Effects of dietary zilpaterol hydrochloride and a non-steroidal growth implant on growth and carcass characteristics of feedlot lambs

By

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I declare that this thesis for the degree M.Sc. (Agric) Production Physiology at the University of Pretoria, has not been submitted by me for a degree at any other university.

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List of abbreviations

ADG	- Average daily gain
β – agonists	- Beta – agonists
β – AR	- Beta – adrenergic receptors
cAMP	- Cyclic adenosine monophosphate
CREB	- cAMP response element binding protein
DES	- Diethylstilbestrol
DMI	- Dry matter intake
EU	- European Union
FAO	- Food and Agriculture Organisation
FDA	- Food and Drug Administration
IGF-1	- Insulin growth factor
LM	- *Longissimus muscle*
MGA	- Melengestrol acetate
NR	- No zeranol (Ralgro®) treatment
NZ	- No zilpaterol hydrochloride treatment
R	- Zeranol (Ralgro®) treatment
SCF	- Subcutaneous fat thickness
ST	- Somatotropin
SRF	- Somatotropin releasing factor
TBA	- Trenbolone Acetate
WHO	- World Health Organisation
ZH	- Zilpaterol hydrochloride
Z	- Zilpaterol hydrochloride treatment

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Abstract

The purpose of this research was to investigate the effect of zilpaterol hydrochloride (ZH) in combination with a non-steroidal growth implant on growth and carcass characteristics of feedlot lambs. The use of ZH is common in commercial beef production, however is not yet registered for use in lamb production without a veterinary prescription on an extra label basis. Lamb producers are looking to a more intensive production system for sheep, which requires a finishing phase and therefore warrants the research of growth promotants that can be used in this phase to increase efficiency.

In this study 180 South African Mutton Merino male-type lambs were selected at a commercial feedlot. After the growing phase 20 lambs were excluded from the group and 160 lambs were used for the finishing phase. Half of the lambs were implanted with zeranol marketed under the name Ralgro® (MSD), a non-steroidal, oestrogen like compound. The lambs were randomly put into 8 groups with different treatments, to test the different combinations of ZH, zeranol and duration of treatment. ZH was either fed for 18 days with a 3 day withdrawal period or for 25 days with a 3 day withdrawal period. The lambs were slaughtered and carcasses were chilled for 3 days before samples were taken and analysed.

ZH had an effect on average daily gain (ADG), cold carcass mass and tenderness (P <0.05). ZH tended to have an effect on hide weight and cooking loss (P <0.10). The lambs treated with ZH grew 16.7g/day more than the control group and had 0.51kg heavier carcasses than the control group. Zeranol significantly affected ADG and cold carcass mass (P <0.05). Zeranol tended to influence subcutaneous fat thickness (SCF) of the 10th rib (P <0.10). Lambs implanted with zeranol grew 18.8g/day more than the control and had 0.35kg heavier cold carcasses.

Duration of treatment significantly affected cold carcass mass, hide weight, pH at 45 minutes post mortem, pH after chilling, fat thickness, tenderness and SCF of the 8th rib (P <0.05). Duration of treatment had a tendency to influence cooking loss, with a longer time on treatment causing more loss (P <0.10).

There were a number of interactions. ZH and zeranol (Z*R) caused significant interactions in ADG, cold carcass mass and hide weight (P <0.05). Z*R also tended to cause interactions in pH after chilling (P <0.10). Zeranol and duration of treatment (R*D) significantly caused an interaction in pH after chilling (P <0.05) and ZH and duration of treatment tended to cause an interaction in pH after chilling (P <0.10). ZH, zeranol and

duration of treatment caused a significant interaction in cold carcass mass (P <0.05) and tended to cause an interaction in cooking loss (P <0.10).

ZH improves ADG, as well as cold carcass mass and did not influence carcass characteristics, probably due to a low dosage. ZH increases the sheer force values of lamb post slaughter and has no practical influence on cooking loss. Feeding ZH for a longer duration also causes tougher meat. Carcass composition did not differ significantly due to ZH treatment as expected, because ZH is known to be a repartitioning agent. ZH has better results in increasing ADG and cold carcass mass, when lambs were implanted with zeranol during the growing phase. The combination of ZH and zeranol seems to have an additive affect on ADG and cold carcass mass. Using Zeranol in combination with ZH for 25 days proved to yield the best results for both ADG and cold carcass mass. ZH tended to decrease the hide weight and therefore shows the repartitioning effect of ZH. A follow up study on different dosage levels of ZH can be helpful to determine if ZH has an effect on carcass composition in lamb.

Chapter 1. Introduction and Motivation

1.1. Project Theme

Animal Production Physiology

1.2. Project Title

Effects of dietary zilpaterol hydrochloride and a non-steroidal growth implant on growth and carcass characteristics of feedlot lambs

1.3. Aim

1. To study the influence of zilpaterol hydrochloride with and without zeranol on growth in male type feedlot lambs
2. To study the influence of zilpaterol hydrochloride with and without zeranol on carcass characteristics in male type feedlot lambs
3. To study the influence of zilpaterol hydrochloride fed to male type lambs with and without zeranol on tenderness and cooking loss after slaughter

1.4. Motivation

Animal scientists need to find more sustainable and efficient ways of producing meat for the growing world population (Roughgarden, 1979). The world's population has an annual growth rate of 1.73%. It is estimated that by the year 2025 there will be approximately 8.5 billion people and is thought to level off in the year 2150 with 11.6 billion people (Daily & Ehrlich, 1992). By the year 2050, the agricultural sector has to increase production by 60% across all sectors (FAO, 2012). It is imperative that the agricultural sector find responsible and sustainable ways of providing food to meet the demands of the growing world population.

The meat industry is expected to grow considerably, because it is a strategic source of protein in the human diet (Montossi *et al*., 2013). Lamb and mutton production are expected to increase by 22% by 2021 due to demand from developing countries (Montossi *et al*., 2013; FAO, 2012). This projected increase in food supply places more emphasis on the need for new growth technologies that promote efficient and affordable meat production. The rate of animal growth and production can be accelerated with a decrease in resources

required, including feed and water, and by the use of modern technology, namely with growth enhancing technology. Growth enhancing technology lead to increases in efficiency, improved carcass composition and live weight gains and can enhance carcass quality (Dunshea, 2005; Barham *et al*., 2003). These advances in technology not only affect the proficiency of the producer, but also the processing, because an improvement in carcass composition, with a resultant increased lean meat yield makes meat packaging more efficient.

The relevance of this present study to the lamb feedlotting industry is the effects of growth technologies on growth, carcass composition and certain meat characteristics. These changes are important in the light of the growing demand for improved efficiency and increased production, mentioned above. The combination of non-steroidal implants and zilpaterol hydrochloride (ZH) has not been investigated in lambs before.

Growth promotants are metabolic modifiers, which are defined as compounds that are fed, injected or implanted in animals to increase feed efficiency, gain, improve the dressing percentage and the carcass meat yield percentage. These compounds can improve the visual meat quality, extend the shelf-life, increase the meats nutritional level or improve meat palatability (Dikeman, 2007). Steroid implants are the best available non-nutritive tool that a producer can use to increase both biological and economical efficiency in livestock (Preston, 1987). Anabolic steroids are steroids which behave like sex hormones to promote growth of the animal. The anabolic effect causes weight gain and generally causes more lean meat development and lower fat content (Hancock et al., 1991). Non-steroidal growth promotants are also used commercially in both sheep and cattle. Non-steroidal growth promotants have a positive effect on growth rate and improved feed efficiency (Perry, 1970).

Beta – agonists (β – agonists) are also growth promotants and can be referred to as repartitioning agents (Ricks *et al*., 1984). In South Africa and Mexico a registered β – agonist, for use in cattle, is Zilmax®. Zilmax® was registered for commercial beef production in 1995 in South Africa. β – agonists stimulate beta – adrenergic receptors (β - AR) on the cell surface and cause protein synthesis and inhibit proteolysis, which in turn encourages cell hyperplasia. The overall response is an improvement in growth efficiency, and a considerable increase in slaughter percentage, making β – agonists an economical product to use (Courtheyn et. al., 2002).

The use of ZH in lamb production is still in its infancy and the US Food and Drug Administration (FDA) has not yet approved its use for commercial lamb production. Lamb

was typically produced on an extensive system in South Africa and didn't warrant a fattening phase in the production process. However, in South Africa more producers are looking to an intensive fattening phase, due to lower growth performance on extensive systems, unpredictable weather, stock theft and a demand for specific carcass grades. According to Montossi *et al*. (2013): the use of accurate technologies that can increase productivity and efficiency with less labour per unit sheep, will play an important role in the future of sheep production. ZH supplementation in lambs is similar to beef, with increases in growth performance, carcass yields and carcass characteristics (Avendaño-Reyers *et al*., 2011; Lopez *et al*., 2011; Lopez *et al*., 2010; Estrada-Angulo *et* al., 2008). There is still very limited information and inconsistencies on the effects, use and efficacy of ZH supplementation on growth performance, carcass yields and carcass characteristics of feedlot lambs, and therefore requires further research (Lopez-Carlos *et al*., 2011; Estrada-Angulo *et al*., 2008; Nourozi *et al*., 2008).

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Chapter 2. Literature Review

ZH is a β – agonist which can be referred to as a repartitioning agent which promotes growth (Ricks *et al*., 1984). Growth promotants or metabolic modifiers are any compounds that are fed, injected or implanted to increase feed efficiency, gain, improve dressing percentage and carcass meat yield percentage, and they can also improve meat quality (Dikeman, 2007). Steroidal implants, non-steroidal implants and β-agonists are all considered growth promotants and will be discussed in detail.

2.1. History of the use of growth promotants

Growth promotants have been used in the beef industry for over 50 years in the form of feed-additives and growth implants (Barham et al., 2003). Refer to Table 1 for a detailed evolution of growth enhancing technology usage in animal production. First use of growth promotants included iodated proteins and oestrogen implants for milk production and broilers respectively. In 1954 the first ‘steroid-like’ hormone was used to increase growth, efficiency and lean meat production in beef and lamb. This product is called diethylstilbestrol (DES). This caused potential carcinogenicity in humans and was banned in 1979 (Preston, 1999).

Table 2.1. The hormones approved by the Food and drug administration (FDA) for cattle and sheep (Preston, 1999)

Year	Hormones approved
1954	Oral DES for cattle
1956	DES implants for cattle
1956	DES implants for sheep
1956	Estradiol Benzoate (EB)/progesterone implants for steers
1957	Oral DES for sheep
1958	EB/testosterone implants for heifers
1968	Oral melengestrol acetate (MGA) for heifers
1969	Zeranol implants for cattle
1969	Zeranol implants for lambs
1970	Oral DES dose range increase for cattle
1979	All use of DES banned
1982	Silicone rubber-estradiol implant for cattle
1984	EB/progesterone implants for cattle
1987	Trenbolone acetate (TBA) implants for cattle
1991	TBA/Estradiol (5:1) implants for steers
1993	Bovine Somatotropin for lactating dairy cows
1994	TBA/Estradiol (10:) implants for steers
1996	Estradiol/TBA (5:1) implants for stocker (growing) cattle

2.2. Mechanisms and metabolisms of growth promotants

Steroids are categorized as oestrogens, androgens and progestagens. Oestrogens are commonly used as implants in the form of estradiol, given as E_2 or Estradiol benzoate. Trenbolone acetate (TBA) is a synthetic androgen and testosterone is the naturally occurring form. It is manufactured as testosterone propionate. The anabolic steroids are circulated in the body via the blood. For the steroids to be effective a certain level needs to circulate to maintain optimum performance (Johnson, 2009). The various anabolic steroids used commercially are shown in Table 2.

2.2.1. Oestrogens

Oestrogens improve protein deposition and decrease the excretion of nitrogen. Growth response works on a dose dependant manner. In heifers on a low dose of oestrogen, growth will be stimulated. On a high dose of oestrogen, calcium retention occurs in the growth plates of the long bones. This results in termination of growth (Meyer, 2001). Response to oestrogens in calves, heifers, lambs and steers has been very positive and has enhanced growth by 5-15% (Meissonneir, 1983). The effect of oestrogens on skeletal muscle is direct and indirect. Primarily oestrogens cause an increase in the size of the pituitary and the proportions of somatotrophins (ST) in the pituitary. This alters the somatotrophic axis as the pituitary is more responsive to somatotrophin releasing factor (SRF). ST and Insulin-like growth factor-1 (IGF-1) production increases in the liver as well as their binding characteristics. Muscle is stimulated to grow as a result of the increased circulating ST and a more effective release pattern (Johnson, 2009; Preston, 1999). Oestrogens also improve protein anabolism and mineral retention in other significant tissues; kidney, skin, rumen and intestinal epithelia (Meyer, 2001).

2.2.2. Androgens

Early use of testosterone (androgens) as growth promotants was generally disappointing, but further research showed that testosterone can be effective if used in combinations with other steroids and if used in the synthetic form (Preston, 1999). Androgens are anabolic in humans, but in bovids they are less active. The reason males have increased muscle size (on specific muscles) is not because of androgens, but because male calves have 2-fold lower concentrations of glucocorticoid receptors on 4 specific muscles (abdominal muscles, neck muscles, shoulder muscles and hindleg musculature). This causes a decreased protein catabolism and an increased protein accretion (Sauerwein,

1991). Androgens cause stimulation in Growth Hormone secretion and IGF-1 levels circulating in the body (Meyer, 2001). Trenbolone acetate (TBA) is 3 – 5 times higher than testosterone in androgenic activity, and it is 8 – 10 times greater in anabolic activity than testosterone (Preston, 1999). The advantage is that trenebolone acts directly on the muscle cell to stimulate muscle protein synthesis and deposition. The indirect effect of androgens is a reduced amount of circulating cortisol and a decreased response to adrenocorticotrophic hormone in TBA – implanted steers. This makes steers more masculine and will increase protein deposition (Johnson, 2009).

2.2.3. Progestins

Progesterone was used in the first founding studies on anabolism of sex steroids and few results indicated growth promoting effects of progesterone. These outcomes weren't confirmed in more recent studies. Progestins reduce teat growth due to oestrogens, and this may be the reasoning that it is included in numerous anabolic preparations (Meyer, 2001).

2.2.4. Non-steroidal growth promotants

Zeranol is a common non-steroidal growth promotant and is sold under the trade name Ralgro® (MSD) for lambs. It is a Resorcyclic acid lactone which is an oestrogen like compound (Perry 1970; Sharp & Dryer, 1971). Zeranol is found in corn mould *Giberella zeae* (Sharp & Dryer, 1971). It is not carcinogenic and results in increased growth rates and feed efficiency (Perry, 1970). Zeranol has a similar mode of action to estradiol, except differs in receptor binding, oral activity and elimination (Meyer, 2001). Zeranol causes increases in live weight gains, due to an increase in feed efficiency and not intake (Nsahlai *et al*., 2002). Zeranol improves feed efficiency and average daily gain in both rams and wethers (Nold *et al*., 1992). Salisbury *et al*. (2007) concluded that Zeranol is not useful to implant in ewes as it resulted in spool joints and prolapses.

Table 2.2. Comparisons of various anabolic preparations (Hoffmann, 1979)

Preparation			
Main Component	Oestrogenic	Non-oestrogenic part	Type of animal production
Endogenous steroids	17-β-oestradiol	Progesterone	Veal calf
	17-β-oestradiol	Testosterone	Veal calf
Extraneous steroids	oestradiolbenzoate	Progesterone	Steer, heifer, lamb
	oestradiolbenzoate	Testosteronepropionate	Steer, heifer, lamb
	oestradiol-monopalmitate		Poultry
	17-β-oestradiol	Trenboloneacetate (TBA)	Veal calf/steer
		TBA	Heifer/cow
Non-steroidal compounds	Diethylstilbestrol (DES)		Steer/heifer/lamb/veal calf
	DES	Testosterone	Steer/heifer
	DES	Methyltestosterone	Pig
	Hexoestrol		Steer/heifer/lamb
	Dienoestroldiacetate		Poultry
	Zeranol		Steer/heifer/lamb/veal calf

2.3. Beta – adrenergic agonists

South Africa was the first country in the world to register a β –agonist. The product is marketed as Zilmax® (MSD). It was registered with the active ingredient being ZH for commercial use in 1995 and has made large contributions to growth performance in livestock. It has helped beef producers increase profit margins (Lopez-Carlos *et al*., 2011; Montgomery *et al*., 2009; Avendaño-Reyes *et al*., 2006).

2.3.1. Beta – adrenergic receptors

β - AR fall under a group of receptors that are known to be the largest and probably the most functionally diverse. This group of receptors couple effector proteins through guanine nucleotide binding (G proteins) (Strader *et al*., 1989). The β - AR is a chain of more than 400 amino acids in length. It has 7 transmembrane hydrophobic domains which anchor it to the cell membrane. As seen in figure 2.1 a ligand binding site is found in the middle of the 7 transmembrane domain, and it involves several of the amino acids from the different domains (Mersmann, 1998). The 7 hydrophobic regions are surrounded by 8 hydrophilic regions that are inconsistent in length, shown in figure 2.2 (Strader *et al*., 1989). β – AR are embedded in the plasma membrane of almost every mammalian cell, therefore the correct β - agonist is vital to manipulate physiological response (Mersmann, 1998; Strader *et al*., 1989). Figure 2.2 also shows the amino terminus of the β – AR exposed extracellularly and the carboxyl terminus intracellularly (Strader *et al*., 1989).

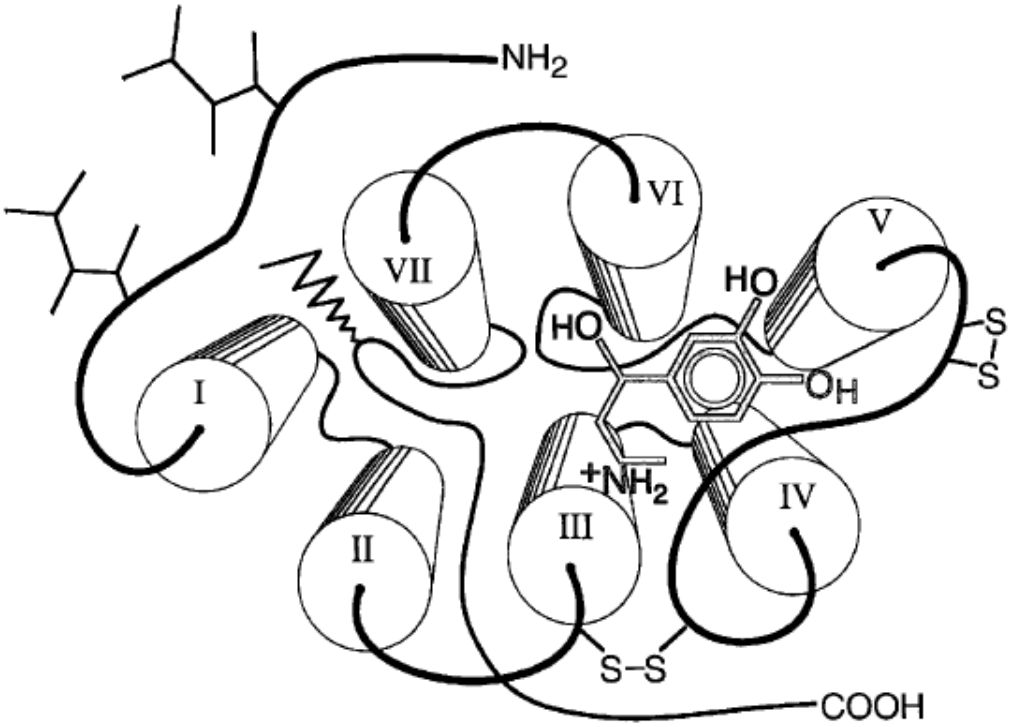


Figure 2.1. Structure of a β – AR. The cylinders represent the seven transmembrane domains. The norepinephrine, in the middle of the cylinders, shows the ligand binding site. The extracellular portions are shown with the thick lines and the intracellular portions are shown by the thin lines (Ostrowski et. al., 1992).

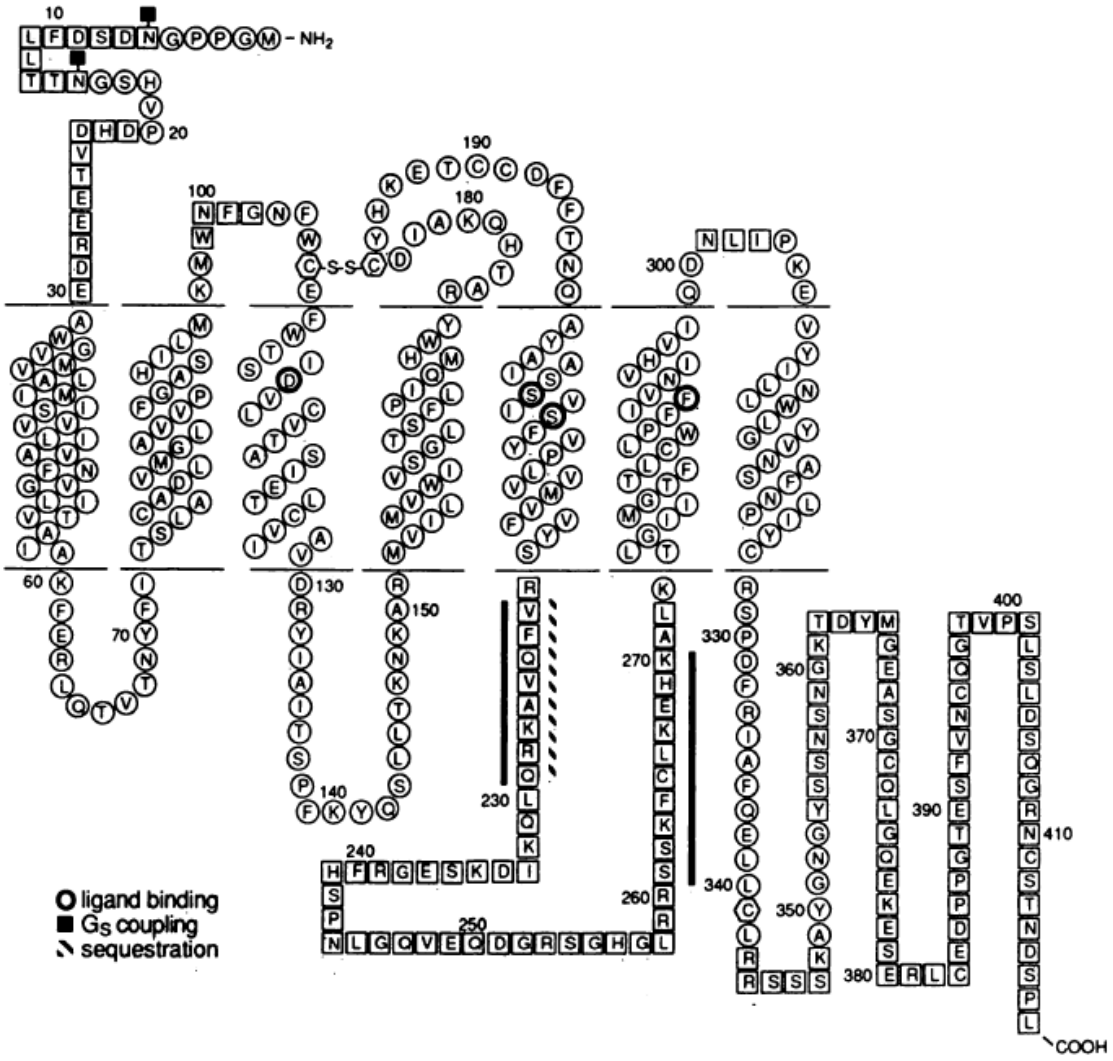


Figure 2.2. A model for the transmembrane topology of the β – AR. The horizontal line represents the plasma membrane of the cell. The top portion above the line represents the extracellular space and the bottom portion below the line represents the cytoplasm of the cell. The solid line shown next to the third intracellular loop is proposed to be involved in G protein coupling. The amino acid residues shown with bold-faced circles are thought to be intricate in interactions with the ligand (Strader *et al*., 1989).

2.3.2. Beta – adrenergic receptor subtypes

There are three β – AR subtypes: β_1-AR, β_2-AR, β_3-AR. The amino acid sequence of each subtype changes between species and the distribution and proportion of subtypes varies between different cells and species (Mersmann, 1998). Differentiation of subtypes is done using functional classification, ligand binding classification and molecular biology

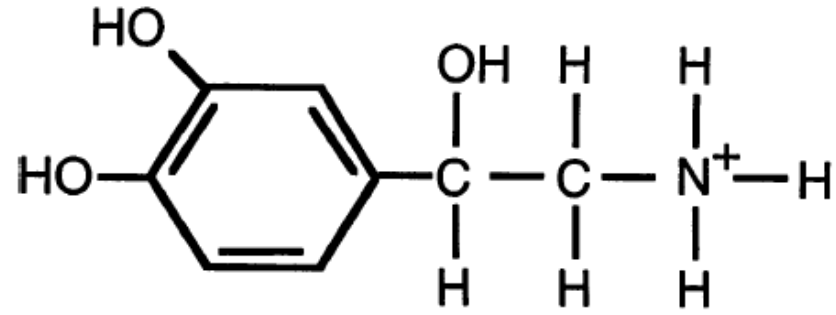
classification. The first separation of subtypes began when physiological function of receptors was being investigated and norepinephrine and epinephrine were used. This led to the idea that there are α and β adrenergic receptors (Mersmann, 1998). Norepinephrine is more potent for β - AR and is more potent for β_1-AR than for β_2-AR. β_1-AR and β_2-AR are among the most thoroughly researched receptor subtypes and are structurally related, however they differ pharmacologically (Frielle *et al*., 1988). According to studies done by Freille *et al.* (1988), transmembrane domains are involved in receptor subtype specificity, by formation of a ligand-binding pocket. The transmembrane domain IV seems to be mostly responsible for determining β_1-AR and β_2-AR properties with regard to agonist binding.

In bovine skeletal muscle β_2-AR is the most abundant subtype. It is also the most abundant subtype on adipocytes (Mersmann, 1998). There is no confirmation of β_3-AR on bovine adipocytes (Van Liefde *et al*., 1994). In sheep, the predominant subtype on adipocytes is β_2-AR (Bowen *et al*., 1992). ZH binds to both β_1-AR and β_2-AR subtypes, with a higher affinity to β_2-AR (Baxa *et al*., 2009).

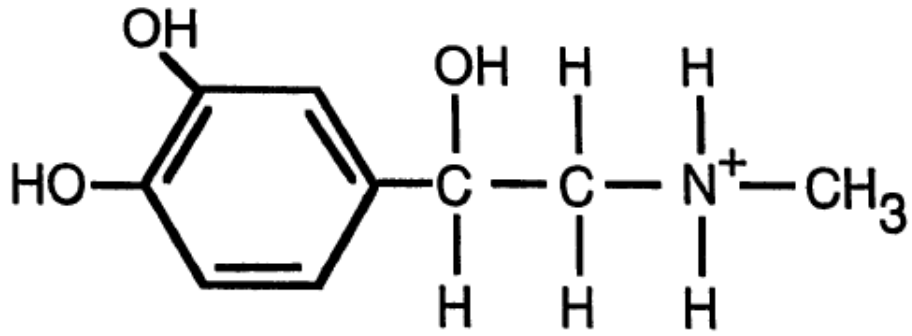
2.3.3. Beta – adrenergic agonists and signal cascade

There is a physiological response when a β – agonist binds to a β – AR. A physiological β – agonist is norephinephrine and epinephrine, shown in Figure 2.3. Synthetic β – agonists are organic molecules, which bind to β – AR and create an agonist-receptor complex which activates the G_s protein. The α – subunit of the G_s activates adenylyl cyclase. Adenylyl cyclase is responsible for producing cyclic adenosine monophosphate (cAMP). cAMP binds to protein kinase A, which in turn stimulates the release of a catalytic subunit which results in the phosphorylation of a number of intracellular proteins. cAMP's response element binding protein (CREB) is also phosphorylated by the kinase A. In a regulatory part of the gene the CREB binds to a response element of the cAMP and transcribes that part of the gene. (Mersmann, 1998). On adipocytes, β – agonists act on β – AR and influence cellular metabolism using signalling cascades. This causes a decrease in lipogenesis and an increase in lipolysis. Growth of adipocytes and adipose tissue mass slows down, resulting in a leaner animal (Dunshea *et* al., 2005). In vitro, it is clear that β – agonists stimulate adipocyte triacylglycerol degradation and inhibit triacylglycerol and fatty acid synthesis in the cells and tissue explants of many species (Mersmann, 1998). β – agonists also bind to certain receptors on the surface of muscle cells and this modifies biochemical processes which cause a decrease in protein degradation and increase protein synthesis (Eisemann *et* al.,1993). β – agonists cause an increase in RNA transcript for many skeletal muscle proteins. Different β – agonists are more potent than others and cells and tissues respond

differently due to differing types of β – AR and the metabolic pathways that are linked to them (Strydom *et al*., 2009). With different doses, duration of treatment, species and type of β – agonist there will be a difference in the magnitude of changes (Dunshea *et al*., 2005).



Norepinephrine



Epinephrine

Figure 2.3. Structures of Norepinephrine and Epinephrine, physiological beta-adrenergic receptor agonists. Norepinephrine circulates plasma at relatively high concentrations, as it is released by the central nervous system and sympathetic nervous system. It is a neurotransmitter substance. Epinephrine is a hormone and is released into the plasma by the adrenal medulla (Mersmann, 1998).

2.3.4. Indirect effects of beta – adrenergic agonists

β – agonists also have indirect effects. There is increased blood flow to the hindquarters of both sheep and cattle when they are treated with β – agonists. This indirect effect could be due to the multiple effects of the number of β - AR found on different cell types. Increased blood flow to skeletal muscle can increase the delivery of substrate for protein synthesis. Increased blood flow to adipose tissue could assist in removing nonesterified fatty acids from the tissue and increase lipolysis (Mersmann, 1998). Feeding β – agonists at chronic levels increases plasma thyroid hormones in sheep (Beerman *et al*., 1987). There is no indication that muscle mass production and fat mass reduction are due to ST (Mersmann, 1998).

2.4. Combining growth promotants

Combining ZH and growth promoting implants is common practice for commercial beef producers. There is limited data available on the combination of these growth promotants in sheep, because no studies have been conducted as yet. Baxa *et al*. (2009) conducted a study on the additive effects of a steroidal implant and ZH on steers. In this trial the combination of ZH and Revelor-S implant had the highest ADG and feed efficiency response. The combination had an additive effect compared with the treatments on their own (Baxa *et al*., 2010). A similar response was found for a study done on cows (Neill *et al*., 2009). Neill *et al*. (2009) also claims: to realize the potential performance benefit of feeding ZH, it is necessary to first implant mature cows.

2.5. Implant matrices

Steroids are delivered to the target tissue through a vessel that is implanted in the ear of the animal (Johnson, 2009; Preston, 1999). Implants can be long-acting or short-acting depending on the carrier (matrix). Matrices differ in their percent solvent, the compression applied during the pelleting process and the type of carrier. The dose released and the dose rate is controlled by the variables in the manufacture of the matrix. Modern techniques make use of lactose, cholesterol or a large polymer of polyethylene glycol as a matrix. Silicone rubber matrices are also being used. Long-acting matrices are cholesterol based and the short-acting matrices are lactose based. Short-acting matrices have to be implanted more than once in a feedlot situation. Theoretically the dose rate over time can be regulated by embedding estradiol in a silicone rubber matrix. And the length of the implants can control

dose rate (Preston, 1999). New matrices have been formed using patented polymers to cover the implant. This has led to implants that are active for 200 days (Revalor-XS, 2013).

2.6 Safety

The use of modern technology, namely growth promotants to produce animal protein has caused much public concern about the effects on human health and the environment. The concern is mainly related to the effects of growth promotant residues in meat. The European Union (EU) has enforced several regulations on the use of steroids and β – agonists because of the possible harmful effect on public health (Regal et al., 2011). Concern about the use of hormones began in the 1970's when DES was banned because of its carcinogenic effects on women. DES was used as a growth promotant in beef cattle and the clinical use of DES in woman was associated with vaginal cancer in their daughters (Health and Welfare Canada, 1973; Heitzman, 1979). The hazards of residues have been reviewed by the World Health Organization (WHO) and they concluded that sex steroids are associated with human and animal cancer. There is evidence that sex steroids can be promoters of tumors in certain organs where hormone receptors are found. These organs are the mammary gland, cervix, prostate gland and uterus (FAO/WHO, 1988). Growth promoters are referred to as 'carcinogens', capable of increasing the risk of cancer. Therefore these sex steroids can be called carcinogens (Waltner-Toews & McEwan, 1994). Although, these compounds cannot be carcinogenic if they are present in volumes less than which is needed to induce a hormonal response (WHO regional office for Europe, 1982).

Estradiol -17β is commonly used as an implant for beef production. It can be carcinogenic, but only when the levels are higher than required for normal physiological purposes (Waltner-Toews & McEwan, 1994). According to a study done by Hoffman and Evers (1986), the amount of Estradiol -17β produced on a daily basis by pre-pubertal boys and pregnant woman was 1000 times higher than that found in 500g of beef from an implanted animal. TBA is a xenobiotic anabolic agent. A proportion of TBA residues can become bound to protein in tissues; however it has no hormonal significance (Burgat-Sacaze *et al*., 1986).

2.6.1. Residues

Residues in meat and the environment have been a concern and still continue to raise questions. Not only because people are worried about health, but also because it is

important to regulate the use of growth promotants. In the EU, there has been vast research into methods of detection, because many growth promotants have been banned from use in animal production (Reig & Fidel, 2008). Rapid screening methods are being developed to detect the presence of illegal substances in the EU. In the past, methods such as thin-layer gas chromatography were used to determine oestrogens and tienic steroids (Hoffmann, 1979). Residue concentrations are in parts per billion and parts per million range. In a study done by Hoffmann (1979), it was found that residues in tissue vary between sex, reproductive status, species and individual animals. The results showed a 100-1000 fold difference between animals. This is illustrated in Table 3.

According to Hoffmann (1979): Based on the daily production of sex steroids in the human and regardless of sex and age, it cannot be expected that the endogenous hormones consumed in food of animal origin will measurably contribute to the steroid levels in the human. This applies to both treated and untreated animals. However, this relates to the proper use of the anabolic steroids. The implantation site of animal does contain very high concentrations of anabolic steroids, even after a long time following the implantation. The implantation site should be the ear and has to be discarded automatically (Hoffman, 1979; Johnson 2009). If an implant is administered properly, the estradiol, progesterone and testosterone are released slowly from the inedible part of the animal namely, the ear. The residues found in meat of animal origin make up an insignificant proportion of the total amount of hormones that humans are exposed to (Waltner-Toews & McEwan, 1994). If the implant is administrated to edible parts of the carcass and that portion of tissue is not disposed of, this can expose the consumer to higher levels of the steroid. This has never been reported before. Therefore, the implantation technique and withdrawal period both play a role in the amount of residue found in edible tissue.

Problems with residues are often short-term, high-level variety frequently as a result of accidents or related with toxicity to livestock. Residue problems can also be long-term, low-level variety, but these are difficult to detect and understand (Waltner-Toews & McEwan, 1994). One example where the effect of β – agonists in food from animal origin has been reported in Portugal. Lamb and bovine tissue contained residues of Clenbuterol, which is the most potent β – agonist and it resulted in 50 people developing gross tremors of the extremities, tachycardia, headaches, nausea and dizziness (Barbosa *et al*., 2005).

β – agonists mainly affects the quality of meat. Large amounts of research have been done on the effect of β – agonists on pork quality and beef quality. From the research, it is reasonable to assume that it decreases the amount of intramuscular fat and increases sheer

force values, pH and drip loss (Dunshea *et al*., 2005). ZH and Ractopamine has favourable aging ability compared to Clenbuterol. Therefore, ZH and Ractopamine results in the meat being slightly more tender than Clenbuterol. The β – agonists are muscle specific and thus it shows that tenderness is associated with β – agonists effect on calpastatin (Strydom et. al, 2009). β – agonists tend to increase toughness in muscle, because β – agonists cause an increase in the inhibitor calpastatin and cause a decrease in calpain activity (Strydom *et al*., 2011)

Table 2.3. Concentrations (pg/g) of various anabolic agents in their free form in tissues of treated and untreated cattle (Hoffmann, 1979)

		Tissue examined			
Compound derived	Animal	Muscle	Liver	Kidney	Fat
Testosterone	Bull	535	749	2783	10950
	Heifer	92	193	595	250
	Veal calf	16	39	256	685
	Veal calf (treated)[a]	70	47	685	340
Progesterone	Pregnant cow				336.2
	Heifer				16.7
	Veal calf				5.8
	Veal calf (treated)[b]				12.5
Oestradiol-17β	Pregnant cow	370-860			
	Veal calf (Untreated and treated‡)	<100	<100	<100	<100
	Steer		19.7	20.7	
	Heifer	12	38.3	39.8	
Oestrone	Pregnant cow	0.12-2.09			
	Veal calf (Treated and untreated[c])	<100	<100	<100	<100
TBOH	Steer[d]	50	230	50	80
	Steer[e]	50	50	20	80
	Veal calf[c]	127	521	235	388
	Veal calf[f]	797	3467	2563	2580
	Veal calf[g]	1673	4930	4083	8893

[a]Slaughtered 77 days after implantation of 20mg oestradiol-17β + 200mg testosterone.
[b]Slaughtered 70 days after implantation of 20 mg oestradiol-17β + 200mg testosterone.
[c]Slaughtered 70-77 days after implantation of 20mg oestradiol-17β + 140mg Trenbolone acetate (TBA).
[d]Slaughtered 60 days after implantation of 40mg oestradiol-17β + 200mg TBA.
[e]Implantation of 40mg oestradiol-17β + 200mg TBA, implant removal after 60 days and slaughter after another 15 days.
[f]Slaughter 70-77 days after implantation of 200mg oestradiol-17β + 1400mg TBA (10 fold of normal dose).
[g]Slaughter 70-77 days after implantation of 500mg oestradiol-17β + 3500mg TBA (25 fold of normal dose).

2.7. Growth implants, zilpaterol hydrochloride and mutton production

The use of both growth implants and ZH have had inconsistent results and the sources of variation still need to be properly established (Nsahlai *et al*., 2002). Growth implants are being used commercially in sheep, however, as mentioned before, β – agonists have not been approved of by the FDA yet.

2.7.1. Growth implants in lamb production

Zeranol, a synthetic oestrogen, increases live weight gain in most cases. In other instances the zeranol does not cause an increase in live weight gain (Mulley *et al*., 1996). Zeranol increases live weight gain generally by increasing feed efficiency and not intake. Zeranol tends to reduce carcass fat and increase carcass protein gain, perhaps due to decreased muscle protein degradation (Nsahlai *et al*., 2002). Studies have commented on animal response due to the effects of dietary properties on the zeranol implant (Peiris *et al*., 1998). Inconsistent results from zeranol may be due to the amount of nutrients at the tissue level, especially protein (Nsahlai *et al*., 2002). Steroid implants have the most consistent results when the sheep are fed a high concentrate diet. In this case a 10 – 15 % increase in average daily gain can be expected (Reinhardt, 2012).

Oestrogen implants can be given to male lambs and this can lead to 5 – 8% improvement in growth response. An oestrogen plus androgen combination is commonly used in cattle and can be effective for up to 150 days. In cattle, bulls have shown to increase growth rate by 2 – 10%. Not only does it make managing male livestock easier, but also reduces damage to the carcass and decreases chances of ‘dark cutting’. Females seem to have the best results with a combination of estradiol, TBA and melengestrol acetate (MGA). However, studies on the effects of MGA have shown reduced gain, feed efficiency and rib eye area. These poor results are overcome by the growth promotion from the suppression of oestrus. Growth implants should not be used on sheep intended for breeding purposes, because an implant such as zeranol causes a decrease in testicular development in rams and decreases the ovulation rate and onset of puberty in female sheep (Reinhardt, 2012).

2.7.2. Effect of zilpaterol hydrochloride on growth, efficiency and carcass characteristics

The effect of β - agonists in sheep is much like the response in cattle, however it is less consistent. β - agonists increase ADG, feed efficiency and hot carcass weight (Nourozi

et al., 2008). ADG and growth rate increase with ZH treatment in cattle (Strydom *et al*., 2009; O'Neill, 2001). Estrada-Angulo *et al*. (2008) conducted one of the first studies investigating ZH in sheep. In this study the effects of ZH on sheep were measured and the results showed increases in gain efficiency (15.8%, $P < 0.03$), apparent energy retention per unit dry matter intake (DMI) (10.9%, $P = 0.03$), ADG (16%, $P < 0.07$), total live weight gain (17.7%, $P < 0.08$) and carcass dressing percentage (2.3%, $P = 0.04$). The kidney pelvic fat weight decreased (36%, $P < 0.01$). The results of growth and efficiency in Estrada-Angulo *et al*. (2008) are shown in Table 2.4.

Similar studies were done with ZH on growth and concluded that ZH improved ADG and growth rate in sheep (Lopéz-Carlos *et al*., 2011, Lopez-Carlos *et al*., 2010). ADG and growth rates also increased with ZH treatment in goat wethers (Lopez-Carlos., 2014).

ZH treatment also causes improvements in carcass characteristics. Estrada-Angulo *et al*. (2008) showed that hot carcass weight increased with an increased dose level, however decreased at 0.25mg/kg of $LW^{-1}d^{-1}$. Chilled carcass weight also had the same quadratic response. *Longissimus muscle* (LM) area and dressing percentage both increased with an increase in the ZH dose. Fat thickness and kidney pelvic fat weight, decreased with an increase in ZH supplementation, therefore ZH improved carcass characteristics (Estrada-Angulo *et al*., 2008). Carcass characteristic results for Estrada-Angulo *et al*. (2008) are shown in Table 2.5. Many studies done on ZH also yielded a similar result, concluding that ZH increases cold carcass mass (Vahedi *et al*., 2014; Lopez-Carlos *et al*., 2011; Lopez-Carlos *et al*., 2010).

In the study done by Estrada-Angulo *et al*. (2008) DMI was not affected by different ZH supplementations. This conflicts with results found by Lopez-Carlos *et al*., (2011). In the test done by Lopez-Carlos *et* al. (2011), DMI decreased linearly over a period of 0, 20, 30 and 40 days. DMI affects the profit margin gained by the producer and therefore it would be beneficial to further investigate the effect of ZH on DMI.

β-agonists cause a rapid growth response at the onset of treatment, however, a plateau is reached due to a desensitisation of β-adrenergic receptors (Moody *et al*., 2000). Growth response over time is not constant with different β-agonists and different species (Lopez-Carlos *et al*., 2011). Lopez *et al.* (2011) reports an increase in ADG over 3 different durations of treatment, namely; 14, 28 and 42 days. In this study the ADG did not increase over time and in fact decreased.

Table 2.4. Effect of zilpaterol supplementation[a] on growth responses in feedlot lambs (Estrada-Angula *et al*., 2008)

Item	Control	Zilpaterol level (mg/kg of LW^{-1} d^{-1})				*P*[b] value		
		0.15	0.20	0.25	S.E.M.	Control vs Zilpaterol	Linear	Quadratic
Days on test	32	32	32	32				
Pen replicates	4	4	4	4				
Performance								
Liveweight (Kg)								
Initial[c]	38.74	38.82	38.90	38.70	0.26	0.87	0.45	0.74
Final[d]	45.67	46.55	47.57	46.76	0.75	0.11	0.08	0.12
Total gain (kg)	6.93	7.73	8.67	8.07	0.49	0.08	0.04	0.14
Daily gain (g)	210	234	263	244	18	0.07	0.12	0.04
DMI (g/d)	1094	1098	1125	1078	42	0.40	0.79	0.20
Gain/DMI	0.222	0.246	0.266	0.259	0.012	0.03	0.01	0.14
Observed:expected DMI	0.98	0.90	0.85	0.87	0.02	0.03	0.01	0.012

[a] Zilmax ® fed first 30 days
[b] *P* observed significance level for effect of level of zilpaterol (Zilmax®, Intervet México, México City) supplementation (control *vs*. zilpaterol) and linear and quadratic effect of dose level (0.15, 0.20, and 0.25 mg/kg of LW−1 d−1).
[c] Initial and final full weight reduced 4% to account for digesta fill.
[d] Computed by using final LW= HCW/0.596, where 0.596 is the dressing percent average observed in trial.

Table 2.5. Effect of zilpaterol supplementation[a] on carcass characteristics in feedlot lambs (Estrada-Angula *et al*., 2008)

Item	Control	Zilpaterol level (mg/kg of LW^{-1} d^{-1})				*P*[b] value		
		0.15	0.20	0.25	S.E.M.	Control vs Zilpaterol	Linear	Quadratic
Hot carcass weight (wt) (kg)	27.05	27.72	28.33	27.65	0.55	0.33	0.27	0.17
Chilled carcass wt (g)	26.66	27.17	27.64	27.21	0.52	0.26	0.95	0.41
Dressing percentage	58.54	59.67	59.77	60.28	0.03	0.04	0.02	0.48
LM area (cm^2)	16.9	16.5	16.5	16.6	0.8	0.67	0.92	0.94
Fat thickness (cm)	0.38	0.29	0.28	0.26	0.08	0.28	0.23	0.38
Kidney-pelvic fat (%)	2.21	1.52	1.43	1.25	0.11	<0.01	<0.01	0.12

[a] Zilmax ® fed first 30 days of trial
[b] *P* observed significance level for effect of level of zilpaterol (Zilmax®, Intervet México, México City) supplementation (control *vs*. zilpaterol) and linear and quadratic effect of dose level (0.15, 0.20, and 0.25 mg/kg of LW−1 d−1).

2.7.3. Effect of duration of feeding zilpaterol hydrochloride

β – adrenergic receptors can become desensitized or down-regulated with too much stimulation. This causes an increase in growth response at the onset of β-agonists feeding, until a plateau is reached, then there will be a linear decline in growth response (Avendaño-Reyes *et al*., 2006; Moody *et al*., 2000). This response over time is not constant between different β-agonists and species (Lopez-Carlos *et al*., 2011). The duration of feeding β-agonists was reviewed by Dikeman (2007) and many of the studies all concluded a duration of the last 30 days on feed is optimal. However, Lopez-Carlos *et al*. (2011) did a study on the effect of feeding two beta adrenergic agonists and the feeding duration in feedlot lambs and found a linear decline in DMI and a linear incline in ADG and feed efficiency during 0, 20, 30 and 40 d of zilpaterol supplementation in the diet. Lopez-Carlos *et al*. (2011) concluded that growth performance and carcass characteristics were enhanced by a longer ZH feeding period. ZH showed a better response than ractopamine, with greater hot carcass weight, dressing percentage and lower back fat thickness (Lopez-Carlos *et al*., 2011).

Recently a study was done on intermittent dietary ZH for 28 days and 42 days by Vahedi *et al*. (2014). It showed that intermittent 42 day treatment was more cost efficient than daily 42 day treatment. Results showed an ADG of 189g d^{-1} for daily 42 day treatment and 178g d^{-1} for 1 day on 1 day off 42 day treatment. ADG differed by 11 grams for half the cost. Vahedi *et al*. (2014) concluded that daily 28 day and intermittent 42 day feeding treatments were the best options for ZH supplementation in lambs.

2.7.4. Effect of zilpaterol hydrochloride on carcass composition

The repartitioning effect of β-agonists has been of interest for over 20 years (Leheska *et al*., 2009). In cattle it has been well documented that ZH has a repartitioning effect, by increasing carcass protein and muscle deposition (Hilton *et al*., 2009; Leheska *et al*., 2009; Rathman *et al*., 2009; Vasconcelos *et al*., 2008; Plascencia *et al*., 1999). In sheep, the repartitioning effect of ZH has also been recognized. In studies done by Lopez-Carlos *et al*. (2011); Lopez-Carlos *et al*. (2010); Estrada-Angulo *et al*. (2008), ZH improved growth performance in lambs as a result of greater muscle accretion and a reduction in fat. There was a reduction in fat thickness and an increase in LM area. In other reports, ZH caused an increase in LM area but did not decrease fat thickness significantly (Vahedi *et al*., 2014; Avendaño-Reyes *et al*., 2011).

2.7.5. Effect of zilpaterol hydrochloride on meat quality

Meat quality is very important for consumer satisfaction and ultimately will influence sales. ZH supplemented to feedlot cattle is known to result in meat toughness, which is thought to be attributed to an increase in the activity of the inhibitor, calpastatin and a decrease in the activity of calpain (Strydom *et al*., 2009, Dikeman, 2007). Hilton *et al*. (2009), reports that ZH does not adversely affect overall consumer acceptance with increased post mortem aging. To date there has not been any reports on whether tenderness is affected in lambs treated with ZH, however, a β-agonist, $L_{644,969}$, when fed to lambs has been shown to increase toughness in lambs (Pringle *et al*., 1993).

A number of studies looked at the effect of ZH on cooking loss in cattle and found that ZH did not significantly influence cooking loss (Hilton *et al*., 2009; Holmer *et al*., 2009; Kellermeier *et al*., 2009; Leheska *et al*., 2009). However, ZH does decrease flavour, juiciness, tenderness and beef flavour intensity; all tested by a sensory panel (Hilton *et al*., 2009). ZH also has the potential to improve meat colour, making it a more red, cherry colour (Hilton *et al*., 2009; Montogomery *et al*., 2009; Avendaño-Reyers *et al*., 2006).

β – agonist supplementation in feedlot cattle may alter fatty acid composition (Webb, 2008). Changes are seen in both the subcutaneous fat and *M. Longissimus dorsi*. In the subcutaneous fat, there is an increase in the deposit of oleic acid (C18:1). In the *M. Longissimus dorsi* there are shifts towards more saturated fats, with effects on palmitic acid (C16:0), palmitoleic acid (16:1) and oleic acid (18:1) (Webb, 1995).

2.8. Environmental effects

The use of growth promotants in feedlots has resulted in a lot of research about the effect on humans and animals, but the effect on the environment still needs to be investigated more thoroughly. In the past a lot of focus was placed on the effects of nitrogenous waste products from feedlots and their environmental toxicity (Randall & Tsui, 2002). It has been known for a few years that oestrogens present in chicken manure can cause hypertension if it fed to cattle (Schiffer *et al*., 2001). Fish can also be affected by the presence of anabolic steroids in manure. In beef feedlots there is more presence of androgens in the manure and if it gets into the water system this can cause dramatic changes on the endocrine functions and reproductive systems of wild fish populations (Khan *et al*., 2008). Oestrogenic hormones have shown to cause feminisation of male fish and this can happen due to waste from dairies. Trenbolone can be a potent androgenic agent to both fish and mammals (Rempel *et al*., 2006).

Steroids are excreted by the animal in a free form or in conjugates with sulphur or glucuronide, which in turn can be hydrolysed to a free form of the steroid. The age, sex, and stage of the female oestrus cycle can affect the amount of steroid that is found in excretions. Hormones go through different metabolisms and pathways to degrade or are transported through the run-off of water. Hormones will slowly degrade in manure, water and soil but the exact mechanisms of their degradation are not fully understood (Khan *et al*., 2009).

Many studies have been done in laboratories where fish were exposed to trenbolone and estradiol and the effect of the compounds caused disruption of the fish endocrine system. 17-β trenbolone caused changes in the gonad morphology, caused male-biased populations and reduced reproductive potency in the fish. The exposure to 17β- estradiol caused alterations in reproduction and sexual development. Although it is clear steroids cause changes in the fish, it is unclear whether using growth promotants actually changes the endocrine activity of cattle wastes (Sellin *et al.*, 2009). Sellin *et al*. (2009) did a comparity

study on the manure of implanted versus non implanted cattle manure, using fathead minnows and exposing them to TBA:E-implanted and unimplanted manure for 7 days. The conclusion was that growth promoting implants alter the endocrine activity of faeces, but does not alter the endocrine activity of the urine.

Fish are very closely associated with their environment and therefore are subject to any environmental changes. Sex determination and differentiation of fish can then be easily changed due to endocrine disrupting chemicals (Orlando & Guillette, 2007).

2.9. Conclusion

Growth promotants are still undergoing changes as scientists find better ways of maximising growth efficiency. Growth promotants in lambs production are not that common in South Africa, but with changes in demand and an increase in intensive lamb production, the use of growth promotants will increase. Residues of steroidal implants are not a concern for human health if implanted correctly. Residues of ZH can cause adverse effects on meat quality and therefore producers must adhere to withdrawal periods. ZH has been found to increase ADG, efficiency and carcass characteristics and shows the potential to be beneficial for the lamb industry. The effect of ZH in combination with other growth promotants has not been investigated yet. More data is needed on the effects of ZH on growth performance, carcass characteristics and meat quality. Moreover, the effect of ZH in combination with growth implants needs to be investigated.

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Chapter 3. Materials and Methods

3.1. Experimental factors

- Phase 1: Growth trial
  - : Slaughter
  - : pH measurements
  - : Sample collection
  - : Carcass measurements
- Phase 2: Cooking loss
  - : Tenderness analysis
- Phase 3: Statistical Analysis

3.2. Experimental Animals

Male lambs were selected at Blokhuis Feedlots in Harrismith, KwaZulu Natal, that had already undergone a regular feed adaption period used by the feedlot. The lambs were South African Mutton Merinos, a late maturing breed type. The lambs used in the trial had an initial average live weight of 31.69kg and all had no teeth. The trial was conducted at Blokhuis feedlot to simulate a real commercial feedlot environment.

3.3. Experimental design

The experimental design as shown in figure 3.1 utilized South African Mutton Merino male lambs X 2 ZH treatments X 2 Implant (zeranol) treatments X 2 duration of treatment X 20 animals per group. The result is 160 experimental animals used in the experimental procedure. During the growing phase 180 animals were used so outliers could be eliminated from the finishing phase (ZH treatment period).

The lambs were randomly allocated to four different pens with two treatment groups per pen, represented in figure 3.2. This meant there were 40 lambs per pen. Ear tags were used to differentiate between the two treatment groups, with odd numbers indicating the lambs had been implanted with zeranol, a non-steroidal growth promotant, marketed under the trade name Ralgro® (MSD).

ZH treatment	Implant treatment	Days of treatment
Zilpaterol hydrochloride treatment	Zeranol	18
		25
	Control	18
		25
No zilpaterol hydrochloride	Zeranol	18
		25
	Control	18
		25

Figure 3.1. Representation of the experimental design

The treatment groups:

- A control with no ZH and no zeranol (Ralgro®) (NZNR) in the feed during a final finishing (last) period of 18 days. This group included 20 male lambs.
- A control with no ZH and no zeranol (NZNR) included in the feed during a final finishing (last) period of 25 days. This group included 20 male lambs.
- ZH and no zeranol (ZNR) included in the feed during the final finishing (last) period for 18 days. This group included 19 male lambs (One lamb died as a result of pneumonia – This was confirmed at post mortem).
- ZH no zeranol (ZNR) included in the feed during the final finishing (last) period for 25 days. This group included 20 male lambs.
- No ZH and zeranol implanted male lambs (NZR) included in the feed during the final finishing (last) phase for 18 days. This group included 20 male lambs.
- No ZH and zeranol implanted male lambs (NZR) included in the feed during the final finishing (last) phase for 25 days. This group included 20 male lambs.
- ZH and zeranol implanted male lambs (ZR) included in the feed during the final finishing (last) phase for 18 days. This group included 19 male lambs (One lamb died as result of Urolithiasis).
- ZH and zeranol implanted male lambs (ZR) included in the feed during the final finishing (last) phase for 25 days. This group included 20 male lambs.

Pen 1	Pen 2	Pen 3	Pen 4
• No ZH • zeranol + no zeranol • 18 days ZH supplementation • 40 lambs	• No ZH • zeranol + no zeranol • 25 days ZH supplementation • 40 lambs	• ZH • zeranol + no zeranol • 18 days ZH supplementation • 40 lambs	• ZH • zeranol + no zeranol • 25 days ZH supplementation • 40 lambs

Figure 3.2. Representation of random allocation of lambs to treatment

3.4. Nutrition and water

Table 3.1. Nutrient inclusion level of the diet

Nutrients	% (DM)
Protein	16.89
Fibre	10.27
Crude fibre	12.41
Net Energy (maintenance)	1.75
Net Energy (growth)	1.19
Calcuim	0.78
STIM	12.55
Fat	4.2
Potassium	0.84
Salt	0.28
Phosphorus	0.39

Nutrition: the lambs were fed a grower ration that is normally used at Blokhuis feedlot (Table 3.1). During the treatment period ZH (Zilmax®) was included in the diet by including ZH in the molasses, the product is called Zilmol®. All the lambs were fed the grower ration for 51 days. Depending on the treatment group, the treatment began either for an additional 18 days or 25 days. Both treatment groups were followed by a compulsory 3 day withdrawal period, where the Zilmol® wasn't included in the ration. The lambs received water *ad lib.*

3.5. Experimental treatments

3.5.1. Growing phase

The male lambs were selected at Blokhuis feedlot to attain a uniform flock. The lambs were ear tagged randomly and received normal processing treatment, explained fully in section 3.9. The lambs with odd numbered ear tags were implanted with zeranol. The lambs were weighed and then were put into two random pens with the feedlots standard grower ration.

After 51 days on the grower ration, all the lambs were weighed and 20 outliers were removed from the experiment. 160 lambs were eligible for the finishing phase of the trial. The lambs were separated into odd and even numbers (zeranol and no zeranol groups) and were randomly allocated to 4 different pens, with 20 zeranol implanted lambs and 20 non zeranol implanted lambs per pen, shown in figure 3.2. 80 lambs received 4ppm ZH (Trade name Zilmax®) treatment during the finishing phase (40mg/animal per day).

Lambs were either fed for one of the two different finishing phases. The one treatment was a finishing phase for 18 days plus 3 days compulsory withdrawal; or the other treatment which included a finishing phase of 25 days plus 3 days compulsory withdrawal. Lambs on ZH treatment for the finishing phase were fed a ration containing Zilmol®, which is ZH included in the molasses and then included in the diet.

The lambs were weighed at Blokhuis feedlots just before slaughter and slaughtered at Blokhuis abattoir in Harrismith.

3.5.2. Slaughter Phase

The lambs were all transported to Blokhuis abattoir for slaughter (Approximately 5 kilometers from the feedlot). The lambs were electrically stunned for 1-4 seconds with a voltage of 60-70 volts/AC 50-60 cycles. The lambs were then slaughtered by cutting the jugular vein. The lambs took approximately 2 minutes to bleed out. The lambs were then hung for the blood to drain and the head was removed. The lamb slaughter order was recorded with the ear tag number to keep track of the carcasses and hides. The carcasses received a serial number which was then recorded with the ear tag number. The lambs were skinned and the hides were kept and weighed. The carcasses were eviscerated and a post mortem inspection was done on each carcass and all abnormalities were recorded.

The carcasses were weighed and approximately 45 minutes after slaughter a pH reading was taken and recorded (mentioned below).

3.6. Carcass measurements post mortem

At the Blokhuis abbotoir the carcasses each received a serial number after they were dressed and graded. This serial number was used as a reference for all the measurements and sample analysis. The serial number was recorded with the ear tag that was initially used to identify the sheep and track their growth and the treatments they received during the growing phase.

The pH was measured using a calibrated (standard buffers pH 4.0 and pH 7.0) portable meat pH meter (Hanna, model HI 99163). The electrode was inserted into the muscle at the 13th rib, 25mm from the mid-line (*M. Longissimus et. lumborum*). The electrode was rinsed with distilled water after each measurement. Photos were taken of the visceral fat of each carcass. The carcasses were then hung in the cold room for 3 days at 4ºC.

3.7. Sample collection

Ten carcasses from each treatment group (80 carcasses) were randomly selected for sample collection. Three days after slaughter the carcasses were delivered to Vereeniging Meat Packers in a refrigerated truck. The carcasses were cut using a band saw on site. Firstly, each carcass was split at the 13th rib and the subcutaneous carcass fat was measured, 2.5cm from the midline on the right side, using a digital caliper (Digital Caliper Vernier Gauge Micrometer) (Johnson *et al*., 1998). The pH was measured again by inserting a meat probe into the rib eye of the 13th rib on the left side of the carcass, using a Hanna Meat pH meter (HI 99163, Romania). The pH meter was calibrated before use (standard buffers pH 4.0 and pH 7.0) and the electrode was rinsed with distilled water after each measurement.

A three rib cut sample was taken from the left side of each carcass (8th – 10th rib). The ventral extremity of the sample was determined by a line drawn from the cranial point of the pubic symphysis to the middle of the first rib, illustrated in figure 3.3. This was to ensure a proper estimate of carcass composition (Webb *et al*., 1990; Casey et. al 1988).

The three-rib cut samples and samples of approximately 5g of visceral fat plus a kidney from each carcass were put in individual polyethylene bags with their serial number and transported in cooler boxes to the University of Pretoria. The samples were stored at the Department of Animal and Wildlife Sciences in the freezer at -22ºC (López-Carlos *et al*., 2014; Byrne *et al*., 2000; Johnson *et al*., 1998; Webb *et al*., 1995).

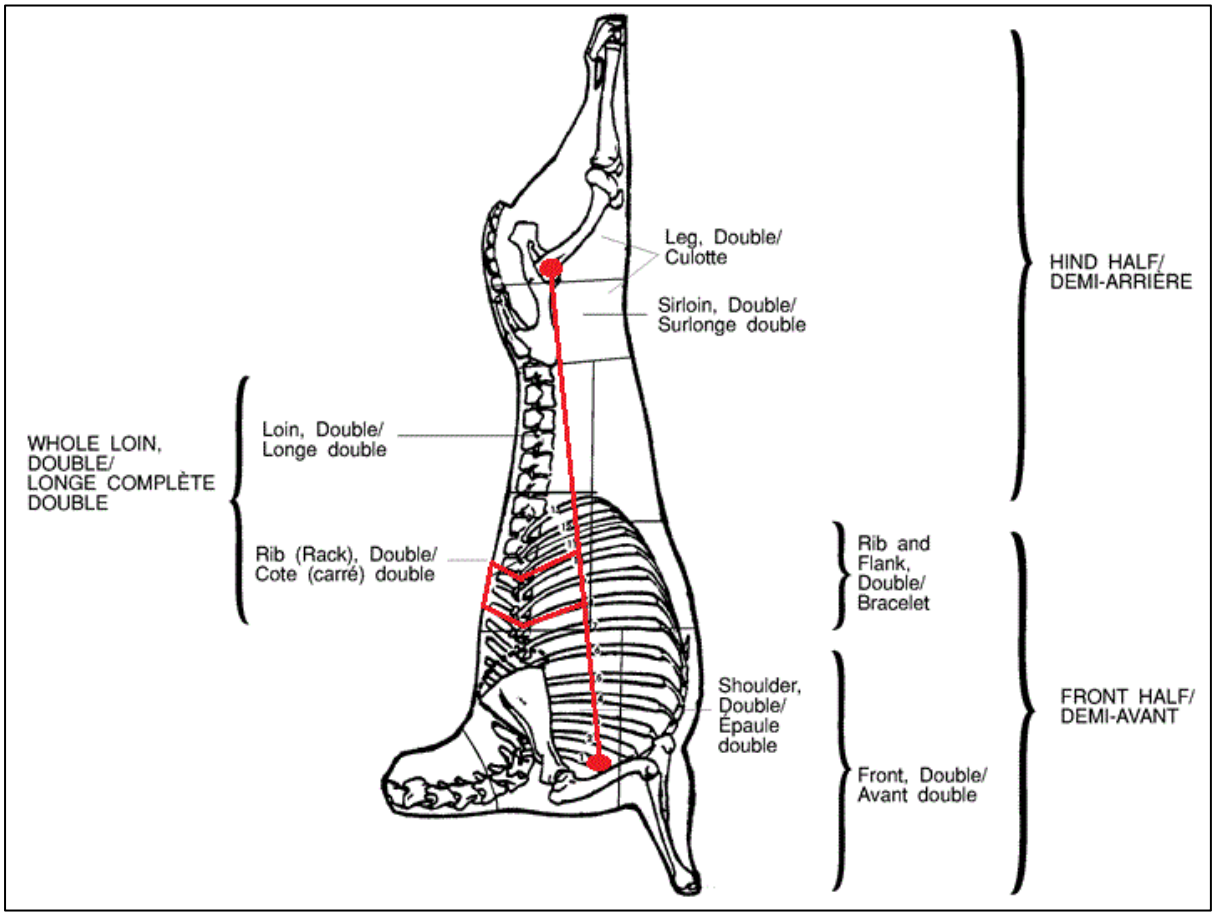

Figure 3.3. An illustration to show sample collection. The red line down the centre of the carcass indicates the line drawn on each carcass, from the cranial point on the pubic symphysis to the middle of the first rib. The blocked section shows the three-rib cut sample that was taken for further analysis.

3.8. Sample Measurements

3.8.1. Carcass composition

The three-rib cut samples were thawed for 24 hours at 4ºC and were then weighed and dissected (López-Carlos *et al*., 2014; Babiker *et al*., 1990). 80 samples were dissected into muscle, fat and bone. Connective tissue was included in the muscle portion. Each portion was weighed and expressed as a percentage of the whole sample.

3.8.2 Cooking loss

The rib-eye was dissected and weighed (included in the muscle portion) and used for measuring cooking loss. Each rib-eye was placed in a polyethylene bag and cooked in a Labcon water bath at 80ºC for 60 minutes (Babiker *et* al., 1990; Kemp *et al*., 1976). The rib-eye was left at room temperature to cool, excess water was removed and was dried by blotting dry the samples with Kimberly-Clark® paper towels. Then the rib-eye was weighed again and total cooking loss was calculated:

Total cooking loss = uncooked rib-eye mass – cooked rib-eye mass

The cooked samples were kept for tenderness analysis.

3.8.3 Tenderness

A minimum of 4 core samples were taken (1.27 cm diameter) parallel with the muscle fiber direction from each rib-eye. Shear force was tested using an Instron Machine (Model 1000), equipped with a Warner-Bratzler blade. The samples were placed parallel to the blade. (Babiker, *et al*., 1990; Bolemann *et al*., 2004). Each sample was sheared at a speed of 100mm/min and the shear force value was recorded in Newtons (N). The mean for each rib-eye sample was calculated and recorded.

3.9 Animal Health

The lambs received inoculations on two occasions. When the lambs were selected they went through their first inoculation process, consisting of: Ovipast Plus (MSD); Coglavax (Ceva); Virbamax First Drench (Virbac) and Prodose Yellow LA (Virbac). Half of

the lambs also received zeranol (Ralgro® (MSD)) implants in their left ear. 14 days later, the lambs had follow up inoculations of: Multivax-P Plus (MSD) and Ovipast Plus (MSD).

It was necessary to remove certain lambs due to illness, poor growth or death. These lambs were eliminated from the experimental group.

3.10 Statistical analysis

The effect of ZH and zeranol on growth performance and carcass characteristics on feedlot lambs was analysed using multifactorial analysis of variance (MANOVA) by means of the General Linear Model (GLM) procedure of SAS (2003). The variables were ZH treatment, zeranol treatment and duration of treatment. The covariate is initial weight of the lambs, because the initial weight of the lamb will influence the rest of the results.

Data was recorded in Microsoft Excel (2010). The results were statistically analysed by analysis of covariance and general linear regression models, using the initial weight of the lambs as the covariate in the model. Significance was tested at a level of 95% certainty (P <0.05).

The Bonferrroni method multiple range test was used for the comparison of means (LS means), to compensate for the different number of observations.

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Chapter 4. Results

4.1. Growth results

4.1.1. Influence of growth promotant treatment on average daily gain

The ADG of the male lambs in this trial was 264,9g/day, shown in Table 4.1. The control group, which received no zilpaterol hydrochloride (NZ-treatment) and no zeranol implants (NR-treatment), had an ADG of 260,4g/day. ADG of lambs in this trial are comparable with ADG in other trials (Deng, *et* al., 2012; Brand & Franck, 2000; Webb, *et al*., 1999).

Treatment of zilpaterol hydrochloride (Z-treatment) significantly influenced the average daily gain (ADG) (P <0.05) and therefore the growth rate of the lambs. Z-treatment had higher ADG's than NZ-treatment. Zeranol treatment (R-treatment) also significantly (P <0.017) affected the ADG, with R-treatment tending to have higher ADG's than NR-treatment. The implant treatment was expected to affect the initial growth phase after implant of Ralgro (60 days) and the ZH was expected to effect the final growth phase. The effect of Z-treatment on growth of the lambs is depicted in Graph 4.2.

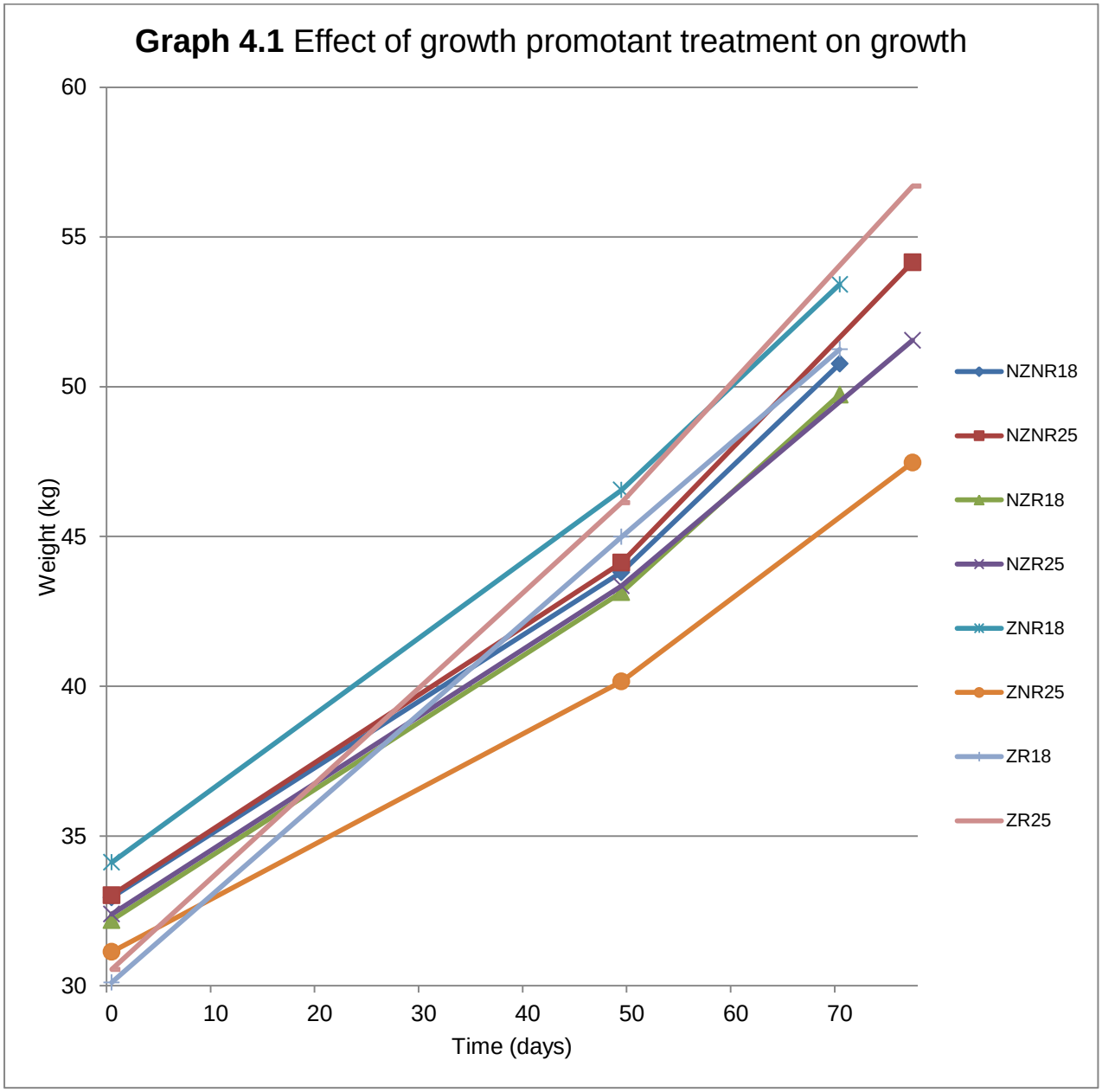
Covariance analysis was done to correct for initial weight and the results are seen in Table 4.6. Table 4.6 shows that ADG differed significantly between Z and NZ-treatment (P <0.05). Likewise, ADG differed significantly between R and NR-treatment (P <0.017). The interaction of Z-treatment and zeranol treatment (Z*R) on ADG was highly significant (P <0.002), showing the combined treatment to yield the best results.

The treatment group that received ZR25-treatment proved to be the group with the highest ADG, shown in Graph 4.1. ADG for this group was 335.3 ± 62.935g/day, summarised in Table 4.1. Similarly, the ADG of the lambs with ZR18-treatment had the next best response, with an ADG of 297.7 ± 36.555g/day.

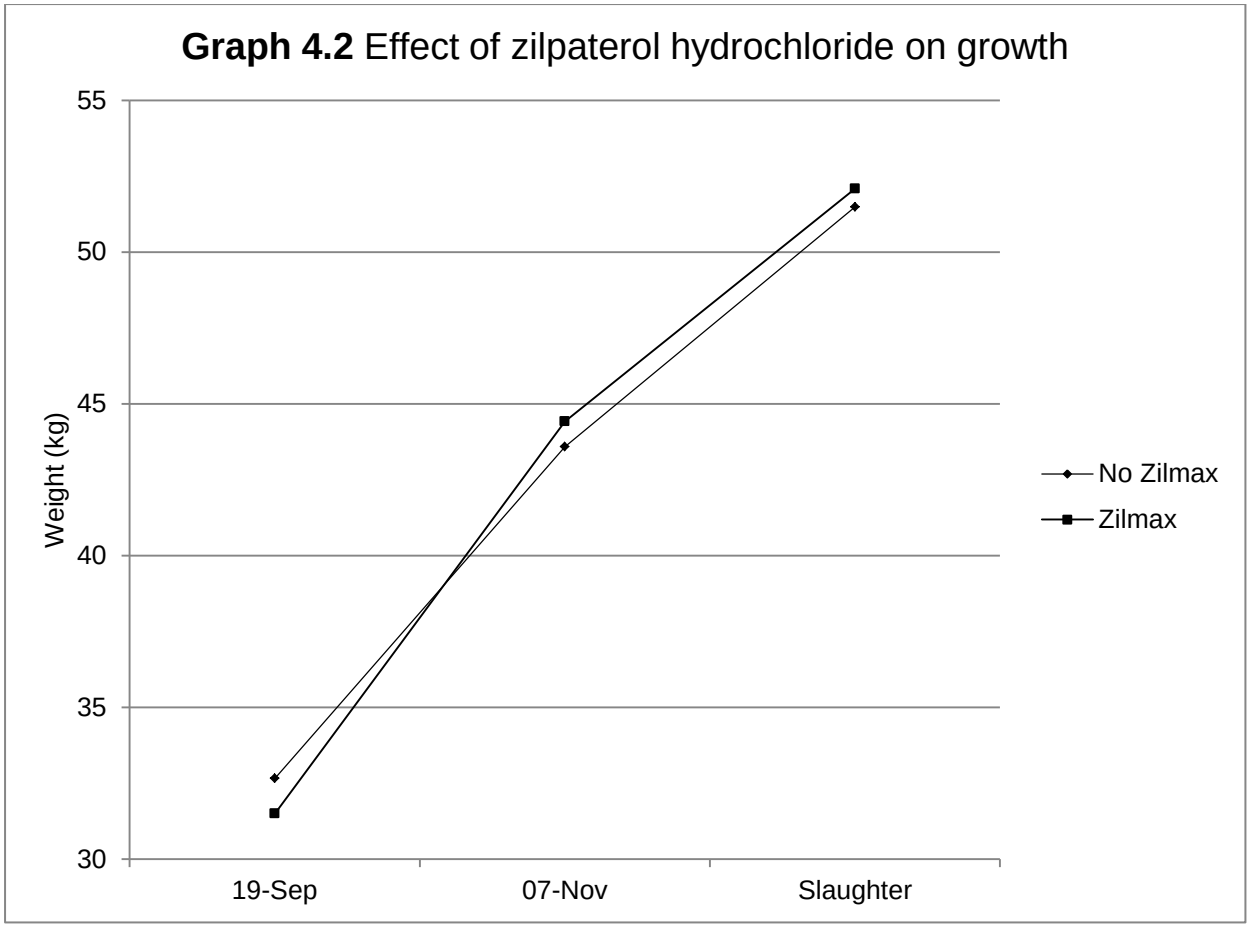
Table 4.1. Effect of zilpaterol hydrochloride, zeranol and duration of treatment on ADG (±SD) of Mutton Merino lambs

Zilpaterol hydrochloride treatment	Zeranol treatment	Duration of treatment (days)	ADG (g/day)	±SD
NZ	NR	18	251.0	79.330
		25	270.9	73.425
		Total	260.4^{w}	75.150
	R	18	247.2	76.525
		25	245.6^{b}	63.379
		Total	246.3^{d}	68.150
	Total	18	249.1	75.886
		25	257.0	67.467
		Total	253.0	70.987
Z	NR	18	271.8	52.481
		25	209.5^{a}	69.838
		Total	240.7^{c}	68.102
	R	18	297.7	36.555
		25	335.3^{ab}	62.935
		Total	315.5^{cdw}	52.917
	Total	18	284.8	45.982
		25	269.1	91.468
		Total	277.1	71.798
Total	NR	18	261.4	66.333
		25	238.6	76.345
		Total	250.3	71.375
	R	18	272.5	63.873
		25	286.0	76.686
		Total	279.2	69.994
	Total	18	266.9	64.517
		25	262.9	79.224
		Total	264.9	71.716

ADG(kg/day) ~ mean average daily gain in kilograms per day for each treatment group
SD ~ Standard deviation
^{abcd} ~ Means with superscripts differ significantly (P <0.05) based on the Bonferroni multiple comparisons test
^{w} ~ Means with suerscripts show trends to differ (P <0.10) based on the Bonferroni multiple comparisons test



Effect of the different growth promotant treatments on growth of the lambs over time is projected in Graph 4.1. Weight of the lambs increased as time progressed, although the rate of the growth differed between different treatments. The treatment with highest growth over time in grams was the ZR25-treatment with 335.3 ± 62.935g/day, as opposed to the lowest growth over time, which was ZNR25-treatment, with 209.5 ± 69.838g/day.



Slaughter ~ Average of weight (kg) of two groups slaughtered at 29 November and 6 December

4.1.2 Influence of growth promotant treatment on cold carcass mass

The effect of the growth promotant treatments on cold carcass mass is summarised in Table 4.2. Covariance analysis results show Z-treatment had a large effect on the cold carcass mass of the lambs and differed significantly (*P* <0.003) from the NZ treatment, as shown in Table 4.6. Mean cold carcass mass of Z-treated and NZ-treated lambs is 25.8 ±3.750kg and 24.9 ±3.695kg respectively. Lambs that received ZH had a cold carcass mass of nearly 1kg heavier. There was a highly significant difference in cold carcass mass between R-treatment and NR treatment (*P* <0.007). Z*R-treatment also differed very significantly (*P* <0.002).

Duration of the treatment period also influenced cold carcass mass, with a longer duration leading to a higher cold carcass mass. This is to be expected as the lambs has a longer time to grow. Duration of treatment differed significantly (*P* <0.008) as shown in Table 4.6. The interaction of Z*R treatment and the duration of treatment was also significant (*P* <0.006). The treatment group that received ZR25-treatment had the highest cold carcass mass. The mean cold carcass mass for ZR25-treatment was 28.722 ± 3.3551kg, shown in Table 4.2.

Table 4.2. Effect of zilpaterol hydrochloride, zeranol and duration of treatment on mean cold carcass mass (±SD) in Mutton Merino lambs

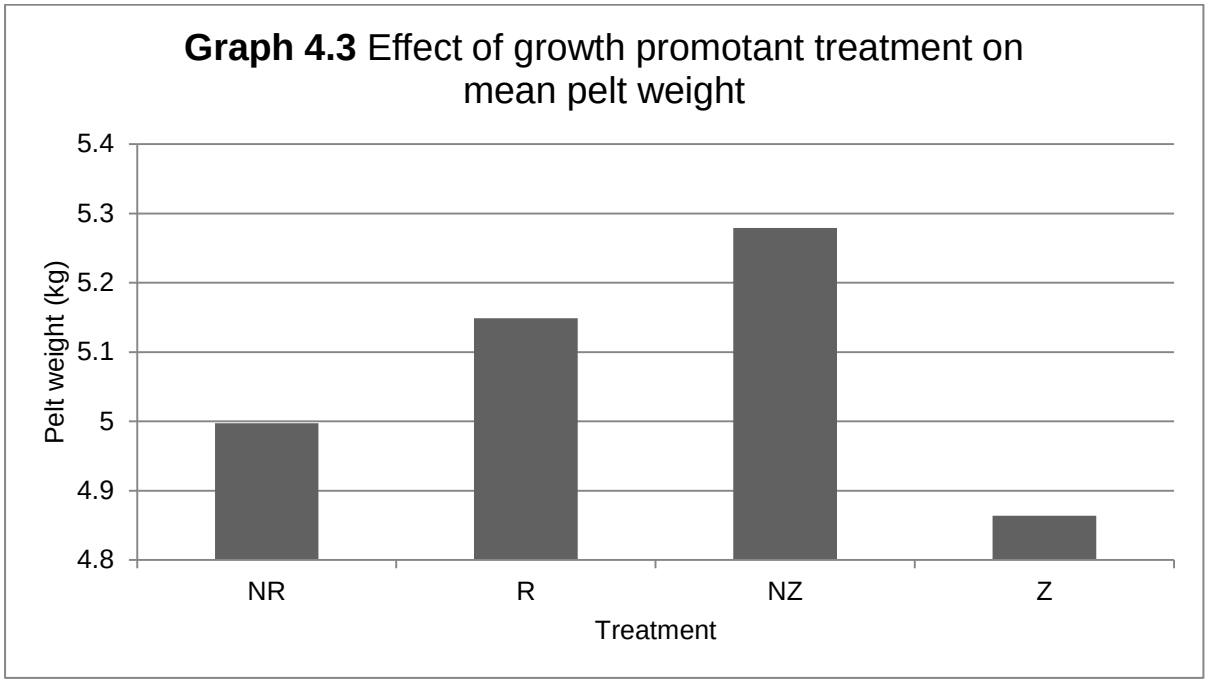
Zilpaterol hydrochloride treatment	Zeranol treatment	Duration of treatment (days)	Cold carcass mass (kg)	±SD
NZ	NR	18	24	3.052
		25	26.58	2.565
		Total	25.22	3.054
	R	18	24.32	4.220
		25	24.79	4.458
		Total	24.57	4.244
	Total	18	24.16	3.588
		25	25.60	3.750
		Total	24.88	3.695
Z	NR	18	26.21	3.355
		25	23.74[a]	3.906
		Total	24.98	3.763
	R	18	24.84	2.899
		25	28.72[a]	3.355
		Total	26.68	3.629
	Total	18	25.53	3.132
		25	26.10	4.378
		Total	25.81	3.750
Total	NR	18	25.11	3.321
		25	25.08	3.560
		Total	25.10	3.394
	R	18	24.58	3.534
		25	26.56	4.385
		Total	25.57	4.057
	Total	18	24.84	3.395
		25	25.84	4.022
		Total	25.34	3.728

Cold mass (kg) ~ mean cold mass in kilograms for each treatment group
SD ~ Standard deviation
[a] ~ Means with superscripts differ significantly (P <0.05) based on the Bonferroni multiple comparisons test

4.1.3 Influence of growth promotant treatment on pelt weight

The effect of growth promotant treatment on pelt weight is summarised in Table 4.3 and Graph 4.3. The covariance analysis results, seen in Table 4.6, show that pelt weight tended to differ with Z-treatment (*P* <0.098). Mean pelt mass of Z-treated lambs and NZ-treated lambs was 4.86 ±0.777kg and 5.28 ±0.9794kg respectively. Duration of treatment differed significantly (*P* <0.001) with a longer duration leading to an increase in pelt weight. This is to be expected as the pelt increases in size as the animal grows, thus a lamb that is alive for longer will have a higher body mass (shown above) and a heavier pelt.

Mean pelt weight of lambs treated for 18 days and 25 days was 4.82 ±0.689kg and 5.34 ±1.024kg respectively. Pelt weight for R and NR-treatment did not differ significantly (*P* <0.117). There was a significant interaction with Z*R on pelt weight (*P* <0.012).



4.1.3.a. The effect of sex on pelt weight

Sex was taken into account because there was a concern of the effect of sex on pelt weight. More force is needed to remove ram pelts than wether pelts, because of the secondary sex characteristics that rams possess (Anderson *et al.*, 1991). Results were analysed and the effect of sex on pelt weight was highly significant (*P* <0.000) (Table 4.12). Therefore, the effect of Z-treatment and zeranol on pelt weight needed to be analysed again, separating the

castrates and the intact males, to see a better effect of these variables on pelt weight. These results are found in Table 4.13 and 4.14. According to the covariance of analysis, the effect of Z-treatment on pelt weight was significant in wethers, but not in rams (P <0.013 and P <0.740, respectively) and the effect of R-treatment on pelt weight for both wethers and rams was not significant (P <0.710, P <0.323, respectively).

There was a significant Z*R interaction in wethers (P <0.022), however not in rams. This interaction was also seen when rams and wethers were analysed together. ZH decreased pelt weight, while zeranol increased pelt weight. In rams, the effect of secondary sex characteristics is high and this decreased the effect of ZH and zeranol on the pelt weight.

Duration of treatment had a significant effect on wether pelt weight and tended to have an effect on ram pelt weight (P <0.002; P <0.091). This is to be expected as seen with the entire group of wethers and rams. As the animal grew the pelts increased in size and therefore pelt weight also increased.

Table 4.3. Effect of zilpaterol hydrochloride, zeranol and duration of treatment on mean pelt weight (±SD) in Mutton Merino lambs

Zilpaterol hydrochloride treatment	Zeranol treatment	Duration of treatment (days)	Pelt mass(kg)	±SD
NZ	NR	18	5.06	1.029
		25	5.75	1.285
		Total	5.38[a]	1.179
	R	18	4.86	0.645
		25	5.48	0.784
		Total	5.18	0.773
	Total	18	4.96	0.842
		25	5.60	1.018
		Total	5.28	0.979
Z	NR	18	4.59	0.548
		25	4.67	0.955
		Total	4.63[a]	0.759
	R	18	4.77	0.398
		25	5.49	0.857
		Total	5.11	0.736
	Total	18	4.68	0.475
		25	5.06	0.979
		Total	4.86	0.777
Total	NR	18	4.82	0.837
		25	5.18	1.224
		Total	5.00	1.045
	R	18	4.81	0.524
		25	5.49	0.795
		Total	5.15	0.747
	Total	18	4.82	0.689
		25	5.34	1.024
		Total	5.07	0.904

Pelt mass(kg/day) ~ mean pelt mass in kilograms per day for each treatment group
SD ~ Standard deviation
[a] ~ Means with superscripts differ significantly (P <0.05) based on the Bonferroni multiple comparisons test

4.2. Carcass characteristics

4.2.1. Influence of growth promotant treatment on pH at 45 minutes post mortem and pH 24 hours of chilling

The effect of growth promotant treatment on pH at 45 minutes post mortem was only significantly influenced with duration of treatment (*P* <0.029), according to covariance of analysis results (Table 4.6). The mean pH for lamb carcasses treated for 18 days and for 25 days were 6.56 ±0.323 and 6.71 ±0.263, respectively. The effect of treatment is summarised in Table 4.4. The effect of lambs growing for a longer period increased the pH of the carcass post mortem. Z-treatment and R-treatment did not influence pH 45 minutes post mortem significantly.

The covariance of analysis results (Table 4.6) show that the effect of duration of treatment differs highly significantly and influences the pH after chilling (*P*<0.000). Mean pH of the lamb carcasses for 18 days and 25 days is 5.42 ±0.130 and 5.54 ±0.125, respectively. This confirms the result above; that lambs that grew for a longer period, had a higher pH.

Z*R treatment had a tendency to influence pH after chilling (*P* <0.081). There was a significant interaction with R*D treatment on pH after chilling (*P* <0.011).

4.2.2. The effect of growth promotant treatment on subcutaneous carcass fat thickness

The effect of growth promotant treatment on carcass fat thickness is summarised in Table 4.5. Z-treatment did not differ significantly as was expected, and duration of treatment was the only treatment that influenced the subcutaneous fat thickness significantly (*P* <0.012), shown in Table 4.6. Merino lambs that were treated for 25 days had thicker subcutaneous fat than lambs treated for 18 days. The mean subcutaneous fat thickness for 18 days and 25 days was 7.27 ±2.296cm and 9.22 ±3.878cm, respectively.

Table 4.4. Effect of zilpaterol hydrochloride, zeranol and duration of treatment on pH (±SD) at 45 minutes post slaughter and pH 24 hours after chilling on Mutton Merino lambs

Zilpaterol hydrochloride treatment	Zeranol treatment	Duration of treatment (days)	pH 45 minutes post slaughter	±SD	pH 24 hours after chilling	±SD
NZ	NR	18	6.51	0.186	5.35[w]	0.067
		25	6.63	0.170	5.52	0.067
		Total	6.57	0.185	5.43	0.109
	R	18	6.51	0.229	5.50[w]	0.088
		25	6.88	0.280	5.47[a]	0.071
		Total	6.70	0.315	5.48	0.079
	Total	18	6.51	0.203	5.42	0.109
		25	6.77	0.262	5.49	0.072
		Total	6.64	0.266	5.46	0.098
Z	NR	18	6.59	0.491	5.41	0.118
		25	6.70	0.311	5.62[a]	0.193
		Total	6.64	0.404	5.52	0.191
	R	18	6.62	0.335	5.42	0.183
		25	6.61	0.193	5.54	0.078
		Total	6.62	0.270	5.48	0.152
	Total	18	6.61	0.410	5.41	0.150
		25	6.65	0.259	5.58	0.153
		Total	6.63	0.341	5.50	0.172
Total	NR	18	6.55	0.364	5.38	0.099
		25	6.67	0.250	5.57	0.154
		Total	6.61	0.315	5.47	0.161
	R	18	6.56	0.286	5.46	0.145
		25	6.76	0.275	5.50	0.080
		Total	6.66	0.294	5.48	0.118
	Total	18	6.56	0.323	5.42	0.130
		25	6.71	0.263	5.54	0.125
		Total	6.63	0.303	5.48	0.140

pH ~ mean pH for each treatment group
SD ~ Standard deviation
[a] ~ Means with superscripts differ significantly (P <0.05) based on the Bonferroni multiple comparisons test
[w] ~ Means with superscripts show trends to differ (P <0.10) based on the Bonferroni multiple comparisons test

Table 4.5. Effect of zilpaterol hydrochloride, zeranol and duration of treatment on subcutaneous carcass fat thickness (cm) (±SD) of Mutton Merino carcasses

Zilpaterol hydrochloride treatment	Zeranol treatment	Duration of treatment (days)	Subcutaneous carcass fat thickness (cm)	±SD
NZ	NR	18	6.55	1.36
		25	8.4	3.293
		Total	7.43	2.578
	R	18	7.75	2.139
		25	10.2	4.69
		Total	9.04	3.825
	Total	18	7.15	1.85
		25	9.39	4.121
		Total	8.27	3.352
Z	NR	18	6.85	2.933
		25	9.72	4.68
		Total	8.28	4.077
	R	18	7.94	2.509
		25	8.27	2.247
		Total	8.09	2.328
	Total	18	7.39	2.715
		25	9.03	3.708
		Total	8.19	3.3
Total	NR	18	6.7	2.23
		25	9.1	4.028
		Total	7.87	3.413
	R	18	7.84	2.271
		25	9.33	3.831
		Total	8.59	3.199
	Total	18	7.27	2.296
		25	9.22	3.878
		Total	8.23	3.305

Subcutaneous carcass fat thickness ~ mean subcutaneous carcass fat thickness in centimetres for each treatment group
SD ~ Standard deviation

4.2.3. Effect of growth promotant treatment on subcutaneous fat thickness of 8th and 10th rib

Z-treatment did not influence subcutaneous fat thickness (SCF) significantly, as was expected. Duration of treatment caused SCF of the 8th rib to differ significantly (P <0.025). SCF was 2.8282 ±1.33519cm for 18 days and 3.5146 ±1.47088cm for 25 days, for the 8th rib. SCF increases with more days on treatment or growing.

R-treatment had a tendency to influence SCF of the 10th rib (P <0.088). SCF was 8.5458 ±2.69577cm for NR-treatment and 7.5802 ±2.34512cm for R-treatment, for the 10th rib. This shows that with R-treatment there was a decrease in SCF. The influence of treatment on SCF is summarised in Table 4.7 and analysis of variance is shown in Table 4.9.

4.2.4. The influence of growth promotant treatment on carcass composition

The effect of treatment on carcass composition was not as expected. A significant difference was expected between fat and muscle portions of the carcass, this was not reflected in the results (P >0.05) (Table 4.9). The effect of treatment on carcass composition is summarised in Table 4.8. Z-treatment was expected to cause a significant increase in the amount of muscle and a decrease in fat.

In Table 4.8, NZ-treatment resulted in 48.34 ±4.75% muscle and Z-treatment resulted in 49.09 ±4.91% muscle, therefore showing an increase in the portion of muscle deposited. Z-treatment had a slight effect on fat deposits with 36.63 ±5.57% and 36.01 ±5.52% for NZ and Z-treatment respectively. These results show the fat decreased by a very small amount with Z-treatment. Lambs on longer treatment period also had more fat deposit, with 35.65 ±4.92% and 37.00 ±6.02% for 18 and 25 days respectively. This is a similar result to SCF results.

Table 4.6. Effect of zilpaterol hydrochloride, zeranol and duration of treatment on subcutaneous fat thickness of the 8th and 10th rib (±SD) of Mutton Merino lambs

Zilpaterol hydrochloride treatment	Zeranol treatment	Duration of treatment (days)	SCF 8th rib (cm)	±SD	SCF 10th rib (cm)	±SD
NZ	NR	18	2.824	1.3112	7.978	1.7098
		25	4.056	1.7439	9.231	2.6229
		Total	3.372	1.5998	8.535	2.1889
	R	18	3.516	1.7383	7.101	2.6951
		25	3.336	1.0896	7.269	2.1916
		Total	3.421	1.4007	7.189	2.3828
	Total	18	3.170	1.5400	7.540	2.2423
		25	3.639	1.4057	8.095	2.5169
		Total	3.399	1.4759	7.810	2.3651
Z	NR	18	2.697	1.1187	7.622	1.9929
		25	3.836	1.4373	9.489	3.8624
		Total	3.267	1.3830	8.556	3.1408
	R	18	2.276	0.9216	8.057	1.8239
		25	2.957	1.6200	7.925	2.7852
		Total	2.617	1.3295	7.991	2.2923
	Total	18	2.487	1.0207	7.840	1.8726
		25	3.397	1.5572	8.707	3.3741
		Total	2.942	1.3789	8.273	2.7291
Total	NR	18	2.761	1.1880	7.800	1.8164
		25	3.934	1.5358	9.374	3.2784
		Total	3.316	1.4700	8.546	2.6958
	R	18	2.896	1.4961	7.579	2.2928
		25	3.155	1.3461	7.581	2.4505
		Total	3.029	1.4094	7.580	2.3451
	Total	18	2.828	1.3352	7.690	2.0447
		25	3.515	1.4709	8.409	2.9646
		Total	3.167	1.4369	8.045	2.5502

SCF (cm) ~ mean subcutaneous fat thickness in centimetres for each treatment group
SD ~ Standard deviation

Table 4.7. Effect of zilpaterol hydrochloride, zeranol and duration of treatment on carcass composition (±SD) of Mutton Merino lambs

Zilpaterol hydrochloride treatment	Zeranol treatment	Duration of treatment (days)	Fat portion	±SD	Muscle portion	±SD
NZ	NR	18	35.34%	5.75%	48.75%	4.90%
		25	36.85%	5.58%	48.55%	4.77%
		Total	36.01%	5.56%	48.66%	4.70%
	R	18	36.51%	4.72%	49.07%	4.42%
		25	37.76%	6.42%	47.14%	5.32%
		Total	37.17%	5.57%	48.06%	4.89%
	Total	18	35.93%	5.15%	48.91%	4.54%
		25	37.38%	5.93%	47.73%	5.01%
		Total	36.63%	5.52%	48.34%	4.75%
Z	NR	18	35.71%	5.50%	49.20%	4.81%
		25	37.40%	7.65%	47.78%	7.09%
		Total	36.55%	6.54%	48.49%	5.94%
	R	18	35.03%	4.22%	50.60%	3.95%
		25	35.89%	4.72%	48.77%	3.31%
		Total	35.46%	4.38%	49.69%	3.66%
	Total	18	35.37%	4.78%	49.90%	4.34%
		25	36.64%	6.23%	48.28%	5.41%
		Total	36.01%	5.52%	49.09%	4.91%
Total	NR	18	35.53%	5.48%	48.97%	4.73%
		25	37.16%	6.63%	48.12%	6.01%
		Total	36.30%	6.02%	48.57%	5.32%
	R	18	35.77%	4.43%	49.84%	4.15%
		25	36.87%	5.61%	47.92%	4.45%
		Total	36.33%	5.04%	48.85%	4.36%
	Total	18	35.65%	4.92%	49.40%	4.42%
		25	37.00%	6.02%	48.01%	5.16%
		Total	36.32%	5.50%	48.72%	4.82%

Fat portion ~ mean fat deposition in 3-rib cut sample as a percent for each treatment group
Muscle portion ~ mean muscle deposition in 3-rib cut sample as a percent for each treatment group
SD ~ Standard deviation

4.3. Meat quality results

4.3.1. Influence of growth promotant treatment on tenderness

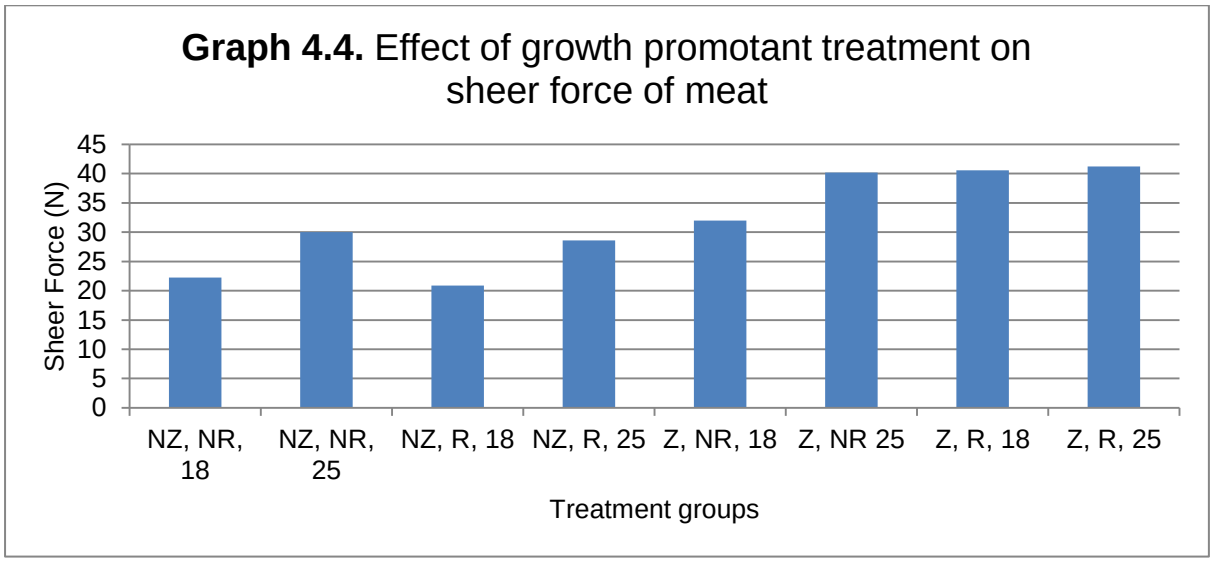
The effect of different growth promotant treatments on the tenderness of the meat is summarised in Table 4.6 and in Graph 4.4. Tenderness was expected to differ between Z-treatment and NZ treatment however such a significant result (*P* <0.000) was not expected.

The analysis of variance results show that the effect of Z-treatment on tenderness was highly significant (*P* <0.000) (Table 4.9). Duration of treatment also significantly differed on tenderness (*P* <0.018). The treatment group with the toughest meat was ZR25-treatment and had a mean sheer force of 41.22 ±14.394N, shown in Table 4.6 and Graph 4.4. The meat that was most tender was from the treatment group NZR18 with a mean sheer force of 20.90 ±3.882N.

Table 4.8. Effect of zilpaterol hydrochloride, zeranol and duration of treatment on sheer force (±SD) of Mutton Merino lambs

Zilpaterol hydrochloride treatment	Zeranol treatment	Duration of treatment (days)	Sheer force (N)	±SD
NZ	NR	18	22.24[a]	7.092
		25	30.03	10.037
		Total	25.70[cdg]	9.163
	R	18	20.90[bw]	3.882
		25	28.57	9.384
		Total	24.92[ef]	8.138
	Total	18	21.57	5.607
		25	29.19	9.415
		Total	25.28	8.519
Z	NR	18	31.98[w]	6.103
		25	40.19	15.360
		Total	36.08[ce]	12.130
	R	18	40.60[ab]	15.784
		25	41.22	14.394
		Total	40.91[dfg]	14.705
	Total	18	36.29	12.458
		25	40.70	14.497
		Total	38.49	13.528
Total	NR	18	27.11	8.149
		25	35.67	13.906
		Total	31.17	11.905
	R	18	30.75	15.075
		25	34.59	13.384
		Total	32.72	14.188
	Total	18	28.93	12.102
		25	35.09	13.457
		Total	31.97	13.079

Sheer force (N) ~ mean sheer force in Newtons of force for each treatment group
SD ~ Standard deviation
[abcdefg] ~ Means with superscripts differ significantly (P <0.05) based on the Bonferroni multiple comparisons test
[wxyz] ~ Means with superscripts show trends to differ (P <0.10) based on the Bonferroni multiple comparisons test



4.3.2. Influence of growth promotant treatment on cooking loss

The influence of treatment on cooking loss is summarised in Table 4.6. Analysis of variance results, (Table 4.9) showed that there was little significance of treatment on cooking loss. Z-treatment and duration of treatment tended to influence cooking loss (P <0.074), (P <0.056), respectively. Z-treatment caused cooking loss to be higher in the lamb rib eye. Z-treated lamb had a cooking loss of 27.01 ±6.078g and NZ-treated lamb; 24.62 ±5.160g. R-treatment was not significantly different (P <0.408). The combination treatment of ZH, zeranol and duration of treatment tended to cause an interaction on cooking loss results (P <0.085).

Table 4.9. Effect of zilpaterol hydrochloride, zeranol and duration of treatment on cooking loss (±SD) in Mutton Merino lambs

Zilpaterol hydrochloride treatment	Zeranol treatment	Duration of treatment (days)	Cooking loss (g)	±SD
NZ	NR	18	25.09	5.304
		25	27.24	3.358
		Total	26.05	4.555
	R	18	23.32	5.629
		25	23.46[w]	5.525
		Total	23.39	5.434
	Total	18	24.20	5.400
		25	25.05	5.003
		Total	24.62	5.160
Z	NR	18	26.47	3.698
		25	26.82	6.584
		Total	26.65	5.200
	R	18	23.89	2.818
		25	30.85[w]	8.221
		Total	27.37	6.966
	Total	18	25.18	3.463
		25	28.84	7.538
		Total	27.01	6.078
Total	NR	18	25.78	4.506
		25	27.01	5.257
		Total	26.36	4.849
	R	18	23.61	4.342
		25	26.98	7.746
		Total	25.33	6.471
	Total	18	24.69	4.505
		25	26.99	6.629
		Total	25.83	5.735

Cooking loss (g) ~ mean cooking loss in grams for each treatment group
SD ~ Standard deviation
[w] ~ Means with superscript show trend to differ (P <0.10) based on the Bonferroni multiple comparisons test

Table 4.10. Analysis of covariance of growth results and carcass characteristics

Treatment	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Weight1909	Weight 7/11	1397.979	1	1397.979	125.296	0
	Weight (18 days/25 days))	1718.215	1	1718.215	74.122	0
	Pelt Weight	10.054	1	10.054	17.257	0
	PH 45 min after slaughter	0.018	1	0.018	0.204	0.653
	Cold Mass	483.853	1	483.853	81.437	0
	PH 24 hours of chilling	0.01	1	0.01	0.719	0.399
	Subcutaneous fat thickness	0.165	1	0.165	0.016	0.901
	ADG	0.015	1	0.015	3.554	0.064
Zilpaterol hydrochloride treatment	Weight 7/11	92.018	1	92.018	8.247	0.005
	Weight (18 days/25 days))	87.988	1	87.988	3.796	0.055
	Pelt Weight	1.639	1	1.639	2.813	0.098
	PH 45 min after slaughter	0	1	0	0.002	0.966
	Cold Mass	58.331	1	58.331	9.818	0.003
	PH 24 hours of chilling	0.024	1	0.024	1.71	0.195
	Subcutaneous fat thickness	0.008	1	0.008	0.001	0.979
	ADG	0.016	1	0.016	3.966	0.05
Zeranol treatment	Weight 7/11	114.541	1	114.541	10.266	0.002
	Weight (18 days/25 days))	144.5	1	144.5	6.234	0.015
	Pelt Weight	1.469	1	1.469	2.521	0.117
	PH 45 min after slaughter	0.05	1	0.05	0.57	0.453
	Cold Mass	45.402	1	45.402	7.642	0.007
	PH 24 hours of chilling	0	1	0	0.017	0.897
	Subcutaneous fat thickness	8.721	1	8.721	0.829	0.366
	ADG	0.025	1	0.025	5.982	0.017

Duration	Weight 7/11	5.336	1	5.336	0.478	0.492
	Weight (18 days/25 days))	70.702	1	70.702	3.05	0.085
	Pelt Weight	6.714	1	6.714	11.524	0.001
	PH 45 min after slaughter	0.435	1	0.435	4.97	0.029
	Cold Mass	44.114	1	44.114	7.425	0.008
	PH 24 hours of chilling	0.267	1	0.267	18.661	0
	Subcutaneous fat thickness	69.437	1	69.437	6.604	0.012
	ADG	5.47E-06	1	5.47E-06	0.001	0.971
Zilpaterol hydrochloride treatment *	Weight 7/11	111.102	1	111.102	9.958	0.002
	Weight (18 days/25 days))	265.877	1	265.877	11.47	0.001
Zeranol treatment	Pelt Weight	3.851	1	3.851	6.609	0.012
	PH 45 min after slaughter	0.098	1	0.098	1.119	0.294
	Cold Mass	63.879	1	63.879	10.751	0.002
	PH 24 hours of chilling	0.045	1	0.045	3.126	0.081
	Subcutaneous fat thickness	13.389	1	13.389	1.273	0.263
	ADG	0.045	1	0.045	10.905	0.002
Zilpaterol hydrochloride Treatment * Duration	Weight 7/11	7.723	1	7.723	0.692	0.408
	Weight (18 days/25 days))	5.268	1	5.268	0.227	0.635
	Pelt Weight	0.074	1	0.074	0.127	0.722
	PH 45 min after slaughter	0.187	1	0.187	2.137	0.148
	Cold Mass	0.096	1	0.096	0.016	0.899
	PH 24 hours of chilling	0.04	1	0.04	2.775	0.1
	Subcutaneous fat thickness	1.391	1	1.391	0.132	0.717
	ADG	0.001	1	0.001	0.311	0.579
Zeranol treatment * Duration	Weight 7/11	13.386	1	13.386	1.2	0.277
	Weight (18 days/25 days))	33.829	1	33.829	1.459	0.231
	Pelt Weight	0.061	1	0.061	0.104	0.748

	PH 45 min after slaughter	0.017	1	0.017	0.194	0.661
	Cold Mass	4.094	1	4.094	0.689	0.409
	PH 24 hours of chilling	0.098	1	0.098	6.837	0.011
	Subcutaneous fat thickness	4.788	1	4.788	0.455	0.502
	ADG	0.005	1	0.005	1.237	0.27
Zilpaterol hydrochloride treatment *	Weight 7/11	18.052	1	18.052	1.618	0.208
	Weight (18 days/25 days))	93.448	1	93.448	4.031	0.049
Zeranol treatment * Duration	Pelt Weight	0.18	1	0.18	0.309	0.58
	PH 45 min after slaughter	0.174	1	0.174	1.989	0.163
	Cold Mass	47.075	1	47.075	7.923	0.006
	PH 24 hours of chilling	0.014	1	0.014	1.003	0.32
	Subcutaneous fat thickness	12.268	1	12.268	1.167	0.284
	ADG	0.014	1	0.014	3.49	0.066

Covariance analysis ~ correct for initial weight

Table 4.11. Analysis of variance for carcass characteristics and meat quality results

Treatment	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Zilpaterol hydrochloride treatment	SCF 8th rib (cm)	4.736	1	4.736	2.455	0.122
	SCF 10th rib (cm)	2.808	1	2.808	0.436	0.511
	Cooking loss (g)	97.762	1	97.762	3.281	0.074
	Fat_perc	7.298	1	7.298	0.227	0.636
	Muscle_perc	9.959	1	9.959	0.408	0.525
	Bone_perc	0.206	1	0.206	0.029	0.866
	Tenderness	3344.248	1	3344.248	27.169	0
Zeranol treatment	SCF 8th rib (cm)	2.164	1	2.164	1.122	0.293
	SCF 10th rib (cm)	19.299	1	19.299	2.994	0.088
	Cooking loss (g)	20.685	1	20.685	0.694	0.408
	Fat_perc	0.017	1	0.017	0.001	0.982
	Muscle_perc	2.086	1	2.086	0.085	0.771
	Bone_perc	1.731	1	1.731	0.24	0.626
	Tenderness	57.454	1	57.454	0.467	0.497
Duration	SCF 8th rib (cm)	10.107	1	10.107	5.24	0.025
	SCF 10th rib (cm)	12.21	1	12.21	1.894	0.173
	Cooking loss (g)	112.927	1	112.927	3.79	0.056
	Fat_perc	34.436	1	34.436	1.069	0.305
	Muscle_perc	35.402	1	35.402	1.45	0.232
	Bone_perc	0.007	1	0.007	0.001	0.976
	Tenderness	723.289	1	723.289	5.876	0.018
Zilpaterol hydrochloride treatment * Zeranol treatment	SCF 8th rib (cm)	1.981	1	1.981	1.027	0.314
	SCF 10th rib (cm)	3.585	1	3.585	0.556	0.458
	Cooking loss (g)	60.195	1	60.195	2.02	0.16
	Fat_perc	22.364	1	22.364	0.694	0.407
	Muscle_perc	14.871	1	14.871	0.609	0.438
	Bone_perc	0.762	1	0.762	0.106	0.746
	Tenderness	190.017	1	190.017	1.544	0.218

Zilpaterol hydrochloride treatment * Duration	SCF 8th rib (cm)	0.723	1	0.723	0.375	0.542
	SCF 10th rib (cm)	0.121	1	0.121	0.019	0.892
	Cooking loss (g)	30.851	1	30.851	1.035	0.312
	Fat_perc	0.056	1	0.056	0.002	0.967
	Muscle_perc	1.546	1	1.546	0.063	0.802
	Bone_perc	2.189	1	2.189	0.303	0.583
	Tenderness	53.854	1	53.854	0.438	0.51
Zeranol treatment *Duration	SCF 8th rib (cm)	4.29	1	4.29	2.224	0.14
	SCF 10th rib (cm)	11.658	1	11.658	1.809	0.183
	Cooking loss (g)	25.91	1	25.91	0.87	0.354
	Fat_perc	1.479	1	1.479	0.046	0.831
	Muscle_perc	5.625	1	5.625	0.23	0.633
	Bone_perc	12.872	1	12.872	1.784	0.186
	Tenderness	72.755	1	72.755	0.591	0.445
Zilpaterol hydrochloride treatment * Zeranol treatment * Duration	SCF 8th rib (cm)	1.117	1	1.117	0.579	0.449
	SCF 10th rib (cm)	1.024	1	1.024	0.159	0.691
	Cooking loss (g)	91.199	1	91.199	3.061	0.085
	Fat_perc	0.377	1	0.377	0.012	0.914
	Muscle_perc	2.18	1	2.18	0.089	0.766
	Bone_perc	0.744	1	0.744	0.103	0.749
	Tenderness	68.516	1	68.516	0.557	0.458

Covariance analysis ~ correct for initial weight

Table 4.12. Analysis of covariance of the effect of sex (wethers / rams) on growth performance and carcass characteristics

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Sex	Weight 7/11	210.589	1	210.589	18.948	.000
	Weight (18 days/25 days))	444.637	1	444.637	19.217	.000
	Pelt Weight	10.380	1	10.380	18.117	.000
	PH 45 min after slaughter	.137	1	.137	1.523	.221
	Fat grading	.288	1	.288	2.170	.145
	Cold Mass	27.516	1	27.516	3.426	.068
	PH 24 hours of chilling	.006	1	.006	.275	.601
	Subcutaneous fat thickness	80.247	1	80.247	7.957	.006
	ADG	.073	1	.073	18.003	.000

Covariance analysis ~ correct for initial weight

Table 4.13. Analysis of covariance of wethers on growth performance and carcass characteristics

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Zilpaterol hydrochloride treatment	Weight 7/11	67.139	1	67.139	6.875	.012
	Weight (18 days/25 days))	105.700	1	105.700	4.486	.040
	Pelt Weight	2.943	1	2.943	6.751	.013
	PH 45 min after slaughter	.012	1	.012	.116	.736
	Cold Mass	45.824	1	45.824	7.265	.010
	PH 24 hours of chilling	.010	1	.010	.511	.479
	Fat thickness	3.038	1	3.038	.284	.597
	ADG	.020	1	.020	4.810	.034
Zeranol treatment	Weight 7/11	57.347	1	57.347	5.872	.020
	Weight (18 days/25 days))	51.400	1	51.400	2.181	.147
	Pelt Weight	.061	1	.061	.140	.710
	PH 45 min after slaughter	.251	1	.251	2.436	.126
	Cold Mass	16.928	1	16.928	2.684	.109
	PH 24 hours of chilling	7.132E-6	1	7.132E-6	.000	.985
	Subcutaneous fat thickness	34.254	1	34.254	3.198	.081
	ADG	.009	1	.009	2.214	.144
Duration	Weight 7/11	3.314	1	3.314	.339	.563
	Weight (18 days/25 days))	30.668	1	30.668	1.301	.261
	Pelt Weight	4.627	1	4.627	10.613	.002
	PH 45 min after slaughter	.301	1	.301	2.924	.095
	Cold Mass	29.048	1	29.048	4.605	.038
	PH 24 hours of chilling	.110	1	.110	5.600	.023
	Subcutaneous fat thickness	73.597	1	73.597	6.871	.012
	ADG	1.974E-5	1	1.974E-5	.005	.945
Zilpaterol hydrochloride treatment * Zeranol treatment	Weight 7/11	84.242	1	84.242	8.626	.005
	Weight (18 days/25 days))	222.428	1	222.428	9.439	.004

	Pelt Weight	2.469	1	2.469	5.663	.022
	PH 45 min after slaughter	4.816E-5	1	4.816E-5	.000	.983
	Cold Mass	54.835	1	54.835	8.693	.005
	PH 24 hours of chilling	.025	1	.025	1.258	.269
	Subcutaneous fat thickness	9.969	1	9.969	.931	.340
	ADG	.038	1	.038	9.202	.004
Zilpaterol hydrochloride treatment * Duration	Weight 7/11	23.789	1	23.789	2.436	.126
	Weight (18 days/25 days))	27.335	1	27.335	1.160	.288
	Pelt Weight	1.215	1	1.215	2.786	.103
	PH 45 min after slaughter	.022	1	.022	.214	.646
	Cold Mass	4.466	1	4.466	.708	.405
	PH 24 hours of chilling	.016	1	.016	.807	.374
	Subcutaneous fat thickness	4.446	1	4.446	.415	.523
	ADG	.006	1	.006	1.414	.241
Zeranol treatment * Duration	Weight 7/11	.605	1	.605	.062	.805
	Weight (18 days/25 days))	1.612	1	1.612	.068	.795
	Pelt Weight	.056	1	.056	.128	.723
	PH 45 min after slaughter	.005	1	.005	.048	.828
	Cold Mass	.007	1	.007	.001	.973
	PH 24 hours of chilling	.081	1	.081	4.129	.049
	Subcutaneous fat thickness	.116	1	.116	.011	.918
	ADG	.000	1	.000	.036	.851
Zilpaterol hydrochloride treatment * Zeranol treatment * Duration	Weight 7/11	2.986	1	2.986	.306	.583
	Weight (18 days/25 days))	61.255	1	61.255	2.599	.115
	Pelt Weight	.599	1	.599	1.374	.248
	PH 45 min after slaughter	.117	1	.117	1.141	.292
	Cold Mass	20.041	1	20.041	3.177	.082
	PH 24 hours of chilling	.008	1	.008	.416	.522

Subcutaneous fat thickness	10.013	1	10.013	.935	.339
ADG	.009	1	.009	2.208	.145

Covariance analysis ~ correct for initial weight

Table 4.14. Analysis of covariance of rams on growth performance and carcass characteristics

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Zilpaterol hydrochloride treatment	Weight 7/11	13.542	1	13.542	1.870	.187
	Weight (18 days/25 days))	3.973	1	3.973	.413	.528
	Pelt Weight	.066	1	.066	.113	.740
	PH 45 min after slaughter	.078	1	.078	2.036	.169
	Cold Mass	7.320	1	7.320	1.443	.244
	PH 24 hours of chilling	.011	1	.011	1.360	.257
	Subcutaneous fat thickness	18.620	1	18.620	3.041	.097
	ADG	.001	1	.001	.343	.564
Zeranol treatment	Weight 7/11	14.983	1	14.983	2.069	.166
	Weight (18 days/25 days))	42.642	1	42.642	4.430	.048
	Pelt Weight	.601	1	.601	1.027	.323
	PH 45 min after slaughter	.275	1	.275	7.133	.015
	Cold Mass	20.657	1	20.657	4.071	.057
	PH 24 hours of chilling	1.870E-5	1	1.870E-5	.002	.962
	Subcutaneous fat thickness	4.861	1	4.861	.794	.384
	ADG	.007	1	.007	3.959	.060
Duration	Weight 7/11	10.270	1	10.270	1.418	.248
	Weight (18 days/25 days))	16.690	1	16.690	1.734	.203
	Pelt Weight	1.848	1	1.848	3.155	.091
	PH 45 min after slaughter	.154	1	.154	3.987	.060
	Cold Mass	7.966	1	7.966	1.570	.225
	PH 24 hours of chilling	.169	1	.169	20.666	.000
	Subcutaneous fat thickness	15.432	1	15.432	2.521	.128
	ADG	.000	1	.000	.210	.652
Zilpaterol hydrochloride	Weight 7/11	.162	1	.162	.022	.883

treatment * Zeranol treatment	Weight (18 days/25 days))	7.555	1	7.555	.785	.386
	Pelt Weight	.197	1	.197	.336	.568
	PH 45 min after slaughter	.495	1	.495	12.855	.002
	Cold Mass	8.143	1	8.143	1.605	.220
	PH 24 hours of chilling	.009	1	.009	1.149	.297
	Subcutaneous fat thickness	8.693	1	8.693	1.420	.247
	ADG	.001	1	.001	.603	.447
Zilpaterol hydrochloride treatment * Duration	Weight 7/11	2.731	1	2.731	.377	.546
	Weight (18 days/25 days))	7.686	1	7.686	.798	.382
	Pelt Weight	.635	1	.635	1.085	.310
	PH 45 min after slaughter	.165	1	.165	4.276	.052
	Cold Mass	6.648	1	6.648	1.310	.266
	PH 24 hours of chilling	.022	1	.022	2.729	.114
	Subcutaneous fat thickness	4.731	1	4.731	.773	.390
	ADG	.001	1	.001	.798	.382
Zeranol treatment * Duration	Weight 7/11	14.049	1	14.049	1.940	.179
	Weight (18 days/25 days))	43.128	1	43.128	4.480	.047
	Pelt Weight	.077	1	.077	.131	.721
	PH 45 min after slaughter	.043	1	.043	1.129	.301
	Cold Mass	9.373	1	9.373	1.847	.189
	PH 24 hours of chilling	.019	1	.019	2.337	.142
	Subcutaneous fat thickness	3.252	1	3.252	.531	.475
	ADG	.007	1	.007	4.230	.053
Zilpaterol hydrochloride treatment * Zeranol treatment * Duration	Weight 7/11	19.651	1	19.651	2.713	.115
	Weight (18 days/25 days))	31.861	1	31.861	3.310	.084
	Pelt Weight	.547	1	.547	.935	.345
	PH 45 min after slaughter	.038	1	.038	.995	.330
	Cold Mass	36.981	1	36.981	7.287	.014

	PH 24 hours of chilling	.003	1	.003	.349	.561
	Subcutaneous fat thickness	.017	1	.017	.003	.958
	ADG	.006	1	.006	3.365	.082

Covariance analysis ~ correct for initial weight

4.4. References

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Chapter 5. Discussion

5.1. Growth results

5.1.1. Influence of growth promotant treatment on average daily gain

South African Mutton Merino's are a late maturing, dual purpose breed. The lambs used in this trial started with an average live weight of 31.69kg and were slaughtered at an average live weight of 51.79kg. The ADG for the entire sample was 264.9g/day. The control group had an ADG of 260.4g/day, which is comparable with previous studies (Deng, *et* al., 2012; Brand & Franck, 2000; Webb, *et al*., 1999). The ADG of lambs that received Z-treatment was 277.1g/day and the influence of Z-treatment on ADG was significant (P <0.05). It is well known that Z-treatment increases growth rate and ADG in cattle (Strydom *et al*., 2009; O'Neill, 2001) Lopéz-Carlos *et al*. (2011), Lopez-Carlos *et al*. (2010), and Estrada-Angula *et al*. (2008) reported an increase in ADG in sheep due to Z-treatment. Lopéz-Carlos *et al*. (2014) also reported an increase in ADG due to Z-treatment in goat wethers.

Zeranol implanted lambs also had significantly higher ADG (P <0.017), which is consistent with the literature (Nsahlai *et al*., 2002; Nold *et al*., 1992). Zeranol implants increase feed efficiency and feed conversion in both rams and wethers (Nold *et al*., 1992).

To date there is no combination study of ZH and zeranol on lambs. In cattle, Baxa *et al*. (2010) did a combination study of ZH and Revelor-S (a steroidal ear implant). In this trial the combination of ZH and the oestrogen/androgen steroid implant had the greatest ADG and feed efficiency response. The combination had an additive effect compared with the treatments on their own (Baxa *et al*., 2010). Neill *et al*., (2009) also found the same response in cows and claimed that for the full performance potential benefit of feeding ZH, it is necessary to first use a combination steroidal ear implant in cows. The current study results show that the combination of zeranol and (Z-treatment) (Z*R) on ADG was highly significant (P <0.0002) and proved to yield the highest ADG and therefore the best growth rates, which is in agreement with the results of Baxa *et al*. (2010) and Neill *et al*. (2009). The highest ADG was for the group ZR25-treatment. ADG for this group was 335.3 ± 62.935g/day and ZR18-treatment had an ADG of 297.7 ± 36.555g/day, this group of lambs had the second best performance in terms of ADG. From this it can be seen that the combination yields very good results. Although, the combination of Z*R on ADG was significant, the combination plus duration on treatment was not significant. Duration of treatment did not affect ADG consistently, however, in the case of Z*R, it seems that duration of treatment did have an effect, due to the big difference in ADG.

It is well known that β-agonists cause a rapid growth response at the onset of treatment, however, a plateau is reached due to a desensitisation of β-adrenergic receptors (Moody *et al*., 2000). Growth response over time is not constant with different β-agonists and different species (Lopez-Carlos *et al*., 2011). Lopez *et al.* (2011) reports an increase in ADG over 3 different durations of treatment, namely; 14, 28 and 42 days. In this study the ADG did not increase over time and in fact decreased. ADG for 18 days treatment was 284.8g/day and for 25 days treatment it was 269.1g/day. As mentioned above, the combination of Z*R did cause an increase in ADG over time and the combination of Z*NR caused a decrease in ADG over time. This shows that the combination is favourable; however more studies need to be done on the duration of feeding ZH in sheep.

5.1.2. Influence of growth promotant treatment on cold carcass mass

Average cold carcass mass for the entire group of lambs was 25.34 ±3.728kg and the average of the control group was 25.22 ±3.054kg. Cold carcass mass in this study was similar to the cold carcass mass in studies done by Estrada-Angulo *et al*. (2008) and Vahedi *et al*. (2014). Z-treatment increased cold carcass mass by 930g (P <0.003). This result is consistent with data on sheep reported in the literature (Vahedi *et al*., 2014; Lopez-Carlos *et al*., 2011; Lopez-Carlos *et al*., 2010; Estrada-Angulo *et al*., 2008). The cold carcass mass also increased with Z-treatment in goats (Lopez-Carlos *et al*., 2014). Cold carcass mass, hot carcass mass and dressing percentage increases with ZH administration in the diet of cattle over the finishing phase of the feeding period (Montgomery *et al*., 2009; Avendaño-Reyers *et al*., 2006).

Cold carcass mass was also affected by zeranol implants (P <0.007). R-treatment yielded a higher cold carcass weight, which is similar to that seen in past studies done on sheep (Hufsteadler *et al*., 1996; Hutcheson *et al*., 1992). Field *et al*. (1993) showed that implanting with zeranol produced lower hot carcass weights and Salisbury *et al*. (2007) also found that dressing percentages had gone down with implanted sheep. Nold *et al*. (1992) also argues that zeranol increases growth rates, but has minimal effects on carcass composition and subprimal weight distribution. In cattle, there are large differences found in hot carcass weights when cattle were implanted with zeranol (King 1992). In the current study the effect of zeranol on cold carcass weight is 0.47kg higher than non-implanted sheep.

The combination treatment of Z*R was also significant (*P* <0.002) on cold carcass mass, and compared with the control, lambs were 1.46kg heavier. This result is similar to what Neill *et al*. (2009) found in cows on concentrate diets, implanted with Revalor-200 and supplemented with ZH in the diet for 70 days. Baxa *et al*. (2010) also found that Revalor-S and ZH had an additive effect on hot carcass weight and dressing percentage in cattle.

Duration of treatment affected cold carcass mass significantly (*P* <0.008). This result was expected as the longer the treatment period continued, the longer the lambs grew and therefore had larger carcasses. Cold carcass mass for lambs that were fed ZH for an extra 7 days, was 1kg heavier. The lambs that were on Z-treatment had cold carcass weights that also differed between different durations of treatment. The cold carcass weights differed by 0.57kg, although the combination of duration of treatment and Z-treatment was not significant. Vahedi *et al*. (2014) and Lopez-Carlos *et al*. (2011) both showed that cold carcass weight increases with increased days on Z-treatment. They also showed that a plateau can be reached in the cold carcass mass because of down-regulation of β - AR, as mentioned above. However, in the current study the treatment period was not long enough to demonstrate this.

The combination of duration of treatment and Z*R treatment was significant and improved cold carcass mass (*P* <0.006). This shows that it is important to implant the lambs before feeding ZH. The results showed that feeding ZH without the use of implants had little effect on improving cold carcass weight if fed for an additional 7 days. The treatment with the highest cold carcass mass was a combination of ZH and zeranol for the longer duration of treatment (25 days on treatment). Cold carcass mass for this treatment was 28.722 ± 3.3551kg, which is 3.502kg higher than the control for the same period of time. This result was expected considering the additive effect of ZH and various nonsteroidal and steroidal growth ear implants, combined with a longer period of time for the animal to grow.

5.1.3. Influence of growth promotant treatment on pelt weight

Pelt weight of the control group of lambs was 5.38 ±1.179kg. Pelt weight of Z-treated lambs tended to differ (*P* <0.098). Z-treated lambs had a mean pelt weight 0.42kg lighter than NZ-treated lambs. A similar result was found in cattle (Scramlin *et al*. (2010). This may be due to the reduction of fat within the pelt, because of the physiological properties of ZH. Duration of treatment also yielded a significant result (*P* <0.001), with a longer period of treatment leading to a heavier pelt. This result was expected because skin area increases with age, therefore the pelt increases in size (and weight) over time (Anderson *et al*., 1991).

R-treatment did not affect pelt weight significantly, but results did show that R-treatment had a mean pelt weight of 0.15kg heavier than NR-treatment, perhaps due to effect of zeranol on live weight and therefore the effect on pelt weight (mentioned above). There was an interaction between Z and R-treatment (P <0.012) on pelt weight. In this study, Z-treatment decreased pelt weight and R-treatment increased pelt weight. The heaviest mean pelt weight was with NZNR25-treatment and weighed in at 5.75kg. The effect of ZH seems to have a positive effect on pelt weight, by decreasing pelt weight and therefore making pelt removal easier and less labour intensive.

5.1.3.a. Influence of sex on pelt weight

Zeranol has been used in rams and wethers and it had a positive effect on pelt removal. Zeranol reduces the weight of pelt in rams (Field *et al*., 1993). More force is needed to remove ram pelts than wether pelts, because of the secondary sex characteristics that rams possess (Anderson *et al*., 1991). Implanting rams with zeranol tended to reduce the difficulty of pelt removal as reported by Field *et al*. (1993). Increased force needed to remove a pelt, means that more labour and equipment is required (Field *et al*., 1993).

In the current trial male South African Mutton Merino's were used and rams and wethers were mixed. The effect of sex on pelt weight became apparent (P <0.000). The effect of ZH, zeranol and duration of treatment on pelt weight was analysed again, with rams and wethers separate. The effect of Z-treatment on pelt weight was significant in wethers, but not in rams (P <0.013 and P <0.740, respectively). There was no significant effect of R-treatment on pelt weight for both wethers and rams (P <0.710, P <0.323, respectively). This was not expected, because Field *et al*. (1993) found that implanting rams decreased the weight of pelt.

Duration of treatment had a significant effect on wether pelt weight and tended to have an effect on ram pelt weight (P <0.002; P <0.091). The same pattern occurred when the group was analysed together (mentioned above). As the animal grew the pelts increased in size and therefore pelt weight also increased.

There was a significant Z*R interaction in wethers (P <0.022), however not in rams. This interaction was also seen when rams and wethers were analysed together. ZH decreased pelt weight, while zeranol increased pelt weight. In rams, the effect of secondary sex characteristics is high and this decreased the effect of ZH and zeranol on the pelt

weight. When analysed together, only ZH tended to have an effect on pelt weight, however, when analysed separately it can be seen that ZH has an effect on pelt weight of wethers, and does not affect pelt weight of rams.

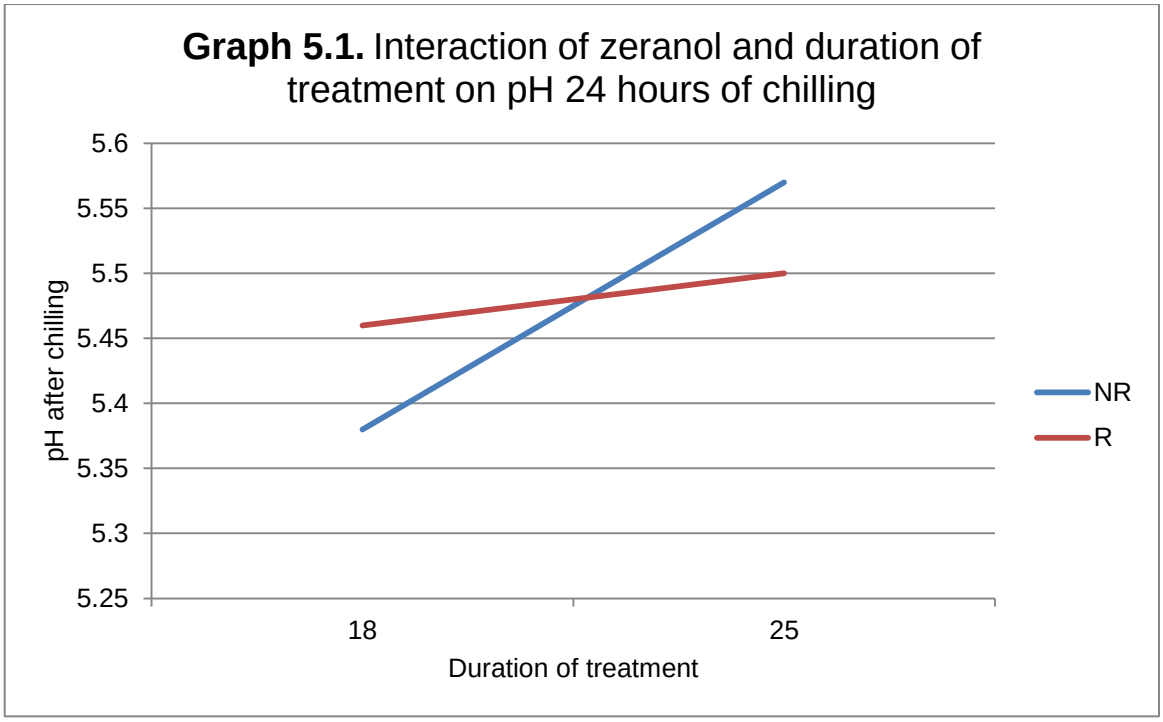
5.2. Carcass characteristics

5.2.1. Influence of growth promotant treatment on pH at 45 minutes post slaughter and pH after chilling

The pH at 45 minutes post slaughter was an average of 6.63 for the entire group of trial lambs and 6.57 for the control group. pH can range from 6.85 to 6.05 in lamb carcasses 45 minutes after slaughter (Okeudo, 1994). ZH did not influence pH at 45 minutes post slaughter and pH 24 hours of chilling (P <0.966; P <0.195, respectively). The same results were found in Hilton *et al*. (2009) and Avendaño-Reyers *et al*. (2006) in cattle treated with ZH. ZH supplemented to hair lambs, in a study done by Dávila-Ramírez *et al*. (2013), significantly increased pH by 3.68%. In a study done on goats, pH tended to decrease with an increase in ZH dosage (López-Carlos *et al*., 2014). These contrasting results may be due to dosage of ZH supplementation as Dávila-Ramírez *et al*. (2013) fed at 10mg per animal per day, and in the current trial the dosage was 40mg per animal per day.

Duration of treatment influences both pH at 45 minutes post slaughter and pH 24 hours of chilling (P <0.029; P<0.000, respectively). The lambs that grew for longer had higher pH values. pH drop is caused by lactic acid production during glycolysis once the animal is slaughtered (Tornburg, 1996). pH is a measure of glycolytic activity according to Okeudo, (1994). Lambs that were fed for longer must have produced less lactic acid, which resulted in higher pH values. This lack of lactic acid is due to less glycolysis and therefore less muscle glycogen. McGeehin *et al*, (2001) found that there was some significance between different ages of lambs and pH values post slaughter, but a relationship between age and pH could not be established.

There was an interaction with zeranol and duration of treatment (P <0.011). Zeranol seemed to reduce the effect of duration of treatment on pH, which is indicated in Graph 5.1. It is difficult to make a conclusion of zeranol on pH, because the results are not consistent and a relationship is not evident.



5.2.2 The effect of growth promotant treatment on carcass subcutaneous fat thickness

ZH had no significant effect on fat thickness and Z-treatment had a slight decreased fat thickness compared to NZ-treatment (0.08cm). In cattle ZH improves carcass characteristics, but in sheep the results are less consistent (López-Carlos *et al*., 2010). In cattle ZH decreases fat thickness and in Elam *et al*. (2009) linear responses were observed for duration of feeding ZH on fat thickness (Vasconcelos *et al*., 2008). Back fat thickness decreased in sheep supplemented with ZH in the study by Vahedi *et al*. (2014) and López-Carlos *et al*. (2010). In contrast, Avendaño-Reyers *et al*. (2011) and Estrada *et al*. (2008) reported that ZH had no effect on fat thickness. Variation in growth performance response to β-agonist supplementation can be attributed to species, sex, age, diet, genetics and dosage inclusion level (Mersmann, 1998). The variation seen in fat thickness might be due to some of these factors.

Duration of treatment affected fat thickness (*P* <0.012). The longer the lambs had time to grow, the fatter they were at slaughter. Lambs that were fed for 7 additional days had 1.95cm thicker fat on the 13th rib. This result was expected, because it is well known that if an animal is on a high concentrate diet for longer, more fat is deposited. Age at slaughter

significantly affects fat content (Cifuni *et al*., 2000). López-Carlos *et al*. (2011) also found a linear increase in fat thickness as feeding duration increased.

5.2.3. Effect of growth promotant treatment on subcutaneous fat thickness of 8th and 10th rib

The results for subcutaneous fat thickness (SCF) are very similar to the results for carcass fat thickness. Duration of treatment affected SCF of the 8th rib (P <0.025), which also affected carcass fat. Feeding the lambs for an additional 7 days increased SCF of the 8th rib by 6.86mm. This result is consistent with López-Carlos *et al*. (2011) who also found a linear increase in fat thickness as feeding duration increased. It is well known that age at slaughter significantly affects fat content (Cifuni *et al*., 2000).

Zeranol has a tendency to decrease SCF of the 10th rib (P <0.088) and it decreased SCF by 9.66mm. This is not consistent with the literature as zeranol used in lambs has been found to have no effect on fat thickness (Field *et al*., 1993; Hutcheson *et al*., 1992; Nold *et al*., 1992).

5.2.4. The influence of growth promotant treatment on carcass composition

An effect on carcass composition was expected, considering the repartitioning effect of ZH. However, there was no significant effect of ZH on fat and muscle portions of the carcass (P >0.05). Avendaño-Reyers *et al*. (2006) also found no difference in carcass composition. In contradiction to this, Leheska *et* al. (2009) and Hilton *et al*. (2009) both found a significant difference in carcass composition due to ZH. A low effect found in this study could be due to a lower dose of ZH in the diet, because Avendaño-Reyers *et al*. (2006) used a low dose on cattle and according to Leheska *et al*. (2009) this could have caused a lack of response.

The results in this study did show an increase in the muscle portion by 0.75% and a decrease in the fat portion by 0.62%. Perhaps with an increase in ZH dose there will be more of a repartitioning response and more significant results.

5.3. Meat quality results

5.3.1. Influence of growth promotant treatment on tenderness

The effect of ZH on tenderness was significant (*P* <0.000). The control group had a sheer force of 25.70N. ZH increased sheer force by 13.21N. ZH is known to negatively affect tenderness in beef (Scramlin *et al*., 2010; Hilton *et al*., 2009; Kellermeier *et* al., 2009; Leheska *et al*., 2009; Avendaño-Reyers *et al*., 2006). Hilton *et al*. (2009), reports that ZH does not adversely affect overall consumer acceptance with increased post mortem aging. To date there has not been any reports on tenderness in lambs treated with ZH, however, a β-agonist, $L_{644,969}$, has been shown to decrease tenderness in lambs (Pringle *et al*., 1993). Z-treatment did increase sheer force by 13.21N to 38.49N; however, this is still considered moderately tender meat according Hilton *et al*. (2009). A trained sensory panel was used in the Hilton *et al*. (2009) study, and meat up to 40N was considered slightly, to moderately tender. Therefore, even though ZH increased sheer force, it is still within consumer acceptance of tender meat.

Duration of treatment also significantly affected tenderness (*P* <0.018) and feeding lambs for 7 additional days resulted in a mean sheer force gain of 6.16N. Several studies have been conducted on age at slaughter and its effect on tenderness, with contradicting conclusions (Veiseth, *et al*., 2004). In Weller *et al*. (1962), lamb age at slaughter was unrelated to tenderness. Some studies have reported a decrease of tenderness with age, however both were studies in cattle (Reagan *et al*., 1976; Hiner & Hankins 1950).

In Rathman *et al*. (2009) and Brooks *et al*. (2009), there was an increase in sheer force as the duration of days fed ZH increased. These results are consistent with the results in the current study, with the least tender meat coming from the treatment group that received ZH for the longest period; ZR25-treatment.

There was no difference in tenderness due to R-treatment. In both Field *et al*. (1993) and Nold *et al*. (1992), there was a decrease in tenderness in wethers and an increase in tenderness in rams due to a zeranol implant. However the differences were not perceived by the sensory panel and were therefore not large differences.

5.3.2. Influence of growth promotant treatment on cooking loss

There was a tendency for Z-treatment and duration of treatment to influence cooking loss, (*P* <0.074), (*P* <0.056), respectively. Z-treatment only decreased cooking loss by 2

grams compared with NZ-treatment, which is practically very little and not likely to make a difference. This result is similar to what is seen in previous studies; where Z-treatment did not influence cooking loss (Hilton *et al*., 2009; Holmer *et al*., 2009; Kellermeier *et al*., 2009; Leheska *et al*., 2009). In Weller *et al*. (1962), cooking losses did not differ to an important degree, due to age at slaughter, which is consistent with the results found in the current study.

Zeranol had no effect on cooking loss and Z*R did not cause an interaction. In the study of Kellermeier *et al*. (2009), there was also no interaction effect with a Revalor-S implant and ZH. Z*R and duration of treatment tended to cause an interaction (P <0.085), however there is no clear relationship between ZH, zeranol and duration of treatment on cooking loss.

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Chapter 6. Conclusion

The aim of this study was to test the use of ZH on growth, carcass characteristics and meat quality in commercial feedlot lambs. This study showed that ZH had an effect on the results.

This study was done under optimal commercial feedlot conditions and simulated a commercial lamb feedlot. Using male lambs is typical of a commercial feedlot, where lambs are mixed and grouped according to age and size. Slaughter conditions were optimal to reduce stress and adverse effects on meat quality, that would be influenced by stress.

6.1. Growth results

ZH increased ADG significantly. Treatment of ZH decreased ADG over time, with ADG being higher for the 18 day treatment than 25 day treatment. Zeranol implants also significantly affected ADG. Combination treatment of ZH and zeranol was also significant and yielded the best ADG results, showing they have an additive effect. When zeranol and ZH were used in combination, ADG did not decrease when ZH was fed for a longer period, indicating that ZH should be used in combination with zeranol to get the full potential use out of the ZH.

Cold carcass mass was influenced by ZH, zeranol and duration of treatment significantly. The combination treatment of ZH and zeranol on cold carcass mass also differed significantly and the combination yielded the best results. Therefore feeding lambs ZH for 25 days after an initial implant of zeranol, results in the largest carcass yield. ZH, zeranol and duration of treatment caused a significant interaction. This shows that it important to implant the lamb before feeding ZH, because feeding ZH without implants decreased cold carcass weight if fed for an additional 7 days.

Pelt weight formed an integral part of this study as the force used to pull pelts off influences the processing in the abattoir. ZH tends to decrease pelt weight, but does not make pelt removal more difficult. Duration of treatment increases pelt weight due to an increase in pelt size. The removal of pelts seems to become difficult in intact males and more research should be done on the effect of ZH on wethers and rams separately.

6.2. Carcass characteristics

ZH administration in the diet did not influence the pH however, this is probably due to the dosage used in this study. Duration of ZH treatment significantly influenced pH at 45 minutes post slaughter and after chilling, resulting in higher pH values for lambs that were fed for longer. Zeranol and duration of treatment had a significant interaction and the conclusion is that zeranol decreased the effect of duration of treatment on pH.

ZH supplementation had no effect on carcass fat thickness. Duration of treatment increased fat thickness significantly.

There was no significant effect of ZH supplementation on carcass composition. The results in this study do suggest a move toward more muscle deposition and less fat deposition and an increase in dosage should be further investigated and will perhaps cause a significant effect in carcass composition.

6.3. Meat quality results

ZH significantly influenced sheer force and increased the sheer force of the meat by a noteworthy amount (18.98N), however it is still below the sheer force considered tender in beef. In this study sheer force significantly increased with increased duration of treatment. Using ZH for 25 days of ZH supplementation resulted in higher sheer force values versus the shorter 18 days of ZH supplementation.

ZH supplementation tended to influence cooking loss, but the difference between samples was so small, that it is probably of no practical significance.

Chapter 7. Critical evaluation

This study was done to evaluate the effect of zilpaterol hydrochloride in combination with a non-steroidal growth implant on growth and carcass characteristics in feedlot lambs. The trial was done at a commercial feedlot under practical conditions, such as bunk space and stocking density. With the environment was as controlled as possible. From an academic point of view, it would have been beneficial to do the trial at a research facility where conditions might have been more controlled.

Certain meat quality traits were investigated however, fatty acid analysis, drip loss and a taste panel were not included. It would've been interesting to include these traits in the investigation and it is recommended that a follow up study be done on these characteristics.

There is inconclusive data on the dosage level of zilpaterol hydrochloride administered in the diet to lambs and the duration of feeding. It would have been favourable to include more variations of duration of zilpaterol hydrochloride administration in the diet and to determine the exact time required until down regulation of beta – adrenergic receptor cells occurs. More research needs to be done on this to maximise the use of zilpaterol hydrochloride in lambs.

The positive response of implanting zeranol in combination with feeding zilpaterol hydrochloride indicates the potential to use growth promotants together to reach full growth potential. It would be interesting to include different growth implants in combination with zilpaterol hydrochloride treatment.

The results in this study agree with results reported on the use of zilpaterol hydrochloride in sheep and adds to our current understanding of the use of beta – agonists.

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