 1,29 kg/day (Elam *et al*., 2009).

e) The effect of the beta-agonist treatments on lean gain

Lean gain (kg) of steers fed zilpaterol HCl (22,7 kg; *P* <0,01) was significantly higher compared to that of steers fed R-salbutamol (19,7 kg). The value of lean gain is a calculated estimate and is notable as an important factor affecting the income of the feedlot due to muscle gain in the finishing phase contributing towards the carcass weight at slaughter. . The mean lean gain of zilpaterol HCl treated steers was 3 kg higher compared to R-salbutamol treated steers.

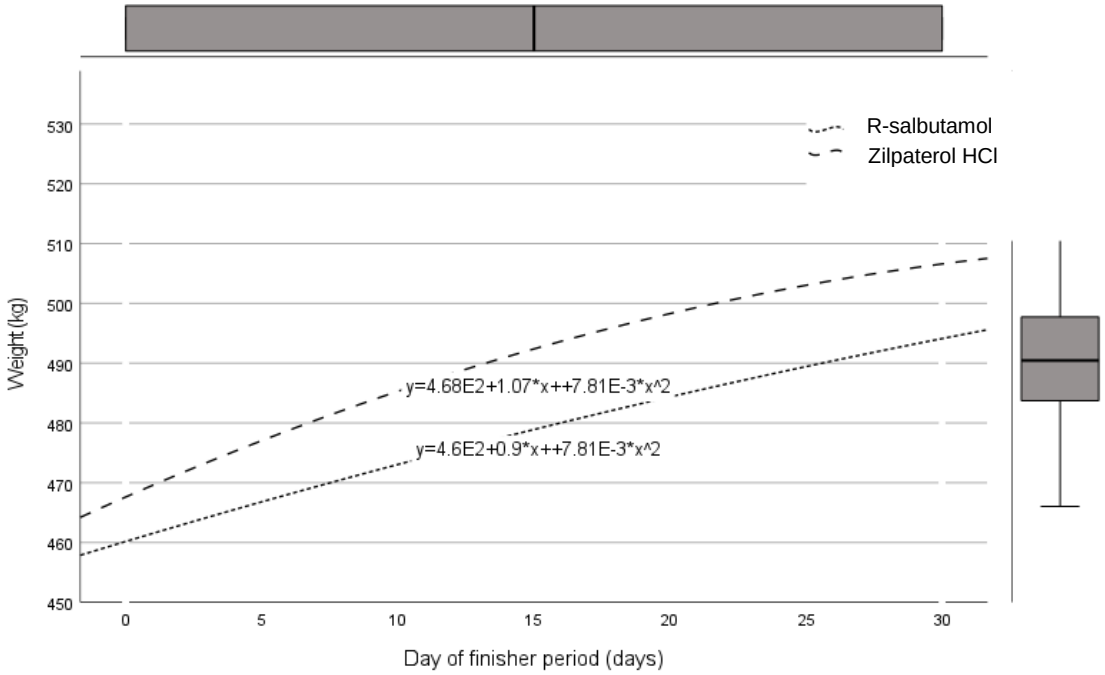

Figure 4.1 The regression of the effect of beta-agonist treatment on growth (weight gain) over the finisher period

The graph in Figure 4.1 is a regression of weight gain over the time during the finisher period which illustrates the higher growth response in cattle supplemented with zilpaterol HCl compared to R-salbutamol as depicted in Table 4.1. R-squared value is 0,128 and *P* <0.000.

4.2 Effects of the interaction between beta-agonist treatment and weight category on growth and feedlot performance

The comparisons between means were done by means of Bonferroni multiple range tests because the number of observations (n) differed between experimental groups, hence the necessity for this statistical method of multiple comparisons. In Table 4.2 the carcasses were separated into medium (240 - 320 kg) and large (321 - 410 kg) weight categories by CCW. This was done to determine the influence of carcass weight or size on the R-salbutamol and zilpaterol HCl experimental groups for each factor.

Table 4.2 Growth and feedlot performance (LS Means ± s.e.) of feedlot steers at different weight categories fed 60 mg zilpaterol HCl per steer per day versus 120 mg R-salbutamol per steer per day

	R-salbutamol		Zilpaterol HCl	
	Medium (n = 75)	Large (n = 39)	Medium (n = 58)	Large (n = 56)
Slaughter weight (kg)	468,0 ± 31,05[a]	544,5 ± 30,46[b]	470,0 ± 27,80[a]	544,5 ± 35,30[b]
Live weight gain (kg)	32,0 ± 14,35[a]	37,6 ± 16,0[a]	36,3 ± 19,6[a]	41,8 ± 14,35[b]
ADG (kg/day)	1,1 ± 0,48[a]	1,3 ± 0,53[ab]	1,2 ± 0,54[ab]	1,4 ± 0,49[b]
Lean gain (kg)	18,7 ± 8,38[a]	21,7 ± 9,44[ab]	21,5 ± 8,22[ab]	24,0 ± 8,54[b]

[a, b] Each superscript is significantly different at a 95% level of significance (*P* <0,05)

The mean slaughter weight (kg) of medium-framed cattle fed zilpaterol HCl (470,0 kg) was similar to the slaughter weight of medium-framed steers fed R-salbutamol (468,0 kg). Similarly, the mean slaughter weight of the large-framed cattle fed zilpaterol HCl (544,5 kg) was similar to that of the large-framed cattle fed R-salbutamol (544,5 kg). Both the medium-framed groups fed R-salbutamol (468,0 kg) and zilpaterol HCl (470,0 kg; *P* <0,05) are significantly lower compared to the large-framed groups fed R-salbutamol (544,5 kg) and zilpaterol HCl (544,5 kg).

Larger-framed cattle fed zilpaterol HCl gained significantly more weight (live weight gain) (*P* <0,05) during the finishing period compared to cattle in the larger-framed cattle fed R-salbutamol and both medium-framed cattle fed zilpaterol HCl or R-salbutamol. This may be due to the fact that such steers were of the later physiologically maturing type, with more physiological capacity to grow more efficiently to a heavier weight as previously reported (Webb & Casey, 2010). The live weight gain observed by medium-framed cattle fed R-salbutamol was similar to that of medium-framed cattle fed zilpaterol HCl.

Larger-framed steers in the zilpaterol HCl experimental group had a significantly higher ADG (1,4 kg/day; *P* <0,05), compared to medium-sized steers treated with R-salbutamol (1,1 kg/day). Although the interaction between the weight category and beta-agonist treatment was not significant in terms of ADG, it was evident from the data in tables 4.1 and 4.2 that zilpaterol HCl treatment resulted in higher mean ADG's (*P* <0,05) when compared to R-salbutamol treated steers.

The mean lean gain (kg) of medium-framed steers fed zilpaterol HCl (21,5 kg) was similar to that of medium-framed steers fed R-salbutamol (18,7 kg), large-framed steers fed zilpaterol HCl (21,7 kg) had a similar mean lean gain to large-framed steers fed R-salbutamol (18,7 kg).

Mean lean gain was significantly higher for larger size steers treated with zilpaterol HCl (24,0 kg; *P* <0,05), compared to medium-sized steers treated with R-salbutamol. Frame size does not have an interaction with the beta-agonist treatment in terms of lean gain, however the zilpaterol HCl fed steers (22,7 kg) did have a significantly higher mean lean gain compared to those fed R-salbutamol (19,7 kg; *P* <0,05) in Table 4.1.

4.3 Effects of zilpaterol HCl and R-salbutamol on carcass characteristics

4.3.1 Results

The following results in Table 4.3 of the carcass characteristics were analysed through a MANOVA with 95% and 99% confidence intervals. WCW is the warm carcass weight after slaughter. CCW is the cold carcass weight which is weighed after the carcass lost any fluids through evaporation. Both hot and cold dressing % were measured values, i.e. cold dressing % was not a calculated value.

Table 4.3 Carcass characteristics (LS Means ± s.e.) of feedlot steers fed 60 mg zilpaterol HCl per steer per day versus fed 120 mg R-salbutamol per steer per day

	R-salbutamol (n = 114)	Zilpaterol HCl (n = 114)	Pooled average (n = 228)
WCW (kg)	313,7 ± 31,47^{A}	325,3 ± 34,23^{B}	319,5 ± 33,32
CCW (kg)	307,4 ± 30,84^{A}	318,8 ± 33,55^{B}	313,1 ± 32,66
Carcass length (cm)	122,4 ± 13,71^{A}	123,0 ± 4,61^{B}	122,7 ± 10,21
Fat thickness (mm)	8,0 ± 4,84	7,6 ± 4,56	7,8 ± 4,70
Hot Dressing %	63,5 ± 2,09^{A}	64,2 ± 1,70^{B}	63,8 ± 1,93
Cold Dressing %	62,2 ± 2,05^{A}	62,9 ± 1,67^{B}	62,6 ± 1,90
Percentage Muscle	58,2 ± 4,60^{A}	58,4 ± 4,20^{B}	58,3 ± 4,40
Carcass compactness	2,7 ± 2,14	2,6 ± 0,20	2,6 ± 1,52

$^{a, b}$ Each superscript is significantly different at a 95% level of significance (*P* <0,05)
$^{A, B}$ Each superscript is significantly different at a 99% level of significance (*P* <0,01)

In Table 4.3 the data was again analysed through a MANOVA with *P* <0,05 and *P* <0,01 for significant differences. There were significant differences found between the means for the experimental groups for WCW, CCW, carcass length, hot dressing %, cold dressing % and percentage muscle, each will be discussed separately below.

4.3.2 Discussion

a) The effect of the beta-agonist treatments on WCW and CCW

The MANOVA showed a that the differences between the WCWs and the CCWs are highly significant (*P* <0,05), the R-salbutamol fed steers (307,4 kg) had significantly lower CCWs compared to zilpaterol HCl fed steers (318,8 kg). In comparative South African feedlot studies, steers fed zilpaterol HCl slaughtered at cold carcass weights ranging from 311 kg (Moholisa et al., 2018) to 353,5 kg (Strydom et al., 2009). The result of the mean CCW for zilpaterol HCl fed steers is within this range at 318,8 kg, however the mean CCW for R-salbutamol fed steers is slightly below this range at 307,4 kg.

b) The effect of the beta-agonist treatments on carcass length

When carcass length was analysed, there was a significant difference (*P* <0,01) between the two treatment groups. Zilpaterol HCl fed steers (123,0 cm) measured mean carcasses that were 0,6 cm longer than R-salbutamol fed steers (122,4 cm).

c) The effect of the beta-agonist treatments on fat thickness

The subcutaneous fat thickness did not differ significantly between the R-salbutamol (8,0 mm) and zilpaterol HCl (7,6 mm) experimental groups. The pooled average of the fat thickness for the trial was 7,8 mm. The adjusted fat thickness for zilpaterol HCl fed steers in a Bonsmara beta-agonist feedlot study measured a fat thickness of 0,91 cm (Strydom *et al*., 2009). A reason for the thinner carcasses obtained compared to the Strydom et al. (2009) study could be that the modern consumer tends towards, i.e. less fat on the carcass (Webb & O'Neill, 2008; Webb & Erasmus, 2013; Webb, 2015).

d) The effect of the beta-agonist treatments on dressing percentage

The hot dressing % of R-salbutamol fed steers (63,5%) was significantly lower (*P* <0,01) compared to zilpaterol HCl fed steers (64,2%). In a study by Montgomery *et al*., (2008), the hot dressing % for steers fed zilpaterol HCl for 35 days during the finisher period was 66%. The higher dressing % in the Montgomery *et al*., (2008) study compared to the presented trial is likely due to the longer feeding of zilpaterol HCl and the trial being conducted in North America and on American feedlot cattle. In a South African study, Bonsmara feedlot steers were fed zilpaterol HCl during the finishing phase for 30 days and produced a hot dressing % of 61,4% (Strydom *et al*., 2009). The presented study resulted in a higher dressing % compared to Strydom *et al*. (2009) which is likely due to feedlots aiming for higher dressing percentages, however still producing a leaner carcass at lower costs (Webb & Erasmus, 2013; Webb, 2015). In a North American study the dressing % of feedlot cattle fed zilpaterol HCl for 30 days was

65,03% (Elam *et al*., 2009). The cold dressing % of R-salbutamol fed steers (62,2%) was significantly lower (*P* <0,01) than that of the zilpaterol HCl fed steers (62,9%).

The observed higher efficiency of zilpaterol HCl compared to R-salbutamol may be associated with the findings by Timmerman (1987), about differences in half-life and receptor sensitivity of different beta-adrenergic agonists. R-salbutamol appears to be faster acting, with a shorter half-life compared to zilpaterol HCl, which may result in receptor downregulation earlier on in the finishing phase, resulting in a slightly lower growth response.

e) The effect of the beta-agonist treatments on percentage muscle

Even though numerically the difference between percentage muscle is only 0,2% (*P* <0,01), there was a significant difference between the R-salbutamol fed steers (58,2%) and the zilpaterol HCl fed steers (58,4%). Zilpaterol HCl treated animals had a higher percentage muscle, this is in line with the higher dressing percentages, carcass weights, live weight gain and lean gain. Because percentage muscle is a calculated amount from the regression equation obtained with Steenekamp's (2012) data, there is no literature to support or compare the presented studies results.

4.3.3 More results and discussions

Table 4.4 Effects of R-salbutamol and zilpaterol HCl treatments on carcass fat classification

			R-salbutamol (n = 114)	Zilpaterol HCl (n = 114)	Total (n = 228)
Fat code	2	Count	83	94	177
		% within Fat code	46,9%	53,1%	100,0%
		% within Treatments	72,8%	82,5%	77,6%
		% of Total	36,4%	41,2%	77,6%
	3	Count	24	17	41
		% within Fat code	58,5%	41,5%	100,0%
		% within Treatments	21,1%	14,9%	18,0%
		% of Total	10,5%	7,5%	18,0%
	4	Count	7	3	10
		% within Fat code	70,0%	30%	100,0%
		% within Treatments	6,1%	2,6%	4,4%
		% of Total	3,1%	1,3%	4,4%

[a, b] Each superscript is significantly different at a 95% level of significance (*P* <0,05)

In Table 4.4 there was no significant difference between the carcass fat classification of the beta-agonist treatments. What Table 4.4 does show is that there was a higher proportion of A2 carcasses (77,6%), then A3 carcasses (18,0%) and with the smallest proportion of A3

carcasses (4,4%). This shows that there was a tendency towards leaner carcass, regardless of the beta-agonist treatment used, which is in line with consumer preferences of a lean carcass and less fatty meat (Higgans, 2004). In feedlot cattle, particularly during the finishing phase there is a risk of carcasses exceeding fat code 2, however the use of beta-agonists aids in reducing maintaining lower carcass fat content, therefore yielding more fat code 2 carcasses. In a recent study that compared the fat codes of steers that weren't fed a beta-agonist (fat codes 3 (67%); 4 (33%)) with those fed zilpaterol HCl (fat codes 2 (13%); 3 (87%)) for 30 days during the finishing phase, the fat codes for zilpaterol HCl fed steers were leaner compared to those that weren't (Moholisa *et al*., 2018). The presented results indicate that there was no significant difference between the fat code of animals treated with different types of beta-agonists, thus in this study carcass fat classification was not affected by the type of beta-agonist treatment, however both beta-agonists resulted in higher proportions of A2 carcasses, thus leaner carcasses and a higher price paid for those carcasses compared to A3/A4 carcasses.

Table 4.5 Effect of R-salbutamol and zilpaterol HCl treatments on carcass conformation classification

			R-salbutamol	Zilpaterol HCl	Total
Carcass classification	3	Count	44[a]	22[b]	66
		% within Carcass classification	66,7%	33,3%	100,0%
		% within Treatments	38,6%	19,3%	28,9%
		% of Total	19,3%	9,6%	28,9%
	4	Count	65[a]	85[b]	150
		% within Carcass classification	43,4%	56,7%	100,0%
		% within Treatments	57,0%	74,6%	65,8%
		% of Total	28,5%	37,3%	65,8%
	5	Count	5	7	12
		% within Carcass classification	41,7%	58,3%	100,0%
		% within Treatments	4,4%	6,1%	5,3%
		% of Total	2,2%	3,1%	5,3%

[a, b] Each superscript is significantly different at a 95% level of significance (P <0,05)

In Table 4.5 the results of carcass conformation classification were that there were significantly (P <0,05) more 4 carcasses in the zilpaterol HCl experimental groups compared to those in the R-salbutamol experimental groups. The carcasses in the zilpaterol HCl group are more consistently 4 (56,7%) compared to that of R-salbutamol, this could be because of

the differences in beta$_2$-agonist receptor effect in the two different molecules however literature on the specific receptor makeup of each molecule could not be found.

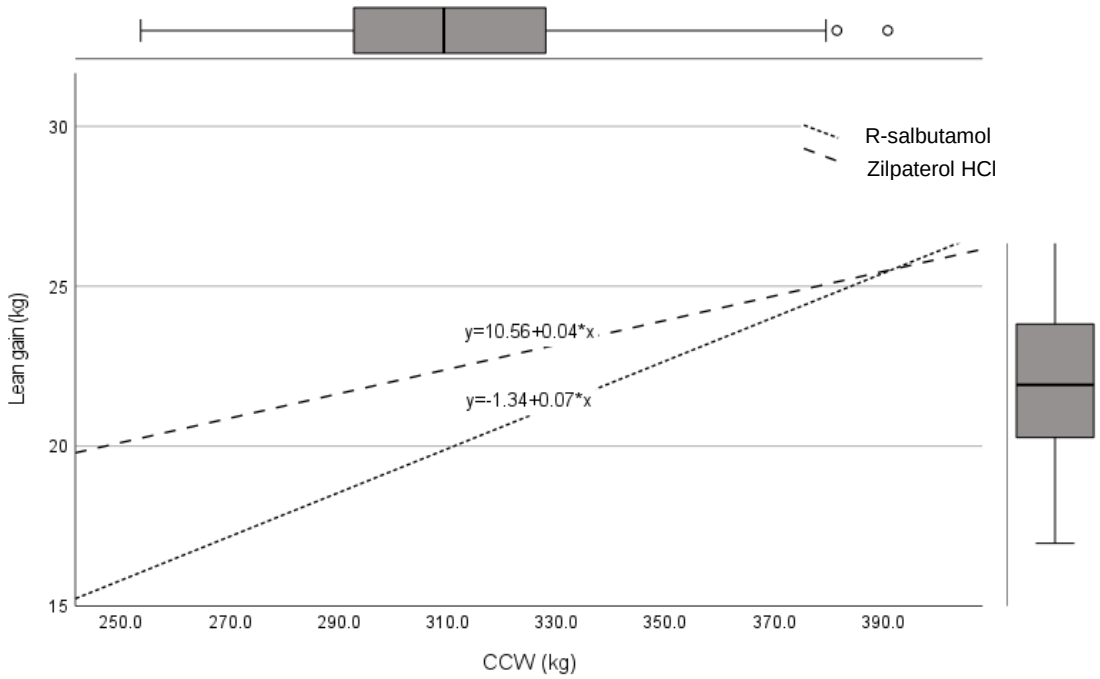

Figure 4.2 The regression of the effect of beta-agonist treatment on lean gain (kg) over the measured CCW (kg)

Figure 4.2 is a representation of the regression of lean gain over the measured CCW in steers treated with R-salbutamol and Zilpaterol HCl, which clearly shows a higher lean gain of steers in the zilpaterol HCl experimental group. This graph gives the regression equations for the lean gains of each of the treatment groups. R-squared value is 0,044 and *P* <0,001**.**

4.4 Effects of the interaction between beta-agonist treatment and weight category on carcass characteristics

Table 4.6 Carcass characteristics (LS Means ± s.e.) of feedlot steers at different weight categories fed 60 mg zilpaterol HCl per steer per day versus fed 120 mg R-salbutamol per steer per day

	R-salbutamol		Zilpaterol HCl	
	Medium (n = 75)	Large (n = 39)	Medium (n = 58)	Large (n = 56)
WCW (kg)	295,6 ± 19,0[a]	348,5 ± 18,92[b]	298,6 ± 18,95[a]	352,9 ± 22,57[b]
CCW (kg)	289,7 ± 18,65[a]	341,5 ± 18,55[b]	292,6 ± 18,58[a]	345,9 ± 22,12[b]
Carcass length (cm)	120,0 ± 16,21[a]	126,9 ± 3,81[b]	120,1 ± 3,36[a]	126,1 ± 3,68[b]
Fat thickness (mm)	8,4 ± 5,04	7,3 ± 4,41	7,3 ± 3,98	8,0 ± 5,1
Hot dressing %	63,2 ± 2,03[a]	64,0 ± 2,12[ab]	63,5 ± 1,46[a]	64,8 ± 1,70[b]
Cold dressing %	62,0 ± 1,99[a]	62,8 ± 2,09[ab]	62,3 ± 1,43[a]	63,5 ± 1,67[b]
Percentage muscle	58,3 ± 4,80	57,9 ± 4,36	59,3 ± 3,55	57,5 ± 4,69
Carcass compactness	2,7 ± 2,64	2,7 ± 0,13	2,4 ± 0,14	2,7 ± 0,14

[a, b] Each superscript is significantly different at a 95% level of significance (P <0,05)

The comparisons between means were done by means of Bonferroni multiple range tests because the number of observations (n) differed between experimental groups, hence the necessity for this statistical method of multiple comparisons.

There was no significant difference between the WCWs of medium-framed feedlot steers fed zilpaterol HCl (298,6 kg) and those fed R-salbutamol (295,6 kg). There was also no significant difference between the WCWs of large-framed steers fed zilpaterol HCl (352,9 kg) and those fed R-salbutamol (348,5 kg). There is therefore no interaction between the frame-size and the beta-agonist treatment in terms of warm carcass weight.

The cold carcass weights of medium-framed cattle fed zilpaterol HCl (292,6 kg) and R-salbutamol (289,7 kg) did not differ significantly. Again, there was no significant difference between the CCWs of large-framed cattle fed zilpaterol HCl (345,9 kg) and R-salbutamol (341,5 kg). The only significant differences (P <0,05) between CCWs were those of different frame-size groups which was to be expected and doesn't show an interaction between the frame-size and the beta-agonist treatment in terms of CCW.

As was observed in both the warm and cold carcass weights, there was no significant difference between carcass lengths of the medium-framed groups of steers that were fed zilpaterol HCl (120,1 cm) and R-salbutamol (120,0 cm). There was no significant difference

between the carcass lengths of the large-framed groups of steers that were fed zilpaterol HCl (126,1 cm) and R-salbutamol (126,9 cm). There were significant differences (P <0,05) between the different frame-size groups, but as mentioned for CCW, this is to be expected and there is no interaction between the frame-size and the beta-agonist treatment in terms of carcass length.

There is no significant difference in fat thickness (cm) between any of the frame-size groups. Therefore there is no interaction between the frame-size and the beta-agonist molecule in terms of the fat thickness.

Dressing percentage observed no significant difference between the medium-framed steer groups, with zilpaterol HCl (63,5%) fed steers recording similar cold dressing % to R-salbutamol fed steers (62,0%). The medium-framed steer groups observed similar cold dressing percentages to the large-framed R-salbutamol fed steers (62,8%), however the medium-framed steers had significantly lower dressing percentages compared to large-framed zilpaterol HCl fed steers (64,8; P <0,05). There might be a slight interaction between the frame-size and beta-agonist in terms of dressing percentage, however it was not significant when comparing only medium- with medium-framed R-salbutamol fed steers and large- with large-framed steers.

The percentage muscle in each of the groups had no significant difference between any of the frame-size groups and the same was observed for carcass compactness.

4.5 References

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Chapter 5. Conclusion and Critical evaluation

5.1 Conclusion

This study aimed to compare the effects of R-salbutamol versus zilpaterol HCl on the growth and feedlot performance of typical South African feedlot steers during the finisher phase. The results of this trial as summarised in Table 4.1, were that zilpaterol HCl fed steers had an overall higher finisher phase live weight growth performance compared to the experimental group of steers that was fed with R-salbutamol. Steers fed zilpaterol HCl had a 12,5 kg (P < 0,05) higher average slaughter weight during the finisher phase compared to the experimental group that was treated with R-salbutamol. Zilpaterol HCl fed steers gained an additional 5,1 kg (P <0,05) of carcass weight and had a higher ADG of 1,3 kg/day compared to the low ADG of 1,1 kg/day that was achieved by R-salbutamol fed steers. The mean lean gain of zilpaterol HCl fed steers was 3 kg higher compared to R-salbutamol fed steers. The slaughter weights, live weight gain and ADG of the feedlot steers fed zilpaterol HCl outperformed R-salbutamol fed steers.

The interaction of beta-agonist treatment with frame-size resulted in larger live weight gains of larger-framed zilpaterol HCl steers compared to the other categories including the larger-framed R-salbutamol fed steers (as seen in Table 4.2), however other than live weight gain the growth and feedlot performance characteristics were not significantly different for the same weight category in different experimental groups.

This study also aimed to compare the effects of R-salbutamol versus zilpaterol HCl on carcass characteristics of typical south African feedlot steers during the finisher phase. The results (as seen in Table 4.3) showed that zilpaterol HCl again outperformed R-salbutamol in terms of carcass characteristics. The difference between the mean CCW of zilpaterol HCl treated steers versus R-salbutamol treated steers was 11,4 kg (P <0,05), which will have a large financial impact. Zilpaterol HCl treated carcasses were on average 0,6 cm (P <0,05) longer compared to R-salbutamol treated carcasses. Percentage muscle was also significantly higher for zilpaterol HCl treated carcasses. Therefore the carcass weights, carcass length, dressing percentages and percentage muscle of zilpaterol HCl fed steers outperformed the steers fed R-salbutamol.

The results (as seen in Table 4.4) show that the use of beta-agonists as feed additive growth promoters shift the carcasses towards A2 carcasses (77,6%), therefore producing leaner carcasses and subsequently meat which is desired by the consumer and is in line with literature as discussed. There were however no significant differences between the experimental groups of the fat codes. The carcass classification results (as seen in Table 4.5) showed significant differences (P <0,05) between the number of zilpaterol HCl carcasses (56,7%) in the 4

conformation classification compared to R-salbutamol carcasses (43,4%), with significantly less zilpaterol HCl carcasses in the 3 classification group.

The interactions between experimental treatment and weight category (as seen in Table 4.6) did not show significant differences that described a possible interaction between carcass weight and size characteristics. There were significant differences (P <0,05) between the dressing percentages of larger-framed zilpaterol HCl carcasses which were higher compared to medium-framed zilpaterol HCl and R-salbutamol carcasses. Larger-framed R-salbutamol carcasses were statistically similar to each of the other size categories.

The overall results of this objective study showed that zilpaterol HCl had higher slaughter weights, live weight gain, ADG, carcass weights, carcass length, dressing percentages and percentage muscle when compared to R-salbutamol, therefore higher performances in both growth and feedlot performance characteristics, as well as carcass characteristics.

5.2 Critical evaluation

The aim of this project was to compare the effects of R-salbutamol at 120 mg per head cattle per day in the feed on feedlot cattle during the last 30 days of the finishing phase preceding slaughter, versus zilpaterol HCl at 60 mg per head per day in the feed for the last 30 days preceding slaughter. This was an objective study conducted at a reputable feedlot with a large sample size to minimize any random effects. There have been previous studies conducted testing the effects of zilpaterol HCl versus R-salbutamol on feedlot steers which did not yield conclusive results and therefore a conclusive and objective study was conducted (Steenekamp, 2014).

The study stratified the starting weights to have two similar experimental groups, however the mean starting weight experimental groups differed by 7,4 kg, this difference was not significantly different. This could have been due to the animals being weighed a few days before the start of the trial. This difference was compensated for by including starting weight as a covariate, however this could not fully compensate for this difference. In future trials the starting weights of the experimental groups should be stratified on the day that the trial begins.

The steers in each replicate group were not recorded and therefore variation due to this external factor could not be compensated for. The replicate groups were however located near each other in the feedlot and likely experienced the same environmental factors. Proper recording of this information in a subsequent trial would enable the minimization of this potential variation.

Despite these improvements, the trial yielded good, trustworthy results and these results can be implemented in the beef cattle feedlot industry. It is not necessary to replicate this trial, the evident higher growth and feedlot performance, as well as carcass characteristics achieved from zilpaterol HCl steers compared to R-salbutamol steers can be substantiated by stratifying the starting weights and then including it as a covariate, using a large sample size (n = 228) and by conducting the trial on typical feedlot steers at one of the best feedlots in the country.