Growth performance and meat characteristics of feedlot cattle fed R-salbutamol or zilpaterol hydrochloride during the finishing period

by

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ABSTRACT

In this study, 14 typical South African feedlot bulls received no beta-adrenergic agonist for the last 30 days of the finishing period (C), 14 received 120 mg R-salbutamol per animal per day for the last 30 days of the finishing period (S30), 13 received 120 mg R-salbutamol per animal per day for the last 40 days of the finishing period (S40) and the last group of 13 bulls received 60 mg zilpaterol hydrochloride per animal per day for the last 30 days of the finishing period (Zh). All animals were slaughtered after a 3-day withdrawal period. Parameters included weight gain, feed intake, feed conversion rate, warm and cold carcass mass, dressing %, subcutaneous fat thickness, hide yield %, internal carcass fat distribution, % bone, % fat and % muscle of the prime rib-cut, carcass classification code, conformation, compactness, post-mortem carcass pH profiles, cooking loss, shear force, blood urea nitrogen, creatinine and residue levels of the beta-adrenergic agonists.

No differences were observed between any of the four treatment groups concerning live feedlot performance. Bulls receiving the S30 and Zh treatments had lower internal carcass fat distribution compared to C bulls (P <0.05). Bulls receiving the S40 treatment had a lower % fat in the prime rib-cut compared to Zh bulls (P <0.05). Carcasses from S30 bulls had higher pH values 24 hours post mortem compared to carcasses from Zh bulls (P <0.01). Meat samples from Zh bulls had higher shear force, which indicates less tender meat, compared to samples from S40 bulls (P <0.05). Change in serum creatinine levels increased only in Zh treated bulls from the start to the end of treatment and may reflect a higher protein turnover in Zh bulls. The results of this study indicate that R-salbutamol has a more pronounced effect on fat metabolism in feedlot bulls compared to zilpaterol hydrochloride, while zilpaterol hydrochloride has a more pronounced effect on protein metabolism. The residue levels in samples of the liver, kidney, muscle and feaces from zilpaterol hydrochloride and R-salbutamol treated bulls were well below acceptable limits.

DECLARATION

I, Stefanie Steenekamp, declare that this dissertation which I hereby submit for the degree MSc (Agric) Animal Science: Production Physiology to the Department of Animal and Wildlife Sciences at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signed: ……

Date: ……

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ABBREVIATIONS

ADG	Average daily gain
BUN	Blood urea nitrogen
C	Control treatment
Ca	Calsium
cAMP	Cyclic adenosine monophosphate
CCM	Cold carcass mass
CFI	Cumulative feed intake
CL	Cooking loss
CREBP	cAMP response element binding protein
DOPA	Dihydroxyphenylalanine
DRESS%	Dressing percentage
FCR	Feed conversion ratio
FDA	United States of America Food and Drug Administration
HY%	Hide yield percentage
ICFD	Internal carcass fat distribution
IVOMD	In vitro organic matter digestibility
LD	*M. longissimus dorsi*
ME	Metabolisable energy
P	Phosphorous
S30	R-salbutamol treatment (for 30 days)
S40	R-salbutamol treatment (for 40 days)
SCF	Subcutaneous fat
SF	Shear force
WCM	Warm carcass mass
Zh	Zilpaterol hydrochloride treatment (for 30 days)

CHAPTER 1. INTRODUCTION

1.1 PROJECT THEME

Growth and Physiology of farm animals

1.2 PROJECT TITLE

Growth performance and meat characteristics of feedlot cattle fed R-salbutamol or zilpaterol hydrochloride during the finishing period

1.3 AIM

The aims of this project were to compare the effects of R-salbutamol at 120 mg per animal per day on South African feedlot bulls for the last 30 or 40 days prior to slaughter, versus zilpaterol hydrochloride fed at 60 mg per animal per day for the last 30 days prior to slaughter on the following parameters:

1) Growth and feedlot performance:
    - Cumulative feed intake (CFI)
    - Average daily gain (ADG),
    - Feed conversion ratio (FCR)

2) Carcass and meat characteristics:
    - Carcass mass (WCM & CCM)
    - Dressing percentage (DRESS%)
    - Hide yield percentage (HY%)

- Carcass classification score
- Carcass conformation
- Post-mortem carcass pH profiles
- Internal carcass fat distribution (ICFD) – amount of visible peri-renal / channel fat
- Carcass compactness
- Subcutaneous fat (SCF) thickness
- Muscle-bone-fat ratio
- Carcass composition
- % Cooking loss (CL)
- Shear force (SF) values of *m. longissimus dorsi* (LD) samples
- Plasma constituents creatine and blood urea nitrogen (BUN)
- Accumulation of residues in the faeces of R-salbutamol and zilpaterol hydrochloride treated bulls to quantify the environmental impact
- Accumulation of residues in the liver, kidneys and muscle tissues of R-salbutamol treated bulls for the safety of humans consuming meat from feedlot bulls treated with R-salbutamol

1.4 MOTIVATION

The meat production industry finds itself in a society of consumers that is becoming more health conscious and environmentally aware. In South Africa, the Consumer Protection Act 68 of 2008 aims to improve access to, and improve quality of, information that is necessary for consumers to make informed choices. Progressive withdrawal or banning of most of the growth agents based on steroid hormones and human antibiotics causes for needs to introduce new and safe production efficiencies. Industry must find ways to support these consumer demands and still maximise productivity and maintain economies of production in a world with escalating population numbers.

Oral synthetic beta-adrenergic agonists like zilpaterol hydrochloride, ractopamine hydrochloride, clenbuterol and R-salbutamol have similar chemical and pharmacological characteristics than natural catecholamines like epinephrine, norepinephrine and dopamine (NRC, 1994; Bell *et al.*, 1998). Beta-agonists bind to and activate specific beta-adrenergic receptors in muscle and fat, causing change in the biochemical growth processes in these tissue types. This change involves increased lipolysis, decreased lipogenesis, increased protein accretion, decreased protein degradation, or a combination of these (Wheeler & Koohmaraie, 1992; Mersmann, 1998). If nutrients are partitioned towards protein synthesis and muscle growth rather than towards fat deposition, profitability can be increased and high feed costs can be decreased through improved carcass leanness and greater dressing percentage (Brooks *et al.*, 2009).

There are three predominant beta-adrenergic receptor subtypes in mammalian tissues, namely a $beta_1$-adrenergic receptor subtype, a $beta_2$-adrenergic receptor subtype and a $beta_3$-adrenergic receptor subtype (Mersmann, 1998; 2002). Different tissues have different proportions of subtypes, and beta-receptor types and metabolic pathways associated with them are differently expressed by cells and tissues. Therefore different beta-agonists will not have exactly the same mechanisms or effects. Type and concentration of beta-agonist treatment, treatment period, and the species involved will also influence the result (Dunshea *et al.*, 2005).

In a study done by Strydom *et al.* (2009), cattle treated with clenbuterol and zilpaterol hydrochloride had a significant advantage over cattle fed no beta-agonist and cattle fed ractopamine (which in South Africa has been approved for the use in pigs) for carcass adjusted feed conversion ratio due to higher dressing percentages. However, clenbuterol is an illegal beta-agonist because it has a strong receptor affinity (Spurlock *et al.*, 1993) and

some of its adverse effects are increased heart rates and depressed appetites during the first part of the treatment period (Ricks *et al*., 1984).

Zilpaterol hydrochloride has been legally used in Mexico and South Africa for longer than 10 years and approved by the FDA to use in USA feedlots in 2006 (Avendaño-Reyes *et al*., 2006; Shook *et al*., 2009). It has been proven in numerous studies to increase weight gain, improve feed efficiency, and increase carcass leanness in cattle fed in confinement for slaughter during the last 20 to 40 days on feed (Avendaño-Reyes *et al*., 2006; Brooks *et al*., 2009; Shook *et al*., 2009). However, many authors also concluded that zilpaterol hydrochloride feeding negatively impacts on meat tenderness (Rathmann *et al*., 2009; Strydom *et al*., 2011; Hope-Jones *et al*. 2012).

R-salbutamol is a beta-adrenergic agonist and a purified derivative of racemic (RS-) salbutamol (also known as albuterol), which has worldwide acceptance for the treatment of human respiratory disorders. R-salbutamol is in the process of registration for use in food-producing animals as a growth modifier. The few official papers that have been published in scientific journals mostly investigate the effects of R-salbutamol on poultry or swine. Marchant-Forde *et al*. (2008) specifically investigated the effect of R-salbutamol on the well-being of finishing pigs, which may be of particular interest as consumers increasingly demand products that meet animal well-being criteria. The study showed little effect on behaviour of finishing pigs that were being supplemented with R-salbutamol over a 4-week period. In a different study, the same author investigated the effects of R-salbutamol on growth, carcass measures and health of finishing pigs. It was found that R-salbutamol had a positive effect on carcass growth and composition (Marchant-Forde *et al*., 2012).

In this study, we investigated the growth performance and selected meat characteristics of feedlot cattle fed R-salbutamol or zilpaterol hydrochloride during the

finishing period. Zilpaterol hydrochloride was used as a positive control in this study, as it is a beta-agonist which has been extensively researched, while the possibility of R-salbutamol as an alternative beta-agonist for finishing cattle was investigated, because this molecule may hold promise as a growth enhancer in feedlot cattle.

1.5 HYPOTHESES

$H1_o$: R-salbutamol has no significant effect on the efficiency and feedlot performance of feedlot bulls compared to zilpaterol hydrochloride or control groups.

$H1_A$: R-salbutamol significantly affects the efficiency and selected feedlot parameters of feedlot bulls compared to zilpaterol hydrochloride or control groups.

$H2_o$: R-salbutamol has no significant effect on the carcass and meat characteristics, selected blood constituent levels and residue levels in selected tissues and faecal samples of feedlot bulls compared to zilpaterol hydrochloride or control groups.

$H2_A$: R-salbutamol significantly affects the carcass and meat characteristics, selected blood constituent levels and residue levels in selected tissues and faecal samples of feedlot bulls compared to zilpaterol hydrochloride or control groups.

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CHAPTER 2. LITERATURE REVIEW

2.1 BETA-ADRENERGIC AGONISTS

Beta-adrenergic agonists (also referred to as beta-agonists) are catecholamines that occur naturally in a mammalian body. These compounds stimulate processes that make energy available for organ systems involved in the response to stress, so that the animal can defend itself against a competitor or flee from a predator. W.B. Cannon has named this phenomenon the 'fight or flight' syndrome in 1932 (Hossner, 2005).

The adrenal medullary hormone epinephrine (also known as adrenaline) and the neurotransmitters of the sympathetic nervous system, norepinephrine (also known as noradrenaline) and dopamine are the main catecholamines found in mammals (Mersmann, 1998; 2002; Hossner, 2005). Figure 2.1 illustrates the synthesis of dopamine, norepinephrine and epinephrine from tyrosine. Epinephrine is the methylation product of norepinephrine and norepinephrine is the hydroxylation product of dopamine. Dopamine is formed from the decarboxylation of DOPA (dihydroxyphenylalanine) which is the hydroxylation product of tyrosine. Epinephrine has a distinguishing amino methyl group, while norepinephrine does not ('nor' means 'without') (Hossner, 2005).

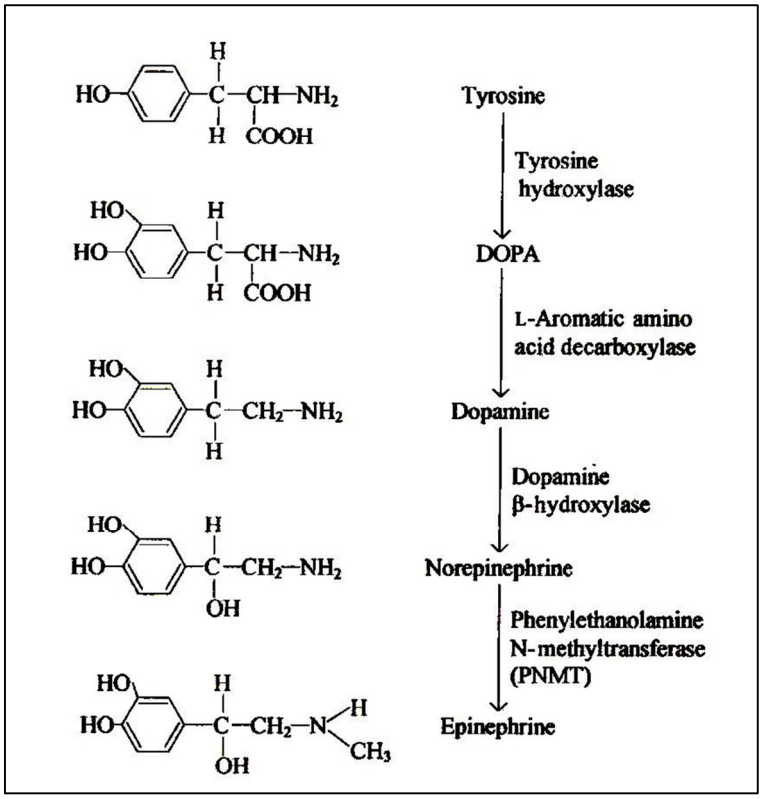

Figure 2.1 The synthesis of catecholamines from tyrosine (Adapted from Hossner, 2005)

Epinephrine, norepinephrine and dopamine all circulate the body at low levels which vary between species (Hossner, 2005). Norepinephrine and dopamine mainly function in the nervous system as neurotransmitters and a very high concentration of norepinephrine is needed to induce an endocrine response. The presence of norepinephrine and dopamine in the circulation can mostly be considered as a “spillover” into the blood when sympathetic activation occurs (Hossner, 2005). Epinephrine circulates at lower concentrations than norepinephrine in most species, but it responds to a greater extent than norepinephrine during stress (Mersmann, 1998). During real or perceived stress from internal or external sources, preganglionic nerve stimulation from the sympathetic nervous system causes the release of epinephrine from the adrenal medulla, which causes beta-adrenergic responses in the whole body (Hossner, 2005).

2.1.1 The general physiological mechanism of beta-agonists

Some of the processes stimulated by beta-agonists during a typical stress response are changes in the cardiovascular, respiratory and gastrointestinal systems (Hossner, 2005). Energy is released through the catabolism of lipids and glycogen and the specific processes involved are lipolysis and glycogenolysis (Casey, 1998; Mersmann, 2002; Hossner, 2005). The heart rate increases, while vasoconstriction reduces blood flow to the gastrointestinal tract and vasodilation increases blood flow to skeletal muscle, the heart and the brain (Morris, 1997; Mersmann, 2002; Dziersk, 2003). In this way, organs involved in the stress response receive optimal concentrations of energy and oxygen (Hossner, 2005).

Hossner (2005) explains the physiological action of epinephrine in a stress response to be as follows: When low blood glucose levels occur due to acute stress, fasting or exercise, epinephrine will increase glycogenolysis and gluconeogenesis in the liver and increase glycogenolysis in the skeletal muscle, thereby rapidly mobilising energy reserves resulting in a quick increase in blood glucose. Epinephrine also causes the suppression of insulin release and the stimulation of glucagon secretion from the pancreas, resulting in the maintenance of a high blood glucose level which can be utilised as an energy source. Also, epinephrine increases lipolysis of adipose tissue, releasing free fatty acids and glycerol which can be used for energy production or the production of glucose through gluconeogenesis.

2.1.2 Beta-adrenergic receptors and the molecular physiology of a beta-adrenergic response

Beta-adrenergic receptors (also referred to as beta-receptors) can be found in the plasma membranes of almost any type of tissue cells and it is through these receptors that catecholamines or beta-agonists act – similarly to hormones. A beta-receptor is complex, consisting of a chain of more than four hundred amino acids; is linked to the Gs protein and

exist in different forms (Mersmann, 1998; 2002; Hossner, 2005). Figure 2.2 illustrates a beta-adrenergic receptor. The receptor is anchored in the membrane by seven relatively hydrophobic transmembrane domains. There are four portions of the amino acid chain on the outside of the cell membrane and three loops on the inside of the cell. The ligand binding site is in the center of the seven transmembrane domains (Ostrowski *et al.*, 1992; Mersmann, 1998; 2002).

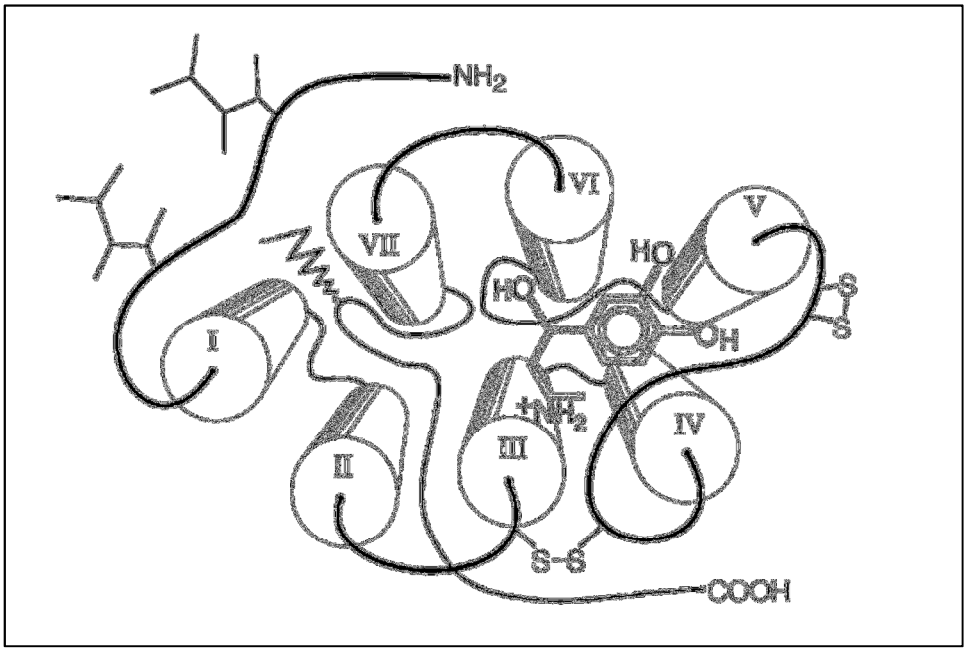

Figure 2.2 A beta-adrenergic receptor with its seven transmembrane domains, indicating the binding site for norepinephrine (Adapted from Mersmann, 1998)

The general molecular action of beta-agonists, natural or synthetic, can be described as follows: Beta-agonists play the role of ligands and bind to beta-receptors in tissue cell membranes (Strosberg, 1992; Mersmann, 1998). The agonist-receptor complex activates the Gs protein in the cell. A subunit of the Gs protein, called the a-subunit, then activates adenyl cyclase, which is the enzyme that catalyses the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) in the cytoplasm (Ostrowski *et al.*, 1992). Cyclic adenosine monophosphate binds to the regulatory subunit of protein

kinase A to release the catalytic subunit that phosphorylates certain proteins in the cell (Strosberg, 1992; Mersmann, 2002). Figure 2.3 is an illustration of the metabolic action of beta-agonists, as described above, on adipose tissue.

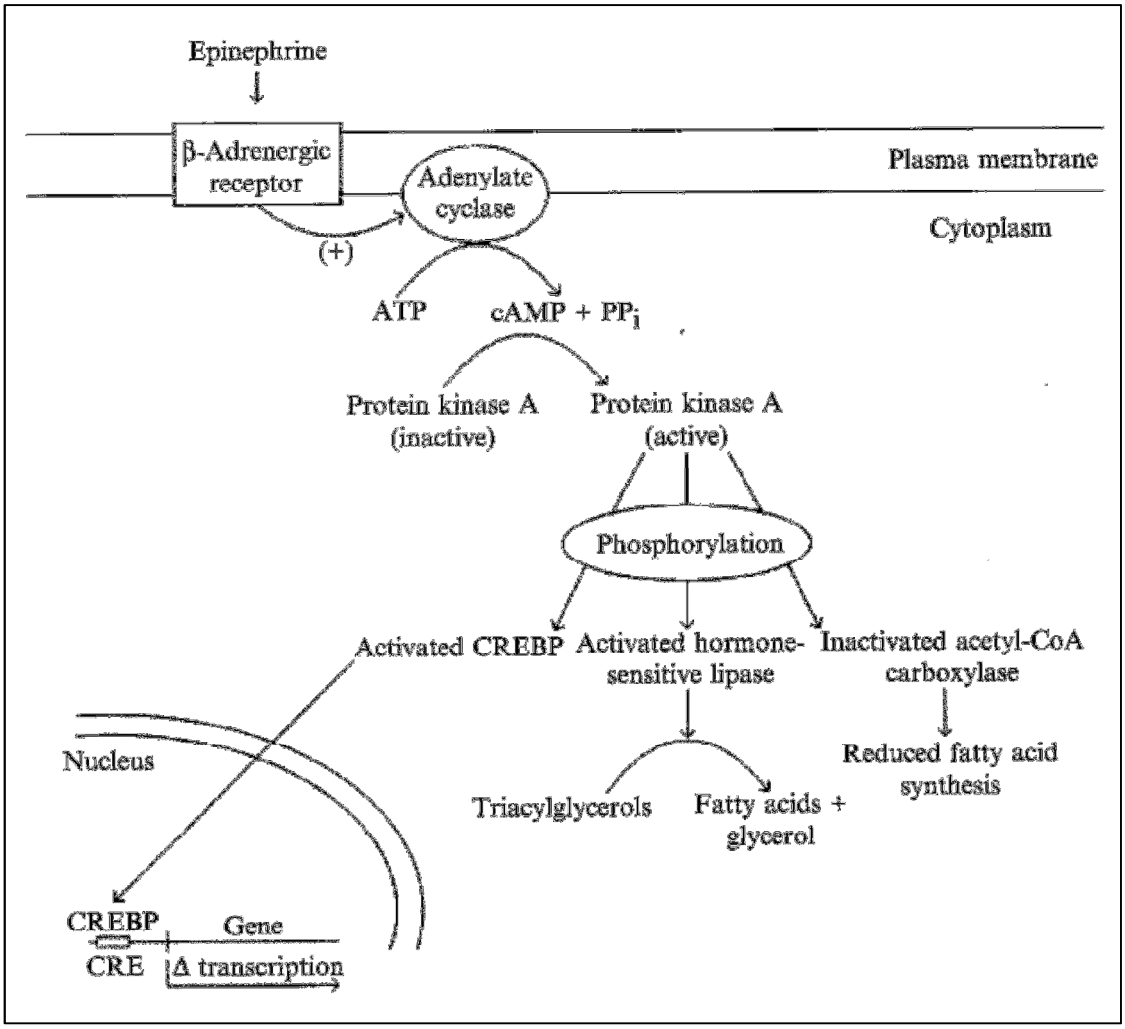

Figure 2.3 The molecular action of beta-adrenergic agonists in adipose tissue (Adapted from Hossner, 2005)

Some of the intracellular proteins are enzymes that are activated when they are phosphorylated (Mersmann, 1998). One protein that is activated when phosphorylated by protein kinase A is the cAMP response element binding protein (CREB). Gene transcription is stimulated when the CREB binds to a cAMP response element in the regulatory part of a

gene (Strosberg, 1992). With the increased occurrence of phosphorylation in the cell, the transcriptional activity of the CREB is increased. In this way, a number of genes can be transcripted via the mechanism initiated by a beta-agonist (Strosberg, 1992). On the other hand, some enzymes can be inactivated when they are phosphorylated, for example acetyl-CoA carboxylase, the rate-limiting enzyme for long-chain fatty acid biosynthesis (Mersmann, 1998; 2002; Hossner, 2005).

There are different ways in which a beta-adrenergic response can be ended: degradation of the beta-agonist or removal of the beta-agonist from the receptor (Mersmann, 1998); inactivation of the receptor by phosphorylation of the receptor by beta-adrenergic receptor kinase or protein kinase A (Strosberg, 1992; Mersmann, 2002) and even removal of the receptor from the plasma membrane, which will result in a reduction of available receptors to facilitate the response (Mersmann, 1998).

The natural beta-agonists norepinephrine and epinephrine are inactivated by two enzymes: catechol-o-methyl transferase, which methylates the catychol-ring hydroxyl groups, and monoamine oxidase, which deaminates the ligand (Mersmann, 1989a; 1998). Norepinephrine can also be reabsorbed at myoneural junctions and synaptic clefts. This lower concentration of norepinephrine will lead to a decreased activation of receptors (Mersmann 1998).

2.1.3 Beta-receptor subtypes

The concept of alpha-receptors and beta-receptors already existed in the 1940's from physiological studies on epinephrine, norepinephrine and other molecules (Mersmann, 1998; 2002). Hossner (2005) points out that the proportions of alpha- and beta-receptors relative to one another in different tissue types will determine how the cells respond to beta-agonist stimulation, and also explains the differences between alpha- and beta-receptors to be as follows: alpha-receptors facilitate responses within the sympathetic nervous system and are involved in vasoconstriction and smooth muscle contraction, while beta-receptors are more

acceptive to beta-agonists, generally mediating smooth muscle relaxation. Beta-receptors are also more acceptive to epinephrine than to norepinephrine.

In the 1960's, the beta-receptors were subclassified into $beta_1$-receptors and $beta_2$-receptors, and in the 1970's, evidence of another subtype was found which was classified as the $beta_3$-receptor subtype (Mersmann, 1998; 2002; Hossner, 2005).

$Beta_1$-receptors play roles in lipolysis and have effects on cardiac muscle and intestinal smooth muscle. They respond to both epinephrine and neural norepinephrine, but they are seen as the main neural system adrenergic receptor and therefore are more sensitive to norepinephrine (Mersmann, 1998; Hossner, 2005). $Beta_1$-receptors are found in the highest ratio on most tissues. According to Hossner (2005), 80% of beta-receptors on adipose tissue, 70% of beta-receptors on the heart, 65% of beta-receptors on the lung, 60% of beta-receptors on skeletal muscle and 50% of the beta-receptors on the liver fall under the $beta_1$-receptor subtype.

$Beta_2$-receptors mainly respond to epinephrine while norepinephrine interacts weakly with $beta_2$-receptors. $Beta_2$-receptors facilitate bronchodilation, vasodilation, uterine smooth muscle relaxation; glycogenolysis in the liver and they are involved in the beta-agonist respons in skeletal muscle (Hossner, 2005).

It has been found from ligand binding experiments that the rat heart has more than 90% $beta_1$-receptors, while the guinea pig tracheal muscle tissue has more than 85% $beta_2$-receptors (Mersmann, 1998; 2002). Tissues like these are used as prototypical tissues which can be used to classify many agonists or antagonists according to their specificity for the receptor subtypes (Mersmann, 1998).

Most tissues have multiple receptor subtypes (Minneman *et al.*, 1979). The complexity in the different ratios and arrays of the receptor types and their subtypes on different tissues cause the response and sensitivity of tissues to beta-agonists to be quite variable – differences in the expression of the same beta-agonist between species and also between

different beta-agonists within a single specie can be expected (Mersmann, 1989b, Dunshea *et al*., 2005; Hossner, 2005).

The ratio between $beta_1$-receptors and $beta_2$-receptors in certain cell types or tissues will differ in the same specie, and the ratio between $beta_1$-receptors and $beta_2$-receptors in the same cell type or tissue but in different species, might also differ (Mersmann, 1998). The stage of differentiation of a cell, or the hormones that influence the cell might cause changes in the ratio between receptor subtypes in the plasma membrane of that cell (Mersmann, 1998).

Different receptor subtypes have different RNA transcripts, different protein sizes and different amino acid sequences (Mersmann, 1998). According to Mersmann (2002), a $beta_1$-receptor has about 460 amino acids, a $beta_2$-receptor has about 420 amino acids and a $beta_3$-receptor has about 410 amino acids. Strosberg (1992), Hall *et al*. (1993) and Pietri-Rouxel & Strosberg (1995) found 50% homology in the amino acid sequence of the three beta-agonist receptor subtypes within a single species, and 75% or more homology in the amino acid sequence of an individual beta-agonist receptor subtype across species. Mersmann (2002) describes the homology in the amino acid sequence within a single species to be 45 - 60%, and the homology in the amino acid sequence of an individual beta-agonist receptor subtype across species to be higher than 70%.

2.1.4 Different direct and indirect physiological effects caused by the supplementation of synthetic beta-agonists

Oral synthetic beta-agonists have the same chemical and pharmacological characteristics than natural catecholamines like epinephrine, norepinephrine and dopamine (NRC, 1994; Bell *et al*., 1998).

In a number of publications (Mersmann 1989a; 1998; 2002) it was discussed how a single beta-agonist can have many direct and indirect effects on an animal, as beta-receptors are present in the plasma membranes of cells in many different tissue types.

Over the years, thousands of molecules that bind to beta-receptors were synthesised due to interest from the biomedical community. Some of the synthesised molecules are called agonists and others are called antagonists (Mersmann 2002). The antagonist also binds to the receptor, but blocks its function, because it does not activate the Gs protein (Mersmann, 1998; Hossner, 2005).

During a stress response, beta-agonists play an important role in the activation of the receptors of bronchialtracheal muscle tissue which results in the relaxation and dilation of the airways, in order to get more oxygen to the brain and the muscles (Morris, 1997; Mersmann, 1998). In human and veterinary medicine, most research concerning beta-agonists has been done towards this specific physiological function of beta-agonists and subsequently, synthetic beta-agonists like salbutamol are and have been well known remedies in the treatment of asthma for many years (Hossner, 2005).

Studies done by Beermann *et al*. (1987), Mersmann (1987) and Eisemann *et al*. (1988) indicate an increased heart rate in animals supplemented with beta-agonists. Beta-agonists are widely used in the moderation of heart rate, contractility and blood pressure (Mersmann, 2002). Elevated heart rate is associated with increased blood flow, and in the case of a beta-adrenergic response which is similar to the response seen with epinephrine and norepinephrine, blood flow is directed towards certain body parts necessary to react in the “fight or flight” situation (Mersmann, 1989a; 1989b). Blood supply might be increased to the skeletal muscles and to adipose tissue, for example. More energy and substrates will be available for the muscle, which can lead to increased protein synthesis; and more nonesterified fatty acids can be removed from adipose tissue, which can aid in fat degradation in order to release energy necessary for the animal to “fight” or “flee” (Zimmerli & Blum, 1990; Moloney *et al*., 1991; Mersmann,1995).

Beta-agonists have endocrine effects. One example is the acute increase in plasma insulin, as reported by Zimmerli & Blum (1990); Webb (1994) and Morris (1997). However, these authors also show that chronic supplementation of beta-agonists to cattle has no effect

on plasma insulin. Beermann *et al*. (1987) even reported a decrease in plasma insulin in sheep after chronic supplementation of beta-agonists. This decrease in plasma insulin might lead to decreased lipogenesis and increased lipolysis.

Other examples of endocrine effects are reported in the following studies: Beermann *et al*. (1987) shows an increase in plasma thyroid hormones in sheep after chronic beta-agonist supplementation while Zimmerli & Blum (1990) did not find the same effect in cattle; Mersmann (1989a) shows an increase in plasma catecholamines after acute beta-agonist supplementation in pigs while Blum & Flueckiger (1988) did not find the same effect in cattle; Morris (1997) found a release of rennin from the kidneys with beta-agonist supplementation and Mersmann (1998) gives an explanation as to why it does not seem that beta-agonists have any influence on somatotropin – and that it is therefore unlikely that protein synthesis and lipolysis arise from the activation of somatotropin by a beta-agonist. Some of his reasons include the following: there is no structural similarities between beta-receptors and somatotropin receptors; somatotropin has a hypertrophic effect in many different organs, while beta-agonists only cause hypertrophy in a few specific tissues like skeletal muscle, the heart and salivary glands according to a study done by Reeds & Mersmann (1991) and Beermann *et al*. (1987), Zimmerli & Blum (1990) and Thomas *et al*. (1994) showed that beta-agonist supplementation actually supresses plasma somatotropin concentration in sheep. Another specific use for beta-agonists involving its endocrine function is to induce uterine relaxation (Mersmann, 2002).

Reductions in feed intake with the supplementation of beta-agonists have been reported in several cases. This might be a central nervous system effect caused by the possibility that some beta-agonists might be able to cross the blood-brain barrier (Ordway *et al*., 1987).

Convincing evidence could not be found to prove that chronic administration of beta-agonists can increase basal metabolic rate (Rikhardsson *et al*., 1991; Yen *et al*., 1991). However, Mersmann (2002) states that beta-agonists may have an effect on the rate of

metabolism and that this might lead to changes in the plasma concentrations of metabolites such as glucose or lactate.

It must be kept in mind that the glucocorticoid or another endocrine status of an animal might influence the effects of a beta-agonist on the animal (Liu *et al.*, 1994b).

2.2. THE EFFECTS OF BETA-AGONISTS ON FEED EFFICIENCY AND CARCASS AND MEAT CHARACTERISTICS

It might seem logical that during a stress response where catecholamines stimulate the mobilisation of energy reserves for the sole purpose of the animal's survival, energy will not be available for growth. However, over the past few decades, it has been discovered that catecholamine derivatives can also be used to improve the body composition and production efficiency of production animals through the repartitioning of nutrients away from adipose tissue towards skeletal muscle (Hossner, 2005). If nutrients are partitioned towards protein synthesis and muscle growth rather than towards fat deposition, profitability can be increased and high feed costs can be decreased through improved carcass leanness and greater dressing percentage (Miller *et al.*, 1988; Brooks *et al.*, 2009).

Cunningham (1965) has investigated the possibility of manipulating mammalian growth with agents like caffeine, theophylline, nicotine and epinephrine. Since the 1980's there have been reports that beta-agonists given orally cause changes in body composition and improvement in carcass growth performance and carcass yield in cattle (Ricks *et al.*, 1984; Fiems, 1987), sheep (Ricks *et al.*, 1984; Thornton *et al.*, 1985), pigs (Ricks *et al.*, 1984; Jones *et al.*, 1985) and turkeys and chickens (Ricks *et al.*, 1984).

Generally, beta-agonist supplementation can have desirable effects on feedlot performance and carcass characteristics such as increased average daily gain, improved feed conversion efficiency, increased protein to fat ratio and improved dressing percentage (Moody *et al.*, 2000). However, some undesirable effects from beta-agonist supplementation

are also evident in literature, mostly concerning meat quality. Many authors found decreased tenderness in meat from beta-agonist supplemented animals (Brooks *et al*., 2009) and some authors also found adverse effects of meat from beta-agonist supplemented animals on consumer sensory scores (Hilton *et al*., 2009; Leheska *et al*., 2009).

Over the past few years, focus in animal production has shifted from growth and production efficiency towards consumer satisfaction and perception, safety and a consistent production of high quality meat (Webb, 2006). Therefore, it is equally important to study the effects of beta-agonists on meat quality than it is to study the effects on production efficiency.

Examples of synthetic beta-agonist molecules that have been extensively studied are clenbuterol, cimaterol, ractopamine and zilpaterol (Hossner, 2005).

2.2.1 Clenbuterol

Ricks *et al*. (1984) specifically studied the change of growth in animals fed the beta-agonist clenbuterol. With oral administration of clenbuterol, an increase in muscle mass and a decrease in fat mass were observed in growing cattle, chickens, turkeys, pigs and sheep. An increase in weight gain and an improvement in the feed conversion ratio were observed in some cases. However, it is not legal to use clenbuterol as a repartitioning agent for meat-producing animals, because it has a strong receptor affinity (Spurlock *et al*., 1993) and some of its adverse effects include increased heart rates and depressed appetites during the first part of the treatment period (Ricks *et al*., 1984). Clenbuterol also show a negative impact on tenderness of meat, which was indicated in several studies by an increase in Warner-Bratzler Shear Force (WBSF) as compared to the control (Mersmann, 1998). Miller *et al*. (1988) found a 13.5% increase, Schiavetta *et al*. (1990) found a 22% increase and Luño *et al*. (1999) found a 113% increase in WBSF of meat from clenbuterol supplemented cattle.

2.2.2 Cimaterol

Cimaterol is a phenethanolamine like clenbuterol, and it achieves similar desirable effects concerning feedlot performance such as increased daily gain, increased carcass mass, decreased intramuscular fat, increased protein content and decreased fat in carcasses (Fiems *et al*., 1990; Chikhou *et al*., 1993; Vestergaard *et al*., 1994). However, it also shows similar undesirable and potentially toxic effects for humans that consume meat or other edible organs from animals that have been supplemented with cimaterol (Dikeman, 2007). It is known to have a negative impact on meat tenderness of bulls and steers (Schiavetta *et al*., 1990), with increases in shear force values such as 27 - 45% (Fiems *et al*., 1990); 55 - 145% (Chikhou *et al*., 1993) and 136 - 250% (Vestergaard *et al*., 1994).

2.2.3 Ractopamine hydrochloride

Ractopamine hydrochloride was the first legal beta-agonist allowed to be used as a production enhancer in meat-producing animals in the USA. It was approved by the FDA for the supplementation of pigs in 2000 and for the supplementation of finishing cattle in 2003 (Hossner, 2005) and is also approved for the use in pig production in South Africa. It has been shown to improve average daily gain, feed conversion rate, dressing percentage and carcass composition in pigs (Dunshea *et al*., 2005; Dikeman, 2007). Ractopamine hydrochloride is regarded to cause less human or animal health or safety concerns, but may not be as efficient to improve animal performance as zilpaterol hydrochloride. In a study done by Strydom *et al*. (2009), cattle treated with clenbuterol and zilpaterol hydrochloride had a significant advantage over cattle fed no beta-agonist and cattle fed ractopamine for carcass adjusted feed conversion ration due to higher dressing percentages. Marchant-Forde *et al*. (2003) found ractopamine hydrochloride fed to pigs to cause elevated heart rates and increased risk of fatigue during handling, and Shroeder *et al*. (2003) reported a

12% increase in Warner-Bratzler Shear Force when beef steers were supplemented with 300 mg/kg ractopamine hydrochloride per day.

2.2.4 Zilpaterol hydrochloride

Zilpaterol hydrochloride (Formula $C_{14}H_{19}N_3O_2$.HCL; Mol.mass 297.78 g/mol) is a beta-agonist which has been extensively researched and much of the research on this molecule has been done at the University of Pretoria in South Africa (Webb, 1994; Webb & Casey, 1995; Maritz, 1996; Morris, 1997; Casey, 1998; Hope-Jones *et al*., 2010; O'Neill *et al*., 2010; Hope-Jones *et al*., 2012). It has been legally used in Mexico and South Africa for longer than 10 years and was also approved in 2006 by the FDA to use in feedlots in the USA (Avendaño-Reyes *et al*., 2006; Brooks *et al*., 2009; Shook *et al*., 2009).

Feeding zilpaterol hydrochloride has been shown to improve final body weight (Morris, 1997; Montgomery *et al*., 2009a; 2009b), improve carcass weights and lean yield (Leheska *et al*., 2009; Montgomery *et al*., 2009a; 2009b), decrease carcass fat and increase carcass moisture and protein (Maritz, 1996; Hilton *et al*., 2009; Rathmann *et al*., 2009), improve average daily gain (Elam *et al*., 2009; Vasconcelos *et al*., 2008; Montgomery *et al*., 2009b), improve feed efficiency and dressing percentage (Elam *et al*., 2009; Montgomery *et al*., 2009a; 2009b) and increase protein-to-bone ratio (Maritz, 1996; Leheska *et al*., 2009).

Rathmann *et al*. (2009) concluded that a similar degree of improvement in carcass yield and composition can be expected with zilpaterol hydrochloride feeding regardless of where the cattle are in their feeding period, while Elam *et al*. (2009) indicated that zilpaterol hydrochloride will have even better effects on carcass fatness if it is fed for a longer period of time.

Many authors conclude that zilpaterol hydrochloride feeding reduces meat tenderness (Kellermeier *et al*., 2009; Strydom *et al*., 2011; Hope-Jones *et al*., 2012). Brooks *et al*. (2009) found that feeding durations of 20, 30 and 40 days on zilpaterol hydrochloride all produce greater WBSF values than the control group (zero days on zilpaterol hydrochloride).

Similarly, Strydom *et al*. (2002) showed that zilpaterol hydrochloride supplementation for 30 to 50 days on South African beef steers increased WBSF values by 20 - 28%. Hope-Jones *et al*. (2010) showed that the increase in Warner-Bratzler Shear Force (WBSF), which indicates increased thoughness of meat from zilpaterol treated steers, is the result of increased calpastatin activity. In the same study, it was found that electrical stimulation of the carcass could improve the tenderness of meat from zilpaterol treated steers by triggering the calpains during the early onset of rigor. However, electrical stimulation could not fully eliminate the toughening effect caused by zilpaterol. Rathmann *et al*. (2009) noticed through WBSF analysis that zilpaterol hydrochloride causes a toughening effect even if the meat was aged for 7, 14 or 21 days, although Brooks *et al*. (2009), Holmer *et al*. (2009) and Shook *et al*. (2009) observed that with appropriate aging, the increased shear force values can be reduced.

Hope-Jones *et al*. (2012) showed that zilpaterol hydrochloride causes an increase in drip loss and also causes reduced redness of meat. Hilton *et al*. (2009), Montgomery *et al*. (2009b) and Vasconcelos *et al*. (2008) indicated that zilpaterol hydrochloride seems to decrease marbling score and quality grade and Leheska *et al*. (2009) noticed that zilpaterol hydrochloride can have a negative effect on other palatability traits.

2.2.5 R-salbutamol

R-salbutamol (Formula $C_{13}H_{21}NO_3$; Mol.mass 239.311 g/mol) is a $beta_2$ -agonist and a purified derivative of racemic (RS-) salbutamol (also known as albuterol), which is widely accepted as a safe, well-known treatment for respiratory disorders in humans and animals alike**.** There is not much literature available on the effect of R-salbutamol in beef cattle. The few official papers that have been published in Animal Science journals mostly investigated the effects of R-salbutamol on poultry or swine. It has more recently become a popular topic for research and is currently in the process of registration.

Salbutamol has two mirror image chemical molecular components (enantiomers). Purifying this mixture of enantiomers to a product containing only a single enantiomer (the R enantiomer) may have several advantages, including a reduction of adverse effects caused by the unwanted enantiomer and preventing the interference with the favourable effects of the active enantiomer (Marchant-Forde *et al*., 2012). The purity of R-salbutamol may yield at least equivalent, physiological responses and efficiencies than racemic beta-agonist products currently in use, but with superior profiles of safety (Marchant-Forde *et al*., 2008) and toxicity.

Marchant-Forde *et al*. (2008) specifically investigated the effect of R-salbutamol on the well-being of finishing pigs, which may be of particular interest as consumers increasingly demand products that meet animal well-being criteria. In this study, the authors showed little effect on behaviour and physiology of finishing pigs that were being supplemented with R-salbutamol over a 4-week period. The heart rate response to transport after 24 or 48 hours of withdrawal was actually decreased in supplemented pigs relative to the control group.

In a study done by Warriss *et al*. (1991) on the palatability of meat from pigs treated with R-salbutamol, a trained taste panel could not detect any increase in toughness from the treated meat. The possible explanation may be that there was an apparent increase in juiciness. The panel therefore gave the meat treated with R-salbutamol the same acceptability score as that from the control meat.

Dunshea *et al*. (2005) mentions that salbutamol shows improved production performance in finisher pigs, that it does not affect marbling score and that there are indications of decreased drip loss with salbutamol supplementation, contrary to increased drip loss caused by cimaterol. However, Marchant-Forde *et al*. (2012) found a lower marbling score as well as lower colour score in meat from salbutamol-supplemented pigs.

Marchant-Forde *et al*. (2012) shows a positive effect on growth and carcass composition of pigs supplemented with R-salbutamol with increased average daily gain, better feed conversion ratio, 2 - 3% higher dressing percentage, 5 - 6 kg heavier warm

carcass mass and 3 - 4 mm less backfat thickness at the 10th rib compared to the control group.

2.3 BETA-RECEPTOR SUBTYPES OF SKELETAL MUSCLE TISSUE AND ADIPOCYTES

Even though it is well known that skeletal muscle tissue and adipocytes are the tissue types that respond best to beta-agonists, there is only limited knowledge about the beta-receptors subtypes on these tissues (Mersmann, 1995).

With the use of competitive ligand binding studies, Sillence & Matthews (1994) found mostly beta$_2$-receptors on skeletal muscle and adipocytes of cattle. Van Liefde *et al*. (1994) found more or less 75% beta$_2$-receptors and 25% beta$_1$-receptors to be on the adipocytes of cattle. According to Van Liefde *et al*. (1994), there is no evidence of beta$_3$-receptors on bovine adipocytes, but Casteilla *et al*. (1994) found transcripts of all three beta-receptor suptypes in bovine adipocytes – the explanation might be that there are some beta$_3$-receptors in the few brown adipocytes from the neonatal period left between the white adipose tissue of the adult (Pietri-Rouxel & Strosberg, 1995). In sheep, Bowen *et al*. (1992) found mostly beta$_2$-receptors on adipocytes. In the skeletal muscle of pigs, saturation ligand binding could only detect one receptor binding site with possibly beta$_1$-receptors and beta$_2$-receptors (Mersmann, 1998). Neither lipolysis nor ligand binding could clearly indicate the proportions of receptor subtypes on porcine adipose tissue (Mersmann, 1998).

2.4 THE EFFECTS OF BETA-AGONISTS ON SKELETAL MUSCLE TISSUE

An increase in muscle mass is a common effect seen in cattle, pigs and sheep fed beta-agonists. Skeletal muscle growth is a result of hypertrophy; therefore it is believed that the mechanism behind the increase in muscle mass from administering beta-agonists can be the following: an increase in muscle protein synthesis, a decrease in muscle protein

degradation, or a combination of both (Wheeler & Koohmaraie, 1992; Mersmann, 1995; Strydom *et al*., 2009).

It is not clear which of these mechanisms contribute the most to an increase in muscle mass, but Dunshea *et al*. (2005) found that the majority of studies support the increase of muscle protein synthesis rather than the decrease in muscle protein degradation. Two studies are discussed by Dunshea *et al*. (2005) in this regard: in the first study, clenbuterol was fed to lambs for a week and protein synthesis was increased in the hindlimb by 45%; in the other study, ractopamine increased skeletal muscle protein synthesis by 46%.

Protein degradation is often measured by the protease activities in the muscle. Protease activities are reduced, or protease inhibitors are increased by beta-agonist treatment (Koohmaraie *et al*., 1991; Bardsley *et al*., 1992; Sainz *et al*., 1993).

An explanation of how a beta-agonist can cause decreased protein degradation, is the increased activity of calpastatin after beta-agonist supplementation. Calpastatin is an inhibitor of the calsium-activated proteases: the calpains (Sensky *et al*., 1996; Dunshea *et al*., 2005). In an experiment where epinephrine was administered to pigs, there was a positive relationship between plasma epinephrine and skeletal muscle calpastatin concentrations (Sensky *et al*., 1996). Pringle *et al*. (1993) indicated that muscle growth in sheep may be stimulated through the effect of the beta-agonist on the calpain-calpastatin system. Strydom *et al*. (2009) noted an association between differences in tenderness and effects on calpastatin in beef cattle.

Dunshea *et al*. (2005) mentions that initial studies with clenbuterol supplementation in lambs show increased muscle weight despite no significant effects on skeletal muscle protein synthesis – which indicates decreased protein degradation.

Beta-agonist treatment increases the number of RNA transcripts for certain skeletal muscle proteins. For example, the mRNA is increased after beta-agonist treatment for the following: the myosin light chain (Smith *et al*., 1989), alpha-actin (Helferich *et al*,. 1990;

Koohmaraie *et al*., 1991; Grant *et al*., 1993) and the calpain protease inhibitor, calpastatin (Higgens *et al*., 1988; Bardsley *et al*., 1992; Killefer & Koohmaraie, 1994).

There have also been negative results in some reports, where beta-agonist administration did not affect rates of protein synthesis or degradation (Bergen *et al*., 1989, Claeys *et al*., 1989; Adeola *et al*., 1992a).

Muscle growth cannot be correlated by the number of receptors on the muscle tissue, because the receptors move to and from the membrane, they are sometimes inactivated by phosphorylation and not all receptors get activated in a response, so there are usually some receptors on the membrane not currently in use (Hoey *et al*., 1995; Mersmann, 1998).

2.5 THE EFFECTS OF BETA-AGONISTS ON ADIPOSE TISSUE

Another very common effect seen from oral supplementation with beta-agonists is the reduction of carcass fat in mammals and birds (Watkins *et al*., 1990; Crome *et al*., 1996; Mersmann., 2002). This can be from decreased lipogenesis or increased lipolysis (Dunshea *et al*., 2005) caused indirectly by beta-agonists. In vitro studies show that beta-agonists stimulate adipocyte triacylglycerol degradation and inhibit fatty acid and triacylglycerol synthesis in vitro (Mersmann, 1998). Few reports have been published on lipid anabolism and catabolism in vivo, but it has been shown that the plasma nonesterified fatty acid concentration increases after the administration of a beta-agonist. This implies that the adipocyte lipolytic system has been activated (Mersmann, 1998; 2002). This elevation in plasma nonesterified fatty acid concentration has been reported in beta-agonist supplementation of pigs (Mersmann, 1987; Hu *et al*., 1988; Adeola *et al*., 1992b), and cattle (Blum & Flueckiger, 1988; Eisemann *et al*., 1988). Dunshea *et al*. (2005) shows that ruminants respond better than pigs to beta-agonists with regards to a decrease in fat deposition. Reasons given by Dunshea *et al*. (2005) for this phenomenon include: a

combination of rapid down-regulation of receptors on adipocytes and insensitivity of porcine adipocytes to beta-agonists.

Mersmann (2002) explains in detail that when a beta-agonist activates a beta-receptor, cAMP activates protein kinase A, which in turns phosphorylates hormone-sensitive lipase, which is then able to start the lipolysis process. During lipolysis, fatty acids are produced and removed from adipose tissue in order to be available as energy sources by other tissues. From this physiological understanding, Mersmann (2002) concludes that during a beta-adrenergic response, fatty acids will neither be synthesised nor esterified into triacylglycerol in order to be stored in the adipose tissue, therefore both of these processes – an increase in lipolysis as well as a decrease in lipogenesis – will lead to an overall decrease in fat deposition.

Similar to studies on the effects of beta-agonists on skeletal muscle tissue, many studies on the effects of beta-agonists on adipose tissue indicate negative results where beta-agonists had no or little effect on lipid metabolism in vitro (Spurlock *et al*., 1993; 1994; Mills & Mersmann, 1995). Also, where the agonist does show an effect, results are not always persistent: ractopamine, when fed to pigs, showed no effect on the deposition or on adipocyte lipogenic rates in vitro (Liu *et al*., 1994a). Dunshea *et al*. (1993a; 1993b) found that ractopamine caused a decrease in fat content but little or no decrease in the daily rate of lipid deposition. However, other researchers found a decreased rate of lipid deposition in pigs fed ractopamine (Mitchell *et al*., 1991) and salbutamol (Oksbjerg *et al*., 1996).

The effects of the response seem to fade with ongoing supplementation of the beta-agonist in cattle (Eisemann *et al*., 1988) and in sheep (Beermann *et al*., 1987).

The number of receptors of adipose tissue decrease to a greater extent than skeletal muscle receptors in pigs (Spurlock *et al*., 1994). This might explain why smaller effects are seen on adipose tissue compared to skeletal muscle tissue in some beta-agonist studies (Mersmann, 1998).

Experimental design, including genetic background of the animals, might cause variable and sometimes negative results in beta-agonist trials (Mersmann, 1998).

2.6 THE RESPONSE OF DIFFERENT SPECIES TO BETA-AGONIST SUPPLEMENTATION

Mersmann (1998) indicates that the same beta-agonist may not activate the beta-receptor of the target tissue with equal efficacy in different species. A few possible reasons noted by Mersmann (1998) include: variations in the agonist affinity for the receptor(s), coupling of the agonist-receptor complex to the signal transduction system, and factors that influence delivery of the compound to the receptor sites. It is also possible that the receptor can become inactivated in a short period of time, or that a specific species may have fewer receptors on a specific target tissue compared to another species, causing a reduced response by the beta-agonist.

With the use of ligandbinding, some researchers found that bovine muscle tissue cells (Sillence & Mathews, 1994), bovine adipocytes (Sillence & Mathews, 1994; Van Liefde *et al.*, 1994) and ovine adipocytes (Bowen *et al.*, 1992) almost only have $beta_2$-receptors. In contrast, pharmacological and mRNA data from Mersmann (2002) shows that porcine adipocytes have over 70% $beta_1$-receptors. According to Mersmann (2002), these different types of receptors, along with receptor subtype tissue distribution and the variation in the pharmacology of receptor subtypes, cause different responses to beta-agonists in the tissues of different species.

A general simplification shows that sheep, cattle and turkeys respond equally well to beta-agonists, pigs have a little lower response to beta-agonists and chickens have the lowest response (Mersmann, 1998; Moody *et al.*, 2000; Mersmann, 2002; Hossner, 2005). A possible explanation may be that some species, e.g. broiler chickens, have been genetically selected for maximum growth over the years. They cannot respond well to beta-agonists in

terms of growth because they are already close to their biological maximum (Mersmann, 1998; 2002). Over the years, there has been intensive selection for lean carcasses in pigs. Therefore, Mersmann (2002) noted that the control pigs are already lean and genetically advanced with little subcutaneous fat and it will be difficult to detect fat deposition differences between control and beta-agonist treated groups.

Mersmann (2002) and Dunshea *et al*. (2005) also noticed that factors other than species may play a role in the response to a beta-agonist, for example breed, age, sex, the type and concentration of beta-agonist, treatment period and diet.

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CHAPTER 3. MATERIALS AND METHODS

3.1 ANIMALS

A total of 62 typical male feedlot cattle (bulls, medium maturity of composite Bonsmara type) were obtained from a large feedlot (Beefcor, Gauteng, South Africa) at an average age of about 12 months old and an average weight of approximately 340 kg. The animals were selected from a larger batch of weaners to ensure a uniform group. The trial was conducted at the feedlot of the University of Pretoria, where a more accurate estimate of feed efficiency could be obtained for different treatment groups with individual electromagnetic feeders (Calan gates, American Calan Inc., Northwood, New Hampshire, USA).

3.2 EXPERIMENTAL DESIGN

A total of 62 animals were stratified according to weight and then randomly allocated to the four treatment groups after an initial adaptation period of 30 days in which the bulls had to learn to use the feeding stations. For each treatment group, there were two pens with eight bulls in each pen. Treatment commenced a week after the allocation of the groups so that the animals could first adapt to the new ranking order in the groups during those seven days. For statistical analyses, data from 54 out of the 62 animals were used after excluding outliers, morbid animals and animals with clinical pathology. No mortalities occurred.

Figure 3.1 provides a schematic illustration of how treatments were randomly allocated to pens. The treatment groups were as follows:

- Negative control with no beta-agonist in the feed over the entire finishing period (C) – 14 bulls (two animals excluded due to an abscess and outlier data).
- R-salbutamol at 120 mg/animal/day in the feed for the last 30 days of the finishing period (S30) –14 bulls (two animals excluded due to liver fluke & outlier data).

- R-salbutamol at 120 mg/animal/day in the feed for the last 40 days of the finishing period (S40) –13 bulls (three animals excluded due to outlier data).

- Zilpaterol hydrochloride at 60 mg/animal/day in the feed for the last 30 days of the finishing period (Zh) –13 bulls (three animals excluded due to hardware disease and outlier data).

10*	PEN 4	PEN 5	1
9*			2
8*	Treatment Zh	Negative Control	3
7*			4
6*	8 bulls initially;	8 bulls initially;	5
5*			6
4*	7 bulls for	7 bulls for	7
3*			8
2*	analyses	analyses	9
1*			10
10	PEN 3	PEN 6	1
9			2
8	Treatment S40	Treatment Zh	3
7			4
6	8 bulls initially;	8 bulls initially;	5
5			6
4	6 bulls for	6 bulls for	7
3			8
2	analyses	analyses	9
1			10
10	PEN 2	PEN 7	1
9			2
8	Treatment S30	Treatment S40	3
7			4
6	8 bulls initially;	8 bulls initially;	5
5			6
4	6 bulls for	7 bulls for	7
3			8
2	analyses	analyses	9
1			10
10	PEN 1	PEN 8	1
9			2
8	Negative Control	Treatment S30	3
7			4
6	8 bulls initially;	8 bulls initially;	5
5			6
4	7 bulls for	8 bulls for	7
3			8
2	analyses	analyses	9
1			10

*The numericals 1 – 10 refer to the electromagnetic feeders. Each bull was given a transponder around its neck with a number between 1 and 10 that corresponded with a specific feeder.

Figure 3.1 Illustration of random allocations of treatments to pens

A General Linear Model was used with a Bonferroni Multiple Comparisons test for each parameter (see section 3.9 for more details regarding the statistical analysis). Carcass classification score and carcass conformation were evaluated by means of a Chi-square analysis. Each individual animal represented an experimental unit, as they were fed individually and did not have any influence on each other.

3.3 EXPERIMENTAL TREATMENTS

The bulls obtained from the Beefcor feedlot have been processed as per usual practice: Upon arrival at Beefcor feedlot, when entering an intial three month phase on natural pasture, they received Ectoshield (25 ml), Bovishield (2 ml), Multisomni (1 ml), Bovitect P (1 ml), Botuhtrax (2 ml), Covexin (2 ml), Multimin (5 ml), Lumpyvax (1 ml), Verbamec L (5 ml), Maxitet (30 ml) and Revalor S. After 3 months on natural pastures, when entering the Beefcor feedlot pens, they received Ivermectin 1% (25 ml), and again Bovishield (2 ml), Multisomni (1 ml) and Revalor S.

The three experimental treatments as well as the negative control were randomly allocated to the different feed pens at Hatfield experimental farm at the University of Pretoria. Two beta-agonist treatments (one 120 mg/animal/day R-salbutamol and 60 mg/animal/day zilpaterol hydrochloride) were applied for the last 30 days of the finishing period and one treatment group received R-salbutamol at 120 mg/animal/day for the last 40 days of the finishing period. No vitamin D_3 was fed for the entire duration of the trial, as it has been shown that vitamin D_3 supplementation can improve meat tenderness (Karges *et al*., 2001) and could therefore have influenced the results. During the first two weeks of the adaptation period very cold and rainy weather occurred. Many animals were treated for respiratory disease and it was decided to check the lungscore of each animal at slaughter to determine if this event had a negative effect on their growth. A withdrawal period of 3 days applied to all bulls after which they were slaughtered at Chamdor abattoir (Gauteng, South Africa).

3.4 FEED AND WATER

The bulls were fed individually through electromagnetically operated feeding stations (Calan gates, American Calan Inc., Northwood, NH, USA). Expected intake (according to data from the feedlot where the bulls were selected from) was approximately 3.0% of live mass per day, but actual intake was 2.5% of live mass per day at the start of the treatment, increasing to 2.9% of live mass per day at the end of the treatment. Beta-agonist treatments were mixed into the feed as part of the standard finisher ration. Intake was recorded accumulatively and good quality water was freely available. All bulls received the same feedlot diet, supplied by Beefcor feedlot. In Table 3.1 the adaptation ration is shown, in Table 3.2 the treatment ration is shown and in Table 3.3 the withdrawal ration is shown.

Table 3.1 Adaptation ration: Standard Grower diet, available ad libitum

Item	Grower kg	Grower %
Hominy Chop	1500	37.5
MVC[1]	140	3.5
Brewer's Grain	800	20
Wheat Bran	250	6.3
Maize (crushed)	400	10
Molbag[2]	820	20.5
Straw	90	2.3
TOTAL	4000	100

[1]MVC: Mineral and vitamin premix. The composition of the MVC is presented in Table 3.4.

[2]Ingredients: Begasse 25%; Molasses Syrup 64.6%; Limestone 8.3%; NaCl 2.1%

Table 3.2 Treatment ration: Standard Finisher diet, with (treatments) or without (negative control) inclusion of the specified concentration of beta-agonist, available ad libitum

Item	Finisher kg	Finisher %
Hominy Chop	1558	39
MVC[1]	140	3.5
Brewer's Grain	750	18.8
Wheat Bran	250	6.3
Maize (crushed)	400	10
Molbag[2]	820	20.5
Straw	70	1.8
Beta-agonist[3]	see below[2]	see below[2]
TOTAL	4000	100

[1]MVC: Mineral and vitamin premix. The composition of the MVC is presented in Table 3.4.

[2]Ingredients: Begasse 25%; Molasses Syrup 64.6%; Limestone 8.3%; NaCl 2.1%

[3]Control: zero beta-agonist; Treatment S30: 120 mg/hd/day R-salbutamol for 30 days feeding prior to slaughter; Treatment S40: 120mg/hd/day R-salbutamol for 40 days feeding prior to slaughter; Treatment Zh: 60mg/hd/day zilpaterol hydrochloride for 30 days feeding prior to slaughter

Note: the nutrient analysis of the standard finisher ration is discussed under section 4.1.

Table 3.3 Withdrawal ration: Standard Finisher Withdrawal diet, without vitamin D_3, available ad libitum

Item	Finisher (withdrawal) kg	Finisher (withdrawal) %
Hominy Chop	1560	39
MVC[1]	140	3.5
Brewer's Grain	750	18.8
Wheat Bran	250	6.3
Maize (crushed)	400	10
Molbag[2]	820	20.5
Straw	70	1.8
TOTAL	3990	99.7*

* 0.3% Vitamin D_3 excluded

[1]MVC: Mineral and vitamin premix. The composition of the MVC is presented in Table 3.4.

[2]Ingredients: Begasse 25%; Molasses Syrup 64.6%; Limestone 8.3%; NaCl 2.1%

Note: the nutrient analysis of the standard finisher ration is discussed under section 4.1.

In Table 3.4 is shown the composition of the mineral and vitamin premix included in the adaptation, treatment and withdrawal diets.

Table 3.4 Mineral and vitamin premix included in all 3 diets above (indicated as MVC)

Ingredients (Composition per unit of Premix)	**Units**	**Bulk 25kg composition per 1kg**	**Bulk 25kg composition per 25kg**	**Composition per 1kg final ration (as is)**
Vitamin A	IU	4,600,000	115,000,000	3243.4
Vitamin E	mg	10,000	250,000	7.1
Vitamin B_1	mg	16,000	400,000	11.3
Niacin	mg	1,500	37,500	1.1
Magnesium	mg	31,000	775,000	21.9
Manganese	mg	24,000	600,000	16.9
Zinc	mg	95,000	2,375,000	67.0
Copper	mg	18,000	450,000	12.7
Cobalt	mg	700	17,500	0.5
Iodine	mg	1,000	25,000	0.7
Selenium	mg	300	7,500	0.2
ZnBac	mg	120,000	3,000,000	84.6
Monensin	mg	42,000	1,050,000	29.6
Tylosin	mg	15,000	3,750,000	10.6
Unit size	kg	1	25	

3.5 FEEDLOT PERFORMANCE

Feedlot performance of bulls was assessed from the start of the treatment period until the end of the withdrawal period. The parameters evaluated included weight gain and feed intake over the experimental period. The results were expressed as average daily gain

(kg/bull/day) and feed conversion ratio (gain (kg) / feed intake (kg "as is")) per treatment group.

3.6 CARCASS MEASUREMENTS AT THE ABATTOIR

All bulls were slaughtered at Chamdor abattoir according to acceptable slaughtering procedures. Carcasses were dressed down hanging from the rail, electrically stimulated for 30 seconds within 30 minutes post mortem (400 V peak, 5 ms pulses at 15 pulses per second) and split in half. Carcass halves were clearly marked and then chilled at 0 – 5°C. Carcass pH was measured with a portable pH meter, code H18424N (Hanna Instruments (Pty) Ltd, 6 Vernon Road, Morninghill, Bedfordview, South Africa) with a glass electrode specifically for measuring carcass pH. Carcass pH was measured about 3 cm deep between the 10^{th} and 11^{th} rib of the *m. longissimus dorsi* at 45 minutes, and then 3, 6, 12 and 24 hours post mortem. Carcass mass (WCM & CCM), dressing percentage (DRESS%), carcass classification score and carcass conformation data were obtained from Chamdor Abattoir (Report: R60203). Internal carcass fat distribution (visible peri-renal / channel fat) was determined by a professional meat classifier at Chamdor abattoir according to the following scale: 0 = no fat; 1 = very lean; 2 = lean; 3 = medium fat; 4 = fat; 5 = overfat and 6 = extremely overfat. Hides were weighed at the abattoir, and hide yield was expressed as the percentage of hide weight per final live weight (HY%). Carcass length was measured from caudal edge of the last sacral vertebra to the dorso-cranial edge of the atlas. Carcass compactness was expressed as the ratio of cold carcass mass per carcass length (Webb, 1992). Sampling for analyses of more carcass measurements commenced 24 hours post mortem.

3.7 SAMPLE COLLECTIONS

Feed samples were taken randomly every fortnight and pooled for proximate analysis to monitor crude protein, crude fibre, crude fat (ether extraction), ash, in vitro organic matter digestibility, calsium and phosphorous content, calsium-to-phosphorous ratio; and to calculate the metabolisable energy (see section 3.8 for calculation).

At the start and at the end of the experiment, 2 x 10 ml blood samples were collected from the jugular vein of each animal into vacuum tubes for plasma and serum analyses respectively. The blood samples obtained at each collection for serum were allowed to coagulate at room temperature for 30 min, whereas the samples obtained at each collection for plasma were immediately placed on ice. All blood samples were centrifuged at 2.75 x 1000 U/min for 10 min, with a Hettich Universal centrifuge (Hettich lab technology, Andreas Hettich GmbH & Co.KG, Tuttlingen, Germany). Serum and plasma samples were immediately decanted using transfer pipettes into 1.5 ml epindorf tubes and stored at –20°C for later analysis.

Once during the trial, a 24 hour challenge was conducted where blood samples were taken from the jugular vein of five animals in each treatment group at hours 1, 4, 7, 10, 13 and 24 after feeding the treatment feed containing R-salbutamol, to determine the half-life of R-salbutamol.

At slaughtering, six kidney, six liver and six muscle tissue samples of carcasses from bulls treated with R-salbutamol were taken and frozen for later analyses of R-salbutamol residues.

Faecal samples from six bulls treated with zilpaterol hydrochloride and faecal samples from six bulls treated with R-salbutamol were taken randomly at the end of the treatment period before beta-agonist withdrawal, and again at the end of the withdrawal period before they were transported to the abattoir. These samples were frozen for later analyses of beta-agonist residues.

Fifty four samples of the prime rib-cut were also collected at slaughter and brought to the University of Pretoria for later analyses.

3.8 SAMPLE ANALYSIS

Proximate analyses were done on the feed samples at Nutrilab, University of Pretoria. Analyses for crude protein, crude fibre, crude fat, ash and in vitro organic matter digestibility were done according to the procedures described by the AOAC (2000). Calsium was determined by a Perkin-Elmer 5100 PC Atomic Absorption Spectrophotometer (Perkin-Elmer Inc., Massachusetts, USA) and phosphorous was detetrmined by an Analytik Jena Spekol 1300 Spectrophotometer (Analytik Jena AG, Jena, Germany). Metabolisable energy was calculated using the following equation:

ME(MJ/kg DM) = 0.82 x (((2.4 x CP) + (3.9 x EE) + 1.8 x (100-Ash-CP-EE)) x IVOMD)/1000

ME~metabolisable energy

DM~dry matter

CP~crude protein

EE~ether extract

IVOMD~in vitro organic matter digestibility

Prime rib-cut samples of *m. longissimus dorsi* samples were taken from each carcass at the abattoir and analysed at the University of Pretoria for subcutaneous fat (SCF) thickness, muscle-bone-fat ratio, % cooking loss (CL), meat tenderness and an estimate of carcass composition.

Subcutaneous fat thickness was measured with a vernier caliper at the 13th rib, 50 mm from the medial plane (Swatland, 1984).

Muscle and fat was carefully separated from the bone of each sample and the muscle, bone and fat from each sample were weighed separately to determine the muscle-to-bone-to-fat ratio. Results were expressed as percentage muscle, percentage bone and percentage fat.

The rib-eye of each sample of *m. longissimus dorsi* was freshly cut along the fibre axis and this cut was used to determine cooking loss. Samples weighed between 143 and 290 grams. For determination of cooking loss, the following procedure was followed as described by Honikel (1998): The freshly cut samples were weighed to obtain the initial weights. They were placed in thin-walled plastic bags in a continuously boiling water-bath with their openings above water level to prevent water from entering the bags. Samples were cooked to an internal temperature of 75℃, after which they were removed from the water bath and put into a bath of ice cold water. The samples were then placed in storage at 4℃ for 12 hours, after which they were blotted dry with papertowel and weighed. The cooking loss was calculated as a percentage of the initial sample weight.

In order to determine tenderness, shear force was used as a parameter and the Warner-Bratzler shear force (WBSF) test was performed following the same procedure as described by Abegniga *et al*. (2013): A hollow, cylindrical metal probe of 1.27 cm in diameter and 8 cm in length were used to cut ten core samples from each cooked meat sample that was used for the determination of cooking loss. The samples were cut along the length of the fibres. An Instron machine, model 1101 was used with the following settings, similar to the specifications given by Abegnina *et al*. (2013): the load transducer was 500 N, the gage speed was 38 mm, the testing speed was 500 mm/min, the load range was 40%, the specimen type was set as round and the specimen dimension was set at 1.27 cm. Ten cuts were made by the blade perpendicular to the direction of the fibre arrangement (Honikel, 1998) to determine the average shear force value for each sample.

Serum, feed, kidney, liver, muscle tissue and faecal samples were analysed at the ARC-OVI Residue Laboratory for R-salbutamol residues using high performance liquid chromatography according to method number RRSAL077 (2011, E. Taljaard, Pers. Comm., ARC-OVI Residue Laboratory, 100 Old Soutpan Road, Onderstepoort, 0110).

Plasma concentrations of creatinine and blood urea nitrogen (BUN) were analysed at the Onderstepoort Physiology laboratory using a Cobas Integra machine, model 400 (Roche,

P.O. Box 1927, Randburg, 2125, South Africa). For creatinine, the method was a buffered kinetic Jaffe reaction without deproteinization and with compensation for serum or plasma. For urea, a kinetic test with urease and glutamate dehydroxynase was performed (2010, C. Muller, Pers. Comm., University of Pretoria, Facultry of Veterinary Science, Clinical Pathology Laboratory, Old Soutpan Road, Onderstepoort, 0110).

3.9 STATISTICAL ANALYSIS

Data was stored in XL-format and transferred to SAS (2004). Data was tested for normality and frequency distributions were done. The effects of treatment and treatment period in the variables measured were analysed by means of General Linear Model procedures of SAS (2004). Carcass classification score and carcass conformation were evaluated by means of Chi-square analysis. Treatment means were compared by means of Bonferroni's multiple range tests. The level of significance was $P < 0.05$ and a tendency towards significance at $P < 0.1$.

3.10 ETHICS, GENERAL HUSBANDRY AND CARE OF EXPERIMENTAL ANIMALS

This trial was conducted in accordance with standard ethical norms of the University of Pretoria. The trial was conducted at the Experimental Farm of the University of Pretoria under typical South African feedlot conditions. General animal health was monitored by Dr. D. Holm, (BVSc, MMedVet). Normal medication (for example bloat control) was given if and when necessary. Bulls that became severely ill or unfit for the rest of the trial received proper veterinary care and their data were not taken into account for the statistical analysis. All experimental animals were managed strictly in accordance with the research protocol.

3.11 REFERENCES

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CHAPTER 4. RESULTS AND DISCUSSION

4.1 FEED ANALYSES: CRUDE PROTEIN, CRUDE FIBRE, CRUDE FAT, CALSIUM, PHOSPHOROUS, CA:P RATIO, IN VITRO ORGANIC MATTER DIGESTIBILITY, ASH AND THE CALCULATED METABOLISABLE ENERGY VALUE

The proximate analyses of feed samples taken during the treatment period of the trial are presented in Table 4.1 on a dry matter basis (pooled results). The same standard feedlot finisher diet was used for all three treatment groups and the control group, with the beta-agonists treatments added to the rations for the treatment groups.

Table 4.1 Feed analyses (LS Means ± SD) on a dry matter basis of the finisher ration given to all animals during the treatment period (pooled results): crude protein, crude fibre, crude fat, calsium, phosphorous, Ca:P ratio, ash, in vitro organic matter digestibility and the calculated metabolisable energy value.

Nutrient	Analysis (DM basis)
Crude protein (g/100g)	14.99 ± 1.54
Crude fibre (g/100g)	11.67 ± 1.28
Fat – ether extraction (g/100g)	5.70 ± 0.32
Calsium (g/100g)	0.83 ± 0.16
Phosphorous (g/100g)	0.42 ± 0.02
Calsium:phosphorous ratio	1.98:1
Ash	6.56 ± 0.73
In vitro organic matter digestibility (g/100g)	75.72 ± 5.22
Calculated metabolisable energy (MJ/kg DM)*	11.74 ± 0.83

Note: the same standard feedlot finisher diet was used for all the treatments and the control.

*Calculation of metabolisable energy is discussed under materials and methods in section 3.8.

The diet was formulated according to the nutrient requirements of typical feedlot cattle in South Africa, for the last 40 days of the finishing period. Generally, the results of the nutrient analyses are acceptable compared to the actual nutrient requirements of South African feedlot cattle, however, the crude protein analysed to be lower than expected.

The crude protein of the ration analysed to be 14.99 g/100g. For a feedlot finisher ration, a crude protein of 16.0 g/100g is more suitable. Also, the standard deviation of 1.54 g/100g between feed samples might explain a small part of the variances observed regarding feed intake, average daily gain and subsequently feed conversion ratio (see section 4.2).

4.2 FEEDLOT PERFORMANCE: WEIGHT GAIN, FEED INTAKE AND FEED CONVERSION RATIO

Feedlot performance of bulls fed 120 mg R-salbutamol for 30 and 40 days respectively, 60 mg zilpaterol hydrochloride for 30 days, and a control group fed no beta-agonist is summarized in Table 4.2. Feedlot performance was determined based on total weight gain (kg), average daily gain (ADG), cumulative feed intake (CFI), average daily intake (ADI), and feed conversion ratio (FCR) over the 40-day trial finishing period and 3-day withdrawal period (43 days in total). Intakes (CFI and ADI) and FCR was determined on an “as is” basis.

Table 4.2 Weight gain, feed intake and feed conversion ratio (LS Means ± SD) of bulls fed 120 mg R-salbutamol for the last 30 or 40 days before slaughter, 60 mg zilpaterol hydrochloride for the last 30 days before slaughter or a control group fed no beta-agonist

Feedlot performance parameter	C (n=14)	S30 (n=14)	S40 (n=13)	Zh (n=13)
Start Weight (kg)	391.0 ± 20.73	388.64 ± 17.73	380.33 ± 21.36	382.46 ± 17.15
Final Live Weight (kg)	462.14 ± 24.18	457.14 ± 19.62	450.85 ± 24.48	453.00 ± 21.33
Total Weight Gain (kg)	71.14 ± 11.15	68.50 ± 8.62	70.46 ± 13.30	70.54 ± 16.32
ADG (kg)	1.65 ± 0.26	1.59 ± 0.20	1.64 ± 0.31	1.64 ± 0.38
CFI (kg)*	494.27 ± 107.58	510.70 ± 117.80	464.27 ± 74.79	461.74 ± 70.04
ADI (kg)*	11.49 ± 2.50	11.88 ± 2.74	10.80 ± 1.74	10.74 ± 1.63
FCR (kg)*	6.96 ± 1.28	7.41 ± 1.45	6.75 ± 1.43	6.75 ± 1.25

C~control treatment, S30~R-salbutamol treatment (30 days), S40~R-salbutamol treatment (40 days), Zh~zilpaterol hydrochloride treatment (30 days), ADG~average daily gain, CFI~cumulative feed intake, ADI~average daily intake, FCR~feed conversion ratio (kg feed consumed per kg body weight gain)

*On an “as is” basis

The data in Table 4.2 was statistically analysed with P <0.05 for differences, but no differences found. After the initial adaptation period of 30 days in which the bulls had to be taught to use the Calan gates, they were stratified according to weight and then randomly allocated to the four treatment groups. The starting weights for all four groups were equal at that time. Seven days later, after they have adapted to the new ranking order in the groups, treatment commenced and the starting weights differed somewhat, as can be seen in Table 4.2. The variation in standard deviations for ADI between treatment groups was higher than expected. Environmental conditions during the onset of this study and experimental period

were characterised by cold spells and rainfall that may in part explain the variation in FI and growth.

Feedlot performance in terms of ADG and FCR in this study per se was not different in beta-agonist fed bulls as opposed to those fed no beta-agonist in the diet. This is in contrast to other studies on the use of beta-agonists in feedlot cattle. Significant improvements in average daily gain and feed conversion efficiency of zilpaterol hydrochloride treated bulls compared to control animals were expected, according to results published by Maritz (1996) and also by Morris (1997) who both did research on zilpaterol hydrochloride supplementation to beef cattle in South African conditions. In the United States of America, where conditions are different, similar results of improved average daily gain and feed conversion efficiency of zilpaterol hydrochloride treated cattle were published by many authors (Vasconcelos *et al*., 2008, Elam *et al*., 2009 and Montgomery *et al*., 2009a; 2009b). Marchant-Forde *et al.* (2012) also reported a positive effect on growth of pigs supplemented with R-salbutamol with increased average daily gain and better feed conversion ratio compared to the control group.

Based on the current data, it appears that the adverse environmental conditions, variation of crude protein content between feed samples and also very likely, that the Calan gates had a major effect on feed intake and therefore subsequent effects on feedlot production. A larger sample size and lower bull variation would likely have increased the chance to show statistical significance. It has also been shown in the literature that bulls implanted with hormones exhibited less improvement in feedlot performance compared to castrated males implanted with steroidal growth modifiers, as intact bulls may be closer to their maximum growth potential (Hunt *et al*., 1991; Lee *et al*., 1990).

4.3 CARCASS QUALITY: WARM CARCASS MASS, COLD CARCASS MASS, DRESSING %, SUBCUTANEOUS FAT THICKNESS, HIDE YIELD %, INTERNAL CARCASS FAT DISTRIBUTION, % BONE, % FAT AND % MUSCLE OF THE PRIME RIB CUT, CARCASS CLASSIFICATION CODE, CONFORMATION AND COMPACTNESS

In Table 4.3 is summarised the carcass quality of bulls fed 120 mg R-salbutamol for 30 and 40 days respectively, 60 mg zilpaterol hydrochloride for 30 days, and a control group fed no beta-agonist. Carcass quality was determined based on warm carcass mass (WCM), cold carcass mass (CCM), dressing %, subcutaneous fat thickness, hide yield %, internal carcass fat distribution, % bone, % fat and % muscle of the prime rib-cut, carcass classification score, conformation and compactness.

Table 4.3 Summary statistics of carcass quality parameters: warm carcass mass and cold carcass mass, dressing %, subcutaneous fat thickness, hide yield %, internal carcass fat distribution, muscle-bone-fat ratio of the prime rib-cut expressed as percentages, carcass classification score, conformation, compactness (LS Means ± SD) and an estimate of carcass value of bulls fed 120 mg R-salbutamol for the last 30 or 40 days before slaughter, 60 mg zilpaterol hydrochloride for the last 30 days before slaughter or a control group fed no beta-agonist

Carcass quality parameter	C (n=14)	S30 (n=14)	S40 (n=13)	Zh (n=13)
WCM (kg)	264.76 ± 16.75	269.37 ± 13.89	268.23 ± 16.41	266.42 ± 16.92
CCM (kg) calculated at 2%	259.91 ± 16.45	263.99 ± 13.60	262.86 ± 16.08	261.11 ± 16.58
DRESS%	56.26 ± 2.41	57.80 ± 3.03	58.32 ± 2.15	57.63 ± 2.17
SCF @ 13th rib (mm)	8.21 ± 3.30	7.89 ± 3.23	8.85 ± 3.17	6.04 ± 2.59
HY%	7.03 ± 0.74	7.40 ± 0.88	6.96 ± 0.61	7.27 ± 0.77
ICFD	3.50^{b} ± 1.29	2.50^{a} ± 0.65	2.69^{ab} ± 0.48	2.54^{a} ± 0.66
Bone %	18.77 ± 2.70	18.25 ± 2.23	18.45 ± 2.15	17.34 ± 1.43
Fat %	24.58^{ab} ± 4.65	24.58^{ab} ± 3.51	22.42^{a} ± 4.62	27.09^{b} ± 3.95
Muscle %	56.64 ± 3.70	57.17 ± 3.55	59.13 ± 3.52	55.57 ± 3.56
Carcass classification score	2.36 ± 0.50	2.43 ± 0.65	2.50 ± 0.67	2.38 ± 0.65
Conformation	3.21 ± 0.43	3.21 ± 0.43	3.17 ± 0.39	3.15 ± 0.38
Compactness (kg/cm)	2.25 ± 0.14	2.24 ± 0.24	2.29 ± 0.13	2.27 ± 0.10
Estimated carcass value (ZAR)	6249.82	6349.57	6324.16	6279.05

C~control treatment, S30~R-salbutamol treatment (30 days), S40~R-salbutamol treatment (40 days), Zh~zilpaterol hydrochloride treatment (30 days), WCM~warm carcass mass, CCM~cold carcass mass, DRESS%~dressing percentage, SCF~subcutaneous fat thickness, HY%~hide yield percentage, ICFD~internal carcass fat distribution, ZAR~South African Rand

^{a,b} LS Means with different superscript letters in the same row differ (P <0.05)

Similar to the feedlot growth performance and probably for the same reason of sample size, bull variation and the effect of the Calan gates, no differences were found between the control group and beta-agonist treatment groups with regards to warm and cold carcass mass, dressing %, subcutaneous fat thickness (SCF) at the 13th rib and hide yield %. There are, however, numerous studies indicating improved carcass weights with the supplementation of zilpaterol hydrochloride to beef cattle (Shook *et al*., 2009; Leheska *et al*., 2009; Montgomery *et al*., 2009a; 2009b) and improved dressing % with the supplementation of zilpaterol hydrochloride to beef cattle (Vasconcelos *et al*., 2008; Elam *et al*., 2009; Montgomery *et al*., 2009a; 2009b). Marchant-Forde *et al.* (2012) reported 2 - 3% higher dressing percentage, 5 - 6 kg heavier warm carcass mass and 3 - 4 mm less backfat thickness at the 10th rib of pigs supplemented with R-salbutamol compared to the control group.

In this trial on average, zilpaterol hydrochloride supplementation improved profit margin of carcasses by 0.47% compared to carcasses from the control group not treated with a beta-agonist. Treating bulls with R-salbutamol for 40 days resulted in 1.19% and 0.72% higher profit margin per carcass compared to carcasses from the control group not treated with a beta-agonist and the carcasses from zilpaterol hydrochloride treated bulls respectively.

Bulls treated with R-salbutamol for 40 days had a lower (P <0.05) percentage fat (4.67%) in the prime rib-cut compared to those fed zilpaterol hydrochloride for 30 days. Bulls fed R-salbutamol for 40 days tended (P =0.087) to have a higher percentage muscle (3.56%) in the prime rib-cut compared to those fed zilpaterol hydrochloride for 30 days. Bulls that were treated with no beta-agonist had more internal carcass fat than bulls treated with R-salbutamol for 30 days and bulls treated with zilpaterol hydrochloride (P <0.05). Bulls that

were treated with no beta-agonist also tended (P =0.093) to have more internal carcass fat than bulls treated with R-salbutamol for 40 days.

The lower proportion of internal carcass fat distribution in bulls (a physiologically earlier maturing fat depot) treated with R-salbutamol for 30 days and bulls treated with zilpaterol hydrochloride for 30 days compared with bulls not treated with a beta-agonist (P <0.05) may be an indication that R-salbutamol, like zilpaterol hydrochloride, plays an effective role in lipid metabolism, especially lipolysis.

The lower percentage fat content in the *m. longissimus dorsi* of bulls that received R-salbutamol for 40 days compared to bulls fed zilpaterol hydrochloride for 30 days (P <0.05), may further suggest that R-salbutamol is a more effective lipolysis agent, which results in more effective depletion of carcass fat.

In addition, bulls that received R-salbutamol for 40 days tended (P =0.087) to show higher percentage of muscle in the carcass compared to bulls that received zilpaterol hydrochloride. This may once again confirm R-salbutamol's efficient effect on lipolysis, altering the carcass composition so that muscle percentage increases with a decrease in the percentage of fat in the carcass.

4.4 MEAT QUALITY: POST-MORTEM CARCASS PH PROFILES, % COOKING LOSS, SHEAR FORCE AND LUNGSCORE VALUES OF *M. LONGISSIMUS DORSI* SAMPLES

Post-mortem carcass profiles, cooking losses and shear force values of meat (determined in the *m. longissimus dorsi* muscle) of carcasses from bulls fed different beta-agonists at different dietary concentrations are presented in Table 4.4.

Table 4.4 Post-mortem carcass pH profiles, % cooking loss, shear force values of *m. longissimus dorsi* samples and lung score (LS Means ± SD) of bulls fed 120 mg R-salbutamol for the last 30 or 40 days before slaughter, 60 mg zilpaterol hydrochloride for the last 30 days before slaughter or a control group fed no beta-agonist

Meat quality parameter	C (n=14)	S30 (n=14)	S40 (n=13)	Zh (n=13)
pH @ 1hr pm	5.67 ± 0.32	5.56 ± 0.29	5.59 ± 0.15	6.12 ± 1.57
pH @ 3hrs pm	5.47 ± 0.15	5.39 ± 0.33	5.40 ± 0.05	5.50 ± 0.14
pH @ 6hrs pm	5.47 ± 0.11	5.39 ± 0.28	5.44 ± 0.03	5.54 ± 0.12
pH @ 24hrs pm	5.62^{ab} ± 0.11	5.68^{b} ± 0.03	5.63^{ab} ± 0.05	5.58^{a} ± 0.07
% Cooking loss	27.96 ± 3.86	26.28 ± 3.54	26.52 ± 1.80	27.53 ± 2.55
Ave Shear Force (N)	41.11^{ab} ± 11.56	40.95^{ab} ± 9.63	38.97^{a} ± 8.13	50.16^{b} ± 7.26
Lungscore	1.93 ± 1.86	1.36 ± 1.65	1.00 ± 1.76	1.77 ± 3.42

C~control treatment, S30~R-salbutamol treatment (30 days), S40~R-salbutamol treatment (40 days), Zh~zilpaterol hydrochloride treatment (30 days), pH @ 1hr pm~pH at 1 hour post mortem, pH @ 3hrs pm~pH at 3 hours post mortem, etc.

^{a,b} LS Means with different superscript letters in the same row differ (P <0.05)

Bulls treated with zilpaterol hydrochloride for 30 days had a significantly (P <0.01) lower pH (0.1) at 24 hours post mortem compared to bulls treated with R-salbutamol for 30 days. The general trend observed was a slower rate of carcass pH decline from 1 hour post-mortem until 24 hours post-mortem for zilpaterol hydrochloride fed bulls compared to carcasses from the other treatment groups.

Meat from bulls treated with zilpaterol hydrochloride recorded significant higher shear force, therefore less tender meat, compared to bulls treated with R-salbutamol for 40 days (P <0.05). The higher shear force values observed for meat samples from zilpaterol hydrochloride fed bulls suggest that this treatment resulted in carcasses with less tender

meat compared to meat from bulls fed R-salbutamol for 40 days. There was only a tendency (P =0.093) observed for bulls treated with zilpaterol hydrochloride to show higher shear force compared to bulls fed no beta-agonist and another tendency (P =0.083) observed for bulls treated with zilpaterol hydrochloride to show higher shear force compared to bulls fed R-salbutamol for 30 days.

The fact that the bulls fed R-salbutamol for 30 days had a higher pH at 24 hours post mortem compared to bulls treated with zilpaterol hydrochloride, suggests differences in muscle energy status possibly due to differences in stress responsiveness of bulls fed zilpaterol hydrochloride as opposed to those fed R-salbutamol. The consequence was a significant drop in pH at 24 hours post mortem in carcasses from zilpaterol hydrochloride fed bulls, which may also partially explain the higher shear force values indicative of tougher meat.

Meat samples from bulls treated with zilpaterol hydrochloride gave significantly higher Warner-Bratzler Shear Force values, indicating tougher meat compared to the control and R-salbutamol groups. This is typical of beta$_2$-agonists such as zilpaterol hydrochloride (Leheska et al., 2009; Rathmann et al., 2009; Strydom et al., 2002, and 2009), which show definite effects on muscle protein metabolism. It has been shown that the most economical way to reduce this toughening effect is by electrical stimulation of the carcass (Hope-Jones et al., 2010). Other suggestions include ageing of the meat (Shook et al., 2009 and Strydom et al., 2009). These methods can reduce the toughening effect of beta$_2$-agonists, but not completely eliminate it (Hope-Jones et al., 2010). R-salbutamol did not cause toughening of the meat, probably because it had a more prominent effect on lipolysis and a smaller effect on muscle metabolism.

4.5 BLOOD UREA NITROGEN (BUN) AND CREATININE

4.5.1 Interaction between treatment and time

A separate analysis was done to study the metabolic effects of blood urea nitrogen (BUN) and creatinine levels in serum samples (see Tables 4.5 and 4.6). The approach followed was to compare blood profiles of samples collected before treatments commenced, as well as samples collected just before the withdrawal period. As expected, there was no effect of treatment on BUN. However, there was a significant (P <0.001) effect of treatment on creatinine levels in the serum. There was no effect of time on serum BUN or serum creatinine concentrations, but an interaction (P <0.05) between treatment groups and time for BUN, and a tendency towards an interaction (P =0.067) between treatment groups and time for creatinine concentrations.

4.5.2 Blood urea nitrogen

Blood urea nitrogen is only dependant on the protein levels in the feed, which were the same for all four groups during the treatment period. In Table 4.5 is shown the blood urea nitrogen levels (mmol/l) tested at the start and the end of the treatment period.

Table 4.5 Blood urea nitrogen levels (mmol/l) in serum at the start and end of treatment (LS Means ± SD) of bulls fed 120 mg R-salbutamol for the last 30 or 40 days before slaughter, 60 mg zilpaterol hydrochloride for the last 30 days before slaughter or a control group fed no beta-agonist

BUN parameters (mmol/l)	C (n=12)	S30 (n=14)	S40 (n=11)	Zh (n=13)
BUN start of treatment	4.38 ± 1.67	5.71 ± 2.42	6.55 ± 1.43	5.03 ± 1.75
BUN end of treatment	6.21 ± 1.28	5.79 ± 0.97	5.72 ± 0.85	5.54 ± 1.07

C~control treatment, S30~R-salbutamol treatment (30 days), S40~R-salbutamol treatment (40 days), Zh~zilpaterol hydrochloride treatment (30 days), BUN~blood urea nitrogen

The data in Table 4.5 was statistically analysed with $P < 0.05$ for differences, but no differences found. As the treatment period progressed and bulls in all four groups received the same ration with the same protein content, the BUN concentration in the serum leveled out to be the same for all four treatment groups. Therefore, as expected, none of the end values in Table 4.5 differed between the treatment groups.

Even though the standard deviation observed for crude protein analysis was high between the feed samples analysed, (see section 4.1), this result where no differences were observed between the treatment groups and control for BUN at the end of the treatment period confirms that overall, the protein in the feed for all the treatments and the control was the same.

The BUN levels were similar in the serum samples collected at the end of the trial for all the treatment groups. This simply means that the dietary protein intakes of the different groups were similar throughout the treatment period and possibly also that stress levels of all animals stabilised.

4.5.3 Creatinine

The creatinine level in the blood serum is an indication of the protein turnover in the muscle tissue. Higher creatinine levels in the serum may therefore indicate a higher protein turnover. In Table 4.6 is presented the creatinine levels (µmol/l) tested at the start and the end of the treatment period, as well as the change in creatinine levels from the start to the end of the treatment (expressed as a percentage of the start level).

Table 4.6 Creatinine levels (µmol/l) in serum at start and end of treatment (LS Means ± SD) of bulls fed 120 mg R-salbutamol for the last 30 or 40 days before slaughter, 60 mg zilpaterol hydrochloride for the last 30 days before slaughter or a control group fed no beta-agonist

Creatinine parameters (µmol/l)	C (n=12)	S30 (n=14)	S40 (n=11)	Zh (n=13)
Creatinine start of treatment	107 ± 15.36	127 ± 24.65	119.82 ± 13.46	115.46 ± 16.65
Creatinine end of treatment	98.08^{a} ± 12.32	112.21^{ab} ± 14.39	118.09^{b} ± 12.96	123.38^{b} ± 12.71
Change from start to end	- 8.33%	- 11.65%	- 1.44%	+ 6.86%

C~control treatment, S30~R-salbutamol treatment (30 days), S40~R-Salbutamol treatment (40 days), Zh~Zilpaterol hydrochloride treatment (30 days)

^{a,b} LS Means with different superscript letters in the same row differ (P <0.05).

Bulls fed zilpaterol hydrochloride and bulls fed R-salbutamol for 40 days had higher final serum creatinine concentrations at the end of the treatment period compared to bulls that received no beta-agonist (P <0.005). However, it is important to note that only bulls fed zilpaterol hydrochloride showed an increase (indicated by the plus “+” sign) in change of serum creatinine levels from start to end of treatment, while all the other treatments and the control group showed a decrease (indicated by the minus “-” sign) in change of serum creatinine levels from the start to the end of the treatment period. This particular data suggests a higher change in rate of protein turnover in bulls fed zilpaterol hydrochloride from

the start to the end of the treatment period, probably due to a higher rate of muscle protein synthesis compared with bulls fed R-salbutamol and bulls fed no beta-agonist.

The present results therefore indicate that treatment with zilpaterol hydrochloride has a more pronounced effect on muscle protein turnover and that the effects of R-salbutamol on muscle protein turnover appear to be less pronounced. The increase in muscle protein turnover from the start to end of the treatment period might explain why meat from zilpaterol hydrochloride treated bulls had higher shear force and therefore less tender meat compared to meat from bulls treated with R-salbutamol for 40 days (see section 4.3).

4.6 RESIDUES

Consumers are becoming more health conscious and environmentally aware. Progressive withdrawal or banning of most of the growth agents based on steroid hormones and human antibiotics causes for needs to introduce new and safe production efficiencies. It is therefore appropriate to study the residue levels of R-salbutamol in consumable tissue as well as in faeces. Residue values (levels) in this study were thus compared with the maximum tolerable residue level for R-salbutamol in muscle, liver, renal fat and kidney tissues which is 0.05 mg/kg as specified in Government Notice No. R. 1387, 1999.

An attempt was made in this study to determine the half-life of R-salbutamol in a 24-hour challenge. The result of this challenge is shown in Fig. 4.1. From this graph it is evident that there was a peak at 0.0043 ng/ml in plasma serum concentration of R-salbutamol at 7 hours after ingestion of R-salbutamol, with a dramatic decrease to 0.0027 ng/ml at 10 hours after ingestion and an omissible serum concentration of 0.0017 ng/ml at 24 hours after ingestion of R-salbutamol.

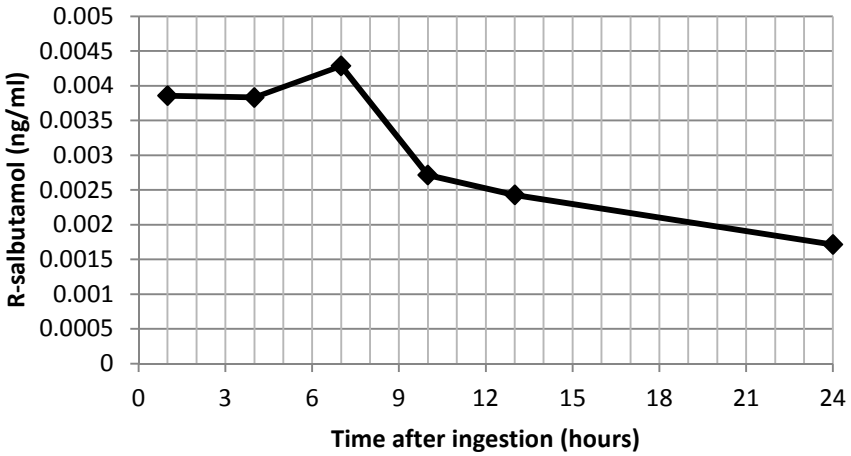

Figure 4.1 Serum values for R-salbutamol residues as determined after feeding 120 mg of R-salbutamol at T_0 and monitoring serum levels every 3 hours over a 24-hour sampling period until T_{24}

Fig. 4.2 shows a residue level in the muscle tissue of bulls treated with R-salbutamol of 0.0736 mg/kg after a withdrawal period of three days. There were almost no traces (≤ 0.0012 mg/kg) of residues found in the kidney and liver samples of the same bulls.

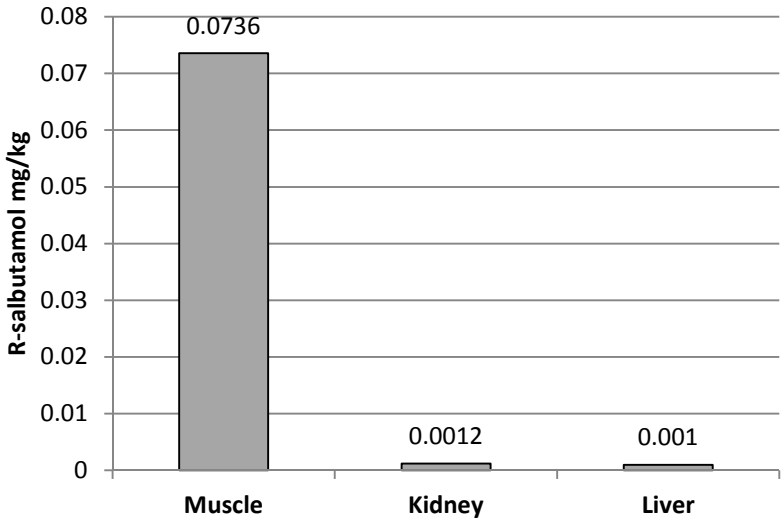

Figure 4.2 Tissue R-salbutamol residues in samples from muscle, kidney and liver from R-salbutamol fed bulls after a withdrawal period of 3 days

In Fig. 4.3 it is clear that almost ten times higher levels of residues were found in the faeces of bulls fed zilpaterol hydrochloride compared to feaces from bulls consuming R-salbutamol. After the withdrawal period this difference decreased in faeces of both zilpaterol hydrochloride and R-salbutamol treated bulls, but faeces of bulls fed zilpaterol hydrochloride still had higher residue levels compared to bulls fed R-salbutamol. This is of great importance when considering contamination of the environment and especially surface water sources, when synthetically produced products are used within animal diets.

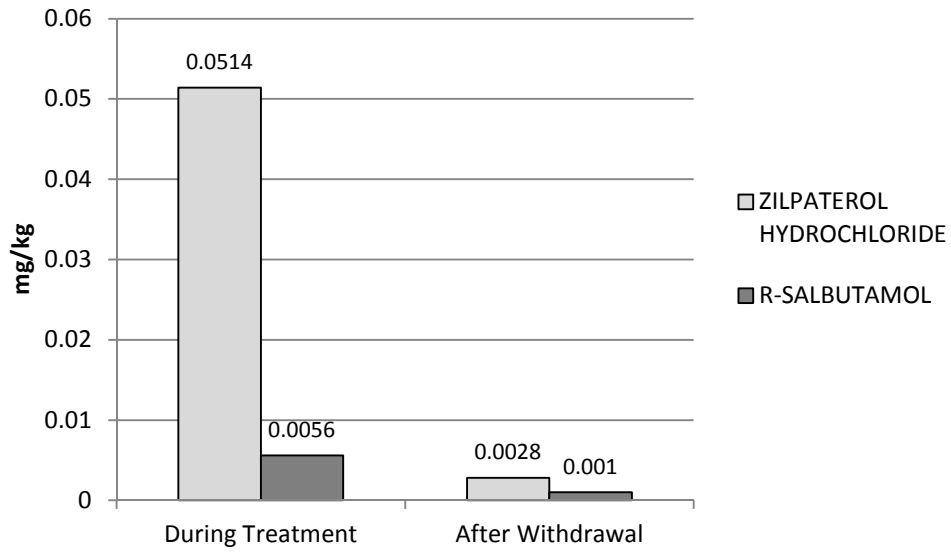

Figure 4.3 Zilpaterol hydrochloride and R-salbutamol residues in faeces

Acceptable residue levels are very important in the use of beta-agonists, as too high levels of beta-agonists in the tissues may have adverse effects on human health when consumed and too high levels of residues in the faeces may contaminate the environment, especially surface water sources around larger feedlot operations. Acceptable residue levels are found in meat from cattle treated with zilpaterol hydrochloride when animals are fed on average 60 mg/head/day in the feed for up to 30 days before slaughter, with a withdrawal period of more than two days as specified in the product details for Zilmax® (SA Reg no. G2180, Act 36/1947). In this trial, the feeding of zilpaterol hydrochloride to bulls complied

under these specifications. The residue levels of R-salbutamol in this study were very low in serum, muscle, kidney, liver samples, and were well below the maximum tissue residue level of 0.05 mg/kg specified for R-salbutamol (Government Notice No. R. 1387, 1999), except for the muscle tissue R-salbutamol residue level that was slightly higher at 0.07 mg/kg. Where residue levels in the faeces were compared, the R-salbutamol samples had much lower residue levels during the treatment (0.0056 mg/kg) and withdrawal period (0.001 mg/kg) compared with the zilpaterol hydrochloride samples. From an environmental perspective, this result shows R-salbutamol to be a beta-agonist that is a safer alternative compared to current beta-agonists in use.

From a mechanistic point of view, when one compares the 30-day with the 40-day data, it appears that R-salbutamol may take somewhat longer and also requires higher feeding levels than zilpaterol hydrochloride before significant physiological effects are observed. It is also possible that R-salbutamol may be metabolised faster due to the shorter half-life of this molecule.

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Zilmax®, SA Reg no. G2180, Act 36/1947. Product Catalogue

CHAPTER 5. CONCLUSION AND CRITICAL EVALUATION

5.1 CONCLUSION

The present results indicate that the physiological modes of action of R-salbutamol and zilpaterol hydrochloride differ somewhat and this will influence the way in which these beta-agonists are to be used. The results in this study show that while zilpaterol hydrochloride decreases fat synthesis and increases protein turnover, as well as causing a toughening effect on the meat, R-salbutamol seems to increase lipolysis very effectively, but the effect on protein turnover is less significant, which may explain why R-salbutamol had no adverse effect on meat tenderness. Due to R-salbutamol's extremely short half-life compared to other beta-agonists, it is a safe beta-agonist to use for animals produced for human consumption and the environment alike, and its residue levels are well below the recommended limits for beta-agonist residues. It appears that the treatment of 120 mg R-salbutamol for 40 days before slaughter showed the most promising animal performance results on bulls when compared to 60 mg zilpaterol hydrochloride treatment for 30 days before slaughter under current South African feedlot conditions. At the same time, feeding similar levels of R-salbutamol for about 10 days longer did not result in detrimental effects on meat quality, and meat- and environmental safety parameters. The results indicate that the feeding of higher levels of R-salbutamol for a longer period can be exploited to further enhance animal performance without compromising meat quality and safety. These results provide good baseline data on the effect of R-salbutamol on carcass and meat quality in South African feedlot bulls. More research is required on R-salbutamol, to optimise treatment period and dosage under commercial feedlot conditions. The differential effects of R-salbutamol in different stages of growth and also between bulls, steers and heifers require further study, particularly due to the shorter half-life and more pronounced effect on lipolysis of internal fat depots as opposed to other depots and muscle protein turnover.

5.2 CRITICAL EVALUATION

The aims of this project were to compare the effects of R-salbutamol at 120 mg per animal per day on South African feedlot bulls for the last 30 or 40 days prior to slaughter, versus zilpaterol hydrochloride fed at 60 mg per animal per day for the last 30 days prior to slaughter on growth and feedlot performance, as well as on carcass and meat characteristics. Much research has been done on zilpaterol hydrochloride supplementation to beef cattle and its effects on feedlot performance and carcass characteristcs, but little information is available on R-salbutamol supplementation to beef cattle. The literature available only focuses on the effects of R-salbutamol on monogastric animals. Zilpaterol hydrochloride was used as a positive control in this study and R-salbutamol was the molecule under investigation, with the intention of providing good baseline data on its effects on growth and feedlot performance and carcass and meat characteristics when supplemented to feedlot cattle. While no significant differences were observed regarding growth and feedlot performance, this study showed pronounced differences between the control group, R-salbutamol treated bulls and zilpaterol hydrochloride bulls regarding carcass and meat characteristics.

Originally, the research protocol stated that only castrated male animals were to be used in the trial, as bull variation would have been excluded and their carcasses would naturally have more fat compared to the carcasses of bulls. Beta-agonists have been shown to have more pronounced effects on animals with higher fat deposition and in older animals, but in South Africa most beef cattle are slaughtered at about fourteen months of age, so the most accurate simulation to the current South African feedlot practice would have been to take castrated male animals of similar young age. However, at the time when the weaners were to be selected at the feedlot, only bulls of medium maturity type and the right age were available, so the protocol had to be amended to only include bulls in this study.

The trial was specifically conducted at the Experimental Farm of the University of Pretoria so that the animals could feed individually from electromagnetic feeders in order to get more accurate data per animal. Expected intake (according to data from the feedlot were the bulls were selected from) was approximately 3.0% of live mass per day. However, the average actual intake for all the animals in the trial was 2.5% of live mass per day at the start of the treatment, increasing to 2.9% of live mass per day at the end of the treatment. No differences were observed between control and treatments concerning feed intakes and the variances were extremely high. This influenced data regarding feed efficiency, and the suggestion would be to rather simulate the typical South African feedlot situation closer by letting cattle, who are social eaters by nature, feed together from open troughs or bunkers. Feed efficiency data will be more accurate when using average feed intakes of pens with open troughs or bunkers and when the number of pens per treatment is increased.

Regarding the addition of beta-agonist treatment to the feed, a suggestion would be to rather add the treatment as a top dressing every day so that the levels of treatment can be controlled according to the daily feed intakes. A clean bunk approach should also be followed when feeding in open troughs or bunkers. All feed must be consumed before fresh feed is given the next morning. In this situation, all animals will approach the trough or bunker at the same time. There must be sufficient feeding space so all the animals in the pen can comfortably feed at the same time.

The adaptation and trial periods were planned to be conducted in a supposedly dry period from the end of autumn to the middle of winter. The unexpected rain and cold spells experienced during this study appears to have influenced adaptation and animal performance. This, together with the effects of using individual feeders and bull variation, contributed to the variation in feedlot performance of experimental animals. The animals that got sick during the cold spell were treated for respiratory disorder and at slaughter the lungs

were inspected for lung lesions. The lungscore data was statistically analysed and no differences were observed between treatment groups, which confirms that all animals in the trial were equally affected by the rain and cold spells.

This study confirms that the beta-agonist zilpaterol hydrochloride decreases fat synthesis and increases protein turnover in feedlot bulls, while R-salbutamol increases lipolysis in feedlot bulls. Zilpaterol hydrochloride has a toughening effect on meat, while the effects of R-salbutamol on meat tenderness were negligible. Both of these molecules showed residue levels in tissue and faecal samples, well below the acceptable limits. It is clear that the physiological modes of action of zilpaterol hydrochloride and R-salbutamol differ and therefore the supplementation strategies should be different for these two molecules. This study makes it clear that performance enhancers, specifically the beta-agonists zilpaterol hydrochloride and R-salbutamol, do not show beneficial effects on feed performance when supplemented to intact male feedlot cattle and also do not show beneficial effects on feed performance when supplemented to animals exposed to unfavourable climatic conditions.