The influence of R-Salbutamol on feedlot performance, carcass characteristics and meat quality in Dorper ram, wether and ewe lambs

by

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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.

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LIST OF ABBREVIATIONS

ADF	Acid detergent fibre
NDF	Neutral detergent fibre
ANOVA	Analysis of Variance
LSMeans	Least Squares Means
SEM	Standard error of Mean
ADG	Average daily gain
FCR	Feed conversion ratio
DMI	Dry matter intake
LD	*Longissimus dorsi* muscle
SM	*Semimembranosus* muscle
pH45	pH at 45 minutes *post mortem*
pH24	pH at 24 hours *post mortem*
WCW	Warm carcass weight
CCW	Cold carcass weight
WBSF	Warner-Brazler shear force
DSA	Descriptive sensory analysis

NOTES

The language and style used in this thesis are in accordance with the requirements of the *South African Journal of Animal Science*. This thesis represents a compilation of manuscripts, where each chapter is an individual entity and some repetition between chapters is therefore unavoidable.

Parts of the thesis were presented as posters at a national congress:

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CHAPTER 1

General Introduction

Sheep farming has been successfully practised across South Africa with the vast majority of these production systems being concentrated around arid parts of the country. This portion of the livestock sector is made up of an estimated 8000 commercial producers and 5800 communal farmers, which account for a total of approximately 24 million sheep (Department of Agriculture Forestry and Fisheries, 2016). Sheep numbers have decreased over the last decade and the price of mutton has doubled in the same period. This, in combination with a higher demand for meat products, warrants intensive rearing and finishing of sheep for the consumer market (DAFF, 2013). The main sheep breeds that are suitable for intensive rearing and finishing in feedlots include Merino types such as the Dohne Merino, South African Mutton Merino, Merino, and non-woolen types such as the Dormer and Dorper (van der Westhuizen, 2010; Cloete *et al.,* 2012b). The Dorper breed contributes a significant percentage to the amount of meat produced annually and is well known as a hardy and extensive, free-range adapted breed but also performs well in intensive feedlot systems (Cloete et al., 2000). Aside from breed, the sex of an animal will also be an important consideration that has to be taken into account, when designing a farming enterprise with specific product standards in sight. Frequently encountered sexes in feedlot systems are rams (entire males), ewes (entire females) and wethers (castrated males), each with different production and meat quality traits (Field, 1971; Crouse et al., 1981; Seideman et al., 1982; Arnold & Meyer, 1988; Dransfield et al., 1990; Vergara et al., 1999). To further improve or manipulate the production figures of these sexes, the producer can incorporate the use of growth agents such as beta-agonists.

Beta-agonists belong to the catecholamine group of compounds and are produced naturally in a healthy mammalian body (Hossner, 2005a). These naturally occurring compounds play a crucial role in the 'fight or flight' syndrome where it takes responsibility for energy release during a period of acute stress experienced by the individual (Cannon & De La Paz, 1911). During this response the body will deploy various tactics that will result in a rapid mobilisation of the internal energy reserves and transport it to the specific site where it is needed (Mersmann, 2002; Hossner, 2005b; Chung et al., 2015). Effects due to administration of synthetically produced beta-agonists such as zilpaterol hydrochloride and ractopamine hydrochloride will invariably differ due to various factors such as treatment dosage, duration of treatment, type of beta-agonist, breed, specie and sex (Mersmann, 1998). Fortunately, beta-agonists have been widely reported to induce a broad range of positive effects on muscle tissue and fat depots (Moody et al., 2000). This molecule will ultimately

reduce the amount of adipose tissue by either increasing fat breakdown or decreasing fat synthesis and improve protein accretion by decreasing protein breakdown and increasing protein accretion thereof (Mersmann, 1998). Previous research indicate that current beta-agonist are capable of increasing production performance, improve carcass composition and do so without negatively effecting the treated animal or consumer of the meat (Carr et al., 2005; Avendaño-Reyes et al., 2006; Apple et al., 2007; Estrada-Angulo et al., 2008; Brooks et al., 2009; Leheska et al., 2009; López-Carlos et al., 2010).

R-salbutamol is a new beta-agonist that has recently been developed and isolated. The latest research suggest that R-salbutamol can also improve production performance in pigs, but without the added negative effect of increasing the toughness of meat produced, the latter being an attribute that was also previously associated with beta-agonists (Marchant-Forde et al., 2012b; Steenekamp, 2014). The main theory that describes the mechanism responsible for this meat toughening is that beta-agonists can influence the activity of specific proteolytic enzymes present in the calpain system. The calpain system is responsible for the turnover of myofibril proteins (natural protein degradation), which is a major group of proteins in striated muscles, and where calpastatin functions as a calpain inhibitor (Goll *et al.,* 1992). Therefore, with beta-agonists having been found to increase the activity of calpastatin and as a result decreasing the calpain activity, it can be expected that meat toughness will increase (Mersmann, 1998; Strydom et al., 2009, 2011).

Currently there is a substantial body of literature on the effect that sex and beta-agonist administration has on lamb meat production, which include feedlot performance, carcass composition and conformation as well as meat quality traits. However, no literature has been published investigating the effect that R-salbutamol has on these aforementioned parameters. This leads to the research question of: at which inclusion level would R-salbutamol improve production performance, carcass characteristics and meat quality traits in Dorper ram, wether and ewe lamb?

The research hypothesis are as follows:

H_0: The inclusion of R-salbutamol to the finishing diets of Dorper ram, wether and ewe lambs will have no effect on their growth, carcass characteristics and meat quality characteristics.

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CHAPTER 2

Literature Review

2.1   Lamb Production in South Africa

It is the responsibility of the Animal Scientist to develop sustainable, effective and ethically acceptable methods of producing, as well as supplying meat to the worlds' growing population. In 2012 the Food and Agricultural Organization (FAO) predicted that the global agricultural sector should increase production by 60 % before 2050 to cope with the expected 39 % increase in world population numbers (Prakash, A. & Stigler, 2012). Globally, there has been a shift in consumer diets which consist of a higher proportion of meat (increase 2.7 percent per annum) and dairy products (increase 3.5 – 4.0 percent per annum) which has driven these sectors to increase production efficiency and product quality (Prakash, A. & Stigler, 2012).

Production efficiency is just as important in South Africa where food security is an additional issue that constantly requires the attention of producers. The livestock sector is furthermore faced with the challenge that large regions of South Africa are defined as desert or semi-desert climates and as a result these areas are predominantly suitable for sheep farming (Brand, 2000). According to the Department of Agriculture, Forestry and Fisheries of South Africa, the national sheep population size is approximately 24 million. This is nearly two-fold the size of the national cattle population and four-fold that of goats. Fortunately, for the small stock producer the price of mutton has increased more than two-fold in the last decade warranting intensive rearing and finishing of sheep for the consumer market (DAFF, 2013). The main sheep breeds that are suitable for intensive rearing and finishing in feedlots include Merino types such as the Dohne Merino, South African Mutton Merino, Merino, and non-woollen types such as the Dormer and Dorper (van der Westhuizen, 2010; Cloete *et al.,* 2012b).

2.1.1   Production challenges using feedlot finishing

It is of paramount importance that the agricultural sector increase production efficiency to combat a decline in available resources such as usable water and animal feed raw materials in combination with the higher demand for food. To achieve this, the producer has to look into increasing production per unit of land by utilizing feedlot systems for finishing of animals for slaughter. Intensification allows the producers to increase stocking density, protect pasture health of the rest of the production system whilst supplying the animals with good quality feed in the feedlot, and limit predation as well as stock theft (Webb, 2013). These intensive systems therefore allow control over most factors that are normally associated with extensive

production systems that may have a detrimental effect on the animal's growth and product quality. Feedlot finishing provides additional advantages, such as a higher growth rate, more consistent carcass weight and carcass quality with an improved dressing percentage (Cloete et al., 2012a; Webb & Erasmus, 2013a).

However, commonly experienced problems in these systems include animals that deposit fat too quickly and not enough muscle, resulting in a carcass downgrade at the abattoir and lower prices for the producer. With high feeding costs it is important that the feed given is utilized efficiently and nutrients are being partitioned towards muscle growth so that heavier animals are produced and deemed ready for slaughter at an younger age (Miller *et al.,* 1988; Brooks *et al.,* 2009). Producers can achieve this objective by the use of growth agents. These growth agents are capable of increasing feed usage efficiency, producing heavier animals with a more ideal carcass composition (Strydom, 2016).

Various maturity type breeds are found in feedlots across South Africa, grouped according to their rate of physiological development. Typically encountered feedlot breeds can be grouped into the three maturity types: Dorper and Dormer (Early maturing); Merino and Dohne Merino (Intermediate maturing); and South African Mutton Merino (Late maturing) (Lawrie, 1998; van der Westhuizen, 2010; Cloete *et al.,* 2012b). Early maturing breeds will start putting on more localized fat and reach maximum potential for fat deposition at an earlier physiological age compared to intermediate- and late maturing breeds (Lawrie, 1998). The window for optimal slaughter fatness is thus smaller in this type of breed. They will also reach their mature weight at an earlier physiological age compared to intermediate- and late maturing breeds. Early maturing breeds will compare to intermediate maturing breeds in a similar manner with which intermediate maturing breeds will compare to late maturing breeds (Lawrie, 1998; Cloete *et al.,* 2004). The Dorper breed is widely distributed in South African feedlots with a significant amount of the commercially produced meat being of Dorper origin (DAFF, 2013). Seeing that the Dorper classifies as an early maturing breed, it exhibits characteristics that can potentially be negative in a feedlot such as early fat deposition. Thus, the administration of a beta agonist such as R-Salbutamol to a feedlot diet of early maturing breeds such as Dorpers may have a value-added effect on production performance due to its ability to repartition nutrients and have a delaying effect on fat deposition (Steenekamp, 2014). Including a beta agonist increases the input cost per animal; so its use will be motivated if the value of the carcass exceeds the input costs. Furthermore, it is important to produce a product according to consumer specifications and recently there has been a health conscious shift towards leaner meat.

2.1.2 Gender effect on feedlot performance and meat quality characteristics

Aside from breed, nutrition and environment, gender is another factor that can have a major effect on growth and meat quality of the animal. Gender is a factor that cannot be changed mid production but can; however, be altered at an earlier time through the process of castration. Castrated individuals exhibit certain characteristics that differ from those associated with intact males and intact females (Field, 1971). Commonly encountered genders in South African feedlots are intact males (rams), intact females (ewes) and castrated males (wethers). Sex hormones play a major role in the growth rate and growth pattern regulation of lambs. Therefore, with each of the aforementioned genders having different levels of various types of circulating sex hormones, differences in production performance and meat quality traits can be expected (Hossner, 2005a). Retaining rams over wethers or ewes has advantages such as faster growth rates, and heavier animals that adhere to carcass characteristics standards. In terms of growth, rams perform better, followed by wethers and finally ewes (Field, 1971; Crouse *et al.,* 1981; Lee, 1986; Cloete *et al.,* 2012a), mainly attributed to the gender's ability to utilize feed better (Lee *et al.,* 1990; Notter *et al.,* 1991; Rodríguez *et al.,* 2011; Craigie *et al.,* 2012). It has also been noted that a gender by nutrition interaction exists. In both extensive free range systems and intensive feedlot systems the rams outperform wethers and ewes but in the feedlot system, where a higher quality feed is supplied, the rams outperform the other two genders by a larger margin (Field, 1971; Crouse *et al.,* 1981; Cloete *et al.,* 2012a). The different levels of testosterone and growth hormone present in the three genders can further explain the differences observed in average daily gain (ADG) and feed conversion ratio (FCR) (Hossner, 2005a). The difference in growth rates and feed usage will have an effect on the fatness of the carcass produced. Butterfield, (1988) postulated that difference in fat between genders was a result of differences in repartitioning of nutrients. The study concluded that rams had less subcutaneous fat but more intramuscular and mesenteric fat compared to ewes and wethers. Surprisingly, no differences were found for total fat weights between genders. In contradiction to this, Afonso & Thompson (1996) found that gender does not affect fat distribution throughout the body fat depots. The majority of research consulted seem to; however, conclude that that rams produce the leanest carcasses followed by wethers and the fattest from ewes (Crouse *et al.,* 1981; Dransfield *et al.,* 1990; Cloete *et al.,* 2007, 2012a).

Further differences can be seen when evaluating slaughter characteristics; ewes generally exhibit higher dressing percentages followed by wethers with the worst performing being rams (Prescott & Lamming, 1964; Vergara *et al.,* 1999; Rodríguez *et al.,* 2011). Lee *et al.* (1990) found that wethers had a higher dressing percentage than both rams and ewes. The weight of the skin and fleece of rams would be higher because of their larger size and

due to the thicker skin associated with mature male animals. Butterfield *et al.* (1984) also note that the heavier reproductive organs would have a negative effect on the dressing percentage associated with an intact animal, although testicles do have a value as a delicacy in certain markets (Hoffman *et al.,* 2013).

There are conflicting results regarding the effect that gender has on tenderness. Some studies found that gender had no effect on meat tenderness (Lee, 1986). While Johnson *et al.* (2005) illustrated that the meat from rams is tougher than that of ewes, Hopkins *et al.* (2007) furthermore showed that the meat from wethers were tougher than that of ewes but more tender than meat originating from rams. According to Young *et al.* (2006) meat toughness in rams increases and become noticeable only after they have reached sexual maturity and this is important to note as the onset of puberty is influenced by growth rate. Craigie *et al.* (2012) evaluated Warner-Bratzler Shear Force (WBSF) values of certain muscles in the carcasses of slaughtered lambs that are important to the consumer. The muscles that were tested include *M. Longissimus dorsi* (LD) and *M. Semimembranosus* (SM).   He found that the peak force for LD muscle in rams were up to 13.3 % higher than that of ewes. These results were supported by Johnson *et al.* (2005) showing that rams produced 14.5% higher shear force values than ewes.   Wojtysiak *et al.* (2010) also found that shear force values for SM in rams were significantly higher than the shear force value for SM in ewes.

Comparing pH levels and meat tenderness between different gender groups it was found that at younger ages (below 20 months) no significant differences occur (Corbett et al., 1973; Okeudo & Moss, 2008). Generally, an increase in age is correlated with a decrease in tenderness (Hiner & Hankins, 1950). This can be explained by the fact that the connective tissue in young animals tend to have less cross-bindings and the amount thereof increase with age (Boucek *et al.,* 1961; Goll *et al.,* 1963). Furthermore, the solubility of the collagen also decrease with age and that decreases tenderness (Lawrie *et al.,* 2006). Meat tenderness is influenced on a structural and biochemical level of skeletal muscle fibres. Further structures in the muscle that plays a role in the perceived tenderness include myofibrils, intermediate filaments, intramuscular connective tissue, as well as the endo- and perimysium (Takahashi, 1996). The more these structures are subject to degradation, the more tender the meat will be and aside from the pre slaughter factors that can influence meat tenderness, the main post mortem factor that will influence this is proteolysis. Proteolysis is an enzymatic pathway responsible for protein degradation and being such it will be subject to pH changes (Belew *et al.,* 2003).

2.2   Beta-agonists

2.2.1   Use of beta-agonists and other growth promoting agents

The dairy production sector was the first to capitalise on the advantages of using growth agents. These agents are classified as compounds or molecules, that when administered can improve certain production parameters in livestock species. The first of such agents used include iodinated proteins that were fed to lactating cows to increase milk production (Preston, 1999). This opened the door for the other livestock sectors to commence using growth agents. In 1954 diethylstilboestrol (DES) was used in beef cattle and sheep production systems for the purpose of increasing not only growth rate and feed usage efficiency, but also the amount of lean meat deposition (Preston, 1999). This product showed potential but due to possible carcinogenic properties to the meat consumer, DES was banned from use in meat production in 1979 (Preston, 1999). Other growth promoting agents that were introduced to the livestock production sector include organic compounds such as ionophores, which has well known antimicrobial properties. According to McGuffey *et al.* (2001) the mode of action of this *Streptomyses* fermentation derived compound is described as facilitating the selective transportation of ions across cell membranes. This will result in a cytoplasmic acidity increase; death of the target Gram-positive bacteria cell and ultimately improved rumen conditions for the animal. Additional advantages of using ionophores include: promotion of propionate production in the rumen (David Baird *et al.,* 1980), decreasing methane production (Schelling, 1984), and having anticoccidial properties (McGuffey *et al.,* 2001). Ionophores have been extensively used in ruminant nutrition. The most commonly encountered one being Monensin, was first approved in 1971 as an anticoccidiostat and in 1975 by the Food and Drug Administration as a feed additive with growth promoting characteristics (McGuffey *et al.,* 2001), such as improving FCR and ADG and also decreasing dry matter intake (DMI) (Duffield *et al.,* 2012).

Producers have also used anabolic steroid implants as growth promoting agents. These can be divided into products containing either male (testosterone) and/or female (estrogen, progesterone) sex hormones or in some cases the derivatives of these. More than 30 countries, including South Africa, USA, Canada and Australia have registered the use of these implants but it has been prohibited in all Western European countries since 1998 (Preston, 1999). Known advantages of using this kind of agent include increasing ADG and FCR with added benefit of decreasing carcass fatness (Preston, 1999), but in some cases a negative effect on meat tenderness was reported (Dikeman, 2007).

In 1995, South Africa registered a beta-agonist (zilpaterol hydrochloride) for commercial use, sold under the trademark name of Zilmax® (MSD). Mexico (1996), USA (2006) and Canada (2009) also approved the use of zilpaterol hydrochloride in livestock production (Delmore *et al.,* 2010). Zilpaterol hydrochloride was shown to improve growth performance parameters in livestock but with the possible negative effect of increasing meat toughness (Rathmann *et al.,* 2009; Strydom *et al.,* 2009, 2011; Hope-Jones *et al.,* 2012; Strydom, 2016). Products like these lead to a significant increase in profit margins for the meat producer due to its positive effect on growth characteristics (Avendaño-Reyes *et al.,* 2006; Lopez-Carlos *et al.,* 2011). Beta-agonists is registered for commercial use in 13 countries but have never been permitted in Europe (Kuiper & Noordam, 1998).

Depicted in Table 2.1 is a summary of the various growth agents, showing when each of these were approved by the Food and Drug Administration (FDA) for use in cattle and sheep production and Table 2.2 gives an overview of some of the major growth agent's specific functions.

Table 2.1 Growth agents approved by the Food and drug administration (FDA) for use in cattle and sheep production (Preston, 1999)

Year	Growth agents approved	Specie(s)
1954	Oral DES	Cattle
1956	DES implant	Cattle
1956	Des implant	Sheep
1956	Estradiol benzoate (EB)/progesterone implant	Steers
1957	Oral DES	Sheep
1958	EB/testosterone implant	Heifers
1968	Oral melengestrol acetate (MGA)	Heifers
1969	Zeranol implant	Cattle
1969	Zeranol implant	Lambs
1970	Oral DES dose range increase	Cattle
1979	All use of DES banned	Cattle and Sheep
1982	Silicone rubber-estradiol implant	Cattle
1984	EB/progesterone implant	Calves
1987	Trenbolone acetate (TBA) implant	Cattle
1991	TBA/estradiol (5:1) implant	Steers
1993	Bovine somatotropin	Lactating dairy cows
1994	TBA/estradiol (10:1) implant	Heifers

1995	Zeranol implant dose increase	Cattle
1995	Zilpaterol hydrochloride approved	Cattle
1996	TBA/estradiol (10:1) implant	Steers
1996	Estradiol/TBA (5:1) implant	Cattle

Table 2.2 The most common growth promoting substances that are currently on the market for use in intensive production systems (adapted from National Research Council, 1994; Kirsty *et al.,* 2016)

Category	Example	Mode of action	Effect
Antimicrobial drugs	• Neomycin • Lasalocid sodium • Monensin • Salinomycin • Oxytetracycline • Chlortetracycline	Prevent the growth or kill harmful bacteria, fungi and protozoa.	• Vital for the treatment of infections and diseases in animals. • Improve growth by enhancing feed efficiency.
Hormonal growth implants based on anabolic steroids (natural and synthetic androgen, oestrogen and progestin)	• Oestradiol E2 • Oestradiol benzoate • Zeranol • Trenbolone acetate • Testosterone propionate	Increase protein accretion and decrease protein degradation rate.	• Enhance growth during the fattening phase.
Beta-adrenergic agonists as a feed additive	• Cimaterol and Clenbuterol • Zilpaterol hydrochloride for cattle and sheep • Ractopamine hydrochloride for pigs • R-Salbutamol for cattle and pigs	Repartition nutrients towards protein accretion over fat synthesis (Mersmann, 1998).	• Improve feedlot performance. • Can increase meat toughness.

2.2.2   Molecular functioning of beta-agonists

Beta-agonists are compounds belonging to the catecholamine group and produced naturally in a healthy mammalian body. Catecholamines play a crucial role in the 'fight or flight' syndrome where it takes responsibility for energy release during a period of acute stress experienced by the individual (Cannon & De La Paz, 1911).

The main catecholamines found in mammals are (Mersmann, 1998; Mersmann, 2002; Hossner, 2005):

- Epinephrine (also known as adrenaline or the adrenal medullary hormone);

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- Norepinephrine (also known as noradrenaline or the sympathetic nervous system neurotransmitter); and

- Dopamine

Illustrated in Figure 2.1 are the enzymatic pathways through which these catecholamines are synthesised from tyrosine. Hydroxylation of tyrosine produces DOPA (dihydroxyphneylalanine), which in turn can undergo decarboxylation to form dopamine. Hydroxylation of dopamine produces norepinephrine and methylation of norepinephrine finally produces epinephrine (Hossner, 2005b).
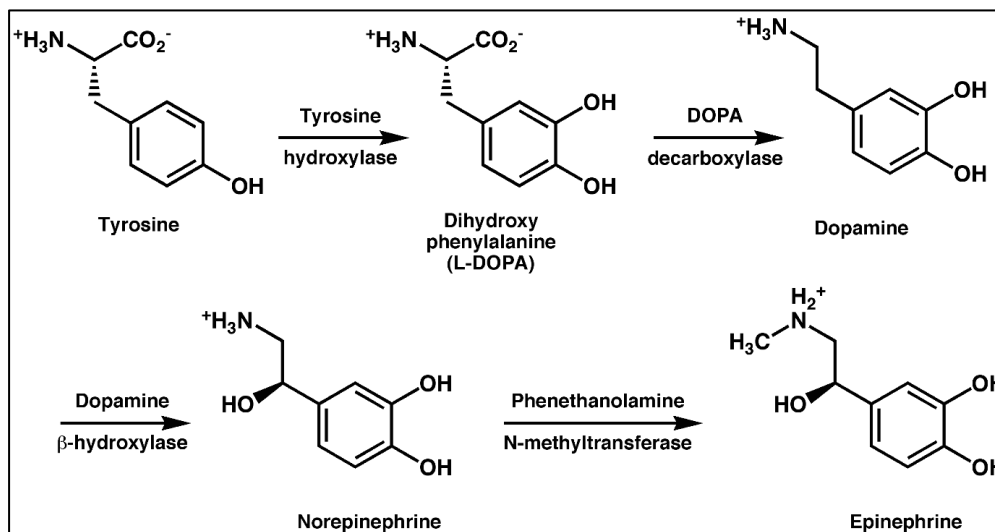
Figure 2.1 Synthesis of catecholamines from tyrosine (Adapted from Schallreuter *et al.,* 1995; Wong & Tank, 2007)

Even though catecholamines circulate in the body in very low levels, higher levels are needed to induce a response. To activate an endocrine response, a high concentration of norepinephrine is needed whereas to stimulate a beta-adrenergic response throughout the body, during a stressful episode, the preganglionic nerve from the sympathetic nervous system needs to be triggered resulting in a release of epinephrine from the adrenal medulla (Hossner, 2005b).

2.2.2.1 Mechanism of a beta-adrenergic agonists

Stimulation of a beta-adrenergic response will result in a rapid mobilisation of the body's energy reserves. The body achieves this by increasing glycogenolysis (process of transforming liver glycogen to glucose) and gluconeogenesis (process of transforming certain non-carbohydrate carbon substrates to glucose) in the liver and increasing glycogenolysis in the skeletal muscle of the animal (Cherrington et al., 1984; Chung et al., 2015). In addition, the body maintains high blood glucose levels by suppressing pancreatic insulin release and inducing glucagon secretion into the bloodstream. Furthermore, the body increases lipolysis

(degradation of lipids through the process of hydrolysis) of adipose tissue to ensure that there are sufficient amounts of free fatty acids and glycerol. These substrates can be used as a readily available energy source or additionally recycled to produce glucose through gluconeogenesis (Östman et al., 1979; Hossner, 2005b). The body deploys multiple tactics to ensure that the organs involved in the beta-adrenergic induced stress response, receive sufficient amounts of energy and oxygen. These include increasing heartrate, decreasing blood flow to the gastrointestinal tract by means of vasoconstriction, increasing blood flow to skeletal muscle as well as to the heart and brain (Mersmann, 2002; Hossner, 2005b).

2.2.2.2 Beta-adrenergic receptors

Since the 1940's it has been known that adrenergic receptors are divided into two main receptor categories namely alpha-adrenergic receptors and beta-adrenergic receptors (Mersmann, 1998). They can be found in many types of body tissues and each of the receptors are responsible for different tissue responses following adrenergic stimulation (Hossner, 2005b). Alpha-receptors' main functions are to facilitate sympathetic nervous system responses and regulate vasoconstriction as well as smooth muscle contractions. Beta-receptors on the other hand regulate smooth muscle relaxation and are generally more receptive to beta-agonists. Beta-receptors also have a higher affinity for epinephrine than norepinephrine (Hossner, 2005b).

These receptors make up part of a group of receptors that are functionally diverse and are large in their size. Their sizes vary but on average it consists of 400 amino acids that contains seven transmembrane hydrophobic domains which are attached to the cell membrane (Mersmann, 1998). According to Strader *et al.* (1989) beta receptors bind to their effector proteins by means of guanine nucleotides.

Figure 2.2 illustrates how the 7 transmembrane domains surrounds the ligand binding site. This domain is further surrounded by amino groups from adjacent domains and eight hydrophilic regions (Mersmann, 1998). Beta-adrenergic receptors have two terminal ends (Strader *et al.,* 1989), one which contains an amino group and is exposed to the extracellular surface of the cell whilst the other terminal end has a carboxyl group that is present inside the cell.

Figure 2.2 Illustration of a beta-adrenergic agonist that contain the seven transmembrane domains. The binding sites in the middle of the cylinders are linked to norepinephrine. The thick lines represent the extracellular portions and the thin lines the intracellular portions (Ostrowski *et al.,* 1992).

Figure 2.3 Model illustrating the transmembrane topology of a beta-adrenergic agonist (Strader *et al.,* 1989)

The plasma membrane is represented by a horizontal line with the portion above the line being the extracellular space and the area below the line indicative of the cytoplasm of the cell. Strader *et al.* (1989) further postulated that the third intracellular loop might be involved in G protein coupling (represented by a solid line) and the amino acid residues (represented by bold circles) with ligand interactions.

2.2.2.3 Beta-adrenergic receptor subtypes

Beta-adrenergic receptors can further be divided into three subtypes. These subtypes are known as beta$_1$, beta$_2$, and beta$_3$ receptors with each of these performing a specific role in the beta-adrenergic response (Mersmann, 1998; Hossner, 2005b) as follows:

- Beta$_1$-adrenergic receptors play an important role in lipolysis of adipose tissue and influences cardiac muscle as well as smooth muscle found in the intestines. Both epinephrine and neural norepinephrine can induce a response in this receptor subtype, which supports the idea that it is considered to be the primary adrenergic receptor of the neural system. Beta$_1$-adrenergic receptors are the most abundant of all receptor subtypes (Mersmann, 1998; Hossner, 2005b).

- Beta$_2$-adrenergic receptors have the primary function of facilitating bronchodilation and vasodilation together with liver glycogenolysis as well as relaxation of the smooth muscle in the uterine tract. It also plays a significant role in eliciting the response between beta-agonists and skeletal muscle (Mersmann, 1998, 2002; Hossner, 2005b). Hossner (2005b) further found that epinephrine has a higher affinity for beta$_2$ receptors than beta$_1$ in contrast to norepinephrine, which interacts poorly with beta$_2$ receptors.

- Beta$_3$-adrenergic receptors make up the smallest proportion of the total number of beta-adrenergic receptors present in the body and are typically found in brown and white adipose tissue. Its functions likely include lipolysis and thermogenesis (Hossner, 2005b).

According to Minneman *et al.,* (1979) these receptor subtypes are present in most tissues. This was confirmed by Mersmann (1989) and Dunshea *et al.* (2005) when they showed that a complex ratio between receptor types and receptor subtypes exists in different tissues and this could possibly explain why certain tissues display varying sensitivity to beta-agonists. Since the early 1990's it is known that the three beta-adrenergic receptor subtypes shared approximately 50 % homology in the amino acid sequences within one species and 75 % homology for a single receptor across multiple species (Strosberg, 1992; Hall *et al.,* 1993). Mersmann (2002) adapted these percentages to respectively 45 % – 60 % and higher than 70 %.

2.2.2.4 Physiological effects caused by beta-adrenergic agonists

Beta-agonists that are administered orally were developed to be similar to the natural catecholamines on the basis of chemical and pharmacological characteristics (National Research Council, 1994; Bell *et al.,* 1998). These synthesised molecules can function either as agonists or antagonists. The difference being that an antagonist will also bind to the

receptor and instead of inducing a reaction like the agonist would, it will block it (Mersmann, 2002).

It has been well documented that beta-agonists have the ability to regulate heart rate, muscle contractions and blood pressure (Beermann *et al.,* 1987; Mersmann, 1987, 2002; Eisemann *et al.,* 1988).   When a "fight" or "flight" response has been triggered, the body will increase blood flow to certain parts of the body to increase the potency of the reaction, also increasing blood pressure and elevating the heart rate (Mersmann, 1989). The primary targets of increased blood supply will be skeletal muscles and adipose tissue for improved mobility and energy supply for the individual. This increased mobility and alertness of the muscles is a result of the improved blood flow that supplied large amounts of energy and substrates for the muscles. An added effect of increased blood flow to those target tissues will be increased protein synthesis in the muscles which may also lead to large amounts of non-esterified fatty acids being removed from the affected adipose tissue (Mersmann, 1998). The favoured fat degradation will supply additional energy to the body's muscles (Zimmerli & Blum, 1990; Mersmann, 1995, 1998).

Besides increasing or decreasing blood flow to certain body tissues, beta-agonist can also trigger increased blood flow to major body parts of an animal, for example the hindquarters of sheep and cattle (Mersmann, 1998). This effect is thought to be the result of the interaction between multiple subtypes of beta-agonist receptors present in different cell types of the body (Mersmann, 1998). Beta-agonist treatment makes it possible for nutrients to be repartitioned away from lipid deposition and rather be deployed for muscle growth by increasing lipid degradation and protein synthesis rate and also decreasing fat synthesis and protein degradation rate. This will increase profitability for the producer by decreasing feeding costs and improving the leanness of the carcass that is produced (Miller *et al.,* 1988; Brooks *et al.,* 2009).

A common effect that beta-agonist supplementation brings about, is an increase in muscle mass in the treated animal (Johnson, 2004). This has been well documented in livestock production (Miller *et al.,* 1988; Vestergaard *et al.,* 1994; Strydom *et al.,* 2009; Webb & Allen, 2015). This apparent increase in muscle mass is due to the mechanism of cell hypertrophy. It can possibly be explained by one of two processes or a combination thereof; namely an increase in muscle protein synthesis or a decrease in muscle protein degradation (Mersmann, 1998; Strydom *et al.,* 2009). It has not yet been decisively proven which mechanism contributes the most to muscle mass increase but the presiding hypothesis is that protein accretion takes preference over decreasing the rate of protein degradation (Dunshea *et al.,* 2005).

Therefore, with beta-agonists having such a major influence on muscle metabolism, it is expected that meat quality can be affected. Dunshea *et al.* (2005) found a decrease in intramuscular fat and an increase in pH and drip loss. Numerous studies have also found that meat tenderness would be negatively influenced with the administration of certain beta-agonists (Strydom *et al.,* 2009; Strydom, 2016). The main theory that describes the possible mechanism responsible for this meat toughening is that beta-agonists can influence the activity of specific proteolytic enzymes present in the calpain system. The calpain system is responsible for the turnover of myofibril proteins (natural protein degradation), which is a major group of proteins in striated muscles, and calpastatin functions as a calpain inhibitor (Goll *et al.,* 1992). Then, with beta-agonists having found to increase the activity of calpastatin and as a result decreasing the calpain activity, it can be expected that meat toughness will increase (Strydom *et al.,* 2009, 2011).

With oral supplementation of a beta-agonist to the diet there is a decrease in carcass fat (Crome *et al.,* 1996; Mersmann, 2002). This can be ascribed to the process of increased lipolysis or decreased lipogenesis (Dunshea *et al.,* 2005). The way that beta-agonists can achieve this was clarified in an *in vitro* study by Mersmann (1998) where it was shown that beta-agonists stimulate adipocyte triacylglycerol degradation and additionally prevent fatty acids and triacylglycerol from being synthesised. Through the limited research that have been published on the subject of *in vivo* lipid anabolism and catabolism it can be postulated that beta-agonist supplementation will increase the plasma concentration of non-esterified fatty acids. This is a result of the activation of the adipocyte lipolytic system (Mersmann 1998; 2002).

In conclusion the rate of lipolysis will be increased while that of lipogenesis will be decreased and that a beta-adrenergic induced response will ultimately lead to a decrease in fat deposition (Mersmann, 2002). Beta-agonist supplementation may also lead to a reduction in feed intake. Ordway *et al.* (1987) postulated that this reduction in feed intake could be a result of some beta-agonists having the ability to cross over the blood brain barrier and this would affect the central nervous system of the treated animal.

2.2.3 Influence of common beta-agonists on production performance

The use of beta-agonist in livestock production have been extensively studied and examples of commonly used synthetic beta-agonists include Cimaterol, Clenbuterol, Ractopomine hydrochloride and Zilpaterol hydrochloride, as depicted in Table 2.2. To conclude this section, the limited available literature on the use of R-salbutamol will also be analysed and discussed.

2.2.3.1 Clenbuterol

Clenbuterol has undergone broad testing as a feed additive in the cattle, chicken, pig and sheep industries. In the majority of these trials it was found that treatment resulted in an improved ADG and FCR (Ricks *et al.,* 1984; Miller *et al.,* 1989; Moloney *et al.,* 1991). Furthermore an increase in muscle mass with an additional decrease in fat content was also observed (subcutaneous and intramuscular) (Strydom *et al.,* 2009). Aside from these financially beneficial implications, clenbuterol was found to have previously unknown negative properties. These include increasing the heart rate of individuals (Ricks *et al.,* 1984), depressing appetites at the onset of treatment, therefore lowering average daily feed intake (ADFI) (Ricks *et al.,* 1984; Strydom *et al.,* 2009) and also notably increasing the Warner-Bratzler Shear Force (WBSF) values, resulting in less tender meat (Miller *et al.,* 1988; Schiavetta *et al.,* 1990; Mersmann, 1998; Strydom *et al.,* 2009). The combination of these negative production characteristics, and due to the fact that it was discovered that clenbuterol has a too strong receptor affinity, led to the banning of this product by the FDA and in the EU from its use in meat producing animals (Spurlock *et al.,* 1993) .

2.2.3.2 Cimaterol

Cimaterol prompts similar responses on production performance characteristics compared to clenbuterol. A multitude of studies found that cimaterol treatment not only lead to heavier and leaner animals being produced with an increased protein content, but it also shortened the period that was necessary for the animal to be in the feedlot (Fiems *et al.,* 1990; Chikhou *et al.,* 1993; Vestergaard *et al.,* 1994). Nonetheless, it also displayed certain negative properties. Dikeman (2007) concluded that the meat originating from an animal that was treated with this product would possibly be toxic for the consumer thereof, due to residues in the meat. Over the last 2 decades, beta-agonists have been exposed to a high level of scrutiny regarding the safety for the animal and consumer alike. Therefore, to protect the consumer from any detrimental effects, that can possibly be caused by beta-agonist residues in the consumable product, various tests have been developed to accurately determine residue levels in a product (Stachel *et al.,* 2003). Furthermore, one can expect meat tenderness to be affected, and again it was proven that meat from animals produced under cimaterol treatment would be tougher (Fiems *et al.,* 1990; Schiavetta *et al.,* 1990; Chikhou *et al.,* 1993; Vestergaard *et al.,* 1994).

2.2.3.3 Ractopamine hydrochloride

Ractopamine hydrochloride was registered by the FDA as a beta-agonist feed additive in pig rations since 2000 and similarly so for cattle in 2003 (Hossner, 2005a). Apple *et al.* (2007) noted that it was evident, from numerous of the 23 published reports, that this growth agent can induce increased ADG, FCR and also produce heavier and leaner animals with an

improved dressing percentage over a broad range of livestock species. Similar to a clenbuterol treatment, there will be a noticeable reduction in ADFI. Apart from the possible increased heart rate in treated animals (Marchant-Forde *et al.,* 2003), ractopamine hydrochloride also increases meat toughness (Aalhus *et al.,* 1990; Stites *et al.,* 1991; Dunshea *et al.,* 1993; Carr *et al.,* 2005; Needham & Hoffman, 2015).

2.2.3.4 Zilpaterol hydrochloride

Zilpaterol hydrochloride is arguably one of the most commonly utilized beta-agonists in feedlot systems today. It has therefore gone through rigorous testing and has been available to the meat producer for more than a decade (Avendaño-Reyes *et al.,* 2006). The molecule has many production performance advantages, including: improved ADG and FCR and higher carcass weights (Estrada-Angulo et al., 2008b; Leheska et al., 2009; Strydom et al., 2009; López-Carlos et al., 2010; Lopez-Carlos et al., 2011b; Webb & Allen, 2015), decreased carcass fatness (Leheska *et al.,* 2009; Strydom *et al.,* 2009) and improved carcass composition (Leheska *et al.,* 2009). Unlike clenbuterol, zilpaterol hydrochloride treatment does not suppress the appetite of animals at the onset of treatment (Estrada-Angulo et al., 2008b). Moody *et al.* (2000) observed a rapid growth response when zilpaterol hydrochloride was administered, but this effect faded after the plateau phase was reached due the beta-adrenergic receptors becoming desensitized. This desensitization is brought on by an over stimulation of the receptors. At the onset of beta-agonist feeding there will be a rapid increase in growth rate where after an increase in rate will be maintained until a plateau phase is reached then finally be followed by a linear decrease in growth rate (Moody *et al.,* 2000; Avendaño-Reyes *et al.,* 2006). As was reported with the previously mentioned growth agents, zilpaterol hydrochloride also increased the toughness of the meat (Brooks *et al.,* 2009; Rathmann *et al.,* 2009; Hope-Jones *et al.,* 2012 and Steenekamp, 2014). Zilpaterol hydrochloride typically has a very prominent effect on muscle protein metabolism and this might result in the tougher meat being produced (Leheska *et al.,* 2009; Rathmann *et al.,* 2009; Strydom *et al.,* 2009).

A possible solution to address the toughening effect caused by a beta-agonist is the use of electrical stimulation following slaughter. It was; however, found that although there was an improvement in tenderness, this did not completely alleviate the problem and that the meat was still classified as tougher than the control (Hope-Jones *et al.,* 2010). Another possible solution would be to apply an appropriate aging time for the meat (Brooks *et al.,* 2009), or a combination of electrical stimulation and aging.

2.2.3.5 R-salbutamol

Salbutamol is a prescribed drug that was originally developed for use in humans to address respiratory problems, but was later used as a growth agent in pig production (Oksbjerg & Agergaard, 1996), lamb production (Bastos *et al.,* 1997), and chicken production (Fawcett *et al.,* 2004). Several cardiovascular side effects were noticed but this was from the racemic mix of R- and S- enantiomers (White *et al.,* 1989) and since then the R-enantiomer has been isolated as it is the active enantiomer (Ameredes & Calhoun, 2009). R-salbutamol has great potential for the future use as a growth promoting agent seeing as, in the limited research that has been published, it does not exhibit the "normal" beta-agonist negative effects such as toxicity from residues in the meat, health implications for the animals (Marchant-Forde et al., 2012b) or producing tougher meat (Steenekamp, 2014). There has been no literature published illustrating the effect that R-salbutamol has on growth, feed efficiency and slaughter characteristics when incorporated into lamb diets. However, there has been research into the effect that R-Salbutamol has on the abovementioned parameters in beef, poultry and swine meat production. Dunshea *et al.* (2005) showed that R-salbutamol inclusion to the diet of finisher pigs led to improved production performance. These findings were further supported by the work from Marchant-Forde *et al.* (2012) where it was shown that the addition of R-salbutamol produced a more ideal carcass composition in combination with improved ADG, FCR, dressing percentage, warm carcass mass and leaner slaughter pigs compared to a control group.

Steenekamp (2014) investigated the effect that R-salbutamol has on growth performance and meat characteristics when fed to cattle in the finishing period. The trial report from the University of Pretoria stated that the addition of R-salbutamol induced no apparent differences for weight gain, ADG and FCR in this trail. Due to the possible negative effect that the feeding system (Calan feeding gates) had on feed intake of individual animals, it is important to repeat a similar trial with a revised feeding system in combination with a larger sample size in order to get a better understanding of the effects on feedlot performance with R-salbutamol in ruminants under feedlot conditions.   These findings are in contrast to similar studies that typically found improved feedlot performance when administering other commercial beta-agonist in the finishing period of ruminants (Strydom *et al.,* 2009) cattle and (Avendaño-Reyes *et al.,* 2011; Brand *et al.,* 2013) sheep. Steenekamp (2014) furthermore looked at meat quality characteristics and found that R-salbutamol did not influence meat tenderness compared to the control group (Steenekamp, 2014). The absence of a toughening effect, when using R-salbutamol, could possibly be explained by a higher incidence of lipolysis and less of an effect on muscle metabolism (Steenekamp, 2014).

2.3 The possibility of incorporating of R-salbutamol in the feedlot industry of South Africa

Recently, there has been an increase in urgency for livestock producers to drastically increase production output. This increase in production not only has to account for product quantity but also product quality. To combat fluctuating feed costs and limited attainable water resources, production challenges associated with extensive systems, a decrease in available farmable land and utilizing the large numbers of livestock in South Africa, production should move towards intensive feedlot systems. Furthermore, combining feedlot systems with the discussed growth promoting agents can increase production output. These agents will bring about advantages such as improved feedlot performance (ADG, FCR, dressing percentage and higher yield percentages regarding protein to fat ratio). Unfortunately, certain negative effects may arise such a reduction in meat quality (tenderness), health status implications for the animal (increased heart rate or influence on social behaviour), and possibly residues in the meat for the consumer (the latter can be addressed with an appropriate feed-withdrawal period, but this has financial implications). Consistency of product quality is another very important production parameter, the major influencing factors being gender and maturity type. Fortunately, due to the repartitioning effect of beta-agonists, it is possible for the producer to manipulate product quality according to consumer specifications especially for all the involved genders and maturity type breeds found in feedlots across South Africa. R-salbutamol is currently in the process of being registered for use in sheep rations in South Africa and as a result, there is no published literature explaining the implications of using this product. To accurately compare the effectiveness of R-salbutamol as a growth-promoting agent against the currently available commercial beta-agonists, it is advisable to commit to further scientific research. Not only is it important to look at its effect on feedlot performance and meat quality characteristics but also to first determine at what levels the molecule should be included at in diets. Furthermore, with the prominent repartitioning effect of beta-agonists and influence on growth rates, it is likely to also have different levels of activity between genders and it will be important to quantify these differences.

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CHAPTER 3

The influence of R-Salbutamol on feedlot performance of Dorper ram, wether and ewe lambs

Abstract

A study on the effects of different R-salbutamol inclusion levels in a commercial finishing diet, on feedlot performance of three Dorper lamb sexes were carried out. The investigation consisted of two individual feedlot performance testing trials following a 2 x 4 factorial arrangement of treatments. Trial 1 consisted of 28 ram and 28 wether lambs, whilst Trial 2 used the same number of wether and ewe lambs. At the start of the trials, the lambs were randomly allocated to one of four R-salbutamol containing finisher feeds (0mg/kg, 110mg/kg, 135mg/kg and 160mg/kg feed). Live weights, feed intake and ort weights were recorded once a week for four consecutive weeks. Results from Trial 1 show that rams had superior average daily gain (ADG) and feed conversion ratio (FCR) values compared wethers. The comparison of ewes and wethers in Trial 2 showed no differences between these two sexes, for any of the feedlot performance attributes (ADG, FCR and dry matter intake (DMI)). In Trial 1 R-salbutamol had an effect only on the efficiency with which the lambs utilised the feed. The 110mg treatment group had superior FCR values compared to the control group. The 135mg and 160mg inclusion levels did however not differ significantly from either the control or 110mg treatment. No differences between beta-agonist inclusion levels were observed for any of the feedlot performance traits in Trial 2.

3.1   Introduction

For the agricultural sector to supply sufficient food to the world's growing population, it will have to increase production efficiency by amongst others; utilizing feedlot finishing systems for livestock (Avendan, 2006; Claasen, 2008; Cloete et al., 2012a). Finishing animals in a feedlot production system can improve production performance characteristics (Cloete et al., 2012a; Webb & Erasmus, 2013a), but to further improve efficiency the producer has to deploy additional strategies in these systems. One of which could be the use of growth agents such as beta-agonists. These compounds are known to increase feed utilisation efficiency in combination with heavier and more ideal carcass compositions (Strydom, 2016). One such compound that has potential use is R-sulbutamol. The salbutamol molecule is found in a

prescribed drug that was originally developed for use in humans to address respiratory problems, but was later used as a growth agent in pig production (Oksbjerg & Agergaard, 1996), lamb production (Bohorov et al., 1987; Del Barrio et al., 1995), and chicken production (Fawcett *et al.,* 2004). Although several cardiovascular side effects were noticed, these were due to the racemic mix of R- and S- enantiomers (White *et al.,* 1989) but since then, the active R-enantiomer has been isolated (Ameredes & Calhoun, 2009). R-salbutamol shows great potential for future use as a growth promoting agent seeing as, in the limited research that has been published, it does not exhibit the "normal" beta-agonist negative effects such as toxicity from residues in the meat, health implications for the animals (Marchant-Forde et al., 2012b) or production of tougher meat (Steenekamp, 2014). There has been no literature published on the effect of isolated R-Salbutamol on feedlot performance characteristics in lamb rations.

The chosen breed for this research project was the Dorper due to the fact that it is a commonly encountered and favoured breed in South Africa. Furthermore it is also classified as an early maturing breed, which makes it the ideal breed for testing the possible nutrient repartitioning effect, which is normally associated with other beta-agonists (Miller *et al.,* 1988; Brooks *et al.,* 2009), or R-salbutamol. The sex of the animal also plays a major role on its production efficiency (Field, 1971; Pommier *et al.,* 1989; Okeudo & Moss, 2008), and therefore, to have a complete understanding of the effect that R-salbutamol might have on feedlot performance, it was important to incorporate rams, ewes and wethers into the trial layout. The manufacturer stimulated the inclusion levels of R-salbutamol in the different diets and the question furthermore was what would its effect be on feedlot performance across the different genders?

3.2   Materials and Methods

The research project was approved by the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUD15-00082). Handling of the animals proceeded according to South African National Standards 10386: 2008. The trials were conducted from August 2015 to January 2016 at the Welgevallen Experimental Livestock Unit in Stellenbosch (Stellenbosch, Western Cape, South Africa).

The project consisted of two individual trials that were completed consecutively. Both trials were conducted in an identical manner and hence, for the purpose of simplifying explanations, the experimental procedures for only one trial will be described. It is therefore important to keep in mind that the following explanations covers a single trial and will be repeated for the second trial. The only difference between the two trials was that in trial one intact males (rams) and physically castrated males (wethers) were used and in trial two

physically castrated males (wethers) and intact females (ewes) were used. The beta-agonist treatment levels were similar for both trials.

3.2.1   Nutrient composition of feed and feed adaptation program

Animals were sourced from an extensive farming system in the Carnarvon district, Northern Cape, South Africa. Upon arrival, the Dorper lambs received a commercial drought pelleted sheep feed (Table 3.1). This feed was given for the first three days of the feeding program to allow the animals to reach a sufficient rumen fill level before a feed with a higher nutritional value was supplied and furthermore to allow them to get habituated to using a feed trough.

Table 3.1 Information provided by manufacturer of the commercial drought cube sheep feed used during the rumen fill phase (day 1-3)

Nutrient	Value (g/kg)	Bounds
Protein	100.0	Min
Fat	25.0	Min
Fat	70.0	Max
Fibre	110.0	Min
Fibre	200.0	Max
Moisture	120.0	Max
Calcium	10.0	Min
Calcium	12.0	Max
Phosphorus	5.0	Min

Bounds represent the maximum or minimum levels of each nutrient

From day four to six the drought cube feed was replaced with a commercial complete sheep adaptation feed (Table 3.2). The animals were monitored closely and regularly to identify and treat any cases of acidosis or other gastro intestinal tract (GUT) disturbances.

Table 3.2 Information provided by manufacturer of the commercial complete sheep adaptation feed used during the adaptation period (day 4-6) and also during the pre-grower phase (day 7-10)

Nutrient	Value (g/kg)	Bounds
Protein	155.0	Min
Fat	25.0	Min
Fibre	200.0	Max
Moisture	120.0	Max
Calcium	10.0	Max
Phosphorus	2.0	Min

Bounds represent the maximum or minimum levels of each nutrient

Day seven to ten is described as the pre-grower phase and here the complete adaptation feed was gradually replaced with the commercial complete sheep finisher feed in varying ratios. The more gradual transition between feeds minimized stress on the rumen microbial populations.

Table 3.3 Information provided by manufacturer of the commercial complete sheep finisher feed used from day 7 to 22 during the pre-grower and grower phase and also during the sample collection period (day 23-50)

Nutrient	Value (g/kg)	Bounds
Protein	120.0	Min
Fat	25.0	Min
Fat	70.0	Max
Fibre	110.0	Min
Fibre	200.0	Max
Moisture	120.0	Max
Calcium	10.0	Max
Phosphorus	2.0	Min

Bounds represent the maximum or minimum levels of each nutrient

From days 11-22 all animals entered the grower phase and received the commercial complete sheep finisher feed *ad lib*. This is also the feed that was used as the control feed (day 23-50) in the experiment. Treatment feeds were identical to this control feed, with the only difference being inclusion level in mg/kg of the chosen beta-agonist (R-salbutamol). The three different beta-agonist inclusion levels were 110mg/kg, 135mg/kg and 160mg/kg of R-salbutamol added to the control feed. Days 23 to 50 represented the sample collection period. For the 28 days

(sample collection period) that the animals were in the individual pens, they had *ad lib* access to one of the four allocated feeds. Table 3.4 contains a summary of the feeding program used during the trial period.

Table 3.4 Summary of the feeding program used during the trial period

Feeding phase	Day	Period (days)	Feed type	Inclusion level (%)
Rumen fill phase	1-3	3	Drought Pellet	100
Adaptation phase	4-6	3	Sheep adaptation feed	100
Pre-grower phase	7-8	2	Sheep adaptation feed	50
			Sheep finisher feed	50
	9-10	2	Sheep adaptation feed	25
			Sheep finisher feed	75
Growth phase	11-22	12	Sheep finisher feed	100
Sample collection phase	23-50	28	Sheep finisher feed + R-salbutamol	100

Feed samples were taken from each of the feed bags upon opening and frozen where after a representative sample was taken from the pooled contents and analysed for proximate chemical composition values on an As-fed basis (Table 3.5). The proximate analysis was done to confirm that the chemical composition of the feed fell within the ranges of feed used in similar studies (Jurgens, 2002).

Table 3.5 Proximate analyses values performed on commercial complete sheep finisher feed

Chemical composition, As fed basis	Value
Average Crude Protein (%)	13.96
Crude Fibre (%)	11.99
Acid detergent fibre (ADF) (%)	14.51
Neutral detergent fibre (NDF) (%)	24.71
Fat (%)	2.33
Average Gross Energy (MJ/kg)	16.358

3.2.2 Animals, housing, feeding and experimental design

Sixty-six animals per trial were sourced from an extensive farming system in the Carnarvon district, Northern Cape, South Africa. The group age was 100 – 110 days with an average live weight of 31.5kg for trial one and 32.5kg for trial two. The trials were conducted from August 2015 to January 2016 at the Welgevallen Experimental Livestock Unit in Stellenbosch (Stellenbosch, Western Cape, South Africa). Prior to use, the testing facilities were washed and cleaned with a high pressure water sprayer and disinfected with F10SC Veterinary Disinfectant (Health and Hygiene (Pty) Ltd, South Africa) which is a SABS approved product (SANS 10228). On arrival all the animals were moved into an indoor group pen and given three days to acclimatize to the new surroundings where after they were processed (dosed and vaccinated), as described in Table 3.6. For the duration of the feed adaptation program (day 1-22) all the sheep were kept and fed in the indoor facility in large group pens. Thereafter (day 23 to 50) animals were moved to the adjacent indoor facility that has the capacity to hold 56 sheep in individual slatted wooden floor pens. Each individual pen is approximately 2m^2 and also contains a steel feed trough and plastic water bucket. Pens were divided with mesh type fencing that allows the animals to socialize through visual interaction but restricts physical interaction.

Table 3.6 The standard processing protocol and commercial products that were used

Name of product	Description	Company, origin
Embavit®	Oral liquid vitamin supplement	Merial, New Zealand
Embamin®	Oral liquid trace element supplement	Merial, New Zeeland
Ivomec®	Sheep drench	Merial, New Zeeland
Valbazen®	Broad spectrum dewormer	Zoetis, USA
OBP Pasteurella®	Control of Pasteurella infections	Onderstepoort, RSA
OBP Enterotoxemia®	Immunization against enterotoxemia	Onderstepoort, RSA
Wound-Sept Plus®	Wound treatment that promote healing	Virbac, RSA

During the sampling period (four weeks) animals were weighed once a week with a Model SI2963 livestock scale measuring to the nearest 0.200kg (Scales Incorporated, South

Africa). At the same time, each feed trough was emptied and contents weighed back with a CTS-30 portion scale (Cape Town Scales, South Africa). Weekly feed intake was calculated by subtracting the weight of the feed refusals that were collected once a week from the total weight of feed that was fed during that week. Topping up of the feedthroughs took place at 07:00, 13:00 and 19:00 every day. At each feeding period, the sheep were checked for any health issues and treated accordingly. Every third day all the pens and water buckets were cleaned.

Lambs were assigned to one of eight gender (ram vs wethers; or ewe vs wethers) and R-salbutamol inclusion level (i.e., 0mg/kg, 110mg/kg, 135mg/kg or 160mg/kg) based treatments in a 2 × 4 factorial arrangement of treatments. R-salbutamol was provided on an As-fed basis in the feed (Table 3.7 and Table 3.8). At the completion of the feed adaptation period (day 22), and before entering the sample collection periods, all 66 animals per trial were weighed and grouped according to sex and ranked for live weight. Five animals per sex, which exhibited outlier characteristics, based on each individual's live weight value deviation away from the live weight mean value for the group, were removed and the remaining 28 animals per sex were once again grouped according to live weight and also blocked accordingly. From this, random allocation of inclusion level could be made. Seven sheep were each allocated to the eight treatment combinations, thus 56 sheep took part in each trial and these combinations were randomly distributed throughout the housing facility. None of the sheep were removed from the trial for any reason and all sheep were slaughtered after completion of the feedlot performance testing period.

Table 3.7 The eight treatment combinations distributed into factors: sex, R-salbutamol inclusion level for Trial 1

Treatment	Sex	Number of animals	R-salbutamol inclusion level
1	Ram	7	0mg/kg
2	Ram	7	110mg/kg
3	Ram	7	135mg/kg
4	Ram	7	160mg/kg
5	Wether	7	0mg/kg
6	Wether	7	110mg/kg
7	Wether	7	135mg/kg
8	Wether	7	160mg/kg

Table 3.8 The eight treatment combinations broken up into factors: sex, R-salbutamol
inclusion level for Trial 2

Treatment	Sex	Number of animals	R-salbutamol inclusion level
1	Ewe	7	0mg/kg
2	Ewe	7	110mg/kg
3	Ewe	7	135mg/kg
4	Ewe	7	160mg/kg
5	Wether	7	0mg/kg
6	Wether	7	110mg/kg
7	Wether	7	135mg/kg
8	Wether	7	160mg/kg

3.3   Statistical analysis

SAS Enterprise guide 9.2 was used to analyse the data and generate the statistics (Version 9.2, SAS Institute Inc., Cary, USA). The two trials were conducted separately and the data were therefore also analysed separately but in an identical manner. The data set was first tested for normality after which multiple Analyses of Variance (ANOVA) were performed following the general linear models (GLM) procedure. The weight of the animal at the start of the sampling period was used as a covariate in each of the variables. The parameters that were tested for include: Dry Matter Intake (DMI), Average Daily Gain (ADG) and Feed Conversion Ratio (FCR) as depicted in Table 3.9 and 3.11. The main factors that were included into the analyses were sex (ram and wether for Trial 1/ wether and ewe for Trial 2), beta-agonist treatment for both trials (control, 110mg/kg, 135mg/kg and 160mg/kg) and also the interaction between main effects. To evaluate the effects that sex and treatment had on body weight gain, a linear regression was fitted to the body weights of each individual over time. The ADG was represented by the slope of each regression. To attempt to further explain the effect of adding R-salbutamol to the diet, an additional statistical analysis was performed on the existing data, where the predetermined treatments were excluded as a main effect and rather the feedlot performance parameters, as a function of true intake in mg R-salbutamol/day, was evaluated as well as the interaction between sex and true intake of R-salbutamol levels. The data was compiled by taking into account the amount of feed ingested by each individual and the amount (mg) of R-salbutamol formulated into the administered treatment. Animals were then ranked according to beta-agonist intake levels and furthermore classified into a zero intake (0mg R-salbutamol/day), low intake (0-200mg R-salbutamol/day), medium intake (200-250mg R-salbutamol/day) and a high intake (>250mg R-salbutamol/day) levels (Table 3.10 and Table 3.12). Fishers LSD comparison of LSMeans was chosen as the

post hoc test where interactions were found to be significant. A significance level of 5% was chosen to report any differences, interactions or effects. The format in which the results are reported is LSMeans ± Standard Error of the mean (SEM).

3.4   Results and Discussion

The results for the various treatments (sex and beta-agonist inclusion level) on DMI, ADG and FCR are reported in Table 3.9 (Trial 1) and Table 3.11 (Trial 2), followed by the results for the same parameters expressed as a function of true intake of R-salbutamol in mg/day in Tables 3.10 (Trial 1) and 3.12 (Trial 2).

Table 3.9 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on the DMI (kg/day), ADG (kg/day) and FCR for Trial 1

Effect		DMI (kg/day)	ADG (kg/day)	FCR
Sex				
	R	1.76 ± 0.03	0.40 ± 0.01	4.46 ± 0.16
	W	1.65 ± 0.03	0.31 ± 0.01	5.54 ± 0.16
	P-value	**0.022**	**< 0.001**	**< 0.0001**
Beta-agonist inclusion level across genders				
	0	1.71 ± 0.05	0.33 ± 0.02	5.43[a] ± 0.23
	110	1.69 ± 0.05	0.38 ± 0.02	4.53[b] ± 0.22
	135	1.67 ± 0.05	0.34 ± 0.02	5.14[ab] ± 0.22
	160	1.75 ± 0.05	0.37 ± 0.02	4.90[ab] ± 0.22
	P-value	0.689	0.206	**0.036**
Beta-agonist inclusion level between genders				
R	0	1.76 ± 0.07	0.38 ± 0.03	4.64 ± 0.33
R	110	1.79 ± 0.07	0.42 ± 0.03	4.32 ± 0.31
R	135	1.73 ± 0.07	0.39 ± 0.03	4.50 ± 0.31
R	160	1.77 ± 0.07	0.41 ± 0.03	4.39 ± 0.31
W	0	1.66 ± 0.07	0.28 ± 0.03	6.22 ± 0.31
W	110	1.59 ± 0.07	0.34 ± 0.03	4.74 ± 0.31
W	135	1.62 ± 0.07	0.29 ± 0.03	5.78 ± 0.31
W	160	1.72 ± 0.07	0.33 ± 0.03	5.42 ± 0.31
	P-value	0.724	0.889	0.308

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different ($p < 0.05$)
R: Rams
W: Wethers

There was no interaction between the main effects of sex and diet for DMI (P = 0.724), (ADG (P= 0.889) or FCR (P = 0.308). Therefore, the main effects can be interpreted individually. Table 3.9 showed that there were clear differences between the ADG values for the two sexes (P < 0.001) with rams (0.40 ± 0.01kg) outperforming wethers (0.31 ± 0.01kg). Sex (P < 0.0001) also had a significant effect on FCR with rams (4.46 ± 0.16) once again performing better than wethers (5.54 ± 0.16). This trend is also confirmed by others (Field, 1971; Crouse *et al.,* 1981; Lee, 1986; Lee *et al.,* 1990; Rodríguez *et al.,* 2011; Cloete *et al.,* 2012; Craigie *et al.,* 2012). The clear differences in ADG, FCR and DMI between wethers and rams in trial one could be because of a sex and nutrition interaction, but not a sex and beta-agonist interaction. Crouse et al. (1981) found that under feedlot conditions where a higher plane of nutrition was supplied, similar to feedlot trial conditions, that rams outperformed wethers with a greater margin than when a low plane of nutrition is supplied. This is a result of the interaction between nutritional plane and the high levels of circulating testosterone found in rams as opposed to the lower specific hormone levels associated with wethers. Rams (1.76 ± 0.03kg) also consumed more (P = 0.022) feed than wethers (1.65 ± 0.03), most probably due to the rams more aggressive nature as a result of circulating sex hormones acting in on the animal (Hossner, 2005a). The higher feed consumption of the ram lambs was converted into significantly better ADG's and FCR's that illustrates a much higher growth efficiency, which is ideal under feedlot conditions.

R-salbutamol treatment had no influence on ADG (P = 0.206), which is in contrast to the findings of López-Carlos et al. (2010) and Lopez-Carlos et al. (2011) that showed an improvement in ADG where other beta-agonists were tested in lamb rations but in agreement with Steenekamp (2014), where R-salbutamol did not improve or decrease ADG in feedlot cattle.

Diet positively affected FCR (P = 0.036). This contradicts the results of Steenekamp (2014) where R-salbutamol had no effect on the FCR of feedlot cattle. Other beta-agonists have also been found to improve feed usage efficacy in treated animals (Apple et al., 2007; Needham & Hoffman, 2015; Strydom, 2016). The 110mg treatment (4.53 ± 0.22) numerically performed better than the control, and as expected the worst FCR was observed with the control group (5.43 ± 0.23). It is important to note that the 135mg and 160 mg groups did not differ from the Control or 110mg treatment.   Diet had no influence on DMI. Other beta-agonists have been known to suppress apatite at the onset of treatment but this was not observed with R-salbutamol (Ricks et al., 1984; Strydom et al., 2009).

Table 3.10 The LSMeans ± SEM for the effect of true intake of R-salbutamol in mg/day and interaction between sex and true intake of R-salbutamol in mg/day on the DMI (kg/day), ADG (kg/day) and FCR for Trial 1

Effect		DMI (kg/day)	ADG (kg/day)	FCR
Beta-agonist true intake level across genders				
	Zero	1.72[a] ± 0.04	0.33 ± 0.02	5.44[a] ± 0.23
	Low	1.50[b] ± 0.06	0.32 ± 0.03	4.76[ab]± 0.33
	Medium	1.72[a] ± 0.04	0.35 ± 0.02	5.18[ab] ± 0.20
	High	1.80[a] ± 0.04	0.39 ± 0.02	4.67[b] ± 0.22
	P-value	**0.002**	0.136	**0.044**
Beta-agonist true intake level between genders				
R	Zero	1.78 ± 0.06	0.39 ± 0.03	4.66 ± 0.33
R	Low	1.51 ± 0.11	0.33 ± 0.05	4.55 ± 0.57
R	Medium	1.78 ± 0.05	0.41 ± 0.02	4.41 ± 0.25
R	High	1.82 ± 0.05	0.42 ± 0.02	4.41 ± 0.29
W	Zero	1.66 ± 0.06	0.28 ± 0.03	6.21 ± 0.31
W	Low	1.49 ± 0.06	0.31 ± 0.03	4.98 ± 0.32
W	Medium	1.66 ± 0.06	0.30 ± 0.03	5.95 ± 0.31
W	High	1.79 ± 0.06	0.37 ± 0.03	4.94 ± 0.33
	P-value	0.787	0.337	0.182

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different (p < 0.05)
R: Rams
W: Wethers
Zero: Individuals with an intake of 0mg R-salbutamol/day
Low: Individuals with an intake of 0-200mg R-salbutamol/day
Medium: Individuals with an intake of 200-250mg R-salbutamol/day
High: Individuals with an intake of >250mg R-salbutamol/day

There was no interaction noted between the main effects of sex and true intake of R-salbutamol for DMI (P = 0.787), ADG (P = 0.337) and FCR (P = 0.182). To avoid repetition, sex as a main effect, was not noted again in Table 3.10 but can be seen in Table 3.9. True intake of R-salbutamol intake level had no influence on ADG (P = 0.105). When true intake groups are compared to each other for FCR (P = 0.044), the lowest (best) FCR was observed in the High intake group (4.67 ± 0.22) which differed significantly (P = 0.019) from the Zero intake group (5.44 ± 0.23), but was very close and not significantly different to the low intake group (4.67 vs. 4.76). Since R-Salbutamol concentration had no effect on DMI (Table 3.9), it is likely that many of the low R-Salbutamol intake lambs were derived from the 110 mg/kg treatment. This supports the finding that the 110 mg/kg treatment resulted in the highest numerical ADG and best FCR. DMI also differed significantly between true intake groups (P = 0.002). Here the Low intake group (1.50 ± 0.06) had a lower DMI value compared to the

Zero, Medium and High intake group values (1.72 ± 0.04, 1.72 ± 0.04 and 1.80 ± 0.04, respectively) that explains the improved FCR's, as there were no differences in ADG.

Table 3.11 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on the DMI (kg/day), ADG (kg/day) and FCR for Trial 2

Effect		DMI (kg/day)	ADG (kg/day)	FCR
Sex				
	E	1.61 ± 0.04	0.28 ± 0.02	6.42 ± 0.33
	W	1.65 ± 0.03	0.29 ± 0.02	6.13 ± 0.32
	P-value	0.369	0.592	0.575
Beta-agonist inclusion level across genders				
	0	1.72 ± 0.05	0.32 ± 0.02	5.67 ± 0.42
	110	1.56 ± 0.05	0.28 ± 0.02	5.70 ± 0.42
	135	1.59 ± 0.05	0.26 ± 0.02	6.82 ± 0.44
	160	1.66 ± 0.05	0.28 ± 0.02	6.71 ± 0.42
	P-value	0.118	0.290	0.146
Beta-agonist inclusion level between genders				
E	0	1.66 ± 0.07	0.31 ± 0.03	5.58 ± 0.64
E	110	1.64 ± 0.07	0.29 ± 0.03	6.09 ± 0.64
E	135	1.56 ± 0.07	0.25 ± 0.03	7.05 ± 0.69
E	160	1.57 ± 0.07	0.26 ± 0.03	6.96 ± 0.64
W	0	1.78 ± 0.07	0.32 ± 0.03	5.60 ± 0.64
W	110	1.49 ± 0.07	0.26 ± 0.03	5.73 ± 0.64
W	135	1.62 ± 0.07	0.27 ± 0.03	6.76 ± 0.64
W	160	1.74 ± 0.07	0.30 ± 0.03	6.43 ± 0.64
	P-value	0.105	0.634	0.979

E: Ewes
W: Wethers

There was no interaction between the main effects of sex and treatment for DMI (P = 0.105), (ADG (P= 0.634) or FCR (P = 0.979) in Trial 2. No statistical differences were found for any of the feedlot performance parameters between wethers and ewes (Table 3.10). This is in contrast to the results of Crouse et al. (1981), Notter et al. (1991) and Hopkins et al. (2007) that found wethers to perform better that ewes. Okeudo & Moss (2008) however found that these two sexes did not differ for feedlot performance values. A comparison of the treatments (and not taking sex into account as an effect), indicated that no differences were observed for ADG (P = 0.290), FCR (P = 0.146) or DMI (P = 0.118).

Table 3.12 The LSMeans ± SEM for the effects of true intake of R-salbutamol in mg/day and interaction between sex and true intake of R-salbutamol in mg/day on the DMI (kg/day), ADG (kg/day) and FCR for Trial 2

Effect		DMI (kg/day)	ADG (kg/day)	FCR
Beta-agonist true intake level across genders				
	Zero	1.72[a] ± 0.05	0.32[a] ± 0.02	5.58 ± 0.45
	Low	1.49[b] ± 0.04	0.24[b] ± 0.02	6.49 ± 0.41
	Medium	1.68[a] ± 0.05	0.29[ab] ± 0.02	6.75 ± 0.47
	High	1.70[a] ± 0.06	0.31[a] ± 0.03	5.87 ± 0.59
	P-value	**0.001**	**0.048**	0.301
Beta-agonist true intake level between genders				
E	Zero	1.66 ± 0.06	0.31 ± 0.03	5.57 ± 0.64
E	Low	1.52 ± 0.06	0.24 ± 0.03	6.93 ± 0.60
E	Medium	1.63 ± 0.06	0.27 ± 0.03	6.93 ± 0.58
E	High	1.67 ± 0.10	0.32 ± 0.04	5.32 ± 0.98
W	Zero	1.77 ± 0.06	0.32 ± 0.03	5.59 ± 0.64
W	Low	1.46 ± 0.06	0.24 ± 0.03	6.06 ± 0.56
W	Medium	1.73 ± 0.08	0.31 ± 0.03	6.56 ± 0.77
W	High	1.74 ± 0.06	0.30 ± 0.03	6.43 ± 0.64
	P-value	0.417	0.778	0.577

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different (p < 0.05)
E: Ewes
W: Wethers
Zero: Individuals with an intake of 0mg R-salbutamol/day
Low: Individuals with an intake of 0-200mg R-salbutamol/day
Medium: Individuals with an intake of 200-250mg R-salbutamol/day
High: Individuals with an intake of >250mg R-salbutamol/day

There was also no interaction between the main effects of sex and beta-agonist intake level for ADG (P = 0.778), FCR (P = 0.577), or DMI (P = 0.417) which means the main effect of true intake level can be reported. True intake level had no influence on FCR (P = 0.301), but an effect manifested for the parameters of ADG (P = 0.048) and DMI (P = 0.001). The Zero intake (1.72 ± 0.05), Medium intake (1.68 ± 0.05) and the High intake (1.70 ± 0.06) groups consumed more feed, compared to the Low intake group (1.49 ± 0.04). This trend continued over to ADG. The Zero intake 0.32 ± 0.02), Medium intake (0.29 ± 0.02) and High intake (0.31 ± 0.03) groups once again had the highest ADG whilst the Low intake group (0.24 ± 0.02) had the lowest ADG however, the Low intake group did not differ significantly from the Medium intake group. Despite the reported statistical effect, the DMI and ADG for the high R-Salbutamol intake group did not differ from the Zero intake group. This finding does not

warrant the use of R-Salbutamol in early maturing ewes or wethers under feedlot conditions, but is in contrast to the findings for rams.

3.5   Conclusion

The results indicate that there was clearly a difference between sexes in Trial 1. Rams outperformed the wethers, whereas there was no clear difference between wethers and ewes for Trial 2. In Trial 1, the 110mg treatment delivered the best feedlot performance values with the poorest coming from the control treatment. In Trial 2, no differences were observed for ADG or FCR between treatments. When the true intake of R-salbutamol was taken into account, for Trial 1, it was observed that including this product was associated with some level of improved feedlot performance. However, no differences were found for FCR between true intake levels in Trial 2 and even though small differences were observed for ADG, no clear trend could be identified for this parameter. It would be advisable to conduct a study where a control feed, without R-salbutamol, is compared to a single treatment feed that contains a fixed level of R-salbutamol, and then the format of expressing true intake of the beta-agonist on feedlot performance can be used with a higher degree of accuracy. The effect that R-salbutamol has on feedlot performance appears to be varied and if further testing of this product is embarked on and additional positive results are found, it will also be necessary to perform an economic comparison study that can establish whether the improvement will be enough to justify the inclusion of R-salbutamol to a feedlot finishing diet.

3.6   References

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CHAPTER 4

The influence of R-Salbutamol on slaughter performance, primal cut yield and carcass composition in Dorper ram, wether and ewe lambs

Abstract

The influence of beta-agonist treatment on slaughter performance and carcass characteristics of three different (ram, wether and ewe) lamb sexes were compared under feedlot conditions over two trial periods. Trial 1 consisted of 28 ram and 28 wether lambs, whilst Trial 2 used the same number of wether and ewe lambs. Each trial followed a 2 x 4 factorial arrangement of treatments. At the start of the trials, the lambs were randomly allocated to one of four R-salbutamol containing finisher feeds (0mg/kg, 110mg/kg, 135mg/kg and 160mg/kg feed). After four weeks of feeding, all animals were slaughtered and various carcass parameters were recorded. Prior to slaughter, the rams weighed significantly more than the wethers but the latter exhibited superior dressing percentage figures. No sex based differences were observed for the carcass composition (%bone; %muscle; fat) or cross-sectional loin surface area measurements. Non-carcass components contributions were similar between rams and wethers, except for the proportions of red offal (rams more), head (rams more), trotters (wethers more) and omental fat (wethers more). Furthermore, the fore shank primal cut contribution towards carcass weight was more in rams and the back cut contribution was more in wether carcasses. Also, slaughter weights and dressing percentages were similar but wethers had a more ideal and leaner carcass composition compared to ewes (Trial 2). All treatment levels (110mg, 135mg and 160mg) were found to suppress of kidney fat deposition. No differences between inclusion levels were observed for any of the feedlot performance or carcass characteristics traits in Trial 2.

4.1   Introduction

Animal growth is the term given for the processes of hyperplasia (amount of cells increase) and hypertrophy (size of cells increase). These processes will continue until the animal reaches mature size and as a result body conformation changes will occur (Lawrie et al., 2006). Knowing what influences these body conformation changes can put the producer in a competitive position.

Lamb meat production systems have used all three sexes (ram, wether and ewe) in the past but it is commonly known that rams outperform wethers and ewes for slaughter parameters but ewes have the highest dressing percentage (Field, 1971; Crouse et al., 1981; Seideman et al., 1982; Arnold & Meyer, 1988; Dransfield et al., 1990; Vergara et al., 1999). To further improve or manipulate the production of these sexes, the producer can also incorporate the use of growth agents such as beta-agonists. Beta-agonist effects do however differ due to various factors such as dosage, duration of treatment, type of cell, type of beta-agonist, breed, specie and sex (Mersmann, 1998). Fortunately beta-agonists have a widely reported and broad range of positive effects on muscle tissue and fat depots (Moody et al., 2000). This molecule will reduce the amount of adipose tissue by either increasing fat breakdown or decreasing fat synthesis and improve protein accretion by either decreasing protein breakdown or increasing the synthesis thereof in a multitude of species (Carr et al., 2005; Avendaño-Reyes et al., 2006; Apple et al., 2007; Estrada-Angulo et al., 2008; Brooks et al., 2009; Leheska et al., 2009; López-Carlos et al., 2010).

The value of a lamb carcass will be based on several factors including slaughter weight, carcass conformation and carcass characteristics. Furthermore, producers have recently become more aware of the value of the "fifth quarter" from the carcass, namely the offal, head, trotters and skin.

There has been no literature published illustrating the effect that R-Salbutamol has on slaughter performance, primal cut yield and carcass composition when incorporated into lamb rations. The chosen breed for this research project was the Dorper, due to the fact that it is a commonly encountered and a favoured breed in South Africa. Furthermore it is also classified as an early maturing breed, which makes it the ideal breed for testing the possible nutrient repartitioning effect, which is normally associated with other beta-agonists (Miller et al., 1988; Brooks et al., 2009), of R-salbutamol.

4.2   Materials and Methods

The research project was approved by the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUD15-00082). Handling of the animals proceeded according to South African National Standards 10386: 2008. The trials were conducted from August 2015 to January 2016 at the Welgevallen Experimental Livestock Unit in Stellenbosch (Stellenbosch, Western Cape, South Africa).

The project consisted of two individual trials that were completed consecutively. Both trials were conducted in an identical manner and hence, for the purpose of simplifying explanations, the experimental procedures for only one trial will be described. It is therefore important to keep in mind that the following explanations covers a single trial and will be

repeated for the second trial. The only difference between the two trials was that in Trial 1 intact males (rams) and physically castrated males (wethers) were used, and in Trial 2, physically castrated males (wethers) and intact females (ewes) were used. The beta-agonist treatment levels were similar for both trials.

4.2.1 Feeding, animals, husbandry and experimental design

The data that were used to generate the results for this chapter was collected after the completion of the sample collection period for the feedlot performance section of the research project (Chapter 3).

The adaptation program (summarised in Table 3.5) as well as the experimental feeds (Tables 3.1 – 3.4) that were used during the trial period are discussed in detail in section 3.2.1 of Chapter 3. Information regarding the trial animals, scientific protocol procedures during the anti-mortem stage of the trial and experimental design can be found in section 3.2.2 of Chapter 3.

4.2.2 Protocol and measurements taken at the slaughter facility

After 28 days of receiving the four experimental diets in the feedlot system all lambs were weighed, loaded and transported to a commercial abattoir (Tomis® abattoir, Hermon, Western Cape, South Africa). Upon arrival, lambs were unloaded and put into lairage (6 hours) to allow sufficient resting and water was provided *ad libitum*. All lambs were weighed again before slaughter and this weight was used as the final live weight. Lambs were rendered unconscious by means of electrical stunning (200V applied for 4 seconds) where after exsanguination and carcass bleeding continued. All slaughter activities were completed according to standard South African techniques as described by (Hoffman et al., 2003). Electrical stimulation with 110V was further applied for 50 seconds just after bleeding. Carcass dressing and classification (the latter according to Agricultural Product Standards Act, 1990. Act No.119 of 1990) were completed at 20 minutes *post mortem* and the carcasses were chilled at 2°C overnight. The carcass classification code that was used, describe the lamb according to age and fatness class. All the sheep that were used in these trials had no permanent incisors and therefore received an A-class grade describing them as lambs. Furthermore, the fat descriptions are divided into groups 0 to 6, with 0 showing no visible fat and 6 being classified as excessively over-fat. In unison with the slaughter activities, certain weight recordings were also made for each carcass. Individual weights were recorded for the head, trotters, skin, testicles (in the case of rams), red offal (heart, lungs, liver, spleen and oesophagus), omental fat and GIT (fasted gastro-intestinal tract). These weights are expressed as a percentage of the slaughter weight. The head was separated between the Atlas and Axis vertebra and the trotters were separated between the Radius/Ulna and Metacarpus bones in the front legs and

between the Tarsus and Metatarsus bones in the hind legs. Before entering the cooling room, each carcass was weighed (warm carcass weight) and pH of the *M. longissimus dorsi* (LD) was measured between the eleventh and thirteenth rib at 45 min and again 24h *post mortem* (McGeehin et al., 2001) using a Crison pH 25 portable pH meter (Lasec Pty Ltd, South Africa). The pH measurements from 45min and 24h post mortem will be discussed in Chapter 5.

After the overnight cooling, the carcasses were weighed (cold carcass mass) and divided into primal cuts, as depicted in Figure 4.1. These include neck (1), shoulder (5), fore shank (6), ribs (4), back (3), hind legs (2), and both shin (7). The first cut was made at the 5th cervical vertebra from the dorsal to ventral side to remove the neck portion. Secondly a cut was made between the last lumbar and first sacral vertebrae, in a dorsal to ventral direction, to divide the posterior portion of the carcass and the anterior portion. This posterior portion was further divided in two to produce the two separate hind legs (with still attached shins). A cut was then made between 4th and 5th thoracic vertebrae in a straight line towards the elbow joint, to produce the fore portion (shoulder and fore shanks) and middle portions (back and ribs). To separate the back and ribs, a cut parallel to the spine was made through the ribs, at a distance away from the midpoint of the spine approximately twice the distance of the diameter of the LD. To separate the shoulder and fore shank, a cut was made following the same imaginary line.

1. Neck
2. Leg
3. Back
4. Rib
5. Shoulder
6. Fore shank
7. Shin
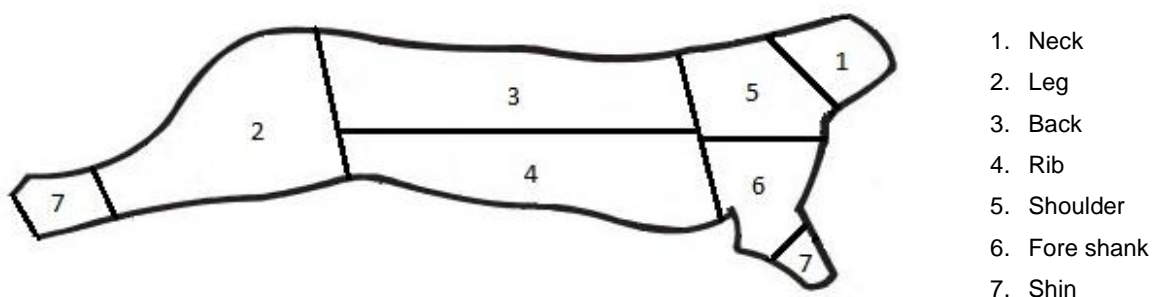
Figure 4.1 Primal cut separation diagram

4.2.3   Sample collection for analysis

Further measuring and processing activities were continued at the meat processing facility of Stellenbosch University. The weight of the cold carcass included the kidneys and kidney fat. The weights of primal cuts, kidney and kidney fat are expressed as a percentage of the cold carcass weight (24h *post mortem*).

On both the left and right side of the back a cut was made on the anterior sides of the 9th and 12th thoracic vertebrae. Following the curvature of the ribs to the spine and then cutting through the spine (including ribs 9 – 11, as well as the associated posterior meat and fat

sections), the three rib sections were isolated and used to predict bone: meat: fat yield of the carcass and measure the eye muscle cross-sectional area. The LD from both sides were also removed, vacuum packaged and stored at 4°C for further meat quality analyses such as cooking loss, Warner-Bratzler shear Force (WBSF) measurements and descriptive sensory analyses (discussed in Chapter 5).

4.2.4 Three rib cut for image analysis and bone: fat: meat yield

To determine the cross-sectional area of the LD muscle, a digital camera was secured with a tripod on a stable surface at a constant height. The three rib cut was placed under the camera and a photo was taken. The scale at the bottom of the image (figure 4.2) was a calibration aid for the computer software program (ImageJ, National Institutes of Health, Wayne Rashand, 1997) that was used to calculate the surface area.

Figure 4.2 Example image of LD muscle surface area determination

Following the capturing of the image, the three rib cut was divided into the major tissue groups of bone, fat and muscle (Babiker et al., 1990; Mantiziba et al., 2014). A cut was made from the spine down to the ribs following the natural separation between muscle and bone. The LD muscle was then removed and separated from the subcutaneous fat layer where after the remaining bones were meticulously cleaned. The removed tissue was further divided and classified as muscle or fat. These groups were weighed individually and the three tissues were expressed as a percentage of the combined weight of the three tissues.

4.3   Statistical analysis

SAS Enterprise guide 9.2 was used to analyse the data and generate the statistics (Version 9.2, SAS Institute Inc., Cary, USA). The two trials were conducted separately and the data were therefore also analysed separately but in an identical manner. The data set was first tested for normality (Shapiro & Wilk, 1965) after which multiple Analyses of Variance (ANOVA) were performed following the general linear models (GLM) procedure. The weight of the animal at the start of the growth period was used as a covariate in each of the variables to correct for the block effect that might occur. The parameters that were tested for include: live weight prior to slaughter (LW), warm carcass weight (WCW), cold carcass weight (CCW) and dressing percentage, depicted in Table 4.1 and Table 4.2. In Table 4.3 and 4.4 summary statistics are given for the different body parts expressed as a percentage of live weight. Furthermore, the primal cut weights are expressed as a percentage of cold carcass weight in Tables 4.5 and 4.6 and carcass conformation data (three rib image analysis and tissue yields) is given in Table 4.7 and 4.8. Factors that were included into the analyses were sex (ram and wether for Trial 1/ wether and ewe for Trial 2), beta-agonist treatment for both trials (control, 110mg/kg, 135mg/kg and 160mg/kg) and also the interaction between main effects.

Fishers LSD comparison of LSMeans was chosen as the *post hoc* test where interactions were found to be significant. A significance level of 5% was chosen to report any differences, interactions or effects. The format in which the results are reported is LSMeans ± Standard Error of the mean (SEM).

4.4   Results and Discussion

This section contains data pertaining to the effect that sex and R-salbutamol inclusion level has, as well as possible interactions, on abattoir slaughter performance (Table 4.1 and 4.2), carcass classification scores (Table 4.3 and 4.4), non-carcass components (Table 4.5 and 4.6), carcass conformation based on primal cut division (Table 4.7 and 4.8) and carcass composition (Table 4.9 and 4.10) for both trials.

Table 4.1 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on the live weight (kg), warm carcass weight (kg), cold carcass weight (kg) and dressing percentage for Trial 1

Effect		LW (kg)	WCW (kg)	CCW (kg)	Dressing %
Sex					
	R	44.29 ± 0.31	22.68 ± 0.17	22.01 ± 0.17	49.89 ± 0.36
	W	41.86 ± 0.31	22.30 ± 0.18	21.62 ± 0.17	51.69 ± 0.36
	P-value	**< 0.0001**	0.138	0.121	**0.001**
Beta-agonist inclusion level across genders					
	0	42.63 ± 0.46	21.95 ± 0.25	21.30 ± 0.24	50.67 ± 0.50
	110	43.11 ± 0.42	22.70 ± 0.24	22.00 ± 0.23	51.03 ± 0.50
	135	42.98 ± 0.42	22.60 ± 0.24	21.93 ± 0.23	50.98 ± 0.50
	160	43.57 ± 0.42	22.72 ± 0.24	22.03 ± 0.23	50.49 ± 0.50
	P-value	0.491	0.079	0.085	0.850
Beta-agonist inclusion level between genders					
R	0	43.90 ± 0.65	21.80 ± 0.34	21.16 ± 0.33	49.29 ± 0.71
R	110	44.21 ± 0.60	23.12 ± 0.34	22.43 ± 0.33	50.68 ± 0.71
R	135	44.45 ± 0.61	22.98 ± 0.34	22.30 ± 0.33	50.10 ± 0.71
R	160	44.60 ± 0.60	22.84 ± 0.34	22.15 ± 0.33	49.50 ± 0.70
W	0	41.35 ± 0.64	22.09 ± 0.37	21.44 ± 0.35	52.05 ± 0.71
W	110	42.01 ± 0.60	22.29 ± 0.34	21.58 ± 0.33	51.38 ± 0.71
W	135	41.51 ± 0.61	22.25 ± 0.35	21.56 ± 0.33	51.86 ± 0.72
W	160	42.54 ± 0.60	22.60 ± 0.34	21.92 ± 0.33	51.49 ± 0.70
	P-value	0.886	0.346	0.313	0.541

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different ($p < 0.05$)
R: Rams
W: Wethers
LW: Live weight (kg)
WCW: Warm carcass weight (kg)
WCW: Cold carcass weight (kg)
Dressing %: Dressing percentage

In Chapter 3, sex clearly influenced feedlot parameters with rams exhibiting superior average daily gain (ADG) and feed conversion ratio (FCR) values. These factors directly influenced the superior live weight observed with rams. Rams (44.29 ± 0.31) weighed on average 2.43 kg heavier prior to slaughter when compared to wethers (41.86 ± 0.31). This significant difference (P < 0.0001) corroborates the findings of various other authors (Field, 1971; Crouse et al., 1981; Seideman et al., 1982; Arnold & Meyer, 1988; Dransfield et al., 1990). No differences were observed between the two genders for WCW (P = 0.138) and CCW (P = 0.121) but as expected wethers had a higher dressing percentage (P = 0.001) than rams (51.69 ± 0.36 and 49.89 ± 0.36, respectively). This confirms the presiding idea that castrating a male animal leads to superior dressing percentages. The reasoning behind this is that the developed sexual organs and thicker skins from mature male animals has a negative effect on dressing percentage (Prescott & Lamming, 1964; Lee, 1986; Butterfield, 1988).

No significant differences could be found for any of the parameters in Table 4.1 when comparing different inclusion levels of R-salbutamol. There were also no existing interactions between the main effects for any of the parameters and therefore the main effects were discussed individually. Supplementation with beta agonists such as zilpaterol hydochloride and ractopamine hydrochloride have been found to increase live weights, carcass weights and also dressing percentages over a wide range of livestock species (Apple et al., 2007; Estrada-Angulo et al., 2008a; Strydom et al., 2009; López-Carlos et al., 2010; Lopez-Carlos et al., 2011b; Webb & Allen, 2015). Marchant-Forde et al. (2012) embarked on the first testing of R-salbutamol as a pig feed additive and found an improvement in WCW and dressing percentages of treated animals but when Steenekamp (2014) tested the same beta-agonist, no effect on slaughter characteristics were observed.

Table 4.2 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on the live weight (kg), warm carcass weight (kg), cold carcass weight (kg) and dressing percentage for Trial 2

Effect		LW (kg)	WCW (kg)	CCW (kg)	Dressing %
Sex					
	E	42.38 ± 0.43	23.62 ± 0.23	22.91 ± 0.23	53.77 ± 0.39
	W	42.93 ± 0.43	23.72 ± 0.23	22.96 ± 0.22	53.98 ± 0.40
	P-value	0.378	0.784	0.885	0.730
Beta-agonist inclusion level across genders					
	0	43.54 ± 0.61	23.81 ± 0.32	23.09 ± 0.32	53.13 ± 0.55
	110	42.53 ± 0.61	23.74 ± 0.32	23.02 ± 0.31	54.18 ± 0.55
	135	41.91 ± 0.61	23.52 ± 0.33	22.75 ± 0.33	53.67 ± 0.55
	160	42.64 ± 0.61	23.59 ± 0.32	22.88 ± 0.31	54.52 ± 0.58
	P-value	0.316	0.921	0.883	0.322
Beta-agonist inclusion level between genders					
E	0	43.23 ± 0.86	23.62 ± 0.45	22.91 ± 0.45	53.08 ± 0.78
E	110	42.83 ± 0.87	23.82 ± 0.45	23.10 ± 0.45	53.97 ± 0.78
E	135	41.33 ± 0.87	23.37 ± 0.49	22.68 ± 0.48	53.43 ± 0.78
E	160	42.13 ± 0.86	23.66 ± 0.45	22.94 ± 0.44	54.59 ± 0.78
W	0	43.85 ± 0.86	24.01 ± 0.45	23.28 ± 0.45	53.17 ± 0.78
W	110	42.23 ± 0.86	23.66 ± 0.45	22.94 ± 0.45	54.38 ± 0.78
W	135	42.48 ± 0.87	23.68 ± 0.45	22.83 ± 0.45	53.90 ± 0.78
W	160	43.15 ± 0.86	23.51 ± 0.45	22.81 ± 0.44	54.45 ± 0.84
	P-value	0.738	0.893	0.921	0.979

E: Ewes
W: Wethers
LW: Live weight (kg)
WCW: Warm carcass weight (kg)
WCW: Cold carcass weight (kg)
Dressing %: Dressing percentage

Table 4.2 shows that there was no difference between the main effects or interactions for the abattoir slaughter parameters in Trial 2. Contradicting results regarding the slaughter performance of wethers and ewes have been published. Some researchers indicated that wethers will produce heavier carcasses than ewes with similar dressing percentages (Cloete et al., 2012a), whilst others indicated no difference at all between the two sexes (Lee et al., 1990b), and finally some state that wethers and ewes will weigh the same with the latter having superior dressing percentages (Vergara et al., 1999). In Chapter 3, it was already evident that

there were no differences between the wether and ewe trial animals for any of the discussed parameters and this trend continued through to this section. It is possible that, as with the rams and wethers of this trial, the young slaughter age and therefore not having reached sexual maturity, could explain the lack of difference between wethers and ewes and also because the circulating sexual hormone makeup in these two sexes are very similar (Hossner, 2005a). The main effect of beta-agonist inclusion level did not have an effect on any of the carcass weights or dressing percentages (Table 4.2).

Table 4.3 Carcass classification scores expressed as a percentage of the total number of carcasses in the group for Trial 1

Carcass classification (%)	A0	A1	A2	A3	A4	A5	A6
Sex							
R	0	0	50.0	39.3	3.6	7.1	0
W	0	0	71.4	25.0	3.6	0	0
Beta-agonist inclusion level across genders							
0	0	0	57.1	28.6	7.1	7.1	0
110	0	0	71.4	21.4	0	7.1	0
135	0	0	64.3	35.7	0	0	0
160	0	0	50.0	42.9	7.1	0	0

R: Rams
W: Wethers

Table 4.4 Carcass classification scores expressed as a percentage of the total number of carcasses in the group for Trial 2

Carcass classification (%)	A0	A1	A2	A3	A4	A5	A6
Sex							
E	0	0	53.6	35.7	7.1	3.6	0
W	0	0	17.9	35.7	21.4	10.7	14.3
Beta-agonist inclusion level across genders							
0	0	0	14.3	57.1	14.3	7.1	7.1
110	0	0	42.9	21.4	21.4	7.1	7.1
135	0	0	50.0	28.6	7.1	0	14.3
160	0	0	35.7	35.7	14.3	14.3	0

E: Ewes
W: Wethers

The majority of lamb carcasses marketed in South Africa will be of A2 or A3 classification. Tables 4.3 and 4.4 give a summary of the carcass classification scores obtained from Trial 1 and 2 respectively. Scores are expressed as a percentage of the total number of carcasses in the specific group. As can be seen, the highest percentages fall into the A2 and A3 groupings with a minor portion being present in the A4, A5 and A6 groups. With Dorper lambs being used in these trials, breed could have influenced the fact that some carcasses were produced with a higher fat classification. In a study by van der Westhuizen (2010) where various different sheep breeds were compared for slaughter parameters, it was found that Dorpers produced the fattest carcasses and this is mainly attributable to the early maturing characteristics of the breed.

In Trial 1 it would appear that rams had a higher proportion of carcasses that were classified as fatter compared to wethers. This contradicts the common knowledge that rams have leaner carcasses than wethers (Crouse et al., 1981; Dransfield et al., 1990; Cloete et al., 2007). The ewes in Trial 2 also had leaner classified carcasses than wethers which opposes the findings of Field et al. (1963) and Seebeck (1966). Carcass classification score is however not the only parameter available for measuring carcass fatness due to possible differences in fat deposition sites. Parameters such as omental fat (Tables 4.5 and 4.6), kidney fat (Tables 4.7 and 4.8), and three rib cut composition (Tables 4.9 and 4.10) can also be used as a measurement of quantifying carcass fatness.

For both Trial 1 and Trial 2 it appears that beta-agonist treatment had little to no effect on carcass classification score. Beta-agonists typically decrease the amount of fat in a carcass due to its influence on delaying fat deposition and increasing nutrient repartitioning towards protein synthesis (Mersmann, 1998), but this was not observed with R-salbutamol treatment in Tables 4.3 and 4.4.

Table 4.5 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on the weights of the head, trotters, skin, testicles (rams only), red offal, GIT and omental fat expressed as a percentage of the cold carcass weight for Trial 1

Effect		Head (%)	Trotters (%)	Skin (%)	Testicles (%)	Red offal (%)	GIT (%)	Omental fat
Sex								
	R	5.17 ± 0.07	2.14 ± 0.03	7.70 ± 0.14	0.40 ± 0.03	4.67 ± 0.07	22.17 ± 0.31	1.47 ± 0.06
	W	4.81 ± 0.07	2.17 ± 0.03	7.56 ± 0.14		4.30 ± 0.07	22.13 ± 0.31	1.73 ± 0.06
	P-value	**0.001**	**0.001**	0.503		**0.002**	0.947	**0.007**
Beta-agonist inclusion level across genders								
	0	4.95 ± 0.10	2.21 ± 0.04	7.71 ± 0.20	0.37 ± 0.06	4.66 ± 0.10	22.25 ± 0.43	1.70 ± 0.09
	110	4.96 ± 0.10	2.17 ± 0.04	7.67 ± 0.20	0.46 ± 0.06	4.55 ± 0.10	22.15 ± 0.43	1.55 ± 0.09
	135	4.91 ± 0.10	2.16 ± 0.04	7.37 ± 0.20	0.39 ± 0.06	4.40 ± 0.10	22.13 ± 0.43	1.67 ± 0.09
	160	5.13 ± 0.10	2.09 ± 0.04	7.77 ± 0.20	0.37 ± 0.06	4.35 ± 0.10	22.08 ± 0.43	1.47 ± 0.09
	P-value	0.432	0.849	0.479	0.658	0.129	0.993	0.261
Beta-agonist inclusion level between genders								
R	0	5.20 ± 0.65	2.21 ± 0.06	8.04 ± 0.28		4.85 ± 0.14	22.45 ± 0.62	1.43 ± 0.12
R	110	5.10 ± 0.60	2.16 ± 0.06	7.62 ± 0.28		4.79 ± 0.15	21.06 ± 0.62	1.50 ± 0.12
R	135	5.03 ± 0.61	2.16 ± 0.06	7.23 ± 0.28		4.63 ± 0.14	22.66 ± 0.62	1.57 ± 0.12
R	160	5.34 ± 0.60	2.04 ± 0.06	7.88 ± 0.28		4.43 ± 0.14	22.23 ±0.61	1.36 ± 0.12
W	0	4.70 ± 0.64	2.22 ± 0.06	7.38 ± 0.28		4.47 ± 0.14	22.16 ± 0.61	1.96 ± 0.13
W	110	4.83 ± 0.60	2.17 ± 0.06	7.71 ± 0.28		4.31 ± 0.14	22.96 ± 0.62	1.60 ± 0.12
W	135	4.79 ± 0.61	2.15 ± 0.06	7.50 ± 0.28		4.16 ± 0.14	21.59 ± 0.63	1.78 ± 0.13
W	160	4.92 ± 0.60	2.13 ± 0.06	7.65 ± 0.28		4.28 ± 0.14	21.92 ± 0.61	1.58 ± 0.12
	P-value	0.781	0.541	0.373		0.627	0.166	0.370

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different (p < 0.05)
R: Rams
W: Wethers
GIT: Gastro intestinal tract

There was no interaction between the treatment main effects for any of the non-carcass components in Table 4.5. The head component contributed significantly more to the 5th quarter weight in rams than wethers (5.17 ± 0.07 and 4.81 ± 0.07, respectively) and also red offal (P = 4.67 ± 0.07 and 4.30 ± 0.07, respectively) but smaller trotter (P = 2.14 ± 0.03 and 2.17 ± 0.03, respectively) and omental fat (P = 1.47 ± 0.06 and 1.73 ± 0.06 respectively) percentages. Skin percentage was not influenced by sex. The fact that the rams' heads were found to contribute more, can be ascribed to the phenomenon of sexual dimorphism (Butterfield, 1988). As male animals move towards reaching sexual maturity they tend to be depositing more weight in the forequarters than castrated males or females, which are inclined to deposit more weight in the hindquarters (Butterfield, 1988). Rams make use of head butting as a way of solving conflict, be it as a form of self-defense or competing for resources, and therefore as rams grow, this dimorphism will develop (Lande, 1980). The larger omental fat percentage of wethers is also in accordance with previous research (Crouse et al., 1981; Dransfield et al., 1990; Cloete et al., 2007).

Beta-agonist bind to the beta-receptors of which there are many different types and varying amounts present in different body tissues. The largest concentration of these receptors are present in skeletal muscle tissue and therefore the strongest effect will be observed in those tissues, as opposed to the tissues listed in Tables 4.5 and 4.6, where lower receptor concentrations will be found (Smith, 1998; Hossner, 2005b). No effect was observed with the administration of R-salbutamol on any of the non-carcass components in Table 4.5. This confirms the findings of Soto & Aguilera Soto (2008) where zilpaterol hydrochloride was the chosen beta-agonist. Macías-Cruz & Álvarez-Valenzuela (2010) reported that the head was the only non-carcass component that significantly increased in size due to zilpaterol hydrochloride treatment.

Table 4.6 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on the weights of the head, trotters, skin, red offal, GIT and omental fat expressed as a percentage of the cold carcass weight for Trial 2

Effect		Head (%)	Trotters (%)	Skin (%)	Red offal (%)	GIT (%)	Omental fat (%)
Sex							
	E	4.38 ± 0.06	2.00 ± 0.03	6.95 ± 0.12	4.21 ± 0.07	21.26 ± 0.33	1.99 ± 0.09
	W	4.54 ± 0.06	2.19 ± 0.03	6.93 ± 0.12	4.31 ± 0.07	21.16 ± 0.34	1.68 ± 0.09
	P-value	0.050	**< 0.0001**	0.802	0.341	0.857	**0.013**
Beta-agonist inclusion level across genders							
	0	4.36 ± 0.08	2.06 ± 0.04	7.08 ± 0.17	4.30 ± 0.10	21.37 ± 0.49	1.90 ± 0.43
	110	4.48 ± 0.08	2.09 ± 0.04	6.84 ± 0.17	4.27 ± 0.10	20.52 ± 0.47	2.00 ± 0.43
	135	4.52 ± 0.08	2.07 ± 0.04	6.84 ± 0.18	4.26 ± 0.11	21.49 ± 0.47	1.71 ± 0.43
	160	4.48 ± 0.08	2.15 ± 0.04	6.99 ± 0.17	4.22 ± 0.10	21.48 ± 0.47	1.73 ± 0.43
	P-value	0.531	0.439	0.721	0.966	0.393	0.284
Beta-agonist inclusion level between genders							
E	0	4.27 ± 0.65	1.99 ± 0.05	7.34^a ± 0.24	4.31 ± 0.14	21.94 ± 0.66	22.45 ± 0.62
E	110	4.42 ± 0.60	2.01 ± 0.05	7.12^{ab} ± 0.24	4.26 ± 0.14	20.34 ± 0.66	21.06 ± 0.62
E	135	4.43 ± 0.61	1.98 ± 0.05	6.54^b ± 0.26	4.20 ± 0.16	21.90 ± 0.66	22.66 ± 0.62
E	160	4.40 ± 0.60	2.01 ± 0.05	6.80^{ab} ± 0.24	4.08 ± 0.14	20.88 ± 0.66	22.23 ±0.61
W	0	4.45 ± 0.64	2.14 ± 0.05	6.82^{ab} ± 0.24	4.28 ± 0.14	20.79 ± 0.72	22.16 ± 0.61
W	110	4.54 ± 0.60	2.17 ± 0.05	6.55^b ± 0.24	4.28 ± 0.14	20.70 ± 0.66	22.96 ± 0.62
W	135	4.60 ± 0.61	2.17 ± 0.05	7.15^{ab} ± 0.24	4.31 ± 0.14	21.08 ± 0.66	21.59 ± 0.63
W	160	4.55 ± 0.60	2.29 ± 0.06	7.19^{ab} ± 0.24	4.37 ± 0.14	22.09 ± 0.66	21.92 ± 0.61
	P-value	0.995	0.641	**0.034**	0.711	0.277	0.599

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different (p < 0.05)
E: Ewes
W: Wethers
GIT: Gastro intestinal tract

Interaction between the main effects were observed for the skin percentage (P = 0.034) parameter (Table 4.6). Here the highest percentage contribution towards the 5th quarter weight for the skin component originated from E0 (7.34 ± 0.24) individuals, intermediate percentages from E110 (7.10 ± 024), E160 (6.80 ± 0.24), W0 (6.82 ± 0.24), W135 (7.15 ± 0.24), W160 (7.19 ± 0.24) individuals and the lowest from W110 (±) individuals. The intermediate groups did not significantly differ from the light or heavy groups but the light and heavy groups did differ from each other. The reason for this interaction is still unclear and warrants further research, providing it can be justified.

For the remaining non-carcass components, there were only sex differences for the trotter (P < 0.0001) and omental fat (P = 0.013) contributions. The trotter components of wethers amounted to a percentage of 2.19 ± 0.03 compared to the ewe's 2.00 ± 0.03. This could possibly be explained by the research of Lee (1986) and Butterfield (1988) that state that castrated male animals will have a larger skeletal structure than female animals and it is important to note that the majority of the trotters are made up of a bone component. Previous research state that ewes can produce fatter carcasses (Field et al., 1963; Seebeck, 1966) and this can also be explained by the clear differences in omental fat between ewes and wethers (1.99 ± 0.09 and 1.68 ± 0.09, respectively). These aforementioned findings, suggesting that a castrated male animal has a larger bone and smaller fat component, will be confirmed in Table 4.9 where a summary of the carcass composition is given. Castrating a male animal will have an effect on the masculinity and as a result reduce the sexual dimorphism effect, associated with intact animals, consequently creating a sex that is more similar to a female animal in hormonal composition. Therefore, the observed similarity in head component percentage between wethers (4.54 ± 0.06) and ewes (4.38 ± 0.06) was expected. Red offal percentages also did not differ between sexes (P = 0.341).

As previously mentioned in the discussion of Table 4.5, research pertaining to the effects that beta-agonists have on non-carcass components are limited due to the fact that there are few beta-adrenergic receptors present in these tissues (Smith, 1998; Hossner, 2005b). No effect was observed with the administration of R-salbutamol on any of the non-carcass components in Table 4.6.

Table 4.7 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on the carcass conformation weights expressed as a percentage of the cold carcass weight for Trial 1

Effect		Kidneys (%)	Kidney fat	Neck (%)	Shoulders	Ribs (%)	Back (%)	Fore shank (%)	Hind legs (%)	Shins (%)
Sex										
	R	0.59 ± 0.01	1.78 ± 0.11	4.03 ± 0.12	16.35 ± 0.23	10.44 ± 0.13	18.47 ± 0.16	13.50 ± 0.22	33.72 ± 33.45	2.38 ± 0.09
	W	0.55 ± 0.01	2.15 ± 0.10	3.75 ± 0.12	16.33 ± 0.23	10.61 ± 0.13	19.59 ± 0.16	12.36 ± 0.22	33.45 ± 0.23	2.26 ± 0.09
	P-value	**0.006**	**0.012**	0.133	0.901	0.347	**0.0001**	**0.001**	0.404	0.366
Beta-agonist inclusion level across genders										
	0	0.58 ± 0.01	2.48[a] ± 0.14	4.07 ± 0.17	16.38[a] ± 0.32	10.56 ± 0.10	19.03 ± 0.43	12.72 ± 0.30	32.94 ± 0.32	2.51 ± 0.12
	110	0.57 ± 0.01	1.88[b] ± 0.15	3.75 ± 0.17	16.92[a] ± 0.31	10.70 ± 0.10	19.31 ± 0.43	12.51 ± 0.30	33.45 ± 0.32	2.15 ± 0.12
	135	0.56 ± 0.01	1.92[b] ± 0.14	3.84 ± 0.17	16.59[a] ± 0.31	10.28 ± 0.10	18.93 ± 0.43	12.99 ± 0.30	34.20 ± 0.32	2.14 ± 0.12
	160	0.56 ± 0.01	1.59[b] ± 0.15	3.91 ± 0.17	15.48[b] ± 0.31	10.56 ± 0.10	18.86 ± 0.43	13.49 ± 0.30	33.76 ± 0.32	2.47 ± 0.12
	P-value	0.700	**0.001**	0.587	**0.015**	0.396	0.504	0.129	0.053	0.057
Beta-agonist inclusion level between genders										
R	0	0.64[a] ± 0.02	2.09 ± 0.20	4.39 ± 0.24	16.83 ± 0.45	10.40 ± 0.25	18.66 ± 0.31	13.18 ± 0.43	32.84 ± 0.45	2.54 ± 0.17
R	110	0.59[b] ± 0.02	1.73 ± 0.22	3.71 ± 0.24	17.24 ± 0.45	11.00 ± 0.25	18.72 ± 0.31	12.77 ± 0.42	33.12 ± 0.45	2.44 ± 0.17
R	135	0.58[b] ± 0.02	2.00 ± 0.20	4.05 ± 0.24	16.03 ± 0.45	10.16 ± 0.25	18.01 ± 0.31	13.73 ± 0.43	34.72 ± 0.45	2.11 ± 0.17
R	160	0.55[bc] ± 0.02	1.29 ± 0.22	3.95 ± 0.24	15.32 ± 0.44	10.22 ± 0.25	18.48 ±0.31	14.32 ± 0.42	34.20 ± 0.45	2.43 ± 0.17
W	0	0.51[c] ± 0.02	2.86 ± 0.20	3.74 ± 0.24	15.94 ± 0.45	10.73 ± 0.25	19.39 ± 0.31	12.26 ± 0.42	33.04 ± 0.45	2.48 ± 0.17
W	110	0.56[bc] ± 0.02	2.02 ± 0.22	3.79 ± 0.24	16.59 ± 0.45	10.40 ± 0.25	19.90 ± 0.31	12.26 ± 0.43	33.79 ± 0.45	1.87 ± 0.17
W	135	0.55[bc] ± 0.02	1.84 ± 0.20	3.63 ± 0.24	17.16 ± 0.45	10.41 ± 0.25	19.84 ± 0.31	12.26 ± 0.43	33.68 ± 0.46	1.17 ± 0.17
W	160	0.57[bc] ± 0.02	1.88 ± 0.20	3.86 ± 0.24	15.64 ± 0.44	10.90 ± 0.25	19.24 ± 0.33	12.66 ± 0.42	33.31 ± 0.45	1.51 ± 0.17
	P-value	**0.006**	0.118	0.410	0.100	0.082	0.265	0.519	0.174	0.192

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different (p < 0.05)
R: Rams
W: Wethers

The phenomenon of heavier forequarters in male sheep is typical of sexual dimorphism (Butterfield, 1988). The fact that this trend is not clearly notable in all of the primal cuts could be a result of the young slaughter age and that a more distinct post-pubertal growth pattern separation had not yet been completed. The proportions of the neck, shoulders, ribs, hind legs and shins did not differ between genders ($P > 0.05$) (Table 4.7). There were however observed differences for kidneys ($P = 0.006$), kidney fat ($P = 0.012$), saddle ($P = 0.0001$) and fore shank ($P = 0.001$) between sexes. The main effect of sex for the parameter of kidney yield will not be interpreted due to the existing interaction ($P = 0.006$) between the main effects. Even though the differences for kidney contributions between treatments were small, it is important to note that it was still significant and that the majority of the significance can possibly be ascribed to gender differences rather than beta-agonist supplementation. The kidneys from the R0 (0.64 ± 0.02) individuals contributed the most towards the CCW, followed by the R110 (0.59 ± 0.02) and R135 (0.58 ± 0.02) individuals, then the R160 (0.55 ± 0.02), W110 (0.56 ± 0.02) and W135 (0.55 ± 0.02) individuals and finally the least from the W0 (0.51 ± 0.02) individuals. Wethers had a higher proportion kidney fat than rams (2.15 ± 0.10 and 1.78 ± 0.10, respectively) and similarly so for the saddle cut (19.59 ± 0.16 and 18.47 ± 0.16, respectively) but a lower fore shank proportion (12.36 ± 0.22 and 13.50 ± 0.22, respectively). This partially confirms the findings of Kemp et al (1970), stating that the fore shank and the kidney fat were the only two primal cut components that were affected significantly by sex in the same manner ($P < 0.05$ and $P < 0.01$, respectively).

Only kidney fat ($P = 0.001$) and shoulder components ($P = 0.015$) differed between beta-agonist treatment groups. The control group (2.48 ± 0.14) had a significantly larger kidney fat proportion contribution than the 110 (1.88 ± 0.15), 135 (1.92 ± 0.14) and 160 (1.59 ± 0.15) groups. This is possibly due to R-salbutamol decreasing fat synthesis (Mersmann, 1998) and confirms the results of Estrada-Angulo et al (2008). Previous research state that beta-agonist tend to increase the size of some of the posterior primal cuts such as the hind quarter and ribs but decrease the size of anterior cuts such as the shoulder, neck and fore quarter (Macías-Cruz & Álvarez-Valenzuela, 2010). Table 4.7 show that none of the remaining primal cuts were influenced by R-salbutamol except the shoulder ($P = 0.015$). The highest inclusion level at 160mg had the smallest proportion (15.48 ± 0.31) whilst the shoulders from the control, 110mg and 135 groups contributed the most to the CCW (16.38 ± 0.32, 16.92 ± 0.31 and 16.59 ± 0.31, respectively). It would appear that only at a high inclusion level would there be an influence on protein repartitioning (shoulder size change), but fat synthesis depression (kidney fat) can be influenced over a wider inclusion range of R-salbutamol.

Table 4.8 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on the carcass conformation weights expressed as a percentage of the cold carcass weight for Trial 2

Effect		Kidneys (%)	Kidney fat	Neck (%)	Shoulders	Ribs (%)	Back (%)	Fore shank (%)	Hind legs (%)	Shins (%)
Sex										
	E	0.52 ± 0.01	2.47 ± 0.12	3.53 ± 0.07	14.68 ± 0.19	10.76 ± 0.17	18.91 ± 0.23	13.29 ± 0.18	34.71 ± 33.45	2.14 ± 0.04
	W	0.52 ± 0.01	2.06 ± 0.12	3.25 ± 0.07	15.31 ± 0.19	11.13 ± 0.17	18.92 ± 0.23	13.62 ± 0.18	33.95 ± 0.23	2.24 ± 0.04
	P-value	0.725	**0.021**	**0.013**	**0.026**	0.128	0.901	0.188	0.074	0.136
Beta-agonist inclusion level across genders										
	0	0.54 ± 0.01	2.34 ± 0.17	3.37 ± 0.10	14.75 ± 0.27	11.35 ± 0.23	19.25 ± 0.34	13.56 ± 0.25	33.85 ± 0.42	2.21 ± 0.06
	110	0.50 ± 0.01	2.54± 0.17	3.22 ± 0.11	14.88 ± 0.27	10.84 ± 0.23	18.93 ± 0.32	13.58 ± 0.25	34.22 ± 0.42	2.11 ± 0.06
	135	0.52 ± 0.01	2.00 ± 0.17	3.58 ± 0.10	15.21 ± 0.27	10.47 ± 0.23	18.33 ± 0.32	13.60 ± 0.25	35.09 ± 0.42	2.15 ± 0.06
	160	0.53 ± 0.01	2.19 ± 0.17	3.39 ± 0.10	15.15 ± 0.27	11.12 ± 0.23	19.16 ± 0.32	13.09 ± 0.25	34.16 ± 0.42	2.29 ± 0.06
	P-value	0.347	0.172	0.127	0.587	0.058	0.204	0.418	0.199	0.206
Beta-agonist inclusion level between genders										
E	0	0.54 ± 0.02	2.39 ± 0.25	3.60 ± 0.15	14.56 ± 0.39	11.11 ± 0.33	19.46 ± 0.50	13.50 ± 0.35	33.91[bc] ± 0.59	2.25 ± 0.09
E	110	0.53 ± 0.02	2.92 ± 0.25	3.41 ± 0.15	14.70 ± 0.39	10.83 ± 0.33	18.97 ± 0.46	13.52 ± 0.35	33.96[bc] ± 0.59	2.03 ± 0.09
E	135	0.52 ± 0.02	2.25 ± 0.25	3.61 ± 0.15	14.84 ± 0.39	10.49 ± 0.33	18.67 ± 0.46	13.13 ± 0.35	35.25[ab] ± 0.59	2.08 ± 0.09
E	160	0.50 ± 0.02	2.34 ± 0.25	3.51 ± 0.15	14.63 ± 0.39	10.64 ± 0.33	18.54 ±0.46	12.99 ± 0.35	35.71[a] ± 0.59	2.21 ± 0.09
W	0	0.54 ± 0.02	2.29 ± 0.25	3.14 ± 0.15	14.94 ± 0.39	11.59 ± 0.33	19.05 ± 0.46	13.62 ± 0.35	33.78[bc] ± 0.59	2.16 ± 0.09
W	110	0.48 ± 0.02	2.16 ± 0.25	3.03 ± 0.16	15.06 ± 0.39	10.86 ± 0.33	18.90 ± 0.46	13.63 ± 0.35	34.49[ab] ± 0.59	2.19 ± 0.09
W	135	0.51 ± 0.02	1.75 ± 0.25	3.56 ± 0.15	15.58 ± 0.39	10.45 ± 0.33	17.98 ± 0.46	14.06 ± 0.35	34.92[ab] ± 0.59	2.23 ± 0.09
W	160	0.59 ± 0.02	2.04 ± 0.25	3.28 ± 0.15	15.66 ± 0.39	11.61 ± 0.33	19.77 ± 0.46	13.19 ± 0.35	32.60[c] ± 0.59	2.37 ± 0.09
	P-value	0.113	0.595	0.536	0.794	0.397	0.181	0.596	**0.019**	0.378

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different ($p < 0.05$)
E: Ewes
W: Wethers

An interaction occurred between the main effects for hind leg contribution. Explaining this interaction is complicated due to the lack of identifiable trend. The biggest proportional contribution was observed in the E160 individuals, but this also did not differ from E135, W110 or W135 individuals. Intermediate contributions came from E110 and W0 individuals, which did not differ from the three previously mentioned groups and similarly so not from the W160 individuals.

Sex had no influence on the proportion of kidneys (P = 0.725), ribs (P = 0.128), saddle (P = 0.901) or shins (P = 0.136). It did however influence the proportions of the kidney fat (P = 0.021), neck (P = 0.013) and shoulders (P = 0.026). Ewes (2.47 ± 0.12) produced a higher proportion kidney fat than wethers (2.06 ± 0.12), which supports the idea that wethers are leaner that ewes when slaughtered at the same weight (Field et al., 1963; Seebeck, 1966). The neck (3.53 ± 0.07 and 3.25 ± 0.07, respectively) and shoulder (14.68 ± 0.19 and 15.31 ± 0.19 respectively) cuts also differed between ewes and wethers.

R-salbutamol had no effect on any of primal cut yields in Trial 2.

Table 4.9 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on the three rib cut composition for Trial 1

Effect		Loin surface area (cm2)	Bone (%)	Muscle (%)	Fat (%)
Sex					
	R	20.79 ± 0.55	23.27 ± 0.40	37.58 ± 0.17	39.01 ± 0.72
	W	20.84 ± 0.55	22.30 ± 0.40	37.27 ± 0.17	40.24 ± 0.72
	P-value	0.969	0.105	0.714	0.247
Beta-agonist inclusion level across genders					
	0	19.97 ± 0.77	22.78 ± 0.55	36.50 ± 0.80	40.62 ± 1.00
	110	21.10 ± 0.76	22.17 ± 0.55	37.12 ± 0.80	40.13 ± 1.00
	135	21.03 ± 0.76	23.13 ± 0.55	38.48 ± 0.80	38.27 ± 1.00
	160	21.15 ± 0.76	23.06 ± 0.55	37.61 ± 0.80	39.49 ± 1.00
	P-value	0.655	0.597	0.356	0.380
Beta-agonist inclusion level between genders					
R	0	19.73 ± 1.09	23.57 ± 0.79	35.74 ± 0.33	40.60 ± 1.42
R	110	21.70 ± 1.08	22.16 ± 0.78	37.81 ± 0.33	39.69 ± 1.41
R	135	20.88 ± 1.09	23.93 ± 0.79	39.24 ± 0.33	36.70 ± 1.42
R	160	20.86 ± 1.08	23.41 ± 0.78	37.54 ± 0.33	39.06 ± 1.41
W	0	20.22 ± 1.08	21.99 ± 0.78	37.25 ± 0.35	40.63 ± 1.41
W	110	20.51 ± 1.09	22.18 ± 0.79	36.43 ± 0.33	40.58 ± 1.42
W	135	21.18 ± 1.10	22.34 ± 0.79	37.72 ± 0.33	39.83 ± 1.44
W	160	21.44 ± 1.08	22.70 ± 0.78	37.68 ± 0.33	39.92 ± 1.41
	P-value	0.826	0.686	0.501	0.724

R: Rams
W: Wethers

There were no significant interactions between main effects of any of the parameters in Table 4.9 or 4.10 and as a result, the main effects will be discussed individually.

The three rib cut analysis have been used as an accurate determination of carcass composition for many years and it describes the ratio in which the main tissues (bone, muscle and fat) are present in a carcass (Berg & Butterfield, 1968). Hankins & Howe (1946) attempted to quantify the accuracy of this method and found significant correlations when predicting total bone ($r = 0.53$), total muscle ($r = 0.83$) and total fat ($r = 0.91$) in a dressed beef carcass. There are various factors influencing the tissue ratios of which include sex, plane of nutrition and growth agents, age of maturity and slaughter weight (Kemp et al., 1970, 1976; Murphy et al., 1994; Johnson et al., 2005; Steenekamp, 2014).

Castrating a male animal would influence the rate at which the aforementioned tissues develop due to circulating sex hormone levels being altered through this process and these play a major role in the growth rate and also growth pattern regulation in animals (Hossner, 2005a). In contradiction to this Table 4.9 shows that there were no differences between the carcass compositions of rams and wethers for this trial. In Table 4.4 it was shown that a bigger proportion of ram carcasses were graded with a high fat score than wether carcasses which contradicts previous research indicating that rams produce leaner carcasses (Crouse et al., 1981; Dransfield et al., 1990; Cloete et al., 2007). In contradiction to Table 4.4, it was observed that Tables 4.5 and 4.7 illustrate wethers had a significantly larger proportion omental fat and kidney fat components. It is possible that a combination of these aforementioned arguments, that indicate a difference in fat deposition sites, and biological variance, could influence the lack of difference seen between sexes for three rib cut composition. Sex also did not influence ($P = 0.969$) the loin surface area and neither did beta-agonist treatment ($P = 0.655$) (Table 4.9).

Beta-agonists administration have been shown to increase the LD surface area (López-Carlos et al., 2010), but as mentioned, no differences were found with R-salbutamol treatment for this parameter. With beta-agonists mainly having an effect on protein turnover and decreasing fat synthesis (Mersmann, 1998) it can be expected to have an profound effect on the carcass composition tissue yield (Apple et al., 2007; López-Carlos et al., 2010), but this was not observed with R-salbutamol in this trial. The carcass composition parameters of bone ($P = 0.597$), muscle ($P = 0.356$) and fat ($P = 0.380$) were not influenced.

Table 4.10 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on the carcass composition for Trial 2

Effect		Loin surface (cm^2)	Bone (%)	Muscle (%)	Fat (%)
Sex					
	E	20.51 ± 0.37	20.04 ± 0.43	35.92 ± 0.46	43.81 ± 0.76
	W	21.01 ± 0.37	21.95 ± 0.43	37.32 ± 0.46	40.56 ± 0.76
	P-value	0.354	**0.003**	**0.039**	**0.004**
Beta-agonist inclusion level across genders					
	0	20.37 ± 0.53	20.79 ± 0.61	35.94 ± 0.65	43.04 ± 1.08
	110	21.59 ± 0.53	20.45 ± 0.61	36.95 ± 0.65	42.38 ± 1.08
	135	21.01 ± 0.53	21.50 ± 0.61	37.83 ± 0.65	40.51 ± 1.08
	160	20.07 ± 0.53	21.23 ± 0.61	35.76 ± 0.65	42.81 ± 1.08
	P-value	0.189	0.626	0.104	0.343
Beta-agonist inclusion level between genders					
E	0	20.14 ± 1.09	19.68 ± 0.86	35.11 ± 0.93	44.91 ± 1.52
E	110	20.56 ± 1.08	18.95 ± 0.89	35.51 ± 0.93	45.27 ± 1.53
E	135	21.05 ± 1.09	21.00 ± 0.86	37.15 ± 0.93	41.72 ± 1.53
E	160	20.29 ± 1.08	20.49 ± 0.86	35.92 ± 0.92	43.34 ± 1.52
W	0	20.60 ± 1.08	21.90 ± 0.86	36.77 ± 0.93	41.17 ± 1.52
W	110	22.61 ± 1.09	21.92 ± 0.86	38.39 ± 0.93	39.50 ± 1.52
W	135	20.96 ± 1.10	22.00 ± 0.86	38.51 ± 0.93	39.30 ± 1.53
W	160	19.85 ± 1.08	21.98 ± 0.86	35.60 ± 0.92	42.28 ± 1.52
	P-value	0.362	0.690	0.395	0.466

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different (p < 0.05)
E: Ewes
W: Wethers

The carcass composition comparison based on sex confirms the findings of previous research done on these parameters (Field et al., 1963; Seebeck, 1966). Significant sex based differences were found for the percentage yields of bone (P = 0.003), muscle (P = 0.039) and fat (P = 0.004). Wethers had a bone yield percentage of 21.95 ± 0.43 compared to the ewes' 20.51 ± 0.37. When looking at lean muscle yield, the wethers had a more favorable composition than ewes. The muscle vs fat distribution percentages were observed to be 37.32 ± 0.46 and 40.56 ± 0.76 for wethers compared to the 35.92 ± 0.46 and 43.81 ± 0.76 of the ewes. For all the "fat based" parameters in Tables 4.6, 4.8 and 4.10, the ewes appeared to be fatter than the wethers, despite the fact that a higher proportion of carcasses were classed

above fat code 4 (Table 4.4). No differences (P = 0.354) were observed for loin surface area between these two genders (20.51 ±0.37 for ewes and 21.01 ± 0.37 for wethers)

It is typically expected that beta-agonist treatment would significantly influence carcass composition by decreasing the proportion fat and increasing the muscularity in the carcass but this was not reflected in the results for Trial 2. R-salbutamol had no influence on the percentage bone (P = 0.626), muscle (P = 0.104) or fat (P = 0.343) in Trial 2 (Table 4.9). The same lack of effect can be seen for loin surface area (P = 0.189).

4.5   Conclusion

In this study, sex had an influence on most of the discussed parameters. The most important differences that need mentioning can be that of live weights, dressing percentages and carcass composition. In Trial 1, rams were heavier than wethers and had lower dressing percentages but no differences were observed between carcass compositions although minor differences did occur between primal cuts and non-carcass components. In Trial 2 no differences were observed for slaughter weights and dressing percentages but wethers had a more ideal carcass composition compared to ewes.

The results from Chapter 3 already indicated that R-salbutamol had limited effects on feedlot performance. This trend seems to continue in Chapter 4 where it is further illustrated that R-salbutamol has limited effect on the carcass and non-carcass components in of this study. It did not influence any of the slaughter weights or dressing percentages, carcass composition values or non-carcass component weights. The only differences were observed for the primal cuts of kidneys and shoulders. It would appear that at a high inclusion level R-salbutamol would influence protein repartitioning (shoulder size change), and fat synthesis depression (kidney fat) can be influenced over a wider inclusion range of R-salbutamol.

4.6   References

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Chapter 5

The influence of R-Salbutamol on meat quality characteristics and descriptive sensory profile in Dorper ram, ewe and wether lambs

Abstract

The measurements and *M. longissimus dorsi* (LD) meat samples that were necessary to generate the results for this chapter were taken from feedlot lambs which formed part of two 2 x 4 factorial arrangement of treatments growth trials. In Trial 1, 28 ram and 28 wether lambs were finished off under feedlot conditions whilst receiving one of four randomly allocated diets. These diets contained different inclusion levels of the beta-agonist R-salbutamol (0mg/kg, 110mg/kg, 135mg/kg and 160mg/kg feed). Trial 2 was designed in an identical manner, with the only differences being wether and ewe lambs were used. Meat samples were evaluated by a trained sensory panel for aroma, flavour and texture attributes whilst physical measurements, including Warner-Bratzler shear force (WBSF) values, were also recorded. In Trial 1, the sensory panel could only distinguish between rams and wethers for lamb meat aroma with rams being the favoured sex. WBSF values revealed that meat from rams were more tender than that of wethers. Furthermore, in Trial 2 the meat from wethers were more tender than that of ewes. R-salbutamol were found to have limited influence on the meat quality traits across both trials. The 110mg significantly decreased WBSF values in Trial 1. Even though the sensory panel scored the 110mg and control groups to be equally the most tender, it is still interesting to note that the higher tenderness value was observed in the 110mg groups. On the other hand, the 160mg treatment group had less tender meat than the control. R-salbutamol did not influence any of the remaining aroma and flavour profile parameters in Trial 1. In Trial 2 the only improvement that was noted, was that the 135mg treatment decreased cooking loss percentage.

5.1   Introduction

Lamb meat has become a more expensive protein source over the last two decades but still remains an important source of red meat for the growing population of South Africa (DAFF, 2013). The Dorper breed contributes a significant portion to the amount of meat produced annually and is well known as a hardy and extensive, free-range adapted breed but also performs well in intensive feedlot systems (Cloete et al., 2000). These feedlot systems are

known to increase production efficiency of an animal by supplying better quality feed and enabling the producer to reduce or even eliminate other external factors that might have a negative impact on production performance (Webb & Erasmus, 2013b). The producer can furthermore make use of growth agents such as beta-agonists to improve growth rates, feed usage efficiency and manipulate carcass conformations according to consumer specifications (Strydom, 2016). These compounds achieve this objective primarily via means of nutrient repartitioning characteristics that result in leaner carcasses but with generally tougher meat being produced (Aalhus et al., 1990; Apple et al., 2007; Leheska et al., 2009; Avendaño-Reyes et al., 2011). Another factor that needs to be taken into account when considering the quality of the meat that will be produced is the sex of the animal that it originates from. As with feedlot performance and carcass characteristics, various differences can be expected for meat quality characteristics between the sexes of rams, wethers and ewes. Furthermore, interactions between these sexes and the aforementioned beta-agonists growth agents on meat quality characteristics possibly exist and knowing what these are place the producer in a profitable position.

When buying a lamb meat product, the consumer would have first assessed the product based on cues such as colour, leanness and perceived value for money as well as wholesomeness but the main determinant for rebuying the product will be factors such as tenderness, juiciness, flavour and aroma (Lawrie et al., 2006; Webb, 2013).

These abovementioned attributes are influenced by various intrinsic factors of which the tenderness is primarily influenced by the amount and type of connective tissue present in the meat (Boucek et al., 1961; Goll et al., 1963). Juiciness can be described as the release of moisture from the piece of meat during the process of chewing, and can be influenced by the quantity of water and also intramuscular fat present (Dryden & Maechello, 1970). A derivative of juiciness is the concept of sustained juiciness and this is particularly influenced by the intramuscular fat which is known for stimulating saliva production. Webb & O'Neill (2008) observed that the consumer correlates this juiciness to the tenderness of the meat.

Furthermore, the flavour and aroma profile of a meat sample will be influenced by the fatty acids present in it (Webb & O'Neill, 2008) and a combination of the non-volatile amino-acids, volatile compounds as well as reducing sugars and nucleotides that are released during the heating process (AMSA, 1995). As mentioned, intensive feedlot systems and the usage of beta-agonists enable an animal to reach slaughter weight at a younger age. This would mean that meat from an animal like this would have less connective tissue (collagen), with fewer cross bindings, resulting in more tender meat, but the fact that a beta-agonist was used could have a detrimental effect on meat tenderness (Brand et al., 2013). The main theory that describes the mechanism responsible for this meat toughening is that beta-agonists can

influence the activity of specific proteolytic enzymes present in the calpain system. The calpain system is responsible for the turnover of myofibril proteins (natural protein degradation), which is a major group of proteins in striated muscles, and where calpastatin functions as a calpain inhibitor (Goll *et al.,* 1992). Then, with beta-agonists having been found to increase the activity of calpastatin and as a result decreasing the calpain activity, it can be expected that meat toughness will increase (Mersmann, 1998; Strydom et al., 2009, 2011).

Previous studies where R-salbutamol has been used in feedlot cattle (Steenekamp, 2014) rations it was found to have no negative effects on meat quality parameters and on a point of interest, no research can be found investigating the possible influence of this beta-agonist in rations fed to Dorper ram, wether and ewe lambs and their meat quality.

5.2   Materials and Methods

The research project was approved by the Research Ethics Committee: Animal Care and Use of Stellenbosch University (SU-ACUD15-00082).   Handling of the animals proceeded according to South African National Standards 10386: 2008. The trials were conducted from August 2015 to January 2016 at the Welgevallen Experimental Livestock Unit in Stellenbosch (Stellenbosch, Western Cape, South Africa).

The project consisted of two individual trials that were completed one after the other. Both trials were conducted in an identical manner and hence, for the purpose of simplifying explanations, the experimental procedures for only one trial will be described. It is therefore important to keep in mind that the following explanations covers a single trial and will be repeated for the second trial. The only difference between the two trials was that in Trial 1 intact males (rams) and physically castrated males (wethers) were used, and in Trial 2, physically castrated males (wethers) and intact females (ewes) were used. The beta-agonist treatment levels were similar for both trials.

The data that were used to generate the results for this chapter was collected after the completion of the sample collection period for the feedlot performance (Chapter 3), and carcass quality (Chapter 4) sections of the research project.

5.2.1   Feeding, animals, husbandry and experimental design

The adaptation program (summarised in Table 3.5) as well as the experimental feeds (Tables 3.1 – 3.4) that were used during the trial period are discussed in detail in section 3.2.1 of Chapter 3.   Information regarding the trial animals that were used, scientific protocol procedures during the anti-mortem stage of the trial and experimental design can be found in section 3.2.2 of Chapter 3.

5.2.2 Measurements taken at the slaughter facility and successive carcass characteristics determination

After 28 days of receiving the four experimental diets in the feedlot system all lambs were weighed, loaded and transported to a commercial abattoir (Tomis® abattoir, Hermon, Western Cape, South Africa). Explanations pertaining to slaughter procedures, sample collections and carcass composition determination can be found in sections 4.2.2 – 4.2.4 of Chapter 4.

5.2.3 Physical measurements taken for meat quality analysis

Before entering the cooling room at the abattoir, the pH of the *M. longissimus dorsi* (LD) was measured between the eleventh and thirteenth rib at 45 min and again 24h *post mortem* (McGeehin et al., 2001) using a Crison pH 25 portable pH meter (Lasec Pty Ltd, South Africa). Following the three rib cut preparation (as described in section 4.2.3 of Chapter 4), the remaining portion of both the left and right LD was removed from the lumbar area, vacuum sealed and kept at 4°C for further meat quality analyses such as cooking loss, Warner-Bratzler shear force (WBSF) measurements and a Descriptive Sensory Analysis (DSA). The DSA was performed on the meat from Trial 1 only wherein rams were compared to wethers.

5.2.4 Sensory analysis

A tasting panel, consisting of 10 judges with relevant experience, was further trained to detect and score specific attributes associated with lamb meat. Training was completed over a period of three days according to standard sensory meat analysis guidelines (AMSA, 1995). For these training sessions the left side LD was used. Before each tasting session, the specified LD muscles were taken out of the vacuum bag, weighed and placed inside an oven bag (Glad®, Clorox Africa Pty Ltd.) with a thermocouple probe attached to a handheld temperature monitor (Hanna Instruments, South Africa) and which was inserted in the centre of the muscle. The oven bag with the LD muscle and temperature probe was then placed inside a 160°C preheated oven (Hobart, France). The sample was later removed from the oven once it was observed that an internal temperature of 72°C had been reached (AMSA, 1995). After a cooling period of 10 minutes the sample was weighed and this weight value in combination with the precooking weight was used to calculate cooking loss percentage (Honikel, 1998). The outer edge of each sample was then trimmed and 1cm^3 blocks were cut out of the muscle. These blocks were individually wrapped in 16cm^2 tinfoil squares and each judge received two wrapped samples. One sample would be used to evaluate the aroma profile as well as initial juiciness score and the second sample was for flavour profile and texture attributes. The samples were placed in glass ramekins and were covered with a petri dish. For the training period the ramekins were clearly marked to allow easy identification of the sample but during the DSA period the ramekins were marked with randomised three-digit codes. Before serving

the judges, the ramekins with the samples inside were heated in an oven at 70°C for 10 minutes and then placed in ceramic cups in a water bath which was also preheated to 70°C. From there each judge could retrieve the ramekins.

The training for the DSA was done on seven reference standard samples, as shown in Table 5.1 and after completion of the training, the judges were able to identify and score a range of aroma, flavour and texture attributes for the DSA, further shown in Table 5.1.

Table 5.1 Sensory attribute detection sheet as well as the reference samples used during training sessions.

Attribute	Description	Reference Sample
Aroma	Impression after a few short sniffs when opening the package (0=None, 100=Prominent)	
Lamb Meat	Aroma associated with cooked lean lamb muscle	Lamb LD muscle
Beefy	Aroma associated with cooked lean beef muscle	Beef fillet steak
Sweet associated	Sweet, sugary aroma from meat	Beef fillet steak
Fatty	Aroma associated with cooked lamb fat	Lamb fat
Flavour	Impression after a few short sniffs when opening the package (0=None, 100=Prominent)	
Lamb Meat	Flavour associated with cooked lean lamb muscle	Lamb LD muscle
Beefy	Flavour associated with cooked lean beef muscle	Beef fillet steak
Sweet associated	Sweet or caramelised sugar flavours from meat	Beef fillet steak
Fatty	Lingering taste or mouth-feel associated with cooked fat	Lamb fat
Metallic	Flat flavour similar to metal coins	Ostrich meat and liver
Texture		
Initial juiciness	Amount of fluid exuded when pressed between thumb and index finger. (0= Dry, 100= Juicy)	Chicken breast and beef forequarter meat
Sustained juiciness	Impression of juiciness formed after first 5 chews using molar teeth. (0= Dry, 100= Juicy)	Chicken breast
Tenderness	Impression of tenderness formed after first 5 chews using molar teeth. (0= Tough, 100= Tender)	Chicken breast and beef forequarter meat
Residue	Amount of residue left in mouth after 10 chews using molar teeth. (0= None, 100= Abundant)	Chicken breast and beef forequarter meat

The DSA took place over three days consisting of 7 sessions. Each session consisted of assessing 1 of each of the 8 treatment combinations (right side LD muscle). Therefore, each judge had to score 8 unknown samples on the aforementioned sensory attributes. The tastings took place at the Food Science Department of Stellenbosch University, South Africa and each panellist was seated in individual judging booths with a desktop computer containing the Compusense® five (Compusense, Guelph, Canada) software. Each panellist received the samples in a completely randomised order and was also supplied with fresh apple slices, water biscuits and still water to cleanse their palates between tastings.

The Warner-Bratzler shear force measuring was used as a means of assessing the objective tenderness of the meat samples. Approximately half of each cooked meat sample was used for the DSA session, and the remaining half was then allowed a resting period of 24 hours (at 4°C) before it was cut up into 1cm x 2cm x 1cm rectangles, to be used for the WBSF determination. From each LD sample a total of 7 rectangles were cut and used for further analysis. The cut was made parallel to the meat fibres to facilitate that the Instron Universal testing machine (Instron model 4444/H1028, Appollo Scientific cc, South Africa) with its 1mm thick V-notch shaped Warner Bratzler attachment cuts the sample perpendicular to the meat fibres. The shear force measuring was done at a speed of 200mm/min with a load cell set at 2 kN and expressed as a Newton (N) value. The mean of the 7 readings was used for further statistical analysis.

5.3   Statistical analysis

SAS Enterprise guide 9.2 was used to analyse the data and generate the statistics (Version 9.2, SAS Institute Inc., Cary, USA).   The data set was first tested for normality (Shapiro & Wilk, 1965) after which multiple Analyses of Variance (ANOVA) were performed following the general linear models (GLM) procedure. The parameters that were tested for include: pH_{45} taken at 45 min post mortem, pH_{24} taken at 24 hours post mortem, WBSF values and cooking

loss percentage, depicted in Table 5.2 and Table 5.3. In Table 5.4 summary statistics is given of the results from the DSA for Trial 1. Analysis of the sensory attributes was done on a line scale, ranging from 0 (low) to 100 (intense). Before running various statistical analyses on the DSA data, the raw data sets were first tested for panel reliability. The model contained factors for panellist, session, treatment effects and treatment interactions (Næs et al., 2010):

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

- μ = overall mean
- b_i = effect due to session
- t_j = effect due to treatment
- bt_{ij} = interaction effect between session and treatment
- p_k = effect due to panellist
- bp_{ik}= interaction effects between panellist and session
- tp_{jk} = interaction effects between panellist and treatment
- ε_{ijk} = the random error associated with the response of panellist k on treatment j in session I

Factors that were included into the analyses were sex (ram and wether for Trial 1), beta-agonist treatment (control, 110mg/kg, 135mg/kg and 160mg/kg) and also the interaction between main effects.

Fishers LSD comparison of LSMeans was chosen as the *post hoc* test where interactions were found to be significant. A significance level of 5% was chosen to report any differences, interactions or effects. The format in which the results are reported is LSMeans ± Standard Error of the mean (SEM).

5.4   Results and Discussion

This section contains data pertaining to the effect that sex and R-salbutamol inclusion level has, as well as possible interactions, on pH_{24}, pH_{45}, cooking loss percentage and Warner-Bratzler shear Force (WBSF) values (Table 5.2 and 5.3) for both trials and on sensory attributes (Table 5.4) tested for in Trial 1.

Table 5.2 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on pH_{45}, pH_{24}, cooking loss percentage and Warner-Bratzler shear Force (WBSF) values for Trial 1

Effect		pH_{45}	pH_{24}	Cooking loss (%)	WBSF value (Nm)
Sex					
	R	6.31 ± 0.07	5.84 ± 0.02	32.92 ± 0.91	28.28 ± 1.08
	W	6.78 ± 0.07	5.74 ± 0.01	33.68 ± 0.91	31.60 ± 1.08
	P-value	**< 0.0001**	**< 0.0001**	0.561	**0.036**
Beta-agonist inclusion level across genders					
	0	6.50 ± 0.09	5.81 ± 0.02	32.56 ± 1.29	30.27^b ± 1.53
	110	6.50 ± 0.09	5.79 ± 0.02	32.88 ± 1.29	25.88^a ± 1.53
	135	6.47 ± 0.09	5.76 ± 0.02	33.49 ± 1.29	30.81^b ± 1.53
	160	6.73 ± 0.09	5.79 ± 0.02	34.25 ± 1.29	32.81^b ± 1.53
	P-value	0.172	0.545	0.803	**0.021**
Beta-agonist inclusion level between genders					
R	0	6.30 ± 0.13	5.88 ± 0.03	31.74 ± 1.83	29.31 ± 2.17
R	110	6.26 ± 0.13	5.84 ± 0.03	32.93 ± 1.83	25.43 ± 2.17
R	135	6.17 ± 0.13	5.78 ± 0.03	34.30 ± 1.83	28.39 ± 2.17
R	160	6.52 ± 0.13	5.84 ± 0.03	32.70 ± 1.83	29.98 ± 2.17
W	0	6.69 ± 0.13	5.73 ± 0.03	33.37 ± 1.83	31.22 ± 2.17
W	110	6.74 ± 0.13	5.74 ± 0.03	32.84 ± 1.83	26.33 ± 2.17
W	135	6.77 ± 0.13	5.74 ± 0.03	32.69 ± 1.83	33.22 ± 2.17
W	160	6.95 ± 0.13	5.74 ± 0.03	35.80 ± 1.83	35.64 ± 2.17
	P-value	0.864	0.317	0.603	0.649

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different ($p < 0.05$)
R: Rams
W: Wethers

Looking at pH_{45} ($P< 0.0001$), it is possible to say that the wethers (6.79 ± 0.07) experienced the slaughter process to be more stressful than the rams (6.31 ± 0.07). The slaughter process (including handling, traveling and lairage at the abattoir) can be perceived as a highly stressful event and could therefore have a result on the animal's metabolism. A stressful event like this would activate the hypothalamic-pituitary- adrenal activity, and increase stress hormones, which in turn will result in a depletion of the animal's glycogen stores, thus resulting in an elevated pH and reduced muscle to meat conversion (Lawrie et al., 2006). Okeudo & Moss (2008) however found that there were no significant differences for pH_{45} between rams (6.51) and wethers (6.45). The same researcher also found that pH_{24} did

not differ between the two sexes (5.73 for rams and 5.67 for wethers). Even though the pH_{24} differences between rams and wethers were small there were still a significant difference (5.84 ± 0.02 and 5.74 ± 0.01, respectively) (Trial 1).

There are conflicting reports regarding the correlation between pH and tenderness (for WBSF values as well as tenderness scores by a DSA). Some report that a high ultimate pH value (pHu >6.0) can be associated with higher shear force values (Devine et al., 1993). Young et al. (1993) found that the relationship between the two parameters are curvilinear but authors such as Hoffman et al. (2003) and Safari et al. (2001) found no significant relationship. Therefore, based on previous research, and even though the pH_{24} of the rams (5.84 ± 0.02) were higher ($P < 0.0001$) than that of the wethers (5.74 ± 0.01), the pH_{24} still falls into the normal range of pH for sheep, but the WBSF values of rams (28.28 ± 1.08 N) were lower ($P = 0.036$) than that of the wethers (31.60 ± 1.08N), it cannot be categorically stated that there was a positive correlation in this trial. No difference ($P = 0.561$) for cooking loss percentage between sexes were observed and confirms previous findings (Okeudo & Moss, 2008).

No difference was found for pH_{45} when R-salbutamol was fed to feedlot cattle (Steenekamp, 2014). Steenekamp (2014) also found that zilpaterol hydrochloride treated animals had a significantly lower pH_{24} when compared to that of the R-salbutamol group. The two beta-agonist treatment groups did however not differ significantly from the control group. Table 5.2 confirms this lack of difference between beta-agonist treatment groups and the control for pH_{45} and pH_{24} (Trial 1). Similarly, no difference in cooking loss percentage was observed in this trial or that of Steenekamp (2014). It is interesting to note that the 110 (25.88 ± 1.53) group had significantly lower WBSF values compared to the control (30.27 ± 1.35), 135 (30.81 ± 1.53) and 160 (32.81 ± 1.53) groups. As previously mentioned, there were no differences for either of the pH measurements between the treatment groups and therefore it is unlikely that the rate of proteolysis (affected by pH decline), which is responsible for post mortem meat tenderisation, is affected by the beta-agonist. The explanation would therefore have to lie within ante mortem factors such as the beta-agonist having a more prominent effect on lipolysis and less on muscle metabolism, but the mechanism responsible for this increased tenderness is still unclear and warrants further research. Typically beta-agonists increase the WBSF values of treated animals (Leheska et al., 2009; Rathmann et al., 2009; Strydom et al., 2009).

However, in Trial 2 when ewes were compared to wethers, there were interactions noted, and treatment effects on these same meat quality measurements (Table 5.3).

Table 5.3 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on pH_{45}, pH_{24}, cooking loss percentage and Warner-Bratzler shear Force (WBSF) values for Trial 2

Effect		pH_{45}	pH_{24}	Cooking loss	WBSF value
Sex					
	E	6.70 ± 0.05	5.39 ± 0.02	32.86 ± 0.54	40.11 ± 1.24
	W	6.45 ± 0.05	5.39 ± 0.02	32.68 ± 0.54	33.72 ± 1.24
	P-value	**0.001**	0.952	0.811	**0.001**
Beta-agonist inclusion level across genders					
	0	6.48[bc] ± 0.07	5.37 ± 0.02	34.66[a] ± 0.76	37.50 ± 1.76
	110	6.44[c] ± 0.07	5.39 ± 0.02	32.72[ab] ± 0.76	32.69 ± 1.76
	135	6.67[ab] ± 0.07	5.43 ± 0.03	30.79[b] ± 0.76	38.25 ± 1.76
	160	6.72[a] ± 0.07	5.39 ± 0.02	32.92[ab] ± 0.76	39.20 ± 1.76
	P-value	**0.005**	0.336	**0.010**	0.057
Beta-agonist inclusion level between genders					
E	0	6.91[ab] ± 0.11	5.38 ± 0.03	34.96 ± 1.08	39.28 ± 2.48
E	110	6.81[ab] ± 0.09	5.40 ± 0.03	32.32 ± 1.08	36.03 ± 2.48
E	135	6.62[bc] ± 0.10	5.43 ± 0.04	30.52 ± 1.08	42.34 ± 2.48
E	160	6.46[c] ± 0.09	5.38 ± 0.03	33.10 ± 1.08	42.78 ± 2.48
W	0	6.06[d] ± 0.09	5.37 ± 0.03	34.70 ± 1.08	35.73 ± 2.48
W	110	6.06[d] ± 0.09	5.38 ± 0.03	33.12 ± 1.08	29.35 ± 2.48
W	135	6.72[abc] ± 0.10	5.43 ± 0.03	31.06 ± 1.08	34.17 ± 2.45
W	160	6.97[a] ± 0.09	5.39 ± 0.03	31.84 ± 1.08	35.62 ± 2.48
	P-value	**< 0.0001**	0.985	0.511	0.809

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different (p < 0.05)
E: Ewes
W: Wethers

In Table 5.3 it can be seen that there was an interaction (P < 0.0001) between the main effects for pH_{45}. The pH values of all 8 treatment combinations falls within the range of 6.06 – 6.97 and even though the differences were significant, it is difficult to identify a clear trend. The W160 group had the highest pH reading, followed by E0 and E110 groups. The last mentioned two groups did not differ from the W160, W135 or E135 groups. Furthermore, the W135 also didn't differ from the W160, E160, E135, E110 or E0 groups. W110 and W0 groups did however differ significantly from the other 6 treatment groups and as a result had the lowest pH_{45} readings. It is possible that, similarly to the two sexes in Trial 1, the ewes perhaps experienced the slaughter procedure to be more stressful and therefore had a higher

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pH_{45} value (Trial 2), but the main effects cannot be interpreted individually due to the above-mentioned interaction. This would have further supported the argument that a high pH_{45} can influence meat tenderness negatively because the ewes (40.11 ± 1.24 N), with a higher pH_{45} were also found to produce meat that was significantly less tender than that of wethers (33.72 ± 1.24 N). Typically the meat from wethers are less tender than that of ewes (Hopkins et al., 2007b; Okeudo & Moss, 2008). No differences were noted for pH_{24} in this trial or that of previous research (Okeudo & Moss, 2008). Cooking loss percentage from wethers have furthermore been found to be higher than that of ewes (Hopkins et al., 2007b; Okeudo & Moss, 2008), but in this trial no differences were observed (P = 0.811).

Supporting the work by Steenekamp (2014) and Strydom et al. (2009), no differences between pH_{24} values of the beta-agonist treatment groups were observed. R-salbutamol inclusion appears to decrease the cooking loss percentage. The 135 (30.79 ± 0.76%) had the lowest percentage and differed significantly from the highest value which was observed in the control (34.66 ± 0.76%) group. The 110 (32.72 ± 0.76) and 160 (32.92 ± 0.76) groups did not differ from either of the previously mentioned groups. WBSF values did not differ significantly between treatment groups and this contradicts the general idea that beta-agonists increase the WBSF values of treated animals (Leheska et al., 2009; Rathmann et al., 2009; Strydom et al., 2009).

Table 5.4 The LSMeans ± SEM for the main effects of sex and beta-agonist inclusion level and interaction on the aroma, flavour and texture attributes as observed in the DSA section for Trial 1

Effect	Lamb meat Aroma	Beefy Aroma	Sweet associated Aroma	Fatty Aroma	Lamb meat Flavour	Beefy Flavour	Sweet associated Flavour	Fatty Flavour	Metallic taste	Initial juiciness	Sustained juiciness	Tenderness	Residue
Sex													
R	77.88±0.25	29.98±0.08	9.99±0.01	10.14±0.12	73.08±0.19	30.04±0.03	10.30±0.05	18.28±0.14	4.79±0.32	37.70±0.65	37.16±0.56	42.95±1.07	29.42±0.95
W	76.84±0.25	29.85±0.08	10.01±0.01	10.16±0.12	72.55±0.19	30.07±0.03	10.32±0.05	17.97±0.14	4.43±0.32	37.09±0.65	36.25±0.56	40.36±1.07	31.86±0.95
P-value	**0.005**	0.270	0.277	0.875	0.060	0.452	0.724	0.124	0.437	0.511	0.257	0.093	0.079
Beta-agonist inclusion level across genders													
0	76.92±0.35	29.87±0.12	10.01±0.01	10.12±0.16	72.67±0.28	30.08±0.04	10.17±0.08	18.29±0.20	4.11±0.46	37.77±0.92	38.27±0.80	43.35^{ab}±1.51	29.75^{bc}±1.35
110	77.37±0.35	29.95±0.12	9.98±0.01	10.18±0.16	72.55±0.28	30.06±0.04	10.38±0.08	18.16±0.20	4.48±0.46	36.67±0.92	36.58±0.80	45.82^{a}±1.51	27.13^{c}±1.35
135	77.30±0.35	29.92±0.12	10.00±0.01	10.18±0.16	72.91±0.28	30.03±0.04	10.37±0.08	17.91±0.20	4.71±0.46	36.99±0.92	36.17±0.80	40.33^{bc}±1.51	31.39^{ab}±1.35
160	77.85±0.35	29.93±0.12	10.01±0.01	10.12±0.16	73.12±0.28	30.07±0.04	10.31±0.08	18.14±0.20	5.14±0.46	38.15±0.92	35.80±0.80	37.12^{c}±1.51	34.29^{a}±1.35
P-value	0.330	0.964	0.221	0.983	0.474	0.890	0.202	0.595	0.453	0.651	0.149	**0.001**	**0.005**
Beta-agonist inclusion level between genders													
R 0	77.06±0.50	30.01±0.17	10.01±0.02	10.06±0.23	73.43±0.39	30.09±0.06	10.15±0.11	18.57±0.28	3.43±0.65	38.04±1.30	39.76±1.13	46.74±2.13	27.20±1.91
R 110	77.86±0.50	29.94±0.17	9.98±0.02	10.07±0.23	72.94±0.39	30.01±0.06	10.52±0.11	18.10±0.28	4.47±0.65	37.06±1.30	36.13±1.13	47.29±2.13	25.87±1.91
R 135	78.18±0.50	30.05±0.17	9.98±0.02	10.27±0.23	72.80±0.39	30.02±0.06	10.26±0.11	18.06±0.28	5.52±0.65	36.98±1.30	36.60±1.13	40.48±2.13	30.76±1.91
R 160	78.40±0.50	29.94±0.17	10.02±0.02	10.14±0.23	73.14±0.39	30.05±0.06	10.26±0.11	18.38±0.28	5.55±0.65	38.72±1.30	36.15±1.13	37.30±2.13	33.87±1.91
W 0	76.77±0.50	29.73±0.17	10.01±0.02	10.17±0.23	71.91±0.39	30.06±0.06	10.20±0.11	18.00±0.28	4.79±0.65	37.49±1.30	36.78±1.13	39.96±2.13	32.30±1.91
W110	76.89±0.50	29.97±0.17	9.98±0.02	10.29±0.23	72.15±0.39	30.10±0.06	10.25±0.11	18.22±0.28	4.30±0.65	36.29±1.30	37.02±1.13	44.35±2.13	28.38±1.91
W135	76.41±0.50	29.79±0.17	10.03±0.02	10.10±0.23	73.02±0.39	30.04±0.06	10.48±0.11	17.75±0.28	3.90±0.65	37.00±1.30	35.73±1.13	40.19±2.13	32.02±1.91
W160	77.29±0.50	29.92±0.17	10.00±0.02	10.09±0.23	73.09±0.39	30.09±0.06	10.37±0.11	17.90±0.28	4.74±0.65	37.58±1.30	35.45±1.13	36.94±2.13	34.71±1.91
P-value	0.532	0.717	0.125	0.833	0.126	0.790	0.158	0.605	0.150	0.975	0.406	0.392	0.680

[a,b] LSMeans within columns for the main effects with different superscripts are significantly different (p < 0.05)
R: Rams
W: Wethers

The summary statistics (Table 5.4) derived from the DSA data show that there were no significant interactions between the main effects for any of the parameters. Main effects will therefore be discussed individually. As pertaining to sex, rams (77.88 ± 0.25) had a significantly stronger lamb meat aroma compared to wethers (76.84 ± 0.25). Previous studies have also shown that ram lamb meat are preferred over that of wethers, due to a favoured aroma profile (Dransfield et al., 1990). Furthermore, no differences were observed in any of the other parameters for the main effect of sex. Claasen (2008) also found no sex based differences for the parameters of initial juiciness, sustained juiciness and aroma profile in Dorper lambs. The aforementioned study did however find that the meat from wethers were perceived to be more tender than that of rams and also more flavoursome.

Beta-agonist have been seen to negatively influence the meat quality in treated animals. Strydom et al. (1998) and Schroeder et al. (2003) reported that beta-agonist treatment resulted in a lower sensory rating for tenderness, juiciness and flavour parameters. This trial also found that R-salbutamol had significant effects on tenderness and residue ratings. For both of these parameters scoring was done on a scale of 0-100. For tenderness a higher score is preferable and for the residue parameter a lower score is desired. In Table 5.4 it can be seen that the 110 (45.82 ± 1.51) group was the most tender but not more so than the control (43.35 ± 1.51). The least tender meat sample was found in the 160 (37.12 ± 1.51) group. Referring back to the WBSF values in Table 5.2, the 110 group was also observed to produce the most tender meat. As pertaining to residue, once again the 110 (27.13 ± 1.35) group was the favoured sample, even though it did not differ significantly from the control (29.75 ± 1.35). The highest residue rating was given to the 160 (34.29 ± 1.35) group. It appears that there is a negative relationship between tenderness and residue scores for beta-agonists in Trial 1. No further differences were seen for any of the sensory attributes.

5.5   Conclusion

In Trial 1 rams had lower pH_{45} values but a higher reading for pH_{24} when compared to wethers. Rams surprisingly also had lower WBSF values but this tenderness was not noticeable in the DSA. Lamb meat aroma was the only parameter where the tasting panel could accurately distinguish between the two sexes with rams being the favoured. The only meat quality characteristic where a differences was observed between wethers and ewes, was with WBSF values; wethers produced significantly more tender meat than ewes.

In Trial 1 it was observed that when R-salbutamol was fed at a rate of 110mg/kg feed, that it would significantly increase the tenderness of meat based on WBSF values and also sensory analysis. R-salbutamol inclusion at 135mg/kg feed, in Trial 2, appeared to significantly decrease the cooking loss percentage compared to the control.

Thus within the current study, sex and R-salbutamol treatment did not have an influence on all the physical meat quality characteristics as well as the sensory profile, but the results found are nonetheless evident that further research is required into the sensory properties of lamb meat resulting from feeding R-salbutamol.

5.6   References

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CHAPTER 6

General conclusion and recommendations

The aim of this study was to evaluate the effect of R-salbutamol on growth, carcass characteristics and meat quality traits in commercial feedlot lambs comprising of three sexes. This study showed that R-salbutamol had an effect on some of the parameters.

The trial was designed to simulate optimal commercial lamb feedlot conditions during the growth period and minimise stress prior to slaughter, thus eliminating any negative impact *anti mortem* handling could have on meat quality.

Results from Trial 1 indicate that rams would clearly be the better choice of sex in a feedlot system. As expected, the wethers had a lower average daily gain (ADG) value and were less efficient at utilising the feed compared to the rams. This inevitably influenced the various slaughter performance parameters. Rams were heavier than wethers and had lower dressing percentages but no differences were observed between carcass compositions, although minor differences did occur between primal cuts and non-carcass components. Rams surprisingly also had lower Warner-Bratzler shear force (WBSF) values but this tenderness was not noticeable in the descriptive sensory analysis (DSA), where similar scores were given. Lamb meat aroma was the only parameter where the tasting panel could accurately distinguish between the two sexes, with rams being the favoured.

During the feedlot performance testing period of Trail 2 no differences were noticed between wethers and ewes. Furthermore, slaughter weights and dressing percentages were similar but wethers had a more ideal and leaner carcass composition compared to ewes. WBSF values also revealed that, for this trial, wethers produced more tender meat.

Beta-agonist treatment had limited effects on the abovementioned parameters. In Trial 1 the 110mg inclusion level treatment group exhibited a superior feed conversion ratio (FCR) compared to the control group. The other two treatment groups (135mg and 160mg inclusion levels) did however not differ significantly from either the control or 110mg treatment. R-salbutamol had no noticeable influence on the rest of the feedlot performance parameters in Trial 1. All inclusion levels were found to suppress of kidney fat deposition but only at 160mg did it decrease the shoulder size. It would appear that at a high inclusion level R-salbutamol would influence protein repartitioning (shoulder size change), whilst kidney fat synthesis can be influenced over a wider inclusion range of R-salbutamol.

It was furthermore observed that at an inclusion level of 110mg, meat would be significantly more tender based on WBSF values. Even though, in the DSA, the tenderness scores for 110mg and control did not differ, it is still interesting to note that the higher tenderness value was observed in the 110mg groups. On the other hand, the 160mg treatment group had less tender meat than the control. R-salbutamol did not influence any of the remaining aroma and flavour profile parameters in Trial 1.

No differences between inclusion levels were observed for any of the feedlot performance or carcass characteristics traits in Trial 2. The only improvement that was noted, was that the 135mg treatment decreased cooking loss percentage.

The effect that R-salbutamol has on feedlot performance, carcass characteristics and meat quality traits appears to be varied or minor, with the exception of the 110mg treatment showing the most promise. Results from this investigation can be used to further develop an effective dosage of R-salbutamol in lamb feedlot diets. It is possible that different inclusion levels that are localised around the 110mg inclusion level or a change in treatment time may further influence production figures.

If further testing of this product is embarked on and positive results are found, it will also be necessary to perform an economic comparison study that can establish whether the improvement will be enough to justify the inclusion of R-salbutamol to a feedlot finishing diet.