THE EFFECT OF DIFFERENT DIETARY IONOPHORES AND INCLUSION LEVELS IN THE FINISHING DIETS OF LAMBS

by

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Melville Maurice Price

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DEDICATED TO MY PARENTS

To my parents, Lawrence and Helena Price, for all the love, guidance and opportunities you gave me in life. Thank you for the interest, encouragement and support throughout my life. I love you.

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ACRONYMS AND ABBREVIATIONS

ABPE	Acute bovine pulmonary emphysema
ADF	Acid detergent fibre
ADG	Average daily gain
AOAC	Association of Official Analytical Chemists
ATP	Adenosine triphosphate
Ba^{++}	Beryllium ions
BC	Buttock circumference
BW	Body weight
Ct	Control
C1	Dried weight of bag with fibre after extraction process
Cs^{+}	Cesium ions
CV	Coefficient of variance
D(-)	Mesotartaric acid
DM	Dry matter
DMI	Dry matter intake
DNA	Deoxyribonucleic acid
EC	European commission
EL	External length
EU	European Union
FCE	Feed conversion efficiency
FCR	Feed conversion ratio
GE	Gross energy
GNR	Global nutritional research
h	hour
HPC	High protein concentrate
HSD	Honest significant difference
Kpa	Kilopascal
L	Lasalocid
L(+)	Dextrotartaric acid
l/h	litre per hour
M	Monensin
ME	Metabolizable energy
MH	Monensin at a high inclusion level

Mg	Magnesium
MI	Methylindole
MIC	Minimum inhibitory concentration
min	minute
ML	Monensin at a low inclusion level
MM	Monensin at an intermediate inclusion level
n	number
ND	Neutral detergent
NDF	Neutral detergent fibre
NE	Net energy
NRC	National Research Council
NSC	Non-structural carbohydrate
OM	Organic matter
PMF	Proton motive force
Rb^+	Rubidium ions
Reg. No.	Registration number
Sl	Salinomycin
SAMM	South African Mutton Merino
SC	Shoulder circumference
SD	Standard deviation
SH	Salinomycin at a high inclusion level
SL	Salinomycin at a low inclusion level
SM	Salinomycin at an intermediate inclusion level
TMR	Total mixed ration
u	Daltons
VFA	Volatile fatty acid
W1	Bag tare weight
W2	Sample weight
W3	Dried weight of bag with fibre after extraction process
β	Beta

CHAPTER 1

GENERAL INTRODUCTION

The livestock industry in South Africa contributes up to 49% of the total agricultural output. South Africa generally produces 85% of its own red meat requirements, while the remaining 15% is imported mainly from Namibia, Botswana, Swaziland, Australia, New Zealand and Europe (http://www.info.gov.za/aboutsa/agriculture.htm#livestock). Estimations suggest the country's population to be approximately 50.5 million in 2011, and it was reported that the South African population increased by an astonishing 12.7% during the last 10 years, from 2001 to 2011 (http://www.southafrica.info/about/people/population.htmm). As most of the world's human population resides in the developing countries (South Africa included), which experience rapid population growth rates, the global demand for meat (animal protein) is projected to increase by more than 60% of the present consumption within the next decade (Page, 2003). This rapid growth in human population of the developing countries with, limited available natural resources, presents a major challenge for agriculture in the future and then for the sustainable supply of animal protein.

Of all mammal species, ruminants have the most differentiated, specialized and complex stomachs, which are influenced by many dietary, environmental and host factors. This complexity regarding the ruminant stomach renders it as one of the most studied aspects by a number of researchers. Although there are many species, as well as great numbers of animals possessing this complicated but very effective digestive system, only three ruminant species, namely cattle, sheep and goats have been domesticated and are commercially used for farming (Hoffman, 1973). The exact knowledge regarding the anatomy and physiology of the digestive system common to these groups of animals is an undisputed and essential requirement for animal scientists in developing new technologies - to increase their production efficiency, produce leaner animals at a lower input cost and hence increase their utilization in human consumption (Hoffman, 1973; Page, 2003; Kart & Bilgili, 2008).

The relationship between the ruminant animal and its resident ruminal population is clearly symbiotic and allows ruminants to utilize fibrous plant material *via* microbial fermentation. However, ruminal fermentation is inherently inefficient, as up to 12% of the dietary carbon

and energy can be converted and lost to the environment as methane and heat energy (Callaway *et al*., 2003). As a result, the conversion of ingested feed into products such as meat, milk and wool by the ruminant animal is not very efficient. Several strategies to improve ruminant feed efficiency, such as heat treatment (to alter protein structure) or the coating of certain nutrients with inert ingredients (e.g. oils or fats) - making them unavailable to microbial fermentation in the rumen and allow them to by-pass rumen fermentation have been developed during the past few decades (Callaway *et al*., 2003). Growth-stimulating agents are also being used on a large scale to improve the efficiency of meat production and to assist in producing leaner meat. There are generally two major types of growth-promoting agents effective in ruminant animals, namely: (i) hormone-like substances which increase the efficiency of utilization of absorbed nutrients, and (ii) antibiotic-like substances, which act on the ruminal microflora, thereby modifying the quantity and quality of the rumen fermentation products (Bondi, 1987).

Finishing lambs in feedlot systems prior to slaughter is a common practice on commercial farms and in feedlots of South Africa. Management practices to improve the growth performance of ruminants include manipulating feed to ensure that the digestion rate is not too rapid - which could result in digestive problems. Nor too slow - which could result in poor feed efficiency rates (Hatfield *et al*., 1997). Therefore, the main objective for all feedlot production systems is to ensure that they remain an economic viable enterprise, and to maximize performance efficiency while minimizing production costs, which result in a possible increased profit margin. Feed costs and production losses associated directly with nutrition are the major factors adding to the production cost inputs and inefficiency of the feedlot enterprise. This highlights the importance of diet formulation, in terms of least cost models - by maintaining optimum weight gain and animal health during the feeding period, without causing metabolic disorders that could decrease feed intake, along with animal production and subsequently financial profits.

Growth promoting agents have revolutionized cattle feeding in the seventies and still play an integral role today in the feedlot industry worldwide. Carboxylic polyether ionophore antibiotics, produced by various strains of *Streptomyces spp.*, are examples of compounds of these rumen metabolic modifiers and include products such as monensin, lasalocid and salinomycin. These metabolic modifiers have been mainly developed to improve the

efficiency and profitability of meat production and subsequently to improve carcass composition (Dikeman, 2007).

Monensin has been extensively characterized and used, since its approval by the FDA in 1975 in ruminant nutrition. Traditionally it has been one of the two most widely used ionophores, the other being lasalocid. However, new products on the market show potential in providing even better results than these stalwarts. One example of these newer products is salinomycin, with a similar mode of action to that of monensin. However, it exhibits properties that may out-perform monensin in terms of efficiency, and have a lesser impact on feed intake of animals than the former (Callaway *et al*., 2003).

With the increasing scarcity of food and feed sources, as well as the effect of global warming, more emphasis should be put on the positive contributions of dietary ionophores in the effectiveness of energy utilization. However, more focussed research should be conducted to determine the real improvement created by ionophores on the efficiency of energy utilization, methane production and mortalities (Bergen & Bates, 1984) - especially in sheep diets, as most of the research in the past has focused on feedlot cattle.

In the available literature, no evidence could be found of the three different ionophores evaluated simultaneously. Some of these ionophores at deviated levels from the mean registered inclusion levels within finishing diets of feedlot lambs could also not be found. This shortfall in research suggests that intensive finishing of lambs in a feedlot is a relatively new (past 20 years) practise which is more common in Southern Africa than in European countries and it still warrants much research. The aim of this study was to determine the effects of different ionophore types and inclusion levels on production and carcass quality characteristics in lambs - in an attempt to evaluate possible efficiency differences between currently registered ionophores in South Africa.

This dissertation is then presented in the form of five chapters that forms a single unit. Firstly the aim of the study is acquainted by a general introduction (Chapter 1), followed by a literature review (Chapter 2). The first production study is reported in Chapter 3, to evaluate three different ionophore types with each other, while two ionophore types at three different inclusion levels (minimum, mean and maximum registered levels according to Act 36/1947)

were evaluated in Chapter 4. The general conclusions regarding the effects of ionophore type and inclusion level are then summarized in Chapter 5.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Veterinary drugs, such as ionophore antibiotics, have become an integral ingredient of the livestock production chain and plays an important role in the maintenance of animal welfare through prevention of diseases, curing of infections, controlling the risk of disease transmission to man and also increasing the productive capacity of animals (Matabudul *et al*., 2001). The improvement in feed efficiency and the production of leaner protein, with rapid growth rates at lower input costs have been the primary objectives in animal husbandry (Kart & Bilgili, 2008).

Antibiotic additives are some of the chemical compounds produced by microorganisms (like fungi) and when provided in small quantities, halt the growth of certain bacteria (McDonald *et al.,* 2002). Antibiotics are primarily used at therapeutic levels in feed and water, or alternatively by injection to treat animals against diseases. However, sub-therapeutic levels are added to ruminant feed to enhance the rate of growth, by reducing specific bacterial numbers in the gut. McDonald *et al.* (2002) classified antibiotics into four groups, according to their specific function, namely:

i. Antibiotics which interfere with bacterial cell wall synthesis. These compounds are of high molecular weight (>1200 u) that act on gram-positive bacteria. They are poorly absorbed by the host and thus are non-toxic, leave no detectable residues and have no withdrawal period. Avoparcin and flavomycin are examples of this type of antibiotic.
ii. Antibiotics which inhibit bacterial protein synthesis (also primarily active against gram-positive bacteria) of medium molecular weight (>500 u). Although they are absorbed to a greater extent than the higher molecular weight compounds, they do not have a withdrawal period. Examples of this type of antibiotic include tylosin and virginiamycin.
iii. Antibiotics which inhibit bacterial DNA synthesis (with a broad spectrum of activity) possess a low molecular weight (approximately 250 u) and require specific

withdrawal periods. Nitrofurans and quinoxaline-N-oxides fall into this category of antibiotics.

Ionophore antibiotics which interfere with the electrolyte balance (Na^+/K^+) of the bacterial cell by transporting potassium into the cell. The bacteria then have to use energy to pump the ions out. Eventually the ion pump fails to operate efficiently and potassium accumulates inside the cell. Water then enters by osmosis and the cell ruptures. Monensin, salinomycin and lasalocid are examples of this type of antibiotic.

According to Wessels (1993), Kart & Bilgili (2008), and Al-Dobaib & Mousa (2009) there are at least 76 different ionophores known, of which monensin (Figure. 2.1), lasalocid (Figure. 2.2) and salinomycin (Figure. 2.3) are probably the best known.

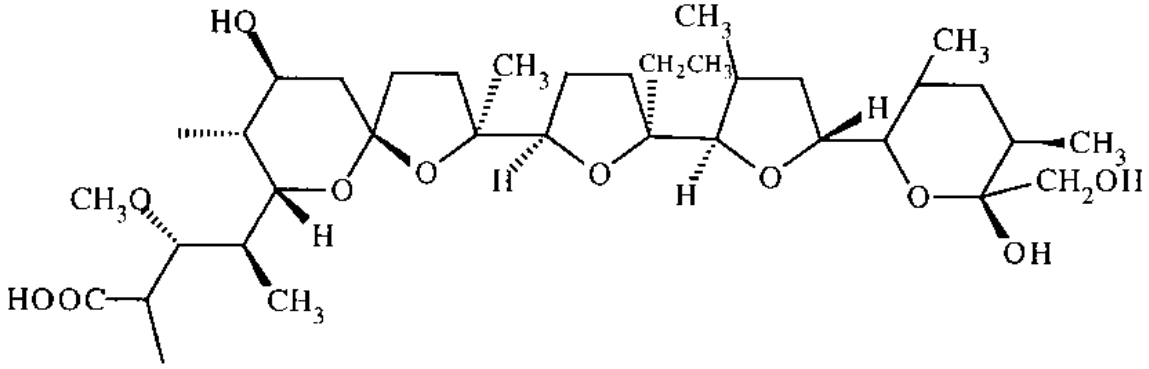

Figure 2.1 Chemical structure of monensin (Shen & Brodbelt, 2000).

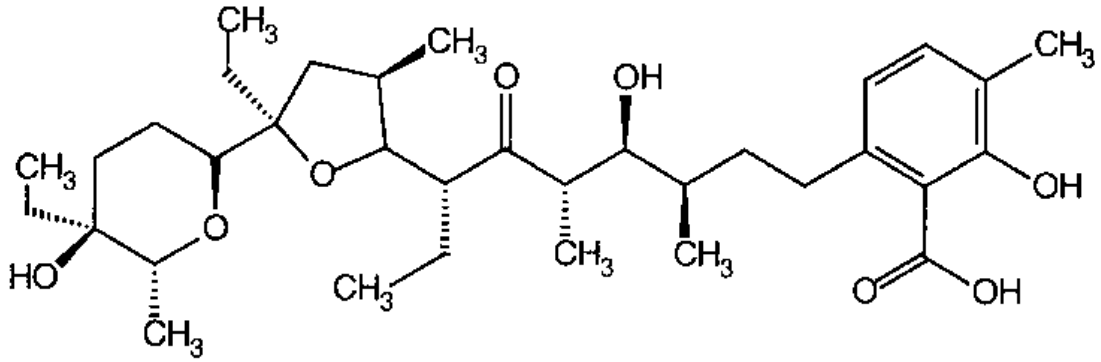

Figure 2.2 Chemical structure of lasalocid (Shen & Brodbelt, 2000).

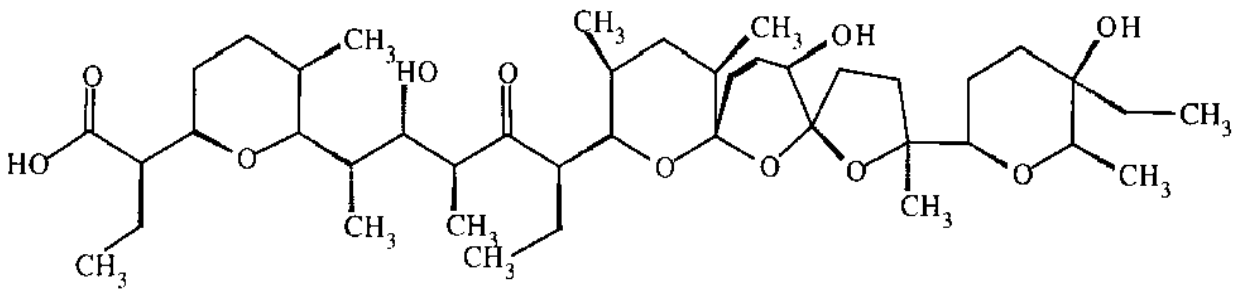

Figure 2.3 Chemical structure of salinomycin (Shen & Brodbelt, 2000).

Monensin, lasalocid and salinomycin are produced by strains of *Streptomyces cinnamonensis*, *Streptomyces lasaliensis* and *Streptomyces albus*, respectively (Van Vuuren & Nel, 1983; Merchen & Berger, 1985; Zinn, 1986b; Martini *et al*., 1996; Page, 2003).

2.2 Ionophore mode of action

Ionophore antibiotics are members of a large and growing group of compounds, possessing the ability to form lipid-soluble complexes with cations and mediate their transport across lipid barriers. They are also called polyether antibiotics because of the multiplicity of the cyclic ethers in the structures of certain ionophores (Schelling, 1984; Nagaraja, 1995; Benson *et al*., 1998; Matabudul *et al*., 2002). Page (2003) quoted the term "polyether" to refer to the unusual structural feature, whereby ionophores possess a considerable number of heterocyclic tetrahydro-pyrans and -furans. Ionophores are divided into two general groups, namely (i) channel formers and (ii) ion carriers, based on the mode of ion transfer across membranes (Kart & Bilgili, 2008).

i. Channel forming ionophores arrange themselves on the inside of the membrane structure, creating a hydrophilic channel for the ions. By this means, ions from outside the cell pass through the provided hydrophilic channel, into the cell. This mode of ion transport is analogue to that of transport proteins found in the cell membranes. A well-known example of this type of ion transport is performed by gramicidin. To form a channel within the membrane, two gramicidin molecules are required to line up across the membrane. When two gramicidin molecules dimerism within the membrane, a hydrophilic channel is formed, with the outside consisting of hydrophobic residues (Kart & Bilgili, 2008).

ii. Ion carriers can be subdivided into neutral ionophores and carboxylic ionophores. Regardless of the subdivisions, both neutral and carboxylic ionophores move ions across the lipid bilayer by diffusing together with ions. These ion carriers act in a way that bind the ions on one side of the cell membrane and allow the ion to bind with the ion carrier. The resulting complex then moves across the lipid bilayer and release the ion on the other side of the cell membrane (Kart & Bilgili, 2008).

While the backbone of ionophores provides an alkyl-rich lipid-soluble exterior, the ether, carboxyl, hydroxyl and carbonyl oxygens are oriented internally to form a cage of potential ligand binding entrapped cations. Monensin, for example, is effectively cyclised by head-to-tail hydrogen bonding, between the carboxyl group at the head, and one or two hydroxyl groups at the tail (Page, 2003). The result is a mobile cation carrier that readily traverses the thick but porous peptidoglycan cell wall of the gram-positive organisms, which is then able to transport cations across the bilaminar lipid cytoplasmic membrane (Bergen & Bates, 1984; Matabudul, *et al*., 2002; Page, 2003).

Some ionophores bind only a single cation (uniporters), while others (which include both monensin and lasalocid), are able to bind more than one cation (antiporters) (Russell & Strobel, 1989; Callaway *et al*., 2003; Khan *et al.*, 2008). Several researchers (Wessels, 1993; Nagaraja, 1995; Wessels *et al*., 1996; Page, 2003) reported the selectivity of cation binding to be a distinguishing feature of each polyether ionophore and to relate to each compound's characteristic dimensions and electro-mechanical properties. So for example, monensin and salinomycin are monovalent polyethers with the following selectivity: (i) Monensin: $Na^+ > K^+ > Rb^+ > Li^+ > Cs^+ > Rb^+$ and (ii) Salinomycin: $Na^+ > K^+ > Cs^+ > Sr^{++} > Ca^{++} > Mg^{++}$.

Monensin`s affinity for Na^+ is approximately tenfold that of K^+, its nearest competitor (Bergen & Bates, 1984). In contrast, lasalocid is a divalent polyether with a monovalent selectivity series of $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$ and a divalent series of $Ba^{++} > Sr^{++} > Ca^{++} > Mg^{++}$. In terms of relative potency, monensin has a 31 fold greater affinity for Na^+ than lasalocid, while lasalocid has a 10 000 fold greater affinity for Ca^{++} than monensin (Page, 2003). Bergen & Bates (1984) also reported that lasalocid had a higher affinity for K^+ and an equal affinity for Ca^{++} as for Na^+.

Bacterial membranes are relatively impermeable to ions, allowing gradients to be utilized as a driving force for nutrient uptake (Callaway *et al*., 2003). Ruminal bacteria then generally maintain high intracellular potassium and low intracellular sodium concentrations. Conversely, the ruminal environment contains high sodium and low potassium concentrations. Thus, ruminal bacteria rely heavily upon ion gradients (both K^+ and Na^+) to take up nutrients and to establish a proton motive force (PMF). Although the ruminal pH is somewhat acidic due to the volatile fatty acid (VFA) concentrations, the intracellular pH of many ruminal bacteria is close to neutral - thus creating an inward directed proton gradient (Callaway *et al*., 2003; Page, 2003).

Ionophores are generally bacteriostatic and not bacteriocidal (Nagaraja, 1995; Rogers *et al*., 1997). The mechanism of bacteriostatic activity of ionophores is related to their ability to alter the flow of cations across the cell membrane (Chow *et al*., 1994; Nagaraja, 1995). So for example, monensin is a metal/proton antiporter that can exchange H^+ for either Na^+ or K^+. Once inserted in the membrane, monensin exchanges the intracellular K^+ for extracellular protons, or extracellular sodium for intracellular protons (Chow *et al*., 1994; Rogers *et al*., 1997; Callaway *et al*., 2003; Kart & Bilgili, 2008). Due to the potassium gradient being greater than the sodium gradient, protons accumulate inside the bacterium. The bacterium then reacts to this cytoplasmic acidification by activating a reversible ATPase-system to pump these protons out of the cell. Additionally, other ATP-utilizing primary pumps for Na^+ removal and K^+ uptake are activated to re-establish ion gradients, resulting in the uncoupling of ATP hydrolysis from growth. This decreases the intracellular ATP pools, leading to cellular death (Chow *et al*., 1994; Benson *et al*., 1998; Matabudul *et al*., 2002; Callaway *et al*., 2003; Page, 2003; Kart & Bilgili, 2008; Khan *et al*., 2008).

2.3 Effect of ionophores on ruminal micro-organisms

2.3.1 Effect on ruminal bacteria

In general, ionophore antibiotics are inhibitory to gram-positive bacteria (see paragraph 2.2) such as *Eubacterium*, *Lactobacillus* and *Streptococcus*, together with those bacteria that often stain gram-negative, however have a gram-positive cell wall structure e.g. *Butyrivibrio*, *Lachnospira* and *Ruminococcus*. The gram-negative bacteria, including the *Anaerovibrio*, *Fibrobacter*, *Megasphaera*, *Prevotella*, *Ruminobacter*, *Selenomonas*, *Succinimonas*, *Succinivibrio* and *Veillonella* species are resistant to ionophores (Nagaraja, 1995).

Katz *et al*. (1986) & White and McGuffy (2006) stated differences recorded in the sensitivity of gram-positive and gram-negative bacteria to monensin or lasalocid to indicate that the cell wall plays a key role in determining the sensitivity of bacteria to a specific type of ionophore. Gram-negative organisms, which are generally resistant to monensin, possess a more complex outer membrane. This membrane then contains protein channels (porins) with a size exclusion limit of approximately 600 daltons, which serves as a protective barrier. Most ionophores are however larger than 600 daltons. Also, the lipid layer in the outer membrane may act as a hydrophobic barrier, trapping the ionophores before they reach the inner cell membrane (Chow *et al*., 1994; Russel & Strobel, 1989; Nagaraja, 1995). Callaway *et al*. (2003) stated that gram-positive bacteria are surrounded by a peptidoglycan layer, which is porous and allows small molecules to pass through reaching the cytoplasmic membrane where the lipophilic ionophores rapidly dissolve. Conversely, gram-negative bacteria are separated from the environment and the antimicrobial agents by a lipopolysachharide outer membrane layer and periplasmic space.

The action of monensin supports the normal biology of the rumen. The sensitive gram-positive bacteria generally produce acetate, butyrate, hydrogen, ammonia and lactate, whereas the resistant gram-negative bacteria produce propionate and succinate (Henderson *et al*., 1981; Bergen & Bates, 1984; Schelling, 1984; Olumeyan *et al*., 1986). Thus, when monensin is fed to ruminants, more propionate is produced and less hydrogen is available for methane production (see paragraph 2.4.2). Again, these biological effects of the ionophores contribute in a varying degree to the observed improvements in animal performance (Bergen & Bates, 1984; White & McGuffy, 2006.

2.3.2 Effect of ionophores on ruminal protozoa and fungi

The significance of antiprotozoal and antifungal activities of ionophore antibiotics in terms of ruminal fermentation is not clear (Nagaraja, 1995). Although ciliate protozoa constitute an important fraction of the total microbial population in the rumen, they are not indispensable for feed digestion. Ruminal fungi possess major properties, such as fibre and protein degradation, but their quantitative significance to the total microbial activity in the rumen is not quantified. It has been estimated that ruminal fungi account for only 8 to 10% of the total ruminal biomass, depending on the dietary cellulose levels (Nagaraja, 1995; McDonald *et al.*, 2002).

According to Nagaraja (1995) ionophore antibiotics are also inhibitory to the ruminal ciliates and anaerobic fungi. This could be attributed to the fact that fungi lack an outer membrane and are sensitive to monensin *in vitro* (Russel & Strobel, 1989). Generally, Isotrichidae (or also commonly known as holotrich) ciliates (*Dasytricha*, *Isotricha* and *Charomina*), are resistant to ionophore supplementation, while Ophryoscolecidae (oligotrichs) (*Entodinium*, *Diplodinium* and *Ophryscolex*) are sensitive to ionophore antibiotics (Nagaraja, 1995). In contrast, Grenet *et al*. (1989) noted that monensin has no effect on the fungal population. Chow *et al*. (1994) reported ruminal protozoa to be inhibited by monensin *in vitro*, but the effects on protozoa numbers *in vivo* are not clear.

The alterations in the rumen flora experienced with ionophore supplementation are partly due to the elimination or reduction of fungi and ciliates, and the associated methanogenic bacteria leading to a change in the hydrogen flow pattern (Nagaraja, 1995). As protozoa produce hydrogen and are colonized by methanogens, their elimination may contribute to the reduction in ruminal methane production (Russell & Strobel, 1989) (see paragraph 2.4.2).

2.4 Manipulation of energy metabolism

2.4.1 Effect of ionophores on ruminal volatile fatty acid (VFA) production

The most consistent and well documented fermentation modifications observed with the feeding of ionophores, are the increased molar proportions of propionic acid and the resulting decrease in molar proportions of acetate and butyrate produced in the rumen (Beacom *et al*., 1988; Marounek *et al*., 1990; Ward *et al*., 1990; Virkel *et al*., 2004; Fujita *et al*., 2007; Quinn *et al*., 2009). Al-Dobaib & Mousa (2009) indicated that the inclusion of ionophores in diets of steers resulted in a 76% increase in propionate production - with a resultant 16% decrease in acetate and 14% decrease in butyrate production. Ricke *et al*. (1984) stated that, although the shift in VFA production may be similar, the depression in acetate and butyrate production was less with lasalocid, than with monensin. It was also found that monensin reduced the total VFA concentration when added to either wheat pastures or a urea-soybean meal for steers (Neto *et al*., 2009). In the absence of monensin De Jong & Berschauer (1983) found that the VFA production increased. In contrast, other researchers (Katz *et al*. 1986; Mbanzamihigo *et al*. 1996; Fujita *et al*., 2007; Gonzalez-Momita *et al*., 2009; Quinn *et al*., 2009) found that neither monensin, lasalocid nor salinomycin administration had any effect on the total VFA concentrations in the rumen.

It seems that changes in molar proportions of both acetate and butyrate, when feeding ionophores, are neither consistent nor linear. The magnitude of the increase in the molar propionate percentage is generally inversely related to the energy content of the diet (Nagaraja, 1995; Maas *et al*., 2001). The relative enhancement is lower in cattle consuming high energy feeds (high concentrate), than those consuming low energy feeds (high roughage). This response could be ascribed to the fact that those animals already have large amounts of propionic acid in the rumen, compared to the roughage fed animals. However, these changes in propionic molar proportions do not accurately reflect the changes in propionate production (Bergen & Bates, 1984; Maas *et al*., 2001).

The concept that propionate is utilized more efficiently than acetate by the host tissue is subject to debate. However, flexibility in the use of propionate (gluconeogenesis or oxidation via the Krebs cycle) by the host tissue offers a distinct advantage over acetate (Schelling, 1984). Also, propionate production in the rumen results in improved fermentation efficiency because of the greater recovery of metabolic hydrogen (Hillaire *et al*., 1980; Russell & Strobel, 1989; Nagaraja, 1995). A shift to propionate production may also lower the heat increment, save amino acids normally destined for gluconeogenesis and promote body protein synthesis (McGuffey *et al*., 2001; Page, 2003), hence improving animal performance.

2.4.2 Effect of ionophores on methanogenesis

Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) are some of the major greenhouse gases (Moss *et al*., 2000; Aluwong *et al*., 2011) that contribute to global warming. Leuning *et al*. (1998) reported that methane accounted for about 20% of the total global radiative forces of all greenhouse gasses, while its release from agricultural sources was estimated to be between 205 and 245 million tons per year (Duxbury & Mosier, 1993). On a global scale, livestock farming may thus contribute up to 18% of the total greenhouse gas emissions (FAOSTAT, 2006). Although methane's contribution is less than 2% of all the factors leading to global warming, it plays an important role, as it is 21 times more effective than carbon dioxide as a greenhouse gas (Johnson & Johnson, 1995; Johnson *et al*., 1996).

Methane emission is a direct result of the fermentation process performed by ruminal micro-organisms and, in particular, the archael methanogens which scavenge the hydrogen ions and use it to produce methane (Song *et al*., 2011). The production of methane in beef cattle is

often as much as 12 *l*/h, where the gas is ultimately lost by means of belching (eructation) (Russell & Strobel, 1989). Since its release to the atmosphere represents an energy loss of 2 to 15% of ingested gross energy (Russell & Strobel, 1989; Van Nevel & Demeyer, 1977), reducing its emission would benefit both production efficiency and the environment (Moss *et al*., 2000).

This loss can however be reduced by as much as 30% when an ionophore is added to the diet (Al-Dobaib & Mousa, 2009). Increased propionic acid accumulation in the rumen of ionophore-fed animals may be the consequence of redirected hydrogen utilization caused by lower methane production (Nagaraja, 1995). However, monensin has been shown to shift the propionate:acetate ratio (Schelling, 1984; Virkel *et al*., 2004) (see paragraph 2.3.1), which suggests that part of the increase in propionate production is independent of monensin`s effect on methane production (Hillaire *et al*., 1980; Thornton & Owens, 1981; Bergen & Bates, 1984; Merchen & Berger, 1985). An increase in rumen propionate concentration due to ionophore supplementation is accompanied by a reduction of 4 to 31% methane produced (Bergen & Bates, 1984; Russell & Strobel, 1989; Mbanzamihigo *et al*., 1996; McGuffey *et al*., 2001; McDonald *et al*., 2002; Quinn *et al*., 2009). As ionophore antibiotics do not inhibit methanogenic forming bacteria, lower methane production is believed to be due to a decreased production rate of its precursors (H_2 and formate). This phenomenon is supported by the observation that when substrates (CO_2 and H_2) are provided, ionophores have no effect on methane production (Van Nevel & Demeyer, 1977; Henderson *et al*., 1981; Mwenya *et al*., 2005). However, monensin inhibits methanogenesis from formate partly due to nickel (Ni) uptake being inhibited in the methanogenic bacteria (Jarrell & Sprott, 1983). Nickel is required for the synthesis of the coenzyme F430 and the hydrogenase enzyme (Daniels *et al*. 1984). In contrast, Oscar *et al*. (1987) has shown that Ni supplementation with or without monensin had no effect on ruminal methane production in cattle.

2.4.3 Effect of ionophores on energy digestibility

A major contribution to enhanced efficiency of feed utilization by ruminants fed diets containing monensin lies in the increased metabolizable energy (ME) content per unit dry matter (DM) feed (Parker & Armstrong, 1987). Both Parker & Armstrong (1987) and Mwenya *et al*. (2005) reported an increase in ME content per unit DM feed when feeding monensin, which resulted mainly from a reduction in methane production, linked to an

increase in the proportion and amount of propionic acid, and to a lesser extent a reduced N excretion *via* the urine (see paragraph 2.5). Mwenya *et al*. (2005) also reported a lower faecal energy loss in steers fed monensin-containing diets, while urinary energy losses were higher for steers fed control diets, without any ionophore inclusion.

Theoretically monensin increases the efficiency of converting feed energy to energy contained within the VFA`s available for absorption in the rumen. Page (2003) and Neto *et al*. (2009) reported that by changing the molar VFA proportions of acetic:propionic:butyric from 60:30:10 to 52:40:8, a gross energy saving of up to 5.6% was accomplished. Bergen & Bates (1984) concluded that approximately 20% more ME was available to sheep when their diet was supplemented with monensin, due to increased VFA production rates. Thus, the ME value of feeds are increased due to increased DM digestibility and increased hydrogen retention in propionic acid (Goodrich *et al*., 1984). In addition, Muntifering *et al*. (1981) reported that monensin caused a greater proportion of starch to be digested in the intestines rather than in the rumen (with possibly a greater resultant metabolic efficiency). These results may account for some of the benefits obtained by feeding this compound in high-grain diets and support the work done by Fujita *et al*. (2007), who found that salinomycin did not affect ME intake in sheep fed a high roughage diet.

Zinn (1986b) demonstrated that salinomycin supplementation increased the estimated net energy (NE) value of a diet fed to steers by 3.2% for maintenance and 2.7% for body weight gain. Hence, about 60% of the improvement in feed conversion with salinomycin supplementation could be attributed to the higher NE derived from the diet. In contrast, Fuller & Johnson (1981) found that energy digestibility remained largely unchanged (≤3%) with either monensin (33 or 44 mg/kg) or lasalocid (32.5, 65 or 130 mg/kg) supplementation.

2.5 Effect of ionophores on protein metabolism

Much of the protein entering the rumen is hydrolysed to peptides and amino acids by rumen microorganisms, but some amino acids are degraded further to organic acids, ammonia and carbon dioxide (McDonald *et al*., 2002). The rate of ammonia production sometimes exceeds the needs of ammonia-utilising bacteria, and excess ammonia is absorbed across the rumen wall into the blood and converted to urea by the liver. Some urea is however recycled, either *via* the saliva back to the rumen, but much of it is excreted in the urine (Russel & Strobel,

1989; McDonald *et al*., 2002). Not only does this constitute a loss, but the re-synthesis of microbial protein after deamination of feed protein is an energetic wasteful process. Therefore, most of the attention paid to manipulate protein metabolism has been focused on decreasing ruminal degradation and increasing bypass protein reaching the small intestine, where it can be digested enzymatically by the host animal and efficiently absorbed (Wessels, 1993).

Since ionophores also inhibit the hydrolysis of protein in the rumen, it appears that deamination rather than proteolysis is mostly affected (Russell & Strobel, 1989; Wessels, *et al*., 1996; McGuffey *et al*., 2001). Deamination of amino acids in the rumen is thus a nutritionally wasteful process, as the rate of ammonia production exceeds the rate of utilization (Tamminga, 1979). Monensin results in a decreased production of ammonia *in vitro* (Van Nevel & Demeyer, 1977), as well as *in vivo* (Dinius *et al*., 1976). Chen & Russell (1991) and Bogaert *et al*. (1991) reported that ammonia nitrogen concentration in the rumen was lower in sheep receiving ionophores. Again, these lower ammonia concentrations are due to decreased proteolysis, degradation of peptides and deamination of amino acids in the rumen (Surber & Bowman, 1998).

Some researchers (Bergen & Bates, 1984; Goodrich *et al*., 1984; Chen & Russell, 1991; Lana *et al*., 1997) support the theory that monensin has a sparing effect on dietary protein from ruminal degradation. Muntifering *et al*. (1981), Merchen & Berger (1985) and McGuffey *et al*. (2001) reported that monensin decreased the fraction of bacterial N to total N digested post-ruminal, and increased the contribution of ruminally undegraded feed N digested enzymatically in the small intestines. This increase in ruminally undegraded N means that the quantity of bypass N is dependent on the protein source (Yang & Russell, 1993). In contrast, Surber & Bowman (1998) found that monensin does not affect the total N flow to the abomasum or microbial N synthesis in cannulated steers. Zinn (1986a) also found that salinomycin supplementation does not significantly influence the passage of either microbial or feed N to the small intestine in feedlot cattle.

Using steers, Parker & Armstrong (1987) reported that both monensin and lasalocid have an effect on urease activity in the rumen fluid, reducing the urea content by as much as 66 and 28%, respectively. Bacterial urease is a nickel-dependent enzyme and monensin has been shown to inhibit the transport of Ni in *Methanobacterium bryantii*, affording a possible

explanation for the decrease in both ammonia and methane concentrations in rumen fluid of treated animals (see paragraph 2.4.2). Zinn (1986b) also reported that the magnitude of urease activity to salinomycin supplementation within feedlot steers tend to decrease with an increase in forage level, averaging 37%, 23% and 15% for diets containing 10%, 15% and 20% forage, respectively.

2.6 Prevention of feedlot disorders

Altered ruminal bacterial fermentation associated with ionophore supplementation may reduce the incidence and severity of certain diseases in ruminants e.g. acidosis, bloat, acute bovine pulmonary edema, emphysema, as well as coccidiosis and liver abscesses (Goodrich *et al*., 1984; Nagaraja, 1995; McGuffey *et al*., 2001). McGuffey *et al*. (2001) and Bagg *et al*. (2005) also reported other health benefits by using dietary ionophore supplementation, which include the reduction in the incidence of subclinical and clinical ketosis, displaced abomasums and retained placenta in dairy cattle.

Management practices to improve the growth performance of weaned ruminants include the manipulation of feed in such a way that digestion is neither too rapid (which may be the case with highly fermentable grains, resulting in digestive problems), nor too slow, which can result in poor feed efficiency (Hatfield *et al*., 1997). Cereal grains are generally the major component of feedlot diets, and situations that lead to the rapid fermentation of starches could lead to an increased accumulation of organic acids in the rumen - which may result in certain disorders (Nagaraja, 1995; Phy & Provenza, 1998b).

2.6.1 Lactic acidosis

Lactic acidosis originates when the diet of ruminants is abruptly changed from forage to concentrates, or when stress causes the animals to reduce their feed intake with a subsequent abnormally high intake of concentrates (Goodrich *et al*., 1984). The incidence of lactic acidosis is more prevalent during nutritional adaptation of animals that are unaccustomed to their new diet (Casey *et al*., 1994). Signs of acidosis include decreased rumen and blood pH, increased rumen and blood lactate levels resulting in clinical signs such as anorexia, diarrhoea, dullness, dehydration (loss of skin elasticity), hyperventilation, mucous in the faeces and loss of coordination (Elam, 1976; Nagaraja *et al*., 1981, 1982; Goodrich *et al*., 1984).

Nagaraja *et al*. (1981) reported that lactic acidosis is initiated by the rapid proliferation of lactic acid-producing bacteria (*Streptococcus bovis* and *Lactobacillus* spp.) in the rumen. Thus, the rate at which lactic acid is produced exceeds the rate at which it is utilized. The excessive production and accumulation of L(+) and D(-) lactic acids lead to ruminal acidosis, which subsequently destroys the normal microbial population of the rumen and produces potentially toxic metabolites (Nagaraja, 1995). Ionophores possess the ideal characteristic of preventing lactic acidosis (Bergen & Bates, 1984; Schelling, 1984). Due to their selectivity toward gram-positive bacteria, major lactic acid-producing rumen bacteria (*Streptococcus bovis* and *Lactobacillus* spp.) are suppressed, but gram-negative lactic acid-fermenting bacteria stay unaffected (White & McGuffy, 2006). Sub-acute or subclinical acidosis, which is characterized by less severe ruminal acidity, may be a more common form of acidosis in grain-fed ruminants (Nagaraja, 1995; Phy & Provenza, 1998). Ionophores generally also provide protection against sub-acute acidosis, by maintaining a favourable ruminal pH with some differences in efficiency (Nagaraja, *et al*., 1982; Bergen & Bates, 1984). Bergen & Bates (1984) found lasalocid to be more effective in inhibiting the growth of several *Streptococcus bovis* strains, as well as depressing L(+) acid accumulation in the rumen.

Cattle often exhibit a quantitative lower feed intake when fed monensin containing diets (see paragraph 2.12), which is mainly due to the fact that monensin is unpalatable, compared to other ionophores (especially salinomycin) (Cheng, *et al*., 1998). This additional effect of ionophores on feed intake variation should potentially be complimentary to the ruminal benefits thereof (Nagaraja *et al*., 1981; Nagaraja, 1995). Meal feeding frequency may be as important as total feed intake in causing acidosis. So for example, cattle with hormonal growth implants typically have higher feed intakes, compared to non-implanted animals. Weather changes, the processing of cattle with hormonal growth implants, or inoculations often disrupt the feeding patterns and may result in overconsumption and subsequent acidosis (Owens *et al*., 1998). Thus, proper timing of animal processing (to prevent feed deprivation), as well as feed intake restriction following the processing of animals, can be beneficial in the prevention of acidosis. Estrogenic implants also increase the eating frequency, which in turn may decrease the potential occurrence of acidosis.

2.6.2 Bloat

Bloat can be classified into two types, namely: (i) gas-free bloat and (ii) froth bloat. Gas-free bloat is most often associated with an obstruction in the oesophagus or trachea. Incompletely processed or chewed feed such as potatoes, sugar beet and turnips can become lodged in the oesophagus and thus prevent the passage of gases from the rumen (Cheng *et al*., 1998). Froth bloat is characterised by excessive foaming of the ruminal contents, and is a common digestive disorder, either caused by legumes like alfalfa or clover (pasture bloat) or high-grain diets (grain or feedlot bloat) in ruminants (Bartley *et al*., 1983; Katz *et al*., 1986; Nagaraja, 1995; McDonald *et al.* 2002; Virkel *et al*., 2004). The froth is caused by a combination of feed and microbial factors (Cheng *et al*., 1998). The major microbial factor includes production of excessive microbial polysaccharides, or slime that contributes to an increased viscosity (surface tension) of the rumen fluid, when coupled with increased gas production, it causes frothy bloat (McGuffey *et al*. 2001; Virkel *et al*., 2004). Ionophores do not generally eliminate the bloat problem completely, but however causes a significant reduction in the number of bloat incidences (Nagaraja, 1995; McGuffey *et al*., 2001; Ruiz *et al*., 2001). The reduction in microbial slime and gas production are attributed to the antibacterial and anti-protozoal effects of monensin and other ionophores (Nagaraja, 1995; Matabudul *et al*., 2001).

As in the case with the prevention of lactic acidosis, not all ionophores are equally effective in preventing bloat (Katz *et al*., 1986; Nagaraja, 1995; Cheng *et al*., 1998; Matabudul *et al*., 2001). Katz *et al*. (1986) and Cheng *et al*. (1998) found that the effectiveness of ionophores to prevent bloat differs. Direct comparisons using *in vitro* techniques have demonstrated that *Streptococcus bovis* is more sensitive to salinomycin than monensin. Salinomycin is about three times as effective against bloat as either monensin or lasalocid. This sensitivity of *Streptococcus bovis* may be related to differences in the solubility between the ionophores (Cheng *et al*., 1998). In contrast, Bartley *et al*. (1983) reported monensin to be more effective than salinomycin in preventing bloat. Cheng *et al*. (1998) suggested that the associated lower feed intake of rapidly fermented carbohydrates due to monensin inclusion (see paragraph 2.6.1), may partially explain the difference in the occurrence of bloat between animals receiving these two ionophores.

However, the different effects of monensin and lasalocid on grain and legume bloat raises some interesting questions concerning the role of the rumen micro-organisms in legume and grain bloat. *Streptococcus bovis* has often been incriminated as an organism responsible for grain bloat. All three strains of *Streptococcus bovis* that were tested were restricted by lower concentrations of lasalocid, compared to that of monensin, which may explain why lasalocid was more effective in controlling grain bloat. As monensin was more effective than lasalocid in controlling legume bloat, it may be inferred that *Streptococcus bovis* is not important in the etiology of legume bloat, as in the case of grain bloat (Bartley *et al*., 1983; McGuffey *et al*., 2001). Poloxalene administrated at the recommended dose levels was found to be 100% effective against bloat, while a combination of poloxalene and monensin however did not provide 100% prevention against bloat (Bartley *et al*., 1983; Katz *et al*., 1986).

2.6.3 Bovine pulmonary emphysema and edema

Acute bovine pulmonary emphysema (ABPE) (also known as fog fever, bovine asthma, acute respiratory distress syndrome, or pulmonary adenomatosis) is an acute non-infectious respiratory distress syndrome in adult beef cattle, clinically characterized by severe respiratory distress (Wessels, 1993; Muhammed *et al*., 2008). In some cases ABPE is accompanied by edema as a complication (Muhammed *et al*., 2008). Moving cattle, particularly adult cows and bulls from dry, sparse grazing onto lush pastures, can be associated with the onset of acute respiratory disease after 4 to 10 days of grazing - with a morbidity rate in some herds of up to 50% and mortality rates of 25 to 50% (Page, 2003; Muhammed *et al*., 2008). The pathogenesis of acute bovine pulmonary emphysema and edema results from the ruminal deamination of elevated levels of tryptophan on lush pastures, to indoleacetic acid which is further metabolised by decarboxylation to 3-methylindole (3-MI) (a toxic metabolite) by *Lactobacillus species* (Potter *et al*., 1984; Nocerini *et al*., 1985; Page, 2003; Muhammed *et al*., 2008). Researchers (Nagaraja, 1995; McGuffey *et al*., 2001; Callaway *et al*., 2003; Page, 2003) have shown that the conversion of tryptophan to 3-MI is prevented by the dietary inclusion of ionophores, demonstrating that acute pulmonary disease could be prevented by these substances. Monensin administration, before and during consumption of lush pasture, decrease this toxic conversion of tryptophan to 3-MI by inhibiting the growth and function of the implicated *Lactobacillus sp*. This could be due to the fact that monensin decreases amino acid deamination by the rumen microbes (Schelling *et al*., 1984) (see paragraph 2.5). Nocerini *et al*. (1985) and Page (2003) reported that lasalocid

administration also reduces rumen 3-MI formation and the development of acute bovine pulmonary emphysema and oedema in cows challenged with an oral dose of L-tryptophan.

2.6.4 Coccidiosis

Ionophores are not only well known for their coccidiostatic properties in the broiler industry, but also in ruminants (Van Vuuren & Nel, 1983; Olumeyan *et al*., 1986; Zinn, 1986a; Wessels, 1993). All types of ionophores seem to be effective in the prevention of coccidiosis in steers and lambs (Thomas *et al*., 1990; McAllister *et al*., 1996; Griffiths *et al*., 1999).
Coccidiosis generally results from the infection of single-cell protozoa of the genus *Eimeria*, which spend most of their lives in the intestinal tract of the host animal (Matabudul *et al*., 2002). These parasites rely on the host cell for energy (Kart & Bilgili, 2008). Upon ingestion of the oocytes by the animals, it develops in the gut epithelial tissue, where they multiply exponentially and destroy the cells. It is frequently observed that the damaged intestines cannot absorb nutrients and the intestinal haemorrhages are responsible for a decline in animal production (Matabudul *et al*., 2002).

As mentioned, the main pharmalogical activities of ionophores depend on their ability to form complexes with lipid soluble polar cations (K^+, Na^+, Ca^{2+} & Mg^{2+}) and the transportation of these cations across the cell membranes (Matabudul *et al*., 2001, 2002) (see paragraph 2.2). Hence, ionophores stimulate the coccidian sporozoite`s Na^+ - K^+ - ATPase as a consequence of ononic disturbance. The rate of ion influx exceeds the capability of the Na^+ - K^+ - ATPase pump to remove the excess Na^+, due to the depletion of energy sources. The increased intracellular Na^+ is followed by an influx of Cl^- to maintain electron neutrality, which brings water from the exterior, causing swelling of the parasite (Kart & Bilgili, 2008) until they distend and burst (Bergen & Bates, 1984; Matabudul *et al*., 2002; Kart & Bilgili, 2008).

Ionophores exhibit a coccidiocidal action against the coccidian, by destroying ("killing") it in contrast to a coccidiostatic action where the coccidia are only prevented from further development (i.e. "non-killing"). The effectiveness of coccidiostatic drugs decreases as soon as it is withdrawn, or if the drug is consumed below the required levels (Matabudul *et al*., 2001). According to Goodrich *et al*. (1984), it is apparent that monensin is effective in the

control of coccidiosis and that dosages necessary to control coccidiosis are similar to those approved for improving feed utilization in beef cattle.

2.6.5 Liver abscesses

All types of ionophores seem to increase the incidence of liver condemnation and liver abscesses of feedlot cattle (Perry *et al*., 1976; Owens *et al*., 1991). In contrast, Delfino *et al*. (1988) and Nagaraja & Chengappa (1998) found that both monensin and lasalocid had no effect on the incidence of abscessed livers in feedlot cattle. It seems that it would therefore be beneficial for the feedlots to combine the use of ionophores with other antibiotics (e.g. tylosin) aimed specifically at combating liver abscesses (Wessels, 1993). Gibb *et al*. (2008) confirmed that a combination of monensin and tylosin reduce liver abscesses. However, the prevalence of severely abscessed livers is not influenced by this combination.

2.7 Face and horn fly control

Although there is no evidence on the mode of action of monensin against either face or horn flies, monensin has been reported to increase larval mortality and decrease pupae weights of both face and horn flies (Goodrich *et al*., 1984; Schelling *et al*., 1984). In a study by Goodrich *et al*. (1984), face and horn fly larval mortality and pupae weight were studied using fresh faeces from grazing steers that received no monensin or 200 mg/animal/day. Fewer face fly and horn fly pupae were recovered from steers fed monensin, than from the faeces of untreated steers, suggesting that monensin supplementation may provide an added benefit of reducing face and horn fly occurrence. In contrast, Rode *et al.* (1993) found that lasalocid does not affect the survival of horn fly larval in fresh manure, compared to manure from the control diet.

2.8 Other ruminal effects

Monensin was found to decrease the rumen turnover rate of solids and liquids, consequently increasing rumen fill (Muntifering *et al*., 1981; Leng *et al*., 1984; Ricke *et al*., 1984; Schelling, 1984; Branine & Galyean, 1990; Sooden-Karamath & Youssef, 1999). However, Armentano & Young (1983) and Rogers *et al*. (1997) reported that monensin had no effect on the rumen liquid turnover or water intake. Certain authors (Muntifering *et al*., 1981; Ricke *et al*., 1984; Branine & Galyean, 1990) stated that the decreased turnover may be independent of the effect of monensin on feed intake, and therefore, probably be because of decreased

feed intake in the forage-fed animals. The decreased turnover may increase the amount of organic matter fermented in the rumen, thereby compensating for reduced microbial activity (De Jong & Bershauer, 1983; Nagaraja, 1995). Hence, monensin decreases the motility of the rumen, thereby providing a physiological basis for the increased ruminal fill and reduced feed intake (Nagaraja, 1995).

2.9 Ionophore potency

The potency of ionophores is generally described by the minimum inhibitory concentration (MIC). The MIC determination is usually performed using unadapted bacteria in batch culture at optimal growth conditions (Nagaraja, 1995). There is evidence that exposure to a low inclusion level of ionophores helps in the selection of resistant bacteria populations (Chen & Wolin, 1979), suggesting that the MIC of rumen fluid from cattle fed ionophores could be different from the MIC for bacteria in cattle fed control diets (Dawson & Boling, 1987).

The cultured pH could also affect the ionophore activity on rumen bacteria. Lasalocid and monensin influenced *Streptococcus bovis* more at a pH of 5.7, than at a pH of 6.7 (Chow & Russell, 1990).

Reports on ionophores (other than monensin or lasalocid) such as salinomycin are limited. However, the overall response appears to be similar and new ionophores (salinomycin, narasin and tetronasin) are generally two to five-fold more potent than either lasalocid or monensin (Schelling, 1984; Funk *et al*., 1986; Olumeyan *et al*., 1986; Cheng *et al*., 1998; Page, 2003). However, the MIC`s of these new ionophores to ruminal bacteria are similar to that of lasalocid or monensin, suggesting that MIC is not a good indicator of the potency of ionophores in altering the ruminal characteristics (Nagaraja & Taylor, 1987).

2.10 Ionophore and mineral interactions

Ionophores could potentially alter the host mineral metabolism by affecting the bioavailability (absorption and retention) (Spears *et al*., 1989) of ions to animal tissue from feed and water. This uptake and transport of ions across biological membranes and tissues, the distribution and storage of ions in tissues and bones, specific mineral to mineral interactions, and homeostatic and regulatory mechanisms govern the intake and excretion of

ions (Nagaraja, 1995). However, results are not consistent, suggesting that the effect of ionophores on mineral metabolism is affected by a combination of dietary, environmental and physiological factors.

Zinn (1986a) stated that the apparent absorption of Ca, P, Mg, K and Cu was not significantly affected by salinomycin supplementation. To the contrary, other researchers (Kirk *et al*., 1985; Greene *et al*., 1986; Spears *et al*., 1989; Beckett, *et al*., 1998; Page, 2003) found that monensin increased the apparent absorption of Ca, Mg, K, Cu, Se and Zn, whilst decreasing the absorption of Na in lambs. According to Kirk *et al.* (1985), both the apparent P absorption, as well as P retention increased following monensin supplementation to lambs. In contrast, Kirk *et al*. (1993) found that monensin did not alter the Ca or P metabolism in lambs. Following the same trend, Van Ryssen (1991) reported that monensin did not have any effect on the serum K or Na content in lambs, whilst Armstrong and Spears (1988) reported a decrease in K, P and Mg plasma concentrations in beef heifers. Lasalocid on the other hand increased the inorganic Ca, P and Na concentrations in the serum of beef steers and lambs (Greene *et al*., 1986; Page, 2003), but did not alter the Ca or P metabolism in lambs (Kirk *et al*., 1993). Greene *et al*. (1986) mentioned that lambs fed lasalocid excreted less Mg, K and Ca in their faeces, indicating an increase in the apparent absorption of these minerals.

Anderson *et al*. (1983) studied the effect of monensin on the selenium status of pregnant ewes and concluded that monensin, either alone or combined with oral Se, increased blood glutathione peroxidase activity in treated ewes and the lambs produced - indicating improved selenium availability. In contrast, Van Ryssen (1991) however found that monensin did not affect the Se status of sheep.

The rumen and duodenum seems to be the major site of Mg absorption in ruminants and that improvement in Mg absorption may be associated with a reduced likelihood of hypomagnesaemia (Page, 2003). The co-administration of monensin with different forms of Mg (with high pre-intestinal availability) may help prevent hypomagnesaemia, once improved absorption appears to be within the pre-intestinal compartment of the digestive tract.

2.11 Ionophore and dietary fat interaction

Ionophores are lipophilic and the inclusion of fat in the diet may alter the ruminal distribution and/or availability of ionophores to the microbes. Also, ionophores and fats have analogous antimicrobial influences on similar populations of microbes, especially the gram-positive bacteria and ciliates. The mechanism of antimicrobial action in both cases involves the alteration of cellular membrane permeability (Nagaraja, 1995). Fats, like ionophores, increase the molar proportion of propionate and lower methane production, thereby improving ruminal fermentation efficiency (Richardson *et al*., 1976; Van Nevel & Demeyer, 1977; Chalupa *et al*., 1984; Clary *et al*., 1993; Depenbusch *et al*., 2008). Korshidi *et al*. (2008) found that monensin and supplemental fat positively affect the final body weight of feedlot lambs, but did not affect the DMI, ADG and FCR. Apparently, supplemental fat increases the threshold level of the ionophore response, but additional research is needed to understand the related effects of supplemental fat and ionophores (Nagaraja, 1995; Depenbusch *et al*., 2008).

2.12 Ionophore effects on animal production

Dikeman (2007) stated rumen metabolic modifiers to have a positive effect on live weight gain, FCR and the resultant decrease in carcass fatness. These metabolic modifiers are thus mainly developed to improve the efficiency and profitability of meat production and subsequently to improve carcass composition. These above mentioned effects are primarily in cattle, as there is considerable less research being performed on metabolic modifiers in lambs. Bergen & Bates (1984), Funk *et al*. (1986) and Nagaraja (1995) reported that the overall effectiveness of these compounds seem to be similar, depending on the dietary inclusion level, diet composition and various inherent animal factors.

As mentioned, changes in fermentation associated with ionophore supplementation have mainly resulted in an increased production of propionate and decreased production of methane, lactic acid and froth formation in the rumen (Bergen & Bates, 1984; Heydari *et al*., 2008). Decreased degradation of protein and deamination of amino acids in the rumen may also contribute to increased production efficiency of the animals (Bergen & Bates, 1984). Due to these changes in rumen fermentation, the efficiency of energy and nitrogen metabolism is improved and the presence of ruminal disorders reduced (Zinn, 1986a). This could also result in a bigger shift to propionate formation, which may lower the heat

production increment (Page, 2003); hence increasing the energy available to the animal for production purposes.

Cattle often exhibit lower feed intake quantities when fed monensin, which is due to the fact that monensin is unpalatable, compared to other ionophores (Cheng, *et al*., 1998). In support of this, Goodrich *et al*. (1984), Merchen & Berger (1985) and Wessels (1993) also found a decline in feed consumption in cattle fed salinomycin. In contrast, some researchers found that neither monensin (Beacom *et al*., 1988; Depenbusch *et al*. 2008; Heydari *et al*., 2008; Salinas-Chavira *et al*., 2010) nor lasalocid (Beacom *et al*., 1988; Heydari *et al*., 2008) had a significant effect on DMI of feedlot lambs.

Various studies (McClure, *et al*., 1980; Owens, *et al*., 1982; Goodrich *et al*. 1984; Merchen & Berger, 1985; Zinn, 1986b) reported enhanced performances in terms of increased body weight gain and feed conversion ratios by using monensin as an ionophore. According to Zinn (1986a) the improvement in feed conversion could be accounted for as either a 5% increase in the net energy value of the diet (see paragraph 2.4.3), or a 10% reduction in the maintenance requirements. Contrary to these findings, Beacom *et al*. (1988) and Heydari *et al*. (2008) found that lasalocid have no significant effect on feed efficiency. However, improved FCR following an 11 to 22 mg/kg levels of salinomycin supplementation were recorded by Funk *et al*. (1986) and Zinn (1986a). Researchers found that monensin (Beacom *et al*., 1988; Zinn & Borques, 1993; Salinas-Chavira *et al*., 2010), lasalocid (Beacom *et al*., 1988), and salinomycin (Funk *et al*., 1986; Zinn, 1986a; Salinas-Chavira *et al*., 2010) did not have a significant effect on ADG, while Heydari *et al*. (2008) and Bagley *et al*. (1988), respectively found that these same ionophores improve the ADG in feedlot lambs. Therefore, differences in literature regarding the responses in production efficiency suggest that much research still needs to be done.

Factors that could affect the variable animal responses to dietary ionophores include the diet cation concentration, microbial adaptation and diet energy level (Zinn *et al*., 1994). As ionophores demonstrate activity against both prokaryotic and eukaryotic cells, part of the performance response may be due to metabolic changes that do not involve alterations in the ruminal microbial fermentation (Nagaraja, 1995). According to Rumsey (1984), Zinn *et al*. (1986b), Rogers *et al*. (1997) and McGuffey *et al*. (2001), the effectiveness of ionophores in achieving improved FCE and ADG is attributed principally to alterations in the ruminal

fermentation. Hence, all improvements in animal productivity caused by ionophore treatment represent a secondary effect caused by the disruption of the normal bacterial membrane physiology (Callaway *et al*., 2003). This in turn is focused on the increased production of propionate, less protein degradation and deamination of amino acids, and the decreased production of methane, lactic acid and froth in the rumen. Ultimately, it leads to a reduction in rumen disorders and generally decreased mortalities (Armstrong & Spears, 1988; Nagaraja, 1995; Rogers *et al*., 1997).

2.13 Ionophore effects on carcass characteristics

From the literature it would seem that the effects of ionophores on carcass characteristics are still open for debate, due to differing results. Dikeman (2007) reported that ionophores have a positive effect on meat production by generally decreasing the carcass fatness - thereby resulting in a more favourable carcass composition, which ultimately leads to an improved efficiency and profitability. However a slight change in carcass characteristics were observed by Zinn & Borques (1993) with monensin, when using crossbred steers. In other studies (Van Vuuren & Nel, 1983; Wessels, 1993), both monensin and salinomycin were found to have no significant effect on the dressing percentage, carcass weight, back fat thickness and area of eye-muscle of steers and feedlot lambs. Berger *et al*. (1981), Delfino *et al*. (1988) and Heydari *et al*. (2008) also concluded that neither monensin nor lasalocid affects the carcass characteristics in feedlot lambs, heifers and/or crossbred steers. Parameters such as cutting ability, loin eye area and average fat cover were not affected by monensin at levels of up to 33 mg/kg, in high concentrate diets (Beacom *et al*., 1988). In contrast, Goodrich *et al*. (1984) indicated that dressing percentage, marbling score, fat depth, grade quality and carcass yield to be negatively affected by monensin.

2.14 Dietary ionophore inclusion levels

2.14.1 Cattle

The inclusion levels of ionophores in diets of feedlot cattle recommended by most manufacturers varies between 11 and 33 g monensin per metric ton of a total mixed ration (TMR) (50 - 300 mg monensin/animal/day), 100 - 333 g lasalocid per ton of complete feed and 167 g salinomycin per ton of complete feed. Adams *et al*. (1981) and Merchen & Berger (1985) quoted monensin at 33 mg/kg and 22 mg/kg to improve the ADG of steers, respectively. Goodrich *et al*. (1984) found that the ADG for cattle fed monensin at 11, 22,

27.5 or 33 g/ton to be similar. However, Goodrich *et al*. (1984) reported that the ADG of cattle fed 33 g of monensin/ton was identical to that of the control group without any monensin.

Berger *et al*. (1981) showed an increase in body weight of steers supplemented with lasalocid at approximately 100 mg/animal/day. Lasalocid inclusion at 30 and 45 g/ton reduced feed intake with a subsequent improvement of feed efficiency by 7.5 and 11%, respectively (Berger *et al.*, 1981). Performance responses to salinomycin supplementation for steers at levels of 0, 5.5, 11, 16.5, and 22 mg/kg on ADG and feed efficiency are similar according to Merchen & Berger (1985) and Zinn (1986a). Zinn (1986a) also recorded an improvement in FCR, by on average 5% at the 11 to 22 mg/kg levels of salinomycin supplementation in steers.

2.14.2 Sheep

Page (2003) fed diets containing 6, 12, or 24 mg salinomycin/kg feed. The lambs in all salinomycin treatments gained body weight, as well as had an improved FCE (other than those in the control group). In contrast, McAllister *et al*. (1996) found lambs randomly assigned to diets containing 0, 4, 10 or 16 mg salinomycin/kg of feed not to differ in DMI, ADG or FCE during the first 21 days of the trial. However, treatment differences were recorded beyond this initial trial period.

Van Vuuren & Nel (1983) supplemented lambs with 15 mg monensin/animal/day in the creep feed, and showed an increased ADG and improved feed conversion efficiency. The improvement in FCE was mainly attributed to a combination of a significant lower feed intake and ADG.

It is evident from the literature that no consistent results were recorded in animal performance of either cattle or sheep, due to different inclusion levels of the same ionophore.

2.15 Ionophore rotation

The rotation of ionophores is an established practice in the broiler industry for some time (Muirhead, 1987). Rotation does not allow sufficient time for microbial resistance to build-up against any one of the ionophores, assuming that some degree of resistance builds up after the

prolonged feeding of a specific ionophore (Morris *et al*., 1990; Casey *et al*., 1994). However, by altering the use of monensin plus tylosin, with lasalocid in feedlot cattle, Russell & Strobel (1989) achieved added benefits over and above that obtained when using only lasalocid or monensin and tylosin. Initial rotation programmes incorporated a weekly change in ionophores, but a daily rotation could improve the feed conversion efficiencies even further (Branine & Galyean, 1990; Wessels, 1993). The greater efficiency achieved by rotation of treatments suggests a possible synergistic effect in combining either salinomycin or lasalocid and monensin. However, a daily rotation of ionophores would seem too frequent to inhibit possible microbial resistance, due to the lag phase and reaction time required for the microbial population to adapt, with the probability that both ionophores would be present in the rumen simultaneously (Morris *et al*., 1990; Casey *et al*., 1994). A combination of monensin and tylosin was also found to be more effective than monensin alone, but not different to tylosin alone (Page, 2003). Stock *et al*. (1994) reported that cattle fed monensin and tylosin had a 3% increase in ADG and a 4% improvement in FCE, compared to cattle fed a control diet. Hence, factors other than fermentive and digestive changes are likely to be responsible for the improvement in feedlot performance of cattle fed a daily rotation of lasalocid and monensin plus tylosin (Morris *et al*., 1990).

2.16 Compatibility of ionophores with other growth stimulants

The lack of an interaction between monensin and anabolic implants in pasturing cattle indicates that the combined response of monensin and 17β-estradiol is additive (Bretschneider *et al*., 2008). Such an additive effect was also observed in feedlot cattle supplemented with monensin and implanted with zeranol, testosterone-estradiol or progesterone-estradiol, which is a common practice (Goodrich *et al*., 1984; Bretschneider *et al*., 2008). No evidence of benefits to their combined use was detected, because of the reduced effect of monensin on ADG of cattle grazing high quality pastures (Bretschneider *et al*., 2008).

2.17 Health considerations

2.17.1 Reproductive benefits

In addition to the benefits of the metabolic disorders listed under paragraph 2.6, monensin may also decrease the risk of ketosis, displaced abomasums, and mastitis (Duffield *et al*., 2008). No significant effects of monensin on milk fever, lameness, dystocia, retained

placenta, metritis or fertility have been recorded by researchers (Beckett *et al*. 1998; Duffield *et al*., 2008).

The use of ionophores (monensin) at conventional dose levels (200 or 600 mg/animal/day) does not adversely affect the average interval to first oestrus, first-service conception rates or pregnancy rates in either heifers, lactating or non-lactating cows (Potter *et al*., 1984; Sprott *et al*., 1988; Webb *et al*., 2001; Duffield *et al*., 2008). In contrast, Page (2003) found that the treatment of pre-pubertal heifers with 200 or 600 mg of monensin/animal/day decreased, either or both, the age and weight at puberty, with conception being advanced between 34 to 38 days, compared to untreated heifers. This indicates that feeding up to 600 mg of monensin/animal/day is safe for growing replacement dairy heifers (Potter *et al*., 1984). Again, some researchers (Hardin & Randall, 1983; Hopman & Weber, 1986; Webb *et al*., 2001) found monensin and lasalocid supplementation to decrease the postpartum interval to oestrus in cows, although some studies have found no effect of ionophore treatment on the postpartum interval. The mechanism of action of ionophores on the heifer reproductive physiology is still unclear, but it may be the result of a complex interaction between bodyweight and body condition changes, combined with an indirect effect, possibly VFA mediated, on the hormonal status (Page, 2003).

Potter *et al*. (1984) reported that monensin supplemented at levels of 200 or 600 mg/animal/day has no apparent effect on scrotal circumference, testicular consistency, libido and semen quality or sperm abnormalities. Furthermore, the fertility of bulls on the basis of sperm numbers and morphology were unaffected by a monensin supplementation level of 200 mg/animal/day (Sprott *et al*., 1988). Monensin supplementation seems therefore safe at this level and is not detrimental to the breeding performance of bulls (Potter *et al*., 1984).

2.17.2 Ionophore toxicity in animals

The toxicity of ionophores has been widely studied in a number of animal species. Reports indicate that horses, cattle, sheep, dogs, cats, pigs and avian species are relatively sensitive to ionophore toxicity. Ionophores are generally safe and effective if used at the recommended inclusion levels for the specific species of animals intended (Kart & Bilgili, 2008). However, ionophore toxicity may occur due to an accidental overdose, misuse and/or a mixing error in the diet (Potter *et al*., 1984; Bastianello *et al*., 1996; Basaraba *et al*., 1999; Kart & Bilgili,

2008). Signs of ionophore toxicity do not have definite general symptoms, with some of the most common signs such being anorexia, hypo activity, leg weakness, ataxia, dyspnea, depression and diarrhoea (Newsholme *et al*., 1983; Potter *et al*., 1984; Benson *et al*., 1998; Kart & Bilgili, 2008).

2.17.3 Human health

The use of veterinary medicines may induce the presence of drug residues in animal products which could be transferred to humans. Therefore, techniques must be available for the determination of any residues in animal products used for human consumption (Matabudul *et al*., 2001). It is a mandatory requirement (96/23/EC) for European Union (EU) member states to monitor the coccidiosis residues in their national residue programmes. Any country exporting to the EU must also have an equivalent national monitoring programme in place (Matabudul *et al*., 2002).

CHAPTER 3

THE EVALUATION OF DIFFERENT IONOPHORES IN FINISHING DIETS ON THE PERFORMANCE AND CARCASS CHARACTERISTICS OF SA MUTTON MERINO LAMBS

3.1 Introduction

Metabolic modifiers are mainly included in ruminant diets to improve the efficiency and profitability of meat production and subsequently to improve carcass composition (Dikeman, 2007). Carboxylic polyether ionophore antibiotics, produced by various strains of *Streptomyces spp.* are compounds of these rumen metabolic modifiers and include products such as monensin, lasalocid and salinomycin (Bergen & Bates, 1984). Various researchers (Bergen & Bates, 1984; Funk *et al*., 1986; Nagaraja, 1995) have reported that the overall effectiveness of these compounds seems to be similar, although the agents may vary depending on the dietary inclusion level, diet composition and various inherent animal factors. Changes in microbial fermentation in the reticulo-rumen, which are associated with ionophore supplementation, mainly result in an increased production of propionate and decreased production of methane, lactic acid and froth forming in the rumen (Schelling, 1984; Russell & Strobel, 1989; Matabudul *et al*., 2001). A decreased degradation of protein and deamination of amino acids in the rumen is also recorded. Due to these changes in rumen fermentation, the efficiency of energy and nitrogen metabolism is improved and the presence of ruminal disorders reduced. With the increasing scarcity of food and feed sources, as well as the aspect of global warming, more emphasis should be put on the positive contributions of dietary ionophores (Bergen & Bates, 1984).

The subsequent lack of literature regarding the effect of dietary ionophore inclusion on production performance and carcass composition of intensive fed lambs is one shortcoming that needs to be addressed - as it hinders the decision-making processes of producers, and feed manufacturers. No literature reports regarding lamb feedlot experiments evaluating the effects of three different ionophores simultaneously on carcass characteristics in South Africa, or elsewhere in the world, could be found in the available literature. The limited reports regarding the effects of ionophores on feedlot and carcass performance of lambs, as

well as contradictory reports regarding ionophore efficiency in beef feedlots and dietary differences between species, necessitates an in-depth investigation regarding the role of ionophores in sheep diets.

The objective of this study was thus to determine the effects of various ionophores (monensin, lasalocid and salinomycin), at standard recommended dietary inclusion levels, on certain feedlot performance parameters and carcass characteristics of S.A. Mutton Merino (SAMM) lambs during an intensive feeding period.

3.2 Materials and Methods

This study was conducted between January and March 2008 on the experimental farm (Paradys) of the University of the Free State. The farm is situated approximately 20 km south of Bloemfontein in the Free State province of South Africa at 29°13'17.45 latitude, 26°12'26.28" longitude and an altitude of 1424 m above sea level. The climate during the study period was the normal seasonal occurrence for the end of summer, and the onset of the autumn season. The maximum temperature fluctuation during the duration of the study was between a minimum of 17.0°C and a maximum of 30.1°C.

3.2.1 Production study

This study was conducted over a period of 63 days (including a 14 day adaptation period) to determine the effects of different dietary ionophores on the dry matter intake (DMI), average daily gain (ADG), feed conversion ratio (FCR), rumen pH and carcass characteristics of lambs fed under pen conditions. All procedures conducted during this study were approved by the Ethical Control Committee for Animal Experimentation at the University of the Free State (Animal Experiment No. 12/07).

3.2.1.1 Experimental design

This trial was compiled according to a complete random design, representing four dietary treatments (n=15 lambs/treatment) (see paragraph 3.2.1.4b), subdivided into five replicates per treatment (n=3 lambs/replicate). The method of random allocation of the treatments used in this study reduced the probability of animals of the same treatment being penned next to each other and thus decreased the probability that a specific treatment may have been affected

either positively or negatively by environmental factors due to pen location and/or stall conditions.

3.2.1.2 Housing

Animals were housed in adjacent pens (n=3 lambs/pen; 4.5 m²) on wooden slatted floors (Figure 3.1), which ensured a clean and hygienic environment within a naturally ventilated building. The elevated slatted floor allowed urine and faeces to accumulate on a concrete floor below. The adjacent constructed pens allowed animals to have visual and limited contact with each other. However, the construction of the pens prevented access to the feed troughs by adjacent animals. The pens were constructed and separated from each other by partitions constructed of steel pipes. All pens were clearly marked with a number according to the specific diet of the animal (Figure 3.2). The house was properly washed and disinfected with a quaternary ammonium compound (Glutabac Plus, GNR 592/30268) before the onset of the study. Each pen was cleaned once a week during the study period to ensure and maintain a good hygienic environment.

Figure 3.1 Pens constructed for housing of the experimental animals.

Figure 3.2 Distinct markings of each pen according to the treatment diet received.

3.2.1.3 Feeding troughs and water buckets

Each pen was equipped with its own single extended feed trough in order to provide feedlot comparable feeding space (27.7 cm/lamb) for the animals (Figure 3.3 and 3.4). The feed troughs were designed to limit feed wastage to a minimum for more accurate results. The feed troughs were placed along the common centre partitioning of each pen and the centre partitioning was reinforced to prevent animals from reaching feed from the adjacent pen (Figure 3.5). Two water buckets were located at the opposite end of each pens` main entrance to minimize the water being contaminated with feed. These buckets were fixed to the side of each pen and cleaned and refilled manually on a daily basis to remove faeces that may contaminate the water.

Figure 3.3 Feed troughs and water buckets used.

Figure 3.4 Feeding space for each animal.

Figure 3.5 Reinforced partitioning between the feed troughs.

3.2.1.4 Experimental diets

a) Preparation of experimental diets

Lucerne hay (*Medicago sativa*) was grounded through a 25 mm screen, while the maize grain was grounded through a 12 mm screen, using an electronically motorised Drotsky hammer mill. The high protein concentrate (HPC) was obtained from a local commercial feed company (Table 3.1) and included in the final diets in the form it was acquired. Feed components (lucerne hay, maize meal and HPC) of the individual diets were accurately weighed and thoroughly mixed with the aid of a stationary mechanical mixer (Figure 3.6) for approximately eight minutes per batch. Each ionophore was included and mixed beforehand

in the respective HPC within each treatment at the feed mill of the supplying company. Each experimental diet contained 20% HPC (differing only in respect to the ionophore included), 15% lucern hay (25 mm) and 65% maize meal (12 mm) on an as-is basis (Table 3.1).

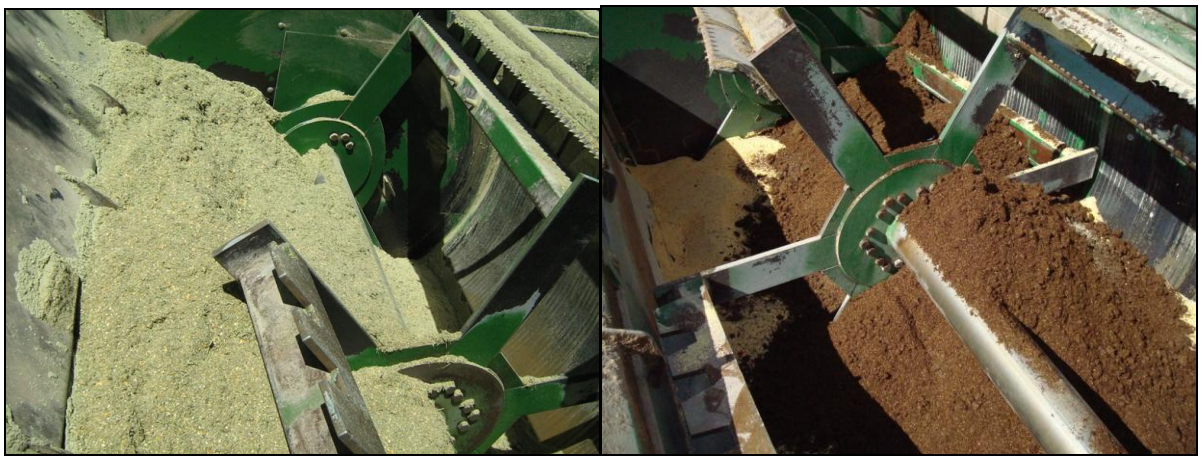

Figure 3.6 Mixing of experimental diets within a feed mixer.

b) Physical and chemical composition of the experimental diets

The four experimental dietary treatments consisted of the same iso-nitrogenous and iso-caloric basal diet, differing only in respect to the additive included i.e. (i) the control (Ct) diet (no additive), (ii) monensin (M), (iii) lasalocid (L) and (iv) salinomycin (Sl) (Table 3.1). As mentioned, monensin, lasalocid and salinomycin were added beforehand to the HPC according to the mean registered levels (Act 36/1947) of the respective suppliers at 16.5, 33.0 and 17.5 mg active ingredient/kg diet, respectively. Accordingly the experimental diet was formulated to obtain maximum live weight gain. The NRC (1985) requirements for finishing lambs in a feedlot were used as guideline. The actual chemical analysis of the diets was determined after composite sampling of intake and refusals (Section 3.2.2).

Table 3.1 Mean calculated physical (%) and chemical (%) composition of the basal diet used during the experimental period

Physical composition (as fed):	**%**
Maize meal	65.0
Lucerne hay	15.0
High protein concentrate[1#]	20.0
Chemical composition (dry matter basis):	
Crude protein	16.4
Neutral detergent fibre	17.9
Ether extract	3.67
Ash	6.7
Calcium	1.07
Phosphorous	0.30

[1] Commercial high protein concentrate with the following specifications (DM-basis): 37.93% crude protein, 24.14% NDF, 4.25% calcium and 0.36% phosphorous.

[#] Ionophores were mixed into the high protein concentrate according to dietary treatment as follows: Control - no ionophore included; Monensin - 16.5 mg active ingredient/kg feed; Lasalocid - 33.0 mg active ingredient/kg feed; Salinomycin - 17.5 mg active ingredient/kg feed.

3.2.1.5 Experimental animals

Sixty (60) SAMM whether lambs (29.7 ± 2.5 kg) (± 3 months of age, which represents the actual age at which lambs enter a commercial feedlot) were randomly allocated to the four dietary treatments. Empty stomach body weights were used to select animals in order to reduce the initial weight variation and to ensure a homogenous group of animals.

The South African Mutton Merino (SAMM) breed was selected because of their versatility and popularity with feedlot operators.

a) Weighing of lambs

At the onset and end of the production study, all the animals were fasted overnight (minimum of 12 hours) and the individual empty stomach body weight recorded the next morning in order to calculate the average daily gain and feed conversion ratios. During the production study the full stomach body weight of all lambs were recorded on a weekly basis, at the same time. Facilities to weigh the lambs are shown in Figure 3.7.

Figure 3.7 Weighing of lambs individually.

b) Preparation of experimental animals

All animals were subjected to a standard health and vaccination program, as practiced in the commercial feedlot sector of South Africa four weeks prior to the onset of this production study. The vaccine used was a 7-in-1 Clostridial plus *Pateurella* vaccine (Reg. No. G3694 Act 36/1947) to aid in the build up of active immunisation. This vaccine also controls lamb dysentery, pulpy kidney, tetanus, blackleg, clostridial metritis (malignant oedema of the uterus), blood gut and infections caused by *Clostridium novyi* type B. All the animals were castrated and weaned about four weeks prior to entering the trial.

At the onset of this study, all animals were implanted with a hormone-free, registered sheep growth promoter (Zeraplix; 72 mg zeranol per animal: Reg. No. G3230 Act 36/1947). All lambs were dosed against tapeworm (Reg. No. G1546 Act 36/1947), and a broad spectrum parasite remedy (Reg. No. G3548 Act 36/1947) was used against round worm, liver fluke and nasal worm. Animals were also injected with a trace mineral optimizer (Reg. No. G1852 Act 36/1947) and received an oral vitamin A (Reg. No. G2264 Act 36/1947).

c) Adaptation of the lambs

At the onset of the study all animals were subjected to a 14-day adaptation period using a stair-step adaptation procedure (Figure 3.8) to correspond with normal feedlot practices. Daily feed provision was calculated at an intake of 1350 g/animal/day, according to live body

weight (BW) (4.5% x 30 kg BW). Day one started at 200 g/animal of the experimental diet, while the remaining 1150 g/animal was composed of grounded (25 mm) lucern hay in a separate feeding trough. The high concentrate diet was gradually increased daily by 100 g/animal, while lucern hay was decreased accordingly until each lamb's individual feed intake reached 1350 g/animal/day. These adaptation procedures ensured that the animals were less prone to metabolic disorders caused by high energy diets, containing high inclusion levels of feed ingredients such as maize meal and molasses, that are easily fermentable in the rumen and have a low roughage content.

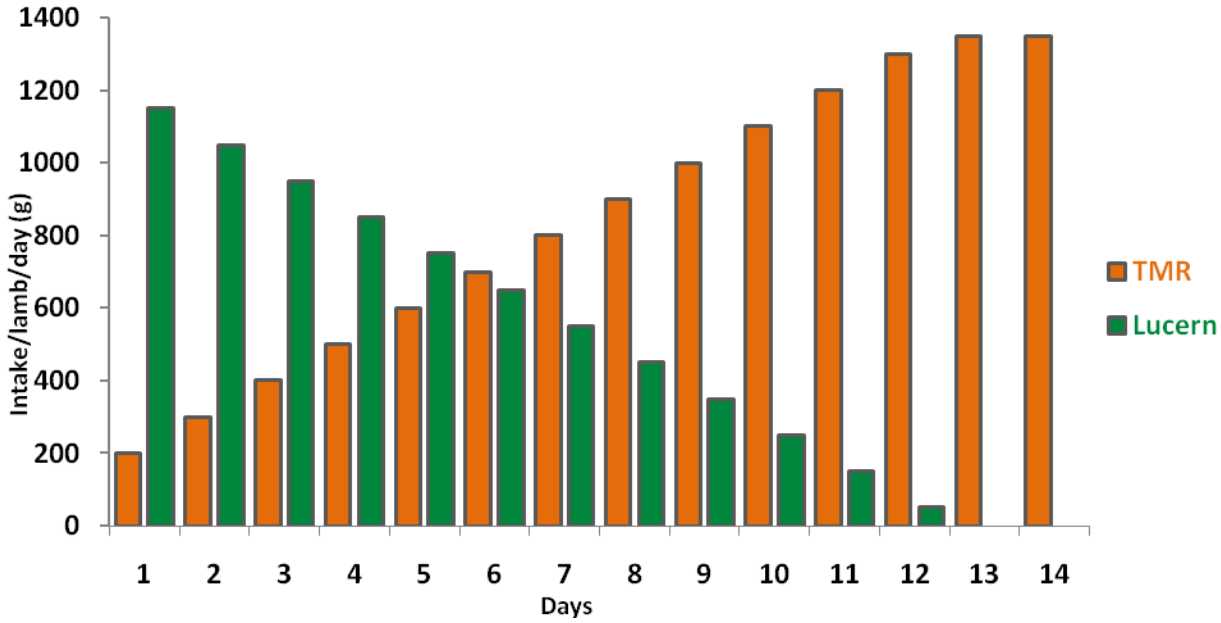

Figure 3.8 Dietary adaptation of the experimental animals according to a stair-step model during a 14-day period.

d) Feeding the lambs

After adaptation, animals were fed the respective experimental diets on an *ad libitum* basis for the remainder of the experimental period (49 days), until the lambs attained an acceptable average live weight of approximately (± 48 kg) prior to slaughter. The animals were fed twice daily (Figure 3.9), at 07:00 and at 16:00, not only to reduce feed wastage, but also to increase the frequency that the animals feed at the troughs, hence increasing the dry matter intake (DMI) of the animals. A composite sample of each of the experimental diets was collected on a daily basis. At the end of the production study a smaller representative sample of feed from

each experimental diet was obtained by the quartering method, ground to pass through a 1 mm sieve, and stored in sealed bottles pending chemical analysis.

Figure 3.9 Feeding of the lambs.

e) Feed refusals

The feed refusals from each pen were collected on a weekly basis (before weighing of the animals), in order to calculate the weekly DMI of each pen. Feed intake was subsequently determined on a weekly basis by subtracting the feed refusal weight from the total amount of feed provided. A composite sample of feed refusals was also collected on a weekly basis. At the end of the production trial a smaller representative sample of refusals from each experimental diet were obtained by the quartering method, ground to pass through a 1 mm sieve and stored in sealed bottles, pending chemical analysis.

f) Water

Fresh, clean water was freely available. The water troughs were cleaned and refilled daily at 8:00 and 16:00.

3.2.1.6 Measuring of ruminal pH

Rumen fluid was collected at three different collection periods (stratified for the onset, middle and end), throughout the duration of the production study. Rumen fluid was collected by means of a stomach tube (Figure 3.10) at five intervals during the day within each collection period - starting before the 08:00 feeding, and continuing three hours apart for the duration of a 12 hour period. Each individual sample was immediately tested using a calibrated pH meter (Cyberscan pH 110; Eutech Instruments) (Figure 3.11).

Figure 3.10 Collection of ruminal fluid.

Figure 3.11 Measuring of ruminal pH.

3.2.1.7 Carcass evaluation

All the lambs were slaughtered at a commercial abattoir at the end of the 74 day feeding period and individual warm carcass weights were immediately recorded. Carcasses were independently graded (Table 3.2) at the abattoir according to the official sheep carcass classification system as described by the South African government legislation no. R 863 (2006). All carcasses were then stored for 24 hours at 4^{o}C and the cold carcass weight recorded according to the methods described by Fisher & De Boer (1993) and Ramsay *et al*. (1991). The cold carcass weight was then used to determine the dressing percentage:

Dressing percentage = [Cold carcass weight (kg) / Live weight (kg)] x100

Table 3.2 Official sheep carcass classification system used in South Africa

Age description (no. of teeth)	Code	Fat description	Grade	Back fat (mm)
0	A	No fat	0	0
1-2	AB	Very lean	1	<1
3-6	B	Lean	2	1-3
>6	C	Medium	3	3-5
		Fat	4	5-7
		Slightly over fat	5	7-10
		Excessively over fat	6	>10

Source: Government legislation no. R 863 (2006).

The fat thickness was measured on the left flank of the carcass between the 12th and 13th rib of the *M. longissimus dorsi* at three different points, using a calliper (Electronic digital caliper; Omni-Tech) (Edwards *et al*., 1989) (Figure 3.12). The mean back fat thickness was calculated accordingly. The external length (EL), shoulder circumference (SC) and buttock circumference (BC) of each carcass were also measured (Carson *et al*., 1999) (Figure 3.13).

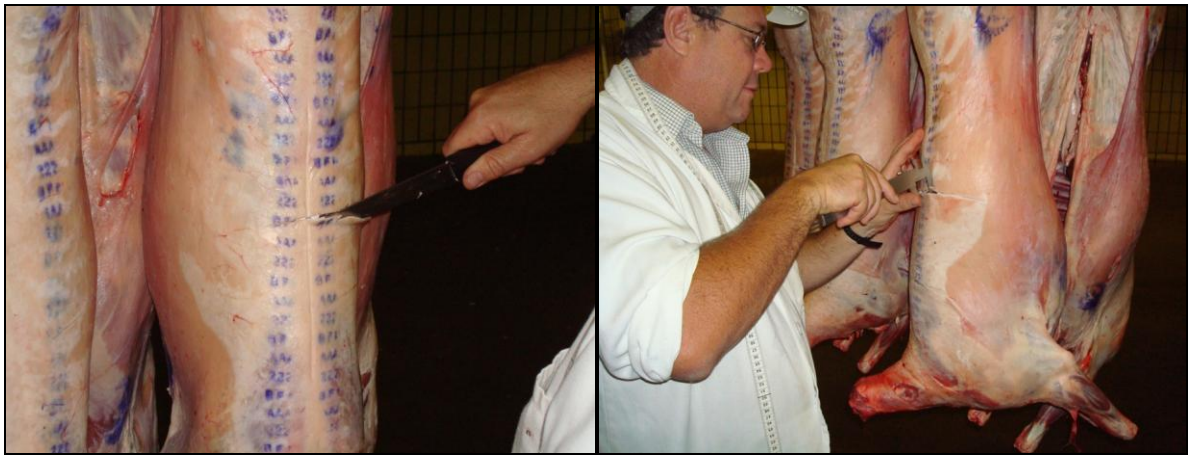

Figure 3.12 Measuring average back fat thickness.

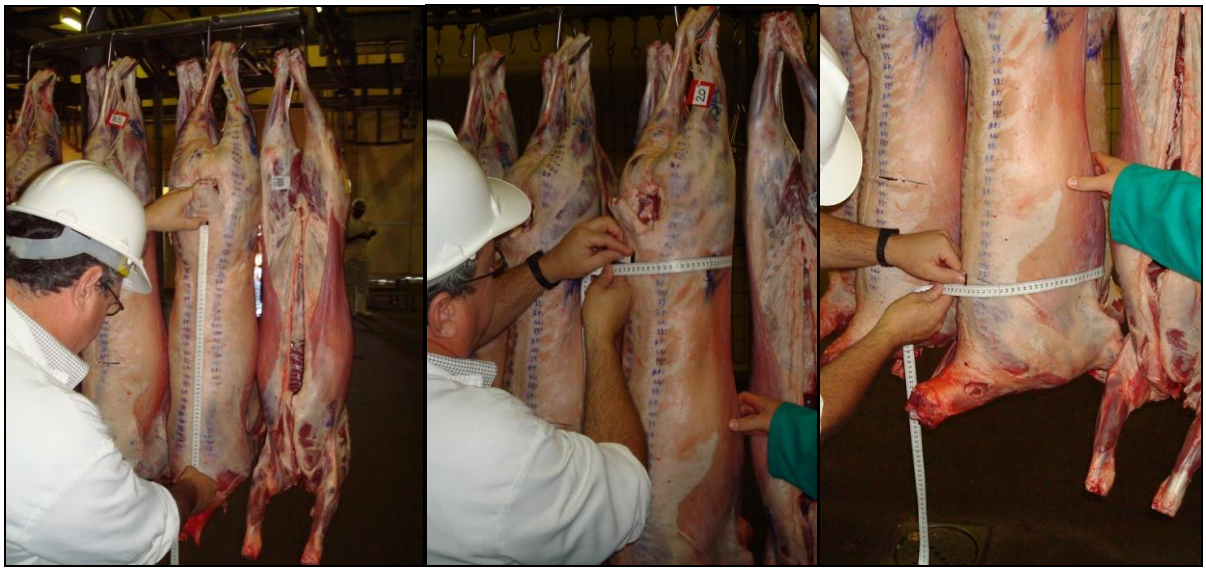

Figure 3.13 Measuring external carcass length, buttocks- and shoulder circumference.

3.2.2 Chemical analysis

Stored feed and refusal samples were analysed for dry matter (DM), crude protein (CP), gross energy (GE), ash, organic matter (OM), neutral detergent fibre (NDF), ether extract (EE), calcium (Ca) and phosphorus (P) content. An analysis was repeated if the value between each duplicate differed more than 3%. The Ca and P content of only the experimental diets were analyzed.

3.2.2.1 Dry matter (DM)

DM content of the feed and feed refusal samples were measured according to the AOAC official method 934.01 (AOAC, 2000). The DM content of the feed was determined in the physical form which the feed was presented to the lambs. Approximately 200 g of each sample was weighed in a porcelain crucible and dried in a force draught oven at 100°C, for a minimum period of 16 hours to a constant weight. After drying, the samples were placed in desiccators to cool and weighed immediately afterwards.

DM was calculated as follows:

% Moisture = [Weight loss after drying (g) / Weight of test sample (g)] × 100

% Dry matter = 100 - % Moisture

The weight of individual crucibles was deducted to determine the weight loss after drying, as all crucibles did not have exactly the same weight.

3.2.2.2 Ash

Ash content of the feed and feed refusal samples were measured according to the AOAC official method 942.05 (AOAC, 2000). Ash content was determined by complete incineration of each sample using a muffle furnace. Approximately 2 g of the ground sample (DM basis) was weighed in porcelain crucibles. Porcelain crucibles containing feed samples were then placed in the cold muffle furnace and pre-heated to a constant temperature of 600°C. Feed samples were kept at this temperature for 3h period, before switching the furnace off, and allowing it to cool until the oven could be open and samples handled. The samples were then transferred to desiccators to cool and immediately weighed afterwards. The ash content was calculated as follows:

% Ash = [Weight of ash (g DM) / Weight of test sample (g DM)] × 100

The weight of the individual crucibles were deducted to determine the weight of the ash and the weight of the test sample, as all crucibles did not have exactly the same weight.

3.2.2.3 Organic matter (OM)

The OM was determined by subtracting the ash content (%) of each sample from 100.

% Organic matter (OM) = 100 - % Ash

3.2.2.4 Crude protein (CP)

The CP content of the feed and feed refusal samples were measured according to the AOAC official method 990.03 (AOAC, 2000) with a Leco FP-528 instrument for N analysis (Leco, 2001). Approximately 0.12 g of each sample (DM) was accurately weighed and placed into aluminium foil cups that were sealed and placed on the carousel of the instrument, which did sample analyses continuously. The principle of the Dumas method is that nitrogen (N_2), freed by pyrolysis and subsequent combustion, and is swept by carbon dioxide (CO_2), as carrier into the nitrometer. The CO_2 is absorbed in potassium hydroxide (KOH) and the residual N_2 volume, measured. The nitrogen content is then converted to the protein equivalent by multiplying the percentage nitrogen with the factor of 6.25. Protein values were recorded on a computer, which was connected to the scale, as well as the analyzing instrument. The protein

equivalent was calculated by the computer program from the numerical factor obtained as described above.

3.2.2.5 Neutral-detergent fibre (NDF)

The neutral-detergent fibre (NDF) of the feed samples and feed refusals was determined according to the method of Van Soest *et al*. (1991), using the ANCOM$^{200/220}$ Fibre Analyser (ANCOM Technology Corp., Fairport, NY, USA).

The experimental procedures for the analyses were as follows: Firstly weigh the filter bag (W1) and zero the balance. Approximately 0.45 to 0.55 g of the prepared sample (W2) is weighed directly into the filter bag. By using a heat sealer, the upper edge of the filter bag is completely sealed, within 4 mm of the top. Weigh one blank bag and include in the run, to determine the blank bag correction. Insert the bag suspender with bags into the fibre analyzer vessel and place a weight on top - to keep it submerged. Add 100 ml/bag of neutral detergent (ND) (use minimum of 1500 ml to ensure bag suspender is submerged). Add 20 g (0.5 g/50 ml of ND solution) of sodium sulphite and 4.0 ml of alpha-amylase to the solution in the vessel. Turn, agitate and heat, setting the timer for 75 min, and close the lid. At end of extraction, turn off the heat and agitator. Open the drain valve (slowly at first), and empty hot solution before opening the lid. After the solution has been drained, close the exhaust valve and open the lid. Add 1900 ml rinse water (70 - 90°C) and 4.0 ml alpha-amylase to the first and second rinses. Turn, stir and rinse for 5 min. The lid may be sealed with the heat on, or left open with the heat off. Repeat the hot water rinses for a total of three times. When the rinsing process is complete, remove the samples and gently press out excess water from the bags. Place bags in a 250 ml beaker and add enough acetone to cover the bags and soak for 3 - 5 min. Remove bags from acetone and place on a wire screen to air-dry. Completely dry the bags in an oven at 102 ± 2°C for 4h, until a constant weight. Remove bags from oven, place directly into a collapsible desiccant pouch and flatten to remove the air. Cool to ambient temperature and weigh bags (W3).

$$\% \text{ NDF (as-received basis)} = \frac{[W3 - (W1 \times C1)]}{W2} \times 100$$

W1 = Bag tare weight (g)

W2 = Sample weight (g)

W3 = Dried weight of bag with fibre after extraction process (g)

C1 = Blank bag correction (running average of final oven-dried weight divided by the original blank bag weight)

3.2.2.6 Gross energy (GE)

A Gallencamp adiabatic bomb calorimeter (CP 400) was used in the determination of the GE values of all samples and the bomb was standardised using benzoic acid. Approximately 0.5 - 0.7 g of each sample was weighed accurately and placed in a steel crucible. A platinum wire (5 cm long) was connected to the electrodes of the bomb calorimeter, the sample carefully placed inside the vessel, where after the bomb was filled with oxygen to a pressure of 3000 Kpa. The temperature of the bomb was then reduced to the temperature of the instrument by means of water cooling. The bomb was then placed inside the instrument, the weight of the sample entered and the GE was determined by the combustion method. Gross energy is expressed as mega joules per kilogram (MJ/kg) (AOAC, 2000).

3.2.2.7 Ether extract (EE)

The EE content of the feed and feed refusal samples were measured according to the AOAC official method 920.39 (AOAC, 2000).

The EE fraction of samples was calculated as follows:

$$\text{EE (g/kg DM)} = \frac{[\text{Dry flask weight (g)} + \text{EE (g DM)}] - [\text{Dry flask weight (g)}]}{\text{Weight of sample (g DM)}} \times 1\,000$$

3.2.2.8 Calcium

The calcium content of the feed samples was measured according to the AOAC official method 935.13 (AOAC, 2000).

3.2.2.9 Phosphorous

The phosphorus content of the feed samples was measured according to the AOAC official method 965.17 (AOAC, 2000).

3.2.3 Statistical analysis

Data was statistically analysed according to a fully randomized design and tested for significant differences using the PROC ANOVA procedures of the SAS programme (1999). Treatment means were compared using a one-way ANOVA model for all data collected. Tukey's honest significant difference (HSD) test of multiple comparisons was used to identify significant differences ($P<0.05$) between treatment means.

3.3 Results and Discussions

3.3.1 Chemical composition of the experimental diets

The analysed chemical composition of the experimental diets containing different ionophores is presented in Table 3.3. Chemical analysis of the experimental diets (Table 3.3) indicated higher CP values than those calculated in Table 3.1. The CP differed from the calculated values with 8.1, 7.6, 9.8 and 4.9% for the diets containing no ionophore (Ct), monensin (M), lasalocid (L) and salinomycin (Sl), respectively. In contrast, the NDF and Ca-content of the mixed diets (Table 3.3) analysed lower than anticipated (Table 3.1). The NDF values differed from the calculated values with 37.2, 17.5, 22.4 and 14.0 % for the diets containing no ionophore (Ct), monensin (M), lasalocid (L) and salinomycin (Sl), respectively.

Table 3.3 Mean DM and chemical composition (%) of the four experimental diets used during the experimental period

	Treatment diets*			
Parameter	**Ct**	**M**	**L**	**Sl**
Chemical composition (dry matter basis):				
Dry matter (%)	82.55	85.52	84.90	84.43
Crude protein (%)	17.73	17.65	18.00	17.21
Energy (MJ GE/kg)	17.15	19.81	18.47	17.37
Neutral detergent fibre (%)	13.05	15.23	14.63	15.70
Ether extract (%)	3.70	3.60	3.90	3.70
Ash (%)	7.13	6.61	6.65	6.35
Calcium (%)	0.76	0.89	0.91	0.80
Phosphorous (%)	0.22	0.24	0.24	0.24

* Treatments: Ct = Control (no ionophore included); M = Monensin (16.5 mg active ingredient/kg feed); L = Lasalocid (33.0 mg active ingredient/kg feed); Sl = Salinomycin (17.5 mg active ingredient/kg feed).

The different actual and calculated CP and NDF values of the experimental diets could be attributed to the fact that it was not practically possible to determine the nutrient concentrations of the feed ingredients, especially lucerne hay, by means of chemical analysis before diet formulation. The lucerne hay used in the present study recorded an average CP content of 21.4% and NDF content of 50.2%. The CP content of the lucerne hay used in this study was higher than the average values given by McDonald *et al*. (2002) of 17.1%, and practically the same NDF content of 49.5%. The HPC used in the present study recorded an average dry matter CP and NDF content of 39.8 and 23.2%, respectively. These values did not indicate a large difference compared to the average values given by the feed manufacturer of 37.9 and 24.1%, on a DM basis, respectively. The maize meal used in the present study had an average dry matter CP content of 9.9%. The CP content of the maize meal used in this study was the same as the average value quoted by McDonald *et al*. (2002).

Roughage sources contribute mainly to nutrient variation, as large differences are found in the nutrient densities within the same roughage due to various factors such as locality, climate, soil and production practices. Scholtz (2001) indicated a NDF variation for *Medicago sativa* hay between 26.70 to 69.82% cultivated in South Africa. The NDF content contained in the bagasse (by-product of the sugar cane industry) of the HPC could also be affected by the locality, climate, soil and production practices. However, this could only hold truth if the primary batch of bagasse was acquired from a different locality (farmer), since all the treatments contained the same inclusion level (20%). Lucerne hay has also been reported to contain between 15 to 22% CP (Hanson *et al*., 1988). This variation in nutrient densities differ from the average used in diet formulations and explains the higher or lower chemical values indicted in the experimental diets. Premixing of all the lucerne hay before mixing the individual diets did not occur for practical and mechanical reasons and the quality of the hay may have differed between the different bales used. However, in this trial, the hay was obtained from a single source and baled at one location from the same cut.

Although feed ingredient inclusion was constant across all treatments, the NDF fractions varied with 20% between the control and salinomycin treatment. In addition, the control diet had the lowest NDF, Ca and P content, and the highest ash content (which should have been reflected in the Ca and P level of the diet). This variation in chemical content could be ascribed to sampling error, or soil contamination of the luserne hay used in this study.

It has also to be kept in mind that the methods used to determine the chemical composition of the treatment diets used in the present study may differ from that used by the feed manufacturer - which may explain part of the chemical differences mentioned above.

3.3.2 Production performance of lambs

3.3.2.1 Feed and chemical constituent intake

The mean (± SD) feed and chemical constituent intake of lambs fed diets containing different ionophores is shown in Table 3.4. No differences (P>0.05) were recorded regarding the DM feed intake between treatments (Table 3.4). Animals receiving the control treatment tended to show the lowest DMI for the duration of the study, compared to the rest of the treatments. This was however reflected in the final live weight of the lambs (Table 3.5). The control diet produced the lightest lambs (P>0.05), whereas salinomycin and lasalocid recorded on average the heaviest lambs. The same results were obtained by Zinn & Borques (1993), who also found no effect regarding the DMI of steers fed a monensin containing diet. Wessels (1993) and Price *et al*. (2009) concluded that the effect of ionophores on DMI may be variable, however most research supports the fact that ionophores negatively affect feed consumption (Van Vuuren & Nel, 1983; Goodrich *et al*., 1984; Merchen & Berger, 1985).

Table 3.4 Mean (± SD) dry matter feed and chemical constituent intake of lambs fed finishing diets containing different ionophores

	Treatments*				Significance	
Intake (g/day)	**Ct**	**M**	**L**	**Sl**	**P-value**	**CV# (%)**
Dry matter	1428 ± 87.54	1477 ± 76.67	1554 ± 132.37	1563 ± 104.48	0.1543	6.81
CP[1]	252 ± 16	258 ± 14	280 ± 27	269 ± 20	0.1624	7.51
Ash	100 ± 7	95 ± 6	102 ± 10	98 ± 8	0.5479	7.98
OM[2]	1318 ± 82	1370 ± 76	1454 ± 136	1465 ± 110	0.1177	7.41
NDF[3]	658[a] ± 40	728[a] ± 40	890[b] ± 82	1000[b] ± 75	<0.0001	7.59

[a,b] Means in the same row followed by different superscripts differ significantly (P<0.05).

* Treatments: Ct = Control (no ionophore included); M = Monensin (16.5 mg active ingredient/kg feed); L = Lasalocid (33.0 mg active ingredient/kg feed); Sl = Salinomycin (17.5 mg active ingredient/kg feed).

Coefficient of variation.

[1] Crude protein.

[2] Organic matter.

[3] Neutral detergent fibre.

No differences (P>0.05) were also recorded regarding the CP, ash and OM intake, between treatments (Table 3.4). This is clearly reflected by the DMI of the lambs between treatments, as was expected. The NDF intake of the salinomycin and lasalocid treatments were higher (P<0.0001) than that of the control and monensin treatments. This was expected due to the difference in NDF content of the experimental diets (Table 3.3), especially for the salinomycin treatment. Due to the fact that the other parameters were not influenced (P>0.05) by DMI, the variation in NDF intake between the treatments could be ascribed to a combination of the variation in the NDF content of the diets and/or the variation in DMI, although not statistical significant.

Of the ionophore treatments, monensin had the lowest DMI (1477 g/lamb/day), crude protein (258 g/lamb/day) and organic matter (1370 g/lamb/day) intake, although none of them were significant (P>0.05). Monensin also had the lowest NDF intake (P<0.0001) of the ionophore treatments. This could be due to the result of the negative effect monensin has on DMI (Goodrich *et al*., 1984; Beacom *et al*., 1988; Salinas-Chavira *et al*., 2010), or it could be attributed to the fact that the lambs fed the monensin treatment (and control treatment) selectively consumed feed constituents with a lower NDF content.

It would seem from the results of the present study that the different ionophores with their respective inclusion levels in finishing diets did not influence (P>0.05) the DM and chemical constituent intake of the lambs.

3.3.2.2 Weight gain and feed conversion ratio

Figure 3.14 represents the weekly live weight gain of the experimental animals during the trial period.

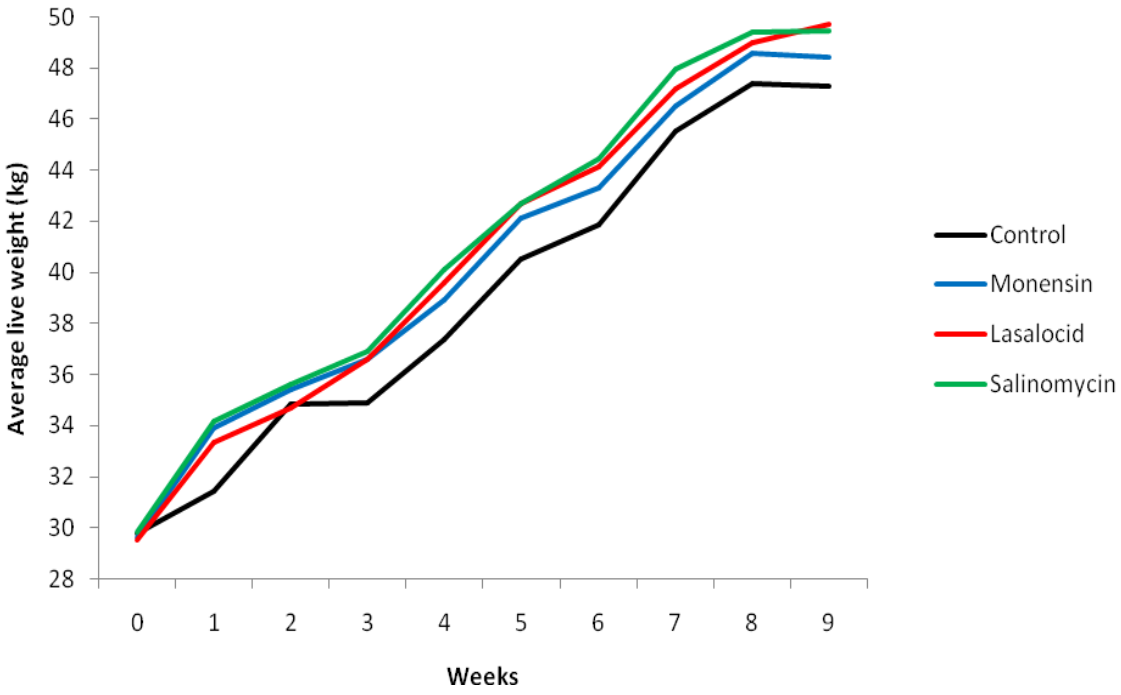

Figure 3.14 Average weekly live body weight (kg) of lambs fed diets containing different ionophores during the experimental period of 63 days (14 day adaptation period included). Day 0 and week 9 (day 63) depict empty stomach body weights.

The effect of dietary ionophore inclusion on lamb weight, ADG and FCR parameters of experimental lambs is presented in Table 3.5. No differences ($P>0.05$) were recorded regarding the final live weight, ADG and feed efficiency between the treatments tested (Table 3.5). Although no differences were observed regarding the production performance of the lambs, the ADG and FCR between the treatments are reflected by the end weight of the same respective treatments. Due to the fact that the DMI of the lambs did not differ between the treatments (Table 3.4), the animals' lack of production response and feed conversion ratio response to the ionophores was expected.

Table 3.5 Mean (± SD) live weight, weight gain and feed conversion ratio of lambs fed finishing diets containing different ionophores

	Treatments*				Significance	
Parameter	**Ct**	**M**	**L**	**Sl**	**P-value**	**CV# (%)**
Start weight[1] (day 0)	29.8 ± 2.53	29.6 ± 2.30	29.6 ± 2.94	29.8 ± 2.58	0.9925	8.73
End weight[1] (day 63)[1]	47.3 ± 3.74	48.4 ± 5.22	49.7 ± 3.76	49.5 ± 4.76	0.4410	9.09
ADG[2]	278 ± 36	298 ± 61	320 ± 38	312 ± 61	0.1452	16.79
FCR[3]	5.15 ± 0.37	4.95 ± 0.18	4.84 ± 0.42	5.03 ± 0.29	0.5336	6.57

* Treatments: Ct = Control (no ionophore included); M = Monensin (16.5 mg active ingredient/kg feed); L = Lasalocid (33.0 mg active ingredient/kg feed); Sl = Salinomycin (17.5 mg active ingredient/kg feed).

Coefficient of variation.

[1] Empty stomach weight (kg).

[2] Average daily gain (g/sheep/day).

[3] Feed conversion ratio (kg DM feed intake/kg live weight gain).

Supportive to the findings of the present study, Funk *et al*. (1986), Nagaraja (1995) and Price *et al*. (2009) reported the overall effectiveness of ionophores to be similar, although the agents may vary, depending on dietary inclusion level, diet composition and various inherent animal factors. Adams *et al*. (1981), Zinn & Borques (1993) and Zinn *et al*. (1994) recorded no effect regarding ADG, DMI and feed efficiency of steers fed a monensin-containing diet. Although monensin may increase FCR and ADG, some researchers mentioned that the effects to be non-significant (Berthiaume *et al*., 2006; Korshidi *et al*., 2008).

Contradictory to the findings in the present study, some researchers found that lasalocid (Funk *et al*., 1986; lambs), salinomycin (Goodrich *et al*., 1984; Merchen & Berger, 1985; Zinn, 1986b; steers) and monensin (Van Vuuren & Nel, 1983; lambs) (Goodrich *et al*., 1984; Merchen & Berger, 1985; steers) inclusion in feedlot diets to significantly improve feed efficiency. Hence, most of the literature sited support the fact that the general effect of ionophores beneficially influences feed efficiency, but with no effect on daily live weight gain (Salinas-Chavira *et al*., 2010). On the other hand, Wessels (1993) found that salinomycin and monensin differ in their effect on ADG and DM feed intake in cattle.

As the different ionophores had no effect ($P>0.05$) on any of the tested parameters, it is of special interest to note the effect of the control diet on the production performance of lambs.

Although the DMI and ADG of the control group were lowest, it did not differ significantly from the ionophore treatments.

It seems from the results of the present trial that the different ionophores with their respective inclusion levels in finishing diets did not influence animal growth performance.

3.3.2.3 Rumen pH

The data in Table 3.6 represents the mean rumen pH (onset, mid and end of study) of lambs fed experimental diets containing different ionophores measured at incremental time intervals (hours) after feeding. Neither ionophore inclusion nor ionophore type had an influence ($P>0.05$) on rumen pH within each time period. This could be ascribed to the fact that the method used to acquire the rumen liquid from each lamb (see paragraph 3.2.1.6) was not effective enough to acquire a representative sample of the rumen content. These findings were in accordance with the performance of the lambs regarding their DMI, growth and feed conversion ratio.

Table 3.6 Mean (± SD) rumen pH of lambs fed finishing diets containing different ionophores, measured at incremental intervals (hours) after feeding

	Treatments*				**Significance**	
Time[1]	**Ct**	**M**	**L**	**Sl**	**P-value**	**CV# (%)**
0 h	6.91 ± 0.27	6.71 ± 0.19	6.78 ± 0.17	6.76 ± 0.18	0.4758	3.06
3 h	6.37 ± 0.26	6.55 ± 0.13	6.20 ± 0.27	6.19 ± 0.31	0.1187	3.94
6 h	6.34 ± 0.26	6.43 ± 0.31	6.30 ± 0.23	6.39 ± 0.24	0.8619	4.10
9 h	6.52 ± 0.33	6.45 ± 0.20	6.41 ± 0.18	6.33 ± 0.23	0.6671	3.81
12 h	6.18 ± 0.13	6.20 ± 0.16	5.94 ± 0.10	6.01 ± 0.27	0.0892	2.94

* Treatments: Ct = Control (no ionophore included); M = Monensin (16.5 mg active ingredient/kg feed); L = Lasalocid (33.0 mg active ingredient/kg feed); Sl = Salinomycin (17.5 mg active ingredient/kg feed).

Coefficient of variation.

[1] Hours after feeding.

A lower rumen pH value was recorded between all the treatments 12 hours after feeding, compared to the 0 h interval before feeding. This could be ascribed to the fact that mastication, due to the feed ingested the previous day, resulted in $NaHCO_3$ being excreted in the saliva, hence increasing the pH of the rumen (Sunagawa *et al*., 2005) at the 0 h interval.

The lower pH after feed ingestion could also be the result of feed fermentation in the rumen resulting in volatile fatty acids (VFA's) being produced, hence lowering the rumen pH (Sunagawa *et al*., 2005; Maheri-Sis & Mirzaei-Aghsaghali, 2011). The ruminal pH value was above 6 in all the diets which indicated a healthy rumen environment. This may be attributed to sufficient fibre (on average 14.65% NDF; Table 3.3) in the diets that was effective in stimulating mastication, which in turn produced adequate saliva to buffer the rumen pH and ensure a healthy rumen environment. On average 10 *l* of saliva (containing phosphates and bicarbonate) is produced per day following mastication in sheep which acts as a buffer (McDonald *et al*., 2002).

As the case in the present study, Patterson *et al*. (1983), Merchen and Berger (1985) and Gonzalez-Momita *et al*. (2009) also found that ruminal fluid pH was not significantly affected by monensin, lasalocid or salinomycin. Bergen & Bates (1984) reported that the intracellular pH of most bacteria appears to be highly regulated and are constant at 7.6 to 7.8, while the pH in the rumen typically ranges from 5.7 to 7.3, with prevailing values being 6.5. In contrast, however, Zinn *et al*. (1993) stated that the ruminal pH tended ($P<0.1$) to be lower with monensin supplementation.

Chow & Russell (1990) explained that the pH could affect the ionophore activity on ruminal bacteria. According to them, lasalocid and monensin for instance influenced *Streptococcus bovis* more at a pH of 5.7, than at a pH of 6.7. This could explain the lack of production response regarding ionophore treatment (Table 3.5) obtained in the present study probably due to the increased propionic acid production resulting from the high energy diet used in the present study.

It would seem from the results of the present study that irrespective of ionophore inclusion, or type of ionophore used in finishing diets or lambs, the rumen pH is not affected ($P>0.05$).

3.3.2.4 Carcass characteristics

The effect of dietary ionophore inclusion on carcass characteristics of experimental lambs is presented in Table 3.7.

Table 3.7 Mean (± SD) carcass characteristics of S.A. Mutton Merino lambs fed finishing diets containing different ionophores

	Treatments*				Significance	
Parameter	**Ct**	**M**	**L**	**Sl**	**P-value**	**CV# (%)**
Cold carcass weight (kg)	23.6 ± 2.0	24.2 ± 2.7	25.2 ± 2.3	24.7 ± 2.7	0.3309	9.87
Fat thickness[1] (mm)	3.90 ± 1.63	3.32 ± 1.06	4.46 ± 1.79	3.52 ± 1.11	0.1632	37.63
Carcass length (cm)	56.7 ± 1.8	57.7 ± 2.5	56.9 ± 2.3	58.1 ± 2.8	0.3662	4.15
SC[2] (cm)	78.3 ± 1.9	77.7 ± 3.0	79.4 ± 2.1	78.7 ± 2.8	0.3296	3.16
BC[3] (cm)	67.1 ± 2.2	66.9 ± 2.8	67.8 ± 3.0	66.5 ± 2.7	0.6425	3.98
Dressing %	50.0 ± 2.0	49.9 ± 1.8	50.7 ± 1.9	49.9 ± 2.0	0.6359	3.82

* Treatments: Ct = Control (no ionophore included); M = Monensin (16.5 mg active ingredient/kg feed); L = Lasalocid (33.0 mg active ingredient/kg feed); Sl = Salinomycin (17.5 mg active ingredient/kg feed).

Coefficient of variation.

[1] Measured between the 12th and 13th rib.

[2] Shoulder circumference.

[3] Buttock circumferences.

The effect of dietary ionophore inclusion on the carcass classification (being an indication of average carcass fatness) of experimental lambs is set out in Table 3.7.

Table 3.8 Carcass classification of S.A. Mutton Merino lambs fed finishing diets containing different ionophores, according to the official sheep carcass classification system [Government legislation no. R 863 (2006)]

	Treatments*			
Parameter # (% of lambs within treatment)	**Ct**	**M**	**L**	**Sl**
A1	-	-	-	-
A2	46.7	33.3	35.7	46.7
A3	46.7	66.7	35.7	53.3
A4	6.6	-	28.6	-
A5	-	-	-	-
A6	-	-	-	-

* Treatments: Ct = Control (no ionophore included); M = Monensin (16.5 mg active ingredient/kg feed); L = Lasalocid (33.0 mg active ingredient/kg feed); Sl = Salinomycin (17.5 mg active ingredient/kg feed).

See Table 3.2.

None of the parameters used in the carcass (Table 3.7) evaluation indicated any statistical differences (P>0.05). No distinct difference, which may be attributed to any of the specific treatments, could be identified within this particular data set.

Lasalocid was responsible for both the highest average fat thickness (4.46 mm) and heaviest carcass weights (25.21 kg) (Table 3.7), although this was not significant. This was also reflected by the carcass grading of the sheep within the same treatment (28.6%; Table 3.8). As in the present study, Clary *et al*. (1993) reported that lasalocid supplementation failed to demonstrate any significant effect on carcass quality or yield. The control group recorded the lightest carcasses, which could be the result of the lower feed intake and performance as described in paragraph 3.3.2.1 and 3.2.2.2, respectively. Monensin resulted in the lowest (P>0.05) fat thickness (3.32 mm) of the ionophore treatments. This could also be the result of the lower (P>0.05) feed and nutrient intake observed in paragraph 3.3.2.1.

Various researchers reported that the carcass characteristics of both feedlot steers and heifers not to be influenced by the dietary inclusion of monensin or salinomycin (Perry *et al*., 1983; Merchen & Berger, 1985; Depenbusch *et al*., 2008) and neither monensin nor lasalocid within feedlot lambs (Delfino *et al*., 1988; Beacom *et al.*, 1988; Murphy *et al*., 2003; Heydari *et al*., 2008; Felix & Loerch, 2011).

In contrast, Zinn & Borques (1993) recorded a significant (P<0.05) increase in fat thickness in a diet containing monensin (33 mg/kg using Holstein steers), compared to the control.

It is evident from most of the literature that dietary ionophore inclusion had either no effect, or variable results regarding its effects on the carcass characteristics of ruminants. It would seem from the results of the present study that the different ionophores with their respective inclusion levels in finishing diets did not affect the carcass characteristics of lambs, especially if compared with the control treatment without any supplementary ionophores.

3.4 Conclusions

It is evident from the results in the present study that the inclusion of ionophores in feedlot finisher diets for lambs had no effect on any of the tested feedlot performance parameters (DMI, ADG and FCR) and carcass characteristics. These results suggest that the overall

effect and type of ionophore, used on lamb performance, is negligible. This lack of response in some of the dietary ionophore supplementations, compared to the control diet, could suggest that the mean registered inclusion levels of the respective ionophores (Act 36/1947) was not adequate to provide a production response. These results are important considering that there is a paucity of research concerning the effect of type of ionophore as a dietary supplement on sheep production performance. The need remains for more research to determine the effect of various ionophore levels on production performance of sheep under feedlot conditions.

CHAPTER 4

THE EVALUATION OF MONENSIN AND SALINOMYCIN AT DIFFERENT INCLUSION LEVELS IN FINISHING DIETS ON THE PERFORMANCE AND CARCASS CHARACTERISTICS OF SA MUTTON MERINO LAMBS

4.1 Introduction

Unanticipated results obtained during the first study (Chapter 3) initiated the planning and development of the current study, whereby the effects of dietary ionophore inclusion levels (low, medium or high) on animal performance and carcass characteristics were evaluated. Due to the lack of literature regarding the effect of dietary ionophore type and subsequently that of ionophore inclusion levels on production performances and carcass composition of intensive fed lambs (as well as the contradictory findings in Chapter 3), the need emerged to determine the effects of ionophore type at the optimal dietary inclusion level in finishing diets of lambs - on certain production and carcass quality parameters.

As monensin, lasalocid and salinomycin had no effect on any of the production and carcass parameters tested in the previous study (Chapter 3), the choice of a single ionophore to evaluate the effect of ionophore dietary inclusion levels on animal performance could be biased. Hence, the objective of this study was to determine the effects of two of the most commonly used ionophores in the animal feed industry (monensin and salinomycin) at the respective minimum, mean and maximum registered inclusion levels – more specifically the effects on certain feedlot performance parameters and carcass characteristics of S.A. Mutton Merino (SAMM) lambs during intensive feeding (similar to a feedlot situation).

4.2 Materials and Methods

This study was conducted between October and November 2008 on the same experimental farm of the University of the Free State, as described in paragraph 3.2 (Chapter 3). The climate during the study period was the normal seasonal occurrence for onset of the summer season. Average temperature fluctuations during the experimental period study were from a minimum of 17°C to a maximum of 30.1°C.

4.2.1 Production study

This study was conducted over a period of 42 days (which included a 14 day adaptation period), to determine the effects of two different ionophores at different inclusion levels, on e.g. dry matter intake (DMI), average daily gain (ADG), feed conversion ratio (FCR) and carcass characteristics of lambs fed under pen conditions. All procedures conducted during this study were approved by the Ethical Control Committee for Animal Experimentation at the University of the Free State (Animal Experiment No. 18/07).

4.2.1.1 Experimental design

This trial layout was according to a complete random design, representing seven dietary treatments (n=12 lambs/treatment) (see paragraph 4.2.1.4 b), subdivided into four replicates per treatment (n=3 lambs/replicate). The method of random allocation of the treatments used in this study reduced the probability of animals of the same treatment being penned next to one another. This decreased the probability that a specific treatment may have been affected either positively or negatively by environmental factors due to pen location and/or housing conditions.

4.2.1.2 Housing

The housing conditions of the lambs used during the present study were identical to that of the previous study, as comprehensively described in paragraph 3.2.1.2 of Chapter 3.

4.2.1.3 Feeding troughs and water buckets

The feeding troughs and water buckets used in the present study was identical to that of the previous study as described in paragraph 3.2.1.3 of Chapter 3.

4.2.1.4 Experimental diets

a) Preparation of the experimental diets

Preparation of the experimental diets used in the present study was the exactly the same as that described in paragraph 3.2.1.4a and b of Chapter 3. The only difference being that the two ionophores were included into the high protein concentrates (HPC) at different inclusion levels, in accordance with their respective registered commercial levels.

b) Physical and chemical composition

The seven dietary treatments consisted of the same iso-nitrogenous and iso-caloric basal diet, differing only in respect to the additive included and the inclusion level i.e. (i) control (Ct) diet (no additive), (ii) monensin at a high inclusion level (MH - 22 mg active ingredient/kg diet), (iii) monensin at a medium (registered) inclusion level (MM - 16.5 mg active ingredient/kg diet), (iv) monensin at a low inclusion level (ML - 11 mg active ingredient/kg diet), (v) salinomycin at a high inclusion level (SH - 20 mg active ingredient/kg diet), (vi) salinomycin at a medium (registered) inclusion level (SM - 17.5 mg active ingredient/kg diet) and (vii) salinomycin at a low inclusion level (SL - 15 mg active ingredient/kg diet) (Table 4.1). The mean registered (Act 36/1947) levels at which monensin (MM - 16.5 mg active ingredient/kg diet) and salinomycin (SM – 17.5 mg active ingredient/kg diet) were included in the diets was used for the calculation of the low and high inclusion levels of the respective ionophores.

Table 4.1 Mean calculated physical and chemical composition (%) of the experimental diets, differing only in ionophore type and -inclusion level

Physical composition (as fed):	**(%)**
Maize meal	65
Lucerne hay	15
High protein concentrate (HPC)[1#]	20
Chemical composition (dry matter basis):	
Crude protein (CP)	16.40
Neutral detergent fibre (NDF)	17.90
Ether extract (EE)	3.67
Ash	6.7
Calcium (Ca)	1.07
Phosphorous (P)	0.30

[1] Commercial high protein concentrate with the following specifications (DM-basis): 37.93% crude protein, 24.14% neutral detergent fibre, 4.25% calcium and 0.36% phosphorous.

[#] Ionophores were mixed into the high protein concentrate according to dietary treatment as follows: Control (no ionophore included); Monensin low (ML; 11 mg active ingredient/kg feed); Monensin medium (MM; 16.5 mg active ingredient/kg feed); Monensin high (MH; 22 mg active ingredient/kg feed); Salinomycin low (SL; 15 mg active ingredient/kg feed); Salinomycin medium (SM; 17.5 mg active ingredient/kg feed); Salinomycin high (SH; 20 mg active ingredient/kg feed).

Ionophores were included beforehand into the HPC mixture at the feed mill by the manufacturer. Accordingly the experimental diet was formulated to obtain maximum live weight gain. The NRC (1985) requirements for finishing lambs in a feedlot were used as guideline.

4.2.1.5 Experimental animals

Eighty-four (84) SAMM whether lambs (30.7 ± 2.7 kg) (± 3 months of age) were randomly allocated to the seven dietary treatments. Animals were selected according to empty stomach body weight (see paragraph 3.2.1.5a; Chapter 3) in order to reduce initial weight variation and to insure the most homogenous group of animals, possible.

All further procedures implemented during the present study such as the preparation of the experimental animals (paragraph 3.2.1.5b), adaptation of the lambs (paragraph 3.2.1.5c) as well as the feeding and daily water supply (paragraphs 3.2.1.5d, e & f) was identical to that discussed in the Materials and Methods section of Chapter 3.

4.2.1.6 Carcass evaluation

The same methods used to evaluate carcasses during the previous trial (paragraph 3.2.1.7) as described in Chapter 3, were also applied during the present study.

4.2.2 Chemical analysis

The diet and feed refusal samples of the present study were analysed according to the same analytic procedures as described in Chapter 3 - for dry matter (paragraph 3.2.2.1), crude protein (paragraph 3.2.2.4), gross energy (paragraph 3.2.2.6), ash (paragraph 3.2.2.2), organic matter (paragraph 3.2.2.3), neutral detergent fibre (paragraph 3.2.2.5), ether extract (paragraph 3.2.2.7), calcium (paragraph 3.2.2.8) and phosphorus (paragraph 3.2.2.9) content.

4.2.3 Statistical analysis

Data was statistically analysed according to a fully randomized design and tested for significant differences using the PROC ANOVA procedures of the SAS programme (1999). Treatment means were compared using a one-way ANOVA model, for all data collected. Tukey's honest significant difference (HSD) test for multiple comparisons was used to identify significant differences ($P<0.05$) between treatment means.

4.3 Results and discussions

4.3.1 Chemical composition of the experimental diets

The analysed chemical composition of the experimental diets containing different ionophores included at three different inclusion levels, is set out in Table 4.2. The chemical analysis of the experimental diets (Table 4.2) indicated a higher crude protein (CP) content (17.1 ± 0.3%) on average, than the formulated diet (16.4%) based on calculated values. The ether extract, Ca and P content of the experimental diets (Table 4.2) compared relatively well with the calculated values (Table 4.1). In contrast to the results of Chapter 3, the neutral detergent fibre (NDF) content of the experimental diets (Table 4.2) recorded values were lower than anticipated (Table 4.1). The average NDF content of the experimental diets (15.1%) differed from the calculated value (17.9%) by 18.4%. However, the standard deviation (± 0.61%) of the analyzed NDF was also the highest of all the nutrients analysed.

Table 4.2 Mean dry matter (DM) and chemical composition (%) of the seven experimental diets, differing with respect to the ionophore type and -inclusion level

	Treatments*						
Parameter	**Ct**	**ML**	**MM**	**MH**	**SL**	**SM**	**SH**
Chemical composition (DM):							
Dry matter (%)	89.12	89.13	88.94	88.98	89.07	89.02	88.96
Crude protein (%)	17.44	16.85	17.19	16.60	17.10	17.34	17.24
Energy (MJ GE/kg)	18.28	18.29	18.54	18.58	18.30	18.44	18.15
Neutral detergent fibre (%)	15.84	14.85	15.16	15.56	15.69	14.41	14.32
Ether extract (%)	3.6	3.4	3.4	3.1	3.6	3.5	3.4
Ash (%)	6.61	6.63	6.02	6.35	6.31	6.34	6.97
Calcium (%)	1.10	1.12	1.16	1.32	1.07	1.20	1.30
Phosphorous (%)	0.25	0.23	0.23	0.23	0.23	0.23	0.24

* Treatments: Ct = Control (no ionophore included); ML = Monensin low (11 mg active ingredient/kg feed); MM = Monensin medium (16.5 mg active ingredient/kg feed); MH = Monensin high (22 mg active ingredient/kg feed); SL = Salinomycin low (15 mg active ingredient/kg feed); SM = Salinomycin medium (17.5 mg active ingredient/kg feed); SH = Salinomycin high (20 mg active ingredient/kg feed).

If the nutrient composition of the current study is compared with that of the previous study (Chapter 3, Table 3.3), it is of interest to notice that both the average NDF and Ca content of the previous study (14.7% and 0.8%, respectively) was lower than that of the present study

(15.1% and 1.2%, respectively). This could be ascribed to the variation in the chemical composition of the lucerne hay, due to large differences recorded in the nutrient density of roughage sources. Factors such as locality, climate, soil type and fertility, production practices and cultivar differences may all contribute to the differences in nutrient composition of roughage sources. The NDF content contained in the bagasse (by-product of the sugar cane industry) of the HPC could also be affected by the locality, climate, soil and production practices. As in the previous study, remixing of all the lucerne hay before mixing the individual diets did not occur during the present study. Due to practical and mechanical reasons and the quality of the hay, lucerne may have differed between the different bales used.

4.3.2 Production performance of lambs

4.3.2.1 Feed and chemical constituent intake

The mean (± SD) feed and chemical constituent intake (g/animal/day) of lambs fed diets containing dissimilar ionophores at three different inclusion levels is set out in Table 4.3.

As in the previous study, no differences (P>0.05) were recorded regarding the dry matter intake (DMI) and organic matter intake (OMI) between the treatments (Table 4.3). This is in agreement with Merchen and Berger (1985), who found that salinomycin included at levels of 5.5, 11 or 22 mg/kg also did not affect (P>0.05) the DMI in sheep. Animals receiving the control treatment tended to demonstrate the highest (P>0.05) DMI for the duration of the study, compared to the rest of the treatments. This was however not reflected in the final live weight of the lambs (see Table 4.4). Zinn & Borques (1992) also found no effect (P>0.05) regarding the DMI of steers fed a monensin containing diet. Again, the effect of ionophores on DMI may be variable (salinomycin vs. monensin) (Wessels, 1993). However most research supports the fact that ionophores negatively affects feed consumption (Van Vuuren & Nel, 1983; Goodrich *et al*., 1984).

Table 4.3 Mean (± SD) feed and chemical constituent intake (g DM/animal/day) of lambs fed finishing diets differing only in ionophore type and -inclusion level

		Treatments*						Significance	
Intake (g/day)	**Ct**	**ML**	**MM**	**MH**	**SL**	**SM**	**SH**	**P-value**	**CV# (%)**
Dry matter	1442 ± 100	1363 ± 48	1334 ± 43	1330 ± 141	1432 ± 45	1409 ± 103	1423 ± 86	0.36	6.33
CP[1]	454[a] ± 32	412[ab] ± 14	405[ab] ± 13	377[b] ± 39	452[a] ± 13	420[ab] ± 30	441[a] ± 27	0.003	6.12
Ash	95[ab] ± 7	90[abc] ± 3	79[c] ± 3	83[bc] ± 9	90[abc] ± 3	89[abc] ± 7	99[a] ± 6	0.002	6.50
OM[2]	1347 ± 94	1273 ± 45	1254 ± 40	1246 ± 132	1343 ± 42	1320 ± 96	1324 ± 81	0.42	6.32
NDF[3]	229 ± 16	203 ± 7	203 ± 6	208 ± 22	225 ± 7	203 ± 15	204 ± 12	0.034	6.30

[a,b] Means within the same row with different superscripts differ significantly (P<0.05).

* Treatments: Ct = Control (no ionophore included); ML = Monensin low (11 mg active ingredient/kg feed); MM = Monensin medium (16.5 mg active ingredient/kg feed); MH = Monensin high (22 mg active ingredient/kg feed); SL = Salinomycin low (15 mg active ingredient/kg feed); SM = Salinomycin medium (17.5 mg active ingredient/kg feed); SH = Salinomycin high (20 mg active ingredient/kg feed).

#Coefficient of variation.

[1]Crude protein.

[2]Organic matter.

[3]Neutral detergent fibre.

Merchen & Berger (1985) reported that salinomycin at a 5.5, 11, 22 or 33 mg/kg inclusion level in feedlot steer diets resulted in a decreased ($P<0.05$) feed intake. In another study, Merchen & Berger (1985) stated that salinomycin inclusion levels of 5.5, 11, 16.5 or 22 mg/kg, or monensin at 22 mg/kg in feedlot steers, did not affect feed intake at all. Although the NDF intake differed ($P<0.05$) between treatments (Table 4.3), the Tukey-Kramer (HSD) test failed to identify the differences between treatment means - even at a relatively low coefficient of variation (6.3%). The variation within a specific treatment, as measured by the standard deviation, may be responsible for the failure of the HSD test to indicate treatment differences. However, as the NDF intake does not follow similar trends as the DM and OM intake of the respective treatments, this data must be interpreted with caution. The dietary selection could possibly be a major determinant for the variation in NDF intake.

In Figure 4.1, the average daily DM intake of the different treatments during the experimental period is illustrated graphically. Although not significant ($P>0.05$), it would seem that sheep fed the salinomycin treatment (irrespective of the inclusion levels), have a higher DM intake on average, than those in the monensin treatment group. Consequently, this higher ($P>0.05$) DM intake of the salinomycin treatment is also reflected in the OM (non-significant) and the crude protein ($P<0.01$) intake. Since this higher DM ($P>0.05$) and CP ($P<0.01$) intake of the salinomycin treatments did not result in a subsequent significant improvement of production performances (Table 4.4), the usage of salinomycin, compared to monensin, would result in a less efficient ionophore option for dietary inclusion in feedlot lambs - from a producers perspective.

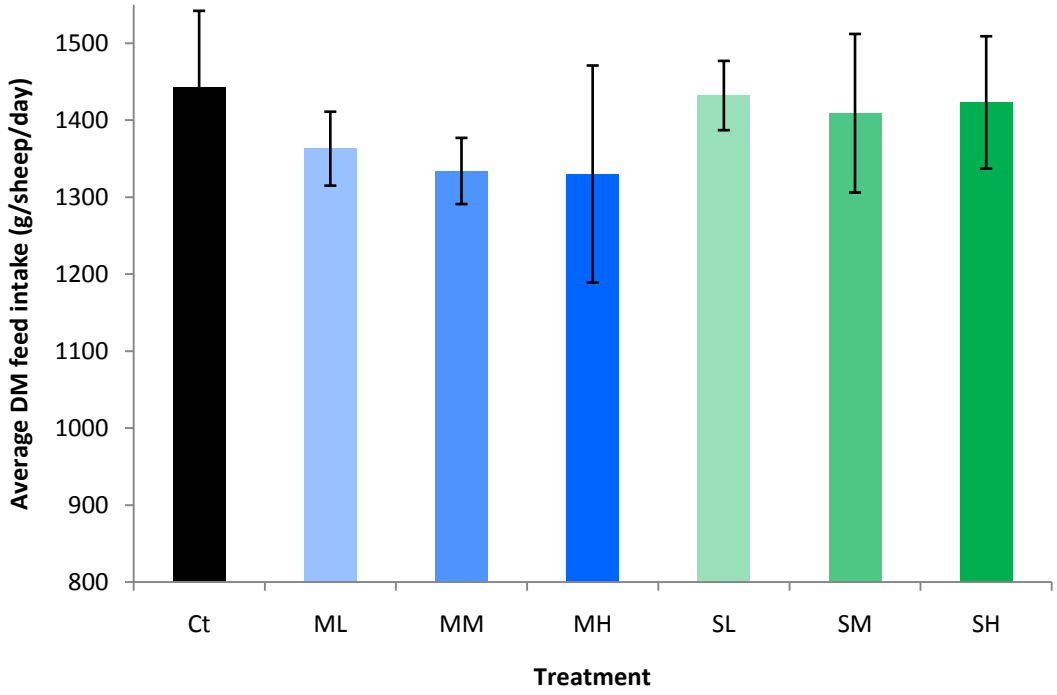

Figure 4.1 Mean (± SD) DM feed intake (g/day) of lambs fed the experimental diets for a 42 day period. [Treatments: Ct = Control (no ionophore included); ML = Monensin low (11 mg active ingredient/kg feed); MM = Monensin medium (16.5 mg active ingredient/kg feed); MH = Monensin high (22 mg active ingredient/kg feed); SL = Salinomycin low (15 mg active ingredient/kg feed); SM = Salinomycin medium (17.5 mg active ingredient/kg feed); SH = Salinomycin high (20 mg active ingredient/kg feed)].

The monensin high treatment recorded the lowest ($P<0.01$; Table 4.3) CP intake, compared to that of the control, the salinomycin low and salinomycin high groups. This could partly be explained by the CP content of the MH (monensin high) treatment that was analyzed to be the lowest (16.6%) (Table 4.1). However, the low CP intake of the MH treatment did not influence any of the production parameters (Table 4.4) in a negative manner. Goodrich *et al*. (1984), Beacom *et al*. (1988) Casey *et al.* (1994) and Salinas-Chavira *et al*. (2010) stated monensin to negatively affect the feed intake of ruminants. Although this phenomenon was not reflected by the DMI between the treatments for the present study, it could hold some truth for the CP intake. Animal nutritionists should give this consideration during dietary ionophore selection.

The MM (monensin medium) treatment had a significant ($P<0.01$) lower ash intake, when compared to that of the control and the SH (salinomycin high) treatment (Table 4.3). This was however neither reflected in the ash content of the experimental diets (Table 4.2), nor the DMI (Table 4.3) of the lambs. This could probably be explained by the selective feeding behaviour of lambs - maybe selecting the more palatable HPC consisting of molasses with its higher ash (10% on a DM-basis) (McDonald *et al*., 2002) and CP (Table 4.1) content. This is reflected by both the CP and ash intake (Table 4.3) of the lambs and partly by the NDF intake (although non-significant). It should also to be kept in mind that sampling error for analytical purposes could also pose a threat for accurate results.

It is of interest to note that the ash intake was not affected by ionophore inclusion in the previous study ($P<0.05$), whereas the NDF intake again differed significantly ($P<0.0001$) (Chapter 3; paragraph 3.3.2.1 Table 3.4). This variation between results of the current two trials seems to support that of other authors (Bergen & Bates, 1984; Funk *et al*., 1986; Nagaraja, 1995) regarding the disparities between studies in terms of ionophore type and inclusion levels. This suggests that future research needs to continue in attempts to address this issue.

It can be concluded from Table 4.3 and Figure 4.1 that higher levels of monensin inclusion in sheep finisher diets affects feed and chemical constituent intake negatively, although the differences were not significant ($P>0.05$). It would seem from the results of the present study that although different ionophores and ionophore inclusion levels did not influence the DM and chemical constituent intake of the lambs significantly, the use of dietary ionophores do depress ($P>0.05$) these parameters when compared to the control diet (no ionophore inclusion).

4.3.2.2 Body weight gain and feed conversion ratio

Figure 4.2 represents the average weekly live body weight gain of the experimental animals during the trial period.

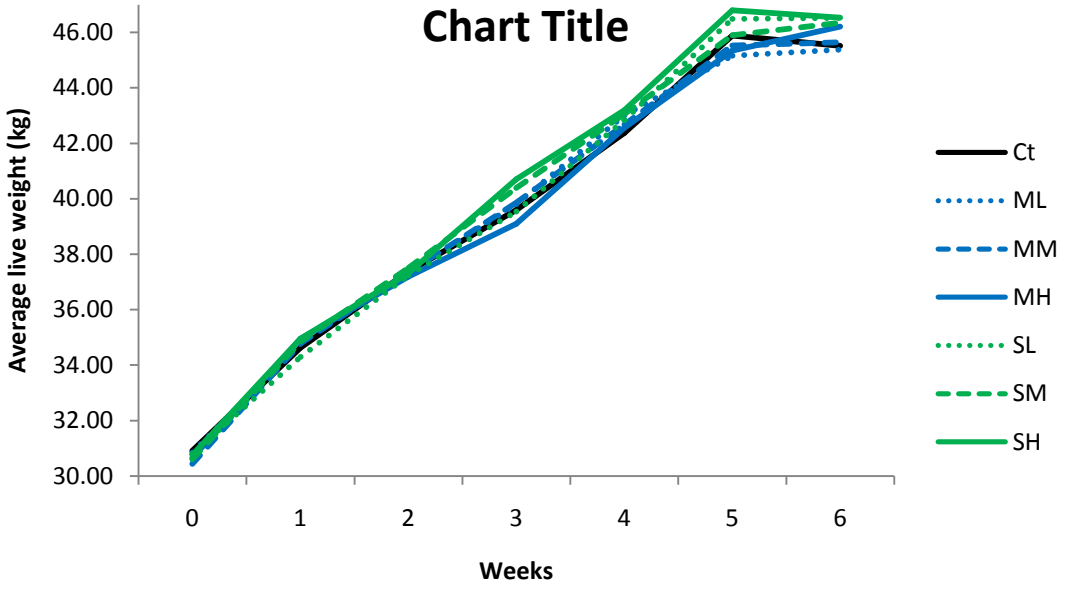

Figure 4.2 Average weekly live body weight (kg) of lambs fed the experimental diets during the 42 day trial period (14 day adaptation period included). [Treatments: Ct = Control (no ionophore included); ML = Monensin low (11 mg active ingredient/kg feed); MM = Monensin medium (16.5 mg active ingredient/kg feed); MH = Monensin high (22 mg active ingredient/kg feed); SL = Salinomycin low (15 mg active ingredient/kg feed); SM = Salinomycin medium (17.5 mg active ingredient/kg feed); SH = Salinomycin high (20 mg active ingredient/kg feed)]. Day 0 and week 6 (day 42) depict empty stomach body weights.

The effect of dietary ionophore inclusion at different inclusion levels on lamb weight and production parameters of the experimental lambs is set out in Table 4.4.

No significant differences (P>0.05) were recorded regarding the final live weight, ADG and feed conversion efficiency between the treatments tested (Table 4.4). This was reflected in the weekly live weights (Figure 4.2) recorded during the trial period. Although no differences were observed regarding the production performance of the lambs, the ADG between the treatments are reflected by the end body weight of the same respective treatments. Due to the fact that DMI of the lambs did not differ (P>0.05) between treatments (Table 4.3), the animals' lack of production response in terms of ADG and FCR was anticipated. Supportive to the findings of the present study, Zinn (1986a) reported that salinomycin (included at 5.5, 11, 16.5, or 22 mg/kg) did not improve the ADG in feedlot steers and heifers.

Table 4.4 Mean (± SD) live weight (begin and end of study), average daily weight gain and feed conversion ratio of lambs fed finishing diets, differing in ionophore type and -inclusion level

		Treatments*						Significance	
Parameter	**Ct**	**ML**	**MM**	**MH**	**SL**	**SM**	**SH**	**P-value**	**CV# (%)**
Start weight[1] (kg)	30.92 ± 2.33	30.84 ± 3.01	30.90 ± 2.55	30.66 ± 2.95	30.80 ± 2.64	31.11 ± 2.63	30.77 ± 2.82	0.99	8.81
End weight[1] (kg)	45.52 ± 3.99	45.38 ± 4.29	46.57 ± 5.06	46.21 ± 3.74	46.53 ± 3.04	48.10 ± 3.54	46.53 ± 3.99	0.77	8.60
ADG[2]	347.62 ± 67.80	346.03 ± 52.44	373.16 ± 81.33	370.24 ± 37.80	374.40 ± 28.86	404.52 ± 39.08	375.40 ± 49.84	0.20	14.52
FCR[3]	4.34 ± 0.5	4.24 ± 0.29	4.03 ± 0.45	3.90 ± 0.15	4.03 ± 0.16	4.01 ± 0.13	4.02 ± 0.14	0.43	7.32

* Treatments: Ct = Control (no ionophore included); ML = Monensin low (11 mg active ingredient/kg feed); MM = Monensin medium (16.5 mg active ingredient/kg feed); MH = Monensin high (22 mg active ingredient/kg feed); SL = Salinomycin low (15 mg active ingredient/kg feed); SM = Salinomycin medium (17.5 mg active ingredient/kg feed); SH = Salinomycin high (20 mg active ingredient/kg feed).

#Coefficient of variation.

[1] Empty stomach weight.

[2] Average daily gain (g/sheep/day).

[3] Feed conversion ratio (kg DM feed intake/kg live weight gain.

Supportive to the findings of the present study, Bergen & Bates (1984), Funk *et al*. (1986) & Nagaraja (1995) reported the overall effectiveness of ionophores to be similar. It was also mentioned that the animal's performance could be affected if the ionophores varied - depending on the dietary inclusion level, the diet composition and various inherent animal factors. However, this was not the case in the present study. Again, the inclusion of ionophores seemed to have no significant effects on animal production (Adams *et al*., 1981; Zinn & Borques, 1993; Zinn *et al*., 1994) with diets containing high levels of readily fermentable carbohydrates (as was the case in the present study). Ionophores generally depress feed intake, without a negative effect on body weight gain, and an improvement in feed conversion ratios (Bergen & Bates, 1984).

In contradiction with findings of the present study, some researchers have reported that salinomycin (Goodrich *et al*., 1984; Merchen & Berger, 1985) and monensin (Van Vuuren & Nel, 1983; Goodrich *et al*., 1984; Merchen & Berger, 1985) inclusion in feedlot diets significantly improved feed efficiency. Zinn (1986a) also found that salinomycin included at 11, 16.5 or 22 mg/kg all improved ($P<0.05$) feed conversion in feedlot steers and heifers. Differences in the results between the current and past studies regarding the effect of ionophores on feed efficiency could partly be ascribed to differences between species (sheep vs. cattle), genotype and age of animal, as well as dietary composition in terms of fibre, starch and sugar components.

From the results of the present study, it would seem that different ionophores and inclusion levels, representing the minimum, mean and maximum dietary inclusion levels of ionophores in finishing diets of lambs, did not influence animal performance significantly.

4.3.2.3 Carcass characteristics

The effect of different dietary ionophore inclusion levels on carcass characteristics of experimental lambs is presented in Table 4.5. None of the parameters used for carcass evaluation indicated any significant differences ($P>0.05$) between treatments. Subsequently, no distinct differences, attributed to any of the specific treatments, could be identified within this particular data set.

Although not significant, all three the salinomycin treatments (Table 4.5) recorded heavier carcass weights than that of the monensin and control treatment groups. This was expected

due to the higher (P>0.05) live body weight at termination of the study and ADG of the salinomycin treatments (SL, SM and SH), as reflected in Table 4.4.

The control treatment resulted in the highest (P>0.05) average fat thickness (4.48 mm) (Table 4.5). However this was not reflected by the carcass weights as the SH treatment had on average the heaviest carcasses (22.6 kg), with the highest dressing percentage (48.4%). These results suggest that back fat thickness is a poor indication of carcass weight, and that dietary ionophore inclusion resulted in improved protein, and decreased lipid carcass deposition. The carcass classification data set out in Table 4.6 did not reflect the measured carcass data in terms of back fat thickness as indicated in Table 4.5. As mentioned, the lowest average back fat thickness (3.7 mm) was obtained in the SM treatment group (Table 4.5). However, according to results presented in Table 4.6, the ML treatment recorded the most carcasses (41.7%) graded as A2 (an average of 1-3 mm fat). These controversial findings regarding data on back fat thickness (Table 4.5) and carcass classification (Table 4.6) could be attributed to differences in the measurement thereof - suggesting that the data in Table 3.6 should be interpreted and used with care, due to its subjective nature.

Table 4.5 Mean (± SD) carcass characteristics of S.A. Mutton Merino lambs fed finishing diets, differing in ionophore type and -inclusion level

		Treatments*						Significance	
Parameter	**Ct**	**ML**	**MM**	**MH**	**SL**	**SM**	**SH**	**P-value**	**CV# (%)**
Cold carcass weight (kg)	21.63 ± 2.13	21.82 ± 1.75	21.75 ± 2.65	21.69 ± 2.17	22.10 ± 1.58	22.13 ± 2.90	22.62 ± 2.05	0.95	10.11
Avg. fat thickness[1] (mm)	4.48 ± 1.39	4.07 ± 1.12	4.18 ± 1.15	4.07 ± 1.33	4.07 ± 1.02	3.70 ± 0.89	4.41 ± 1.16	0.74	28.03
Carcass length (cm)	57.08 ± 1.49	57.54 ± 1.84	58.38 ± 2.30	57.58 ± 2.29	58.39 ± 2.79	57.96 ± 1.70	58.86 ± 1.66	0.41	3.55
SC[2] (cm)	75.71 ± 2.47	76.08 ± 1.74	76.54 ± 3.04	75.59 ± 2.77	76.17 ± 1.47	75.83 ± 3.34	76.45 ± 2.56	0.96	3.37
BC[3] (cm)	63.08 ± 1.82	63.67 ± 1.93	64.08 ± 2.65	63.72 ± 2.23	63.42 ± 2.08	63.46 ± 3.70	64.09 ± 2.70	0.95	3.96
Dressing %	47.51 ± 1.48	48.16 ± 1.36	47.70 ± 1.29	46.81 ± 1.62	47.50 ± 1.13	47.68 ± 1.68	48.42 ± 1.29	0.20	2.98

*Treatments: Ct = Control (no ionophore included); ML = Monensin low (11 mg active ingredient/kg feed); MM = Monensin medium (16.5 mg active ingredient/kg feed); MH = Monensin high (22 mg active ingredient/kg feed); SL = Salinomycin low (15 mg active ingredient/kg feed); SM = Salinomycin medium (17.5 mg active ingredient/kg feed); SH = Salinomycin high (20 mg active ingredient/kg feed).

#Coefficient of variation.

[1] Measured between the 12th and 13th rib.

[2] Shoulder circumferences.

[3] Buttock circumferences.

Table 4.6 Carcass classification of S.A. Mutton Merino lambs fed finishing rations containing ionophores at different inclusion levels according to the official sheep carcass classification system [Government notice no. R 863 (2006)]

	Treatments[*] (% of lambs within a treatment)						
Parameter	**Ct**	**ML**	**MM**	**MH**	**SL**	**SM**	**SH**
A1							
A2	25.0	41.7	25.0	25.0	33.3	33.3	33.3
A3	58.3	33.3	33.3	41.7	25.0	50.0	58.3
A4	16.7	25.0	41.7	33.3	41.7	16.7	8.4
A5							
A6							

[*]Treatments: Ct = Control (no ionophore included); ML = Monensin low (11 mg active ingredient/kg feed); MM = Monensin medium (16.5 mg active ingredient/kg feed); MH = Monensin high (22 mg active ingredient/kg feed); SL = Salinomycin low (15 mg active ingredient/kg feed); SM = Salinomycin medium (17.5 mg active ingredient/kg feed); SH = Salinomycin high (20 mg active ingredient/kg feed).

The results of various researchers indicate that carcass characteristics of feedlot steers and heifers (Perry *et al*., 1983; Beacom *et al.*, 1988; Depenbusch *et al*., 2008; Felix & Loerch, 2011), as well as feedlot lambs (Delfino *et al*., 1987; Murphy *et al*., 2003; Heydari *et al*., 2008) are not influenced by the dietary inclusion of ionophores (monensin and/or salinomycin). Also, Merchen & Berger (1985) found that carcass characteristics of feedlot steers were not affected by salinomycin inclusion levels of 5.5, 11, 16.5, 22 or 33 mg/kg, or monensin at a 22 mg/kg inclusion level. Results of the present study are supportive to these findings and suggest that neither ionophore type nor inclusion level results in significant improvements of carcass characteristics in lambs. However, it must also be kept in mind that ionophores (especially monensin) may increase the fat thickness of ruminants as recorded by Zinn & Borques (1992). Another aspect that needs consideration during the selection of ionophore type and/or inclusion level is the variation in terms of inherent animal and dietary factors that could affect these results (Nagaraja, 1995).

As discussed in Chapter 3, most of the literature has stated that dietary ionophore inclusion had either no effect, or variable results regarding its effects on the carcass characteristics of ruminants. Results in the present study seem to be supportive of these findings on carcass characteristics. Firstly, since ionophore type as well as inclusion levels fail to provoke response differences in treatment means. Secondly, since no definite trend regarding dietary ionophore inclusion levels on carcass characteristics could be observed.

4.4 Conclusions

It is evident from results of the present study that dietary treatment with ionophores at the recommended registered minimum, mean and maximum dietary inclusion levels in feedlot finisher diets for lambs had no effect on any of the feedlot performance parameters (DMI, ADG and FCR) and carcass characteristics tested. These results suggest that the overall effect of ionophore type and inclusion level on lamb performance is negligible. This lack of response in dietary ionophore supplementation could suggest that none of the inclusion levels (minimum and maximum), compared to the mean registered level of each respective ionophore (Act 36/1947) were adequate to provide any production response. As the ionophore inclusion level again had no effect on lamb feedlot performance, it can be concluded that formulating diets with any ionophore included will pose no additional benefits to the feedlot operator. These results are important, considering that there is a scarcity of documented literature concerning the effect of ionophore type on sheep production performance. On the other hand, care should be taken that ionophores are also included in feedlot diets for their additional benefits in terms of ruminal acidosis, feedlot bloat and coccidiosis. In general, although the ionophore treatments (both type and inclusion levels) failed to result in significant differences, it could be suggested that these rumen modifiers do have a beneficial (and limited) response in feedlot lambs, when compared to the control treatment.

CHAPTER 5

GENERAL CONCLUSIONS

Results of this study indicate that the inclusion of different dietary ionophores, even at altered inclusion levels in feedlot finisher diets for lambs, had generally no ($P>0.05$) effect on any of the tested performance parameters (DMI, ADG and FCR) as well as carcass characteristics (dressing percentage, back fat thickness and carcass measurements). This lack of response in dietary ionophore supplementation could suggest that none of the inclusion levels, compared to the mean registered level of each respective ionophore (Act 36/1947), was adequate to provide any added production response. Even though both ionophore type and -inclusion levels failed to provoke significant differences between treatments, the dietary inclusion of ionophores did result in a limited response ($P>0.05$) in terms of production performances of lambs. However, these results were not statistical significant and should therefore be interpreted with care. Generally, the good health of lambs during the experimental period, as well as good adaptation practices of the animals to the feedlot comparable diets, may have limited the possible beneficial effects of ionophores on production and carcass traits measured during the present trial.

The chemical constituent intake was largely unaffected, but it is of interest to note that the NDF intake in the first study was affected ($P<0.0001$) by ionophore type during the first study (Table 3.4; Chapter 3). However, the most reasonable explanation for differences in NDF intake, as reported in Chapter 3, was due to differences in dietary NDF content (Table 3.3). Dietary differences in terms of the formulated diets (Table 3.1) and the chemical analyzed diets (Table 3.3) could most probably be ascribed to variation in roughage sources (both lucerne and bagasse), or mixing and sampling errors.

In the second study, different dietary ionophore inclusion levels only affected the CP ($P<0.01$) and ash ($P<0.01$) intake (Table 4.4), whereas monensin included at 22 mg/kg (MH treatment) resulted in the lowest CP (377 g/animal/day; $P<0.01$) and ash (83 g/animal/day; $P<0.01$) intake of all the ionophore treatments. Irrespective of the fact that the ionophore inclusion level had no effect ($P=0.36$) on the DMI of the animals. Similar to the previous explanation, the variation in CP and ash content of the experimental diets (Table 4.2), in

combination with the lowest (not significant) DMI (Table 4.3) of the MH treatment, could be responsible for these results. However, these differences in results (Table 3.4; 4.3) were not reflected in the growth and feed conversion ratios (Tables 3.5; 4.4) of the lambs. Although not statistically significant, the overall results regarding the DMI of animals on the three different inclusion levels of salinomycin during the second study, were on average higher than that of monensin. Which was also reflected by the respective carcass weights within the treatments. However, this tendency was not similarly reflected in any of the production responses (Table 4.4) of the animals. These results were expected to a certain extent, as most of the literature proposes that monensin lowers feed intake, by maintaining the same ADG compared to other ionophore types.

The lack in production response regarding different dietary ionopores, with their respective minimum, mean and maximum inclusion levels, seems to pose no added benefits to the small stock (lamb) feedlot industry. Although numerical differences could be detected, little statistical differences could be identified. The main focus of feedlot operators should be to make an informed decision if the added benefits (decreasing ruminal acidosis occurrence, feedlot bloat and coccidiosis) of these substances outweigh the cost of its dietary inclusion. These proposed added benefits could pose as a possible explanation for the numerical benefits of some ionophores (lasalocid and salonomycin at registered inclusion levels) as observed in the first study (Chapter 3). However, even with limited significant responses in terms of production and carcass quality enhancement during the present study, these results remain important - considering the paucity of research concerning the effect of ionophores as dietary supplements in sheep production performance. Carcass weight difference might still be of economical value to the producer seeing that lamb is quite expensive in most countries.

Considering the results of the present study, it can be concluded that formulating diets with different dietary ionophores and inclusion levels for finishing lambs in feedlots, does not pose any additional benefits to the feedlot operator. Since the effect of different ionophore inclusion levels was similar, the financial implications, health benefits and/or ionophore residual levels would rather influence the inclusion levels of ionophores in sheep feedlot diets. However, the effect in terms of combinations of ionophores with other beneficial feed additives in the finishing diets of lambs warrants further investigation.

ABSTRACT

The aim of this study was to evaluate the effects of different ionophore types and inclusion levels in feedlot finisher diets on production performance and carcass characteristics of S.A. Mutton Merino lambs. To address the aim of the study, it was divided in two phases namely: firstly, to evaluate the effect of different ionophores on animal production and carcass characteristics and secondly, to evaluate the effects of different dietary ionophore inclusion levels on the same experimental parameters. During the first study (63 days) 60 lambs (29.7 ± 2.5 kg) were randomly allocated to four treatment groups (n=15/treatment) and further subdivided into five replicates (n=3/replicate). Monensin, lasalocid and salinomycin was included into a commercial high protein concentrate (379 g CP/kg DM) at 16.5, 33.0 or 17.5 mg active ingredient/kg feed, respectively. The fourth treatment, namely the control diet was without any ionophore inclusion. Treatment diets (164 g CP/kg DM; 179 g NDF/kg DM) consisted of this protein concentrate (200 g/kg; containing an ionophore or not), maize meal (650 g/kg) and lucerne hay (150 g/kg) representing the four dietary treatments: control (C: no ionophore inclusion), monensin (M), lasalocid (L) and salinomycin (S). During the second study (42 days) 84 (30.7 ± 2.7 kg) lambs were randomly allocated to seven dietary treatment groups (n=12/treatment) and further subdivided into four replicates (n=3/replicate). Treatment diets of the second study consisted of the same basal diet used during the first study, differing only in respect of ionophore type and inclusion level; representing the control (no ionophore included), monensin low (ML: 11 mg active ingredient/kg feed), monensin medium (MM: 16.5 mg active ingredient/kg feed), monensin high (MH: 22 mg active ingredient/kg feed), salinomycin low (SL: 15 mg active ingredient/kg feed), salinomycin medium (SM: 17.5 mg active ingredient/kg feed) and salinomycin high (SH: 20 mg active ingredient/kg feed) treatments. Generally, neither ionophore type nor inclusion level had an effect ($P>0.05$) on any of the production performance parameters, as well as carcass characteristics determined in both studies. However, in the first study, salinomycin resulted in the highest ($P<0.0001$) NDF intake (1000 g/lamb/day), whereas monensin included at 22 mg/kg resulted in the lowest ($P<0.01$) CP (377 g/animal/day) and ash (83 g/animal/day; $P<0.01$) intake during the second study. In general, the efficiency of monensin, lasalocid and salinomycin, even at different levels, in sheep finisher diets, seem to be similar and does not affect rumen fermentation in such a way to improve feedlot performance and carcass characteristics of lambs when compared with the control treatments.

OPSOMMING

Die doel van die studie was om die effek van verskillende ionofoortipes en -insluitingspeile binne afrondingsdiëte op die produksie- en karkaseienskappe van Suid-Afrikaanse Vleismerinolammers te evalueer. Om hierdie doel te bereik was die studie in twee fases opgedeel: eerstens, om die invloed van verskillende ionofore op die produksierespons en karkaseienskappe van die lammers te bepaal, en tweedens, om die die effek van verskillende ionofoorinsluitingspeile op bogenoemde maatstawwe te evalueer. Tydens die eerste produksiestudie (63 dae) was ‘n totaal van 60 lammers (29.7 ± 2.5 kg) ewekansig ingedeel tussen vier dieetbehandelings (n=15 diere/behandeling), waarna elke behandeling verder onderverdeel is in vyf replikasies (n=3 diere/replikasie). Monensin, lasalocid en salinomycin is elk onderskeidelik vooraf ingesluit in ‘n kommersiële hoë-proteïen konsentraat (379 g RP/kg DM) teen 16.5, 33.0 en 17.5 mg aktiewe bestanddeel/kg voer. Die vierde behandeling, naamlik die kontrole dieet, was sonder enige ionofoor insluiting. Die rantsoene (164 g RP/kg DM; 179 g NBV/kg DM) het uit hierdie hoë-proteïen konsentraat (200 g/kg; met ‘n ionofoor ingesluit of nie), mieliemeel (650 g/kg) en lusernhooi (150 g/kg) bestaan wat die vier behandelings verteenwoordig naamlik: kontrole (C: geen ionofoor ingesluit), monensin (M), lasalocid (L) en salinomycin (S). Tydens die tweede studie (42 dae) was ‘n totaal van 84 lammers (30.7 ± 2.7 kg) ewekansig ingedeel tussen sewe behandelings (n=12 diere/behandeling), waarna elke behandeling verder onderverdeel is in vier replikasies (n=3 diere/replikasie). Die diëte wat tydens die tweede produksiestudie gebruik is, het bestaan uit dieselfde basale dieet as tydens die eerste studie, maar het slegs verskil rakende die ionofoor tipe en dienooreenkomstige insluitingsvlakke: wat die kontrole (C: geen ionofoor ingesluit), monensin laag (ML: 11 mg aktiewe bestanddeel/kg voer), monensin medium (MM: 16.5 mg aktiewe bestanddeel/kg voer), monensin hoog (MH: 22 mg aktiewe bestanddeel/kg voer), salinomycin laag (SL: 15 mg aktiewe bestanddeel/kg voer), salinomycin medium (SM: 17.5 mg aktiewe bestanddeel/kg voer) en salinomycin hoog (SH: 20 mg aktiewe bestanddeel/kg voer) behandelings verteenwoordig. Oor die algemeen, het beide die ionofoor tipe sowel as -insluitingsvlak nie ‘n effek ($P>0.05$) op enige van die produksieparameters of karkaseienskappe wat gemeet is tydens beide studies gehad nie. Ongeag die beperkte respons rakende beide ionofoor tipe sowel as –insluitingspeil op produksie –en karkaseienskappe, het lammers van die salinomycin behandeling tydens die eerste studie die hoogste ($P<0.0001$) NBV-inname gehad (1000 g/lam/dag). Tydens die tweede studie het die MH behandeling

(monensin teen 22 mg/kg diet) die laagste ($P<0.01$) RP-inname (377 g/lam/dag) en as-inname (83 g/lam/dag; $P<0.01$) gehad. As algemene gevolgtrekking wil dit voorkom of die effektiwiteit van monensin, lasalocid en salinomycin, selfs teen verskillende insluitingsvlakke binne skaapafrondingsdiëte, baie soortgelyk is en nie rumenfermentasie tot so ‘n mate beïnvloed om die produksierespons en karkaseienskappe van lammers noemenswaardig te verbeter nie.

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The effect of dietary ionophores on feedlot performance of lambs

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Abstract

This study was conducted to evaluate the effect of different rumen fermentation modifiers (ionophores) in feedlot finisher diets on the production performance of S.A. Mutton Merino lambs. Monensin (16.4 mg/kg), lasalocid (33.0 mg/kg) or salinomycin (17.5 mg/kg) was incorporated into a commercial high-protein (398 g CP/kg DM) concentrate. Treatment diets consisted of maize meal (650 g/kg), lucerne hay (150 g/kg) and a protein concentrate (200 g/kg; containing an ionophore or not) to supply isonitrogenous (177 g CP/kg DM) total mixed diets during the experimental period. Sixty lambs (29.7 ± 2.5 kg) were randomly allocated to the treatment groups (n = 15/treatment) and each treatment was further subdivided into five replicates (n = 3/replicate). Individual body weight and average feed intake per replicate were recorded weekly and used to calculate the feed conversion ratio (FCR) and average daily gain (ADG). Ionophore treatment had no effect on any of the feedlot performance parameters measured (feed intake: 1379, 1434, 1534 and 1559 g DM/day; ADG: 298, 314, 340 and 329 g/day; FCR: 4.66, 4.58, 4.51 and 4.74 g DM intake/kg live weight gained for the Control, Monensin, Lasalocid and Salinomycin treatments, respectively. The results suggest the efficiency of the different rumen fermentation modifiers to be similar and financial implications and/or animal preference would influence their usage in sheep diets.

Keywords: Feed efficiency, ionophores, lambs, production
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Introduction

Management practices to improve growth performance of ruminants include manipulating feed so that the rate of digestion is not too rapid - which could result in digestive problems, nor too slow - which could result in poor feed efficiency rates (Hatfield *et al.*, 1997). Rumen metabolic modifiers (ionophores) have been shown to have a positive effect on live weight gain, feed conversion ratio and a resultant decrease in carcass fatness. These metabolic modifiers are thus mainly developed to improve the efficiency and profitability of meat production and subsequently to improve carcass composition (Dikeman, 2007). Carboxylic polyether ionophore antibiotics, produced by various strains of *Streptomyces* spp., are compounds of these rumen metabolic modifiers and include products such as monensin, lasalocid and salinomycin. Bergen & Bates (1984) and Nagaraja (1995) also reported that the overall effectiveness of these compounds seem to be similar, although they may vary, depending on the dietary inclusion level, diet composition, and various inherent animal factors.

Changes in fermentation, associated with ionophore feeding, have mainly result in an increased production of propionate and decreased production of methane, lactic acid and froth formation in the rumen. A decreased degradation of protein and de-amination of amino acids in the rumen has also been recorded (Bergen & Bates, 1984). Due to these changes in rumen fermentation, the efficiency of energy and nitrogen metabolism is improved, and the presence of ruminal disorders reduced. Ionophores (especially salinomycin) have also exhibited effectiveness in the treatment of coccidial infections in poultry and cattle (Zinn, 1986).

Even though all ionophores are fermentation products, it is important that they should be viewed as discrete chemical entities with their own distinct properties, rather than as a uniform group with uniform effects, in order to obtain maximum benefits (Wessels, 1993). The subsequent lack of literature regarding the effect of dietary ionophore inclusion on production performances and carcass composition of intensively fed lambs is one shortcoming that needs to be addressed, since it hinders the decision-making processes of producers and feed manufacturers.

The aim of this study was to determine the effect of various ionophores on certain feedlot performance parameters of S.A. Mutton Merino (SAMM) lambs during an intensive feeding period.

Materials and Methods

All procedures conducted during this study were approved by the Ethical Control Committee for Animal Experimentation at the University of the Free State (Animal Experiment No. 12/07). Dietary treatments consisted of monensin, lasalocid, salinomycin or a control diet with no ionophore inclusion. The dietary inclusion (as fed) of monensin, lasalocid or salinomycin (16.5, 33.0 and 17.5 mg active ingredient/kg diet, respectively) into the high protein concentrate (398 g CP/kg DM) of each treatment was according to the mean registered (Act 36/1947) levels of the respective suppliers. Each experimental diet consisted of a protein concentrate (200 g/kg), lucerne hay (150 g/kg) and maize meal (650 g/kg) containing on average 177 ± 0.6 g CP/kg DM, 18.2 ± 1.8 MJ GE/kg DM, 147 ± 1.6 g NDF/kg DM, 8.4 ± 0.1 g Ca/kg DM, 2.4 ± 0.02 g P/kg DM, 0.4 ± 0.0 g EE/kg DM and 67 ± 0.5 g ash/kg DM. The only difference between the four diets was therefore the ionophore (type) included or not (Control).

Sixty (60) S.A. Mutton Merino (SAMM) lambs were weighed (29.7 ± 2.5 kg) and randomly allocated to the four treatments (n = 15/treatment), each with five replicates. Animals (n = 3/replicate) were housed in pens (6.5 m^2) on slatted floors.

At the onset of the study all animals were implanted with a hormonal growth promoter (Zeraplix; 72 mg zeranol per animal: Reg. no. G3230) and subjected to a standard health and vaccination programme as practiced in the commercial feedlot sector of South Africa. Animals were subjected to a dietary adaptation period of 14 days, after which feed allocation was on an *ad libitum* basis for the remainder of the experimental period (60 days). Individual body weights and average feed intake per replicate was recorded weekly and used to calculate the feed conversion ratio (FCR) and average daily gain (ADG). At the start and end of the trial all animals were fasted for 10 h before recording the live body weight (48.7 ± 4.4 kg).

Representative samples (treatment diets and feed refused) were collected from each replicate within a treatment and dried in a force draught oven at 100 °C for at least 16 hours to determine the DM content. After thorough mixing, these samples were ground to pass through a 1 mm sieve and stored, pending chemical analyses. The composite feed sample from each treatment offered was collected on a daily basis and the feeds refused on a weekly basis. The DM, NDF, EE, Ca, P and gross energy (GE) of samples were determined according to the procedures described by the AOAC (2000). Crude protein was determined using a Leco Nitrogen analyzer (Leco, 2001). A factor of 6.25 was used to convert the nitrogen (N) content of the samples to CP content.

The effect of dietary ionophore inclusion on certain feedlot performance parameters was statistically analyzed using a fully randomized one-way ANOVA design. The PROC ANOVA procedures of the SAS program (SAS, 1999) were used to test for significant differences between treatments.

Results and Discussion

The effect of dietary ionophore inclusion on DM feed intake and certain feedlot performance parameters of S.A. Mutton Merino lambs is presented in Table 1.

Table 1 The effect of dietary ionophore inclusion on the mean (± s.d.) dry matter feed intake (DMI) and certain feedlot performance parameters of S.A. Mutton Merino lambs

Parameter	Treatments				Significance	
	Control	Monensin	Lasalocid	Salinomycin	P[1]	CV (%)
DMI (g/sheep/day)	1379 ± 84	1434 ± 84	1534 ± 149	1559 ± 115	0.07	7.5
Final body weight (kg)	47.3 ± 3.7	48.4 ± 5.2	49.7 ± 3.8	49.5 ± 4.8	0.44	9.1
ADG (g/day)	298 ± 24	314 ± 29	340 ± 35	329 ± 17	0.11	8.4
FCR[2]	4.66 ± 0.44	4.58 ± 0.26	4.51 ± 0.28	4.74 ± 0.22	0.68	6.7

[1] Means tested for significance at P =0.05.
[2] feed conversion ratio = kg DM feed intake/kg live body weight gained.

No differences (P >0.05) were recorded regarding DM feed intake, live weight gain and feed efficiency between the treatments tested (Table 1). These findings in the present study are contradictory with

the results of Funk *et al*. (1986). They found that lasalocid improved feed efficiency ($P < 0.10$) of feedlot lambs. According to Zinn (1986) salinomycin seems not to influence rate of gain, though feed conversion was improved ($P < 0.05$) by an average of 5% at the 11 to 22 mg/kg levels of supplementation. Wessels (1993) found that even though salinomycin and monensin improved feed efficiency of cattle to apparently the same extent, these ionophores differ in their effect on ADG and DM feed intake. According to Goodrich *et al*. (1984) and Merchen & Berger (1985), feed consumption in cattle fed a monensin or salinomycin treatment decreased while an improvement ($P < 0.05$) in feed efficiency was recorded. The same effects were observed by Van Vuuren & Nel (1983) with a monensin treatment. Factors that could have affected the variable animal responses to dietary ionophores include the diet cation concentration, microbial adaptation and diet energy density (Zinn *et al*., 1994).

Supportive to the findings of the present study, Bergen & Bates (1984), Funk *et al*. (1986) and Nagaraja (1995) reported the overall effectiveness of ionophores to be similar, although the agents may vary depending on dietary inclusion level, diet composition and various inherent animal factors. Also, no effect was recorded regarding ADG, DM intake and feed efficiency of steers fed a monensin containing diet (Zinn & Borques, 1992).

As the different ionophores had no effect ($P > 0.05$) on any of the tested parameters, it is of special interest to note the effect of the Control diet (no ionophore inclusion) on the production performances of the lambs. Although the DM intake and ADG of the Control group was lowest, it did not differ significantly from the ionophore treatments. This lack of response in the dietary ionophores supplementation, compared to the Control diet, could suggest that the mean registered inclusion levels of the respective ionophores (Act 36/1947) was not adequate to provide a production response. Therefore, the need remains for more research to determine the effect of various ionophore levels on production performance of sheep under feedlot conditions.

Conclusions

Dietary treatment with ionophores in feedlot finisher diets for lambs had no effect on any of the feedlot performance parameters (DMI, ADG and FCR) tested. These results suggest that the overall effect of type of ionophore on DM feed intake, live weight gain and feed efficiency is negligible. These results are important considering that there is a paucity of research concerning the effect of type of ionophore as a dietary supplement on sheep production performance. More research is warranted in this regard.

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