The effect of r-salbutamol on apparent digestibility, feed efficiency, growth, carcass characteristics, and meat quality of feedlot lambs

by

Martin Johannes de Klerk

Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in Animal Science in the Faculty of AgriScience at Stellenbosch University

Supervisor: Prof L.C. Hoffman

Co-supervisor: Prof P.E. Strydom

Co-supervisor: Prof V. Muchenje

Co-supervisor: Dr J.vE. Nolte

December 2016

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2016

Table of contents

Chapter 1:

General introduction

The world population is predicted to increase to 9 billion by 2050 and to meet the demand for food, production of especially commodities associated with high incomes such as meat, will have to increase (FAO, 2009). As feed ingredients are expensive and animals compete with humans for the same feed ingredients, the aim of current research worldwide is to improve the efficiency with which animals utilize their feeds (Parr *et al*., 2016). The modern meat production industry also finds itself in a society that is becoming more environmentally aware and also health conscious. This has led to withdrawal and banning of most steroid hormone-based growth agents and also a demand for healthier meat with more lean tissue and less fat. The industry thus requires new safe production efficiencies to maximise productivity to feed the growing world population (Steenekamp, 2014; Parr *et al*., 2016).

Oral synthetic beta-adrenergic agonists such as ractopamine hydrochloride, zilpaterol hydrochloride and r-salbutamol are examples of growth agents that are considered safe to use in feedlot production systems (Steenekamp, 2014). The goal of beta-agonists is to increase utilization of feed that results in an increase of lean carcass weight (Parr *et al*., 2016). These beta-agonists have pharmacological and chemical properties similar to natural catecholamines such as dopamine, norepinephrine and epinephrine (Bell *et al.,* 1998). Beta-agonists bind to beta receptors activating receptors in muscle and fat tissue causing a change in biochemical growth in these tissue types (Mersmann, 1998). This results in repartitioning effects which seems to involve a reduction in lipogenesis, an increase in lipolysis and a reduction in protein breakdown which favours protein synthesis (Warriss, 2010). If nutrients are partitioned towards muscle growth rather than fat deposition resulting in a better dressing percentage, profitability and carcass leanness can be improved while feed costs can be decreased (Brooks *et al*., 2009). The improved carcass leanness can also appeal to more health conscious consumers.

Zilpaterol hydrochloride is one the most researched beta-agonists and has been used legally in South Africa and Mexico for more than ten years and since 2006 in USA feedlots (Shook *et al*., 2009). It was shown in numerous studies to improve weight gain, feed efficiency and carcass leanness when administered to feedlot cattle and sheep (Brooks *et al*., 2009; Shook *et al*., 2009; Lopez-Carlos *et al*., 2010; Strydom *et al.,* 2009). However, zilpaterol hydrochloride has been shown to have a negative impact on meat tenderness in various studies (Strydom *et al*., 2011; Rathmann *et al*., 2008). Zilpaterol hydrochloride and ractopamine hydrochloride have been observed to increase lameness in cattle when administered in high doses and also increase death losses (Whay, 2010; Longeragan *et al*., 2014;

Montgomery *et al*., 2009). Furthermore, zilpaterol hydrochloride can increase heat stress in sheep and make pigs more susceptible to stress when handled roughly (Macias-Cruz *et al*., 2010; James *et al*., 2013).

The beta-agonist r-salbutamol is a purified derivative of racemic (RS-) salbutamol or albetrol which is used for treatment of respiratory disorders in humans all over the world. However, the few official papers that have been published on its use in animals were on pigs, poultry (Marchant-Forde *et al*., 2008) and one on feedlot cattle (Steenekamp, 2014). The effects r-salbutamol had on finishing pigs were investigated from an animal welfare perspective which may be of special interest as consumers are more aware of animal well-being and demand products from such systems (Steenekamp, 2014). R-salbutamol showed little effect on behaviour of finishing pigs over a four week period (Marchant-Forde *et al*., 2008) and also had a positive effect on growth and carcass composition (Marchant-Forde *et al*., 2012). However, no research has been conducted on the use of this agent in sheep under feedlot conditions. When the effect of zilpaterol hydrochloride was tested on sheep meat quality specifically, a decrease in colour parameters, a decrease in intramuscular fat, an increase in shear force, an increase in connective tissue and harder meat was observed (Dávila-Ramírez *et al.,* 2013). Feedlotting of sheep is on the increase in amongst others, an attempt to increase productivity and meat production by removing lambs earlier from grazing systems and finishing them off in feedlots.

The aim of this study was to test the effects r-salbutamol has on apparent digestibility, feed efficiency, growth, and carcass characteristics of feedlot sheep. As beta-agonists' action differs with different types and concentration of beta-agonists, different species and different breeds within species (Moody *et al*., 2000; NRC, 1994), two sheep breeds (medium and late maturing) were supplemented with three different concentrations of r-salbutamol.

1.1 References

Bell, D. G., I. Jacobs, and J. Zamecnik. 1998. Effects of caffeine, ephedrine and their combination on time to exhaustion during high-intensity exercise. Eur. J. Appl. Physiol. Occup. Physiol. 77(5): 427-433.

Brooks, J., H. Claus, M. Dikeman, J. Shook, G. Hilton, T. Lawrence, J. Mehaffey, B. Johnson, D. Allen, and M. Streeter. 2009. Effects of zilpaterol hydrochloride feeding duration and post mortem aging on warner-bratzler shear force of three muscles from beef steers and heifers. J. Anim. Sci. 87(11): 3764-3769.

Cochrane, K., C. De Young, D. Soto, and T. Bahri. 2009. Climate change implications for fisheries and aquaculture. FAO Fisheries and Aquaculture Technical Paper. 530: 212.

Dávila-Ramírez, J. L., L. Avendaño-Reyes, U. Macías-Cruz, N. G. Torrentera-Olivera, L. Zamorano-García, A. Peña-Ramos, and H. González-Ríos. 2013. Effects of zilpaterol hydrochloride and soybean oil supplementation on physicochemical and sensory characteristics of meat from hair lambs. Small Rumin. Res. 114(2): 253-257.

James, B., M. Tokach, R. Goodband, J. Nelssen, S. Dritz, K. Owen, J. Woodworth, and R. Sulabo. 2013. Effects of dietary L-carnitine and ractopamine HCl on the metabolic response to handling in finishing pigs. J. Anim. Sci. 91(9): 4426-4439.

Longeragan, G., D. Thomson, and H. Scott. 2014. Increased mortality in groups of cattle administered the B-adrenergic agonist ractopomine hydrochloride and zilpaterol hydrochloride. PLoS One. 9(3): e91177.

López-Carlos, M., R. Ramírez, J. Aguilera-Soto, C. Aréchiga, F. Méndez-Llorente, H. Rodríguez, and J. Silva. 2010. Effect of ractopamine hydrochloride and zilpaterol hydrochloride on growth, diet digestibility, intake and carcass characteristics of feedlot lambs. Livest. Sci. 131(1): 23-30.

Macías-Cruz, U., F. Álvarez-Valenzuela, N. Torrentera-Olivera, J. Velázquez-Morales, A. Correa-Calderón, P. Robinson, and L. Avendaño-Reyes. 2010. Effect of zilpaterol hydrochloride on feedlot performance and carcass characteristics of ewe lambs during heat-stress conditions. Anim. Prod. Sci. 50(10): 983-989.

Marchant-Forde, J., D. Lay, R. Marchant-Forde, K. McMunn, and B. Richert. 2012. The effects of R-salbutamol on growth, carcass measures, and health of finishing pigs. J. Anim. Sci. 90(11): 4081-4089.

Marchant-Forde, J., D. Lay, R. Marchant-Forde, K. McMunn, and B. Richert. 2008. The effects of R-salbutamol on behavior and physiology of finishing pigs. J. Anim. Sci. 86(11): 3110-3124.

Mersmann, H. J. 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76(1): 160-172.

Montgomery, J., C. Krehbiel, J. Cranston, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, R. Swingle, and T. Montgomery. 2009. Effects of dietary zilpaterol hydrochloride on feedlot performance and carcass characteristics of beef steers fed with and without monensin and tylosin. J. Anim. Sci. 87(3): 1013-1023.

Moody, D., D. Hancock, and D. Anderson. 2000. Phenethanolamine repartitioning agents. Farm Animal Metabolism and Nutrition: 65-96.

National Research Council (US). 1994. Metabolic modifiers: Effects on the nutrient requirements of food-producing animals. National Academy Press.

Parr et al, T., M. H. Mareko, K. J. Ryan, K. M. Hemmings, D. M. Brown, and J. M. Brameld. 2016. The impact of growth promoters on muscle growth and the potential consequences for meat quality. Meat Sci: 93-99

Steenekamp, S. 2014. Growth Performance and Meat Characteristics of Feedlot Cattle Fed R-Salbutamol Or Zilpaterol Hydrochloride during the Finishing Period. Master's Thesis. Department Animal and Wildlife Sci. University of Pretoria. South Africa.

Strydom, P., L. Frylinck, J. Montgomery, and M. Smith. 2009. The comparison of three β-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Sci. 81(3): 557-564.

Strydom, P. E., M. Hope-Jones, L. Frylinck, and E. C. Webb. 2011. The effects of a beta-agonist treatment, vitamin D 3 supplementation and electrical stimulation on meat quality of feedlot steers. Meat Sci. 89(4): 462-468.

Vasconcelos, J., R. Rathmann, R. Reuter, J. Leibovich, J. McMeniman, K. Hales, T. Covey, M. Miller, W. Nichols, and M. Galyean. 2008. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing diet on feedlot cattle performance and carcass traits. J. Anim. Sci. 86(8): 2005-2015.

Warris, P. D. 2010. Meat Science. An Introductory Text. 2nd edition. CABI Publishing. Wallingford UK.

Whay, H. R., and D. C. Main. 2010. Improving animal welfare: Practical approaches for achieving change. Improving Animal Welfare: A Practical Approach. Wallingford: CABI. : 227-251.

Chapter 2:

Literature review

2.1. Beta-adrenergic agonists

2.1.1 Catecholamines

Catecholamines are mostly neurotransmitters that function within the nervous system of an animal's body. These catecholamines stimulate processes that make energy available for organ systems so the animal can react to a predator or threat. This phenomenon is called the "fight or flight syndrome", named by W.B. Cannon in 1932 (Hossner, 2005).

The three main catecholamines found in the mammalian body are the adrenal medullary hormone, epinephrine and two neurotransmitters of the sympathetic nervous system called norepinephrine and dopamine. Epinephrine is also called adrenaline while norepinephrine is called noradrenaline (Mersmann, 1998; Hossner, 2005). Dopamine, epinephrine and norepinephrine are all synthesised from tyrosine. The decarboxylation of dihydroxyphenylalanine or DOPA forms dopamine, the hydroxylation product of tyrosine. Norepinephrine is the hydroxylation product of dopamine. Epinephrine is then the methylation product of norepinephrine (Fig 2.1.1). Epinephrine thus has an added methyl group distinguishing it from norepinephrine which does not (Hossner, 2005).

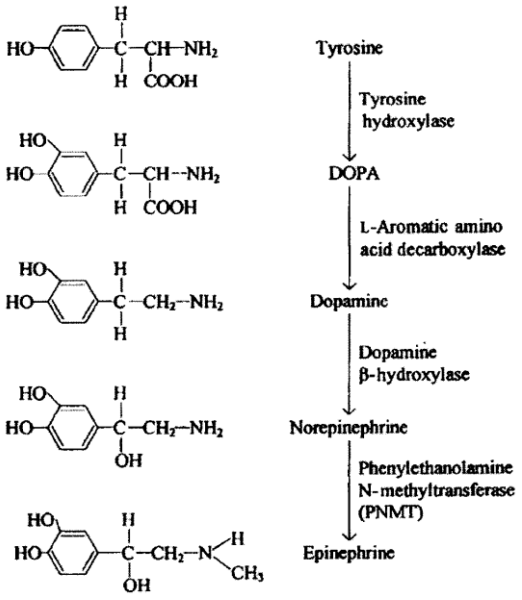

Figure 2.1.1: Biological synthesis of catecholamines (Adapted from Fiems, 1987).

The levels in which these catecholamines (epinephrine, norepinephrine and dopamine) circulate in the blood vary between species but are normally low. Dopamine and norepinephrine function as neurotransmitters in the nervous system and only a very high level or concentration of norepinephrine

is needed to induce an endocrine response. When epinephrine and dopamine are present in the blood circulation, it is usually due to the occurrence of sympathetic activation which causes the catecholamines to spill over into the blood (Hossner, 2005). The concentration in which epinephrine circulates is lower than the concentration of norepinephrine in most species. During stress however, epinephrine responds to a larger extent than norepinephrine (Mersmann, 1998). When an animal experiences stress from either internal or external sources, the sympathetic nervous system stimulates the preganglionic nerve, which causes the adrenal medulla to release epinephrine (Muchenje *et al*., 2008). Alpha and beta-receptors are the main receptors that these catecholamines act on as they act through specific receptors on their target tissues. The proportions of these receptors in various tissues determine its response to adrenergic stimulation (Smith, 1998; Parr *et al.*, 2016).

2.1.2 Beta-adrenergic receptors

Beta-adrenergic receptors can be found in the plasma membrane of almost any type of tissue cells and like with hormones the receptors can act with either beta-agonists or catecolamines. Beta-receptors are complex molecules and can consist of a chain of more than 400 amino acids (Smith, 1998). These beta-receptors are linked to G proteins and occur in different forms (Hossner, 2005). Seven relatively hydrophobic transmembrane domains anchor the receptor in the plasma membrane (Fig 2.1.2). Four of these amino acid loops are on the outside of the cell membrane while three of the portions are on the inside of the cell. The site where the ligands bind is right in the centre of the seven domains (Ostrowski *et al*., 1992).

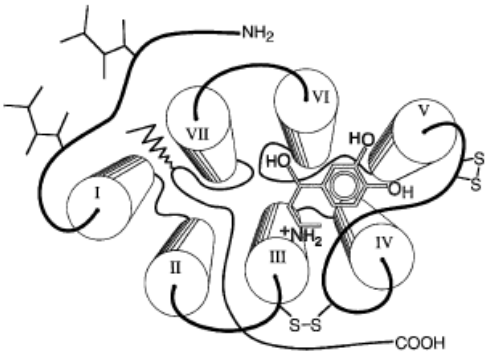

Figure 2.1.2: A beta-adrenergic receptor with its seven domains, indicating the binding site for norepinephrine (Adapted from Mersmann, 1998).

Beta-adrenergic receptors are subdivided into three subtypes. The subtypes are $beta_1$, $beta_2$ and $beta_3$ receptors, each more abundant in different tissue types where they mediate different responses (Hossner, 2005; Smith, 1998). Receptor subtypes will be discussed more thoroughly in the category; Beta-receptor subtypes.

2.1.3 Beta-adrenergic agonists

Beta-agonists are compounds that occupy a beta-receptor and mimic a natural, biological mediator's activity (Fiems, 1987; Parr *et al.*, 2016). The beta-agonist is generally more potent than the endogenous mediator or catecholamine and usually stimulates the system maximally (Stiles *et al.,* 1984). The action of Beta-adrenergic agonists is in relation with the catecholamines, epinephrine and norepinephrine. Beta-agonists are chemically synthesized and show strong similarity with epinephrine as shown in Figure 2.1.3 (Fiems, 1987).

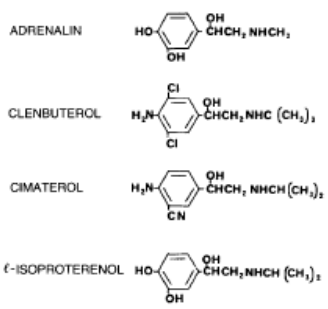

Figure 2.1.3 Chemical formula of epinephrine or adrenaline and some well-known beta-agonists (Adapted from Fiems, 1987).

In veterinary and human science beta-agonists are used as bronchodilators. They treat asthma, stimulate both rate and strength of cardiac contraction, and induce uterine relaxation. Beta-agonists are active when orally administered and can be used in farm animal production systems. The beta-agonist compounds used in animal production systems have advanced effects on skeletal muscle and adipose tissue. In the doses used however, it has reduced effects on the cardiovascular system. When beta-agonists are used in animal production systems, it is referred to as repartitioning agents. This is due to their effects on redirecting nutrients for use in skeletal muscle at the expense of adipose tissue. Beta-agonists are effective in varying degrees when used in several farm animal species such as cattle, sheep, poultry and swine (Dunshea *et al.,* 2005; Strydom *et al*., 2009).

2.1.4 Physiological mechanism of beta-adrenergic agonists

Beta-agonists have no effect on circulating hormones in an animal's body but rather act on the beta-receptors in tissues affected by the treatment (Dunshea *et al*, 2005).

In order for a beta-agonist to have biological activity, it needs a hydroxyl group bonded to the beta-carbon in the R configuration, a substituted six-membered aromatic ring, nitrogen with a positive charge in the ethylamine side chain, and also to have specificity for the beta-receptor, a substituent on the aliphatic nitrogen (Carlström *et al*., 1973). The catecholamines epinephrine and norepinephrine

which are natural adrenergic neurotransmitters however, have an exception of a bulky group on the aliphatic nitrogen (Smith, 1998).

Beta-agonists bind to beta-adrenergic receptors at three points on the molecule: the charged aliphatic amine, the beta hydroxyl group and the aromatic ring or substituents of it (Wallis, 1993; Easson & Stedman, 1933). Wallis, (1993) also showed that specific amino acids within the beta-adrenergic receptor are responsible of interacting with these three binding sites. Substituents of any of these sites or regions may have adverse effects on receptor binding and thus agonistic activity (Ruffolo, 1991).

The physiological activity a beta-agonist has is dependent on its activity at the receptor and thus the rates of metabolism, absorption, elimination and the resulting distribution to target tissues. This means that the chemical characteristics influencing a beta-agonist's receptor activity may also influence the absorption, metabolism, elimination and distribution of that beta-agonist. The pharmacokinetic properties of individual molecules thus determine the physiological mechanism of beta-agonists to an extent (Smith, 1998).

2.1.5 Molecular physiology of beta adrenergic receptors in a beta-adrenergic response

When a beta-agonist binds to the receptor the beta-agonist receptor complex activates the Gs protein. The Gs protein's α-subunit, adenylyl cyclase, the enzyme that produces cyclic adenosine monophosphate or cAMP is one of the major intracellular signalling molecules. cAMP produces its effects by binding to the regulatory subunit of protein kinase A. This then releases the catalytic subunit that phosphorylates intracellular proteins. These proteins include enzymes that are activated when phosphorylated (Altarejos and Montminy, 2011). The CREB (cAMP response element binding protein) is phosphorylated by protein kinase A, when this happens, the CREB binds to a response element of cAMP in the regulatory part of the gene and then stimulates the transcription of that gene. The transcriptional activity of the CREB is increased by phosphorylation, providing the mechanism of beta-agonist receptor mediated gene transcription in the mammalian cell. Some enzymes, however get inactivated when phosphorylated (Strosberg, 1992; Parr *et al.*, 2016).

Beta adrenergic responses can be ended in various ways. Beta-agonists can be removed from the receptor, it can be degraded (Mersmann, 1998), the receptor can be removed from the plasma membrane, which will decrease the amount of receptors available to facilitate the response (Strosberg, 1992), or the receptor can be inactivated. The last mentioned takes place when phosphorylation of the receptor occurs by protein kinase A or beta-adrenergic receptor kinase (Altarejos and Montminy, 2011).

Two enzymes inactivate the natural beta-agonists epinephrine and norepinephrine. The enzymes are catechol-o-methyl transferase and monoamine oxidase. The catechol-o-methyl transferase methylate the hydroxyl group with the catechol-ring, while the monoamine oxidase deaminates the ligand (Mersmann, 1998). Norepinephrine can also be absorbed at the synaptic clefts and myoneural junctions. When the concentration of norepinephrine lowers it will lead to a decreased activation of receptors (Dunshea *et al*., 2005; Smith, 1998).

2.1.6 Beta-receptor subtypes

The concept of α-adrenergic receptors and β-adrenergic receptors comes from the late 1940's when epinephrine and norepinephrine were used to investigate physiological functions. Both α- and β-adrenergic receptors are stimulated by epinephrine and norepinephrine, epinephrine however is more effective in lower concentrations for α-adrenergic receptors than norepinephrine is. The classification of these receptors allows better understanding of the complexities associated with adrenergic functions (Mersmann, 1998; Smith, 1998).

How tissue types respond to beta-agonist stimulation is a result of the different proportions of α- and β-receptors relative to one another in the cells of the tissue. Alpha-adrenergic receptors involve the responses associated with the sympathetic nervous system and thus affect smooth muscle contraction as well as vasoconstriction. Beta-receptors on the other hand are more reactive with beta-agonists and normally aid relaxation of smooth muscle, it is also more receptive to epinephrine than norepinephrine (Hossner, 2005).

In 1967 it was found by Lands *et al* that beta-adrenergic receptors also had subtypes. This study led to the sub classification of beta receptors into $Beta_1$- and $Beta_2$-adrenergic receptors (Minneman *et al*, 1979). In the 1970's another subtype was found and classified as the $Beta_3$-adrenergic receptor subtype (Hossner, 2005).

$Beta_1$-adrenergic receptors exert its effect on intestinal smooth muscle and cardiac muscle and play a major role in lipolysis. $Beta_1$-receptors are seen as the main adrenergic receptor of the neural system. That is why norepinephrine is more potent for $Beta_1$-adrenergic receptors than for $Beta_2$-receptors (Mersmann, 1998). These receptor subtypes are the most abundant in various tissues in the body. $Beta_1$-receptors account for 80% of beta-receptors in adipose tissue, 70% of beta-receptors in the heart, 65% in the lung, 60% in skeletal muscle and 50% in the liver (Hossner, 2005).

$Beta_2$-receptors respond weakly to norepinephrine and thus respond mainly to epinephrine. These receptors are involved in vasodilation, bronchodilation, relaxation of uterine smooth muscle, glycogenolysis in the liver and finally, also in the beta-agonist response of skeletal muscle (Altarejos and Montminy, 2011).

Tissue response and sensitivity to beta-agonists can be variable (Dunshea *et al*., 2005; Strydom et al., 2009). As tissues can have multiple receptor subtypes (Minneman *et al*., 1979). Tissues thus have different ratios of receptor types and subtypes making its response to beta-agonists very complex. The same beta-agonist can be expressed differently between species and a different beta-agonist can then again be expressed differently within a single species (Dunshea *et al*., 2005).

In a single species the ratio of $Beta_1$- to $Beta_2$-receptors will differ between certain cell and tissue types, while the ratio of $Beta_1$- to $Beta_2$-receptors in the same cell and tissue type but in different species may also differ. The ratio in which receptor subtypes are present on the plasma membrane of a cell can be influenced by the cell's stage of differentiation and also the hormones interacting with it (Mersmann, 1989).

Different beta-receptor subtypes have different RNA transcripts, possess different size proteins and also different sequences of amino acids (Mersmann, 1998). In 2002, Mersmann reported that the receptor subtypes have different length amino acid chains. $Beta_1$-receptors have a chain length of about 460 amino acids, $Beta_2$-receptors a chain length of about 420 amino acids and $Beta_3$-receptor a chain length of about 410 amino acids. In a single species, the three beta-receptor subtypes have 50% homology in their amino acid sequence. With a single Beta-receptor subtype across species, a 75% or more homology in the amino acid sequence was found (Strosberg, 1992; Hall *et al*., 1993; Pietri-Rouxel & Strosberg, 1995). Mersmann (2002) reports that the homology in amino acid sequence within a species is 45-60% and the homology in amino acid sequence for the same beta-agonist across species is higher than 70%.

2.1.7 Physiological effects caused by the supplementation of synthetic beta-agonists, both direct and indirect

Oral synthetic beta-agonists are similar to natural catecolamines like dopamine, epinephrine and norepinephrine in terms of chemical composition and physiological characteristics (NRC, 1994). As for the differences a single beta-agonist has on an animal, both direct and indirect, reporting effects are complicated. Due to beta-agonists being present on plasma membranes of cells, in a wide variety of tissue types (Mersmann, 1995).

Over the years, due to large interest from the biomedical community, molecules binding to beta-receptors were synthesised by the thousands. These molecules include both agonists and antagonists (Mersmann, 2002). Both bind to the receptor but the antagonist does not activate the Gs protein like the agonist does and therefor blocks its function (Hossner, 2005; Smith, 1998).

Beta-agonists have the potential to increase blood flow to particular parts of the body. When the increase in blood flow is directed to the skeletal muscle it may enhance hypertrophy. This means that

increased energy sources and substrates are available for protein synthesis in skeletal muscles. Blood flow in adipose tissue also increases. This however may carry nonesterified fatty acids away from the tissue to enhance the degradation of lipids in adipose tissue. These mechanisms account for the more direct effects of oral beta-agonists on adipocytes and muscle cells (Dunshea *et al.,* 2005; Mersmann, 1998). Blood flow is likely increased to a number of organs in the mammalian body due to the increased heart rate many beta-agonists induces. There is also an increased blood flow to the hind limb of cattle with chronic or acute beta agonist administration (Eisemann *et al*., 1988), to the hind limp of sheep with chronic beta-agonist administration (Beerman *et al*., 1987) and to the adipose tissue and skeletal muscle of pigs with acute administration of beta agonists (Mersmann, 1989).

During stress responses in animals, beta-agonists can also play an important role in relaxing and dilating the airways by acting on the receptors of the bronchial tracheal muscle tissue. When the receptors are activated, more oxygen can be distributed to the brain and muscles (Mersmann, 2002). In human and veterinary medicine, beta-agonists like salbutamol have been well researched and are used as remedies for the treatment of asthma (Steenekamp, 2014).

Another effect of these beta-agonists is the modulation of endocrine substances circulating in the body (Parr *et al.*, 2016). In sheep, plasma thyroid hormones are increased by chronic administration of beta-agonists Beermann *et al*., 1987); in cattle however, plasma thyroid hormones are not increased (Zimmerli & Blum, 1990). When exogenous beta-agonists were acutely administered in pigs, the endogenous plasma catecholamines were elevated. The elevated endogenous catecholamines could then mediate effects in different tissues (Mersmann, 1989). In a similar experiment in cattle by Blum & Flueckiger (1988), plasma concentrations of epinephrine and norepinephrine were not modified.

Somatotropin is believed to not aid in the increase of muscle mass and the decrease of fat mass when administered (Dunshea *et al.,* 2005). This is due to 3 main reasons. The first being the fact that somatotropin receptors and beta-agonist receptors have no structural relationship with one another. Secondly, no evidence suggests that the intracellular signalling systems of the two receptors are related and the third is that somatotropin produces hypertrophy in many organs while beta-agonists produce hypertrophic effects restricted to cardiac and skeletal muscle as well as salivary glands (Reeds and Mersmann, 1991). Somatotropin further causes a major reduction in feed intake while beta-agonists have little or no effect at all (Mersmann, 1998). Oral beta-agonist administration does not increase plasma somatotropin secretion and when administered in sheep, it actually lowers plasma concentrations of somatotropin (Beermann *et al*., 1987; Thomas *et al.*, 1994).

Modified plasma concentrations of metabolites such as lactate or glucose can arise in different tissues due to altered rate of metabolic pathways. This happens when exogenous beta-agonists work on pathways controlled by beta-receptors (Mersmann, 1998).

Little evidence exists that beta-agonists increase basic metabolic rate when chronically administered (Rikhardsson *et al*., 1991; *Yen et al*., 1991). Reduced feed intake observed with certain beta-agonists function in that specific species when prescribed conditions exists (Dunshea *et al*., 2005).

2.2 Effect of beta-agonist supplementation on feed passage rate and digestibility

The effect of beta-agonist supplementation on rumen fermentation, passage rate and nutrient digestibility has been scarcely studied and little literature is available. Beta-agonists binding to beta-adrenergic receptors along the gastrointestinal tract have been found to affect motility and secretions within the tract. Motility is affected when adrenoreceptor stimulation by direct beta-receptor mediated action on smooth muscle, reduces contraction of intestines (except at sphincters). Tonic activity also occurs in the beta-adrenergic pathway, as contraction pressures tends to be increased by beta-antagonists (McIntyre and Thompson, 1992). In humans, a beta-adrenergic pathway has shown to control secretomotor function. *In vitro*, contraction of isolated smooth muscle cells and muscle strips that are electrically stimulated is inhibited whilst beta-antagonists on the other hand enhance contraction (McIntyre and Thompson, 1992).

In a study to evaluate the effects of ractopamine on *in vitro* fermentation, it was found that ractopamine hydrochloride affected microbial populations, stimulated microorganism fermentation in the rumen and improved dry matter disappearance (Lopes-Carlos *et al.,* 2010). When cimaterol was administered in sheep it was found that only crude fibre decreased while other digestibility coefficients were unaffected. Rumen fluid however contained significantly lower concentrations of butyric acid and higher concentrations of acetic and propionic acids compared to the control (Fiems *et al*., 1991). Sheep fed zilpaterol-hydrochloride for 30 days had greater neutral detergent fibre (NDF) and gross energy (GE) intake and a tendency for higher organic matter (OM), dry matter (DM), crude protein (CP), acid detergent fibre (ADF) and ether extract intake than sheep fed zilpaterol hydrochloride for 15 days when apparent digestibility was measured. However, the DM, OM, CP, and GE were less in lambs fed zilpaterol-hydrochloride than that of controls (Macias-Cruz *et al*, 2010).

In feedlot lambs supplemented with ractopamine- and zilpaterol hydrochloride, DM, CP, ADF, and NDF of both treatments had no significant differences over a control (Lopes-Carlos *et al*., 2010). Feedlot steers treated with ractopamine-hydrochloride, zilpaterol-hydrochloride and clenbuterol digested the same amounts of DM and CP. Lambs supplemented with cimaterol also had similar nutrient digestibility (Kim *et al*., 1989; Rikhardsson *et al.*, 1991). Romero *et al*. (2011) noted that rumen

fermentation activities such as pH, VFA, NH_3-N, and DM degradability in the rumen were unaffected by zilparerol-hydrochloride treatment in feedlot steers.

2.3 The effects of beta-agonists on growth, feed efficiency and product yield

The first person to present data indicating that use of agents such as caffeine, nicotine, and epinephrine could change mammalian growth, was Cunningham in 1965. These agents can function by directly or indirectly changing the intracellular concentration of cAMP (Cunnigham, 1965). In the early 1980's data was published on the modulation of growth in animals fed a beta-agonist, clenbuterol. Animals had better growth performance, a better carcass yield and also a change in body composition (Ricks *et al.*, 1984). Most of the above mentioned effects were observed in cattle, pigs, chickens and sheep with oral administration of clenbuterol (Fiems *et al*., 1987; Thornton *et al*, 1985; Jones *et al*., 1985). In the years that followed, several other beta-agonists were fed to different species with similar effects (Mersmann, 1998). To date, new beta-agonists are still being tested and used.

When an animal has a stress response where catecholamines stimulate the process of energy mobilization for its own survival, it seems logical that the energy will then not be available for growth. The catecholamine derivatives such as beta-agonists however, can improve body composition and production efficiency of farm animals (Hossner, 2005). Beta-agonists' potential value lies in its repartitioning effects. The amount of fat in the body is decreased while protein accretion is increased. Increased protein accretion promotes muscle development (Warris, 2010). Beta-agonists appear to do this by reducing lipogenesis, increasing lipolysis and also by reducing protein degradation and thereby favouring protein synthesis (Warris, 2010). When nutrients are partitioned to muscle development rather than fat deposition, the greater dressing percentage and carcass leanness can increase profitability and decrease feed costs (Brooks *et al*., 2009).

Beta-agonists supplementation in general showed desirable effects on growth and feedlot performance. The effects include increased average daily gain, better feed conversion ratio or efficiency and better carcass yield or conformation (Moody *et al*, 2000). Carcass yields can be increased by up to 1-2% in poultry and pigs and up to 5-6% in sheep and cattle. Evidence suggests that this is due to both the decrease in the size of viscera and increase in carcass weight (Warris, 2010).

The following are examples of beta-agonists that have been extensively tested with their effect on growth and feed efficiency.

2.3.1 Clenbuterol

The beta-agonist Clenbuterol induce a decrease in total body fat content and an increase in lean tissue content of farm animals in general (Baker *et al*., 1984). This is true for species such as cattle, sheep,

pigs, chickens and turkeys. In some cases, an increase in weight gain and feed conversion efficiency were also observed (Ricks *et al*., 1984).

In broiler chickens, clenbuterol has shown to increase daily weight gain, final weight and feed conversion ratio. The carcass yield however, was shown to be only slightly higher. The gain in carcass protein was also markedly higher for both males and females. The effect of Clenbuterol on growth was more apparent in males whereas carcass composition was more apparent in females (Rehfeldt *et al*., 2007).

Young *et al* (1995) found that lambs that were treated with Clenbuterol showed no increases in carcass weight. The weight of individual muscles such as the *semimembranosus*, *gastrocnemius* and *biceps femoris* however, were increased.

Clenbuterol have strong receptor affinity and its adverse effects such as increased heart rate and depressed appetites, makes it illegal to use as repartitioning agent for farm animals in production systems in most countries (Spurlock *et al*., 1993).

2.3.2 Cimaterol

Cimaterol is a phenethanolamine, the same as clenbuterol (Fiems *et al*, 1987). It can increase protein gain, decrease body fat, (especially intramuscular fat), increase daily gain, increase total carcass mass and also enhance muscle growth (Sainz and Wolff, 1988). This increase in muscle growth has also been reported to increase saleable meat in livestock (Reeds *et al*., 1986).

Cimaterol too has undesirable effects like clenbuterol does. It shows potential toxic effects in humans when meat is consumed from animals that were supplemented with cimaterol (Dikeman, 1991). Cimaterol, like clenbuterol is a phenethanolamine while zilpaterol hydrochloride and ractopamine hydrochloride is synthetic. Cimaterol residues in tissues can thus potentially be toxic (Dikeman, 2007).

2.3.3 Ractopamine hydrochloride

Ractopamine hydrochloride was the first beta-agonist approved as a growth stimulant in meat producing animals in the USA (Hossner, 2005). It was approved by the FDA for use in pig production systems and feedlot cattle in the USA. It is also approved for the use in pig production systems in South Africa (Hossner, 2005).

Ractopamine hydrochloride can increase average daily gain, final body weight, feed conversion efficiency, and dressing percentage while it decreases carcass fat. Ractopamine hydrochloride has less safety concerns for both humans and animals than beta-agonists such as clenbuterol and cimaterol (Scramlin *et al.*, 2010). It is however, not as efficient as zilpaterol hydrochloride, and steers treated with zilpaterol hydrochloride showed significantly better performance in the last 33 days of feeding

than steers fed ractopamine hydrochloride (Scramlin *et al*., 2010). Marchant-Forde *et al,* (2003) also reported elevated heart rates and fatigue when handling pigs treated with ractopamine hydrochloride.

2.3.4 Zilpaterol hydrochloride

Zilpaterol hydrochloride is one of the most researched beta-agonists and has undergone extensive research. It was approved for feedlot use in the USA in 2006 and has been used legally in Mexico and South Africa for more than 10 years (Shook *et al*, 2009). Feeding of zilpaterol hydrochloride has many benefits including; increased final body weight (Montgomery *et al*., 2009), higher lean yield and carcass weights (Montgomery *et al*., 2009; Leheska *et al*., 2009), increased average daily gain (Strydom *et al*., 2009; Montgomery *et al*., 2009; Elam *et al.,* 2009), increased carcass moisture and protein with a decrease in carcass fat (Maritz, 1996; Hilton *et al*., 2009), increased dressing percentage and feed efficiency (Strydom *et al.,* 2009; Montgomery *et al*., 2009; Elam *et al.,* 2009) and improved protein to bone ratio (Maritz, 1996; Leheska *et al*., 2009).

Research has also shown that Zilpaterol hydrochloride can improve carcass yield and composition in cattle, regardless of the feeding period (Rathmann *et al*., 2009). Also when fed for longer periods of time, it will have an even better effect on carcass fat (Elam *et al*., 2009).

2.3.5 r-salbutamol

R-salbutamol is a beta-agonist and a purified derivative of racemic salbutamol or RS salbutamol (also known as albetrol). It is accepted as safe and has been used as treatment for respiratory disorders in both humans and animals for several years (Merchant-Forde *et al*., 2012). Not much research has been done on the use of r-salbutamol in ruminants however and the limited literature published in animal science journals are mostly of poultry and swine (Steenekamp, 2014). R-salbutamol is currently considered as a popular research topic and has only recently been registered for use in feedlot cattle.

Salbutamol has two enantiomers, which are described as two chemical molecules that represents mirror images of each other. There are several advantages of purifying these enantiomers to a product only containing the R-enantiomer. The advantages include the absence of the unwanted enantiomer which can interfere with the positive effects of the active enantiomer and there is also a reduction in negative or adverse effects caused by the unwanted enantiomer (Merchant-Forde *et al*., 2012). The pure form of r-salbutamol may cause the same physiological responses with the same efficiency as products containing the beta agonist racemic, but with superior safety and levels of toxicity (Merchant-Forde *et al*., 2008).

When Merchant-Forde *et a*l. (2008) investigated the influence of r-salbutamol on the well-being of finishing pigs it was found that there was little effect on the physiology and behaviour of pigs

supplemented with the beta-agonist over a four week period. With consumers demanding products of animals with better animal welfare, this study could be of particular value. It was further found that after 24 or 48 hours of transport, pigs that were supplemented, actually had lower heart rates than the control group (Merchant-Forde *et al*., 2008).

R-salbutamol had positive effects on growth of supplemented pigs with improved average daily gain, better feed conversion efficiency, better dressing percentage of up to 2-3%, 5-6 kg heavier warm carcass weight and a decrease in back fat thickness of up to 3-4mm over the control group (Merchant-Forde *et al*., 2012).

When r-salbutamol and zilpaterol hydrochloride were compared in feedlot bulls, no significant differences in growth and feed efficiency was found in either of the beta-agonist supplemented animals compared to the control. R-salbutamol decreased internal carcass fat when supplemented for 40 days in feedlot bulls compared to control animals. Percentage fat in the prime cut was lower than bulls fed zilpaterol hydrochloride for 30 days. A tendency for r-salbutamol treated bulls to have higher percentages of muscle in the prime-cut compared to zilpaterol hydrochloride treated bulls was also observed. This suggests that r-salbutamol is an effective agent in lipid metabolism, successful in depleting carcass fat. Zilpaterol hydrochloride treated bulls had a significantly lower pH 24 hours post-mortem than r-salbutamol treated bulls when treated for the same period of time. The trend observed was a slower rate of pH decline from 1 hour until 24 hours post-mortem for zilpaterol hydrochloride treated bulls compared to r-salbutamol treated bulls and the control (Steenekamp, 2014).

2.4 The effect of beta-agonists on meat quality

Literature suggests that beta-agonists have undesirable or negative effects on meat quality. It was found that when supplemented with beta-agonists meat can decrease in tenderness (Brooks *et al*., 2009). Adverse effects on sensory panel scores have also been documented (Hilton *et al*., 2009; Leheska *et al*., 2009).

Over the last couple of years, focus has shifted from growth and production to consumer demands and perception. It is therefore just as important to study the effects of beta-agonist supplementation on meat quality as it is to study its effect on growth and feed efficiency (Webb, 2010). The following are extensively studied beta-agonists with its effects on meat and carcass characteristics.

2.4.1 Clenbuterol

Clenbuterol has a negative impact on meat tenderness. Animals treated with clenbuterol had higher Warner-Bratzler shear force values when compared to control groups (Mersmann, 1998). In cattle, Miller *et al*. (1988) found a 13.5% increase in Warner-Bratzler shear force, a 22% increase was found by Schiavetta *et al* in 1990 and 113% increase was found by Luno *et al* in 1999. A lower subcutaneous

fat thickness and marbling score was found in feedlot cattle (Strydom *et al*., 2009). The same author also found higher Warner-Bratzler shear forces in beef due to a higher calpastatin activity among others.

2.4.2 Cimaterol

As with Clenbuterol, Cimaterol also has a negative effect on meat tenderness. It shows an increase in shear force values of 27-45% as reported by Fiems *et al*. (1987), 55-145% as found by Chikhou *et al.* (1993) and 136-250% as noted by Vestergaard *et al*. (1994). Cimaterol also reduced fat contend in sheep carcasses (Rikhardsson *et al*., 1991).

2.4.3 Ractopamine hydrochloride

Ractopamine hydrochloride like other beta-agonists mentioned increases toughness of meat. A Warner-Bratzler shear force increase of 12% has been reported when supplemented to beef steers at 300mg/kg per day (Shroeder *et al*., 2003). Increased calpastatin activity has been found to contribute to the higher shear force in meat from feedlot cattle (Strydom *et al*., 2009). Ractopamine hydrochloride further decreased cooling loss, USDA yield grade and fat thickness when administered on feedlot lambs (Lopez-Carlos *et al*., 2010).

2.4.4 Zilpaterol hydrochloride

The effects of Zilpaterol hydrochloride have been extensively researched and literature also concludes an increase in the toughness of meat from feedlot steers fed Zilpaterol hydrochloride (Kellermeier *et al*., 2009; Strydom *et al*., 2011). It was found that even with feeding durations of 20 to 40 days, zilpaterol hydrochloride treated steers and heifers showed greater Warner-Bratzler shear force values compared to control groups (Brooks *et al.,* 2009). When supplemented for 30 to 50 days, meat from beef steers had 20-28% higher Warner-Bratzler shear force values (Strydom *et al*., 2011). The increase in shear force values have been reported to be due to increased calpastatin activity. By electrically stimulating meat treated with zilpaterol hydrochloride its toughness can be decreased by triggering calpains during the early onset of rigor (Hope-Jones *et al*., 2012). Another technique used for decreasing toughness of zilpterol hydrochloride treated meat is aging (Brooks *et al.*, 2009; Holmer *et al.,* 2009). However, even when electrical stimulation and aging is used, meat treated with zilpaterol still has higher shear force values than meat from control groups. When meat was aged for 7, 14 and 21 days Warner-Bratzler shear force values were still higher and meat was still tougher than untreated meat (Rathmann *et al*., 2009).

Zilpaterol hydrochloride causes an increase in drip loss and a reduced redness in meat (Hope-Jones *et al*., 2012). Zilpaterol also decreases marbling scoreand subcutaneous fat thickness, decreases quality grade (Hilton *et al*., 2009; Montgomery *et al*., 2009) and has a negative effect on palatability (Leheska *et al*. 2009).

2.4.5 R-salbutamol

No literature suggesting an increase in toughness of meat from animals supplemented with r-salbutamol could be found. In a study on the palatability of pork from pigs treated with r-salbutamol, no increase in toughness could be detected. The possible reason for this can be the apparent increase in the juiciness of the meat, creating a false sensation of tenderness in the mouth when eaten (Warriss *et al*., 1991). It seems that for the above mentioned reason the panel gave meat with r-salbutamol the same acceptability score as control meat.

R-salbutamol further seems to decrease drip loss and show no effect on marbling score in finisher pigs (Dunshea *et al*., 2005). This is in contrast to other beta-agonists such as Zilpaterol hydrochloride and cimaterol which seems to increase drip loss. Conflicting results were found by Merchant-Forde *et al*. (2012) however, which suggests lower marbling and colour score in meat from finisher pigs when treated with r-salbutamol.

In a study comparing zilpaterol-hydrochloride and r-salbutamol in feedlot cattle, it was found that Warner-Bratzler Shear Force values for samples treated with zilpaterol-hydrochloride were significantly higher than r-salbutamol samples. Furthermore, no difference in shear force values were found in samples treated with r-salbutamol and control groups without a beta-agonist. This means that when treated with r-salbutamol, meat is not only less tough than zilpaterol-hydrochloride meat but also show no increase in toughness over control groups when administered in feedlot cattle. It is suggested that this is due to r-salbutamol having a more prominent effect on lipolysis and a smaller effect on muscle metabolism (Steenekamp, 2014).

2.5 Beta-agonists' effect on skeletal muscle tissue

Beta-agonists generally increase muscle mass in farm animals such as pigs, cattle and sheep. The increase in muscle mass is considered to be mainly due to hypertrophy as there is no increase in DNA content in affected skeletal muscle (Hossner, 2005). Muscle hypertrophy has been a consistent result in animals supplemented with beta-agonists. When cattle were treated with zilpaterol-hydrochloride, a drastic increase in longissimus muscle fibre diameter over the controls were observed (Kellermeier *et al*., 2009). Zilpaterol increases the mRNA abundance of myosin heavy chain IIx. This myosin isoform would then be responsible for the larger diameter fibres, classified then as fast glycolytic (Baxa *et al.*, 2009). These findings are supported by other authors suggesting that cimaterol also increase muscle fibre diameter (Vestergaard *et al*., 1994). An increase in muscle fibre diameter results in an increase in the toughness of meat. Meat will thus be less tender (Dunshea *et* al., 2005).

There is some confusion however, due to why the increases in muscle mass by hypertrophy occur. Authors found differences in protein synthesis and degradation, suggesting a combination of these (Dunshea *et al*., 2005). Initial studies on lambs found that despite an increase in muscle mass no

significant changes in protein synthesis were observed, suggesting reduced protein degradation (Bohorov, 1987). In another study where clenbuterol were fed for one week, an increase in protein synthesis of 45% were found in the hind limb of lambs, but only an 8% increase in protein degradation. The net result was an increase in muscle accretion of 130% in the hind limb (McDonagh *et al.,* 1999). In a study by Bergen *et al*. (1989), dietary ractopamine increased protein synthesis of skeletal muscle by 46%. With zilpaterol-hydrochloride, proteolysis was not altered in cattle when compared to controls. The rate of protein degradation in steers was also shown to occur at rates similar to animals without a beta-agonist supplemented (Kellermeier *et al*., 2009).

Many differences thus occur between individuals treated with beta-agonists. Apart from a few exceptions, most beta-agonists however, cause an increased protein accretion due to a reduction in protein degradation (Hossner, 2005).

Protein degradation in the muscle is often measured by its protease activities. A decrease in both lysosomal protease activity (cathepsin B) and non-lysosomal proteinase activity (calpain μ) is observed with treatment of beta-agonists (Hossner, 2005). In ruminants, the activity of calpastatin, the major skeletal muscle inhibitor of protease, is increased by a variety of beta-agonists (Koohmaraie *et al*., 1990; Kretchmar *et al.,* 1990). The increase in calpastatin reduces activity of calpain μ (Hossner, 2005). This also suggests that beta-agonists tend to decrease protein degradation (Dunshea *et al*., 2005). Stimulation of muscle growth in sheep has been shown to be due to beta-agonist effects on the calpain-calpastatin system (Pringle *et al*., 1993).

These controversial results can be due to different beta-agonists used and also due to the different species used in various studies (Dunshea *et al.*, 2005). However, the effects of beta-agonists on skeletal muscle are time dependant; it shows rapid early growth that is later attenuated. This may be due to the downregulation of beta-receptors in skeletal muscle tissue (Hossner, 2005). Studies on rodents suggest that receptor concentration is reduced by 50% after clenbuterol was administered for 18 days. Attenuation however, can be avoided by intermittent treatment (Hossner, 2005).

2.6 Beta-agonists' effect on adipose tissue

Beta-agonists have a pronounced effect on adipose tissue and fat deposition, especially in ruminants (National Research Council, 1994). It acts directly on adipocytes through beta-adrenergic receptors to affect cellular metabolism via signalling cascades. When a beta-receptor is activated by a beta-agonist, protein kinase A is activated by cAMP. Protein kinase A then phosphorylates hormone sensitive lipase which starts the process of lipolysis (Mersmann, 2002). Beta-agonists thus indirectly lead to increased lipolysis and decreased lipogenesis (Dunshea *et a*l, 2005; Mersmann, 1998). The rate of fat tissue growth and fat storage in adipocytes then slows, especially in ruminants, resulting in a leaner carcass

(Dunshea *et al*, 2005). The extent of this process depends on the duration and dose of supplementation with the beta-agonist, the type of beta-agonist used and the species it is used on (Beerman, 1993; Mersman, 1998).

When used in *in vitro* trials, beta-agonists also inhibit triacylglycerol and fatty acid synthesis and stimulate degradation of adipocyte triacylglycerol in tissues or cells from several species (Mersmann, 1998). However, beta-agonists that bind to beta-receptors and cause a decrease in fat when fed may have small effects on fat metabolism in adipocytes *in vitro* when administered in the same specie (Mersmann, 1995). In some cases, animals have an increased rate of lipolysis and a decreased rate of lipogenesis in its adipose tissue after chronic beta-agonist administration (Dunshea *et al.,* 2005). Little research has been done on lipid catabolic and anabolic processes *in vivo*, but elevation of plasma fatty acid concentration that is not esterified when a beta-agonist is administered suggests that the adipocyte lipolytic system is activated (Dunshea *et* al., 2005; Mersmann, 1998).

Ractopamine in pigs caused a decrease in fat content but little to no increase in the rate of lipid deposition (Dunshea *et al*., 1993). Other sources however, found a decrease in fat deposition when pigs were fed ractopamine and salbutamol (Oksbjerg*, et al*., 1996; Mitchell *et al*., 1991).

When administered in sheep and cattle, the effects of the beta-agonist's response on adipose tissue seem to be less pronounced (Beermann *et al*., 1987; Eisemann *et al*., 1988). However, fat accretion was still reduced in sheep due to a decrease in total fat cell number when supplemented with beta-agonists (Coleman *et al*., 1986). Beta-agonists in heifers also depressed lipogenesis in subcutaneous - but not in intramuscular adipose tissue (Miller *et al*., 1988; Coleman *et al*., 1986). In ovine adipose tissue, fatty acid uptake and esterification was inhibited while lipolysis was increased by clenbuterol (Thornton *et al.,* 1985).

In vitro, lipolysis in porcine adipose tissue was not stimulated by clenbuterol, blood glycerol concentrations and plasma free fatty acids however, were increased (Mersmann, 1987). This indicate that the *in vivo* mechanism differ between species. In pigs it has an indirect effect and in sheep, either a direct effect or indirect effect (Mersmann, 1998).

Beta-agonists are more anti-lipogenic than lipolytic, or has equal anti-lipogenic and lipolytic activity (Duquette and Muir, 1985). In growing animals, the anti-lipogenic function is of more importance than the lipolytic function. This is because 50-60% of energy deposited is protein in animals during early growth while 85-90% of energy deposited is fat in animals near maturity. Fat is thus more predominant in mature animals. An inhibition of lipogenesis is thus of more importance than lipolysis for meat animals still approaching maturity (Fiems, 1987; Van Es, 1977).

2.7. Factors affecting an animal's response to beta-agonists

It has been reported that differences in beta-agonist response between species, breeds, and age within a species also occur (Mersmann, 2002; Dunshea *et al*., 2005). The differences in response between species, breed and age of animals will be discussed below.

2.7.1 Species differences in beta-agonist response

The effects of beta-agonists in sheep are more pronounced than in chickens, while the effects in swine are intermediate and the effects in cattle more or less the same as in sheep. The possible reason for this is that some species such as chickens are more intensely selected and therefore closer to their maximal biological growth rate. There is thus less potential to improve growth. Species such as sheep on the other hand, have not been selected for growth rate with the same intensity and the potential for increased growth rate is higher. There is also the fact that particular beta-agonists are more effective in some species than in other (Parr *et al.,* 2016). The given beta-agonist can thus react differently to the target tissue beta-receptors and can then be either more or less effective. Possible mechanisms responsible for this include; the beta-agonist's affinity for the receptor, binding of the agonist-receptor complex to the signal transduction system, and also factors that influence the agonist's delivery to the receptor sites. Additionally, beta-agonists may be inactivated at target tissues or the species may even have a limited number of receptors on cells in target tissues, all reducing the response (Dunshea *et al*., 2005; Mersmann, 1998). Too many factors can influence a response from beta-agonist treatment and to compare species, experiments needs to run simultaneously in a dose x response manner.

2.7.2 The influence of breed on beta-agonist response

It was found that when clenbuterol was administered, muscle growth of laboratory rats was depended on the strain of rat used (Berne *et al*., 1985). In Ghezel lambs, subcutaneous fat was increased at the 8th rib by a beta-agonist metaproterenol while subcutaneous fat was decreased at the 12th rib in Mehran lambs due to a too high dose (Zamiri *et al*., 1995). This indicates that a breed X treatment interaction exists. Buyse *et al.* (1991) also noted that beta-agonist action might be influenced by factors such as breed. Beta-agonists have proven to be effective in a wide variety of genetic backgrounds (Moody *et al*., 2000). Cimaterol and ractopamine have both proven to be equally effective in a variety of genetically diverse pig breeds (Mills *et al*., 1994; Yen *et al*., 1991). However, a significant interaction has been reported between ractopamine and genotype for lean carcass growth in pigs (Gu *et al.,* 1991). A significant difference in basal lipolytic activity was found in Suffolk and Southdown sired lambs (Sidhu *et al*., 1973). Also in cattle, lower insulin levels were found in double muscled animals compared to conventional animals (Michaux *et al*., 1982). Insulin and CAMP levels as discussed in the mode of action section are involved in beta-agonist activity. Finally, an interaction

between line and cimaterol treatment was observed with mice (Eisen *et al*., 1988). Little other literature either confirming or denying these findings can be found.

2.7.3 The influence of age on beta-agonist response

In several species there is an improved response to beta-agonist supplementation as the animal becomes older (Fiems, 1987). When comparing clenbuterol treated lambs with an initial starting weight of 40 kg and 37.5 kg respectively, the smaller lambs showed no effect while the larger lambs gained weight faster than controls (Baker *et al*., 1984). Also in two different experiments with 10 ppm cimaterol, lambs in the first experiment with 17 kg starting weight showed no effect on growth rate while lambs in the second experiment with a starting weight of 28 kg showed a significant effect on growth rate (Beerman *et al.,* 1986). In a study by Hanrahan *et al.* (1986), cimaterol increased live weight gain in steers with a starting weight of 530 kg but Ricks *et al*. (1984) reported no response of clenbuterol treated steers with starting weights of 350 kg. In veal calves, clenbuterol had no effect on growth rate (Williams *et al.,* 1987). In all of the above investigations carcass fat however, was reduced.

There are several reasons or possibilities suggested as to why beta-agonists have a reduced effect in younger animals. Animals from different ages can have different pharmocodynamical properties. This means that if beta-agonist absorption and metabolism differ in young compared to old animals, the optimal dose will not be the same for the different age groups (Fiems, 1987). Another possibility is that beta-receptor number for younger animals is too low. Lai *et al*. (1981) found that beta-receptors can increase in number by 60-70% as an animal ages; 3T3-L1 preadipocytes differentiating into adipocytes causes this increase in receptor number (Lai *et al*., 1981). It is also suggested that alteration of the endocrine status can cause this reduced effect of beta-agonists in young animals (Fiems, 1987). Older animals have lower growth hormone secretion and this could lead to an increased effect of beta-agonists (Fiems, 1987). In cattle and lambs, plasma growth hormone concentration is lower in older animals than it is in younger animals (Joakimsen & Blom, 1976; Johnsson *et al*., 1985). In a study with cimaterol, it was found that endogenous anabolic factors block the anabolic effect in young animals, since more tyrosine was converted to muscle in larger lambs and not in smaller ones (Wilson *et al*., 1988). Suggestions that sex hormones are involved in the regulation of beta-receptors also exist (Stiles *et al*., 1984). If this is true beta-agonists will have a different response in animals before puberty than after puberty (Fiems, 1987).

On the other hand, feeding of beta-agonists to young cattle improves performance. An increase in body weight of 5.5% (Plascencia *et al*., 1999) and an increase in average daily gain of 26% (Avendano-Reyes *et al*., 2006) have been recorded with steers fed zilpaterol hydrochloride. When fed cimaterol, growth, composition and eating quality were the same for both bull calves and young bulls

(Vestergaard & Sejrsen, 1994). Chikhou *et al.* (1993) also found very few interactions between cimaterol treatment and live weight at slaughter between different slaughter weight groups.

2.8. References:

Altarejos, J. Y., and M. Montminy. 2011. CREB and the CRTC co-activators: Sensors for hormonal and metabolic signals. Nature Reviews Molecular Cell Biology. 12(3): 141-151.

Avendaño-Reyes, L., V. Torres-Rodríguez, F. Meraz-Murillo, C. Pérez-Linares, F. Figueroa-Saavedra, and P. Robinson. 2006. Effects of two β-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J. Anim. Sci. 84(12): 3259-3265.

Beermann, D., W. Butler, D. Hogue, V. Fishell, R. Dalrymple, C. Ricks, and C. Scanes. 1987. Cimaterol-induced muscle hypertrophy and altered endocrine status in lambs. J. Anim. Sci. 65(6): 1514-1524.

Bergen, W. G., S. E. Johnson, D. M. Skjaerlund, A. S. Babiker, N. K. Ames, R. A. Merkel, and D. B. Anderson. 1989. Muscle protein metabolism in finishing pigs fed ractopamine. J. Anim. Sci. 67(9): 2255-2262.

Berne, R., J. Novakofski, and P. Bechtel. 1985. Effects of beta-agonist clenbuterol on body and tissue weights in four strains of rats. J. Anim. Sci. 61: 256.

Blum, J. W., and N. Flueckiger. 1988. Early metabolic and endocrine effects of perorally administered ß-adrenoceptor agonists in calves. Eur. J. Pharmacol. 151(2): 177-187.

Bohorov, O., P. Buttery, J. Correia, and J. Soar. 1987. The effect of the β-2-adrenergic agonist clenbuterol or implantation with oestradiol plus trenbolone acetate on protein metabolism in wether lambs. Br. J. Nutr. 57(01): 99-107.

Brooks, J., H. Claus, M. Dikeman, J. Shook, G. Hilton, T. Lawrence, J. Mehaffey, B. Johnson, D. Allen, and M. Streeter. 2009. Effects of zilpaterol hydrochloride feeding duration and post mortem aging on warner-bratzler shear force of three muscles from beef steers and heifers. J. Anim. Sci. 87(11): 3764-3769.

Buyse, J., E. Decuypere, G. Huyghebaert, and M. Herremans. 1991. The effect of clenbuterol supplementation on growth performance and on plasma hormone and metabolite levels of broilers. Poult. Sci. 70(4): 993-1002.

Carlström, D. 1973. The structure of the catecholamines. IV. The crystal structure of (−)-adrenaline hydrogen ( )-tartrate. Acta Crystallographica Section B: Struct. Crystallography & Crystal Chem.. 29(2): 161-167.

Chikhou, F., A. Moloney, P. Allen, J. Quirke, F. Austin, and J. Roche. 1993. Long-term effects of cimaterol in Friesian steers: I. growth, feed efficiency, and selected carcass traits. J. Anim. Sci. 71(4): 906-913.

Cunningham, H. 1965. Effect of epinephrine and nicotine on protein and fat metabolism in pigs. Energy Metabolism. Academic Press. New York.Págs. : 29-36.

Dalrymple, R. H., P. K. Baker, P. E. Gingher, D. L. Ingle, J. M. Pensack, and C. A. Ricks. 1984. A repartitioning agent to improve performance and carcass composition of broilers. Poult. Sci. 63(12): 2376-2383.

Dikeman, M. 2007. Effects of metabolic modifiers on carcass traits and meat quality. Meat Sci. 77(1): 121-135.

Dikeman, M. 1991. Growth, carcass composition and meat quality. Proceedings of the 37th international congress on meat science and technology.

Dunshea, F., D. D'souza, D. Pethick, G. Harper, and R. Warner. 2005. Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. Meat Sci. 71(1): 8-38.

Dunshea, F., R. King, R. Campbell, R. Sainz, and Y. Kim. 1993. Interrelationships between sex and ractopamine on protein and lipid deposition in rapidly growing pigs. J. Anim. Sci. 71(11): 2919-2930.

Duquette, P., and L. Muir. 1985. Effect of the beta-adrenergic agonists isoproterenol, clenbuterol, L-640,033 and BRL-35135 on lipolysis and lipogenesis in rat adipose tissue in vitro. J. Anim. Sci. 61(Suppl. 1): 265A.

Easson, L. H., and E. Stedman. 1933. Studies on the relationship between chemical constitution and physiological action: Molecular dissymmetry and physiological activity. Biochem. J. 27(4): 1257-1266.

Eichhorn, J., L. Coleman, E. Wakayama, G. Blomquist, C. Bailey, and T. Jenkins. 1986. Effects of breed type and restricted versus ad libitum feeding on fatty acid composition and cholesterol content of muscle and adipose tissue from mature bovine females. J. Anim. Sci. 63(3): 781-794.

Eisemann, J., G. Huntington, and C. Ferrell. 1988. Effects of dietary clenbuterol on metabolism of the hindquarters in steers. J. Anim. Sci. 66(2): 342-353.

Eisen, E., W. Croom, and S. Helton. 1988. Differential response to the β-adrenergic agonist cimaterol in mice selected for rapid gain and unselected controls. J. Anim. Sci. 66(2): 361-371.

Elam, N., J. Vasconcelos, G. Hilton, D. Van Overbeke, T. Lawrence, T. Montgomery, W. Nichols, M. Streeter, J. Hutcheson, and D. Yates. 2009. Effect of zilpaterol hydrochloride duration of feeding on performance and carcass characteristics of feedlot cattle. J. Anim. Sci. 87(6): 2133-2141.

Fiems, L. 1987. Effect of beta-adrenergic agonists in animal production and their mode of action. Ann. Zootech. 36(3): 271.

Geesink, G. H., and M. Koohmaraie. 1999. Effect of calpastatin on degradation of myofibrillar proteins by mu-calpain under post mortem conditions. J. Anim. Sci. 77(10): 2685-2692.

Gu, Y., A. Schinckel, J. Forrest, C. Kuei, and L. Watkins. 1991. Effects of ractopamine, genotype, and growth phase on finishing performance and carcass value in swine: I. growth performance and carcass merit. J. Anim. Sci. 69(7): 2685-2693

Hall, J. M., M. P. Caulfield, S. P. Watson, and S. Guard. 1993. Receptor subtypes or species homologues: Relevance to drug discovery. Trends Pharmacol. Sci. 14(10): 376-383.

Holmer, S., D. Fernández-Dueñas, S. Scramlin, C. Souza, D. Boler, F. McKeith, J. Killefer, R. Delmore, J. Beckett, and T. Lawrence. 2009. The effect of zilpaterol hydrochloride on meat quality of calf-fed Holstein steers. J. Anim. Sci. 87(11): 3730-3738.

Hope-Jones, M., P. E. Strydom, L. Frylinck, and E. C. Webb. 2012. Effect of dietary beta-agonist treatment, vitamin D 3 supplementation and electrical stimulation of carcasses on colour and drip loss of steaks from feedlot steers. Meat Sci. 90(3): 607-612.

Hossner, K. L. 2005. Hormonal regulation of farm animal growth. CABI Publishing. Wallingford UK.

Joakimsen, Ø., and A. K. Blom. 1976. Growth hormone concentration in jugular blood plasma in relation to growth rate and age in young bulls. Acta Agric. Scand. 26(4): 239-242.

Johnsson, I., I. Hart, and B. Butler-Hogg. 1985. The effects of exogenous bovine growth hormone and bromocriptine on growth, body development, fleece weight and plasma concentrations of growth hormone, insulin and prolactin in female lambs. Anim. Prod. 41(02): 207-217.

Jones, R., R. Easter, F. McKeith, R. Dalrymple, H. Maddock, and P. Bechtel. 1985. Effect of the β-adrenergic agonist cimaterol (CL 263,780) on the growth and carcass characteristics of finishing swine. J. Anim. Sci. 61(4): 905-913.

Kellermeier, J., A. Tittor, J. Brooks, M. Galyean, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, B. Johnson, and M. Miller. 2009. Effects of zilpaterol hydrochloride with or without an estrogen-trenbolone acetate terminal implant on carcass traits, retail cutout, tenderness, and muscle fiber diameter in finishing steers. J. Anim. Sci. 87(11): 3702-3711.

Kim, Y., Y. Lee, W. Garrett, and R. Dalrymple. 1989. Effects of cimaterol on nitrogen retention and energy utilization in lambs. J. Anim. Sci. 67(3): 674-681.

Kjelsberg, M. A., S. Cotecchia, J. Ostrowski, M. G. Caron, and R. J. Lefkowitz. 1992. Constitutive activation of the alpha 1B-adrenergic receptor by all amino acid substitutions at a single site. Evidence for a region which constrains receptor activation. J. Biol. Chem. 267(3): 1430-1433.

Kretchmar, D., M. Hathaway, R. J. Epley, and W. Dayton. 1990. Alterations in post mortem degradation of myofibrillar proteins in muscle of lambs fed a beta-adrenergic agonist. J. Anim. Sci. 68(6): 1760-1772.

Lai, E., O. Rosen, and C. S. Rubin. 1981. Differentiation-dependent expression of catecholamine-stimulated adenylate cyclase. roles of the β-receptor and G/F protein in differentiating 3T3-L1 adipocytes. J. Biol. Chem. 256(24): 12866-12874.

Leheska, J., J. Montgomery, C. Krehbiel, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, J. Blanton, and M. Miller. 2009. Dietary zilpaterol hydrochloride. II. carcass composition and meat palatability of beef cattle. J. Anim. Sci. 87(4): 1384-1393.

López-Carlos, M., R. Ramírez, J. Aguilera-Soto, C. Aréchiga, F. Méndez-Llorente, H. Rodríguez, and J. Silva. 2010. Effect of ractopamine hydrochloride and zilpaterol hydrochloride on growth, diet digestibility, intake and carcass characteristics of feedlot lambs. Lives. Sci. 131(1): 23-30.

Luño, M., J. Beltrán, I. Jaime, and P. Roncalés. 1999. Textural assessment of clenbuterol treatment in beef. Meat Sci. 51(4): 297-303.

Macías-Cruz, U., F. Álvarez-Valenzuela, N. Torrentera-Olivera, J. Velázquez-Morales, A. Correa-Calderón, P. Robinson, and L. Avendaño-Reyes. 2010. Effect of zilpaterol hydrochloride on feedlot performance and carcass characteristics of ewe lambs during heat-stress conditions. Anim. Prod. Sci. 50(10): 983-989.

Marchant-Forde, J., D. Lay, R. Marchant-Forde, K. McMunn, and B. Richert. 2012. The effects of R-salbutamol on growth, carcass measures, and health of finishing pigs. J. Anim. Sci. 90(11): 4081-4089.

Marchant-Forde, J., D. Lay, R. Marchant-Forde, K. McMunn, and B. Richert. 2008. The effects of R-salbutamol on behavior and physiology of finishing pigs. J. Anim. Sci. 86(11): 3110-3124.

Marchant-Forde, J., D. Lay, E. Pajor, B. Richert, and A. Schinckel. 2003. The effects of ractopamine on the behavior and physiology of finishing pigs. J. Anim. Sci. 81(2): 416-422.

McDonagh, M., C. Fernandez, and V. Oddy. 1999. Hind-limb protein metabolism and calpain system activity influence post-mortem change in meat quality in lamb. Meat Sci. 52(1): 9-18.

McIntyre, A., and D. Thompson. 1992. Review article: Adrenergic control of motor and secretory function in the gastrointestinal tract. Aliment. Pharmacol. Ther. 6(2): 125-142.

Mersmann, H. J. 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76(1): 160-172.

Mersmann, H. 2002. Beta-adrenergic receptor modulation of adipocyte metabolism and growth. J. Anim. Sci. 80(E-Suppl. 1): E24-E29.

Mersmann, H. 1989. Potential Mechanisms for Repartitioning of Growth by β-Adrenergic Agonists. Animal Growth Regulation. Springer: (337-357)

Mersmann, H. J. 1995. Species variation in mechanisms for modulation of growth by beta-adrenergic receptors. J. Nutr. 125(Suppl. 6): 1777S-1782S.

Mersmann, H. J. 1989. Acute changes in blood flow in pigs infused with beta-adrenergic agonists. J. Anim. Sci. 67(11): 2913-2920.

Michaux, C., R. van Sichem-Reynaert, J. Beckers, M. De Fonseca, and R. Hanset. 1982. Endocrinological Studies on Double Muscled Cattle: LH, GH, Testosterone and Insulin Plasma Levels during the First Year of Life. Pages 350-367 in Muscle Hypertrophy of Genetic Origin and its use to Improve Beef Production. Springer.

Miller, M., D. Garcia, M. Coleman, P. Ekeren, D. Lunt, K. Wagner, M. Procknor, T. Welsh, and S. Smith. 1988. Adipose tissue, longissimus muscle and anterior pituitary growth and function in clenbuterol-fed heifers. J. Anim. Sci. 66(1): 12-20.

Minneman, K. P., A. Hedberg, and P. B. Molinoff. 1979. Comparison of beta adrenergic receptor subtypes in mammalian tissues. J. Pharmacol. Exp. Ther. 211(3): 502-508.

Mitchell, A., M. Solomon, and N. Steele. 1991. Influence of level of dietary protein or energy on effects of ractopamine in finishing swine. J. Anim. Sci. 69(11): 4487-4495.

Montgomery, J., C. Krehbiel, J. Cranston, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, D. Bechtol, E. Johnson, and T. TerHune. 2009. Dietary zilpaterol hydrochloride. I. feedlot performance and carcass traits of steers and heifers. J. Anim. Sci. 87(4): 1374-1383.

Montgomery, J., C. Krehbiel, J. Cranston, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, R. Swingle, and T. Montgomery. 2009. Effects of dietary zilpaterol hydrochloride on feedlot performance

and carcass characteristics of beef steers fed with and without monensin and tylosin. J. Anim. Sci. 87(3): 1013-1023.

Moody, D., D. Hancock, D. Anderson, and J. D'Mello. 2000. Phenethanolamine repartitioning agents. Farm Anim. Metabolism and Nutr. : 65-96.

Muchenje, V., K. Dzama, M. Chimonyo, P. Strydom, and J. Raats. 2009. Relationship between pre-slaughter stress responsiveness and beef quality in three cattle breeds. Meat Sci. 81(4): 653-657.

National Research Council (US). 1994. Metabolic modifiers: Effects on the nutrient requirements of food-producing animals. National Academy Press.

Oksbjerg, N., J. Fernandez, H. Jorgensen, O. Olsen, T. Rolph, and N. Agergaard. 1996. Effects of salbutamol on protein and fat deposition in pigs fed two levels of protein. J. Anim. Physiol. Anim. Nutr. 75(1): 1-12.

Parr, T., M. H. Mareko, K. J. Ryan, K. M. Hemmings, D. M. Brown, and J. M. Brameld. 2016. The impact of growth promoters on muscle growth and the potential consequences for meat quality. Meat Sci: 93-99

Pietri-Rouxel, F., and A. Strosberg. 1995. Pharmacological characteristics and species-related variations of β3-adrenergic receptors. Fundam. Clin. Pharmacol. 9(3): 211-218.

Plascencia, A., N. Torrentera, and R. Zinn. 1999. Influence of the agonist zilpaterol on growth, performance and carcass characteristics of feedlot steers. American society of Anim. Sci. Vol 50.

Pringle, T., C. R. Calkins, M. Koohmaraie, and S. J. Jones. 1993. Effects over time of feeding a beta-adrenergic agonist to wether lambs on animal performance, muscle growth, endogenous muscle proteinase activities, and meat tenderness. J. Anim. Sci. 71(3): 636-644.

Quirke, J., P. Allen, A. Moloney, M. Sommer, J. Hanrahan, W. Sheehan, and J. Roche. 1988. Effects of the beta-agonist cimaterol on blood metabolite and hormone concentrations, growth and carcass composition in finishing Friesian steers. J. Anim. Physiol. Anim. Nutr. 60(1-5): 128-136.

Rathmann, R., J. Mehaffey, T. Baxa, W. Nichols, D. Yates, J. Hutcheson, J. Brooks, B. Johnson, and M. Miller. 2009. Effects of duration of zilpaterol hydrochloride and days on the finishing diet on carcass cutability, composition, tenderness, and skeletal muscle gene expression in feedlot steers. J. Anim. Sci. 87(11): 3686-3701.

Reeds, P., and H. Mersmann. 1991. Protein and energy requirements of animals treated with beta-adrenergic agonists: A discussion. J. Anim. Sci. 69(4): 1532-1550.

Rehfeldt, C., R. Schadereit, R. Weikard, and K. Reichel. 1997. Effect of clenbuterol on growth, carcase and skeletal muscle characteristics in broiler chickens. Br. Poult. Sci. 38(4): 366-373.

Ricks, C. A., R. Dalrymple, P. K. Baker, and D. Ingle. 1984. Use of a-agonist to alter fat and muscle deposition in steers. J. Anim. Sci. 59(5): 1247-1255.

Rikhardsson, G., K. Johnson, and D. Johnson. 1991. Effects of cimaterol on energetics and carcass characteristics of Suffolk ewe lambs. J. Anim. Sci. 69(1): 396-404.

Ruffolo, R. R. 1991. Chirality in α-and β-adrenoceptor agonists and antagonists. Tetrahedron. 47(48): 9953-9980.

Sainz, R., and J. Wolff. 1990. Evaluation of hypotheses regarding mechanisms of action of growth promotants and repartitioning agents using a simulation model of lamb metabolism and growth. Anim. Prod. 51(03): 551-558.

Schiavetta, A., M. Miller, D. Lunt, S. Davis, and S. Smith. 1990. Adipose tissue cellularity and muscle growth in young steers fed the beta-adrenergic agonist clenbuterol for 50 days and after 78 days of withdrawal. J. Anim. Sci. 68(11): 3614-3623.

Schroeder, A., D. Polser, S. Laudert, and G. Vogel. 2003. Effects of optaflex on sensory properties of beef. " Optaflex Exchanges" Elanco Technical Bulletin AI9253, Elanco Animal Health, Greenfield, Indiana.

Scramlin, S., W. Platter, R. Gomez, W. Choat, F. McKeith, and J. Killefer. 2010. Comparative effects of ractopamine hydrochloride and zilpaterol hydrochloride on growth performance, carcass traits, and longissimus tenderness of finishing steers. J. Anim. Sci. 88(5): 1823-1829.

Sidhu, K., R. Emery, A. Parr, and R. Merkel. 1973. Fat mobilizing lipase in relation to fatness in lambs. J. Anim. Sci. 36(4): 658-662.

Smith, D. 1998. The pharmacokinetics, metabolism, and tissue residues of beta-adrenergic agonists in livestock. J. Anim. Sci. 76(1): 173-194.

Spurlock, M., J. Cusumano, S. Ji, D. Anderson, C. Smith, D. Hancock, and S. Mills. 1994. The effect of ractopamine on beta-adrenoceptor density and affinity in porcine adipose and skeletal muscle tissue. J. Anim. Sci. 72(1): 75-80.

Steenekamp, S. 2014. Growth Performance and Meat Characteristics of Feedlot Cattle Fed R-Salbutamol Or Zilpaterol Hydrochloride during the Finishing Period. Master's Thesis. Department Animal and Wildlife Sci. University of Pretoria. South Africa

Stiles, G. L., M. G. Caron, and R. J. Lefkowitz. 1984. Beta-adrenergic receptors: Biochemical mechanisms of physiological regulation. Physiol. Rev. 64(2): 661-743.

Strosberg, D. 1992. Biotechnology of beta-adrenergic receptors. Pathol. Biol. (Paris). 40(8): 767-772.

Strydom, P., L. Frylinck, J. Montgomery, and M. Smith. 2009. The comparison of three β-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Sci. 81(3): 557-564.

Strydom, P. E., M. Hope-Jones, L. Frylinck, and E. C. Webb. 2011. The effects of a beta-agonist treatment, vitamin D 3 supplementation and electrical stimulation on meat quality of feedlot steers. Meat Sci. 89(4): 462-468.

Thomas, G., C. Scott, J. Cummins, and I. Clarke. 1994. Adrenergic regulation of growth hormone secretion in the ewe. Domest. Anim. Endocrinol. 11(2): 187-195.

Thornton, R., R. Tume, T. Larsen, and G. Johnson. 1985. The influence of the beta2 adrenergic agonist, clenbuterol, on long chain fatty acid metabolism of isolated ovine subcutaneous adipocytes.[conference paper]. Proceedings of the Nutrition Society of Australia (Australia). Vol 10.

Thornton, R., R. Tume, G. Payne, T. Larsen, G. Johnson, and M. Hohenhaus. 1985. The influence of the (32-adrenergic agonist, clenbuterol, on lipid metabolism and carcass composition of sheep. Proceedings of the New Zealand Society of Anim. Prod. Vol. 43.

Van Es, A. 1977. The energetics of fat deposition during growth. Annals of Nutrition and Metabolism. 21(1-3): 88-104.

Vestergaard, M., K. Sejrsen, and S. Klastrup. 1994. Growth, composition and eating quality of *longissimus dorsi* from young bulls fed the β-agonist cimaterol at consecutive developmental stages. Meat Sci. 38(1): 55-66.

Wallis, C. 1993. Salbutamol, salmeterol - A chemist's perspective. Actualites De Chimie Therapeutique. 20: 265-265.

Warris, P. D. 2010. Meat Science. An Introductory Text. 2nd edition. CABI Publishing. Wallingford UK.

Warriss, P., G. Nute, T. Rolph, S. Brown, and S. Kestin. 1991. Eating quality of meat from pigs given the beta-adrenergic agonist salbutamol. Meat Sci. 30(1): 75-80.

Webb, E. C., and N. H. Casey. 2010. Physiological limits to growth and the related effects on meat quality. Livest. Sci. 130(1): 33-40.

Williams, P., L. Pagliani, G. Innes, K. Pennie, C. Harris, and P. Garthwaite. 1987. Effects of a β-agonist (clenbuterol) on growth, carcass composition, protein and energy metabolism of veal calves. Br. J. Nutr. 57(03): 417-428.

Wilson, M., C. Zhong, N. Forsberg, R. Dalrymple, and C. Ricks. 1988. Effects of cimaterol on protein synthesis, protein degradation, amino acid transport and acetate oxidation in sheep external intercostal muscle. Nutr. Res. 8(11): 1287-1296.

Yen, J., J. Nienaber, J. Klindt, and J. Crouse. 1991. Effect of ractopamine on growth, carcass traits, and fasting heat production of US contemporary crossbred and Chinese Meishan pure-and crossbred pigs. J. Anim. Sci. 69(12): 4810-4822.

Young, O., S. Watkins, J. Oldham, and J. Bass. 1995. The role of insulin-like growth factor I in clenbuterol-stimulated growth in growing lambs. J. Anim. Sci. 73(10): 3069-3077.

Zamiri, M., and J. Izadifard. 1995. Effects of metaproterenol, a beta-adrenergic agonist, on feedlot performance and body composition of two fat-tailed breeds of sheep. Small Ruminant Research. 18(3): 263-271.

Zimmerli, U., and J. Blum. 1990. Acute and long term metabolic, endocrine, respiratory, cardiac and skeletal muscle activity changes in response to perorally administered β-adrenoceptor agonists in calves1. J. Anim. Physiol. Anim. Nutr. 63(1-5): 157-172.

Chapter 3:

An *in vivo* digestibility study to determine the effect of the beta-agonist r-salbutamol on apparent digestibility in Dohne Merino feedlot lambs

3.1 Abstract

An *in vivo* digestibility study to determine the effects of r-salbutamol on the apparent digestibility of a standard feedlot diet was conducted on Dohne Merino feedlot lambs. Lambs were housed individually in indoor pens on Welgevallen experimental farm in Stellenbosch. Two treatment groups were used; a control fed commercial finisher pellets and a group fed the same finisher pellets but with 135 mg r-salbutamol added per kg feed (on an as fed basis). Lambs from the control group had an average starting weight of 38.34 ± 2.80 kg whilst the 135 mg/kg r-salbutamol group had an average weight of 37.60 ± 2.85 kg. Faeces collection bags and urine collection harnesses were secured to each lamb for quantitative faecal and urine collection. The trial ran for 7 days during which lambs had access to *ad libitum* feed and water; daily feed intake was measured and feed, faeces (10% of total daily faeces) and urine (5% of total daily urine) samples were collected daily. A proximate (organic matter, ash, NDF and crude protein) analysis on the feed, faecal matter and urine (nitrogen content) samples showed no differences between treatment groups. R-salbutamol had no significant effect on % organic matter disappearance, % neutral detergent fibre, % crude protein and % nitrogen retention between Dohne Merino feedlot lambs. The control group had an organic matter disappearance of 70.13 ± 7.51 % compared to the 71.27 ± 4.42 % of 135mg S/kg group, a neutral detergent fibre digestibility of 15.73 ± 3.32 % compared to 15.45 ± 2.75 %, a crude fibre digestibility of 4.56 ± 1.25 compared to the 4.30 ± 4.56 % and a nitrogen retention of 1.15 ± 0.31 g/kg $BW^{0.75}$ compared to 0.94 ± 0.29 g/kg $BW^{0.75}$, none of which show significant difference. It may thus seem that digestibility functions are unaffected by r-salbutamol supplementation.

3.2 Introduction

The rumen has a symbiotic relationship with rumen microorganisms that are able to ferment and degrade polysaccharides in plant cell walls. This allows ruminants to utilize forages more effectively (Hungate, 1966). Digestion, and especially fibre digestion of ruminants is considered to be a limiting factor in a wide range of ruminant production systems. Much research has been done on manipulating rumen activities in an attempt to maximise ruminant production by increasing digestion (Titi & Tabbaa, 2003). An increase in digestibility is mainly due to the stimulation of the microbial populations in the rumen (Beauchemin *et al*., 2000). By increasing fibre digestibility, synthesis of microbial protein in the rumen can be enhanced (Hristov *et al.,* 1998).

A poorly researched compound that may have these positive effects is beta agonists. Beta-agonists such as ractopamine hydrochloride has been shown to affect microbial populations, stimulate fermentation by microorganisms and increase dry matter disappearance in series of *in-vitro* studies done by Walker & Drouillard, (2010). A decrease in crude fibre but not in other digestibility coefficients were observed in cimaterol treated sheep. Rumen fluid also had higher levels of propionic and acetic acid with lower levels of butyric acid in cimaterol treated sheep when compared to control groups (Fiems *et al*., 1987). On the other hand, others reported that beta-agonists had no effect on nutrient digestibility (Kim *et al*., 1989; Li *et al*., 2000; Strydom *et al*., 2009).

Beta-agonists are known for their effect as repartitioning agents as well as the fact that they further bind to beta-receptors along the gastro intestinal tract which affects the secretory functions and motility of the tract (McIntyre & Thompson, 1992). As beta-agonists directly influence digestion by stimulating microbial activity and indirectly by changing passage rate, improved performance might not only be due to redistribution of absorbed nutrients but also due to improved digestibility (Macias-Cruz *et al*., 2010).

However, very little research has been done on the effect beta-agonists have on ruminant digestibility, and limited information is available on the positive effects they may have. The aim of this study was therefore to determine apparent digestibility of nutrients in feedlot sheep fed the beta-agonist r-salbutamol, specifically looking at its influence on crude protein and Neutral Detergent Fibre (NDF) digestibility.

3.3 Materials and Methods

3.3.1 Animals

The animals chosen for the digestibility trial were castrated male Dohne Merino lambs. Twenty 6-month old lambs with a weight range of 35 to 38 kilograms were selected from a larger flock of feedlot lambs. Lambs were adapted to Meadow® complete sheep finisher pellets in a feedlot environment.

3.3.2 Experimental procedure

On arrival animals were processed as per usual practise and received an oral dose of Embavit® (Oral liquid vitamin supplement, Merial, New Zealand), Embamin® (Supplementary provision of trace elements, Merial, New Zealand), Ivomac® (Drench for sheep, Merial, New Zealand) and Valbazen® (Broad sectrum dewormer, Zoetis, USA) according to the suppliers' directions. They were further also inoculated against pasteurella (OBP® Pasteurella vaccine for sheep and goats, Onderstepoort, South Africa) and clostridium diseases (OBP® enterotoxaemia vaccine for sheep and goats, Onderstepoort, South Africa).

After complete adaptation of 7 days lambs were randomly divided and housed in group pens for three days to adapt to the new environment. The digestibility study was performed in two consecutive trials which were repetitions of one another. This was done to minimize workload and also for more accurate feeding and sample collection. Ten animals (per trial) were randomly selected and moved to individual pens. Pens were designed with fenced sides to simulate a feeling of being in a group while individual intake could also be measured accurately. Faecal bags and urine collection harnesses were secured to each sheep and the wethers were allowed five days to adapt. After the adaptation period, the animals were weighed and the collection period of the trial started. Treatments were allocated as shown in Figure 3.1. Two treatment groups were used (5 sheep per treatment per trial); Meadow® complete sheep finisher pellets as the control and Meadow® complete sheep finisher pellets containing the beta-agonist r-salbutamol in the concentration of 135 mg/kg feed on an as fed basis (Table 3.1). The dosage was chosen according to the manufacturer's recommendations after a pilot study was done. Animals were fed three times a day, at 07:00, 13:00 and 19:00 hours. Feed was weighed out in the morning and refusals weighed back the following morning after 24h to determine daily intake. Faeces were weighed twice daily and a sample taken for analyses while urine was measured once every day and a sample also taken for analysis. The collection period ran for 7 days, where after animals were weighed and removed from the individual pens. The procedure was repeated for the second batch of animals as soon as the first batch was removed. Samples were frozen to be analysed at a later stage.

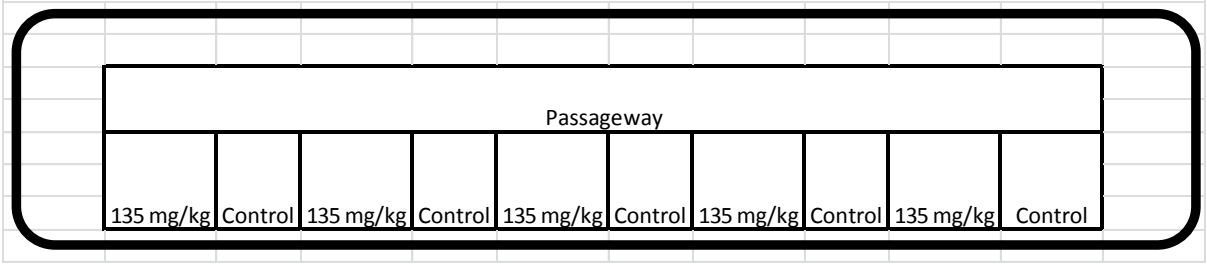

Figure 3.1 Shed layout with the treatment allocation in the individual pens

Table 3.1 Nutritional information of Meadow® complete sheep finisher pellets

Nutrient	Quantity (g/kg)	Max/Min
Protein	120	Min
Fat	25	Min
Fat	70	Max
Fibre	110	Min
Fibre	200	Max
Moisture	120	Max
Calcium	10	Max
Phosphorus	2	Min
Urea	10	Max

3.3.4 Sample collection

A 10% faecal matter sample of each sheep as well as a 5% urine sample was taken daily for proximate analysis. Feed samples (50 g) were also taken daily and pooled for both treatments. The analysed nutrients of the feed did not differ substantially as shown in Table 3.2. The fat, ash, crude protein, crude fibre and average gross energy on a Dry Matter (DM) basis of the two diets are summarised in Table 3.2.

Table 3.2 Nutrients of the control diet containing no beta-agonist and the diet containing 135 mg r-salbutamol per kg of feed

Nutrients	Treatment	
	Control	135 mg S/kg
Fat (%)	3.79	3.78
Ash (%)	7.62	7.52
Moisture (%)	9.91	10.12
Average crude protein (%)	14.83	14.12
Crude fibre (%)	14.95	13.87
Average Gross energy (MJ/kg)	18.23	17.77
NDF (%)	32.15	31.98

3.3.5 Sample preparation

Feed

The sample was mixed on a clean non-absorbent surface and sub-sampled by quartering. Quartering was done by dividing the flattened heap into quarters and rejecting the two diagonally opposite portions. The remaining material was then re-mixed and the process repeated until a 200-300g sample was obtained.

Faeces

The wet faeces were weighed accurately in a clean, dry and empty tinfoil tray. Approximately 500g of the sample was dried at 60°C for three days. The moisture free tray and sample were then re-weighed.

All feed and faecal samples were ground in a hammer mill with a 1mm diameter sieve to ensure all samples were homogenised.

3.3.6 Chemical analyses of samples

Faecal and feed samples were analysed for moisture, ash, Neutral Detergent Fibre (NDF) and crude protein while urine was analysed for nitrogen content. The proximate analysis was done in duplicate on all twenty faecal samples as well as the two feed samples. Analysis for nitrogen content in urine was also done in duplicate.

Moisture

AOAC (2002) official method 934.01: For determination of moisture content

For moisture analysis, a 2g sample was weighed accurately into a moisture free crucible. After samples were dried in the drying oven at 105°C for 24 hours they were placed in a desiccator to cool down. The moisture free crucible was then weighed accurately. % Moisture was calculated with the following formula:

$$\%\ Moisture = \frac{(Dry\ crucible + Sample\ weight)}{Sample\ weight} - (Dry\ sample)\ x\ \frac{100}{1}$$

$$\%\ \text{Dry Matter} = 100 - \%\ \text{Moisture}$$

Organic Matter Disappearance

Organic matter disappearance was calculated for each sheep with the formulae as follows:

$$\%\ OMD = \frac{Feed\ OM\ intake\ (g) - Faeces\ OM\ excretion(g)}{Feed\ OM\ intake\ (g)}\ X\ \frac{100}{1}$$

Ash

AOAC (2002) official method 942.05: For determination of ash content

Crucibles with the dry samples were places in an ashing furnace at 500°C for 6 hours. The samples were left to cool for 30 minutes before the crucible with ash was weighed accurately. The % ash was calculated by:

$$\%\ Ash = \frac{(Crucible\ with\ ash) - (Clean\ crucible\ weight)}{Sample\ weight}\ X\ \frac{100}{1}$$

Neutral Detergent Fibre (NDF)

Method used as described by Van Soest *et al*., (1991) for NDF determination

NDF is determined gravimetrically, after chemical digestion and solubilisation of compounds such as protein, starch and other digestible carbohydrates with a NDF solution. An ANKOM^{220} Fibre Analyser (ANKOM Technologies, Fairport, NY, USA) was used for determining % NDF. A 1g sample was boiled (65°C) with a NDF solution and 0.1 ml heat stable α-Amylase in a hot extraction unit, washed, dried for 24 hours at 100°C and ashed at 500°C for 6 hours. Finally the sample was cooled and weighed accurately. Calculations for lab values of feed and faeces were done with the following formulae:

$$\%\ NDF = \frac{(Mass\ of\ crucible\ after\ drying, in\ g) - (Mass\ of\ crucible\ after\ ashing, in\ g)}{Sample\ mass\ in\ g}\ X\ \frac{100}{1}$$

To determine the % NDF digested, the following formulae was used:

$$Digestibility\ coefficient = \frac{\left((DM\ Intake\ in\ g)\ X\ \frac{(\%\ NDF\ on\ DM\ basis\ of\ feed)}{100}\right) - \left((DM\ Excretion\ in\ g\ )\ X\ \frac{(\%\ NDF\ on\ DM\ basis\ of\ faeces)}{100}\right)}{(DM\ Intake\ in\ g)\ X\ \frac{(\%\ NDF\ on\ DM\ basis\ of\ feed)}{100}}$$

% NDF = Digestibility Coefficient X % NDF on DM basis of feed

Crude Protein

AOAC (2002) official method 968.06: For determination of crude protein content

For crude protein (CP) determination, the Dumas combustion method was used with a LECO FP 528 for quantitative determination of nitrogen in the samples. The LECO was calibrated. A foil cup with sample weight of 0.1000g was placed in the carousel to be analysed. The calculation for lab values of crude protein in feed and faeces is done with the following formulae:

$$\%\ Crude\ protein = \%\ Nitrogen\ X\ Protein\ conversion\ factor\ of\ 6.25$$

% CP digested is calculated with the following formulae:

$$Digestibility\ coefficient = \frac{\left((DM\ Intake\ in\ g)\ X\ \frac{(\%\ CP\ on\ DM\ basis\ of\ feed)}{100}\right) - \left((DM\ Excretion\ in\ g\ )\ X\ \frac{(\%\ CP\ on\ DM\ basis\ of\ faeces)}{100}\right)}{(DM\ Intake\ in\ g)\ X\ \frac{(\%\ CP\ on\ DM\ basis\ of\ feed)}{100}}$$

$$\%\ CP = Digestibility\ Coefficient\ X\ \%\ CP\ on\ DM\ basis\ of\ feed$$

Nitrogen excretion was corrected for metabolic faecal nitrogen (MFN) and endogenous urinary nitrogen (EUN) according to McDonald *et al.* (1988) and nitrogen retention calculated with the following formulae:

MFN = 5 g N/kg dry matter intake

EUN = 0.18g N/kg $BW^{0.75}$

Nitrogen retention (g N/kg $BW^{0.75}$/day) =

$$[(Nitrogen\ intake\ -(Nitrogen\ in\ faeces\ -\ MFN) - (Nitrogen\ in\ Urine - EUN)]/BW^{0{\cdot}75}/Days$$

3.3.7 Statistical analysis

Animals were regarded as random repetitions for the different diets and a univariate analysis of variance (Anova) was performed for apparent digestibility variables. Shapiro-Wilk test was performed to test for normality (Shapiro, 1965). Least squares means (LS-means) were computed and compared using pairwise p-values. A probability level of 5% was considered significant for all significance tests. Univariate analyses (Anova and regression) were performed using SAS software (Version 9.2, SAS Institute Inc, Cary, USA).

3.3.8 Care and general husbandry of experimental animals

Standard ethical norms of Stellenbosch University were used in this trial (SU-ACUD15-00082). The trial was conducted at Welgevallen Experimental Farm in Stellenbosch. Animals were housed in single

indoor pens for a controlled environment as well as protection against extreme weather conditions. Since animals were constricted, great care was taken to ensure animals had minimum levels of discomfort and were under constant surveillance during the day. General health was monitored by the student, animal technician and Professor LC Hoffman (distinguished Professor in the Animal Science Department at Stellenbosch University). When any abnormal conditions occurred, the animal received veterinary care. Two animals (one from each treatment) were taken out of the trial because they did not seem to adapt to the specific conditions as their intake was lower than the others. These animals were removed before they started losing weight. The data of the animals were not used in the statistical analysis.

3.4 Results and discussion

3.4.1 Animal weights

At the start of the trial, starting weights were recorded for each animal. End weights were also taken when the trial finished. The average of the starting weight and end weight was used to calculate the average weight which was then used to determine metabolic weight for each animal (Average weight $^{0.75}$). The results of the two treatment groups are summarised in Table 3.3.

Table 3.3 Summary statistics (LSMeans ± SD) of Starting weight, End weight, Average weight and Metabolic weight of both the control and 135 mg/kg r-salbutamol treatment groups.

Parameters measured	Treatment		P-value
	Control (N=9)	135 mg S/kg (N=9)	
Start weight (kg)	38.34 ± 2.80	37.60 ± 2.85	0.57
End weight (kg)	39.70 ± 2.64	38.18 ± 2.79	0.24
Average weight (kg)	39.02 ± 2.63	37.68 ± 2.67	0.29
Metabolic weight (kg)	15.61 ± 0.79	15.20 ± 0.81	0.28

No significant differences were found between the control and the treatment group. None of the weights from the two groups differed significantly from each other and can thus be considered as being statistically similar.

3.4.2 Proximate analysis values on faeces

Proximate values (on a DM basis) were determined in the lab for the two treatments (Table 3.4). The percentages of organic matter (OM), neutral detergent fibre (NDF), crude protein (CP) and Ash were similar (P> 0.05) between treatments.

Table 3.4 Summary statistics (LSMeans ± SD) of proximate analyses of faeces.

Parameters measured *(%)	Treatment		P-value
	Control (N=9)	135 mg S/kg (N=9)	
Ash in faeces	14.33 ± 1.21	13.39 ± 0.89	0.06
CP in faeces	15.05 ± 1.19	14.93 ± 1.16	0.82
NDF in faeces	56.92 ± 2.70	58.09 ± 1.77	0.29
DM of faeces	31.55 ± 3.95	33.87 ± 4.65	0.86

*Values expressed as a percentage of total faeces weight

3.4.3 Intake, excretion and digestion of nutrients

In Table 3.5 the total kg of nutrients ingested, excreted and apparently digested calculated for organic matter, neutral detergent fibre (NDF) and crude protein (CP) is summarised for the whole 7-day trial period. Digestibility coefficients of NDF and CP as calculated in 3.3.6 under NDF and CP respectively are also included. No differences (P> 0.05) were observed between treatments.

Table 3.5 Nutrients ingested, excreted and apparently digested on a DM basis for the 7-day period

Nutrients	Organic matter		NDF		Crude protein	
Parameters measured	Treatment					
	Control (N=9)	135 mg S/kg (N=9)	Control (N=9)	135 mg S/kg (N=9)	Control (N=9)	135 mg S/kg (N=9)
Intake (kg per period)	9.33 ± 1.31	8.74 ± 1.15	3.00 ± 0.42	2.79 ± 0.37	1.46 ± 0.21	1.30 ± 0.17
Excreted (kg per period)	2.52 ± 0.45	2.52 ± 0.57	1.42 ± 0.21	1.45 ± 0.34	0.39 ± 0.09	0.38 ± 0.09
Apparently Digested (kg per period)	6.54 ± 1.09	6.22 ± 0.86	1.50 ± 0.40	1.35 ± 0.27	1.03 ± 0.18	0.93 ± 0.14
Apparent Digestibility Coefficients	No value	No value	0.49 ± 0.10	0.48 ± 0.09	0.71 ± 0.08	0.71 ± 0.05

[a,b] means in a row with different superscript letters differ (P≤ 0.05).

No differences in organic matter intake were observed with r-salbutamol supplementation. Excretion and digestion of organic matter too showed no differences between treatments. This is in line with other studies suggesting that beta-agonists have no effect on intake (Montgomery *et al.,* 2009; Elam *et al.,* 2009). Intake, excretion and digestion of both NDF and CP also showed no differences between treatments. This suggests that r-salbutamol supplementation in Dohne Merinos does not affect apparent digestibility when measured in kg per period. In accordance with this study, Fiems *et al.* (1991) reported that except for a decrease in crude fibre, nutrient digestibility coefficients were unaffected when sheep were treated with cimaterol. When sheep were treated with zilpaterol hydrochloride and ractopamine hydrochloride, digestibility coefficients were also similar (Lopez-Carlos *et al*., 2010).

3.4.4 Digestibility results: Organic Matter Disappearance, NDF, Crude Protein and Nitrogen retention

Organic matter disappearance, NDF and crude protein, all on a dry matter basis were compared between the control and 135 mg/kg treatment group. To determine the % organic matter disappearance, % NDF and % crude protein, further calculations were conducted using the values of the proximate analysis calculated as described in 3.3.6.

The calculated values for the 18 animals are summarised in Table 3.6. The four calculated values were compared with $P \leq 0.05$ for both the control and 135 mg/kg r-salbutamol group.

Table 3.6 Summary statistics (LSMeans ±SD) for % Organic Matter Disappearance, % NDF digested and % crude protein digested on a DM basis of the two treatment groups.

Parameters measured	Treatment		P-value
	Control (N=9)	135 mg S/kg (N=9)	
% Organic Matter Disappearance	70.13 ± 7.51	71.27 ± 4.42	0.70
% NDF	15.73 ± 3.32	15.45 ± 2.75	0.85
% Crude Protein	11.08 ± 1.25	10.61 ± 0.71	0.60

No significant differences were found between the control and treatment group of any parameters measured. R-salbutamol thus had no significant effect on % OMD, % NDF and % CP of Dohne Merino feedlot lambs. Only a few studies show significant effects of beta-agonists on digestibility coefficients; Walker and Drouillard (2010) reported and increased dry matter disappearance *in vitro* while Fiems *et al*., (1991) reported that only crude fibre decreased when sheep were treated with cimaterol. Most results are thus in line with this study with similar digestibility coefficients observed for neutral

detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP) and dry matter (DM) in feedlot lambs supplemented with both zilpaterol hydrochloride and ractopamine hydrochloride (Lopez-Carlos *et al*., 2010). Feedlot steers administered zilpaterol hydrochloride, ractopamine hydrochloride and cimaterol showed no differences in either CP or DM digestibility (Strydom *et al*., 2009). When lambs were administered beta-agonists such as cimaterol (Kim *et al*., 1989; Rikhardsson *et al*., 1991) and L-644,969 (Li *et al*., 2000) no effects were observed on nutrient digestibility.

3.4.5 Nitrogen retention

In Table 3.7 the statistics for nitrogen intake, nitrogen excretion and nitrogen retention as calculated in 3.3.6 for the two groups of lambs receiving a feedlot diet, is summarised. No differences (P> 0.05) were observed between treatments.

Table 3.7 Summary statistics (LSMeans ±SD) for nitrogen retention of the two treatment groups.

Parameters measured	Treatment		P-value
	Control (N=9)	135 mg/kg (N=9)	
N intake (g/day)	30.07 ± 4.23	26.78 ± 3.53	0.09
Faecal N (% of N intake)	29.14 ± 7.99	28.84 ± 4.77	0.92
Urinary N (% of N intake)	26.47 ± 8.78	33.27 ± 14.01	0.24
Total N excreted (% of N intake)	55.62 ± 11.89	62.11 ± 14.37	0.31
N retention (% of N intake)	62.22 ± 13.38	53.20 ± 19.53	0.27
N retention (g/kg $BW^{0.75}$)	1.15 ± 0.31	0.94 ± 0.29	0.16

All parameters for nitrogen intake, excretion and retention are unaffected by r-salbutamol supplementation in Dohne Merino feedlot lambs. The similar N intake between treatments with the corresponding similarity of N excretion may suggest that there are no differences in nitrogen digestion and absorption between treatments (Nolte and Ferreira, 2004). With urinary nitrogen being similar, ammonia production in the gut of lambs from both treatments is also unaffected (Cole, 1999). Mammals have no endogenous urease which means excretion is the only means of removing urea from the body (Nolan, 1993). Therefore, the similar nitrogen retention may indicate that lambs from both treatments had the same nitrogen circulation and/or nitrogen utilization (Ørskov, 1992). Very few studies have been done on the effect beta-agonists have on nitrogen retention in sheep. However, Kim *et al*. (1989) found that cimaterol treated sheep had greater nitrogen retention due to reduced nitrogen loss in urine. Rikhardsson *et al*. (1991) also found an 18% increase in N retention due to a lower nitrogen excretion in urine when sheep were treated with cimaterol. This contradicts the current study which showed no differences in either N excretion in urine or N retention with r-salbutamol supplementation in feedlot lambs.

3.5 Conclusion

R-salbutamol supplementation had no effect on apparent digestibility in Dohne Merino feedlot lambs when administered at 135 mg r-salbutamol per kg of feed. All digestibility coefficients were similar. Higher doses of r-salbutamol may have an effect on apparent digestibility and further research is needed before recommendations can be made.

3.6 References

Beauchemin, K., L. Rode, M. Maekawa, D. Morgavi, and R. Kampen. 2000. Evaluation of a nonstarch polysaccharidase feed enzyme in dairy cow diets. J. Dairy Sci. 83(3): 543-553.

Cole, N.A., 1999. Nitrogen retention by lambs fed oscillating dietary protein concentrations. J. Anim. Sci. 77, 215-222.

Elam, N., J. Vasconcelos, G. Hilton, D. Van Overbeke, T. Lawrence, T. Montgomery, W. Nichols, M. Streeter, J. Hutcheson, and D. Yates. 2009. Effect of zilpaterol hydrochloride duration of feeding on performance and carcass characteristics of feedlot cattle. J. Anim. Sci. 87(6): 2133-2141.

el-Shazly, K., and R. E. Hungate. 1966. Method for measuring diaminopimelic acid in total rumen contents and its application to the estimation of bacterial growth. Appl. Microbiol. 14(1): 27-30.

Feldsine, P., C. Abeyta, and W. H. Andrews. 2002. AOAC international methods committee guidelines for validation of qualitative and quantitative food microbiological official methods of analysis. J. AOAC Int. 85(5): 1187-1200.

Fiems, L., C. Boucque, B. Cottyn, and G. Van de Voorde. 1991. Effect of dietary cimaterol on performance and carcass traits in bulls and on aspects of digestion in cattle and sheep. Ann. Zootech. 40: 191-200.

Fiems, L. 1987. Effect of beta-adrenergic agonists in animal production and their mode of action. Ann. Zootech. 36(3): 271.

Hristov, A. N., T. A. McAllister, and K. Cheng. 1998. Effect of dietary or abomasal supplementation of exogenous polysaccharide-degrading enzymes on rumen fermentation and nutrient digestibility. J. Anim. Sci. 76(12): 3146-3156.

Kim, Y., Y. Lee, W. Garrett, and R. Dalrymple. 1989. Effects of cimaterol on nitrogen retention and energy utilization in lambs. J. Anim. Sci. 67(3): 674-681.

Li, Y., R. Christopherson, B. Li, and J. Moibi. 2000. Effects of a beta-adrenergic agonist (L-644,969) on performance and carcass traits of growing lambs in a cold environment. Canadian J. Anim. Sci. 80(3): 459-465.

López-Carlos, M., R. Ramírez, J. Aguilera-Soto, C. Aréchiga, F. Méndez-Llorente, H. Rodríguez, and J. Silva. 2010. Effect of ractopamine hydrochloride and zilpaterol hydrochloride on growth, diet digestibility, intake and carcass characteristics of feedlot lambs. Livest. Sci. 131(1): 23-30.

McDonald, P., Edwards, R.A. & Greenhalgh, J.F.D., 1988. Evaluation of foods. In: Animal Nutrition (4th ed.). Longman Scientific & Technical, Essex, UK. pp. 260-283.

McIntyre, A., and D. Thompson. 1992. Review article: Adrenergic control of motor and secretory function in the gastrointestinal tract. Aliment. Pharmacol. Ther. 6(2): 125-142.

Montgomery, J., C. Krehbiel, J. Cranston, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, R. Swingle, and T. Montgomery. 2009. Effects of dietary zilpaterol hydrochloride on feedlot performance and carcass characteristics of beef steers fed with and without monensin and tylosin. J. Anim. Sci. 87(3): 1013-1023.

Nolan, J.V., 1993. Nitrogen kinetics. Quantitative aspects of ruminant digestion and metabolism. Eds. Forbes, J.M. & France, J., CAB International, Wallingford, UK. pp. 123-143.

Nolte, J. v. E., and A. Ferreira. 2004. Energy and nitrogen retention of merino and Dohne merino lambs receiving a feedlot diet. S. Afr. J. Anim. Sci. 34: 2.

Ørskov, E.R., 1992. Protein nutrition in ruminants. Academic Press Limited, Oval Road, London, UK.

Rikhardsson, G., K. Johnson, and D. Johnson. 1991. Effects of cimaterol on energetics and carcass characteristics of Suffolk ewe lambs. J. Anim. Sci. 69(1): 396-404.

Shapiro, S.S. and Wilk, M.B., 1965. An analysis of Variance Test for Normality (complete samples), Biometrika 52, 591-611.

Strydom, P., L. Frylinck, J. Montgomery, and M. Smith. 2009. The comparison of three β-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Sci. 81(3): 557-564.

Titi, H., and M. Tabbaa. 2004. Efficacy of exogenous cellulase on digestibility in lambs and growth of dairy calves. Livest. Prod. Sci. 87(2): 207-214.

Van Soest, P. v., J. Robertson, and B. Lewis. 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74(10): 3583-3597.

Walker, C. E., and J. Drouillard. 2010. Effects of ractopamine hydrochloride are not confined to mammalian tissue: Evidence for direct effects of ractopamine hydrochloride supplementation on fermentation by ruminal microorganisms. J. Anim. Sci. 88(2): 697-706.

Chapter 4:

The effect of r-salbutamol on growth, feed efficiency, carcass characteristics and meat quality of Dohne Merino feedlot lambs

4.1 Abstract

The effect the beta-agonist r-salbutamol has on feed efficiency, growth, carcass characteristics and meat quality of Dohne Merino feedlot lambs was evaluated. Fifty-six lambs were housed in individual pens indoor at Welgevallen experimental farm, Stellenbosch. Four treatments (14 animals per treatment) received a control diet or a diet containing one of three concentrations of r-salbutamol (S). The control were fed Meadow complete sheep finisher® pellets and the three treatment groups were fed the same finisher pellets but with 110mg, 135mg and 160mg added r-salbutamol per kilogram feed (on an as fed basis), respectively. Lambs were blocked according to initial live weight and randomly allocated to treatments. The trial ran for 28 days during which wethers had *ad libitum* access to feed and quality water. After the trial, animals were slaughtered at a commercial abattoir where individual offal weights and primal cut weights were determined. A sample from the *longissimus dorsi* muscle from each animal was taken for physical measurements that included cooking loss, pH and shear force as well as for descriptive sensory analysis. A 3-rib cut (between the 9th and 12th rib) from the right side of each animal was taken for image analysis of both *longissimus dorsi* muscle area and fat thickness, the ratios of muscle to fat to bone of each animal were also determined. R-salbutamol at 160 mg/kg (on an as fed basis) improved the FCR significantly. Few significant differences were observed between offal and primal cuts from the different treatments. R-salbutamol also had no significant effect on either meat tenderness or carcass fat. The most pronounced effect r-salbutamol (160 mg S/kg diet) had on the carcass was the increase in % carcass muscle (P-value of 0.02).

4.2 Introduction

The livestock and meat industry are constantly looking for methods to promote efficient and rapid growth of the animals followed by improved carcass characteristics (Etherton, 2009; Beerman, 2009; Lopez-Carlos *et al*., 2010). This has led to extensive research on compounds such as beta-agonists that promote growth and improve carcass quality in meat producing species (Johnson and Chung, 2007). Beta-adrenergic receptors are present on almost all types of cells in mammalian tissue (Mersmann, 1998). Although beta-agonists' potency differ due to a variety of factors such as type of cell, dose, length of dose, type of beta-agonist, species and breed supplemented in, it has a range of positive effects on muscle and adipose tissue (Moody *et al*., 2000; NRC, 1994). Beta-agonists reduce adipose tissue and increase muscle mass by improving muscle metabolism in poultry, pigs, cattle and sheep

(Apple *et al*., 2008; Carr *et al*., 2005; Allen *et al*., 2009; Schaivone *et al*., 2004; Lopez-Carlos *et al.,* 2010; Pringle *et al*., 1993; Parr *et al*., 2016).

The beta-agonist zilpaterol hydrochloride has been used legally on feedlot cattle in South Africa and Mexico for more than ten years. It was also approved and has been used in USA feedlots since 2006 (Avendano-Reyes *et al.*, 2006; Shook *et al*., 2009). However, sales in the USA were suspended by the suppliers in 2013 due to reports of lameness in cattle. It has also been reported in numerous studies that zilpaterol hydrochloride has a negative effect on meat tenderness in beef (Rathmann *et al*., 2009; Strydom *et al*., 2011; Hope-Jones *et al*., 2012; Van Donkersgoed *et al*., 2014) and lamb meat (Dávila-Ramírez *et al.,* 2013).

R-salbutamol is a purified derivative of albuterol (RS-salbutamol) which is used worldwide for treatment of respiratory disorders in humans. R-salbutamol has been investigated in feedlot cattle and a few scientific papers have also been published on its use in poultry, swine and feedlot cattle (Steenekamp, 2014). However, R-salbutamol has not been tested on feedlot sheep. It was found that r-salbutamol had a positive effect on carcass growth and composition in pigs (Merchant-Forde *et al.,* 2012).

Dual purpose breeds such as Mutton Merino and Dohne Merino are favoured in South African feedlot production systems due to their better growth and being medium maturity types (Beukes *et al*., 2014). The Dohne Merino breed was thus chosen for this experiment. The aim of this study was to determine the effect three different concentrations of r-salbutamol have on the growth and carcass characteristics of feedlot lambs.

4.3 Materials and methods

4.3.1 Animals

A total of 65 castrated Dohne Merino lambs at an average age of four months were obtained from a producer in the Malmesbury area of the Western Cape, South Africa. Lambs with a weight range of 28 to 32 kg were selected from a larger flock of the same producer. Lambs were housed at Welgevallen experimental Farm in Stellenbosch in indoor pens.

The 65 Dohne Merino lambs were randomly divided into 4 group pens for adaptation to the group pen facilities. All animals were processed as per standard commercial practise: each lamb received an oral dose of Embavit® (Oral liquid vitamin supplement, Merial, New Zealand), Embamin® (Supplementary provision of trace elements, Merial, New Zealand), Ivomec® (Drench for sheep, Merial, New Zealand) and Valbazen® (Broad sectrum dewormer, Zoetis, USA) according to the suppliers' directions while they were also inoculated against pasteurella (OBP® Pasteurella vaccine for sheep and goats,

Onderstepoort, RSA) and clostridium diseases (OBP® enterotoxaemia vaccine for sheep and goats, Onderstepoort, RSA). It should be noted that the lambs had not been shorn since birth.

While in the group pens, lambs were fed commercial Meadow drought pellets for three days where-after Meadow adaptation pellets were fed for another three days (Meadow Feeds®, PO Box 262, Paarl, 7620,Tel: +27 21 807 8700). As soon as animals were adapted to the adaptation pellets, 56 animals were blocked according to their weight and selected to be moved to individual pens for the trial. These individual pens (1.1m X 1.8m) were constructed with fenced sides simulating a feeling of being in a group while individual feed intake could be measured accurately. In the individual pens, animals were adapted to Meadow complete finisher pellets. After 7 days, the animals were fully adapted to the pellets. Nutritional information of the different pellets is summarised in Table 4.1. Sheep were randomly assigned to four treatment groups within the sheep shed. The groups were: Standard Meadow sheep finisher as the control and Meadow sheep finisher with 160g/kg, 135g/kg and 110g/kg of R-salbutamol, respectively as the three treatments. Dosages were assigned to treatments according to the manufacturer's recommendations and also a pilot study previously done on sheep. For 14 days the lambs received the standard Meadow sheep finisher pellets. On day 21 the trial started and animals were fed the four different treatment diets for 28 days before being slaughtered. No withdrawal of r-salbutamol is required.

Table 4.1 Nutritional composition of Meadow Drought Cubes 10®, Meadow Complete Sheep Adaptation® and Meadow Complete Sheep Finisher® fed to the Dohne Merino wethers

	Sheep drought pellets	Sheep adaptation pellets	Sheep finisher pellets	
Nutrient		Quantity (g/kg)		Max/Min
Protein	100	155	120	Min
Fat	25	25	25	Min
Fat	70	Not specified	70	Max
Fibre	120	Not specified	110	Min
Fibre	200	200	200	Max
Moisture	120	120	120	Max
Calcium	3	Not specified	Not specified	Min
Calcium	15	10	10	Max
Phosphorus	3	2	2	Min
Urea	10	2	10	Max

Animals had *ad libitum* access to feed and clean quality water throughout the trial. Buckets of feed were weighed out for every animal at the start of each week. At the end of the week the refusals and

the feed left over in the feeding bucket were weighed back to determine weekly intake. Animals were fed 3 times a day; at 7am, 1pm and 7pm to stimulate regular feed intake.

4.3.2 Care and general husbandry of experimental animals

Standard ethical norms of Stellenbosch University were used in this trial (SU-ACUD15-00082). Animals were housed indoors for a controlled environment and protection against extreme weather conditions. General health was monitored by the student, animal technician and Professor LC Hoffman distinguished Professor in the Animal Science Department at Stellenbosch University. When any abnormal conditions occurred, animals received veterinary care; although none of these experimental animals required this care.

4.3.3 Feedlot performance

Feedlot performance of lambs were assessed by means of weekly gain of unfasted sheep (converted back to average daily gain), total weekly feed intake (converted to daily intake) and feed conversion ratio (calculated from both parameters mentioned).

A feed sample of approximately 50 grams was taken from each 50 kg bag of the experimental feed fed to the animals which was then pooled and stored in an airtight container. Samples were chilled between 0-5°C before a proximate analysis was done on the feed. Feed was analysed for % moisture, % fat, % crude protein, % crude fibre and average gross energy. The results are summarised in Table 4.2.

Table 4.2 Nutrients of the experimental feed fed to all the animals (on a DM basis)

Nutrients	
Moisture (%)	13.55
Ash (%)	7.48
Fat (%)	3.74
Average Gross energy (MJ/kg)	17.99
Average crude protein (%)	14.44
Crude fibre (%)	14.4

4.3.4 Carcass measurements at the abattoir

After having received the experimental diets for 28 days, all lambs were slaughtered at a registered commercial abattoir (Tomis® abattoir, Hermon, Western Cape Province, SA) according to commercial slaughtering procedures. Electrical stimulation had been applied (110V for 50 seconds). Live mass before slaughter (With a 6 hour withdrawal period), warm carcass mass, cold carcass mass and carcass

classification data were obtained from the abattoir. Carcasses were classified according to South African legislation (Agricultural Product Standards Act, 1990. Act No.119 of 1990). Most carcasses had A2 classifications with odd animals reaching an A3 classification as indicated in Table 4.3. Weights were also taken of the head, feet, skin, pluck, omental fat and intestines of each animal. The head was removed from the spinal column at the occipito-atlantal junction while the feet were removed at the joint between the humerus and the scapula in the forelimbs and the joint between the femur and the tibia in the hind limbs. Carcasses were then chilled overnight at 0-5°C, cut up into primal cuts (shoulders, neck, back, ribs, fore legs, hind legs, and shins) by the same butcher as shown in Figure 4.1 and weighed. The hind legs were removed at the position between the last lumbar and the first sacral vertebrae. The neck was removed at a right angle to the spine at the seventh cervical vertebrae. The front legs were removed by cutting the scapula and its associated muscles from the muscles on the thorax (*Serratus ventralus*). To remove the back, a cut was made along the spine from the seventh cervical vertebrae (where the neck was removed) all the way to the last lumbar vertebrae. A cut was then made from the 1st rib perpendicular to the spine on both sides, to remove the ribs, flank and shoulder. The thorax part was then divided at the first rib to split the shoulder and rib cut. The weight of the chilled kidneys and kidney fat were also recorded.

Table 4.3 Carcass classification according to South African legislation of carcasses from each treatment

Classification	Treatment			
	Control	110 mg S/kg	135 mg S/kg	160 mg S/kg
	n=14	n=14	n=14	n=14
A2	12	13	13	13
A3	2	1	1	1

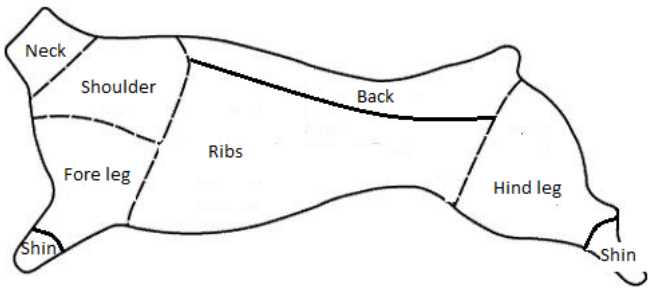

Figure 4.1 Diagram showing how the carcass was devided into primal cuts

4.3.5 Sample collection and analysis

On the day after slaughter, the left and right side of the back was divided into 3 parts of which the loin and 3-rib cut was removed for analyses. The *longissimus dorsi* (LD) muscle of both loins were taken between the 2nd- 3rd last thoracic vertebrae and the 4th -5th lumbar vertebrae, vacuum packed and chilled between 0-5° for sensory analysis as well as cooking loss and Warner-Bratzler shear Force measurements. The 3-rib cut was removed between the 9th and 12th rib (includes the 9th, 10th and 11th rib) and extended from the spinal cord to where the plane of the ribs started to curve inwards. It was then frozen at -18 °C for image analysis and to determine the percentage bone, muscle and fat (carcass conformation) by dissection at a later stage. Subcutaneous fat was removed from the *longissimus dorsi* and categorised as fat where after the *longissimus dorsi* muscle was removed from the spine and rib bones and categorised as muscle. The bones were cleaned carefully, the remaining tissue divided into muscle and fat and added to the other muscle and fat tissue to be weighed.

4.3.6 Image analysis

A digital camera was secured on a tripod and a photo was taken of each of the 3-rib cuts on the 10th rib in the exact same manner. A ruler was also placed in the photo for accurate calibration with ImageJ. The fat thickness was measured 90° to the angle of the bone, where the fat attached to the centre point of the eye muscle (as illustrated in Figure 4.2) for each of the right 3-rib cuts. Eye muscle area was also determined for the right 3-rib cut of each animal using ImageJ.

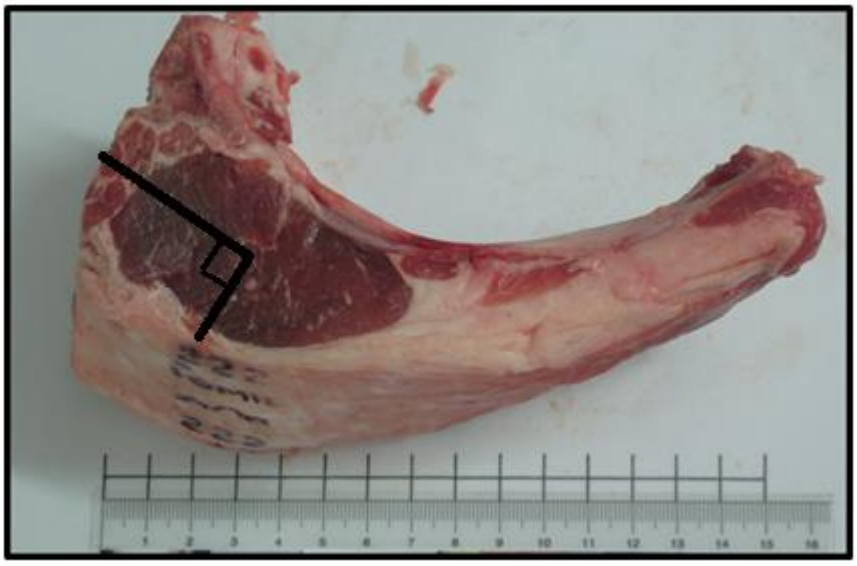

Figure 4.2: Graphic image of the 10th rib cut indicating the point of measurement of the fat thickness

4.3.7 Physical rib cut composition or carcass composition

For determination of the muscle to fat to bone ratio, each of the 3-rib cuts was carefully dissected into muscle, fat and bone. The three different tissue types were weighed and expressed as a percentage of the three rib cut's total weight.

4.3.8 Physical measurements

The pH of the four samples was measured for each of the eight replications. pH using a Crison pH 25 portable pH meter (Lasec (Pty) Ltd, South Africa) was measured immediately after removal from the packaging before cooking for the DSA session. The pH meter was calibrated using the standard buffers (pH 4.0 and pH 7.0) provided by the manufacturer before each session.

The cooking loss of the all the meat samples used for DSA were determined (AMSA, 1995).

The Warner Bratzler shear force test as described by Honikel (1998) was used to measure the instrumental shear force of all cooked meat samples. Samples included the eight replications in the DSA as well as the six replications per treatment used for training the panellists. From the centre of each LD muscle two adjacent meat strips was cut parallel to the muscle fibre direction. These strips were then wrapped in aluminium foil and refrigerated at 4°C for 24 hours. A total of six rectangular cubes of 2 cm by 1 cm by 1 cm each were then cut from the respective meat strips. An Instron Universal Testing Machine (Instron UTM, Model2519-107) with a Warner-Bratzler fitting attached was used to test the force needed to shear the meat cubes perpendicular to the muscle fibre direction. The fitting used was a 1 mm thick blade with a V-notch and a cutting edge that was semi-circular. The Instron was operated with a compression load cell of 2 kN and the speed the shear was performed at was 200mm/min. The shear force value was recorded in Newton and the mean of the six readings was used for statistical analysis.

4.3.9 Sensory analysis

Seven reference standards were prepared during the training phase of the descriptive sensory analysis (DSA; Geldenhuys *et al*., 2014). This included lamb meat, lamb fat, Karoo lamb meat, chicken breast meat, beef fillet, C-grade beef and lamb liver. These reference samples made it possible for panellists to calibrate their pallets for the different attributes. For the DSA of the experimental lambs' LD muscles, eight samples from each treatment were specifically chosen; the LD muscle of the three heaviest as well as the three lightest animals (within each treatment) were removed from the sensory test to try and eliminate the effects the higher and lower intake of r-salbutamol may have had. The LD muscles of the six discarded carcasses were used to train the sensory panel.

Sensory analysis was conducted on carcasses of all four dietary treatments. The two LD muscles from each carcass was treated as an entity and cooked in the same oven bag (Glad®, Clorox Africa (PTY) LTD). Throughout the DSA no additives or seasoning were added to the meat samples during the cooking. The meat samples and oven bags were placed on a stainless steel grid fixed to a roasting pan. Thermocouple probes connected to a handheld digital temperature monitor (Hanna Instruments, South Africa) were carefully inserted in the centre of each meat sample. The samples were then placed in a conventional oven (Defy, Model 835) already preheated to 160°C and removed when an internal temperature of 72°C was reached. After it was left to cool for 10 minutes, the LD was cut up in 1 cm^3 cubes and individually wrapped in aluminium foil. Two cubes of each sample were placed in coded glass ramekins. The ramekins were placed in a preheated industrial oven (Hobart, France) at 100°C for 10 minutes and served to the panellists immediately thereafter.

A panel of nine judges experienced in meat DSA, were selected for the analysis. The panel were trained according to the "Guidelines for meat sensory analyses" (AMSA, 1995) using the techniques for generic descriptive analysis as described by Lawless and Heymann, (2010). The panel had six training sessions where they received 1 cm^3 cubes of meat from both the four treatments and the seven reference samples. Reference samples were chosen to illustrate the aromas and flavour normally associated with lamb meat. The attributes and definitions for each as defined by the panel are described in Table 4.4. Panellists were seated in individual booths fitted with computers containing the Compusense five (Compusense, Guelph, Canada) software program. The four treatments were received in a complete randomised order. DSA lasted for 3 days of which two sessions were on the first day and 3 sessions on each of the second and third day. Each session had two cubes from a carcass of each treatment. An unstructured line scale was used to analyse the respective attributes. Zero on the scale indicated low intensity while 100 indicated high intensity (AMSA, 1995).

Table 4.4 Definition and scale of each attribute used for descriptive sensory analysis of the wether meat

Sensory attribute	Description	Scale
Lamb meat aroma	Aroma associated with cooked lean muscle from lamb meat	0- Extremely bland/100- Extremely intense
Fatty aroma	Aroma associated with cooked lamb fat	0- Extremely bland/100- Extremely intense
Beef aroma	Aroma associated with cooked lean muscle from beef meat	0- Extremely bland/100- Extremely intense
Sweet-associated aroma	Sweet aroma of sugar	0- Extremely bland/100- Extremely intense
Fatty flavour	Flavour associated with cooked lamb fat	0- Extremely bland/100- Extremely intense
Lamb meat flavour	Flavour associated with cooked lean muscle from lamb meat	0- Extremely bland/100- Extremely intense
Beef meat flavour	Flavour associated with cooked lean muscle from beef meat	0- Extremely bland/100- Extremely intense
Sweet-associated flavour	Flavour of sugar	0- Extremely bland/100- Extremely intense
Metallic flavour	Flavour similar to coins, ferro-sulphate of liver	0- Extremely bland/100- Extremely intense
Sustained juiciness	The level of juiciness perceived after 5 chews with molar teeth	0- Extremely bland/100- Extremely intense
First bite tenderness	The level of tenderness with the first bite	0- Extremely bland/100- Extremely intense
Residue	The amount of residue left in the mouth after 10 chews	0- Extremely bland/100- Extremely intense

4.3.10 Statistical analysis

The trial experimental design was a randomised block with the four diets replicated at random within 14 blocks of animals of similar weight 14 days before the onset of the trial. For the growth data, average daily gain (ADG in kg/day) for each animal was estimated using the slope of a linear regression of weight change (kg) over the trial period (days). Feed conversion ratio (FCR) was calculated as the quotient of DMI over the trial period (kg/day) and ADG (kg/day). The data for individual or primal cuts were expressed as a percentage of the cold carcass weight. For carcass conformation and image analysis the right three rib cuts were used.

The trial experimental design for the sensory analysis and corresponding physical measurements consisted of a randomised block design with four treatments and a selected eight of the fourteen replications. For sensory analysis panel performance was monitored using Panelcheck software (Version 1.4.0, Nofima; Tromsø, Norway). Sensory analysis data was pre-processed to test for panel

reliability using a model that includes panellist, session (corresponding to blocks) and treatment effects and interactions (Næs et al. 2010) indicated by the following equation:

$$y_{ijk} = \mu + b_i + t_j + bt_{ij} + p_k + bp_{ik} + tp_{jk} + \varepsilon_{ijk}$$

where terms of the model are defined as μ, the overall mean, b_i the effect due to session (block), t_j the effect due to treatment, bt_{ij} the interaction effect between session and treatment, p_k the effect due to panellist, bp_{ik} and tp_{jk} the interaction effects respectively between panellist and session or treatment and ε_{ijk}, the random error associated with the response of panellist k on treatment j in session i.

The Shapiro-Wilk test for normality was performed on the standardised residuals from the model. If there was significant deviation from normality, outliers were removed when the standardised residual for an observation deviated more than three standard deviations from the model value. Following the confirmation of panel reliability and normality, subsequent statistical analyses on sensory data were conducted on means over panellists.

Univariate analysis of variance (Anova) was performed on data for intake of r-salbutamol as main effect, data of individual cuts as well as sensory and physical variables according to the model for the experimental design indicated by the following equation:

$$y_{ij} = \mu + b_i + t_j + \varepsilon_{ij}$$

Where terms of the model are defined as μ, the overall mean, b_i the effect due to block, t_j the effect due to treatment and ε_{ij}, the random error associated with the response on treatment j in block i.

For growth data (DMI, ADG and FCR) and carcass data a covariance analysis was performed using animal weight at the start of the trial as covariate (instead of block effect) to adjust for initial weight differences between individual animals.

Shapiro-Wilk test was performed to test for normality (Shapiro, 1965). As FCR distribution deviated from normality, an inverse transformation was done to improve normality. Least squares means (LS-means) were computed and compared using pairwise p-values. A probability level of 5% was considered significant for all significance tests. Univariate analyses (Anova and regression) were performed using SAS software (Version 9.2, SAS Institute Inc, Cary, USA).

4.4 Results and Discussion

4.4.1 Feedlot performance: Average daily gain, Dry matter intake and feed conversion ratio

In Table 4.5, the growth data is summarised for the 28 day feeding period of the four treatment groups.

Table 4.5 Summary statistics (LSMeans ± SD) of dry matter intake (DM intake), average daily gain (ADG), average daily gain calculated from the slope (ADG from slope), feed conversion ratio (FCR) and feed conversion ratio calculated from the slope (FCR from slope) of the four treatment diets containing different concentrations of r-salbutamol.

Parameter measured	Treatment				P-value
	Control (n=14)	110 mg S/kg (n=14)	135 mg S/kg (n=14)	160 mg S/kg (n=14)	
DM intake (kg/day)	1.81 ± 0.26	1.76 ± 0.19	1.82 ± 0.18	1.83 ± 0.16	0.74
ADG (kg)	0.29 ± 0.07	0.31 ± 0.08	0.32 ± 0.06	0.33 ± 0.05	0.64
ADG from slope (kg)	0.29 ± 0.07	0.31 ± 0.07	0.33 ± 0.06	0.33 ± 0.05	0.50
FCR (kg)	6.50^{a} ± 1.33	5.95^{ab} ± 1.25	5.76^{ab} ± 0.65	5.70^{b} ± 0.92	0.03
FCR from slope (kg)	6.43^{a} ± 1.21	5.91^{ab} ± 1.12	5.65^{b} ± 0.59	5.64^{b} ± 0.85	0.01

^{a,b} means in a row with different superscript letters differ (P≤ 0.05).

Lambs fed 160 mg S/kg r-salbutamol required significantly less feed per kg weight gain (5.70 ± 0.92 kg) than the control (6.5 ± 1.33 kg).

Feed conversion ratios calculated from the slope also showed significant differences with both 135 and 160 mg S/kg performing significantly better than the control group (Table 4.4). This indicates that in r-salbutamol treated groups, less feed was required (5.65 ± 0.59kg/5.64 ± 0.85kg) for each kg body weight gain compared to the control group (6.43 ± 1.12kg). This is in accordance with results from others who also found an improved FCR in feedlot cattle and sheep treated with the beta-agonist zilpaterol hydrochloride (Vasconcelos *et al.,* 2008; Elam *et al*., 2009; Montgomery *et al.,* 2009; Lopez-Carlos *et al.,* 2010; Salinas-Chavira *et al*., 2011). R-salbutamol also showed improved FCR when administered in pigs (Marchant-Forde *et al.,* 2012). When r-salbutamol and zilpaterol hydrochloride were compared by Steenekamp (2014) in feedlot bulls however, no significant improvements in FCR was found between the beta-agonist fed bulls and the control.

R-salbutamol had no significant effect on DM intake, ADG and ADG calculated from the slope which agreed with previous studies with regard to DM intake (Montgomery *et al*., 2009; Elam *et al*., 2009).

However, in studies with other beta-agonists on cattle, sheep and also in pigs treated specifically with r-salbutamol, an increased ADG was observed (Vasconcelos *et al.,* 2008; Montgomery *et al*., 2009; Elam *et al*., 2009; Marchant-Forde *et al.,* 2012; Lopez-Carlos *et al.,* 2010; Salinas-Chavira *et al*., 2006).

4.4.2 Feed Conversion Ratio and Average Daily Gain calculations with actual r-salbutamol intake as main effect

As an addition to the normal growth and performance data with treatment as main effect (Table 4.5), the dietary treatments were ignored and individual FCR and ADG values were also recalculated with the actual intake of the of active ingredient, r-salbutamol. Thereafter, the wethers were re-categorised as zero, low, medium, and high. The control group was taken as zero r-salbutamol and for the other 3 groups, the total r-salbutamol was according to the intake classified as low with a range of 4299.79<x<6082.01mg intake of r-salbutamol, medium with a range of 6088.23<x<7446.88mg intake of r-salbutamol and high with a range of 7467.68<x<9814.56mg intake of r-salbutamol. This method of calculation was used as variation in feed intake, and therefor of r-salbutamol, was high amongst and within the treatments. This variation made groups formed based on equal intervals too uneven for statistical analysis. The ranges are thus unequal to have balanced groups (n=14). Data summarised in Table 4.6.

Table 4.6 Summary statistics (LSMeans ± SD) of average daily gain (ADG) and feed conversion ratio (FCR) calculations with mg r-salbutamol intake as main effect

Parameter measured	Treatment				P-value
	Zero (n=14)	Low (n=14)	Medium (n=14)	High (n=14)	
ADG (kg/day)	0.29 ± 0.07	0.29 ± 0.07	0.33 ± 0.04	0.34 ± 0.07	0.12
FCR (kg feed/kg weight gain)	6.48 ± 1.85	6.27 ± 3.56	6.53 ± 2.56	6.17 ± 2.08	0.98

No significant differences were observed for ADG and FCR between groups with different intake levels of the active ingredient, r-salbutamol. Improved ADG and FCR was expected as the intake of r-salbutamol increased. The results are in contrast with results from other authors that have administered beta-agonists to feedlot lambs. When feedlot lambs were treated with zilpaterol hydrochloride and ractopamine hydrochloride in a comparative study, both beta-agonists showed improved ADG and FCR compared to a control group (Lopez-Carlos *et al.,* 2010). Other authors

suggested the same improvement in beta-agonist supplemented lambs (Salinas-Chavira *et al*., 2006). R-salbutamol treated pigs also had an improved ADG and FCR (Marchant-Forde *et al.,* 2012).

4.4.3 Carcass and slaughtering data

In Table 4.7 the carcass data is summarised. Feeding of r-salbutamol had no effect ($p>0.05$) on any of the slaughter parameters.

Table 4.7 Summary statistics (LSMeans ± SD) of live weight at slaughter, warm carcass mass, cold carcass mass, dressing out percentage and warm carcass pH45 (45 minutes post mortem) of the four treatment groups.

Parameters measured	Treatment				P-value
	Control (N=14)	110 mg S/kg (N=14)	135 mg S/kg (N=14)	160 mg S/kg (N=14)	
Live weight before slaughter (kg)	42.70 ± 4.52	43.66 ± 3.82	42.70 ± 2.89	43.89 ± 3.09	0.22
Warm Carcass mass (kg)	21.36 ± 2.29	21.89 ± 2.04	21.93 ± 1.32	21.99 ± 1.89	0.19
Cold Carcass mass (kg)	20.71 ± 2.24	21.29 ± 2.01	21.27 ± 1.28	21.33 ± 1.84	0.21
Dressing out percentage	48.5 ± 2.02	48.8 ± 1.78	48.4 ± 1.37	48.6 ± 2.00	0.94
pH_{45}	6.34^{b} ± 0.41	5.96^{c} ± 0.43	6.45^{ab} ± 0.44	6.76^{a} ± 0.50	<0.01

^{a,b} means in a row with different superscript letters differ ($P \leq 0.05$).

Beta-agonist supplementation normally results in heavier carcass mass and better dressing out percentage (Moody *et al*., 2000). The extent of the response however, depends on the type of beta-agonist used and is influenced by factors such as age, sex, species, breed and diet. When ractopamine hydrochloride was administered in sheep, none of the carcass characteristics improved (Robles-Estrada *et al*., 2009). In another study, carcass characteristics such as warm carcass mass and dressing out percentage increased linearly as ractopamine hydrochloride increased (Lopez-Carlos *et al.,* 2010).

Felix *et al.* (2005) also showed that results of carcass characteristics in sheep are inconsistent where zilpaterol hydrochloride had no effect on carcass characteristics of Pelibuey lambs.

Also, when feedlot bulls were supplemented with r-salbutamol, no effect on carcass characteristics was observed (Steenekamp, 2014). However, an improved warm carcass mass and dressing out percentage was observed in pigs when treated with r-salbutamol (Marchant-Forde *et al.,* 2012).

4.4.4 Data of individual cuts and organs for determination of muscle distribution in the carcass

In Table 4.8 the data of the different body parts and organs collected on the day of slaughter are summarised. The values are expressed as a percentage of the animal's live weight immediately before slaughter. In Table 4.9, the data of the primal cuts taken 24 hours after slaughter is summarised.

Table 4.8 Summary statistics (LSMeans ± SD) of different body parts expressed as a percentage of live weight of wethers fed different levels of r-salbutamol (S).

Parameters measured (%)*	Treatment				P-value
	Control (N=14)	110 mg S/kg (N=14)	135 mg S/kg (N=14)	160 mg S/kg (N=14)	
Head	4.94^{b} ± 0.53	5.26^{a} ± 0.44	5.23^{ab} ± 0.27	5.06^{ab} ± 0.31	0.14
Feet	2.59 ± 0.26	2.57 ± 0.23	2.67 ± 0.26	2.55 ± 0.14	0.55
Skin	10.19 ± 0.75	10.00 ± 0.72	9.73 ± 0.99	9.77 ± 0.62	0.38
Pluck	4.63^{b} ± 0.29	4.65^{ab} ± 0.31	4.88^{a} ± 0.31	4.79^{ab} ± 0.34	0.12
Intestines	21.24 ± 1.41	20.84 ± 1.83	20.61 ± 1.27	20.32 ± 1.70	0.47
Omental fat	1.27^{ab} ± 0.37	1.15^{b} ± 0.31	1.30^{ab} ± 0.17	1.40^{a} ± 0.24	0.17

^{a,b} means in a row with different superscript letters differ (P< 0.05).

* The values are expressed as a percentage of the live weight.

The effect of beta-agonists on offal is rarely studied as it has little value compared to the rest of the carcass. It was found however, that beta-agonists including zilpaterol hydrochloride has no effect on non-carcass components in sheep when expressed as a percentage of live weight (Li *et al*., 2000; Aguilera-Soto *et al*., 2008). This is due to the low hypertrophic effect of beta-agonists on visceral organs (Li *et al.*, 2000). The type, number and activation of receptors differ in different tissues and the largest concentration is found in skeletal muscle where hypertrophic action occurs. In a study with ewe lambs, zilpaterol hydrochloride also had no effect on internal fat of the carcass (Macias-Cruz *et al*., 2010).

In this study, from the different body parts and organs, only the percentages of the head, pluck and omental fat differed significantly between treatment groups. The percentage omental fat was larger

for the group treated with the highest concentration of r-salbutamol. This is in contrast with previous beta-agonist studies, which had no effect on non-carcass components and also tend to decrease fat in the carcass. As in other studies, no further significant differences between treatment groups were observed for the percentage weights of the intestines, skin and feet.

Table 4.9 Summary statistics (LSMeans ± SD) of different primal cuts as a percentage of cold carcass mass of wethers fed different levels of r-salbutamol (S).

Parameter measured (%)*	Treatment				P-value
	Control (N=14)	110 mg S/kg (N=14)	135 mg S/kg (N=14)	160 mg S/kg (N=14)	
Neck	$4.15^{b} \pm 0.52$	$4.61^{a} \pm 0.41$	$4.10^{b} \pm 0.41$	$4.24^{b} \pm 0.32$	0.01
Shoulders	$16.19^{a} \pm 1.66$	$14.82^{b} \pm 0.95$	$15.73^{a} \pm 0.77$	$15.48^{ab} \pm 0.97$	0.02
Forelegs	13.88 ± 1.38	14.41 ± 1.09	14.10 ± 1.07	14.28 ± 0.83	0.61
Ribs	10.33 ± 0.99	10.59 ± 0.65	10.37 ± 0.71	10.34 ± 0.59	0.77
Back	$18.69^{a} \pm 0.82$	$17.82^{b} \pm 0.62$	$18.25^{ab} \pm 0.64$	$18.52^{a} \pm 0.50$	0.01
Hind legs	31.95 ± 1.03	32.56 ± 1.17	32.76 ± 1.33	32.27 ± 1.16	0.30
Shins	$3.14^{b} \pm 0.33$	$3.27^{ab} \pm 0.32$	$3.46^{a} \pm 0.16$	$3.47^{a} \pm 0.24$	0.01
Kidneys	0.61 ± 0.08	0.61 ± 0.07	0.64 ± 0.05	0.62 ± 0.06	0.68
Kidney fat	2.53 ± 0.75	2.31 ± 2.31	2.22 ± 2.22	2.21 ± 2.21	0.50

^{a,b} means in a row with different superscript letters differ (P≤ 0.05).

*The values are expressed as a percentage of cold carcass mass

Of the primal cuts, only percentage weights of the shoulders, neck, back and shins showed differences (P≤0.05) between treatment groups. The other primal cuts and organs such as kidneys, ribs, forelegs, hind legs and kidney fat did not differ (P> 0.05) between treatments. Percentage shoulder of the control group was the heaviest followed by the 135mg S/kg group. These two groups did not differ (P> 0.05) from each other but both groups differed from the 110 mg S/kg group which had the lightest percentage shoulders. The percentage neck was the heaviest for the 110 mg S/kg group and differed significantly from the other three treatment groups. Zilpaterol hydrochloride was shown to decrease forequarter % but increase hind quarter % in a study on feedlot lambs as the Control groups had greater shoulder and neck percentages than the zilpaterol treated lambs (Macias-cruz *et al*., 2010). However, R-salbutamol in this study did not decrease the % shoulders and in contrast increased the % neck when administered in low concentrations (135mg S/kg). It also had no effect on the hind quarters as hind leg yields did not differ across treatment groups. Percentage shins was the heaviest

for the 160 mg S/kg group, followed by the 135 mg S/kg group; both groups differed significantly from the control. The % back of the control group and 160 mg S/kg group did not differ ($p > 0.05$) and was the heaviest across the four treatment groups. Both groups differed significantly from the 110 mg S/kg group which had the lightest % back. Zilpaterol hydrochloride treated feedlot lambs had similar percentages of average loin yields as the control group according to Macias-cruz *et al,* (2010); a result similar to the r-salbutamol in the higher concentrations in this investigation. The 110 mg S/kg group seem to increase the percentage of less valuable cuts such as the neck while it decreases the percentage of more usable and valuable cuts such as the shoulders and back. With higher concentrations of r-salbutamol this phenomenon seems to disappear as the 160 mg S/kg group and 135mg S/kg group did not show any differences in the % shoulders and back compared to the control group ($p > 0.05$).

4.4.5 Image analysis of eye muscle area and fat thickness

Table 4.10 Summary statistics (LSMeans ± SD) for image analysis of eye muscle (*m. longissimus muscle*) area and subcutaneous fat thickness (in cm) of the 3-rib cut. No significant differences in eye muscle area and fat thickness were found between treatment groups.

Parameter measured	Treatment				P-value
	Control (n=14)	110 mg S/kg (n=14)	135 mg S/kg (n=14)	160 mg S/kg (n=13)	
Eye muscle area (cm^2)	24.26 ± 3.91	26.89 ± 4.40	26.69 ± 3.71	24.57 ± 2.87	0.14
Fat thickness right (cm)	0.41 ± 0.12	0.46 ± 0.12	0.41 ± 0.12	0.41 ± 0.20	0.64

Many authors found an increase in eye muscle area with beta-agonist supplementation (Lopez-Carlos *et al*., 2010; Macias-Cruz *et al*., 2010). As discussed previously beta-agonists tend to decrease carcass fat; subcutaneous fat thickness was decreased in feedlot lambs when administered both zilpaterol hydrochloride and ractopamine hydrochloride (Lopez-Carlos *et al*., 2010). In contrast, no significant effect on subcutaneous fat thickness was found when feedlot bulls were supplemented with r-salbutamol (Steenekamp, 2014). In this study the same lack of an effect on subcutaneous fat was observed in feedlot lambs with r-salbutamol treatment.

4.4.6 Carcass composition (percentage muscle, bone and fat)

In Table 4.11 the carcass conformation data of the right 3-rib cut (taken between the 9^{th} and 12^{th} rib) is summarised.

Table 4.11 Summary statistics (LSMeans ± SD) of carcass composition (percentage muscle to bone to fat) of the 3-rib cut.

Parameter measured	Treatment				P-value
	Control (n=14)	110 mg S/kg (n=14)	135 mg S/kg (n=14)	160 mg S/kg (n=13)	
Percentage muscle	34.67^{b} ± 3.31	36.42^{ab} ± 3.36	36.55^{ab} ± 2.71	37.37^{a} ± 2.58	0.02
Percentage fat	33.84^{ab} ± 4.55	32.30^{b} ± 5.07	35.01^{ab} ± 3.60	36.33^{a} ± 4.51	0.02
Percentage bone	31.49^{a} ± 3.36	31.28^{ab} ± 4.00	28.44^{bc} ± 4.45	26.30^{c} ± 3.56	<0.01

^{a,b} means in a row with different superscript letters differ (P≤ 0.05).

Percentage muscle is significantly higher for the 160 mg S/kg group compared to the control group indicating that beta-agonist supplementation has an overall effect on skeletal muscle in the wethers. Beta-agonists can stimulate hypertrophy through decreased protein degradation and increased protein synthesis depending on the type of beta-agonists, the breed, species and also the nutritional status of the animal (Mersmann, 1998). In a study comparing zilpaterol hydrochloride and ractopamine hydrochloride on feedlot lambs, both beta-agonists improved muscularity (Lopez-Carlos *et al.,* 2010). When r-salbutamol was administered in feedlot bulls it not only had larger percentage muscle than the control group but also a larger percentage muscle than zilpaterol hydrochloride treated bulls (Steenekamp, 2014). This is all in accordance with the results of the current study which suggests that r-salbutamol is an effective agent in skeletal muscle metabolism.

Percentage fat was also the highest for the 160 mg S/kg treatment group and differed significantly from the 110 mg S/kg group. Although both the 135 mg S/kg and the 160 mg S/kg group had a larger fat percentage than the control group, it did not differ significantly. This means that percentage fat is decreased in low concentrations of r-salbutamol but the effect disappears as the concentration increases. Only the 110 mg S/kg can thus be seen as having a significant effect on lipid metabolism. This contradicts previous studies which suggest reduced fat in sheep carcasses with beta-agonist supplementation (Rikhardsson *et al*., 1991; Salinas-Chavira *et al.,* 2004). R-salbutamol administered in feedlot bulls also showed lower proportions of internal carcass fat and a lower percentage fat in the *longissimus dorsi* muscle compared to control bulls (Steenekamp, 2014). The lower percentage fat suggests that r-salbutamol is an effective lipolysis agent in feedlot bulls. In feedlot sheep however, this seems not to be the case although it could be argued that the results of this investigation are inconclusive and thus warrants further research.

With the percentage bone the 160 mg S/kg group again performed the best with the lowest percentage followed by the 135 mg S/kg group; these groups did not differ significantly. The 160 mg S/kg group differed significantly however from the control as well as the 110 mg S/kg group. The 135 mg S/kg group also had significantly lower percentage bone than the control group. The control group performed the worst with the largest percentage bone. This means that r-salbutamol can decrease percentage bone in the carcass resulting in more usable and valuable meat in lamb carcasses.

4.4.7 Physical measurements

Summarised in Table 4.12 are the pH values of the uncooked samples of the *longissimus dorsi* muscle, the percentage cooking loss, and also the average shear force value of six repetitions measured on the cooked samples.

Table 4.12 Summary statistics (LSMeans ± SD) of muscle pH before cooking, percentage cooking loss and average shear force values.

Parameter measured	Treatment				P-value
	Control (n=14)	110 mg S/kg (n=14)	135 mg S/kg (n=14)	160 mg S/kg (n=14)	
pH Raw	5.73 ± 0.12	5.69 ± 0.10	5.65 ± 0.06	5.68 ± 0.10	0.37
% Cooking loss	31.79 ± 8.34	30.04 ± 8.36	29.88 ± 4.91	31.18 ± 8.55	0.95
Instron (N)	25.29 ± 9.41	29.59 ± 5.57	31.27 ± 10.56	29.32 ± 3.82	0.49

[a,b] means in a row with different superscript letters differ (P< 0.05).

No differences (P >0.05) in the pH of the raw muscle, the average shear force value (N) nor the percentage cooking loss were observed. In a study on feedlot bulls, a trend was observed where r-salbutamol treated bulls had slower pH declines than zilpaterol hydrochloride treated bulls. This was suggested to be due to differences in muscle energy status possibly due to differences in stress responsiveness of zilpaterol supplemented bulls compared to r-salbutamol supplemented bulls (Steenekamp, 2014). Unfortunately, pH decline over time during the chilling process was not measured in this investigation and may warrant further research.

Beta-agonists have been reported by many authors to increase toughness of meat, especially beta$_2$-agonists such as zilpaterol hydrochloride which has a pronounced effect on muscle metabolism (Leheska *et al*., 2009; Strydom *et al*., 2009; Rathmann *et al.,* 2009; Strydom *et al*., 2011). This is due to changes in muscle fibre size and proportions linked to changes in proteolytic activity (Dunshea *et* al., 2005). Steenekamp (2014) also reported higher shear force values of feedlot bulls treated with zilpaterol hydrochloride when compared to control and r-salbutamol treated bulls although the R-

salbutamol treated bulls did not differ in shear force values from the control group. This was suggested to be due to the more prominent effect r-salbutamol has on lipolysis and the minor effect it has on muscle metabolism (Steenekamp, 2014). This is in accordance with the current study where no increase in shear force values of r-salbutamol treated feedlot lambs were noted, regardless of the concentration of beta-agonist fed/consumed.

4.4.8 Descriptive sensory analysis

In Table 4.13 the statistics of the sensory attributes are summarised as scored by the nine judges on the descriptive sensory panel. Significant differences (P<0.05) were only noted for the attributes metallic taste and sustained juiciness.

Table 4.13 LSMeans ± SD of Sensory data scored on a scale from 0-100 with zero representing low intensity and 100 representing high intensity.

Parameter measured	Treatment				P-value
	Control (n=8)	110 mg S/kg (n=8)	135 mg S/kg (n=8)	160 mg S/kg (n=8)	
Lamb meat aroma	59.3 ± 0.8	59.1 ± 0.9	59.3 ± 1.0	59.2 ± 1.0	0.95
Beefy aroma	50.2 ± 1.4	50.3 ± 1.2	50.4 ± 0.5	50.5 ± 1.3	0.95
Sweet associated aroma	10.1 ± 0.1	10.2 ± 0.2	10.3 ± 0.3	10.3 ± 0.3	0.63
Fatty aroma	10.1 ± 0.3	10.1 ± 0.2	9.9 ± 0.3	10.1 ± 0.3	0.29
Initial juiciness	41.6 ± 4.9	39.8 ± 3.5	41.7 ± 3.6	41.2 ± 3.7	0.76
Lamb meat flavour	59.5 ± 0.9	59.5 ± 0.8	59.7 ± 0.5	59.0 ± 0.8	0.32
Beefy flavour	50.4 ± 0.8	50.5 ± 0.6	50.2 ± 0.6	50.5 ± 0.5	0.77
Sweet associated flavour	10.3 ± 0.2	10.2 ± 0.2	10.3 ± 0.3	10.1 ± 0.1	0.25
Fatty Flavour	10.4 ± 0.6	10.7 ± 0.9	10.2 ± 0.8	9.9 ± 0.6	0.30
Metallic Taste	0.9^{b} ± 0.9	1.9^{ab} ± 1.3	2.3^{a} ± 1.2	2.0^{ab} ± 1.5	0.04
Sustained juiciness	43.2^{ab} ± 4.4	39.1^{b} ± 3.7	41.4^{ab} ± 3.0	43.3^{a} ± 5.1	0.03
Tenderness	53.0 ± 8.9	47.2 ± 8.7	52.9 ± 8.2	46.8 ± 7.6	0.28
Residue	26.9 ± 5.0	29.4 ± 7.1	27.7 ± 5.4	29.7 ± 5.4	0.73

^{a,b} means in a row with different superscript letters differ (P< 0.05).

A significant difference was found in metallic taste between the 135 mg S/kg group (higher value) and the control group. However, the metallic flavour was not very distinct as it was scored 2.3 ± 1.17 on a scale from zero to 100. The 160 mg S/kg group had a significantly higher (P<0.05) sustained juiciness than the 110 mg S/kg group. However, there were no significant differences in sustained juiciness between the control group, the 135 mg/kg r-salbutamol group and the 160 mg/kg r-salbutamol group.

In low concentrations, r-salbutamol can reduce sustained juiciness making meat seem dry when eaten. When administered in higher concentrations however, this is not the case as both the 135 and 160 mg S/kg treatment group has higher sustained juiciness than the control, although not significant. This could have been due to the higher fat content or percentage fat in the 135 and 160 mg S/kg groups.

The sensory analysis indicates that meat not only has similar shear force values (Table 3.8) but the perceived toughness when eaten is also the same. Perceived toughness can sometimes be influenced by other factors such as juiciness. This is not the case with r-salbutamol and no differences in toughness or residue could be detected by the sensory panel. It is interesting that the other attributes especially tenderness and residue showed no differences ($P>0.05$) between treatment groups. The use of B-agonists and other growth stimulants are frequently associated with increased toughness (Montgomery *et al*., 2009; Elam *et al*., 2009).

4.5 Conclusions

In this study, r-salbutamol seemed most effective in Dohne Merinos when administered in the highest concentration of 160 mg S/kg. This group had a better feed conversion ratio compared to the control but no effects were observed on daily gain and lipid metabolism as other beta-agonists tend to have. R-salbutamol did not affect carcass characteristics at any concentration and effects on offal and primal cuts were minimal. The most prominent effect on offal and primal cuts were the lack of difference in omental as well as kidney fat suggesting that r-salbutamol may have reduced effect on fat in the abdominal area. Furthermore, no differences were observed in percentage carcass fat and subcutaneous fat thickness which suggests that r-salbutamol was ineffective in lipid metabolism in this study. Percentage muscle in the 160 mg S/kg group was higher than the control while percentage bone was lower. R-salbutamol is thus effective in muscle metabolism. Beta-receptor numbers for younger animals are often low and can increase in number as an animal ages. The fact that lambs in this study were still very young could thus explain the reduced effect on fat and lipid metabolism in Dohne Merino lambs. R-salbutamol, other than other beta-agonists tested has no effect on shear force of meat or perceived toughness as tested for in descriptive sensory analysis when administered in any concentration. This suggests that r-salbutamol does not increase toughness of meat.

R-salbutamol needs to be tested on a later maturing breed which normally go into feedlot systems at a higher age, to see if the same lack of differences in fat metabolism is observed. Growth and skeletal muscle may also show more effects due to increased beta receptors.

4.6 References

Aguilera-Soto, J., R. Ramirez, C. Arechiga, F. Mendez-Llorente, M. Lopez-Carlos, J. Silva-Ramos, R. Rincon-Delgado, and F. Duran-Roldan. 2008. Zilpaterol hydrochloride on performance and sperm quality of lambs fed wet brewers grains. J. Appl. Anim. Res. 34(1): 17-21.

American Meat Science Association. 1995. Research guidelines for cookery, sensory evaluation and instrumental tenderness measurements of fresh meat. National Live Stock and Meat Board, Chicago.

Apple, J., C. Maxwell, B. Kutz, L. Rakes, J. Sawyer, Z. Johnson, T. Armstrong, S. Carr, and P. Matzat. 2008. Interactive effect of ractopamine and dietary fat source on pork quality characteristics of fresh pork chops during simulated retail display. J. Anim. Sci. 86(10): 2711-2722.

Avendaño-Reyes, L., V. Torres-Rodríguez, F. Meraz-Murillo, C. Pérez-Linares, F. Figueroa-Saavedra, and P. Robinson. 2006. Effects of two β-adrenergic agonists on finishing performance, carcass characteristics, and meat quality of feedlot steers. J. Anim. Sci. 84(12): 3259-3265.

Beermann, D. 2009. ASAS centennial paper: A century of pioneers and progress in meat science in the United States leads to new frontiers. J. Anim. Sci. 87(3): 1192-1198.

Beukes, J. A. 2013. Maize Silage Based Diets for Feedlot Finishing of Merino Lambs. Master's Thesis. Faculty of AgriScience. University of Stellenbosch. South Africa.

Brooks, J., H. Claus, M. Dikeman, J. Shook, G. Hilton, T. Lawrence, J. Mehaffey, B. Johnson, D. Allen, and M. Streeter. 2009. Effects of zilpaterol hydrochloride feeding duration and post mortem aging on warner-bratzler shear force of three muscles from beef steers and heifers. J. Anim. Sci. 87(11): 3764-3769.

Carr, S., D. Ivers, D. Anderson, D. Jones, D. Mowrey, M. England, J. Killefer, P. Rincker, and F. McKeith. 2005. The effects of ractopamine hydrochloride on lean carcass yields and pork quality characteristics. J. Anim. Sci. 83(12): 2886-2893.

Dunshea, F., D. D'souza, D. Pethick, G. Harper, and R. Warner. 2005. Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. Meat Sci. 71(1): 8-38.

Elam, N., J. Vasconcelos, G. Hilton, D. Van Overbeke, T. Lawrence, T. Montgomery, W. Nichols, M. Streeter, J. Hutcheson, and D. Yates. 2009. Effect of zilpaterol hydrochloride duration of feeding on performance and carcass characteristics of feedlot cattle. J. Anim. Sci. 87(6): 2133-2141.

Etherton, T. 2009. ASAS centennial paper: Animal growth and development research: Historical perspectives. J. Anim. Sci. 87(9): 3060-3064.

Felix, A., A. Estrada-Angulo, F. Rios, C. Ramos, and A. Perez. 2005. Effect of zilpaterol clorhidrate on growth performance and carcass traits in finishing sheep. J. Dairy Sci.

Geldenhuys, G., L. Hoffman, and M. Muller. 2014. Sensory profiling of Egyptian goose (*Alopochen aegyptiacus*) meat. Food Res. Int. 64: 25-33.

Honikel, K. O. 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49(4): 447-457.

Hope-Jones, M., P. E. Strydom, L. Frylinck, and E. C. Webb. 2012. Effect of dietary beta-agonist treatment, vitamin D 3 supplementation and electrical stimulation of carcasses on colour and drip loss of steaks from feedlot steers. Meat Sci. 90(3): 607-612.

Johnson, B. J., and K. Y. Chung. 2007. Alterations in the physiology of growth of cattle with growth-enhancing compounds. Veterinary Clinics of North America: Fd Anim. Pract. 23(2): 321-332.

Lawless, H. T., and H. Heymann. 2010. Sensory evaluation of food: Principles and practices. Springer Science & Business Media.

Leheska, J., J. Montgomery, C. Krehbiel, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, J. Blanton, and M. Miller. 2009. Dietary zilpaterol hydrochloride. II. Carcass composition and meat palatability of beef cattle. J. Anim. Sci. 87(4): 1384-1393.

Li, Y., R. Christopherson, B. Li, and J. Moibi. 2000. Effects of a beta-adrenergic agonist (L-644,969) on performance and carcass traits of growing lambs in a cold environment. Canadian J. Anim. Sci. 80(3): 459-465.

López-Carlos, M., R. Ramírez, J. Aguilera-Soto, C. Aréchiga, F. Méndez-Llorente, H. Rodríguez, and J. Silva. 2010. Effect of ractopamine hydrochloride and zilpaterol hydrochloride on growth, diet digestibility, intake and carcass characteristics of feedlot lambs. Livest. Sci. 131(1): 23-30.

Macías-Cruz, U., F. Álvarez-Valenzuela, N. Torrentera-Olivera, J. Velázquez-Morales, A. Correa-Calderón, P. Robinson, and L. Avendaño-Reyes. 2010. Effect of zilpaterol hydrochloride on feedlot performance and carcass characteristics of ewe lambs during heat-stress conditions. Anim. Prod. Sci. 50(10): 983-989.

Marchant-Forde, J., D. Lay, R. Marchant-Forde, K. McMunn, and B. Richert. 2012. The effects of R-salbutamol on growth, carcass measures, and health of finishing pigs. J. Anim. Sci. 90(11): 4081-4089.

Marchant-Forde, J., D. Lay, R. Marchant-Forde, K. McMunn, and B. Richert. 2008. The effects of R-salbutamol on behavior and physiology of finishing pigs. J. Anim. Sci. 86(11): 3110-3124.

Mehaffey, J., J. Brooks, R. Rathmann, E. Alsup, J. Hutcheson, W. Nichols, M. Streeter, D. Yates, B. Johnson, and M. Miller. 2009. Effect of feeding zilpaterol hydrochloride to beef and calf-fed Holstein cattle on consumer palatability ratings. J. Anim. Sci. 87(11): 3712-3721.

Mersmann, H. J. 1998. Overview of the effects of beta-adrenergic receptor agonists on animal growth including mechanisms of action. J. Anim. Sci. 76(1): 160-172.

Montgomery, J., C. Krehbiel, J. Cranston, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, D. Bechtol, E. Johnson, and T. TerHune. 2009. Dietary zilpaterol hydrochloride. I. feedlot performance and carcass traits of steers and heifers. J. Anim. Sci. 87(4): 1374-1383.

Montgomery, J., C. Krehbiel, J. Cranston, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, R. Swingle, and T. Montgomery. 2009. Effects of dietary zilpaterol hydrochloride on feedlot performance and carcass characteristics of beef steers fed with and without monensin and tylosin. J. Anim. Sci. 87(3): 1013-1023.

Moody, D., D. Hancock, and D. Anderson. 2000. Phenethanolamine repartitioning agents. Farm Animal Metabolism and Nutrition. : 65-96.

Næs, T., Brockhoff, P.B. & Tomic, O. (2010). Statistics for sensory and consumer science. New York, USA: Wiley.

National Department of Agriculture. 1990. Agricultural Product Standards Act, 1990 (Act No. 119 of 1990) No. R. 342. Regulations Regarding the Classification and Marking of Meat.

National Research Council (US). 1994. Metabolic modifiers: Effects on the nutrient requirements of food-producing animals. National Academy Press.

Parr et al, T., M. H. Mareko, K. J. Ryan, K. M. Hemmings, D. M. Brown, and J. M. Brameld. 2016. The impact of growth promoters on muscle growth and the potential consequences for meat quality. Meat Sci: 93-99

Pringle, T., C. R. Calkins, M. Koohmaraie, and S. J. Jones. 1993. Effects over time of feeding a beta-adrenergic agonist to wether lambs on animal performance, muscle growth, endogenous muscle proteinase activities, and meat tenderness. J. Anim. Sci. 71(3): 636-644.

Rikhardsson, G., K. Johnson, and D. Johnson. 1991. Effects of cimaterol on energetics and carcass characteristics of suffolk ewe lambs. J. Anim. Sci. 69(1): 396-404.

Robles-Estrada, J., A. Barreras-Serrano, G. Contreras, A. Estrada-Angulo, J. Obregón, A. Plascencia, and F. Ríos. 2009. Effect of two β-adrenergic agonists on finishing performance and carcass characteristics in lambs fed all-concentrate diets. J. Appl. Anim. Res. 36(1): 33-36.

Salinas-Chavira, J., M. Domínguez-Muñoz, R. Díaz-Martínez, P. Cruz-Bautista, M. Montaño-Gómez, and C. Arzola-Alvarez. 2006. Effect of duration of zilpaterol hydrochloride treatment on carcass characteristics and weight gain in grazing pelibuey lambs. J. Appl. Anim. Res. 29(1): 25-28.

Salinas-Chavira, J., R. Ramírez, M. Domínguez-Muñoz, R. Palomo-Cruz, and V. López-Acuña. 2004. Influence of zilpaterol hydrochloride on growth and carcass characteristics of pelibuey lambs. J. Appl. Anim. Res. 26(1): 13-16.

Schiavone, A., M. Tarantola, G. Perona, S. Pagliasso, P. Badino, R. Odore, B. Cuniberti, and C. Lussiana. 2004. Effect of dietary clenbuterol and cimaterol on muscle composition, β-adrenergic and androgen receptor concentrations in broiler chickens. J. Anim. Physiol. Anim. Nutr. 88(3-4): 94-100.

Shapiro, S.S. and Wilk, M.B. 1965. An analysis of Variance Test for Normality (complete samples), Biometrika 52, 591-611.

Steenekamp, S. 2014. Growth Performance and Meat Characteristics of Feedlot Cattle Fed R-Salbutamol or Zilpaterol Hydrochloride during the Finishing Period. Master's Thesis. Department Animal and Wildlife Sci. University of Pretoria. South Africa.

Strydom, P., L. Frylinck, J. Montgomery, and M. Smith. 2009. The comparison of three β-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Sci. 81(3): 557-564.

Strydom, P. E., M. Hope-Jones, L. Frylinck, and E. C. Webb. 2011. The effects of a beta-agonist treatment, vitamin D 3 supplementation and electrical stimulation on meat quality of feedlot steers. Meat Sci. 89(4): 462-468.

Van Donkersgoed, J., J. Berg, G. Royan, J. Hutcheson, and M. Brown. 2014. Comparative effects of zilpaterol hydrochloride and ractopamine hydrochloride on growth performance, carcass characteristics, and longissimus tenderness of feedlot steers fed barley-based diets. The Professional Anim. Scientist. 30(1): 56-61.

Vasconcelos, J., R. Rathmann, R. Reuter, J. Leibovich, J. McMeniman, K. Hales, T. Covey, M. Miller, W. Nichols, and M. Galyean. 2008. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing diet on feedlot cattle performance and carcass traits. J. Anim. Sci. 86(8): 2005-2015

Chapter 5:

The effect of r-salbutamol on growth, feed efficiency, carcass characteristics and meat quality of Merino feedlot lambs

5.1 Abstract

The effect the beta agonist r-salbutamol (S) has on growth, feed efficiency and carcass characteristics of late maturing Merino feedlot lambs was evaluated. Fifty six lambs were housed indoors at Welgevallen experimental farm, Stellenbosch. Four treatments were fed (14 animals per treatment), a control or one of three concentrations of r-salbutamol (S). The control received Meadow complete sheep finisher® pellets and the three treatment groups received the same finisher pellets but with 110mg S/kg, 135mg S/kg and 160 mg S/kg feed, respectively. The animals were blocked and randomly assigned to treatments according to starting weight before the onset of the trial. The trial ran for 28 days of which animals had *ad libitum* access to feed and quality water. After the trial, lambs were slaughtered where individual weights of offal and primal cuts were determined. A sample of the *longissimus dorsi* muscle was taken for physical measurements such as, pH, cooking loss and shear force. A 3-rib cut of the right side of each animal was taken to determine *longissimus* muscle area and fat thickness. It was also used to determine the ratio of muscle to fat to bone of each animal. No sensory analysis was done as was the case in Chapter 4. No differences in growth or carcass characteristics were observed and only a few minor differences were observed between offal and primal cuts from different treatments. R-salbutamol had no significant effect on toughness of meat. The most pronounced effect r-salbutamol had on Merino lambs was the increase in percentage muscle and the lack of difference in percentage fat of the 160mg S/kg group. This suggests that r-salbutamol was effective in muscle metabolism but not as effective in fat metabolism. However, results are conflicting as subcutaneous fat thickness was reduced for the 160 mg S/kg group compared to the control. Lipid metabolism is thus also involved but is not as pronounced as muscle metabolism.

5.2 Introduction

Feedlot production systems in South Africa prefer medium maturing dual-purpose breeds such as Dormer and Dohne Merino because of their better growth and younger slaughter age (Beukes *et al*., 2013). However, the Merino which is late maturing breed, accounts for almost half of the sheep population in South Africa as reported by Champher *et al.,* (1998) and cited by Van der Westhuizen (2010). The Merino thus plays an important role in meat production of feedlot systems. Being a late maturing breed, the Merino has a higher dressing percentage and lower fat content when slaughtered at a younger age, making it more acceptable to the modern consumer (Beukes *et al*., 2013). Merino sheep can also enter the feedlot at an older age due to their late maturity. Animals with a higher age

tend to show better response to beta-agonists (Fiems, 1987; Liu *et al*., 1981). In the earlier study with r-salbutamol on Dohne Merino lambs (Chapter 4), the lower age of animals (4 months vs 6 months) seemed to result in reduced effects of the beta-agonist r-salbutamol. With beta-agonists playing a major role in South African feedlot systems due to their ability to improve growth, feed efficiency and carcass characteristics, it is important to evaluate the effects of beta-agonists on Merino sheep (Vasconcelos *et al.,* 2008; Elam *et al*., 2009; Montgomery *et al.,* 2009; Lopez-Carlos *et al.,* 2010; Salinas-Chavira *et al*., 2006). The aim of this study was thus to test the effect the beta-agonist r-salbutamol has on growth, feed efficiency and carcass characteristics of the late maturing Merino breed.

5.3 Materials and methods

5.3.1 Animals

A total of 65 castrated Merino lambs with an average age of eight months were obtained from the Swellendam area in the Western Cape. Animals with a weight range of 35-40 kilograms were selected from a larger flock and housed in the same pens on Welgevallen experimental farm as in the previous trial (Chapter 4). All lambs were shorn to improve feedlot performance. The protocol and methods used in Chapter 4 were repeated for this trial, with 56 from the 65 lambs blocked according to weight and divided into individual pens prior to commencement of the trial.

5.3.2 Experimental design and treatments

Although the trial had the same experimental design as in Chapter 4; it differed in that the wethers were randomly divided into individual pens for the first 14 days where after they were blocked to be assigned to treatments for the start of the feeding trial (rather than being blocked 14 days before the start of the trial). This was done to limit live weight differences between the lambs when the animals started receiving the experimental diets. Treatment diets again consisted of Meadow® complete sheep finisher pellets with 0, 110, 135, and 160 mg S/kg feed (detail of feed composition, etc. in Chapter 4). Treatments were also allocated as in the previous trial.

All data collection as pertaining to feed intake, performance, carcass measurements, etc. were similar to that described in Chapter 4 with carcasses also being classified according to South African legislation (Agricultural Product Standards Act, 1990. Act No.119 of 1990). Fifty one of the lambs received an A2 classification and five reached an A3 classification (Table 5.1).

Table 5.1 Carcass classification according to South African legislation of the carcasses from each treatment

Classification	Treatment

	Control n=14	110 mg S/kg n=14	135 mg S/kg n=14	160 mg S/kg n=14
A2	13	13	13	12
A3	1	1	1	2

Only physical measurements however, were done on the *Longissimus Dorsi* (LD) muscle and no sensory analysis.

The same statistical analyses were also performed as described previously.

5.4 Results and discussion

5.4.1 Feedlot performance: Average daily gain, Dry matter intake and Feed conversion ratio

In Table 5.2 the growth data is summarised for the 28 day feeding period as in the previous chapter (Chapter 4) but for Merino sheep. No significant (P> 0.05) differences were observed for any of the growth parameters in Merinos whilst the 160 mg S/kg feed fed to Dohne Merinos had a better FCR (Table 4.5).

Table 5.2 LSMeans ± SD of dry matter intake (DM intake), average daily gain (ADG), average daily gain calculated from the slope (ADG from slope), feed conversion ratio (FCR) and feed conversion ratio calculated from the slope (FCR from slope) of the four treatment diets containing different concentrations of r-salbutamol.

Parameter measured	Treatment				P-value
	Control (n=14)	110 mg S/kg (n=14)	135 mg S/kg (n=14)	160 mg S/kg (n=14)	
DM intake (kg/day)	1.62 ± 0.21	1.63 ± 0.20	1.68 ± 0.15	1.62 ± 0.17	0.22
ADG (kg)	0.21 ± 0.06	0.21 ± 0.06	0.21 ± 0.06	0.22 ± 0.08	0.65
ADG from slope (kg)	0.21 ± 0.06	0.21 ± 0.06	0.21 ± 0.06	0.22 ± 0.09	0.67
FCR (kg)	8.31 ± 1.93	8.21 ± 1.93	8.72 ± 2.24	8.48 ± 2.92	0.13
FCR from slope (kg)	8.33 ± 2.01	8.28 ± 1.89	8.67 ± 2.18	8.50 ± 3.12	0.19

As found in Dohne Merinos (Chapter 4), dry matter intake and average daily gain was unaffected by r-salbutamol. However, differences were found between Dohne Merinos and Merinos when comparing FCR. In this study Merinos showed no significant differences between treatments while Dohne Merinos showed a difference in FCR between the control and 160 mg S/kg for FCR over time and for FCR calculated from the slope, the control differed from both the 135 mg S/kg and the 160mg S/kg.

Previous studies also suggest no effect on intake but improvements in ADG and FCR when cattle and sheep are supplemented with beta-agonists (Vasconcelos *et al.,* 2008; Montgomery *et al.,* 2009; Elam *et al.,* 2009; Lopez-Carlos *et al.,* 2010; Salinas-Chavira *et al*., 2006). However, Steenekamp (2014) found no differences in FCR with both r-salbutamol and zilpaterol hydrochloride when fed to feedlot bulls.

5.4.2 Feed Conversion Ratio and Average Daily Gain calculations with mg r-salbutamol intake as main effect

Actual intake of the active ingredient r-salbutamol was calculated as in Chapter 4 with the control group taken as zero r-salbutamol and for the other 3 groups, the total intake in mg was classified as low with a range of 3926.23<x<5688.98mg intake of r-salbutamol, medium with a range of 5745.85<x<6848.28mg intake of r-salbutamol and high with a range of 6859.88<x<8682.40mg intake of r-salbutamol. Data were analysed as in Chapter 4 (Table 4.6).

Table 5.3 Summary statistics (LSMeans ± SD) of average daily gain (ADG) and feed conversion ratio (FCR) calculations with actual r-salbutamol intake as main effect.

Parameter measured	Treatment				P-value
	Zero (n=14)	Low (n=14)	Medium (n=14)	High (n=14)	
ADG (kg/day)	0.18 ± 0.22	0.22 ± 0.12	0.21 ± 0.23	0.17 ± 0.19	0.92
FCR (kg feed/kg weight gain)	7.02 ± 5.91	6.74 ± 4.80	6.28 ± 7.44	5.72 ± 8.13	0.96

No significant differences were found between the four groups of different intake levels of r-salbutamol. FCR decreased as r-salbutamol increased but it was non-significant. This is mainly due to the large variation between lambs within the four groups. In Chapter 4, Dohne Merinos had similar results with no differences observed between treatments. However, other authors suggest increased ADG and FCR with beta-agonist supplementation in lambs as discussed in Chapter 4 (Lopez-Carlos *et al.,* 2010; Salinas-Chavira *et al*., 2006).

5.4.3 Carcass and slaughtering data

The carcass and slaughtering data of the four treatments which except for cold carcass mass, were collected on the day of slaughter, is summarised in Table 5.4. Live weight, warm carcass mass, cold carcass mass, dressing out % and pH_{45} did not differ (P> 0.05) between treatment groups with varying concentrations of r-salbutamol.

Table 5.4 LSMeans ± SD of live weight at slaughter, warm carcass mass, cold carcass mass, dressing out percentage and carcass pH_{45} (45 minutes post mortem) of the four treatment groups.

Parameters measured	Treatment				P-value
	Control (N=14)	110 mg S/kg (N=14)	135 mg S/kg (N=14)	160 mg S/kg (N=14)	
Live weight before slaughter (kg)	44.04 ± 3.51	44.07 ± 3.44	44.93 ± 2.50	43.93 ± 3.29	0.56
Warm Carcass mass (kg)	23.83 ± 1.66	23.76 ± 1.68	23.38 ± 1.60	23.82 ± 2.02	0.83
Cold Carcass mass (kg)	23.44 ± 1.64	23.43 ± 1.72	23.00 ± 1.56	23.47 ± 1.98	0.77
Dressing out percentage	54.2 ± 2.74	54.0 ± 2.59	52.1 ± 3.71	54.3 ± 3.02	0.22
pH_{45}	6.47 ± 0.59	6.59 ± 0.79	6.51 ± 0.78	6.31 ± 0.61	0.75

Results are similar to that in Chapter 4; R-salbutamol had no-effect on carcass parameters in Merinos as was also observed in Dohne Merinos (Table 4.7). Normally, beta-agonist supplementation in sheep and cattle result in higher dressing out percentages and heavier carcass mass (Moody *et al*., 2000; Lopez-Carlos *et al.,* 2010). However, results in sheep are inconsistent as described in Chapter 4 (Felix *et al.,* 2005; Robles-Estrada *et al*., 2009). R-salbutamol also had no effect on carcass characteristics when fed to feedlot bulls (Steenekamp, 2014). However, improved warm carcass mass and dressing out percentage was observed with r-salbutamol supplementation in pigs (Marchant-Forde *et al.,* 2012).

5.4.4 Data of individual cuts and organs for determination of muscle distribution in the carcass

The data of individual body parts and organs collected on the day of slaughter are summarised in Table 5.5. All values are expressed as a percentage of live weight as determined immediately before slaughter. In Table 5.6 the individual or primal cuts taken 24 hours after slaughter are summarised.

Table 5.5 LSMeans (± SD) of organs and individual body parts expressed as a percentage of live weight to wethers fed various concentrations of r-salbutamol.

Parameters measured (%)*	Treatment				P-value
	Control (N=14)	110 mg S/kg (N=14)	135 mg S/kg (N=14)	160 mg S/kg (N=14)	
Head	5.54^{ab} ± 0.38	5.35^{ab} ± 0.46	5.28^{b} ± 0.35	5.60^{a} ± 0.43	0.05
Feet	2.66 ± 0.20	2.64 ± 0.21	2.60 ± 0.20	2.68 ± 0.24	0.74
Skin	10.10 ± 0.83	9.59 ± 0.71	9.63 ± 0.97	9.93 ± 0.72	0.28
Pluck	5.01^{a} ± 0.18	4.70^{b} ± 0.20	5.02^{a} ± 0.23	4.86^{ab} ± 0.30	<0.01
Intestines	19.20 ± 1.31	18.52 ± 1.34	18.37 ± 1.88	18.59 ± 1.76	0.55
Omental fat	1.58 ± 0.37	1.81 ± 0.40	1.62 ± 0.40	1.56 ± 0.45	0.37

^{a,b} means in a row with different superscript letters differ (P≤ 0.05).

* The values are expressed as a percentage of the live weight.

Again the head and pluck differed between treatments as in Chapter 4 (Table 4.8). However, the percentage omental fat showed no significant differences. Dohne Merinos had a significantly larger percentage omental fat for the group with the highest concentration of r-salbutamol. This is in contrast with other studies suggesting a reduction in carcass fat with beta-agonist administration. In other studies it was found that beta-agonists such as zilpaterol hydrochloride has no effect on non-carcass components in sheep when expressed as a percentage of live weight (Li *et al.*, 2000; Aguilera-Soto *et al*., 2008). This is due to beta-agonists having a low hypertrophic effect on visceral organs and a higher effect on skeletal muscle (Li *et al.*, 2000).

Table 5.6 LSMeans ± SD of individual or primal cuts of Merino wethers fed the different concentrations of r-salbutamol.

Parameters measured (%)*	Treatment				P-value
	Control (N=14)	110 mg S/kg (N=14)	135 mg S/kg (N=14)	160 mg S/kg (N=14)	
Neck	3.58 ± 0.35	3.64 ± 0.38	3.73 ± 0.43	3.64 ± 0.33	0.77
Shoulders	16.71 ± 1.03	17.05 ± 1.03	17.15 ± 0.82	17.24 ± 1.14	0.56
Forelegs	11.68 ± 0.76	12.29 ± 1.21	12.46 ± 1.00	12.11 ± 1.19	0.30
Ribs	10.55^{a} ± 0.95	10.04^{ab} ± 0.55	9.92^{b} ± 0.87	10.03^{ab} ± 0.57	0.03
Back	18.81 ± 1.19	19.61 ± 0.85	18.82 ± 1.21	19.20 ± 0.88	0.15
Hind legs	31.71 ± 0.95	31.58 ± 0.98	32.10 ± 0.93	32.12 ± 1.04	0.36
Shins	2.29 ± 0.17	2.43 ± 0.31	2.51 ± 0.40	2.42 ± 0.35	0.36
Kidneys	0.56^{b} ± 0.05	0.58^{ab} ± 0.03	0.61^{a} ± 0.06	0.56^{b} ± 0.04	0.04
Kidney fat	2.74^{ab} ± 0.79	2.92^{a} ± 0.67	2.50^{ab} ± 0.61	2.28^{b} ± 0.73	0.05

^{a,b} means in a row with different superscript letters differ (P≤ 0.05).

* Values expressed as a percentage of cold carcass weight

The only parameters showing significant differences are % kidneys, % kidney fat and % ribs. Parameters such as % shoulders, % fore legs, % hind legs, % neck, % back and % shins showed no significant differences between treatment groups. The percentage kidney fat was significantly higher for the group with the lowest concentration of r-salbutamol compared to the highest concentration of r-salbutamol. However, the largest concentration does not differ from the control. In Dohne Merinos, kidney fat also did not differ between different treatments of r-salbutamol (Table 4.9). This further suggests that r-salbutamol may not decrease fat in the carcass as other beta-agonists do. Zilpaterol hydrochloride increased hind quarter percentage and decreased forequarter percentage in a study on feedlot lambs (Macias-cruz *et al*., 2010). As on Dohne Merinos in Chapter 4, r-salbutamol showed no effect on the hindquarter percentages but also showed no decrease in forequarter percentages in Merino lambs. Zilpaterol hydrochloride further had no effect on the loin section when fed to feedlot lambs (Macias-cruz *et al*., 2010). Similar results were found in Dohne Merinos (Table 4.9) and now also in Merinos.

5.4.5 Image analysis of eye muscle area and fat thickness

Table 5.7 Summary statistics (LSMeans ± SD) for image analysis of eye muscle (*m. longissimus muscle*) area and subcutaneous fat thickness (in cm) of the 3-rib cut. No significant differences were found in eye muscle area but fat thickness however, did show significant differences.

Parameters measured	Treatment				P-value
	Control (n=14)	110 mg S/kg (n=14)	135 mg S/kg (n=14)	160 mg S/kg (n=13)	
Eye muscle area (cm^2)	25.10 ± 4.28	23.80 ± 3.98	23.71 ± 2.99	23.36 ± 4.91	0.77
Fat thickness (cm)	0.84^{a} ± 0.28	0.75^{ab} ± 0.15	0.74^{ab} ± 0.19	0.66^{b} ± 0.15	0.03

^{a,b} means in a row with different superscript letters differ (P≤ 0.05).

Subcutaneous fat depth became thinner as the amount of r-sulbutamol consumed increased. In Dohne Merinos no significant effects was observed with neither eye muscle area nor fat thickness. Authors suggest that beta-agonists increases eye muscle area in feedlot lambs which contradicts the current results (Lopez-Carlos *et al*., 2010; Macias-Cruz *et al*., 2010). However, in Merinos r-salbutamol decreases fat thickness. This is in accordance with results from other beta-agonists and also r-salbutamol in bulls, but contradicts results with r-salbutamol in Dohne Merino lambs (Table 4.10). This may be a result the fact the Merino sheep were older and more energy would normally be partitioned towards subcutaneous fat deposition.

5.4.6 Carcass composition (percentage muscle to fat to bone)

In Table 5.8 the percentage dissected muscle to fat to bone of the 3-rib cut is summarised for determination of carcass composition.

Only the percentage muscle showed differences (P≤ 0.05) between treatments. In Dohne Merinos (Table 4.11), percentage muscle and percentage fat were highest for the group with the highest concentration of r-salbutamol (160mg S/kg; Table 4.9), percentage bone was lowest. In Merinos the percentage muscle was also significantly higher than the control for both the 160mg S/kg and the 135 mg S/kg. This is in accordance with other studies suggesting an increase in muscularity with beta-agonist supplementation (Lopez-Carlos *et al.,* 2010). R-salbutamol when compared to zilpaterol hydrochloride in feedlot bulls, had larger percentages muscle than the control as well as zilpaterol hydrochloride treated bulls (Steenekamp, 2014). However, no significant differences were observed for either the percentage fat or percentage bone as was seen with Dohne Merinos. This again contradicts other studies suggesting a reduction in carcass fat, resulting in a leaner carcass (Rikhardsson *et al*., 1991; Salinas-Chavira *et al.,* 2005).

Table 5.8 Summary statistics (LSMeans ± SD) of carcass composition (percentage muscle to bone to fat) done on the 3-rib cut.

Parameter Measured (%)*	Treatment				P-value
	Control (n=14)	110 mg S/kg (n=14)	135 mg S/kg (n=14)	160 mg S/kg (n=13)	
Percentage muscle	35.91^{b} ± 3.01	38.16^{a} ± 3.13	36.93^{ab} ± 2.07	38.31^{a} ± 3.10	0.05
Percentage fat	41.02 ± 5.19	39.67 ± 4.69	39.97 ± 2.94	39.37 ± 5.16	0.72
Percentage bone	23.07 ± 2.74	22.17 ± 3.79	23.10 ± 2.89	22.32 ± 3.19	0.52

^{a,b} means in a row with different superscript letters differ (P≤ 0.05).

* calculated as % of 9th-12th 3-rib cut's weight

5.4.7 Physical measurements

The pH values of an uncooked sample of the *longissimus dorsi* muscle, the percentage cooking loss, and the average shear force value are summarised in Table 5.9.

Table 5.9 LSMeans ± SD of pH before cooking, percentage cooking loss and average shear force values

Parameters measured	Treatment				P-value
	Control (n=14)	110 mg S/kg (n=14)	135 mg S/kg (n=14)	160 mg S/kg (n=14)	
pH Raw	5.61 ± 0.07	5.61 ± 0.04	5.63 ± 0.08	5.63 ± 0.08	0.88
% Cooking loss	32.05 ± 6.56	30.35 ± 5.37	30.73 ± 8.19	28.92 ± 8.19	0.75
Instron (N)	23.49 ± 4.93	24.70 ± 5.89	24.57 ± 4.84	23.42 ± 5.03	0.87

No significant differences (P> 0.05) between treatments were observed with the raw pH and the % cooking loss of the 56 samples. As also observed in the Dohne Merinos (Table 4.12), average shear force values between treatments did not differ significantly in Merinos. This further suggests that r-salbutamol does not have a significant effect on shear force values of meat indicating no increase in toughness as has been reported for other beta-agonists (Montgomery *et al*., 2009; Elam *et al.,* 2009). Zilpaterol hydrochloride being a beta$_2$-agonist has a more pronounced effect on muscle metabolism and results in bigger muscle fibres which makes it tougher (Dunshea *et al.*, 2005; Leheska *et al*., 2009; Strydom *et al*., 2009). R-salbutamol seems to have a less aggressive effect on muscle metabolism than

zilpaterol hydrochloride as R-salbutamol treated bulls also showed no effect on shear force of meat compared to control bulls and had significantly lower shear force values compared to zilpaterol treated bulls (Steenekamp, 2014).

5.5 Conclusion

In this study with Merinos, no significant differences were found in growth, feed efficiency and carcass characteristics with r-salbutamol supplementation. With offal and primal cut weights, minimal differences were found. The concentration of r-salbutamol that seemed to perform best is the 160 mg S/kg group as seen with Dohne Merinos in Chapter 4. This group exhibited a higher percentage of carcass muscle in this study, suggesting that r-salbutamol may be effective in skeletal muscle metabolism (P-value of 0.05). Although fat seemed unaffected in the kidneys and abdominal area, subcutaneous fat thickness was reduced in the 160 mg S/kg group. Different r-salbutamol concentrations had no effect on shear force of meat. Meat tenderness is thus unaffected by r-salbutamol supplementation.

5.6 References

Aguilera-Soto, J., R. Ramirez, C. Arechiga, F. Mendez-Llorente, M. Lopez-Carlos, J. Silva-Ramos, R. Rincon-Delgado, and F. Duran-Roldan. 2008. Zilpaterol hydrochloride on performance and sperm quality of lambs fed wet brewers grains. J. Appl. Anim. Res. 34(1): 17-21.

Beukes, J. A. 2013. Maize Silage Based Diets for Feedlot Finishing of Merino Lambs. Master's Thesis. Faculty of AgriScience. University of Stellenbosch. South Africa.

Dunshea, F., D. D'souza, D. Pethick, G. Harper, and R. Warner. 2005. Effects of dietary factors and other metabolic modifiers on quality and nutritional value of meat. Meat Sci. 71(1): 8-38.

Elam, N., J. Vasconcelos, G. Hilton, D. VanOverbeke, T. Lawrence, T. Montgomery, W. Nichols, M. Streeter, J. Hutcheson, and D. Yates. 2009. Effect of zilpaterol hydrochloride duration of feeding on performance and carcass characteristics of feedlot cattle. J. Anim. Sci. 87(6): 2133-2141.

Felix, A., A. Estrada-Angulo, F. Rios, C. Ramos, and A. Perez. 2005. Effect of zilpaterol clorhidrate on growth performance and carcass traits in finishing sheep. Journal of dairy science. Vol. 88.

Fiems, L. 1987. Effect of beta-adrenergic agonists in animal production and their mode of action. Ann. Zootech. 36(3): 271.

Leheska, J., J. Montgomery, C. Krehbiel, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, J. Blanton, and M. Miller. 2009. Dietary zilpaterol hydrochloride. II. carcass composition and meat palatability of beef cattle. J. Anim. Sci. 87(4): 1384-1393.

Li, Y., R. Christopherson, B. Li, and J. Moibi. 2000. Effects of a beta-adrenergic agonist (L-644,969) on performance and carcass traits of growing lambs in a cold environment. Canadian J. Anim. Sci. 80(3): 459-465.

Liu, C., J. Boyer, and S. Mills. 1989. Acute effects of beta-adrenergic agonists on porcine adipocyte metabolism in vitro. J. Anim. Sci. 67(11): 2930-2936.

López-Carlos, M., R. Ramírez, J. Aguilera-Soto, C. Aréchiga, F. Méndez-Llorente, H. Rodríguez, and J. Silva. 2010. Effect of ractopamine hydrochloride and zilpaterol hydrochloride on growth, diet digestibility, intake and carcass characteristics of feedlot lambs. Lives. Sci. 131(1): 23-30.

Macías-Cruz, U., F. Álvarez-Valenzuela, N. Torrentera-Olivera, J. Velázquez-Morales, A. Correa-Calderón, P. Robinson, and L. Avendaño-Reyes. 2010. Effect of zilpaterol hydrochloride on feedlot performance and carcass characteristics of ewe lambs during heat-stress conditions. Anim. Prod. Sci. 50(10): 983-989.

Marchant-Forde, J., D. Lay, R. Marchant-Forde, K. McMunn, and B. Richert. 2012. The effects of r-salbutamol on growth, carcass measures, and health of finishing pigs. J. Anim. Sci. 90(11): 4081-4089.

Marchant-Forde, J., D. Lay, R. Marchant-Forde, K. McMunn, and B. Richert. 2008. The effects of r-salbutamol on behavior and physiology of finishing pigs. J. Anim. Sci. 86(11): 3110-3124.

Montgomery, J., C. Krehbiel, J. Cranston, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, D. Bechtol, E. Johnson, and T. TerHune. 2009. Dietary zilpaterol hydrochloride. I. feedlot performance and carcass traits of steers and heifers. J. Anim. Sci. 87(4): 1374-1383.

Montgomery, J., C. Krehbiel, J. Cranston, D. Yates, J. Hutcheson, W. Nichols, M. Streeter, R. Swingle, and T. Montgomery. 2009. Effects of dietary zilpaterol hydrochloride on feedlot performance and carcass characteristics of beef steers fed with and without monensin and tylosin. J. Anim. Sci. 87(3): 1013-1023.

Moody, D., D. Hancock, and D. Anderson. 2000. Phenethanolamine repartitioning agents. Farm Animal Metabolism and Nutrition. : 65-96.

National Department of Agriculture. 1990. Agricultural Product Standards Act, 1990 (Act No. 119 of 1990) No. R. 342. Regulations Regarding the Classification and Marking of Meat.

Rikhardsson, G., K. Johnson, and D. Johnson. 1991. Effects of cimaterol on energetics and carcass characteristics of Suffolk ewe lambs. J. Anim. Sci. 69(1): 396-404.

Robles-Estrada, J., A. Barreras-Serrano, G. Contreras, A. Estrada-Angulo, J. Obregón, A. Plascencia, and F. Ríos. 2009. Effect of two β-adrenergic agonists on finishing performance and carcass characteristics in lambs fed all-concentrate diets. J. Appl. Anim. Res. 36(1): 33-36.

Salinas-Chavira, J., M. Domínguez-Muñoz, R. Díaz-Martínez, P. Cruz-Bautista, M. Montaño-Gómez, and C. Arzola-Alvarez. 2006. Effect of duration of zilpaterol hydrochloride treatment on carcass characteristics and weight gain in grazing Pelibuey lambs. J. Appl. Anim. Res. 29(1): 25-28.

Salinas-Chavira, J., R. Ramírez, M. Domínguez-Muñoz, R. Palomo-Cruz, and V. López-Acuña. 2004. Influence of zilpaterol hydrochloride on growth and carcass characteristics of Pelibuey lambs. J. Appl. Anim. Res. 26(1): 13-16.

Steenekamp, S. 2014. Growth Performance and Meat Characteristics of Feedlot Cattle Fed R-Salbutamol or Zilpaterol Hydrochloride during the Finishing Period.

Strydom, P. 2011. Quality related principles of the South African beef classification system in relation to grading and classification systems of the world. S. Afr. J. Anim. Sci. 41(3): 177-193.

Strydom, P., L. Frylinck, J. Montgomery, and M. Smith. 2009. The comparison of three β-agonists for growth performance, carcass characteristics and meat quality of feedlot cattle. Meat Sci. 81(3): 557-564.

Van der Westhuizen, E. 2010. The Effect of Slaughter Age on the Lamb Characteristics of Merino, South African Mutton Merino and Dorper Lambs. Master's Thesis. University of Stellenbosch. South Africa.

Vasconcelos, J., R. Rathmann, R. Reuter, J. Leibovich, J. McMeniman, K. Hales, T. Covey, M. Miller, W. Nichols, and M. Galyean. 2008. Effects of duration of zilpaterol hydrochloride feeding and days on the finishing diet on feedlot cattle performance and carcass traits. J. Anim. Sci. 86(8): 2005-2015.

Chapter 6

General conclusion

It was hypothesized that r-salbutamol would improve digestibility, increase growth and feed efficiency and also improve carcass characteristics of feedlot lambs without the added disadvantages of increasing toughness of meat as alternative beta-agonists tend to do. The improved carcass characteristics include better dressing out percentages, increased percentage of carcass muscle and a decreased fat percentage or carcass fat. The potential increase in digestion was expected due to r-salbutamol influencing the beta-receptors on smooth muscle and also the effect it has on rumen microorganisms.

In the digestibility trial (Chapter 3) r-salbutamol had no effect on the apparent digestibility of Dohne Merino feedlot lambs. All digestibility coefficients (OMD, NDF, CP, ash and N-retention) were unaffected when supplemented with 135 mg r-salbutamol per kg of feed. Higher doses of r-salbutamol may have an effect on apparent digestibility of feedlot lambs and a concentration of at least 160 mg S/kg is suggested for further research before any recommendations are made.

In Dohne Merinos (Chapter 4), feed efficiency was improved in the group with the highest concentration of 160 mg r-salbutamol per kg of feed but no effects were observed on ADG between treatments. Carcass characteristics such as warm carcass mass, cold carcass mass and dressing out percentage did not differ between treatments. Minimal effects were observed with offal and primal cut yields, with fat in the abdominal area and around the kidneys unaffected by r-salbutamol in the highest concentration. Percentage muscle was increased while percentage bone was decreased and percentage fat unaffected in carcasses treated with the highest concentration of r-salbutamol. Subcutaneous fat thickness was also unaffected in all concentrations of r-salbutamol fed. This suggests that r-salbutamol was effective in muscle anabolism but less effective in lipid metabolism. This may be due to younger animals having lower receptor numbers. R-salbutamol also had no effect on perceived toughness as evaluated by the descriptive sensory analysis or on toughness tested mechanically by an Instron machine.

In the later maturing Merinos (Chapter 5), no differences were observed in growth (ADG), feed efficiency (FCR) and carcass characteristics such as warm carcass mass, cold carcass mass and dressing out percentage between various concentrations of r-salbutamol. The change of pharmocodynamical properties in older animals are suggested to be the reason animals did not perform as expected. Minimal effects were found in offal and primal cuts between treatments. Again the optimal concentration seemed to be the 160 mg S/kg group as seen in Dohne Merinos (Chapter 4) where a

higher percentage of carcass muscle was exhibited. Percentage fat, abdominal fat and kidney fat was once more unaffected by r-salbutamol supplementation. However, fat thickness was reduced in the group with the highest concentration of r-salbutamol. Again, R-salbutamol did not affect the tenderness (measured via shear force) of the meat, as r-sulbutamol did not increase the toughness of the meat in either the late maturing Merinos or medium maturing Dohne Merinos, it can be concluded that r-salbutamol does not increase the toughness of lamb meat. As for digestibility, further research needs to be done before recommendations can be made.