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Effects of steroidal growth implants alone or in combination with a beta-adrenergic agonist feed additive on the gonadal development of South African Dohne Merino rams and ewes

By

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Submitted in partial fulfilment of the requirements for the degree M.Sc. (Agric) Animal Science with specialisation in Production Physiology and Product Quality, in the Department of Animal and Wildlife Sciences, University of Pretoria

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

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Signature

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List of abbreviations

AA	- Amino acids
ADG	- Average daily gain
AI	- Artificial insemination
βAA	- Beta – adrenergic agonists
CL	- *corpus luteum / corpora lutea*
CZ	- Zilmax control
DES	- Diethylstilbestrol
EU	- European Union
E_2	- Estradiol
FCE	- Feed conversion efficiency
FCR	- Feed conversion ratio
FI	- Feed intake
G:F	- Gain to feed ratio
GH	- Growth hormone
GHR	- Growth hormone receptor
GRE	- Glycopeptide – resistant enterococci
GRF	- Gonadotrophin releasing factor
IGF – 1	- Insulin – like growth factor 1
L_R	- Left and right treatment
MANOVA	- Multivariate analysis of variance
Ral	- Ralgro
SC	- Scrotal circumference

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TBA	- Trenbalone acetate
RevG	- Revalor G
RevH	- Revalor H
Ral	- Ralgro
T_4	- Thyroxin
WHO	- World Health Organization
ZH	- Zilpaterol hydrochloride (Zilmax)
Zil	- Zilmax

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Abstract

The purpose of this research was to investigate the effects of different combinations of steroidal growth implants alone or in combination with a beta–adrenergic agonist feed additive on the gonadal growth, relative to ADG per treatment group of feedlot rams and ewes.

The trial was conducted at the University of Pretoria's Hatfield experimental farm. A total of 240 Dohne Merino lambs of 8 months old were randomly selected into 8 different treatment groups and fed for a period of 70 days following 3 days of adaptation. Zilpaterol hydrochloride was included into the finisher ration of half of the treatments for the last 18 days prior to slaughter (with 3 days withdrawal before slaughter). On arrival, the lambs were split by weight, first removing the outliers, and then split per sex (rams and ewes). Both sex groups were then randomly allocated into six treatment groups and then implanted as per treatment group, with one group receiving no implants only Zilmax and one group receiving no growth enhancers at all. Following the 70 days feeding period the lambs were taken to the Cavalier Group abattoir where they were humanely slaughtered, and the reproductive organs kept aside for further sampling and analysis. Data collection and statistical analysis was done on 68 selected lambs (22 rams and 46 ewes).

The results indicate that the effects of steroidal growth implant and Zilmax treatments differed between ewes and rams. The results for the ewes show that Revalor G, Revalor H and Ralgro all caused a significant decrease in the number of ovarian follicles in the ewes ($P<0.05$) when compared to the control of 12.3 ± 4.12 follicles, with Revalor G, Revalor H and Ralgro being 6.2 ± 3.86, 7.0 ± 2.87 and 8.6 ± 2.81, respectively. No changes were observed for the length or width of the ovaries, nor in the number of corpora lutea ($P>0.05$). The feed supplementation with Zilpaterol hydrochloride (Zilmax) to the ewes decreased the growth of the gonads in terms of length, width and number of follicles ($P<0.05$) but no change was seen in the number of corpora lutea ($P>0.05$).

The results for the rams indicate that the feed supplementation with Zilmax did not cause changes in the physical size or shape of the testes (in terms of length, width, volume and weight) ($P>0.05$). Revalor G significantly decreased the weight, volume, length and width of the testes ($P<0.05$) when compared to sheep in the control group and those implanted with Ralgro. Ralgro did not result in any physical changes ($P>0.05$) in the growth of the testes.

Results from the growth part of the trial show that the ewes responded positively to the addition of Revalor G and Revalor H. The subcutaneous fat thicknesses of the ewes

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decreased from 2.11 ± 0.564mm in the control to 1.68 ± 0.565mm for both the Revalor G and Revalor H treatment groups (P<0.05), which was not seen in the rams. The final mass of the ewes also did not respond consistently to the addition of the steroids (Revalor G, Revalor H) and Ralgro (Non-steroidal), with only small numerical improvements seen in the final masses (P>0.05). No increases were seen in final mass of the rams due to the supplementation of Zilmax (P>0.05), but significant decreases in carcass subcutaneous fat thicknesses were observed with the feed supplementation of Zilmax in rams decreasing the subcutaneous fat thickness from 1.67 ± 0.564mm to 1.36 ± 0.564mm (P<0.05). No significant improvement was seen in the final mass of rams due to implant supplementation. Observations in the ewes, however, showed the opposite trend; Zilmax improved the ewes' final mass by 800g (P<0.05) but no change in carcass subcutaneous fat thickness was observed (P>0.05).

Additional studies are needed to test the effects of the age at which lambs are implanted as well as the effects of different combinations of exogenous growth promotants and supplementation of different dosages of Zilpaterol hydrochloride on sperm counts, morphogenesis, motility and other related fertility estimates in male ruminants as well as on folliculogenesis and patterns of oestrous in females.

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Chapter 1: Introduction and motivation

1.1. Project theme

Production Animal Physiology

1.2. Title of study

Effects of steroidal growth implants alone or in combination with a beta-adrenergic agonist feed additive on the gonadal development of South African Dohne Merino rams and ewes

1.3. Aim of project

1. To determine the effect of exogenous steroidal growth implants on growth of rams and ewes especially in relation to subcutaneous fat thickness
2. To determine the effect of Zilmax feed supplementation on growth of rams and ewes especially in relation to subcutaneous fat thickness
3. To determine the effect of exogenous steroid treatment on gonadal development of rams and ewes with regards to gonadal size, mass and follicle and CL development
4. To determine the effect of Zilmax feed supplementation on gonadal development of rams and ewes with regards to gonadal size, mass and follicle and CL development

1.4. Motivation for project

This chapter of the dissertation serves to discuss the effect that exogenous growth promotants have on the growth of small stock as well as the growth and macroscopic development of the gonads in rams and ewes, namely the testes and ovaries. The focus will be on sheep (experimental models) but reference will also be made to cattle and other livestock as well as other animal species.

Due to the ever-growing population of the world, meat producers are continuously searching for more efficient methods of farming; from improved nutrition and enhanced genetics and breeding to the use of exogenous growth promotants and enhancers. There is a delicate balance and need for all these aspects of production. By the year 2050, the population is expected to grow to 9.15 billion people, with most of this growth occurring in the developing

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countries (FAO, 2012). More protein sources (meat, eggs and milk) are needed to feed this rapidly growing population. Challenges such as changes in climate patterns; increasing temperatures, severe droughts and infrequent rainfall patterns, as well as over population and socio-economic problems have led to scientists believing that an increase in livestock productivity is needed rather than an increase in animal numbers (Turk, 2016). The amount of arable land is declining making the need for the efficient ruminant more necessary.

The South African Dohne Merino breed is a popular sheep breed in South Africa and very well known for its dual purpose, being both a meat and wool producer. It is a medium to large framed breed that can be farmed under intensive or extensive conditions, making it very popular. The SA Dohne Merino lambs are hardy sheep with good performance in the sour grass veld conditions with fast growth rates in lambs (South African Dohne Merino Society, 2016), which explains why they are a suitable choice for South African feedlot producers.

It is well known that the growth and development of the gonads are directly related (Bearden, Fuquay & Willard, 2004) but there is a low level of understanding of the effects of exogenous steroids and endocrine-related effects on the gonads. 'Growth promotants' is a general term given to endogenous as well as exogenous products causing efficient growth in production animals. Exogenous growth promotants can include products of nutritional origin (products given via the diet) or products administered via implant or injection. Endogenous growth promotants include natural hormones such as testosterone, oestrogen and progesterone, these hormones can also be given subcutaneously, implants placed at the back of the ear. Exogenous growth promotants such as zilpaterol hydrochloride (ZH) is a popular choice for finishing feedlot lambs, increasing feed efficiency, weight gain, improving dressing percentage and carcass yield (Webb, *et al.* 2018). ZH is commonly referred to as a "β-agonist" which functions as a repartitioning agent as it channels the energy from feed to promote protein deposition instead of fat deposition, allowing for an extremely low FCR to result in highly lean carcasses. Steroidal implants are given subcutaneously as they are less effectively absorbed through the GIT.

The use of exogenous growth promotants in animal production has resulted in the efficient production of more meat, milk and eggs to feed the growing population, by using fewer natural resources (water, crops/grains and arable lands). But the effect of exogenous growth promotants on development of the gonads is yet to be researched, along with the effect on future breeding. It is only assumed that the use of exogenous growth promotants decreases production of endogenous hormones which directly impacts on breeding and reproductive health of stud animals.

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Chapter 2: Literature Review

Animal and human bodies are composed of a variety of endogenous hormones, including several growth and sex hormones. These hormones are found naturally in the body and each has an important role to play in the regulation and maintenance of bodily functions.

The primary task of livestock producers is to obtain the most output from the least input, in other words, using the lowest amount of resources, time and money. The efficiency of the animal is manipulated using feed additives or exogenous growth promotants to improve animal performance. Several feed additives have been discovered over the past few decades that improve the efficiency and thus performance of production animals through promoting favourable intestinal microflora, some of which include: prebiotics and probiotics. More recently combinations of growth promotants have been used in the industry showing considerable results. Exogenous growth promotants may act via alteration of the animals' metabolism or by changing the gastrointestinal microflora thereby causing changes in the deposition of protein and fat – the end results that livestock producers want to see (Al-Dobaib & Mousa, 2009).

There is a strong positive relationship between the supply of food and the availability of nutrients and growth in animals. As the plane of nutrition increases so does the level of glucose and volatile fatty acids in the blood. When growth promotants are used in food-producing animals, the dosage of steroid/hormone determines the performance response observed, but when primary nutrients, such as energy or protein, are limited the end response seen from the steroid/hormone will not be as expected (Johnson & Reinhardt, 2009).

2.1. History of exogenous steroidal hormone use

DES (diethylstilbesterol) is a stilbene derivative that has high biological activity. This non-steroidal synthetic oestrogen was used in the 1940's as a treatment for preterm birth and miscarriage in prepartum women. Unfortunately, after a series of clinical trials done in the 1950's it was found to be ineffective and only years later it was discovered that it had adverse side effects on women exposed prepartum. Studies were done on prepartally exposed mice to DES, These studies show signs of infertility as well as reproductive tumours, and the results were consistent with the outcomes seen in exposed women. DES was also associated with other adverse pregnancy outcomes such as preeclampsia, infertility and earlier menopause. (Hatch, et al, 2010).

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Oestrogen was first administered to cattle in a growth promotion study conducted by W.E Dinnusson on February 9, 1947. The stilbene derivative was first administered as an exogenous hormone diethylstilbesterol (DES). It was hypothesized that DES improved the growth rate of heifers because spayed heifers had a lower growth rate than intact ones. The first experiment contained five treatments; Control, spayed, DES (42mg implant), testosterone (50mg testosterone propionate initially and 32.5mg after 56 days) and thiouracil (given in the feed at 4g/animal/day). A second study was started on December 11, 1947 with the DES implant containing higher doses than the first study (48mg) as well as a testosterone propionate implant (50mg) and a thyroprotein given orally at 11mg/kg BW. The authors of the two experiments concluded that DES increased gain and feed conversion as well as increased length of leg, back and width of back and carcass results with a more mature-looking carcass. The reproductive results included pronounced mammary and teat development, vulvar swelling and extended oestrus, amongst others (Raun & Preston, 2002).

2.2. Growth and development

Growth is a complex process involving interactions between genetics, the environment and different hormones with their receptors located within tissues (Spencer, 1985; Trenkle, 1997). Growth of mammals is accompanied by development, with these two processes going hand in hand. Growth is defined as an increase in weight and is expressed quantitatively while development is defined as the changes in conformation and shape following growth and is expressed qualitatively. The degree to which conformation occurs depends on the environmental influences of the animal. (Batt, 1979).

There are several factors regulating growth, some of which include: genetic, environmental and nutritional factors. The efficiency of growth is greatest in the young animal when very little energy and nutrients are deposited as fat as opposed to the mature animal with over 60% of the body weight gain is being deposited as fat. Postnatal growth of the animal occurs differently for different types of tissues, with the number of muscle fibres being fixed prior to birth, growth of muscle fibres can only occur through hypertrophy because the muscle fibres and nuclei within the muscle fibres are postmitotic and have therefore lost the capability to divide (Johnson & Chung, 2007), while the growth of fat occurs through both hyperplasia and hypertrophy throughout the mature life of the animal (Buttery, *et al.* 1986). Postnatal muscle growth requires an external source of DNA, muscle satellite cells are responsible for the postnatal growth occurring within muscle fibres. A positive correlation exists between

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number of satellite cells and efficiency of muscle growth, as more protein is then synthesized within each muscle fibre causing faster rate of growth. The process of transdifferentiation can be explained by using figure 2.1 (fig 1. Johnson & Chung, 2007). Muscle fibres are surrounded by plenty satellite cells, during division of these cells they donate their DNA to the existing muscle fibre to allow for skeletal hypertrophy. (Johnson & Chung, 2007). Satellite cells are mononucleated myogenic cells located within the muscle cell between the sarcolemma and the basal lamina.

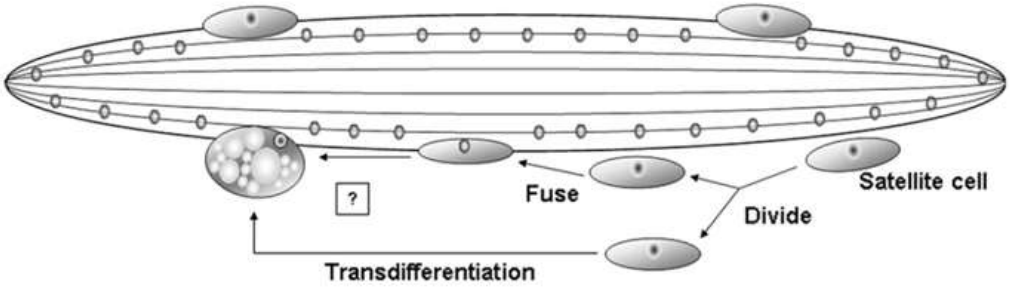

Fig 2.2. The role of muscle satellite cells in post-natal growth (From Johnson & Chung, 2007).

The process of transdifferentiation is critical in newborn animals because 30% of muscle DNA lies within satellite cells nuclei while mature animals have less than 10%. This theory explains the limitations of muscle growth in older mature animals (Johnson & Chung, 2007). The process of the cell cycle becomes crucial when it comes to muscle accretion, the cell cycle consists of a series of ordered events; the two main phases are interphase and mitotic phase. During interphase the cell undergoes normal cell growth. Interphase consists of three phases: G_1, S and G_2. When cells are needed for muscle growth, they are removed from the cell cycle and enter G_0 phase. G_0 phase is defined as a state of dormancy which causes growth of the cell to plateau. IGF-1 and fibroblast growth factor-2 can initiate cells to carry on in the cell cycle. IGF-1 promotes muscle cell differentiation while fibroblast growth factor-2 does just the opposite; it inhibits differentiation (Johnson & Chung, 2007). During the cell cycle these two growth factors stimulate the cell to divide, therefore stimulating satellite cell proliferation and thus muscle growth. Due to the fact that the number of muscle fibres is fixed at birth, further muscle growth (size) requires that the satellite cells donate their DNA (nuclei) to support the increase in size (Johnson & Chung, 2007).

The endocrine system plays a large role in coordinating this growth and development. It is well known that cattle that have been implanted with anabolic steroids have higher concentrations of plasma growth hormone (GH), Studies have shown that this is due to a

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higher secretion of GH rather than a decreased clearance from the vascular system (Trenkle, 1997). GH is a strong regulator of post-natal growth in mammals, it acts indirectly (via IGF-1) to result in an increased uptake of AA and therefore increased protein synthesis and muscle deposition. A review done by Blache, *et al.* (2000) shows a strong correlation that exists between GH and IGF-1. External stimuli (positive stimuli promoting growth) cause the release of GRF from the hypothalamus, these releasing factors act on the pituitary to release GH which then directly stimulates the peripheral tissues (e.g. Liver, fat cells, etc) causing growth through the release of IGF-1 and IGF-2. A similar action exists for a decrease in growth, the negative stimuli act on the hypothalamus to release GH inhibiting factors which causes the pituitary to respond by decreasing the amount of GH acting on peripheral tissues. IGF contributes towards skeletal muscle growth by stimulating protein synthesis as well as decreasing protein degradation.

2.2.1. Overall growth and development

Whole body growth and development requires several processes at the cellular and tissue level; (i) hypertrophy – which is the increase in cell size, (ii) hyperplasia – increase in cell number due to mitosis, (iii) differentiation – gives rise to different tissue structures and functions and differentiation of cells into types, and (iv) organization – of (i), (ii), (iii) of all tissue levels. In summary, hypertrophy and hyperplasia ensures growth while differentiation of cells determines the degree of development of mammals (Batt, 1979). Animal growth occurs both quantitatively and qualitatively. Body weight is the simplest way to measure animal growth although the weight obtained does not take gut fill or water accumulation into consideration and therefore does not reflect the true weight of the animal.

It is important to remember that while body weight is an important indicator of growth, it is the total measurement of all body tissue types, Tissue types respond differently to growth and development.

Before the discovery of growth hormone (GH) in the 1930's and 1940's many earlier experiments explained the discovery of an alkaline extract from the anterior pituitary shown to promote growth in rats, these discoveries led to the conclusion of a substance being present in the pituitary responsible for causing growth and affecting carcass composition (Etherton, 2004). The first experiment was done by Evans and Simpson in the 1920's when they discovered that an extract from the bovine anterior pituitary caused growth in rats, this extract was then named "growth hormone" (Hossner, 2005). GH is a polypeptide hormone produced by the anterior pituitary gland. The secretion of GH from the anterior pituitary is pulsatile and

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is related to the species, sex and age of the animal. Among the many functions of GH are bone and skeletal muscle growth, lipolysis, milk production and IGF-1 gene expression (Jiang & Ge, 2014). One of the major target organs of GH is the liver, which when under GH stimulation, synthesizes and secretes IGF-1. IGF-1 is the hormone responsible for carrying out the effects of GH on bone, muscle, and adipose tissue. High concentrations of circulating IGF-1 inhibit GH secretion (Hossner, 2005). IGF-1 exerts a negative feedback mechanism on the hypothalamus and pituitary. Figure 2.2.1a accurately depicts this feedback mechanism.

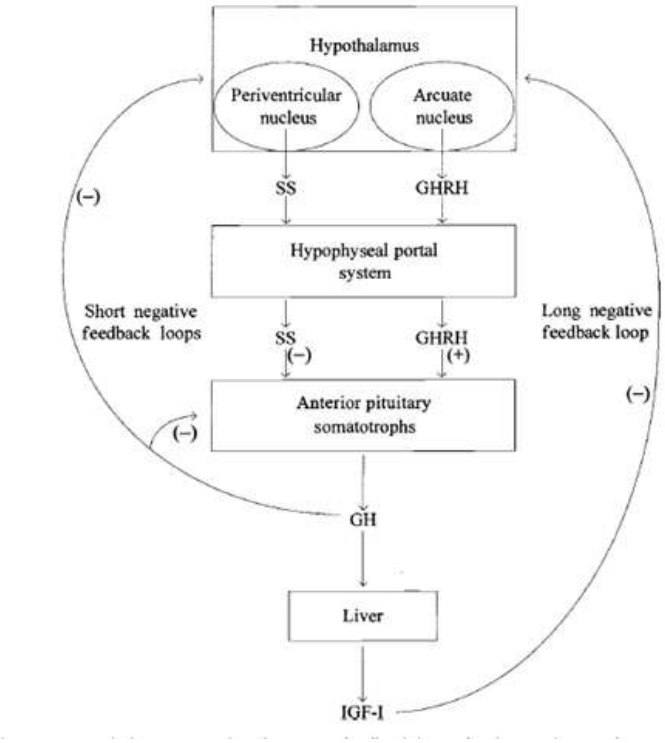

Fig. 2.2.1a. Hypothalamic control of IGF-1 and negative feedback loops (From Hossner, 2005)

Allometry is defined, according to Gayon (2000), as “the changes in relative dimensions of parts of the body that are correlated with changes in overall size’. In 1936, the term, “Allometry” was given to avoid confusion in the field of relative growth by Huxley and Teissier (Gayon, 2000). Gayon (2000) also pointed out the importance of the relationship between the changes in shape and size. In another paper, Gould (1996) referred to the term ‘allometry’ as the “differences in proportions correlated with changes in absolute magnitude of the total organism or specific parts under consideration”. He also refers to Bertalanffy (1964) who described that any change in size should be followed by a change in proportion in order

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to remain functional. Figure 2.2.1b refers to a diagram adapted from Hammond (1955) showing the rate of weight gain (y-axis) versus time (x-axis). Hossner (2005) refers to this graph as the sequential growth of different tissues from anterior to posterior during the development phases from conception to maturity. In the prenatal phases (A) the first tissues to develop are the brain and spinal cord (tissues of the central nervous system), shortly after that the next phase (B) starts developing while phase A is still busy, but at a slower rate. This second phase is the phase of skeletal growth. Phase C allows for the growth and development of muscle and other soft tissues. The last phase (D) is the adipose tissue phase with the slowest growth rate, it takes the longest to establish and starts primarily postnatally. The adipose tissue phase is generally associated with a more mature type animal.

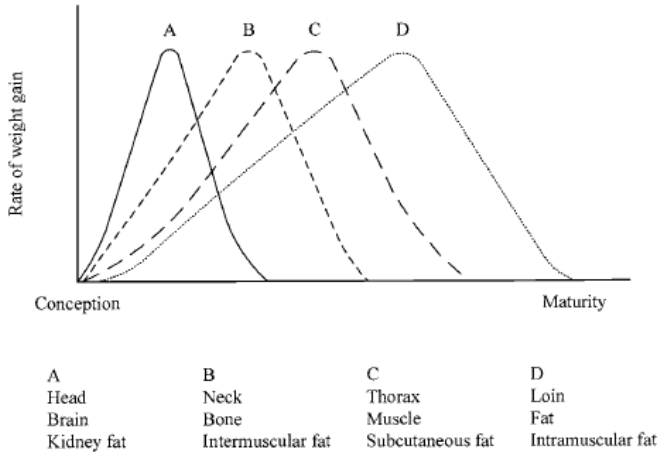

Fig. 2.2.1b. Allometric growth (taken from Hammond, 1955)

Comparison of implanted vs non-implanted cattle show that the implanted cattle gain faster than non-implanted cattle, but the rate of fat accumulation is not proportional to the rate of increased growth (Johnson & Reinhardt, 2009). There are products on the market (β-agonists as well as steroidal implants) that cause changes in the ratio of muscle to fat deposition. β-agonists and steroidal implants become important in feedlot situations allowing the animal to become more feed efficient, because about 2.25 times more energy is required to deposit fat than the other tissues, resulting in increased protein synthesis and less protein degradation. Products like zilpaterol hydrochloride cause a shift in the energy metabolism of the animal allowing more energy to be directed to proteogenesis and less to lipogenesis, and are therefore referred to as repartitioning agents (Webb, Allen & Morris, 2018. This results in a leaner animal with more muscle and less fat.

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2.2.2. Gonadal growth, development and function

The ovaries are considered the primary reproductive organs in females. The ovaries of ewes are almond shaped and are responsible for the secretion of hormones important in the female, namely progesterone, and oestrogen. Females are born with a fixed number of primary follicles, which after puberty, are in constant stages of growth and maturation. A primary follicle contains only a single layer of granulosa cells, proliferation of these granulosa cells results in the primary follicle becoming a secondary follicle containing more than one layer of granulosa cells. Between the granulosa cells, a fluid-filled cavity is formed and starts to separate the granulosa cells: the follicle is now a tertiary follicle. This tertiary follicle matures to become the Graafian follicle (looks like a blister on the surface of the ovary) and is responsible for the production of oestrogen. During ovulation, the mature follicle is ruptured and releases the potential ovum toward the infundibulum of the fallopian tubes. After the mature follicle is released some blood is left over, this blood starts to clot and forms the *corpus hemorrhagicum* which later become the *corpus luteum*. The *corpus luteum* (CL) produces progesterone. The CL will then reduce in size until a white scar is left on the ovary; this is the *corpus albicans (*Bearden, H.J., *et al.* 2004).

The development of the gonads is positively correlated with desirable traits for breeding in rams such as libido, seminal quality, scrotal circumference and thus number of offspring. The development of well sized, normal gonads leads to higher chances of pregnancies as opposed to underdeveloped gonads. Makarechian, *et al.* (1984) did a scrotal circumference test using 119 young bulls where the scrotal circumference was measured before and after a test period done in feedlot cattle that were separated into four breeding groups (treatments). The study aimed to determine the association between growth parameters to testis size. It was found that scrotal circumference is positively correlated to body weight due to the mutual association between age and growth traits. The most important growth factor identified in terms of the association between body weight and scrotal circumference was the pre weaning gain, followed by birth weight and age. Breed and maturing type (age at which the bulls reach maturity) also played a significant role in the scrotal circumference change. Breeds of a heavier weight had a smaller scrotal circumference change during the test period than in the lighter breeds, due to the ages and rate at which they mature. Makarechian, *et al.* (1984) further explained that for the lighter breeds, a minimum testes size would be required for adequate fertility, and that other factors such as season and year and breed by year interactions had a significant influence on the scrotal circumference and thus testes size. They concluded that since pre-weaning gain is an important factor when bulls are

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selected for breeding, that the chances of selecting a bull with a high pre weaning gain but small testes size is unlikely due to the positive correlation between the two variables.

Sheep are seasonal breeders, so they are affected by changes in daylength. Bearden, et al. (2004) explains that during periods of decreasing daylength the pineal gland in rams is stimulated to cause an increase in the GnRH pulses increasing the LH surge which act directly on the Leydig cells of the testes to release more testosterone. The exact opposite occurs during periods of increasing daylength.

In the review by Blache *et al* (2000), the significant effect of nutrition on reproductive function was emphasised. There findings in mature merino rams indicate that nutritional effects can be divided into short term and long-term effects. Short term effects cause changes in the neuroendocrine system; responsible for controlling testicular activities while the long-term effects impact growth of the testes as well as sperm production. Changes in the diet affect the concentrations of glucose, insulin and leptin in the blood and cerebrospinal fluid. Short term changes (flushing) may be used to improve their condition and body condition score in rams and maximise testis size and ensure maximum sperm production. Also, feeding a diet high in grains should be avoided to prevent high levels of fat accumulation in the scrotum; rather, a diet high in energy (or supplementing some of the grain sources with fat) and bypass protein (RUP) should be fed to promote testes development and spermatogenesis. Similar results about scrotal fat accumulation were reported by Du Preez et al. (2021). When the diet is changed to promote a higher energy and protein intake an increase in the frequency of GnRH and LH pulses is seen as well as a higher FSH secretion (Blache, *et al.*, 2000).

A study was done by Rae *et al*. (2002), to determine what the effects of maternal undernutrition are on adult reproductive function in the male and female offspring. The ewes were separated into treatments where they were fed either a high (H) or low (L) diet, with the H diet meaning the ration provided 100% metabolizable energy requirements needed for pregnancy, while the L diet only met 50% of the requirements for pregnancy. LH and FSH were tested in the offspring, as well as the response to exogenous GnRH. The authors concluded that there was no response to pre-natal undernutrition in the ewe in terms of the male offspring's reproductive function, the testicular size or on the mean birth weights of the lambs. Male L-offspring displayed higher FSH levels than male H-offspring ($P<0.05$), while FSH levels did not differ ($P>0.05$) in the female L- or H-offspring. There was no difference between H- or L-offspring LH levels in males or females. There was, however, a negative response in the ovulation rate in the female L-offspring ($P<0.05$), showing that maternal undernutrition prenatally has a greater effect on female than male offspring.

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GnRH is synthesized by neuroendocrine cells in the hypothalamus, it is a peptide releasing hormone with an important reproductive function. GnRH is secreted through a series of pulses; the activity of the pulses is significant in that it either promotes gonadal activity during a high frequency or causes gonadal activity to decrease during periods of low frequency. (Martin, *et al.*, 2004). GnRH travels to the anterior pituitary through the hypophyseal portal system causing secretion of GH hormones (Bearden *et al.*, 2004); namely LH (luteinizing hormone, which is pulsatively secreted in rams) and a continuous secretion of FSH (follicle-stimulating hormone) (Blache, et al., 2000). The secretion of GnRH differs between males and females; in males, GnRH is secreted from the hypothalamus at a constant rate while in female's GnRH secretion varies according to the oestrous cycle of the ewe. Moenter, *et al.* (1991) studied the GnRH secretion in the preovulatory ewe; the data demonstrated that the GnRH secretion differed during the different phases of oestrous, with slower pulses during the luteal phase leading to much higher frequency pulses during the follicular phase resulting in strong surges of GnRH.

In the male ruminant a negative feedback loop exists, when a GnRH pulse is released it causes a surge in LH which acts on the Leydig cells in the testes. The Leydig cells are stimulated to release testosterone, the more testosterone released the stronger the negative feedback signal is on the hypothalamus to stop the stimulation and release of GnRH (Martin, *et al.*, 2004). While, in the female ruminant, a positive feedback loop exists between FSH and the hypothalamus. When GnRH is released from the hypothalamus is stimulates the anterior pituitary to release FSH which directly acts on the ovaries to release oestrogen (oestradiol), the more oestradiol released the more the hypothalamus is stimulated to carry on releasing GnRH (Bearden, *et al.*, 2004). Figure 2.2.2 depicts the interactions between the hypothalamus, gonadotrophins and other important tissues/organs involved in reproduction. Nutrition, changes in daylight length and sociosexual stimuli (such as presence of the ram/ewe) cause changes in GnRH pulse frequency directly influencing on reproduction.

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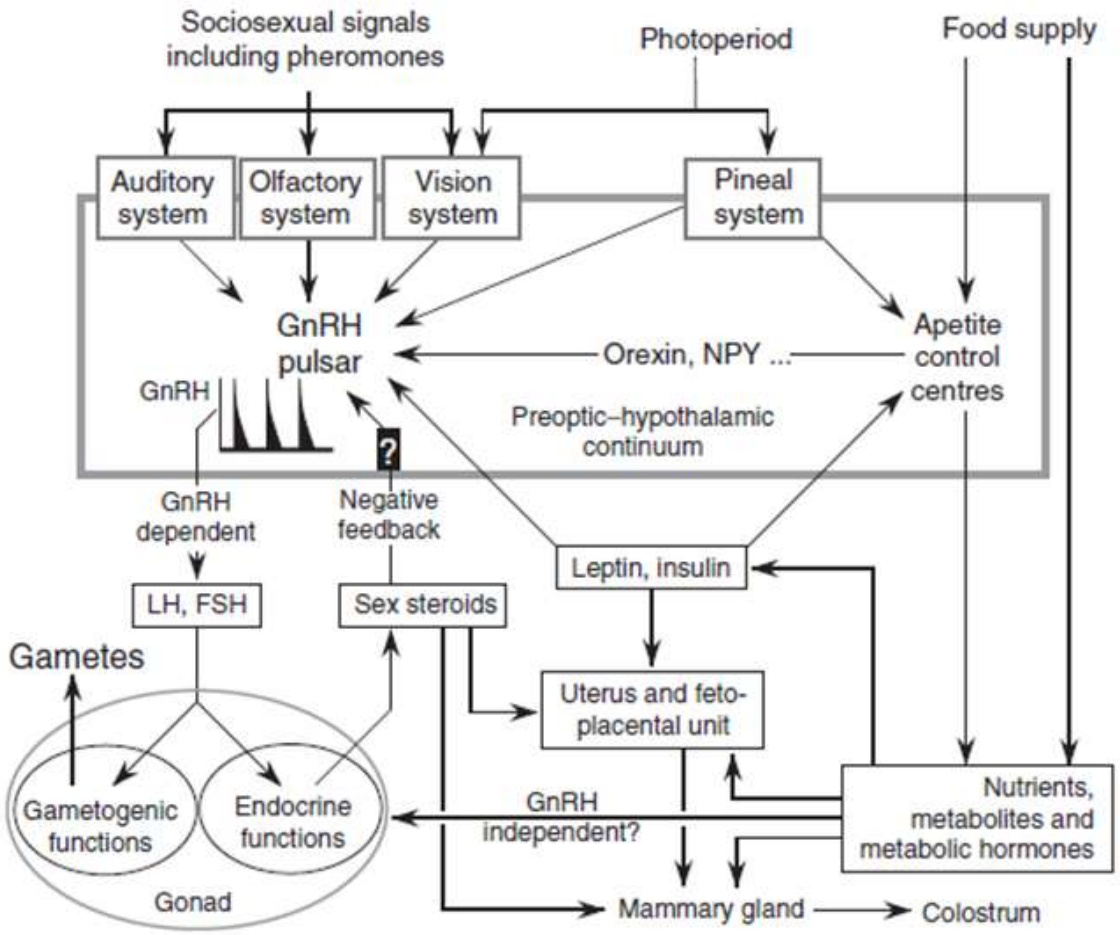

Figure 2.2.2. Gonadotrophin feedback loop (Martin, *et al.*, 2004). There are several environmental stimuli that cause changes to the GnRH pulse frequency, including changes in daylength, nutrition and sociosexual cues.

2.2.3. Interaction between animal growth and gonadal growth

There is a direct relationship between animal growth and growth of the gonads. The gonads are recognized for their ability to cause growth. In a study done by Bhatta, *et al.* (2011) the gonads of the Mozambiquan tilapia (Oreochromis mossambicus) were surgically removed to study the effects that the gonads have on the growth of the fish. They reported that the presence of the gonads is a requirement for a normal growth process after finding that the fish without their gonads had lower body weights than the controls. The same information can be extrapolated to livestock species, Raun & Preston (2002) discussed that intact heifers had higher weight gains than spayed heifers and more pronounced secondary sex characteristics.

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When using exogenous steroidal implants, the physiological age of the animal is altered, causing a later age at maturity and thus a leaner carcass than without the use of implants (Reinhardt, 2007). It is thought, that by altering the physiological age of the animal you are interfering with the age at which an animal is regarded as sexually mature.

2.3. Exogenous growth promotants

Exogenous growth promotants is a broad term used to describe a variety of compounds used for growth promotion in production animals; it includes anabolic steroids, beta-adrenergic agonists, GH, antimicrobials and ionophores (Du, 2014). Sex hormones such as oestrogen, testosterone and progesterone are also commonly used. (Al-Dobaib & Mousa, 2009). Since the beginning of growth promotants use, their purpose has been to enhance the efficiency of production and to decrease the cost of production thus improving the overall profitability (Reinhardt, 2007). Growth promotants are a valuable resource to livestock producers to get more output (carcass weights, ADG, feed : gain ratio) using less inputs (less food, land, water).

Antibiotic Growth Promotants (AGP) have been used in the production animal industry for over 50 years (Dibner & Richards, 2005), they are valuable tools ensuring the highest possible efficiencies. Many studies indicate the benefits and improvements in efficiency in both poultry and swine in the 1940's and 1950's. According to Visek (1978), the mechanism by which antibiotic growth promotion works has not yet been clearly defined but some mechanisms have been proposed by Luckey (1963), Jukes and Willams (1953), Francois (1962) and Francois and Michel (1968). The proposed mechanisms are; (i) Microorganisms that are responsible for milder infections are suppressed to low levels; (ii) Growth of growth-depressing toxins is reduced; (iii) Possible increased synthesis of vitamins or other growth factors or antimicrobial factors that reduce destruction or loss of essential nutrients; (iv) Increased efficiency of absorption and utilization of essential nutrients because of a thinner intestinal wall. All of these mechanisms are due to actions caused by the gut microflora.

2.3.1. Exogenous steroidal growth promotants

Steroids are a class of lipids with a tetracyclic conformation, the precursor to all steroids is cholesterol. Steroids are not soluble in water but are soluble in fats. Steroids are less effectively absorbed through the gastrointestinal tract than by systemic administration. The natural sex hormones testosterone, oestradiol-17β and progesterone are commonly used in animal production to enhance growth and improve feed conversion efficiency in both cattle

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and sheep. These hormones are used either individually or in combination with one another, with combinations of oestrogen and progesterone more commonly being used in bulls and steers while a combination of testosterone and oestrogen being used in heifers and cows (Bearden, *et al.*, 2004).

The silicone-based growth promoting implant was first approved for use in 1982, it was a significant development at the time as it allowed for a more uniform release of hormone (Preston, 1999). The beneficial effects of the growth promoting implants had hugely beneficial results to producers with an increase of 5 to 10% for feed intake, 10 to 20% ADG and an improvement in feed efficiency of 5 to 14% (Beerman, 1995).

The rate of release of hormone from the implant is biphasic, with more hormone being released in the earlier stages of the implant than later. The response of growth to the implant is directly correlated to the dose of the hormone (dosage circulating) and the period that the hormone is released from the implant, with more hormone causing a greater growth response. (Reinhardt, 2007). The reduction of circulating hormones towards the end of the feeding period in the feedlot means a decrease in efficiency and thus performance compared to the initial period after implantation. It is for this reason that most producers use combination implants with different active hormones to prevent having to administer another implant while another is still ‘paying out’ (Johnson & Becket, 2014).

Synthetic anabolic agents can be divided into two main classes based on their effects on metabolism; the first class being the anabolic agents with androgynous activity; TBA (trenbolone acetate) and Testosterone, and the second being those with oestrogenic activity; Estradiol (E2), E2 Benzoate and Zeranol. The naturally occurring androgens have a direct anabolic effect on metabolism when they combine with cytoplasmic muscle receptors (Van Doorn, et al. 1976). The androgenic hormone implants directly affect the muscle by causing an increase in protein synthesis and a decrease in muscle catabolism (Reinhardt, 2007) but do not affect secondary sex characteristics (Buttery, et al. 1986). Skeletal muscle contains androgen receptors which can bind both testosterone and TBA thus provides a direct pathway for increased skeletal muscle growth (Hossner, 2005). It may be important to note that the androgens also have significant indirect mechanisms by altering glucocorticoid activity (cortisol as well as cortisol-binding activity is decreased). Oestrogens have a non-direct effect in that they induce specific proteins in tissues such as the uterus and liver thereby altering the pattern of endogenous anabolic hormones (Buttery, et al. 1978) i.e. stimulating the production and release of the secondary hormones somatotropin (Growth hormone) and insulin-like growth factor 1 (Reinhardt, 2007). Oestrogen receptors are present on skeletal muscle in sheep in very low concentrations, this makes the direct effect on skeletal tissue non-significant

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compared to the effect of androgens on skeletal muscle (Hossner, 2005). It is important to note that ruminants have a special relationship with oestrogen, which causes increases in plasma hormone concentrations of GH, thyroxin and insulin (Preston, 1975) causing an increase in the uptake of amino acids in the tissues. In a study done by Sinnet-Smith, et al (1983) male castrate lambs and female lambs were treated with combinations of TBA, oestradiol and/or zeranol. Increased growth rate, weight gain and feed conversion efficiency (FCE) resulted from female lambs being treated with TBA and/or zeranol, with a decrease in DNA and RNA concentrations in muscle. When the male castrate lambs were treated with TBA and oestradiol they showed increased muscle rate, FCE with deceased RNA muscle concentrations. They hypothesized that the increased growth rate of the female lambs due to TBA or zeranol treatment was due in part to the decrease in muscle protein degradation.

When steroidal implants are used in cattle, higher plasma concentrations of GH were found. The increased concentration is said to be from an increased secretion and not a decreased clearance of GH from the vascular system. Cattle with implants have a greater number of GH secreting cells in the pituitary glands, the liver of these cattle also have more GH receptors for increased binding capacity of GH. The greater secretion of GH also results in more mRNA in the liver to produce IGF-1 and IGF-1 binding proteins (Trenkle, 1997).

Due to a greater hormone response, a combination of these hormones is often used instead of single-hormone implants. When oestrogenic and androgenic hormone implants are used their effects are not only synergistic, but additive responses have also been observed (Reinhardt, 2007). According to a number of studies; during the administration of a TBA/E_2 implant an ‘anabolic steroid-mediated muscle hypertrophy’ mode of action is initiated which alters the activity of muscle satellite cells due to the effect of increased production of IGF-1 after administration of steroidal implants, and because IGF-1 causes cells to carry on in the cell cycle (and increasing satellite cell activity) it results in more cell division and thus donation of nuclei to muscle cells to cause skeletal muscle hypertrophy (Johnson, *et al*. 1998; Kamanga-Sollo, *et al.* 2004).

DES (diethylstilbesterol), an oestrogenic hormone, caused an increase in the weight of the anterior pituitary (Trenkle, 1997). When MGA (melengestrol acetate) is used in females it suppresses oestrus and results in a favourable change in behaviour, with regards to feedlot behaviour and performance. When MGA is used in combination with TBA it creates an additive effect, although when used with E_2 the result seen is minimal. The MGA causes a reduction in satellite cell numbers as well as DNA synthesis hypothesizing that progestins act directly

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on muscle cells to cause a reduction in skeletal muscle growth, therefore also causing an increase in physiological maturity (Johnson & Reinhardt, 2009).

2.3.2. Exogenous non-steroidal growth promotants

Certain growth promotants are classed as, “non-steroidal” because of their chemical structure being different to that of the steroids, Non-steroidal growth promotants do not contain cholesterol. The mode of action also differs to steroids.

Zeranol

Zeranol [6-(6, 10-dihydroxy-undecyl)-β-resorcylic acid-μ-lactone] is a non-steroidal anabolic agent of fungal origin (Sinnet-Smith, et al, 1983), more specifically from the *Giberella zeae* fungus (Velle, 1978). Zeranol has oestrogenic activity, it is produced from zearalenone which has an entirely different structure to the synthetic oestrogen, DES. It is not classified as a steroid because it does not have a cholesterol ring as its base.

According to Richards et al. (1987) using Zeranol in intact male cattle caused a significant reduction in the testes size. A similar study was done by Silcox, R.W., *et al.* (1986) to determine if the age of implantation had a significant effect on testes and carcass traits as well as weight gain. The study was divided into two experiments: with experiment 1 testing the effects of age at first Zeranol implantation. Silcox, R.W., *et al.* (1986) found that when Zeranol was implanted at 100 or 150 days of age there was no reduction in testis growth ($P<0.05$), also no reduction in serum testosterone concentration ($P<0.01$) or in sperm production ($P<0.01$). What they did find was that the group that was implanted with Zeranol at 200 days of age has testis growth, sperm production and serum testosterone concentration that was partially suppressed, but cattle implanted at 250 days of age had no change in the testis growth, sperm production or serum testosterone concentration, Indicating that the age of implantation of Zeranol can have a significant impact on testis growth in a younger animal (<200 days of age), Body weights were not affected by age of implantation. A second experiment was also done where trial animals that received implants at 175 and 231 days of age received a second single implantation of Zeranol, testosterone or a combination of Zeranol and testosterone. Once again, body weights were not affected, carcass traits were also similar to the control (no Zeranol). But treatments implanted with Zeranol showed a reduction in the sperm production and testis weight ($P<0.05$), while implantation of testosterone only reduced sperm production ($P<0.05$). Therefore, the results indicate that Zeranol negatively affects the

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production of testosterone and spermatogenesis in the intact bull when implantation occurs before 200 days of age. It was also found that implantation of both Zeranol and testosterone together is of no value.

Eckerman, *et al.* (2013) did a study to determine the effect of the increased dosages of Zeranol on growth and carcass characteristics in feedlot lambs, the study consisted of two phases, the study resulted in there not being a significant difference between the dosages of Zeranol for body weights, average daily gain, dry matter intake or feed conversion, but what they did find was an increase in the incidence of prolapse and mortality in feedlot lambs with the increasing dose of Zeranol being directly related to the increase in the incidence of prolapse (without increasing growth and performance).

β-adrenergic agonists

β-adrenergic agonists (βAA) (also known as β-agonists or repartitioning agents) are organic molecules available for use in cattle, they repartition nutrients to increase the lean : adipose tissue ratio by binding to adrenergic receptors. β-adrenergic agonists have been studied in livestock species since the 1980's (Anderson, et al, 2005). Two compounds have been approved for use in livestock, Zilpaterol (Intervet / Merck Animal Health) approved for use in South Africa and Mexico for cattle and Ractopamine (Instavet) approved for use in swine in 21 different countries. These compounds are only orally active and are therefore used as feed additives (Anderson, et al, 2005). β-adrenergic agonists have a significant effect on lipid metabolism as they cause stimulation of lipolysis as well as a depression of lipogenesis (Blackman, 1990) leaving more energy available for growth processes.

In the sheep feedlotting industry, Zilpaterol hydrochloride is used (off label use) at 80g/ton inclusion into the diet of finisher or rounding off animals. Other than the withdrawal period (3 days) of Zilmax, the Zilpaterol hydrochloride based product is fed for approximately 25 days to produce a lean carcass. Zilpaterol hydrochloride is a repartitioning agent increasing protein synthesis (the deposition of protein as muscle) and decreasing lipogenesis (the deposition of fat). A script is required when including zilpaterol hydrochloride into the diet.

According to Johnson & Chung (2007) feeding βAA to ruminants for a period of three to five weeks (depending on whether cattle or sheep are being fed) causes a dramatic increase in both skeletal muscle mass and the cross-sectional area of individual muscle fibres, but during this period there is no change in the number of nuclei (as with steroidal implants). The number of nuclei (amount of DNA) is constant in relation to the increase in protein

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accumulation and thus muscle mass compared to the control (no βAA), therefore βAA act by binding to their receptors to increase protein accretion and decrease protein degradation. The already existing nuclei within muscle fibres basically become more efficient to result in more protein accretion to occur without the support of additional nuclei from neighbouring satellite cells. One negative effect of the lack of excess DNA is that the effect of the βAA is time limited due to the lowered content of DNA, or available machinery, necessary to support skeletal muscle hypertrophy. Zilmax causes neurostimulation and if used over time leads to receptor down regulation and a blunted response. Beta-agonists cause both a decrease in lipogenesis and well as an increase in lipolysis and therefore are regarded as having a dual role in metabolism (Blackman, 1990). It is for this reason that zilpaterol hydrochloride will have a better response in animals with a higher fat content, possibly females because they naturally have more fat deposition.

2.4. Efficacy of use of different types and combinations of exogenous growth promotants

Many combination implants are used which contain both oestrogenic and androgenic hormones resulting in a greater response (additive response) than a single-hormone implant. According to Reinhardt (2007), the oestrogenic portion of the implant stimulates the release of secondary hormones, such as somatotrophin and IGF-1, stimulating muscle protein deposition. While androgenic implants stimulate protein accretion by directly acting on the muscle tissue. Interestingly these two contrasting mechanisms of actions are assumed to have an additive response to the implant, as seen in the Revalor products.

2.5. Conclusion

The endocrine system plays a large role in coordinating growth, which is such a critical phase of any young animals' life. Exogenous steroidal implants and repartitioning agents are commonly used nowadays to ensure the farmer can keep up with their competitors. The use of growth promotants in food-producing animals has many advantages, mainly in increasing efficiency of animals resulting in more meat/milk/eggs per kg of feed eaten and therefore decreasing costs for farmers allowing consumers to pay less for the final product. It also means less negative impact on the environment. With the total world population growing to 9.15 billion people by 2050, food producers will need to continue to search for more efficient way of producing animal protein sources to feed this growing population, however, the impact

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of exogenous steroidal implants and β-adrenergic agonists on the growth of the gonads and the future impact on the breeding of animals will need to be addressed.

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Chapter 3: Materials and Methods

This research formed part of a larger exogenous growth molecule study in sheep, in which the effects and interactions between steroidal growth implants and a zilpaterol hydrochloride feed additive (Zilmax) was assessed (Le Riche, 2018). This current part of the study focussed mainly on the effects of the same exogenous growth molecules and feed additive on the gonadal development of ram and ewe lambs. A total of 240 lambs, approximately 8 months old, were used to evaluate the effects of different types of growth promotants on the development and growth of the gonads of male and female sheep. The experiment was carried out at the Hatfield Experimental Farm of the University of Pretoria for approximately 10 weeks. Dohne Merino lambs were used in the trial where the animals were treated with various combinations and types of exogenous growth promotants, namely steroidal implants; Revalor H, Revalor G and Ralgro and supplementation of the beta-adrenergic agonist Zilmax in the feed, as well as combinations thereof.

The steroidal implant was placed directly under the skin in the middle third of the right ear, therefore reducing the risk of any edible meat still containing residues - the implant was placed subcutaneously in the middle third of the back of the right ear, between the cartilaginous ribs of the ear (Johnson & Reinhardt, 2009). Care was taken to ensure that the ear was sterile by wiping clean with a brush first and then applying alcohol/ chlorhexidine solution (Reinhardt, 2007).The steroidal implant concentrations used were as follows: Revalor H implant- TBA (Trenbolone Acetate) 200mg; Oestradiol 17-β 20mg, Revalor G implant- TBA 40mg; Oestradiol 17-β 8mg, Ralgro implant; 36mg Zeranol. Zilmax is a feed additive, added directly to the feed (during the production of the feed), in which the pellets that the lambs receive contain 80g/ton Zilpaterol Hydrochloride which is equivalent to 0.15mg/kg/lamb/day.

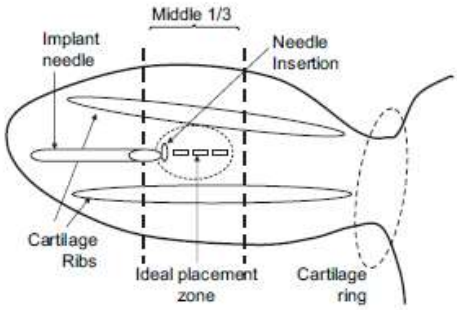

Figure 3. Placement of steroidal implant under the skin of the back of the right ear in the middle third using an implant gun (From Reinhardt, 2007)

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The lambs arrived at the Experimental farm in a custom-built sheep carrier where all animals were processed in the same manner. The lambs were split by weight, first removing the outliers, and then split per sex (rams and ewes). Both sex groups were then randomly allocated into treatment groups and then implanted as per treatment group, with one group receiving no implants only Zilmax and one group receiving no growth enhancers at all. The lambs also received Cidectin, Multivax P, Ovipast and a preparation against tapeworm and liver fluke. Sheep were identified by means of colour coded ear tags to indicate which treatment group they belong to. The lambs belonging to the steroidal implant groups received their implant under the skin of the right ear.

The lambs were kept at the Sheep Feedlot at the Hatfield Experimental farm of the University of Pretoria. A total of 10 Dohne Merino type lambs were allocated per sheep holding pen and were from the same farm of origin, age, breed and processing method as described by Le Riche (2018). The only dietary differences were due to the inclusion of Zilmax in specific treatment groups during the last 21 days, notably the inclusion of Zilmax in the feed for the last 18 days and 3 days withdrawal before slaughter. All the lambs entered the feedlot at 8 months of age (approximately 30kg) and were weighed using the Experimental farm's weighing facilities available upon entry into the feedlot. The lambs were stratified according to weight and then randomly allocated to treatment groups in a completely randomized study design (Le Riche, 2018). The treatment groups were as follows (left refers to the ear tag in the left ear; right refers to the ear tag in the right ear);

- Rams
  - -Control (Purple left)
  - -Ralgro (Blue left)
  - -Revalor G (Green left)
  - -Revalor H (Red left)
  - -Zilmax (Orange left)
  - -Ralgro and Zilmax (Yellow left)
  - -Revalor G and Zilmax (White left)
  - -Revalor H and Zilmax (Navy left)

- Ewes
  - - Control (Purple right)
  - -Ralgro (Blue right)
  - -Revalor G (Green right)
  - -Revalor H (Red right)
  - -Zilmax (Orange right)
  - -Ralgro and Zilmax (Yellow right)

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-Revalor G and Zilmax (White right)

-Revalor H and Zilmax (Navy right)

Of the 240 lambs, 22 rams and 46 ewes were sampled to monitor the gonadal development, the wethers were excluded from this objective for the gonadal development of the lambs. The required samples were collected during the slaughter process and clearly identified. The ovaries were collected with the uterus and cervix to clearly identify the left from the right ovary. The testes were collected still in the scrotum to clearly identify the left from right testis. The samples were then placed into a polystyrene cooler box and transported to the University of Pretoria to be stored in the fridge until samples could be measured. After measuring the samples and all data collection the samples were placed into the freezer and kept at -22ºC.

3.1. Experimental factors

- Phase 1 consisted of:
  - Experimental trial
  - Slaughter of animals with collection of all samples for analysis and measurement of all carcass as well as growth-related traits
- Phase 2 consisted of:
  - Analysis of samples
- Phase 3 consisted of:
  - Statistical analysis

3.2. Experimental Animals

240 eight month-old Dohne Merino lambs of approximately 30kg were kept at the University of Pretoria's Experimental Farm's sheep feedlot facilities with 10 lambs per sheep holding pen which were all from the same farm of origin, age and breed (to remove any source of bias) (Le Riche, 2018). Once they arrived, they were processed and received colour coded ear tags for easy identification. The ear tags were numbered to easily identify animals.

3.3. Experimental design (Treatments)

The treatment groups are presented in Table 3.1.

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Table 3.1 Treatment groups of experimental design

Rams	Ewes
10 Control (maroon)	10 Control (maroon)
10 Ralgro (light blue)	10 Ralgro (light blue)
10 Revalor G (green)	10 Revalor G (green)
10 Revalor H (red)	10 Revalor H (red)
10 Zilmax (orange)	10 Zilmax (orange)
10 Ralgro & Zilmax (yellow)	10 Ralgro & Zilmax (yellow)
10 Revalor G & Zilmax (white)	10 Revalor G & Zilmax (white)
10 Revalor H & Zilmax (navy)	10 Revalor H & Zilmax (navy)

The lambs were randomly allocated to treatment groups for a completely randomized study based on the initial weights of the lambs and their sex. Rams and ewes were separated to prevent any behavioural or dominance effects as well as to compare rams versus ewes. It follows that there was a total of 8 treatment groups for rams and the same for the ewes.

3.4. Nutrition and feeding

The feed and water were available *ad lib.* The pelleted feed given to the lambs was formulated according to an accredited feed company (ANH – Animal Nutrition & Health) and was based on a standard feeding regime consisting of a starter ration (14 days), grower ration (35 days) which was also the finisher ration (21 days) and three days before beginning the trial an adaptation phase, therefore making the feeding time approximately 70 days. In order to stimulate normal feed intake, the lambs were kept in pens with at least 13cm of feeding space/sheep/pen. The only feed additive given was Zilmax which was included at 80grams per metric tonne into the pelleted feed for the treatments receiving Zilpaterol hydrochloride; this is equivalent to 0.15 mg/kg /day/ lamb for 18 days with three days Zilmax withdrawal during the finisher phase as prescribed. The diets given to all treatments were isonutritional to prevent nutrient inclusion levels interfering with the responses seen from the trial.

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3.5. Health

As the health of the animals was imperative to the growth trial, any animals that fell ill over the growing phase were immediately pulled out of the trial and put into a sick pen to be treated by the vet.

When the animals arrived at the Experimental Farm's sheep feedlot facilities, they were all processed in a similar manner receiving; Cidectin, Multivax P, Ovipast and a preparation against tapeworm and liverfluke.

The steroidal implant concentrations used were as follows: Revalor H implant- TBA (Trenbalone Acetate) 200mg; Oestradiol 17-β 20mg, Revalor G implant- TBA 40mg; Oestradiol 17-β 8mg, Ralgro implant- Zeranol (36mg per implant) – Ralgro will fall under the steroidal treatment group for the purposes of this study, even though it is regarded as non-steroidal.

3.6. Experimental Phases

3.6.1. Growth Trial

240 Dohne Merino type lambs were obtained from a local farmer for the growth trial. The animals were transported to the University of Pretoria's Experimental Farm and were split by weight and sex and then allocated to treatment groups to be processed and allocated into randomly assigned pens, with 10 animals per pen (Le Riche, 2018). All lambs received Cidectin, Multivax P, Ovipast and a preparation against tapeworm and liverfluke (as mentioned in 3.5).

After the three days adaptation phase the lambs received the starter, then grower and then finisher ration as explained in 3.5. Weekly weights were recorded for all lambs individually. The lambs then had the final weighing before being transported to the abattoir for slaughter.

3.6.2. Slaughter

Once the trial was completed the lambs were transported in a custom-built sheep carrier to the Cavalier Group abattoir, which is HACCP accredited with SABS, where they were all handled in a humane manner during the slaughter process. The lambs were slaughtered according to treatment group. Each treatment is lined up in preparation for electrical stunning prior to slaughter, the lambs were then electrically stunned until unconsciousness is achieved, a chain is put around the lower leg of the lamb and it is hoisted up in preparation for slaughter. During slaughter, the jugular vein is severed to allow for effective bleeding out, after this the pelt is removed as well as the head. Each carcass received

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a serial number to be recorded together with the ear tag number for full traceability. All abnormalities seen during slaughter were also recorded.

3.6.3. Sample Collection

Of the 240 Dohne Merino type lambs, 22 rams and 46 ewes were sampled to monitor the gonadal development; the wethers were excluded from this objective. The required samples were collected during the slaughter process and clearly identified. The ovaries were collected with the uterus and cervix to clearly identify the left from the right ovary. The testes were also collected still in the scrotum to clearly identify the left from right testis. The samples were placed into plastic bags and were clearly identified with the animals' ear tag number, as well as the carcass serial number, and then placed onto ice in a polystyrene cooler box and transported to the University of Pretoria to be stored in the sample fridge until samples could be measured. After measuring the samples, they were placed into the freezer and kept at -22ºC.

3.6.4. Analysis of Samples

The following gonadal parameters were measured:

In the ewes:

- ovary length (measurements were done on the longest diameter using callipers)
- ovary width (measurements were done on the widest diameter using callipers)
- the number of follicles
- number of *corpora lutea*

In the rams:

- testis length (measurements were done on the longest diameter using callipers)
- width (measurements were done on the widest diameter using callipers)
- volume (measurements were done using a displacement test; displacement of water in a volumetric flask)
- weight (measurements were done using a scale)

The following growth parameters were measured:

- Final mass (kg) (final live mass was taken the day before slaughter using a scale)

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- Subcutaneous fat thickness (mm) (measurements taken using callipers after the sheep were slaughtered, carcasses were hanging into the freezers for storage)

All data was recorded using Microsoft Excel (2010).

3.6.5. Statistical analysis

The experimental design was a completely randomised control study, but the final numbers of animals per treatment group differed somewhat due to differences in the number of rams and ewes that could be obtained for the study and the number that could be included into the control treatment groups due to financial constraints. A multivariate analysis of variance (MANOVA) was used to determine the effect of the factors on the variables using the programme IBM SPSS Version 26. Differences between treatment means were determined by means of the Bonferronic multiple range test to compensate for the small differences in the number of animals per treatment group. Differences were tested at a level of significance of $P<0.05$.

Analysis of data for the ewes and rams was done separately.

- Ewes:
  - Variables :Final mass (kg)
    :Subcutaneous fat thickness (mm)
    :Ovary length (mm)
    :Ovary width (mm)
    :Number of follicles
    :Number of *corpora lutea*
  - Fixed Factors :Steroidal treatment
    :Zilmax treatment

- Rams:
  - Variables :Final mass (kg)
    :Subcutaneous fat thickness (mm)
    :Testis weight (g)
    :Testis volume (mm^3)
    :Testis length (mm)
    :Testis width (mm)

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- Fixed Factors :Steroidal treatment
  :Zilmax treatment

The mean, standard deviation, standard error, type III sum of squares, degrees of freedom, mean square, F and p-values were calculated with a 95% level of significance used ($P<0.05$). The Bonferroni test was used to compare the means, this was done because steroid treatment groups are not equal, therefore this test corrects for the number of observations.

The final weights or mass referred to in this dissertation show the final mass received prior to slaughter (this was the last week of growth and measurements taken prior to slaughter). LS means of final mass (kg) and subcutaneous fat thickness (mm) were used.

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Chapter 4: Results

The results of this study will be presented in two main parts namely, growth data and gonadal data. In these two parts the effects of different combinations of exogenous steroidal implants with or without the feed supplementation of Zilmax will be presented for both rams and ewes separately. Tables and graphs will be used to present the findings. Interpretations of the results will be discussed in Chapter 5. It is important to note that the growth data was part of another postgraduate study of which I assisted with the sample collection and slaughter procedures.

The final masses referred to in this chapter (Chapter 4) show the final mass received prior to slaughter (this was the last week of growth and measurements taken prior to slaughter). LS means of final mass (kg) and subcutaneous fat thickness (mm) were used.

In this study Ralgro is sometimes referred to as a ‘steroidal treatment’ or ‘steroid’ even though it is chemically regarded as a ‘non-steroidal’ growth implant.

4.1. Growth data

In the growth data section, the rams were not separated from the ewes as the effects of sex were evaluated, whereas, in the section on the gonadal growth, data the rams and ewes are separated to test the effects of the steroidal and Zilmax treatments on gonadal development within sexes (e.g. the variables tested in one sex did not apply to the other sex). All results represent the LS means ± SD for both final mass, measured in kg, and subcutaneous fat thicknesses, measured in mm.

4.1.1. Effect of exogenous steroidal treatment on growth of rams

Table 4.1.1 shows the results of the steroidal treatments on the final mass and subcutaneous fat thickness of rams treated with one of the three different steroids tested; namely Ralgro, Revalor G and Revalor H compared to the control. The results for final mass show that Ralgro had the lowest numerical growth response (42.6 ± 1.57kg) followed by the control, Revalor H and lastly Revalor G (42.8 ± 1.56kg, 43.2 ± 1.58kg and 43.3 ± 1.54kg respectively). No significant differences were observed between treatment groups at $P<0.05$ level. The results show a slight numerical increase in final mass due to the use of Revalor H and Revalor G but this difference was not statistically significant ($P>0.05$).

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Additional results presented in Table 4.1.1 show the differences in subcutaneous fat thickness (mm) due to the three steroidal treatments compared to the control in rams. Rams treated with steroidal implants show a slight numerical increase in subcutaneous fat thickness compared to those in the non-implanted control group namely 1.45 ± 0.564mm, with Ralgro, Revalor G and Revalor H being 1.55 ± 0.564mm, 1.56 ± 0.564mm and 1.51 ± 0.564mm, respectively. No significant differences were observed (P>0.05).

Table 4.1.1 LS means [± SD] showing the effect of the exogenous steroidal treatment on final mass (kg) and subcutaneous fat thickness (mm) of rams.

Steroid treatment	n	Final mass [± SD] (kg)	Subcutaneous fat thickness [± SD] (mm)
Control	20	42.8 ± 1.56	1.45 ± 0.564
Ralgro	20	42.6 ± 1.57	1.55 ± 0.564
RevalorG	20	43.3 ± 1.54	1.56 ± 0.564
RevalorH	20	43.2 ± 1.58	1.51 ± 0.564

[a, b]Column means without a superscript do not differ significantly for the treatments LS mean (P>0.05)

4.1.2. Effect of exogenous steroidal treatment on growth of ewes

Table 4.1.2 shows the results of implanting exogenous steroidal implants on final mass and subcutaneous fat thicknesses in ewes. The use of exogenous steroidal implants in ewes did not show a clear trend for final mass to increase (compared to the control of 41.2 ± 1.57kg) with Ralgro and Revalor H showing a lower final mass of 41.0 ± 1.53kg and 40.9 ± 1.58kg respectively, the only steroid treatment to show increased final mass compared to the control was Revalor G with 41.6 ± 1.52kg compared to 41.2 ± 1.57kg for the control. Only small numerical changes were seen in the final mass of the ewe.

The use of Revalor G and Revalor H showed a significant decrease (P<0.05) in subcutaneous fat thickness (1.68 ± 0.564mm and 1.68 ± 0.564mm Revalor G and Revalor H respectively versus 2.11 ± 0.564mm for the control). In contrast to the use of Ralgro in ewes, which showed a small numerical decrease (P>0.05) in subcutaneous fat thickness from 2.11 ± 0.564mm in the control to 1.86 ± 0.564mm for Ralgro.

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Table 4.1.2 LS means [± SD] showing the effect of the exogenous steroidal treatment on final mass (kg) and subcutaneous fat thickness (mm) in ewes.

Steroid treatment	n	Final mass [± SD] (kg)	Subcutaneous fat thickness [± SD] (mm)
Control	20	41.2 ± 1.57	2.11[a] ± 0.564
Ralgro	20	41.0 ± 1.53	1.86[a] ± 0.564
RevalorG	20	41.6 ± 1.52	1.68[b] ± 0.565
RevalorH	20	40.9 ± 1.58	1.68[b] ± 0.565

[a, b]Column means with different superscripts differ significantly for the treatments LS mean (P ≤0.05) + column means without a superscript do not differ significantly for the treatments LS mean (P>0.05)

4.1.3. Effect of Zilmax supplementation in the feed on growth of rams

The effect of Zilmax supplementation on final masses and subcutaneous fat thicknesses are summarized in Table 4.1.3. A slight numerical increase (P>0.05) was observed for the final mass of rams supplemented with Zilmax in the feed (43.3 ± 1.66kg) compared to those in the control group without Zilmax supplementation. This observation was surprising as a more significant increase was expected in the group with dietary Zilmax supplementation.

The subcutaneous fat thickness of the Zilmax supplemented rams decreased (P<0.05) in comparison to the control, with values of 1.36 ± 0.564mm compared to 1.67 ± 0.564mm for the control, respectively. This significant difference in fat thickness clearly confirms the repartitioning of energy away from fat deposition towards other components of growth.

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Table 4.1.3 LS means [± SD] showing the effect of supplementation of Zilmax on final mass (kg) and subcutaneous fat thickness (mm) in rams.

Zilmax treatment	n	Final mass [± SD] (kg)	Subcutaneous fat thickness [± SD] (mm)
Control	40	42.7 ± 1.57	1.67[a] ± 0.564
Zilmax	40	43.3 ± 1.66	1.36[b] ± 0.564

[a, b]Column means with different superscripts differ significantly for the treatments LS mean (P ≤0.05) + column means without a superscript do not differ significantly for the treatments LS mean (P>0.05)

4.1.4. Effect of Zilmax supplementation in the feed on growth of ewes

Zilmax supplementation in the feed of the ewes had a significant effect on their final mass (P<0.05), as was recorded in their male counterparts. The treatment group that received Zilmax weighed 41.6 ± 1.56kg versus the control ewes of 40.8 ± 1.58kg. As in rams a similar trend was noted of a slightly lower subcutaneous fat thickness in ewes fed Zilmax compared to the control group, but this difference was not statistically significant. This trend fits the previously reported effects of beta-adrenergic agonists, namely, to repartition the ingested energy more to protein synthesis and increased lipolysis (Webb, *et al.* 2018).

Table 4.1.4 LS means [± SD] showing the effect of supplementation of Zilmax on final mass (kg) and subcutaneous fat thickness (mm) in ewes.

Zilmax treatment	n	Final mass [± SD] (kg)	Subcutaneous fat thickness [± SD] (mm)
Control	39	40.8[a] ± 1.58	1.96 ± 0.565
Zilmax	39	41.6[b] ± 1.56	1.71 ± 0.565

[a, b]Column means with different superscripts differ significantly for the treatments LS mean (P ≤0.05) + column means without a superscript do not differ significantly for the treatments LS mean (P>0.05)

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4.1.5. Effects of the interaction between exogenous steroidal implants and Zilmax supplementation on growth of rams and ewes

Table 4.1.5 shows the effects of treating rams and ewes with exogenous steroidal implants and supplementation of Zilmax on final mass (kg) and subcutaneous fat thickness (mm). Results of the rams in Ralgro x Zilmax group showed a significant increase in final mass compared to the Ralgro x Control group, with the mass increasing by 1,7kg when Zilmax was supplemented to the diet ($P<0.05$). A similar observation was seen in the ewes treated with Revalor G and supplemented with Zilmax, final mass increased by 1,1kg ($P<0.05$).The use of steroidal implants in rams shows a clear trend for higher final masses than the ewes ($P<0.05$).

The inclusion of Zilmax into the feed yielded an improved response in the ewes that were implanted with Revalor G simultaneously, therefore increasing their final mass to 42.2 ± 1.52kg compared to 41.0 ± 1.52kg for the Revalor G ewes not supplemented with Zilmax ($P<0.05$).

When comparing final mass for rams and ewes it would be expected for the rams to have a higher final mass, this is seen in all the results ($P<0.05$) except for the Ralgro without Zilmax group and Revalor G with Zilmax group where only slight numerical increases are seen ($P>0.05$). It seems that Ralgro suppresses growth in rams but not in ewes.

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Table 4.1.5 LS means [± SD] showing the effect of the interaction between exogenous steroidal treatment, Zilmax supplementation and sex on final mass (kg) and subcutaneous fat thickness (mm) in rams and ewes (n = 158).

Steroidal treatment	Zilmax treatment	Sex	Final mass [± SD] (kg)	Subcutaneous fat thickness [± SD] (mm)
Control	Control	♀	40.5^{a} ± 1.56	2.01^{a} ± 0.564
		♂	43.0^{b} ± 1.52	1.28^{b} ± 0.564
	Zilmax	♀	41.9^{a} ± 1.53	2.22^{a} ± 0.564
		♂	42.6^{b} ± 1.56	1.62^{b} ± 0.564
Ralgro	Control	♀	40.6^{a} ± 1.52	1.86^{a} ± 0.564
		♂	41.7^{a} ± 1.55	1.88^{a} ± 0.564
	Zilmax	♀	41.4^{a} ± 1.53	1.87^{a} ± 0.564
		♂	43.4^{b} ± 1.54	1.22^{b} ± 0.564
Revalor G	Control	♀	41.0^{a} ± 1.52	2.02^{a} ± 0.564
		♂	42.9^{b} ± 1.52	1.87^{a} ± 0.564
	Zilmax	♀	42.2^{b} ± 1.52	1.34^{b} ± 0.564
		♂	43.7^{b} ± 1.54	1.25^{b} ± 0.564
Revalor H	Control	♀	42.0^{a} ± 1.54	1.94^{a} ± 0.564
		♂	43.1^{b} ± 1.53	1.65^{b} ± 0.564
	Zilmax	♀	40.8^{a} ± 1.55	1.43^{b} ± 0.564
		♂	43.3^{b} ± 1.58	1.36^{b} ± 0.564

♀ –Ewe

♂ –Ram

$^{a, b}$Column means with different superscripts differ significantly for the treatments LS mean (P ≤0.05) + column means without a superscript do not differ significantly for the treatments LS mean (P>0.05)

The use of exogenous steroidal treatment alone in ewes did not show a change in subcutaneous fat thickness (P>0.05), with the results for Ralgro, Revalor G and Revalor H being 1.86 ± 0.564mm, 2.02 ± 0.564mm and 1.94 ± 0.564mm compared to 2.01 ± 0.564mm for the control. When Zilmax is introduced into the diet a significant decrease in subcutaneous fat thickness is seen in both Revalor G (1.34 ± 0.564mm) and Revalor H (1.43 ± 0.564mm) treatment groups (P<0.05) compared to 2.01 ± 0.564mm for the control group and 2.22 ± 0.564mm for the control group with Zilmax.

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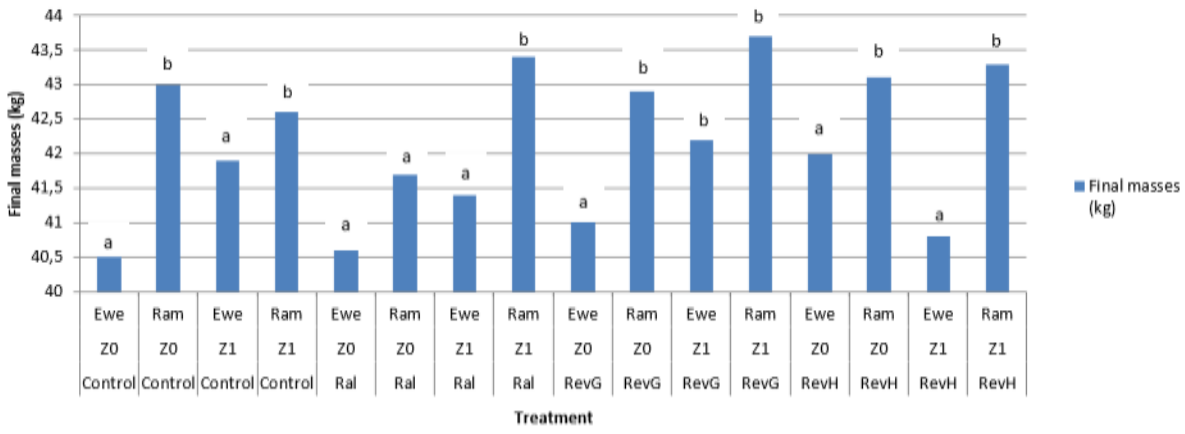
The supplementation of Zilmax together with a steroid in rams caused a decrease ($P<0.05$) in subcutaneous fat thickness for the Ralgro and Revalor G groups (1.22 ± 0.564mm and 1.25 ± 0.564mm) compared to just the respective steroidal treatments of Ralgro and Revalor G alone (1.88 ± 0.564mm and 1.87 ± 0.564mm). Revalor H and Zilmax only shows a slight numerical decrease in subcutaneous fat thickness ($P>0.05$) compared to the steroid (1.36 ± 0.564mm compared to 1.65 ± 0.564mm).

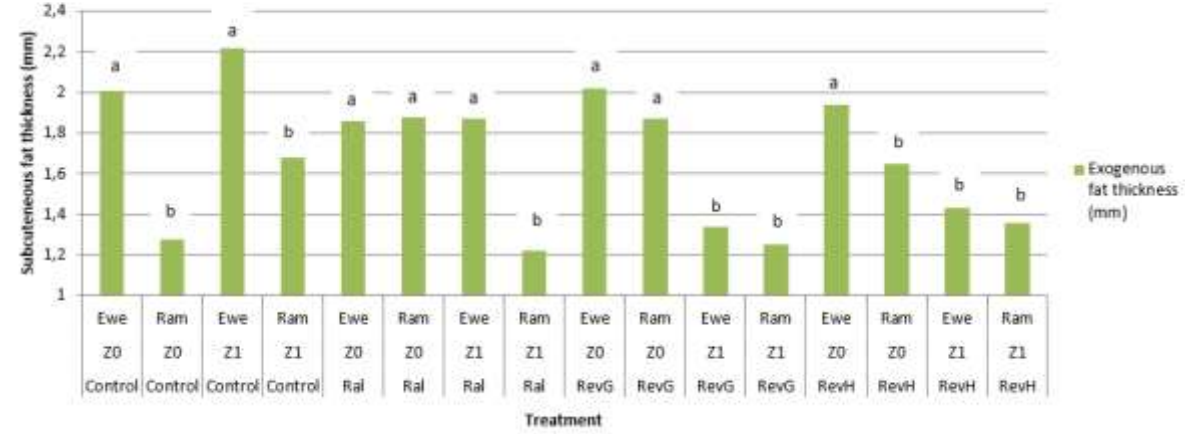
A comparison between the rams and ewes subcutaneous fat thicknesses shows that the rams almost always had a lower subcutaneous fat thickness than the ewes, which was to be expected (except for the Ralgro group which was only slightly numerically higher ($P>0.05$)).

Graphs 4.1 and 4.2 illustrate the interactions of exogenous steroidal treatment with Zilmax supplementation with sex differences for final masses (kg) and subcutaneous fat thicknesses (mm) respectively.

Graph 4.1 LS means showing the interaction of exogenous steroidal treatment with Zilmax supplementation with sex on final mass (kg) of rams and ewes



Graph 4.2 LS means showing the interaction of exogenous steroidal treatment with Zilmax supplementation with sex on subcutaneous fat thickness (mm) of rams and ewes



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The group of rams that received Ralgro but no Zilmax showed the lowest final mass of 41.7 ± 1.55kg of all the ram treatments (P<0.05). Although, what was also seen was that the Ralgro and Zilmax supplemented rams' final mass was lower than the true control but higher than rams receiving Ralgro without Zilmax supplementation, so when Zilmax was supplemented with Ralgro implantation it improved final mass as well as decreased subcutaneous fat thickness (P<0.05), Zilmax is overpowering the effects of Ralgro in rams, as seen when the Ralgro rams received Zilmax supplementation resulting in decreased subcutaneous fat thicknesses compared to Revalor G rams not supplemented with Zilmax (P<0.05). For subcutaneous fat thickness, a similar decreasing trend is seen in the Revalor G rams supplemented with Zilmax (P<0.05). This trend is also seen in the control rams with and without Zilmax supplementation.

4.2. Gonadal growth results of rams

4.2.1. Effect of exogenous steroidal treatments on gonadal development of rams

The effects of steroidal treatments on gonadal measurements of rams are presented in Table 4.2.1. The use of Revalor G significantly retarded gonadal development in terms of testicular weight, volume and testicular dimensions (length and width) compared to rams in the control group and Ralgro treatment group (P<0.05). Testicular weight and volume were almost 60% higher in rams in the control group, compared to the rams treated with Revalor G. Although the testicular weight and volume were about 8% less in rams treated with Ralgro compared to those in the control group, the differences were not statistically significant.

The results show that the use of Revalor G has a negative impact on the physical development of the gonads in rams. Revalor G significantly retarded testis length and width compared to both the control and Ralgro treated rams. This finding suggests that Revalor G affects testicular growth and testicular measurements (length, width and volume). Table 4.2.1 also shows that when rams were treated with Ralgro small numerical differences were seen in the testicular weight, volume and testicular dimensions, but these differences were not statistically significant compared to the control.

Table 4.2.1 LS means [± SD] showing the effect of the exogenous steroidal treatment on testis weight (g), testis volume (mm^3), testis length (mm) and testis width (mm) in rams (n = 44).

Steroid treatment	Testis weight [± SD] (g)	Testis volume [± SD] (mm^3)	Testis length [± SD] (mm)	Testis width [± SD] (mm)
Control	151.5^a ± 12.86	140.7^a ± 16.04	153.4^a ± 9.88	59.7^a ± 2.07

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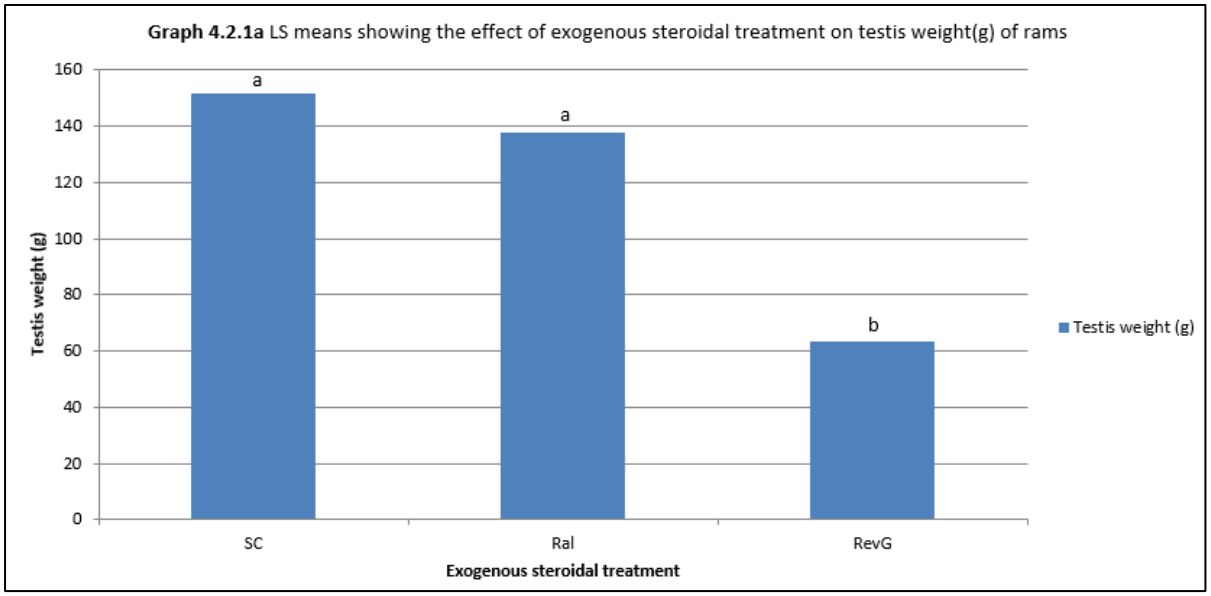
Ralgro	137.6[a] ± 23.37	130.9[a] ± 24.26	145.8[a] ± 10.43	55.3[a] ± 6.97
RevalorG	63.3[b] ± 33.20	52.3[b] ± 28.00	121.7[b] ± 16.04	41.9[b] ± 8.19

[a, b]Column means with different superscripts differ significantly for the treatments LS mean (P ≤0.05) + column means without a superscript do not differ significantly for the treatments LS mean (P>0.05)

The decrease in the development of the testes of rams implanted with exogenous steroidal growth molecules is demonstrated in Graphs 4.2.1a-d. A significant difference (P<0.05) can be seen between Revalor G and the other treatments. Revalor G causes a significant reduction in testis weight, testis volume, testis length and testis width. While Ralgro causes only a small, numerical decrease in testis weight, testis volume, testis length and testis width, though not statistically significant.



C = control group; Ral = Ralgro; RevG = Revalor G

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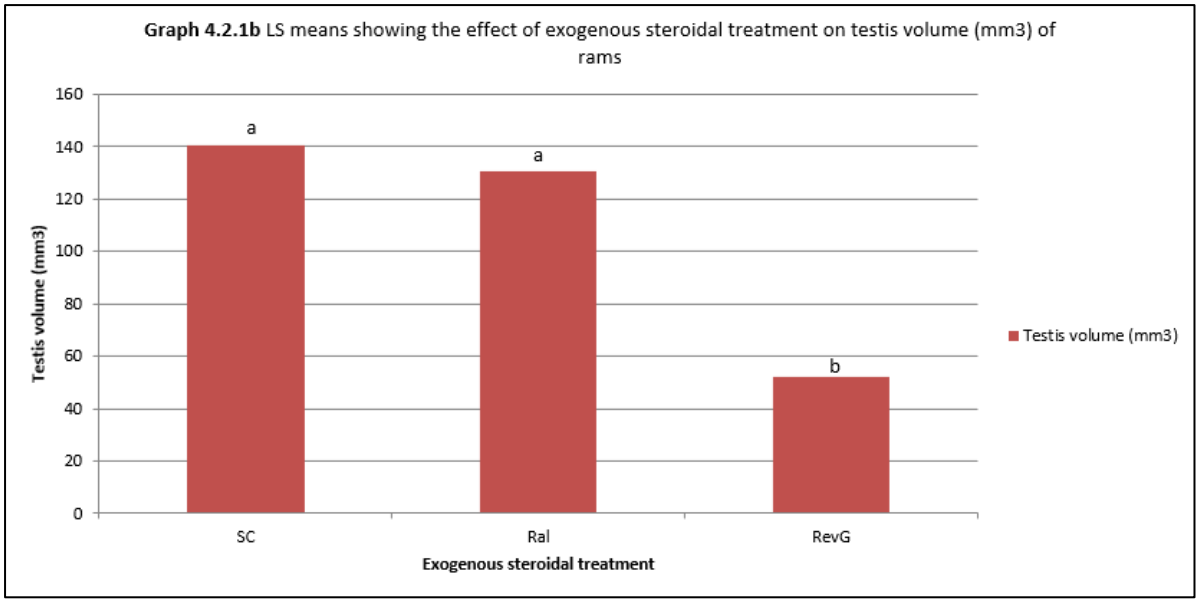
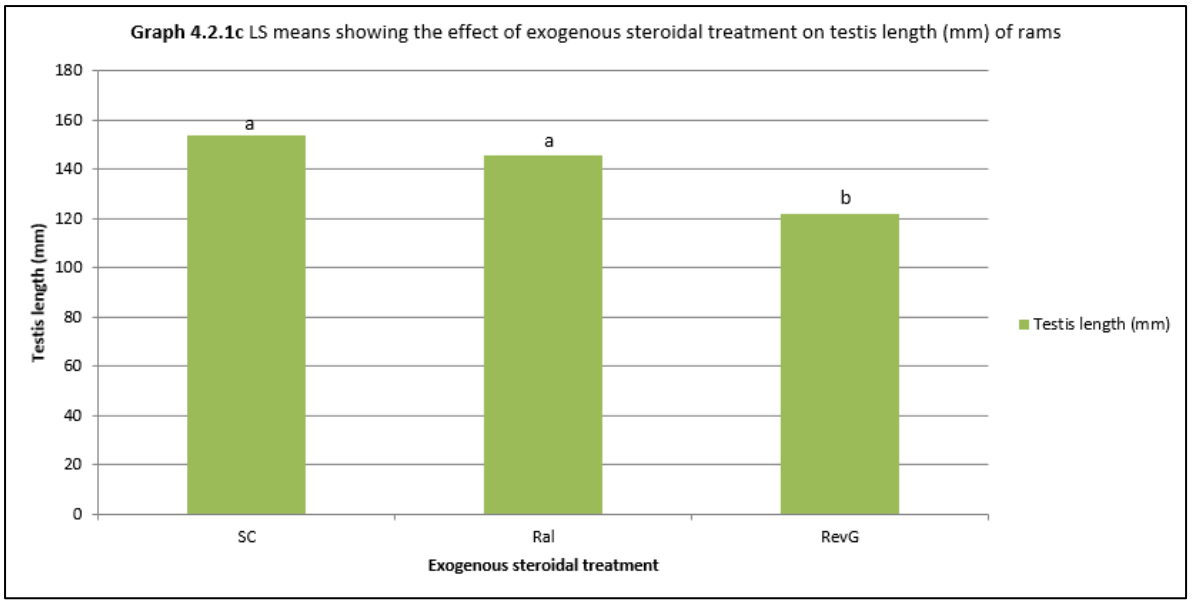


C = control group; Ral = Ralgro; RevG = Revalor G



C = control group; Ral = Ralgro; RevG = Revalor G

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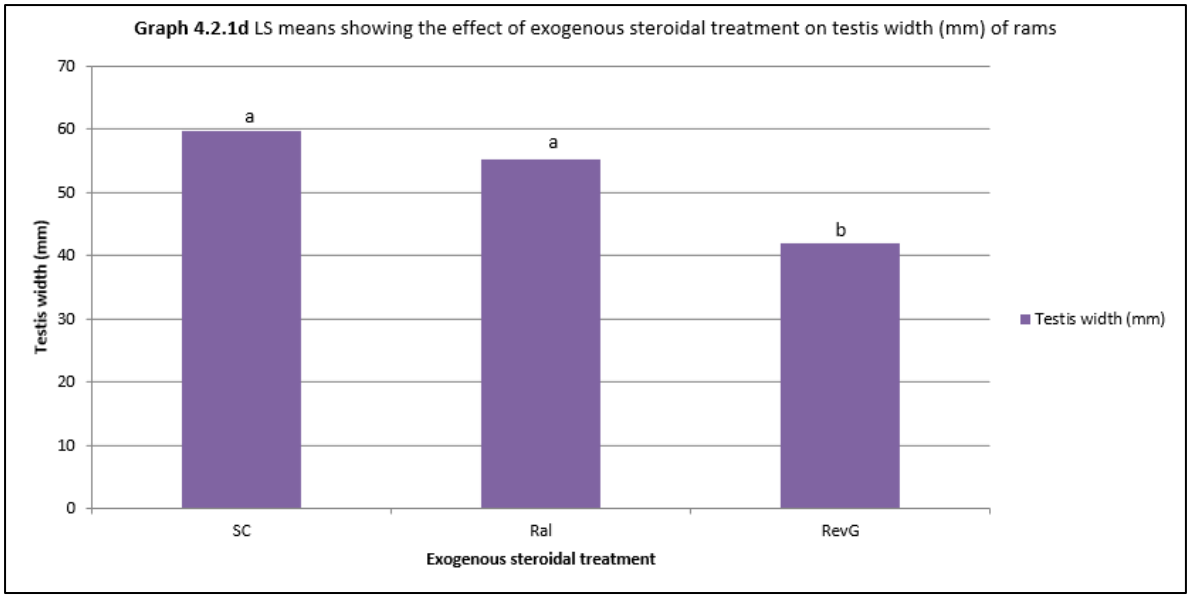

C = control group; Ral = Ralgro; RevG = Revalor G

4.2.2. Effect of Zilmax supplementation in the feed on gonadal development of rams

The effects of Zilmax supplementation in the feed on gonadal growth of rams is summarized in Table 4.2.2. The use of Zilmax did not significantly stunt the testicular growth and development; in terms of weight, volume and testicular dimensions (P>0.05) as only numerical decreases were seen in Table 4.2.2 where the testicular dimensions were compared in rams supplemented with Zilmax versus the control. The minor numerical depression in testicular growth and development suggests that energy is not shunted away from growth and development of the testes and towards skeletal muscle growth. Zilmax is not a reproductive steroid therefore no major effects on gonadal growth was expected.

Table 4.2.2 LS means [± SD] showing the effect of the Zilmax supplementation on testis weight (g), testis volume (mm^3), testis length (mm) and testis width (mm) in rams (n = 44).

Zilmax treatment	Testis weight [± SD] (g)	Testis volume [± SD] (mm^3)	Testis length [± SD] (mm)	Testis width [± SD] (mm)
Control	143.8 ± 24.33	133.9 ± 25.82	150.8 ± 11.48	57.9 ± 4.29
Zilmax	123.3 ± 37.90	116.7 ± 39.10	139.0 ± 13.53	51.9 ± 9.76

[a, b]Column means without a superscript do not differ significantly for the treatments LS mean (P>0.05)

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4.2.3. Interaction between exogenous steroidal treatment with Zilmax supplementation in the feed on gonadal development of rams

Table 4.2.3 shows the effect of the interaction of the steroid treatment with the Zilmax treatment. The results for testis weight, testis volume, testis length and testis width for Steroidal Control x Zilmax Control were as follows: 151.5 ± 12.86g, 140.7 ± 16.04mm^3, 153.4 ± 9.88mm and 59.7 ± 2.07mm, respectively. The rest of the treatments all showed a trend to decrease compared to the Steroidal Control x Zilmax Control interaction even though statistical significance was not proven (P>0.05).

Revalor G x Zilmax had a large impact on all four variables with the testis weight, testis volume, testis length and testis width being 63.3 ± 33.20g, 52.3 ± 27.99mm^3, 121.7 ± 16.04mm and 41.9 ± 8.19mm respectively. Differences (P<0.05) could not be proved, although a large numerical difference was seen. Ralgro x Zilmax resulted in larger testis weights and testis volumes (140.5 ± 14.13g and 135.1 ± 13.18mm^3) compared to Ralgro x Zilmax Control (134.8 ± 31.40g and 125.8 ± 32.88mm^3).

Table 4.2.3 LS means [± SD] showing the interaction of exogenous steroidal treatment with Zilmax supplementation on testis weight (g), testis volume (mm^3), testis length (mm) and testis width (mm) in rams (n = 44).

Steroid Treatment	Zilmax Treatment	Testis weight [± SD] (g)	Testis volume [± SD] (mm^3)	Testis length [± SD] (mm)	Testis width [± SD] (mm)
Control	Control	151.5 ± 12.86	140.7 ± 16.04	153.4 ± 9.88	59.7 ± 2.07
	Zilmax	-	-	-	-
Ralgro	Control	134.8 ± 31.40	125.8 ± 32.88	147.8 ± 12.89	55.9 ± 5.29
	Zilmax	140.5 ± 14.13	135.1 ± 13.18	143.9 ± 7.84	54.8 ± 8.32
RevalorG	Control	-	-	-	-
	Zilmax	63.3 ± 33.20	52.3 ± 27.99	121.7 ± 16.04	41.9 ± 8.19

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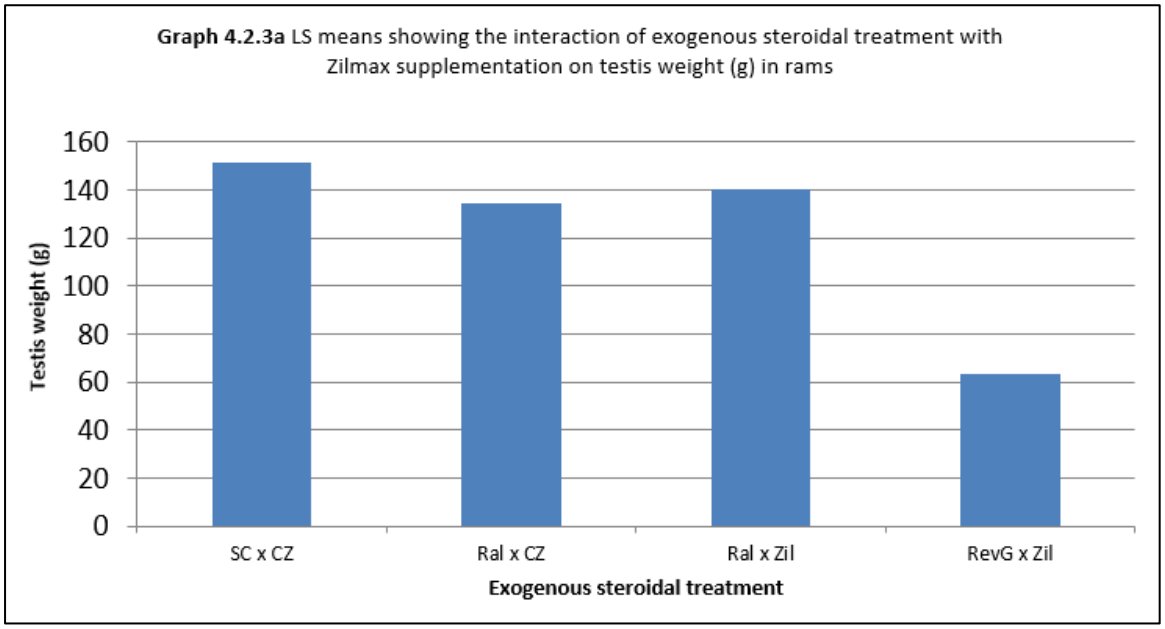
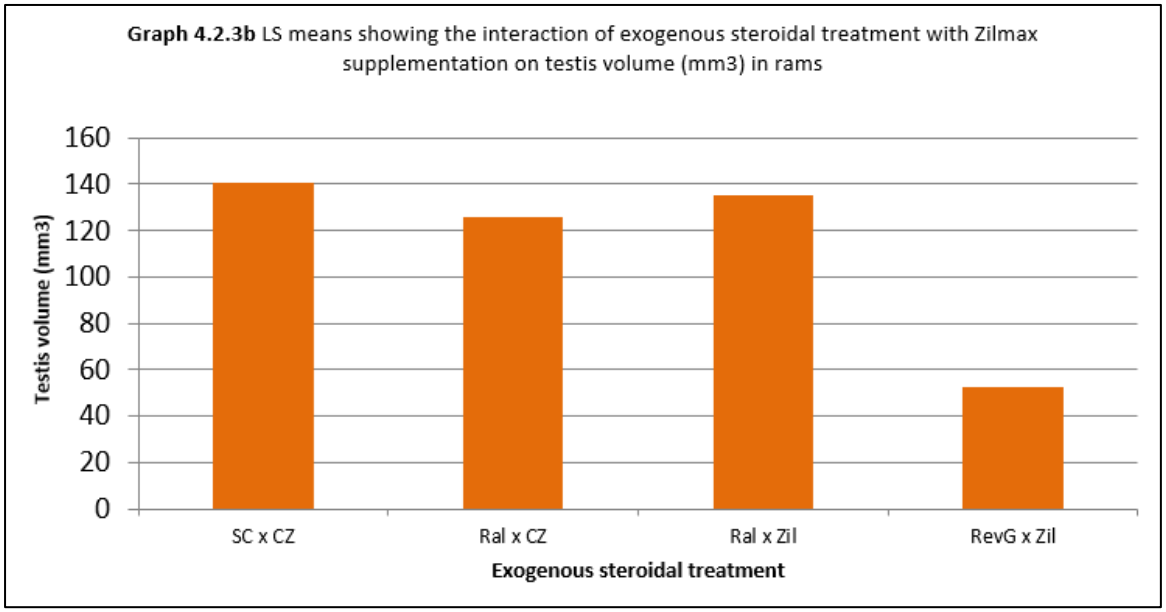
[a, b]Column means with different superscripts differ significantly for the treatments LS mean (P ≤0.05) + column means without a superscript do not differ significantly for the treatments LS mean (P>0.05)

- This level combination is not observed; thus the corresponding population mean is not estimable.

Graphs 4.2.3a-d showing the effect of the exogenous steroidal treatment with Zilmax supplementation on testis weight (g), testis volume (mm^3), testis length (mm) and testis width (mm) in rams.



SCxCZ = Control x No-Zilmax; Ral x CZ = Ralgro x No-Zilmax; Ral x Zil = Ralgro x Zilmax; RevG x Zil = Revalor G x Zilmax



SCxCZ = Control x No-Zilmax; Ral x CZ = Ralgro x No-Zilmax; Ral x Zil = Ralgro x Zilmax; RevG x Zil = Revalor G x Zilmax

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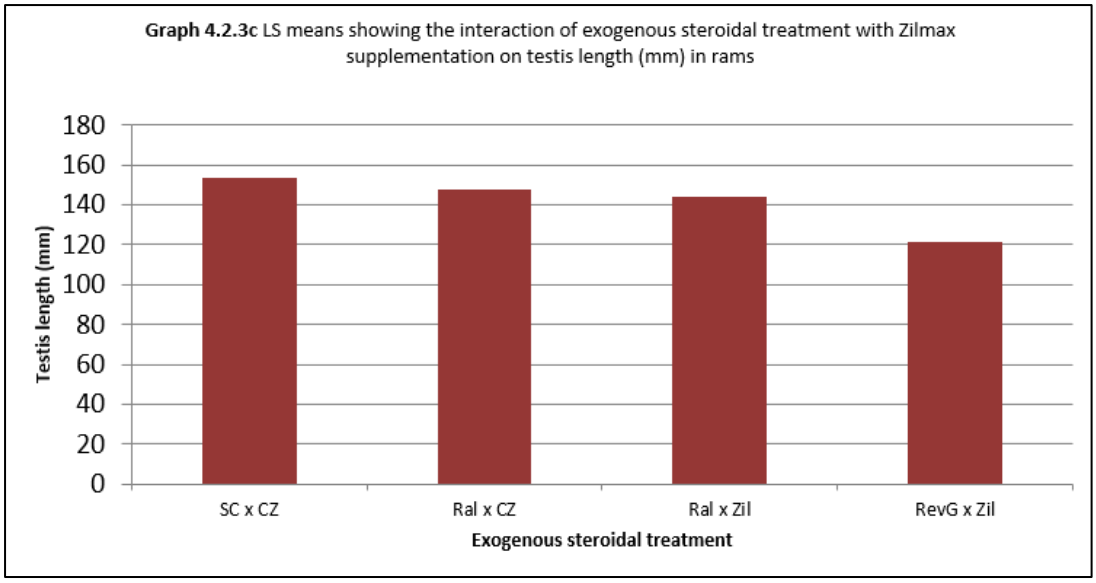
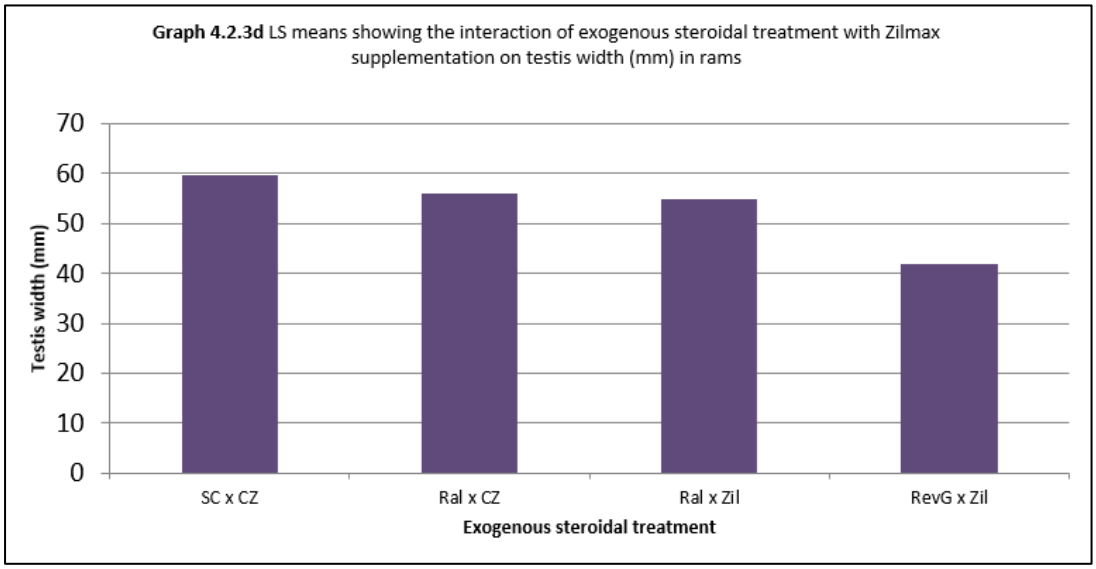


SCxCZ = Control x No-Zilmax; Ral x CZ = Ralgro x No-Zilmax; Ral x Zil = Ralgro x Zilmax; RevG x Zil = Revalor G x Zilmax



SCxCZ = Control x No-Zilmax; Ral x CZ = Ralgro x No-Zilmax; Ral x Zil = Ralgro x Zilmax; RevG x Zil = Revalor G x Zilmax

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4.3. Gonadal growth results of ewes

4.3.1 Effect of exogenous steroidal treatments on gonadal development of ewes

Table 4.3.1 shows the effect that the steroid treatment had on the ovary length (mm), ovary width (mm), number of follicles and number of corpora lutea in ewes. Ovary length, width and number of corpora lutea were not affected by the steroidal treatment of Ralgro, Revalor G nor Revalor H ($P>0.05$). The number of follicles on the ovary was significantly decreased by the steroidal treatment ($P<0.05$) of Ralgro, Revalor G and Revalor H. Revalor G had the greatest negative impact on number of follicles (6.2 ± 3.86 follicles) followed by Revalor H (7.0 ± 2.87 follicles) and lastly by Ralgro (8.6 ± 2.81 follicles) when compared the steroidal control which had a LS mean of 12.3 ± 4.12 follicles. When comparing Ralgro, Revalor G and Revalor H to each other; no significant differences were observed between these treatments ($P>0.05$).

No significant trend or change was observed for ovary length (mm), ovary width (mm) or number of corpora lutea in ewes were treated with steroidal implants, namely Ralgro, Revalor G or Revalor H ($P>0.05$).

Table 4.3.1 Summary statistics (LS means [± SD]) of the effect of exogenous steroidal treatments on ovary length (mm), ovary width (mm), number of follicles and number of corpora lutea in ewes.

Steroid treatment	n	Ovary length [± SD] (mm)	Ovary width [± SD] (mm)	Number of follicles [± SD]	Number of corpora lutea [± SD]
Control	24	12.9 ± 3.05	9.4 ± 2.11	12.3^{a} ± 4.12	0.5 ± 0.59
Ralgro	24	12.0 ± 1.68	8.8 ± 1.76	8.6^{b} ± 2.81	0.3 ± 0.57
Revalor G	24	12.4 ± 2.60	9.0 ± 2.00	6.2^{b} ± 3.86	0.2 ± 0.38
Revalor H	20	11.9 ± 1.36	8.6 ± 1.39	7.0^{b} ± 2.87	0.2 ± 0.49

[a, b]Column means with different superscripts differ significantly for the treatments LS mean ($P \leq 0.05$) + column means without a superscript do not differ significantly for the treatments LS mean ($P>0.05$)

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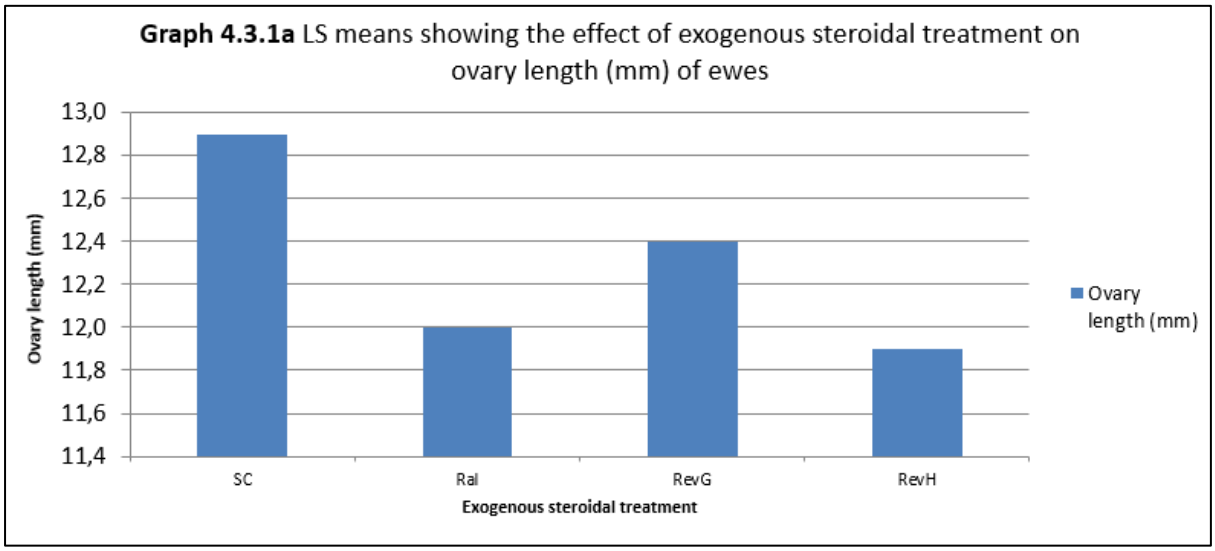
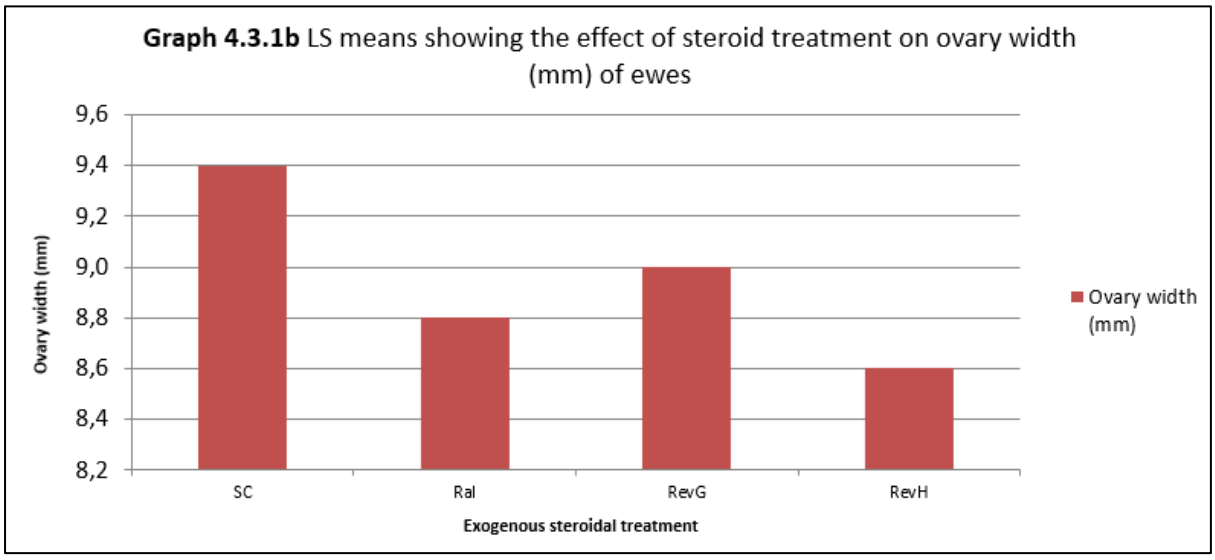
Graphs 4.3.1a-d below refers to Table 4.3.1 which shows the effect of different exogenous steroidal treatments on the number of follicles (P<0.05) compared to the steroidal control. A numerical decrease in the number of corpora lutea was also observed but the main effect was not proved significant. Ovary length and width was not affected by the steroidal treatment of Ralgro, Revalor G nor Revalor H (P>0.05).



SC = Control; Ral = Ralgro; RevG = Revalor G; RevH = Revalor H



SCxCZ = Control x No-Zilmax; Ral x CZ = Ralgro x No-Zilmax; Ral x Zil = Ralgro x Zilmax; RevG x Zil = Revalor G x Zilmax

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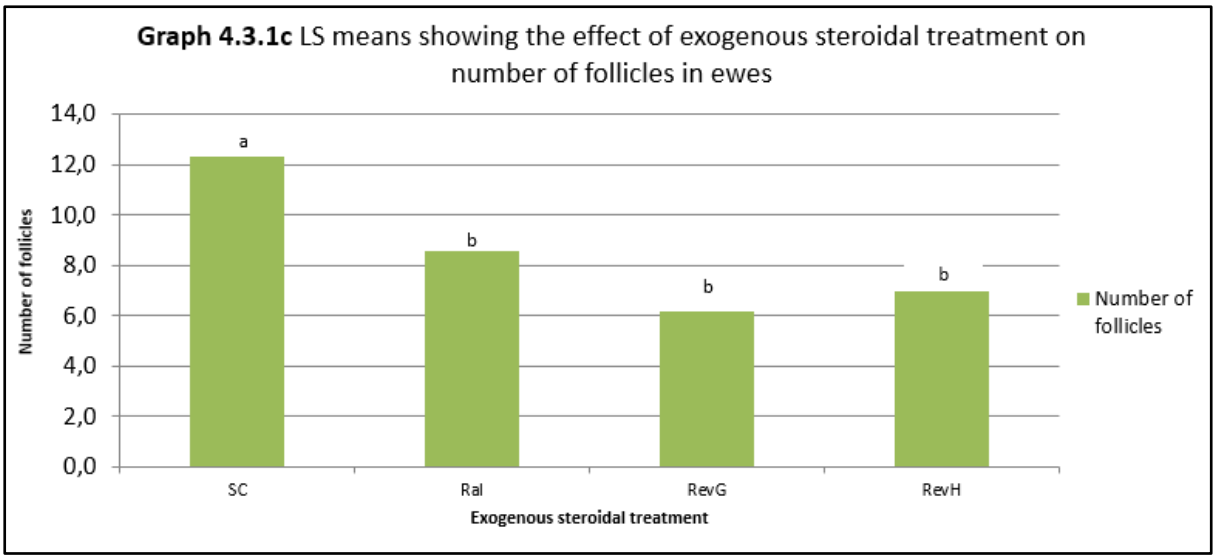
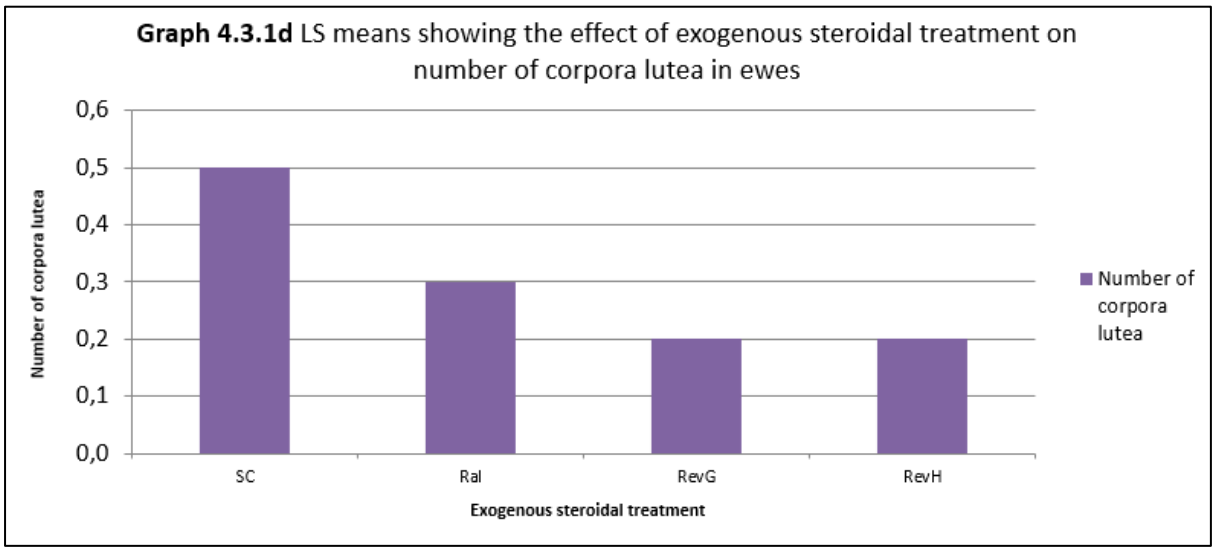


SCxCZ = Control x No-Zilmax; Ral x CZ = Ralgro x No-Zilmax; Ral x Zil = Ralgro x Zilmax; RevG x Zil = Revalor G x Zilmax



SCxCZ = Control x No-Zilmax; Ral x CZ = Ralgro x No-Zilmax; Ral x Zil = Ralgro x Zilmax; RevG x Zil = Revalor G x Zilmax

4.3.2. Effect of Zilmax supplementation in the feed on gonadal development of ewes

Table 4.3.2 shows the effect of Zilmax supplementation in the feed on ovary length (mm), ovary width (mm), number of follicles and number of corpora lutea in ewes. From this Table significant differences ($P<0.05$) are observed between the Zilmax and control treatments for ovary length (11.8 ± 2.01mm vs 14.0 ± 3.54mm), width (8.3 ± 1.46mm vs 10.5 ± 2.16mm) and number of follicles (10.3 ± 3.26 vs 14.3 ± 4.07), while no difference is observed for the number of corpora lutea ($P>0.05$).

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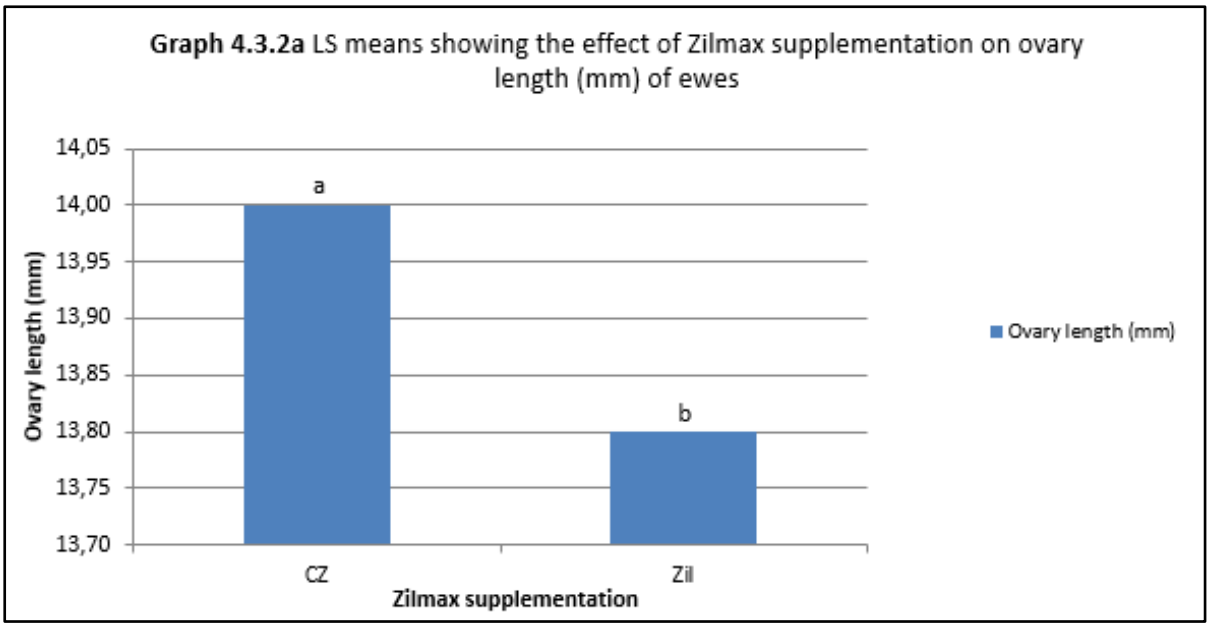
Table 4.3.2 LS means [± SD] showing the effect of the Zilmax supplementation in the feed on ovary length (mm), ovary width (mm), number of follicles and number of corpora lutea in ewes.

Zilmax treatment	n	Ovary length [± SD] (mm)	Ovary width [± SD] (mm)	Number of follicles [± SD]	Number of corpora lutea [± SD]
Control	48	$14.0^{a} \pm 3.54$	$10.5^{a} \pm 2.16$	$14.3^{a} \pm 4.07$	0.3 ± 0.45
Zilmax	44	$11.8^{b} \pm 2.01$	$8.3^{b} \pm 1.46$	$10.3^{b} \pm 3.26$	0.7 ± 0.65

$^{a, b}$Column means with different superscripts differ significantly for the treatments LS mean ($P \leq 0.05$) + column means without a superscript do not differ significantly for the treatments LS mean ($P > 0.05$)

Graphs 4.3.2a-d show the significance of Zilmax supplementation on gonadal development in ewes.



CZ = Control; Zil = Zilmax

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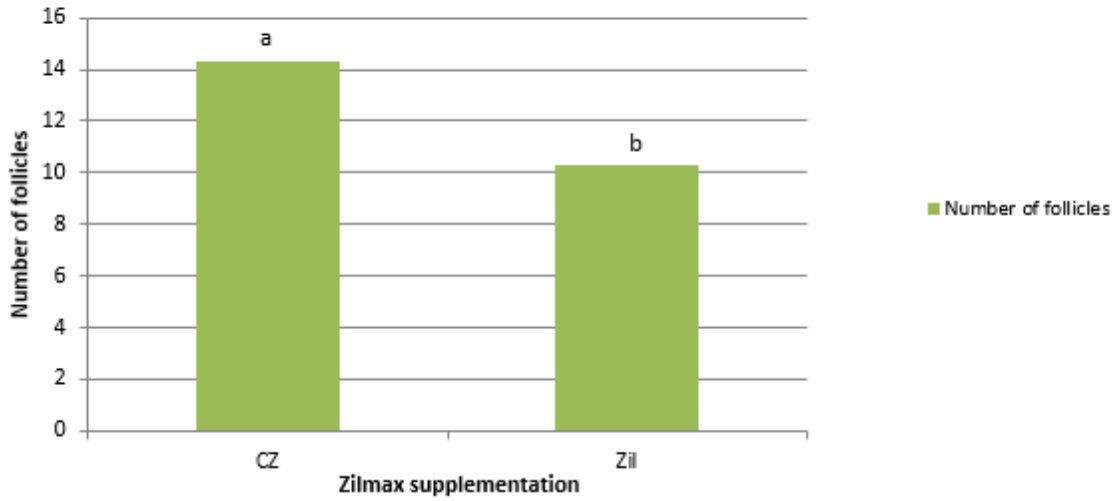
Graph 4.3.2b LS means showing the effect of Zilmax supplementation on ovary width (mm) of ewes

CZ = Control; Zil = Zilmax

Graph 4.3.2c LS means showing the effect of Zilmax supplementation on number of follicles of ewes



CZ = Control; Zil = Zilmax

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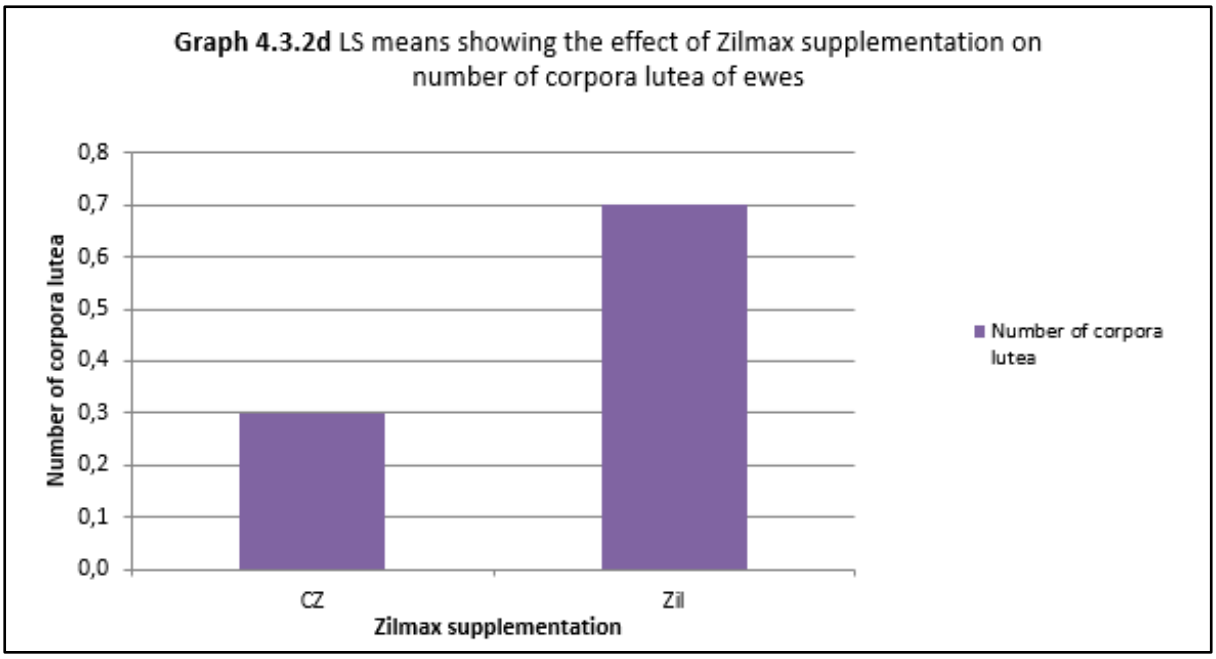

CZ = Control; Zil = Zilmax

4.3.3. Interaction effects of exogenous steroidal treatment with Zilmax supplementation in the feed on gonadal development of ewes

Table 4.3.3 shows the differences and interaction effects of steroid and Zilmax treatments on ovary length (mm), ovary width (mm), number of follicles and number of corpora lutea in ewes. It is interesting to note that in all the Steroidal implant x Zilmax combinations, the treatments supplemented with Zilmax had lower responses than the steroid treatments alone. The interaction between Revalor G and Zilmax was noticeable, but not significantly different (P>0.05).

The Steroidal Control x Zilmax Control (e.g. no Zilmax) treatment compared to the Steroidal control x Zilmax treatment which shows a slight decrease in all variables (P>0.05) overall resulting in smaller ovaries. Zilmax Control refers to the animals that did not receive Zilmax. Zilmax treatment refers to animals that received Zilmax.

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Table 4.3.3 LS means [± SD] showing the interaction effects of exogenous steroid treatment with Zilmax supplementation in ewes on ovary length (mm), ovary width (mm), number of follicles and number of corpora lutea (n = 92).

Steroid x Zilmax interactions	Ovary length [± SD] (mm)	Ovary width [± SD] (mm)	Number of follicles [± SD]	Number of corpora lutea [± SD]
Steroidal Control x No Zilmax	14.0 ±3.54	10.5 ±2.16	14.3 ±4.07	0.3 ±0.45
Steroidal Control x Zilmax	11.7 ±2.01	8.3 ±1.46	10.3 ±3.26	0.7 ±0.65
Ralgro x No Zilmax	12.5 ±1.54	9.6 ±1.73	9.3 ±2.99	0.5 ±0.67
Ralgro x Zilmax	11.5 ±1.73	7.9 ±1.40	7.9 ±2.58	0.2 ±0.39
Revalor G x No Zilmax	12.8 ±2.92	9.8 ±1.39	8.5 ±3.53	0.1 ±0.29
Revalor G x Zilmax	12.1 ±2.31	8.2 ±2.25	3.8 ±2.62	0.3 ±0.45
Revalor H x No Zilmax	12.1 ±1.28	9.1 ±1.33	8.4 ±2.71	0.3 ±0.62
Revalor H x Zilmax	11.6 ±1.48	7.8 ±1.16	4.8 ±1.46	0.0 ±0.00

[a, b]Column means with different superscripts differ significantly for the treatments LS mean (P ≤0.05) + column means without a superscript do not differ significantly for the treatments LS mean (P>0.05)

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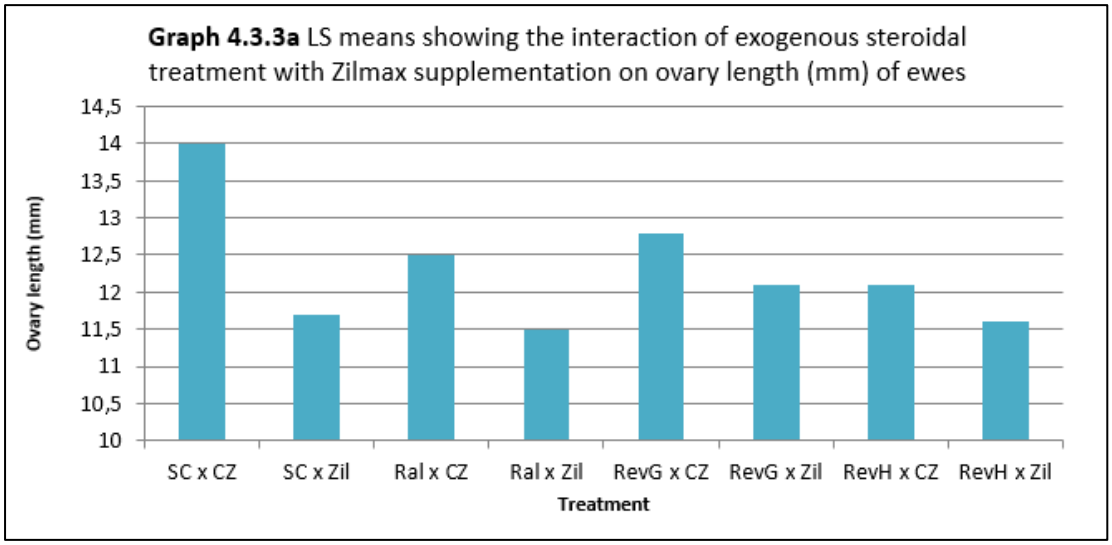
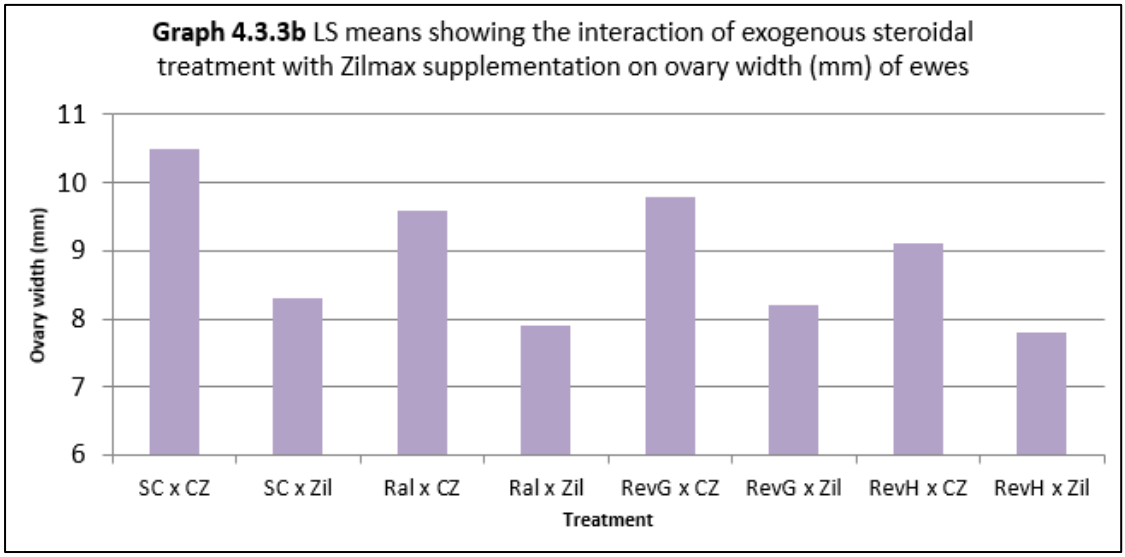
The effects of different combinations of steroid implants and Zilmax have on gonadal development in ewes are presented in Graphs 4.3.3a-d. A decrease in number of follicles when Revalor H and Revalor G are used in combination with Zilmax is seen. There were numerical decreases observed in the other treatments compared to Steroidal Control x Zilmax Control but not to the degree of Revalor H x Zilmax and Revalor G x Zilmax observations.



Graph 4.3.3a LS means showing the interaction of exogenous steroidal treatment with Zilmax supplementation on ovary length (mm) of ewes

SC x CZ = Control x No-Zilmax; SC x Zil = Control x Zilmax; Ral x CZ = Ralgro x No-Zilmax; Ral x Zil = Ralgro x Zilmax; RevG x CZ = Revalor G x No-Zilmax; RevG x Zil = Revalor G x Zilmax; RevH x CZ = Revalor H x No-Zilmax; RevH x Zil = Revalor H x Zilmax



Graph 4.3.3b LS means showing the interaction of exogenous steroidal treatment with Zilmax supplementation on ovary width (mm) of ewes

SC x CZ = Control x No-Zilmax; SC x Zil = Control x Zilmax; Ral x CZ = Ralgro x No-Zilmax; Ral x Zil = Ralgro x Zilmax; RevG x CZ = Revalor G x No-Zilmax; RevG x Zil = Revalor G x Zilmax; RevH x CZ = Revalor H x No-Zilmax; RevH x Zil = Revalor H x Zilmax

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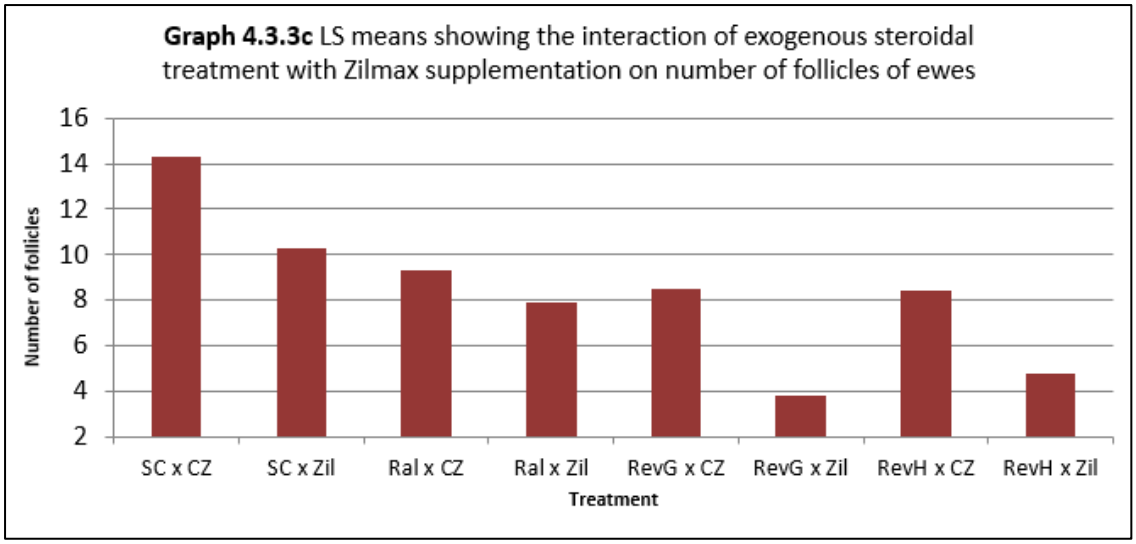
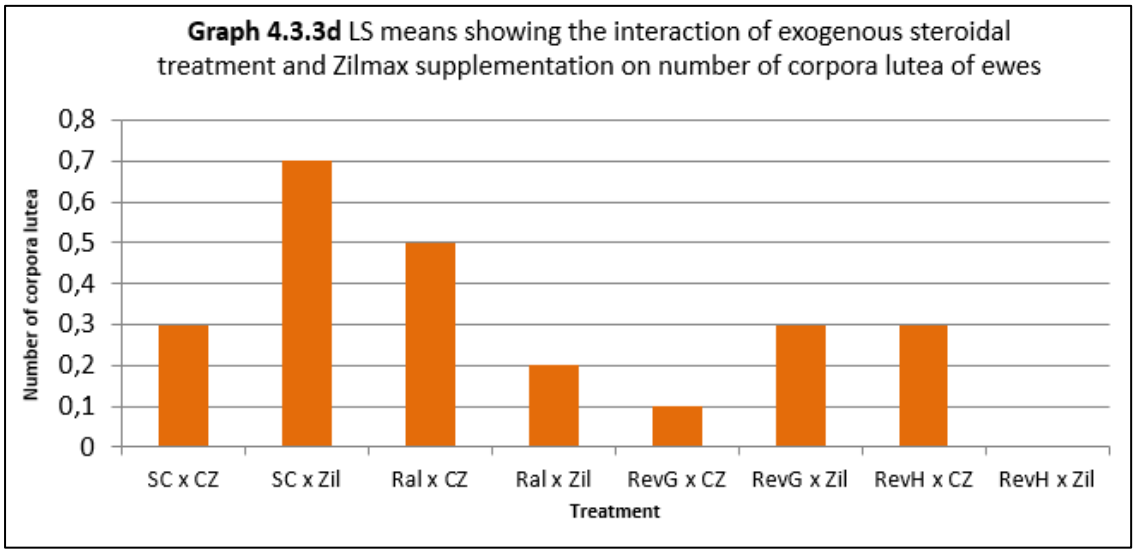


SC x CZ = Control x No-Zilmax; SC x Zil = Control x Zilmax; Ral x CZ = Ralgro x No-Zilmax; Ral x Zil = Ralgro x Zilmax; RevG x CZ = Revalor G x No-Zilmax; RevG x Zil = Revalor G x Zilmax; RevH x CZ = Revalor H x No-Zilmax; RevH x Zil = Revalor H x Zilmax



SC x CZ = Control x No-Zilmax; SC x Zil = Control x Zilmax; Ral x CZ = Ralgro x No-Zilmax; Ral x Zil = Ralgro x Zilmax; RevG x CZ = Revalor G x No-Zilmax; RevG x Zil = Revalor G x Zilmax; RevH x CZ = Revalor H x No-Zilmax; RevH x Zil = Revalor H x Zilmax

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Chapter 5: Discussion

In the present study, feedlot lambs were implanted with a combination of exogenous steroidal growth implants and Zilmax supplementation in the feed to determine the effect such treatments may have on the growth and physical development of the gonads of Dohne Merino rams and ewes.

5.1. Sheep growth data

Johnson & Reinhardt (2009) stated that the responses seen from the use of exogenous steroidal implants in animals are determined by the dosages of the active hormones, which implies that a higher dose will result in an improved performance. This remains true as long as the main nutrients in the diet (energy and protein) are not limiting, which may partly explain the lack of a response in animals treated with a higher dose of hormone. The genetic potential of the animal is another important determinant of growth, the higher the non-implanted animal's growth rate the better the results seen when implanted. Other research shows that when important nutrients are limiting in the diet the response seen by the growth promotant is greater than expected (Johnson & Beckett, 2014).

5.1.1. Final mass

When considering the effect of exogenous steroidal implants on the growth and development in rams, the results often show that the use of these exogenous steroidal growth promotants results in a marginally higher final mass ($P>0.05$) compared to the control. The ram growth results from Table 4.1.1 agree with this observation, with the control being 42.8 ± 1.56kg followed by Revalor H (43.2 ± 1.58kg) and Revalor G (43.3 ± 1.54kg). The final mass of Ralgro treated rams decreased slightly ($P>0.05$) compared to the control (42.6 ± 1.57kg), demonstrating a small non-significant increase in final mass. Parameters of efficiency such as feed conversion ratio, average daily gain and feed intake may be more useful to demonstrate the beneficial effects of exogenous steroidal implants.

The results for the ewes are presented in Table 4.1.2; the final mass (kg) for the ewes show no difference in final mass ($P>0.05$) due to exogenous steroidal implants, this is in agreement to Eckerman, *et al.* (2012) where they found that implanting even 12mg Zeranol

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(and increasing dosages) did not cause improvements in final mass and average daily gains. Cooper (1981) found similar results when implanting 12mg of Zeranol into 169 ewe lambs over two years, this resulted in heavier lambs, although not statistically significant. These findings contradict those of Sinnett-Smith, *et al.* (1983) who reported that lambs implanted with TBA or Zeranol resulted in increased weight gains ($P<0.05$). The mean final mass of the ewes was lower than that of the rams which was expected. Eckerman, *et al.* (2012) also stated that the majority of previous research done indicate that implanting lambs with 12mg Zeranol does increase weight gains.

It was expected that the supplementation of Zilmax in the diets would cause significant increases in the final mass in both the rams and ewes, Tables 4.1.3 and 4.1.4 show the results of the effect of Zilmax supplementation versus the control (no Zilmax) on final mass for the rams and ewes respectively. The addition of Zilmax to the diets of the rams did not cause a significant improvement in the final mass ($P>0.05$), with the control being 42.7 ± 1.57kg and the Zilmax treatment being 43.3 ± 1.66kg. The reason for the non-significant increase may be due to number of observations (N), or possibly too much variation caused by other uncontrolled factors. The study was conducted in the summer so it is possible that the high temperatures caused depressed intakes and therefore a suboptimal feed intake. By contrast, the results of the ewes fed Zilmax showed a significant increase in final mass ($P<0.05$) with the Zilmax group gaining approximately 800g more over the treatment period. Rams were expected to show a greater response to the Zilmax treatment than the ewes. Montgomery, *et al*. (2009) conducted a study to determine the effects of dietary Zilpaterol hydrochloride on performance (body weights) and carcass traits in heifers and steers. What they found was that when Zilpaterol hydrochloride was fed to heifers the final body weights of the heifers increased ($P<0.01$) by 6.7kg and by 11.6kg when fed to steers, this agrees with what Avendano-Reyes, *et al.* (2011) found; the ewe lambs that were fed Zilmax had greater ADG, final body weight and gain : feed ratio.

The results from the current study indicate that the combined effects of steroidal growth implants and Zilmax supplementation in feedlot lambs did not increase final mass significantly. Nevertheless, the nutritional quality of the diet fed to the animals was adequate for feedlot lamb growth and development as the animals were in a state of hypernutrition to encourage growth which yielded acceptable carcasses.

Table 4.1.5 shows a nice comparison of the use of steroids with and without Zilmax between the two sexes (rams and ewes). These results confirm that rams achieved a higher final mass compared to the ewes ($P<0.05$), which fits the basic biological differences between sexes. Table 4.1.5 clearly shows the differences between sexes was true for all treatment

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combinations except for the Ralgro without Zilmax group and Revalor G with Zilmax group where only slight numerical improvements in final mass were seen (P>0.05). A possible explanation for the Ralgro without Zilmax group having lower masses than the ewes could be because Ralgro is a non-steroidal growth promotant with oestrogenic activity, when Ralgro is fed directly to intact males it results in an increase in body fat and does not have direct effect on skeletal muscle accretion as TBA or testosterone does. Zeranol has oestrogenic properties; in ruminants the oestrogens have a special and indirect effect on skeletal muscle growth; oestrogens act by inducing GH and thus IGF-1 which in turn has a direct effect on skeletal muscle by increasing protein synthesis therefore resulting in higher body weights (Hossner, 2005). Perhaps the results should rather be seen from the point that the ewes outperformed the rams and not that the rams underperformed compared to the ewes. Revalor G contains 40mg TBA and 8mg Oestradiol-17 β and Revalor H contains 140mg TBA and 14mg Oestradiol-17 β and are known to be potent growth promotants causing an increase in gains and efficiency by increasing secretions of GH into the vascular system and directly stimulating protein synthesis through skeletal muscle receptors.

The mechanism of action of steroids on skeletal growth is divided into a direct and indirect response. Oestrogens have an indirect response on the skeletal muscle by altering the somatotrophic axis, the hypothalamus is stimulated to release more GHRF which stimulates the anterior pituitary to produce and release more GH causing increase muscle protein synthesis as well as affecting the nitrogen balance in ruminants, increasing the nitrogen stores in muscles to allow for more lean muscle tissue deposition. The increased GH stimulates the release of IGF-1 and IGF-2 (Hossner, 2005). Increases in IGF-1 results in the activation of inactive satellite cells (allows the satellite cells to carry on in the cell cycle) found in skeletal muscle as well. The more satellite cells available means more sources of DNA are available for donation to muscle cells to allow for hypertrophy (Johnson & Chung, 2007). Reinhardt (Chpt 124) also discusses the proliferation and differentiation of muscle satellite cells to cause skeletal hypertrophy.

Skeletal muscle contains androgen receptors allowing the testosterone and/or TBA to bind directly to the muscle to cause hypertrophy via protein synthesis and deposition (Johnson & Reinhardt, 2009). Other indirect effects of TBA/testosterone also exist; TBA can suppress the effects of the adrenal corticosteroids; corticosteroids reduce protein synthesis and cause degradation. Another hormone involved in protein metabolism in skeletal muscle is thyroxin (T_4); the function of T_4 is the metabolism in cells which results in degradation of those cells, how the androgens are involved is that they decrease the concentrations of T_4 therefore less protein degradation occurs (Hossner, 2005).

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5.1.2. Subcutaneous fat thickness

According to Bradley & Reinhardt (2009), steroidal implants typically do not have direct effects on adipose tissue. Oestrogen receptors that are present are only present in low concentrations and there is still no evidence of androgen receptors in adipose tissue, therefore, the changes seen in fat deposition may be due to changes in concentrations of other hormone profiles, GH and the impact they have on lipolysis. This could be the reason for the rams' treatment groups not responding to the implantation of steroids while the ewes did show that steroids cause a more significant effect on subcutaneous fat thickness due to the presence of low concentrations of oestrogen receptors. Reinhardt (Chpt 124) also states that exogenous steroids do not largely impact fat deposition rate in livestock. In the ewe results we see a significant decrease ($P<0.05$) in the subcutaneous fat thicknesses of Revalor G treated ewes and Revalor H treated ewes. This observation is not seen in the rams' treatment group receiving Revalor G and Revalor H implants.

The results seen are reversed when the subcutaneous fat thickness in the control group are measured against the Zilmax treated rams. The Zilmax treated rams had significantly lower subcutaneous fat thicknesses (1.36 ± 0.564mm) than the control group (1.67 ± 0.564mm) ($P<0.05$). While in the ewes no difference was seen in subcutaneous fat thickness between Zilmax treated ewes and control ewes ($P>0.05$). It was expected for the ewes to have significant differences in subcutaneous fat thickness when treated with Zilmax, mainly because of the higher initial subcutaneous fat thickness than the rams and therefore a stronger observation would be seen. It is possible that the duration of time in the feedlot can affect the effect of Zilmax on animals; the longer an animal spends in the feedlot the more mature and thus more fat deposition occurs. Female animals are also not as efficient as the males so while Zilmax causes lipolysis the effect of the Zilmax is not as potent as in the males.

The use of growth promoting agents, such as exogenous steroidal implants, on lambs is expected to have a significant impact on the subcutaneous fat thickness, as well as many other traits (such as carcass quality, hot carcass weight, dressing percentage, etc). Tables 4.1.1 and 4.1.2 show the impact that the steroidal implants had on the subcutaneous fat thickness in rams and ewes. The rams and ewes do not show the same trend, in the rams when Ralgro, Revalor G and Revalor H are compared to the control no difference in subcutaneous fat thickness ($P>0.05$) was observed. The results in the ewes, however, do differ. The ewes in the control group had the largest subcutaneous fat thicknesses (2.11 ±

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0.564mm) followed by Ralgro (1.86 ± 0.564mm) and then Revalor G and H (having the same subcutaneous fat thickness of 1.68 ± 0.564mm), P<0.05. When comparing the control to Ralgro there was no difference seen (P>0.05), but there were differences seen between the control and both Revalor G as well as the control and Revalor H (P<0.05). Ralgro was the only exogenous steroidal implant to not cause a significant decrease in subcutaneous fat thickness in ewes. A study was done by Vanderwert, *et al.* (1985) where they looked at the influence of zeranol implants on growth and carcass traits in bulls, what they found was that bulls that were implanted did not outgain control bulls, this was probably due to age at implantation, which shows that an earlier implant may be necessary to increase efficiency and rate of gain in intact animals. Vanderwert, *et al.* (1985) also found that the Zeranol decreased marbling in bulls.

The results from tables 4.1.3 and 4.1.4 show that the subcutaneous fat thickness in the rams seemed to be more sensitive to dietary Zilmax (P<0.05) (subcutaneous fat thickness of 1.36 ± 0.564mm in Zilmax treatment compared to 1.67 ± 0.564mm in control group), while in the ewes the final masses were more sensitive to Zilmax (P<0.05) than the subcutaneous fat thicknesses were (P>0.05) (Zilmax treated ewes final masses were 41.6 ± 1.56kg compared to 40.8 ± 1.58kg in the control). A study done by Allen (2015) found that Zilmax had no effect on subcutaneous fat thicknesses in Zilmax treated male lambs, this current study's results agree with those from Avendano-Reyes, *et al.* (2011) who found that supplementing Zilmax to the diets of finishing ewes did not cause a change (P>0.05) in subcutaneous fat thicknesses.

Blackman (1990) briefly discussed the improved FCE and carcass quality characteristics and reduced subcutaneous fat deposits due to the use of beta agonists (clenbuterol and cimaterol) in lambs. What was important to note was that the subcutaneous fat deposits were lower while the intramuscular fat deposit was similar to the control; the authors mention the influence of beta agonist being site specific. In another study summarized by Blackman (1990), beta agonists cause increased muscle hypertrophy in type II fibres, more so than in type I muscle fibres. It may be necessary to look deeper into the influence of beta agonists being site specific.

An important note to take into consideration is that the animals tested were all young (immature) animals that were still growing, so the difference seen in the final masses or subcutaneous fat thicknesses are not only due to the Zilmax or exogenous steroidal implants given but due to allometric growth that occurs naturally. Zilmax has a substantial effect on the allometric curve. During the progression of growth we know that fat deposition occurs later on in the growth phases but when Zilmax is supplemented to lambs it causes a shift in the allometric curve delaying the lipogenesis process and thus decreasing the amount of fat

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deposition occurring. This process also redirects energy for proteogenesis and thus to skeletal muscle growth, therefore away from lipogenesis (Batt, 1979). For this reason, Zilmax is known to be a repartitioning agent.

5.2. Gonadal data

The results of this study indicate that Dohne Merino rams and ewes treated with steroidal growth implants, alone or in combination with the supplementation of Zilmax in the feed, the growth and development of the gonads (testes and ovaries) are stunted. Analysis of the main effects and interactions between the steroidal growth implants and Zilmax indicates that the decrease ($P<0.05$) in the length, volume, weight and width of the testes is was mainly due to the effects of steroidal implants and not the β-agonists.

Foote (2003) made an important statement regarding the ability to estimate fertility; Since males are responsible for hundreds of thousands of pregnancies per year and due to the ease of measuring scrotal circumference as an indicator of semen production capacity, it follows that, there is more research being done on bulls and rams compared to cows and ewes. For this reason, fertility estimates are more accurate for males than females. It is also important to remember that, apart from the effects from exogenous growth promotants, there are several genetic as well as environmental factors that contribute to the gonadal development (Johnson & Chung, 2007)

The measurement of testis size is highly repeatable ($r>0.9$), sperm output is also highly correlated with testes circumference ($r=0.81$) and the testes size is correlated with total sperm production (Foote, 2003). These genetic correlations allow us to make the conclusion that if the implantation of steroids affects the physical attributes of the male, then we can accurately estimate that fertility could be negatively affected.

5.2.1. Effect of exogenous steroidal treatments on gonadal development

Rams

Implantation of steroidal implants containing TBA and E_2 is known to result in improved feed conversion efficiency, increased ADG and growth as well as carcass leanness (Kongsuwan, *et al.* 2012). When Ralgro is used it results in increased gains and an improved and more desirable meat flavour (Vanderwert, *et al.* 1985), however, compared to Eckerman,

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et al. (2013) who did not see any differences in lamb growth, ADG, FCE or carcass characteristics due to Ralgro use. The result of using these exogenous growth promotants on the development of the gonads is not very well documented. Results from this study on the effect of the exogenous steroidal treatment (Ralgro and Revalor G) on testis weight (g), testis volume (mm^3), testis length (mm) and testis width (mm) in rams show that when compared to the control, no significant effect ($P>0.05$) can be seen when Ralgro is implanted, but the physical development did show a trend for less growth and development than the control. This is contrast to what Richards, *et al.* (1987) found; where using Ralgro (Zeranol) in entire cattle caused a significant decrease in testis size, similar results were found by Vanderwert, *et al.* (1985) who also found a decrease in both scrotal circumference and testes weight when implanted with Zeranol. Studies done by Richards, *et al.* (1986) found that when entire male calves were implanted with Ralgro it resulted in decreased testicular weights ($P<0.05$) as well as decreased scrotal circumference ($P<0.05$). It is important to note that these results show that there are differences seen between bovines and ovines. The previously reported bovine studies of Vanderwert, *et al.* (1985) and Richards, *et al.* (1986) show a more significant effect than the current study on rams. Another possibility for the differences observed could be because the rams and ewes were implanted at approximately 245 days old in this trial. Blackman, (1990) summarized the findings of Gary *et al*. (1986) of hormonal growth promotants in lambs and cattle, studies done on cattle in the USA found that implanted (Zeranol implanted) bulls above the age of 12 months had higher ADG and higher carcass subcutaneous fat thickness compared to non-implanted bulls under 12 months of age. In contrast Blackman (1990) also summarized a trial done by Richards, *et al.* (1987) where the age and interval at which the cattle received the implants was essential; in this study the cattle received four Zeranol implants at 84 day intervals, results showed an insignificant increase in total weight gain and only slight changes in carcass composition, but what was reported by the authors was a distinct decrease in testicular development when Ralgro was implanted. The physiological maturity may have affected the response observed due to Ralgro implantation in this study. The general trend for steroidal treatments in males would be a negative feedback effect beyond or above a certain blood level of steroid.

Silcox, et al. (1986) did a similar experiment where it was found that the age of implantation with Zeranol had a significant effect on the development of the gonads, in terms of testes size (as well as other carcass traits – which is to be expected). The group that was implanted with Zeranol at an earlier age (before or at 200 days of age) showed depressed testis growth ($P<0.05$), while the group that was implanted with Zeranol at 250 days showed no decrease in testis growth. So, even though these experiments were conducted in cattle we would expect the same/similar results in sheep. A possible explanation for no response being

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seen in this trial is because the lambs entered the feedlot at approximately 245 days of age, which is when they received their implants, and, according to Silcox, *et al.* (1986) cattle receiving the implant after 200 days of age were not affected by the Zeranol. It is possible that there are differences within species as well as breeds with regards to the effects seen due to age/days at implantation of Ralgro. A possible limitation of this study was that all the lambs were implanted with exogenous growth promotants on the same day, it would possibly be of added value to extend the study further and implant the exogenous growth promotants at different ages, as well as do multiple implantations at different ages, to then determine the effect of age of implant as well as the effect of the implant on the gonads.

The decrease in testicular size ($P>0.05$) was to be expected in the rams treated with Ralgro. The active ingredient in Ralgro is Zeranol; a synthetic product produced from zearalenone (a toxin produced from fungus). Zeranol has oestrogenic properties because of its source and therefore binds to oestrogen receptors. Sinnet-Smith, *et al.* (1983) mentioned that oestrogenic exogenous implants may act via alteration of the levels of normal endogenous hormones by possibly creating a negative feedback loop to the hypothalamus.

When Revalor G and the control are compared; Revalor G showed a decrease ($P<0.05$) in all four variables tested (testis weight, testis volume, testis length and testis width) in rams. Table 4.2.1 shows the LS means of the effect of the exogenous steroidal treatments (Revalor G and Ralgro) on the variables. The rams receiving Revalor G implants had significantly lower testicular development ($P<0.05$) with values of 63.3 ± 33.20g, 52.3 ± 28.00 mm^3, 121.7 ± 16.04mm and 41.9 ± 8.19mm for testis weight, volume, length and width, respectively, compared to the steroidal control (SC) of 151.5 ± 12.86g, 140.7 ± 16.04mm^3, 153.4 ± 9.88mm and 59.7 ± 2.07mm. These results were expected because Revalor G is a more aggressive implant (in terms of its concentrations of trenbolone acetate and oestradiol). Blackman (1990) reports on the use of a combination of trenbolone and oestradiol on pasture and feedlot groups compared to Zeranol; in all the trials the trenbolone and oestradiol groups resulted in significant increases in liveweight gain over the Zeranol groups. Blackman (1990) also reported on trials done in lambs that revealed a 5% increase in total weight gain, but most importantly, a significant reduction in pubertal development, the authors then go on to mention the possibility of using combinations of trenbolone and oestradiol as an alternative method to castration through the suppression of gonadotrophin secretion. Suppression of GnRH would eventually result in less testosterone production. Sexual activity of both rams and ewes depends on the secretion on GnRH (Bearden, *et al.* 2004).

The size of the testes in rams is important for the selection of future breeding animals, Preston, *et al.* (2012) mentions two interesting points, i) the positive association between the

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testicular size and titres of testosterone as well as the association between the testicular size and the amount of spermatogenic tissue available for sperm production and ii) a 'testosterone-mediated link' that exists between testosterone and mate-seeking behaviour in rams. Overall, the amount of testosterone could be associated with the size of the testis and amount of sperm producing tissue and thus directly impact breeding in rams. A study done by Vanderwert, *et al.* (1985) also found that bulls implanted with Ralgro had lighter weight testicles and small scrotal circumferences when compared to non-implanted bulls.

Leydig cells are the cells responsible for the production of testosterone in the male and are found on the seminiferous tubules, they are the source of endogenous testosterone in the male and function via means of a negative feedback mechanism; high levels of circulating testosterone inhibit the release of GnRH, FSH and LH. These hormones stimulate the testes to produce more testosterone when circulating levels are low and inhibit negative feedback when levels are high. When exogenous testosterone is added to the body the body will down regulate the production of natural endogenous testosterone – further research is needed to confirm this hypothesis. Testosterone is necessary for maintenance of the male duct system, healthy functioning of accessory glands, spermatogenesis and many other critical functions linked to male reproduction (Bearden, *et al.* 2004). Therefore, a down-regulation in circulating testosterone will have potentially negative effects on male reproduction physiology and thus fertility.

Ewes

The major finding from comparison of the effect of different steroidal treatments on ovary length, ovary width, number of follicles and number of corpora lutea was the significant effect of exogenous steroidal treatment on the number of follicles; when Ralgro, Revalor G and Revalor H are compared to the control (SC) no significant effect was seen on ovary length, ovary width or number of corpora lutea ($P>0.05$). There was, however, a significant difference seen on the number of follicles when Ralgro, Revalor G or Revalor H implants were used ($P<0.05$). It was expected that Revalor H would have the greatest impact on the number of follicles since it had the highest doage of hormones of the three implants, Revalor H contains 14mg of oestradiol and 140mg of trenbolone acetate compared to 8mg oestradiol and 40mg trenbolone acetate in Revalor G. Conversely, the results indicated that Revalor G had the greatest effect with 6.2 ± 3.86 follicles compared to 12.3 ± 4.12, 8.6 ± 2.81 and 7.0 ± 2.87 for SC, Ralgro and Revalor H respectively. Only slight numerical differences are seen between Revalor G and Revalor H on number of follicles when comparing to each other ($P>0.05$). Also, very slight decreases were seen in ovary length and width as well as number of corpora lutea ($P>0.05$). The number of follicles seems to be very sensitive to the use of exogenous steroidal

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treatment in ewes as shown in Table 4.3.1. Graphs 4.3.1c clearly depicts the effect of the exogenous steroidal treatment on the number of follicles on the ovary, the strong downwards trend of the steroidal implants. The reason why a decrease in the number of follicles is important in ewes is because of the basic reproductive biology in the ewe; immature follicles are the precursors to an ovum, and once the ovum is fertilized becomes the next offspring. Thus, if there is a significant decrease in follicle (Graafian follicle) number it results in a decrease in potential ova being shed and thus decreases reproductive efficiency and the chance for fertilization.

It was expected for a similar trend to be seen in the number of CL, due to the CL being part of the follicular cycle, but the results don't show this, possibly because the number of CL seen is very small and difficult to quantify. No clear pattern can be seen in the results of the CL between the exogenous steroidal implants or between Zilmax treatments, indicating that steroids and Zilmax do not affect number of CL ($P>0.05$).

Reproductive biology shows that FSH promotes follicle growth and oestrogen production in the follicle in the female. At the same time inhibin is produced in the follicle, the role of inhibin is to selectively inhibit the release of FSH, which then ensures that the dominant follicle is further developed and smaller non dominant follicles are regressed (Bearden, *et al.* 2004), but with the continuous stimulation of follicles by FSH due to increase circulating estradiol it is not known what the effect will be.

A study done by Moran, *et al.* (1990) revealed that when implants such as TBA, zeranol and oestradiol are used long term it resulted in a decrease in the reproductive efficiency of heifers. TBA, oestradiol and Zeranol were all found to inhibit secretion of LH by the anterior pituitary. LH is an important hormone necessary for ovulation, corpus luteum formation and maintenance in the female and testosterone release in the male, necessary for spermatogenesis (Bearden, 2004). The authors mention that a great deal of research is still needed to be done on the reproduction of heifers after long term implant use. Implanting heifers with TBA and Zeranol did in fact delay the onset of puberty and thus ovulation in beef heifers since the time of ovulation coincided with the time at which the implants active ingredients (TBA and Zeranol) expired. It was also found that TBA was more effective in delaying first ovulation than Zeranol; this may be due to the higher dose of active ingredient in the TBA implant (Moran, *et al.* 1990) or even due to chemical properties of Zeranol being oestrogenic. (Moran, *et al.* 1990) also found that the vulva length was affected by treatments and attributed this increase to the oestrogenic compounds (Zeranol and oestradiol) and decrease to the antiestrogenic compounds (TBA), meaning that oestradiol is necessary for the normal growth of these reproductive tissues, meaning that at the end of the trial very few

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of the TBA x oestradiol heifers had reached puberty before slaughter. What is important to note from this trial is that these heifers were reimplanted and did not follow the 'normal' feedlot cycle before slaughter. Exposure of heifers to oestradiol implants causes a reduced effect of delayed puberty and thus decreased fertility (Moran, *et al.* 1990).

The implantation of 12 mg Zeranol to ewe lambs was tested by Cooper (1981a) to determine the value of Zeranol on animals retained for breeding purposes. After trials done for two years it was concluded that it is possible that the animals implanted during August of 1978 and 1979 had delayed onset of puberty and it was concluded that, 'the use of Zeranol in ewe lambs retained for breeding purposes is contra-indicated'. The study also states a declining level of residue (Zeranol) and that normal puberty is only attained once these residues disappear. The author does refer to similar trials done in mature ewes where infusing 4.8mg of Zeranol per day for five days had no effect on oestrous and that these trials suggested that Zeranol would not have adverse effects on oestrogenic activity. Cooper (1981b) then continued his trial to test the effect of 12mg of Zeranol on ovary weight, ovulation rate and puberty and gonadotrophin levels. Ovary weights were only affected (decreased) by double implantation ($P<0.001$). Implantation caused a significant decrease in ovulation rate and thus the number of animals failing to attain puberty, FSH levels were lowered ($P<0.05$) and LH levels also decreased following double implantation ($P<0.05$) while single implantation caused variable results to be seen in LH concentrations. The significance of the depressed gonadotrophin levels supports the idea that Zeranol causes decreased gonadotrophin synthesis necessary for critical gonadal development. In another follow up trial Cooper (1985) tested the effect of Zeranol implantation on plasma LH levels, concluding that residual levels of Zeranol for up to 70 days post implantation can cause decreased plasma LH levels. The present study shows that the implantation of exogenous steroidal growth promotants interrupts the endogenous gonadotrophin hormone levels in the ewe, negatively influencing follicle development. So even though there was no difference seen in the physical development of the ovaries, the ovaries proved to be less active gonadotrophically in terms of the production of follicles.

5.2.2. Effect of Zilmax supplementation in the feed on gonadal development

Rams

Table 4.2.2 shows the results of the effects of the supplementation of Zilmax to the diets of rams; Zilmax mean control results of testis weight, volume, length and width to be

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143.8 ± 24.33g, 133.9 ± 25.82mm^3, 150.8 ± 11.48mm and 57.9 ± 4.29mm, respectively. It was expected for the supplementation of Zilmax to cause a significant stunt in gonadal growth and development, however, the results of the Zilmax treatment shows a trend for the means to decrease, with the testis weight, volume, length and width to be 123.3 ± 37.90, 116.7 ± 39.10mm^3, 139.0 ± 13.53mm and 51.9 ± 9.76mm, respectively. From this study, using Zilmax did not negatively impact (P>0.05) gonadal development (more specifically decrease the weight, volume or size of the testes) in rams versus a control of no Zilmax. Different results were seen when Zilmax is used in combination with a steroid, especially Revalor G. The non-significant decrease in gonadal development as expected may be due to several factors which will need to be further investigated.

Blackman (1990) briefly discussed the improved FCE and carcass quality characteristics and reduced subcutaneous fat deposits due to the use of beta agonists (clenbuterol and cimaterol) in lambs. What was important to note was that the subcutaneous fat deposits were lower while the intramuscular fat deposit was similar to the control; the authors mention the influence of beta agonist being site specific. In another study summarized by Blackman (1990), beta agonists cause increased muscle hypertrophy in type II fibres, more so than in type I muscle fibres. It may be necessary to look deeper into the influence of beta agonists being site specific. Webb, *et al.* (2018) studied the effect of Zilpaterol hydrochloride on growth, carcass characteristics and meat quality on commercial feedlot lambs. When Zilpaterol hydrochloride and Zeranol implants were treated together it resulted in an additive effect to yield the highest ADG results, when compared to Zilpaterol hydrochloride and Zeranol alone. Zilpaterol hydrochloride had no effect on carcass fat thickness.

Ewes

Table 4.3.2 shows the results of the Zilmax supplementation on ewes, the results for ovary length, ovary width, number of follicles and number of corpora lutea being 14.0 ± 3.54mm, 10.5 ± 2.16mm, 14.3 ± 4.07 and 0.3 ± 0.45 for the Zilmax control, respectively, while the Zilmax supplemented ewes results were: 11.8 ± 2.01mm, 8.3 ± 1.46mm, 10.3 ± 3.26 and 0.7 ± 0.65 for ovary length, ovary width, number of follicles and number of corpora lutea. There is a significant difference (P<0.05) between the results received for ovary length (14 ± 3.54 vs 11.8 ± 2.01), ovary width (10.5 ± 2.16 vs 8.3 ±1.46) and number of follicles (14.3 ± 4.07 vs 10.3 ± 3.26) for the control compared to the ewes treated with Zilmax respectively.

The results seen were to be expected originating from the knowledge that Zilmax causes significant decreases in fertility as well as gonadal development and size, although, it was expected that the number of corpora lutea would be significantly decreased, although this was not observed with the mean results being 0.3 ± 0.45 compared to 0.7 ± 0.65 for the control

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compared to the Zilmax treated ewes respectively. These results were unexpected but perhaps are the result of the inaccurate method of sample collection specifically for number of *corpora lutea,* also, it is important to keep in mind that c*orpora lutea* are further-developed follicles and are possibly past the point of being affected by Zilpaterol hydrochloride.

5.2.3. Interaction of exogenous steroidal treatment with Zilmax supplementation in the feed on gonadal development

Rams

The interaction between exogenous steroidal and Zilmax treatments in rams are presented in Table 4.2.3, it was found that when Revalor G is used in combination with Zilmax it caused a strong decrease in all variables; with testis weight, testis volume, testis length and testis width being 63.3 ± 33.20g, 52.3 ± 27.99mm^3, 121.7 ± 16.04mm and 41.9 ± 8.19mm respectively, compared to the Steroidal Control x Zilmax Control interaction being 151.5 ± 12.86g, 140.7 ± 16.04mm^3, 153.4 ± 9.88mm and 59.7 ± 2.07mm, statistical significance was not tested, only a strong interaction was seen.

The Ralgro x Zilmax treatment results were not expected with the testis weights and volumes being slightly larger (P>0.05) than the Ralgro x Zilmax Control treatment (140.5 ± 14.13g and 135.1 ± 13.18mm^3 compared to 134.8 ± 31.40g and 125.8 ± 32.88mm^3 for Ralgro x Zilmax Control). When comparing Ralgro x Zilmax Control to the true control, a numerical decrease (P>0.05) is seen in the Ralgro x Zilmax Control for testicular development (testis weights and volumes being 134.8 ± 31.40g and 125.8 ± 32.88mm^3 compared to 151.5 ± 12.86g, 140.7 ± 16.04mm^3). The reason a decrease is seen in the Ralgro x Zilmax Control treatment is the same reason why the rams treated with Ralgro saw a decrease in testis weight and size; Zeranol has oestrogenic properties causing the testes to be less developed. Results from the interaction of Ralgro with Zilmax were not expected, we saw that the use of Ralgro in rams depressed the gonadal development in rams in all four variables while the use of Ralgro x Zilmax caused a slight increase in testis weight and volume only. It is possible that the interaction of the Zilmax possibly overshadows/cancels out the use of the Ralgro, or that the Ralgro and Zilmax cancel each other out. So where we expected the Ralgro x Zilmax combination to cause a further decrease in development (compared to Ralgro x Zilmax Control) the Zilmax overpowered the negative effect of the Ralgro and decreased the reduction in the size of the testes and volume, but did not get to the results seen in the true control treatment (151.5 ± 12.86g and 140.7 ± 16.04mm^3 for testis weight and testis volume,

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respectively). A possible reason for the increase in Ralgro x Zilmax results compared to Ralgro x Zilmax Control is that the Zilmax results in improved body weights, ADG and feed conversion efficiency and resulted in higher overall body gains and because we know that scrotal circumference has a high correlation to body weight – the increase in testes weight and volume could be due to this.

Table 4.2.3 shows the results for Revalor G x Zilmax: there is a dramatic decrease in testicular development especially for testis weight and volume compared to Steroidal Control x Zilmax Control (63.3 ± 33.20g and 52.3 ± 27.99mm^3 for Revalor G x Zilmax and 151.5 ± 12.86g and 140.7 ± 16.04mm^3 for SC x CZ respectively) and only a small decrease in testis length and width (121.7 ± 16.04mm and 41.9 ± 8.19mm compared to 153.4 ± 9.88mm and 59.7 ± 2.07mm for Steroidal Control x Zilmax Control) ($P>0.05$). What this means is that there may not be a significant change in size of the testes but the amount of spermatogenic tissue is affected – why this occurs warrants further research. We know that testosterone is the male sex hormone and is responsible for many physical as well as reproductive changes, some of which include; function of the accessory gland as well as function of the *tunica dartos* muscle and spermatogenesis (Bearden, H.J., 2004) and the Leydig cells are responsible for the production and secretion of testosterone (which has a negative feedback mechanism on the hypothalamus), we can then question that the testosterone from the implant will down-regulate the feedback loop resulting in less testosterone being produced. We assume that this will then have a negative effect on spermatogenesis, regulation of temperature, etc. It is then found that the Revalor G has a potent effect on the development of the testis or more specifically the development of spermatogenic tissue or sperm producing capacity than Ralgro. Revalor G contains 8mg estradiol and 40mg trenbolone acetate while Ralgro contains 12mg Zeranol. It is possible that the added TBA could be the reason for the potent effect on the testicular development or the combination of the TBA and E_2.

Ewes

Table 4.3.3 shows that the major finding from the exogenous steroidal implantation x Zilmax supplementation was the reduction in the number of follicles, the Steroidal Control x Zilmax Control treatment resulted in 14.3 ± 4.07 follicles compared to the Ralgro x Zilmax, Revalor G x Zilmax and Revalor H x Zilmax treatments (7.9 ± 2.58, 3.8 ± 2.62 and 4.8 ± 1.46 respectively). While in the Zilmax treatments resulted in a significant decrease in the ovary length, ovary width and number of follicles ($P<0.05$). When evaluating the exogenous steroidal x Zilmax treatments a possible undesirable additive effect is seen between the steroid and Zilmax treatment on the ovary length, ovary width and number of follicles because the results of the Steroidal x Zilmax treatments are all lower than steroid treatment or Zilmax treatments

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alone. From the results it is seen that Zilmax negatively impacts the growth and development of ovaries while the steroids only affected the follicular activity.

Graphs 4.3.3 indicate that all steroidal and Zilmax treatments decreased the growth of ovaries compared to the negative control animal that did not receive a steroidal treatment or supplementation with Zilmax. Although some of the results were not statistically significant, it does stress the point that steroidal implants and Zilmax should not be used in breeding animals.

It is well known that females are born with a limited, or rather pre-determined, number of follicles. If the addition of growth promotants given to her affect the number of follicles, then you are decreasing the reproductive ability of this female and decreasing the chances of potential offspring.

5.3. Interaction effects of growth results and gonadal results

This study shows the role that exogenous steroidal implants and Zilmax supplementation have on the growth and development of the gonads, rams seem to be severely affected by the implantation of Revalor G; causing significant retardation in the testes weight, volume, length and width ($P<0.05$), meanwhile implantation of Revalor G does not seem to significantly improve on the final masses nor does it influence subcutaneous fat thickness ($P>0.05$). Zilmax supplementation did not show typical results that were expected, with a significant decrease in subcutaneous fat thickness but no retardation of testes growth and development. Meanwhile, the Revalor G x Zilmax treatment showed a decrease in all testicular growth variables, although not significant.

The results of the ewe implanted with exogenous steroids show that subcutaneous fat thickness is significantly decreased (by Revalor G and Revalor H) ($P<0.05$) and only number of follicles is decreased while no other physical changes are seen in the ovaries of these ewes. When Zilmax is supplemented into the diet the ovaries' length, width and number of follicles decreases ($P<0.05$), the interaction of the steroidal implants and Zilmax shows an even further retardation of the ovary length, width and number of follicles, although not significant, for Revalor G and Revalor H.

From this study we have seen a positive correlation between growth and gonadal development, once the animal reaches 95% of its growth, we then start to see negative correlations between growth and gonadal development. The reason for this is because we have pushed the animal passed its 'normal' growth to the point that the body simply cannot

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keep up and this is when we see a drop in fertility and reproductive function, this is observed as soon as you push past its genetic capabilities.

It is important to note that testicular function is affected by long term nutritional changes as well as changes in photoperiod. What was not recorded was the plane of nutrition of the lambs prior to the study and then when moving to the research facilities they were placed on the feedlot high protein and energy diet – this may act as a 'long-term effect of nutrition' as described by Blache, et al. (2000) where long term changes in nutrition cause changes in the GnRH pulse frequency and therefore LH and FSH secretion from the anterior pituitary. When rams are fed a high protein diet the GnRH pulse frequency is increased, stimulating the increase of more LH and FSH, LH stimulates the Leydig cells to produce testosterone; from basic biology we know that testosterone is a secondary sex hormone necessary for the growth and development of the testes. Blache, et al. (2000) described that the effect of the high protein diet fades after three weeks but what is interesting is that even after the fading the testicular mass and sperm production were seen to increase long after the high protein diet was stopped, Blache, *et al.* (2000) summarized that part of the 'long-term effect of nutrition' may be independent of changes in GnRH pulse frequencies, other causes for changes in testicular growth and development may be correlated to changes in body weight and metabolic endocrine systems. There are receptors for both IGF-1 and GH in the testes; this allows the somatotrophic axis to bring about the effects of nutrition on the testis, according to Monget & Martin (1997) a similar process also occurs in the ovary of ewes.

Steroids are involved in the control of skeletal growth. They can have both a positive and negative impact directly on skeletal growth; positive by increasing the rate of bone growth and negative by causing cessation of skeletal growth by closing of the epiphyseal growth plates in long bones (Bradley & Reinhardt, 2009). Producers need to be aware of when to use steroidal implants and for how long. Another negative point against the use of steroidal implants is the possible decrease in the tenderness of meat, resulting in a decrease in customer satisfaction (Johnson & Reinhardt, 2009).

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Chapter 6: Conclusion

Growth data shows that the use of exogenous steroidal implants in ewes does not show a clear trend for the final mass to improve significantly; however, both Revalor G and Revalor H show significant decreases in subcutaneous fat thicknesses- this observation is not seen in the rams. Zilmax treatments on rams show no improvement in final mass ($P>0.05$) but significant decreases in carcass subcutaneous fat thicknesses. The opposite observation was observed in the ewes, with the final mass showing a positive improvement to the addition of Zilmax ($P<0.05$) but no change in carcass subcutaneous fat thicknesses ($P>0.05$).

Results seen from implantation of steroids to the rams only shows Revalor G to have caused a significant decrease to the weight, volume, length and width of the testes ($P<0.05$). Ralgro only resulted in small numerical ($P>0.05$) changes to the physical size of the testes. Interestingly Zilmax supplementation to the rams did not cause significant changes to the testes.

Results observed in the ewes can confirm that Revalor G, Revalor H and Ralgro all caused a significant decrease in the number of follicles in ewes, but the physical changes in the size (length or width) were not seen due to the addition of the steroidal implants, even though earlier trials (mentioned above) found changes in ovary physical development due to Zeranol implantation. The supplementation of Zilmax to the diets of the ewes also caused significant changes in the length, width and number of follicles of ewes, however, Zilmax did not cause changes in the number of *corpora lutea*. When Zilmax and Revalor G are used together it results in a substantial decrease in the number of follicles in ewes and in rams a reduction in testicular size, especially the weight and volume ($P<0.05$).

Based on the results from this study it is recommended against the use of Zilmax**,** Zeranol (Ralgro) or Revalor to breeding animals or animals intended to be kept at stud for future breeding purposes, but for animals passing through a feedlot before slaughter it is not be a problem since development of the gonads is not important. More research is needed to confirm the exact effect of Revalor H and Zilmax in rams. Zilmax should only be used in animals entering the feedlot and intended for slaughter.

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Chapter 7: Critical evaluation

Firstly, it is agreed that the study must be replicated to determine repeatability, although the present study does provide a good starting point for further investigations. Further research is needed to determine the effects of these combinations of growth promotants on spermatogenesis and sperm counts, morphogenesis and/or motility in the male ruminant as well as on folliculogenesis and patterns of oestrous in the female. The purpose of this study was to compare commercial growth promotants as used in the industry and only to determine the effect of exogenous growth promotants on gonadal growth and development and not on factors influencing fertility. It is recommended to consider all the factors contributing to fertility in one study to understand the complete picture, although that goes beyond the scope of a normal Master's degree study. More research into the level of inclusion/implantation of the different treatments as well as testing different dosages in treatment would be advantageous, for example: The effect of Zilmax was only tested at one inclusion level (80g/ton) and is not registered for use in sheep diets in South Africa. It would therefore be interesting to study the effects of Zilmax at different inclusion levels in the feed. The inclusion of Zilmax at higher levels may result in a higher growth rate or more efficient growth, but more studies are required in South African conditions. It has been documented previously that overdosing of Zilmax causes increased heart rate and potentially death.

It is unfortunate that the Zilmax control and Revalor G treatment in the rams was not tested. The interaction treatment of Revalor G x Ralgro was also not tested in this study, due to issues in sampling. It would be of value to see the results since Sillcox, *et al.* (1986) found that there is no value in implanting Zeranol and testosterone together.

Because sheep are seasonal breeders, measurement of testicular size and development must be made during the breeding season (or at a fixed time). Specifically, in ovaries, the size of the ovaries can change according to the stage in the oestrous cycle; active ovaries are larger than inactive ovaries (Bearden, 2004). Also, the season should be taken into consideration as high summer temperatures may have caused depressed intakes. The counting of the *corpora lutea* was quite difficult on the ovaries, especially once they have lost their blood supply and ovaries lose their healthy 'pink' colour, so it was difficult to count the blister-like CL. Measuring the weight and volume of the ovaries could also be an important variable to take into consideration.

Other treatments in the study could have been different ages of implantation – to see if the age of implantation has a significant effect.

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As already mentioned, the plane of nutrition of the lambs was not noted at entry into the feedlot, it was assumed that the lambs were all of a healthy weight for their age. It is, however, known that if the lambs were either underweight or underdeveloped that during the feedlot phase ‘compensatory growth’ occurred which could have skewed then results for the development of the gonads. Other important nutritive components of the diet include energy, protein (macro nutrients) and vitamins and minerals (micronutrients), when looking at the whole picture we ideally want all the macro and micronutrients to be balanced. A deficiency or excess of any of these components can decrease reproductive ability, for example: excess protein fed to dairy cows results in an increase in open days, weak expression of estrous or even the birth or premature calves/lambs. An excess energy diet fed to rams or bulls results in fat deposition around the reproductive organs causing detrimental effects on fertility (Bearden, *et al.* 2004).

Ralgro is a non-steroidal growth implant because it contains Zeranol, unlike Revalor G and H which contain TBA and oestradiol. It is a non-steroidal oestrogen that is also referred to as a phytoestrogen due to its plant origin. Ralgro was found to be a less aggressive steroidal implant, especially when used in combination with Zilmax as the Zilmax overshadows the effects of the steroids. The effects of combining Zilmax and monensin together in the diet must also be taken into consideration. This is done in commercial feedlot situations with the monensin added as an anticoccidial and to improve growth in feedlot lambs. It is believed that the monensin gives a synergistic advantage, especially upon entry into the feedlot during the stressful first two weeks of adaptation.

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