

**The potential of buffers (Acid Buf) or plant extracts (Xtract 7035) as
natural alternatives for ionophores in lamb feedlot diets**

By

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Contents

Declaration.....	iv
Acknowledgements	v
Summary.....	vi
List of abbreviations	viii
List of tables.....	ix
List of figures.....	x
Chapter 1	1
Introduction.....	1
Chapter 2	3
Literature review	3
2.1 Ionophores	5
2.1.1 Introduction.....	5
2.1.2 Mode of action	5
2.1.3 Effect on animal performance	6
2.2 Plant extracts.....	7
2.2.1 Introduction.....	7
2.2.2 Mode of action	8
2.2.3 Effects on animal performance	8
2.3 Calcified marine algae (AB)	9
2.3.1 Introduction.....	9
2.3.2 Mode of action	10
2.3.3 Effects on animal performance	10
2.4 Objectives.....	11
Chapter 3	12
Materials and Methods.....	12
3.1 Facilities	12
3.2 Pilot trial	12
3.3 Experimental trial.....	12
3.3.1 Treatments.....	12
3.3.2 Animals	13
3.3.3 Feeding and feed analysis.....	14
3.3.4 Health	15
3.3.5 Rumensampling.....	15
3.3.5.1 Rumenocentesis sampling procedure.....	16

3.3.6 Slaughter and rumen scoring.....	16
3.3.7 Parameters measured	16
3.3.8 Statistical analyses	17
Chapter 4	18
Results and discussion	18
4.1 Pilot trial	18
4.2 Experimental trial.....	18
4.2.1 Average daily gain.....	19
4.2.2 Total gain	20
4.2.3 Dry matter intake and feed conversion ratio.....	21
4.2.4 Dressing percentage	22
4.2.5 Health	23
4.2.6 Rumen health and pH.....	24
4.2.7 Economics	27
Chapter 5	28
Conclusion	28
References.....	29

Declaration

I, Ruben Frederick Gouws, declare that this dissertation, which I hereby submit for the degree MSc (Agric) Animal Science: Animal Nutrition at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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Summary

The potential of buffers (calcified marine algae) or plant extract (capsicum) as natural alternatives for ionophores in lamb feedlot diets.

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Ionophore antibiotic supplementation is standard practice in almost all feedlots in South Africa and many other countries due to its effectiveness in increasing feed efficiency and modulating feed intake. Public concern about the emergence of antibiotic resistant bacteria and consumers' demand for safe, high quality nutritious food has stimulated the search for natural alternatives to ionophores in ruminant diets. A study was conducted to evaluate the effect of a buffer (calcified marine algae) and/or plant extract (capsicum) in combination with or replacing an ionophore on the performance of lambs in a commercial feedlot. A total of 2340 lambs were randomly allocated to six different treatments with six pens per treatment; with a pen as the experimental unit. Treatments were as follows: (1) Ionophore (18 ppm) (Monensin); (2) Calcified marine algae (5 kg/ton) (AB); (3) Capsicum (25 g/ton) (Caps); (4) AB (5 kg/ton) + Mon (18 ppm); (5) Caps (25 g/ton) + Mon (18 ppm); and (6) AB (5 kg/ton) + Caps (25 g/ton). All supplement concentrations were measured on an "as is" basis. Average starting weight of lambs was 30.9 kg \pm 5 kg. The lambs were individually weighed on days 0, 10, 21, 35, 50, and at slaughter. All lambs were slaughtered at a pre-determined end weight of \pm 48 kg. Average daily gain, dry matter intake, feed conversion ratio, cold carcass mass, dressing percentage, rumen fluid pH, and rumen scoring were among the parameters that were

determined. The basal diets (starter, grower, and finisher) were the same for all treatments albeit with adjustments to the specific supplementation treatments. Days on feed were significantly longer for the AB + Caps treatment compared to the other treatments ($P < 0.001$). Rumen pH values between day 1 and day 3 as well as days 13 and 30 were different between treatments ($P < 0.05$). Other performance parameters measured did not differ between treatments. Results suggest that an ionophore such as monensin can be successfully replaced in lamb feedlot diets with natural alternatives (AB and/or Caps), with minor differences in production parameters (ADG, FCR, DMI, carcass weight and Dressing %). Further research, however, is needed to determine the potential adaptation of rumen microbial populations to the experimental treatments over time. Furthermore, the cost-benefit ratio should be determined under the prevailing conditions in different countries.

List of abbreviations

AB	Acid Buf (The commercial name of Calcified marine algae)
ADF	Acid detergent fibre
ADG	Average daily gain
AMTS	Agricultural Modelling and Training Systems
Ca	Calcium
Caps	Capsicum
CCM	Cold carcass mass
CP	Crude protein
DM	Dry matter
DMI	Dry matter intake
DOF	Days on feed
EE	Ether extract
EO	Essential oils
FCR	Feed conversion ratio
FDA	Food and Drug Administration
LSD	Least significance difference test
MJ	Megajoules
Mon	Monensin
NDF	Neutral detergent fibre
NRC	National Research Council
P	Phosphorus
PMF	Positive motive force
SARA	Sub-acute rumen acidosis
SOP	Standard operating procedure
UDP	Undegradable protein
VFA	Volatile fatty acid

List of tables

Chapter 3

Table 3.1 Different coloured ear tags were used to distinguish between different treatments. 12

Table 3.2 Ingredient and nutrient composition (%) of the three lamb feedlot diets. 13

Chapter 4

Table 4.1 Pilot trial – effect of different levels of Capsicum on DMI of feedlot lambs 16

Table 4.2 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on production performance of feedlot lambs. 16

Table 4.3 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on morbidity and mortality 21

Table 4.4 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on rumen health. 23

Table 4.5 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on rumen pH of feedlot lambs. 24

Table 4.6 Economic calculation comparing the cost effectiveness of the feed additives used in this study.....27

List of figures

Chapter 4

- Fig 4.1.** The effect of monensin, calcified marine algae and Acid Buf supplemented in different combinations on the performance of feedlot lambs. 20
- Fig 4.2.** The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on DMI of feedlot lambs over the total trail period. 21
- Fig 4.3.** The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on the weight gain of feedlot lambs for different periods. 23
- Fig 4.4.** The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on the live weight change of feedlot lambs over the total feeding period..... 23
- Fig 4.5.** The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on rumen pH of feedlot lambs. 27

Chapter 1

Introduction

South Africa is well known for its active feedlot industry in beef as well as mutton. Annually 2.3 million head of cattle are slaughtered in South Africa and 5.1 million head of sheep. The sheep feedlot industry produces in the region of 106 000 metric tons of mutton per annum according to Steyn (2017) from the Red Meat Producers Organization (RPO). The gross turnover of the sheep industry is R 7 800 000 000 annually which makes it a significant contributor to revenue and potential employment. South Africa is still a net importer of mutton with 1.1 million carcasses being imported from Namibia annually, also 650 000 live sheep are imported from Namibia annually. The feedlot industry plays an important role to satisfy the ever-growing demand of protein due to the growing population which would not be possible in the extensive production systems. Statistics by the RPO showed an expected increase of 19% in beef consumption, 23% increase in chicken consumption and 5% increase in mutton consumption from the year 2016 to 2026. This indicates that production performance would have to increase to keep up with demand leading the industry to use performance enhancers such as feed additives and growth implants as stated by Coetzee (2019). Other advantages that can be attributed to feedlots as stated by Coetzee (2019) from Voermol, a leading feed manufacturer in South Africa, are less pressure on pastures due to lambs being removed from the ewes earlier allowing faster recovery of pastures during rainy season and more lambs due to ewes that can recover faster after weaning to be mated again.

There are 10 major mutton feedlots throughout South Africa ranging from 7000 head standing to 30 000 head standing. Currently a positive feeding margin allows for favourable feeding of lambs although a low profit margin is expected due to a lower price margin caused by low number of lambs available and unfavourable carcass prices for feedlots, however these conditions are ever changing during the course of the year. Most of the feedlots make use of feed additives and growth implants to increase production for maximum profit.

Some of the challenges faced daily by the industry are climate changes, poor water quality and supply, poor bunk management, stock theft, predation and metabolic disorders according to Le Riche (2019, Dr. A. Le Riche, Pers.Comm., Cavalier foods, Private Bag X680, Cullinan, 1000) who is heading up the team at one of the larger mutton feedlots in South Africa. Predation causes a significant loss of R2.6 million per annum and stock theft more so causing a loss of R900 million per annum across all animal production sectors as stated by the RPO.

One of the major challenges of a mutton feedlot is metabolic disorders of which the most significant are rumen acidosis and bloat. It is not uncommon to be as high as 10% which cause a significant loss in ADG leading to less profit for the feedlot, one of the main aims of feedlot production. According to Le Riche (2019, Dr. A. Le Riche, Pers.Comm., Cavalier foods, Private Bag X680, Cullinan, 1000), proper bunk management and a scientifically formulated diet the incidence could be reduced significantly. In order to reduce the incidence of metabolic disorders and allow feeding of high concentrate diets feed additives are added to the feedlot ration. Feed additives,

specifically ionophores, has been used in the feedlot industry for a long period of time and has been approved by the FDA in 1976 (Russel & Houlihan, 2003). In short in feed anti biotics contributes to reduce undesirable bacteria in the rumen to allow better feed efficiency (Ipharraguerre & Clark, 2003).

Recently law has been passed by the EU that prohibit the use of ionophores in animal feed due to concerns about food safety for human consumption (Cardozo et al., 2006). This poses a significant problem for feedlots as natural alternatives has to be used as alternatives to in feed anti biotics. Currently in South Africa in feed anti biotics are still allowed, however doing research pertaining to this matter is essential as in future the ban might be imposed as well. This has led to renewed interest in research, searching for natural and safe alternatives to ionophore antibiotics in ruminant diets.

The following literature review will review the feed additives relevant to this study, namely buffers (calcified marine algae), plant extracts (capsicum), and ionophores (monensin) and will be discussed in terms of mode of action and effect on animal performance. Thereafter the materials and methods, results, a discussion, and conclusion chapters will follow. The overall aim of this study was therefore to investigate the potential of natural feed additives (calcified marine algae and capsicum) to replace ionophore antibiotics (monensin) in lamb feedlot diets.

Chapter 2

Literature review

Ruminants and rumen bacteria form a symbiotic relationship in which a favourable rumen environment and nutrients are provided by the ruminant to the micro-organisms, and the micro-organisms in the rumen in turn assist in feed digestion and fermentation of feeds. This provides protein and energy for the ruminant through by-products of fermentation in the form of microbial protein and volatile fatty acids. This source of energy and protein is thought to be the most important to the host animal (Adesogan, 2009). However, these processes are not always as efficient as one would expect and can lead to loss of energy and protein that otherwise could have improved animal performance (Calsamiglia et al., 2005). Feed additives have been used for many years as rumen modifiers in order to decrease methane emission or N excretion, modulate rumen pH, improve fibre digestibility, increase feed intake and feed efficiency, and decrease the risk for milk fat depression in dairy cows. The aim of feed additive supplementation is therefore to improve production performance in ruminant production systems. Some additives, however, have both advantages and disadvantages. In recent years it has been found that the use of ionophores may lead to antibiotic resistance in bacteria due to the sub-therapeutic levels being used, and this can have a negative effect on the safety of food produced for human consumption (Russel & Houlihan, 2003). Cardozo et al., (2006) also emphasised the need for research in this field, the reason being the ban on the use of feed antibiotics by the European Union in 2006. Williams-Nguyen et al., (2016) reported that after the use of antibiotics in animals there is residue and resistant bacteria that are excreted into the environment, either directly or by animal fertilisers. This antibiotic presence may also contribute to the selection for resistance. Examples of such natural alternatives are directly fed microbials, buffers, plant extracts, essential oils, and organic acids (Haasbroek et al., 2014).

Rumen bacteria are classified as either gram-positive or gram-negative bacteria. The end products of fermentation of gram-positive bacteria are lactate, ammonia, acetate, formate, and hydrogen ions (Russel, 1996). Most of these end products are linked to the production of methane which is undesirable since it leads to a waste of energy that could have been directed towards production (Owens & Goetsch, 1988). Gram-negative bacteria on the other hand will ferment dietary nutrients to more useful products such as propionate and succinate, used for gluconeogenesis; therefore, gram-negative bacteria are more desirable. Lactic acid producing bacteria with its acidic pH tend to have a significant role in causing rumen acidosis (Ipharraguerre & Clark, 2003). The mode of action of some feed additives is such that it reduces the number of these undesirable bacteria and thereby increases animal performance. Rumen modifiers are used in animal management practices to improve growth by manipulating the rate of digestion, slowing it down sufficiently to avoid digestive problems and increasing the rate of digestion to keep a good feed efficiency (Hatfield et al., 1997).

Acidosis is a metabolic disease that is caused by high starch or rapidly fermentable carbohydrates content in diets with insufficient roughage to buffer the effect in the rumen. Both starch and fermentable carbohydrates are converted to glucose in the rumen by rumen bacteria which

in turn is converted to pyruvate. Pyruvate is then converted to VFA and lactate which will decrease rumen pH (Galyean & Rivera, 2002). In a review of acidosis Owens et al. (1998) stated that the increase in free glucose will stimulate bacterial growth of bacteria species that have smaller population under normal circumstances. These bacteria, such as coliforms and amino acid decarboxylating microbes, produce lactate that could lower the rumen pH. The higher concentration of glucose in the rumen also cause an increase in the osmolality of the rumen environment which in turn decrease the uptake of VFA adding to the acidic load in the rumen (Galyean & Rivera, 2002). High intake of starch and fermentable carbohydrates in feedlots are usually the result during the adaptation of animals to the high concentrate diets. These diets tend to focus on chemostatic fill rather than mechanical fill for intake regulation causing animals to over eat (Owen et al., 1998). According to Owens et al. (1998) acidosis incidence can be controlled through management of the intake as well as sufficient roughage in the diets.

Another common metabolic disorder found in feedlots is bloat (Galyean & Rivera, 2002). Bloat can be classified in two main groups. One is build-up of gas due to a physical obstruction that prevent the eructation of gas. The other group which is more commonly the case in feedlots is frothy bloat where a foam layers forms on top of the rumen digesta which also prevents eructation of gas (Galyean & Rivera 2002). In both cases the gas build-up causes the rumen to distend and place pressure on the lungs and diaphragm suffocating the animal. Frothy bloat is typical during adaptation to high concentrate diets, however could occur at any time during the feeding period for sometimes unexplainable reasons according to Galyean & Rivera (2002). As mentioned with acidosis adding enough roughage to the diet will help to reduce the incidence of bloat in the feedlot due to lower rate of fermentation and enough stimulation of saliva release through mastication (Cheng et al., 1998). Another strategy to control bloat is by supplementing feed additives to the diet. One such additive is ionophores which will be discussed further on in more detail (Branine & Galyean, 1990).

In this study combinations (AB + Mon; Caps + Mon; AB + Caps) was compared against the single additive treatments (Mon; Caps; AB) with the purpose of investigating whether any synergistic, additive or complimentary effects could be observed. For example, AB has a positive effect on rumen pH stabilisation (Cruywagen et al., 2015) and with an improved rumen pH it can create a rumen environment where other feed additives can present more efficiently and thereby possibly improve production. Another possible complementary effect can be that monensin reduces lactic acid production while AB could increase rumen pH, which is unfavourable for lactic acid production. The AB and capsicum combination could also potentially have a complementary effect. Acid Buf creates a more favourable rumen environment due to a more consistent pH which will increase DMI. Capsicum could also lead to a more consistent DMI resulting in a more stable rumen pH (Rodríguez-Prado et al., 2012). One of the negative effects of monensin is a possible reduction in DMI during the adaptation period; however, capsicum could stimulate DMI and can therefore counteract the negative effects of monensin (Fandino et al., 2007).

2.1 Ionophores

2.1.1 Introduction

Ionophores are organic compounds produced by bacterial fermentation of actinomycetes (Ipharraguerre & Clark, 2003), in particular *Streptomyces spp.* The original purpose of ionophores was to act as an anticoccidiostat in poultry production. Therefore, it was introduced into other animal production systems due to the possible improvement in efficiency and the profitability of meat production systems brought about by its antibacterial action which contributes to the improvement of animal performance through the reduction in methane production, its positive effects on live weight gain, feed conversion ratio, decrease carcass fatness, and increase in propionate production. The commonly used ionophores are Rumensin® (Monensin sodium), Bovatec® (Lasalocid sodium), Salinomycin® and Cattlyst® (Laidlomycin propionate potassium) (Adesogan, 2009).

2.1.2 Mode of action

A number of excellent review papers on the mode of action and performance of ionophore supplementation to ruminants have been published and will be the basis of the discussion below.

The lipophilic nature of ionophores could cause a flux of ions into and out of the cells of bacteria, fungi, and protozoa. There are two main effects initiated by the different types of ionophores. For example, monensin will form complexes with ions which act as ion-selective mobile carriers. In addition, monensin creates pores in the membranes that cause the influx and outward flux of ions (gramicidin) from the rumen environment. Rumen bacteria maintain a high concentration of potassium and a low concentration of sodium in the cells. It is this ion balance that helps the bacterial cell to absorb nutrients from the environment by establishing a positive motive force (PMF) (Callaway et al., 2003). The pH in the rumen is slightly acidic although near neutral in many ruminal bacterial cells. This pH difference causes an inward proton gradient. Ionophores on the other hand are antiporters transporting protons and ions in the opposite direction as the normal cell membrane porters. Due to its metal/ion binding capacity ionophores will exchange potassium in the cell for protons outside the cell, and also exchange sodium outside the cell with protons inside the cell. This can result in the disruption of the ion balance in the cell resulting in the cell activating the reversible ATPase to pump protons out of the cell, which would lead to the depletion of its energy to counter the ion imbalance (Callaway et al, 2003). The cell wall complexity determines how effective ionophores will be against an organism. Gram-positive bacteria are much more sensitive than gram-negative bacteria due to the fact that gram-positive bacteria have a much simpler membrane (Russel, 1996). Ionophores will penetrate the gram-positive bacteria cell membrane and cause an outward flux of potassium ions and an influx of sodium and hydrogen ions. The ATPase pump of bacteria cells will expend a significant amount of energy to alleviate this problem, and limited energy will be available for production or reproduction. Gram-negative bacteria have a more complex membrane and therefore do not allow ionophore molecules to pass into the cells (Ipharraguerre & Clark, 2003). Ionophores also inhibit the activity of obligate amino acid and proteolytic bacteria, which contribute to the reduction of ammonia production because of the decline in proteolysis. To conclude, ionophores cause a reduction in the gram-positive bacterial count which can lead to reduced methane and acetate production, lowering the acetate to propionate ratio. It also inhibits lactic acid producing

bacteria (*Streptococcus bovis* and *Lactobacillus species*) through the selection of succinate producers and lactate fermenters. This action can help reduce the prevalence of ruminal acidosis. Monensin also inhibits proteolytic bacteria, potentially increasing the flow of undegradable protein (UDP) from the rumen (Dennis, 1981).

2.1.3 Effect on animal performance

Most studies on ionophore supplementation have been conducted in beef feedlots. An overall increase in performance has been reported in terms of ADG (average daily gain) FCR (feed conversion ratio), and a decrease in incidences of ruminal disturbance.

Ionophores reduce the production of undesired metabolites such as methane, acetate, hydrogen ions, and ammonia. This improves the efficiency of energy utilisation in the rumen. In a study by Joyner et al. (1979), monensin was fed to feedlot lambs in varying concentrations. Monensin tends to decrease feed intake from days 5 to 15 during adaption. Incidences of diarrhoea were also observed during that period. This appeared to be dose related as the higher doses of 20 ppm and 30 ppm have shown the most pronounced effects. At the 30 ppm level the feed efficiency was at its greatest; however, gain was slightly lower indicating that 30 ppm was excessive. In a study by Nockels et al. (1978) using feedlot lambs, different inclusion levels of monensin were tested to evaluate the effects on animal performance. As in the previously mentioned study, Nockels et al. (1978) found an increase in average daily gain as well as feed intake.

The reduction in methane production is not due to the reduction in methanogenic bacteria but rather to the reduction in the production of the precursors for this process (Adesogan, 2009). According to Bickell et al. (2014), there tends to be a linear relationship between methane production and feed intake. Thus, if monensin increases the feed intake it will increase methane production; however, monensin reduces methane production as well. In most studies, however, monensin decreases feed intake without reducing performance, thereby increasing the efficiency of feed utilisation and improving FCR.

By increasing the production of propionate and reducing the loss of energy as methane, more energy is available to the animal for productive purposes. Propionate is the precursor for gluconeogenesis in the liver. This means that more propionate could lead to an increase in glucose production which could improve animal performance and reduce incidences of metabolic disorders. For example, a shortage of dietary energy in early lactating dairy cows can lead to ketosis due to excessive fat mobilisation to combat the energy deficit caused by the increased energy demands of lactation (Ipharraguerre & Clark, 2003). Feeding ionophores could improve energy balance and therefore increase feed efficiency, milk production, and protein yield (Ipharraguerre & Clark, 2003). According to Adesogan (2009), ionophores reduce gram-positive bacteria in the rumen which could lead to an increase in energy efficiency, protein utilisation, milk production, and feed efficiency. However, a decrease in dry matter intake (DMI), milk fat, and protein was observed. Duffield et al (2012) conducted a meta-analysis that included 77 dairy cattle trials in which they compared the results of the studies for DMI, milk fat percentage, milk protein percentage, milk yield, body condition score, and milk production efficiency. According to the results they found improvements in efficiency in milk yield, body condition score, and milk production efficiency. They also noted from

the results a tendency for a decrease in DMI, milk fat percentage, and milk protein percentage. In an experiment conducted by De Jong & Berschauer (1983) where the VFA profile was tested in adapted and unadapted lambs to monensin De Jong & Berschauer (1983) found an increase in the propionate levels and a decrease in the levels of butyrate; however, no effect on acetate levels was observed.

2.2 Plant extracts

2.2.1 Introduction

Plant extracts are not a new concept as it was widely used before the discovery of synthetic drugs. Evidence of the use of plant-based medicine could be dated as far back as 2600 BC in Mesopotamia (Greathead, 2003). Synthetic drugs were easier to manufacture and patent; however, with the ever-increasing pressure of EU legislation against antibiotic use for animal performance it is probably the main reason for the renewed interest in research on plant extracts to replace the synthetic additives used. According to Greathead (2003), if it is considered that (1) adding feed additives to pig diets led to an increase of 4% in gain and 2% in feed conversion ratio, and (2) over the last 30 years, 50 to 90% of pig feed contained feed additives and that the value of the industry was valued at $\$237 \times 10^6$, it shows that banning these additives could have a significant impact on the pig production industry. This emphasises the importance of supporting research into the use of naturally occurring alternatives to ionophore antibiotics.

Plant extracts (also referred to as essential oils) are secondary metabolites with a hydrophobic nature that are extracted from various plant parts such as the leaves, flowers, stem, seeds, roots, and bark through different processes, including steam distillation. There is a variety of these plant extracts some of which have an anti-nutritional effect leading to less effective absorption of ingested nutrients while others have an enhancing effect (Greathead, 2003). The composition of essential oils can vary depending on the part of the plant from which the oils are extracted and at what stage of the growth process (Benchaar et al., 2009). Plant extracts are also volatile compounds and have an oily texture. These secondary compounds are not “essential” in plants but constitute the essence and fragrance of the plant that act as chemical messengers in the environment through odour and colour; some also show antimicrobial activity (Calsamiglia et al., 2007a). Photosynthesis, glycolysis, and the citric acid cycle are the primary processes and building blocks for these secondary metabolites or essential oils. The building blocks of some of the essential oils are acetyl-CoA, shikimic acid, mevalonic acid, and deoxyxylulose. There are two basic chemical groups in which plant extracts can be classified, namely terpenes and phenylpropenes (Benchaar et al., 2009). If oxygen is added to these groups they are converted to terpenoids which include mono- and sesquiterpenoids, and phenylpropanoids (Hart et al., 2007). Terpenoids are the most commonly found organic chemicals and consist of around 15000 groups. The basic structure comprises a number of isoprene units which determine the compound. Each of these consists of five carbons. A phenylpropanoid has an aromatic ring of six carbons with three carbons attached to it. The antimicrobial activity of both these groups emanate from interaction with the membrane of the microorganism. There are two different hypotheses relating to the manner in which this could occur (Calsamiglia et al., 2007a). This aspect will be discussed in more detail below.

2.2.2 Mode of action

Plant extracts exhibit a hydrophobic nature which assists the passage of bacteria across the membranes, specifically gram-positive bacteria which has a simpler membrane. The plant extracts accumulate in the lipid bilayer and cause disruption in the membrane by damaging the membrane proteins (Hart et al., 2007). Plant extracts also affect the micro-organisms through altering the permeability of the membrane and acting directly on the cytoplasmic components. The effects caused by the essential oils depend on the secondary compound used (Benchaar et al., 2009) by damaging the membrane proteins or changing the permeability of the membrane. It could lead to fluid efflux of ions causing the bacterial cell to spend most of its energy in maintaining the ion balance through the use of ion pumps. Plant extracts do not kill the bacteria but will force it to utilise its energy in maintaining the processes which then limit the growth of bacteria. Hydroxyl groups are also a mode of action that acts as transporters carrying ions across the membrane, similar to that of ionophores (Calsamiglia et al., 2007b). Due to their lipophilic nature, most plant extracts cannot penetrate the cell membranes of gram-negative bacteria. However, the membranes of gram-negative bacteria are not completely impermeable and molecules of low molecular weight can associate with water and diffuse over the cell membrane, but not to the same extent as compared to gram-positive bacteria (Calsamiglia et al., 2007a). This can be seen as negative since it will reduce the selectivity of plant extracts.

The effects caused by certain plant extracts in the rumen are similar to those of ionophores. It has been seen that there could be a reduction in the activity of the total population of microbes in the rumen due to plant extracts. This is a disadvantage of plant extracts as they allow for less selectivity of ruminal bacteria types (Hart et al., 2007). Plant extracts, similar to ionophores, could reduce protein deamination which reduces ammonia production in the rumen. It could also reduce the gram-positive bacterial count and thereby reduces the amount of acetate production, thus shifts the acetate-propionate ratio downwards (Calsamiglia et al., 2007b). According to a review done by Calsamiglia et al. (2007a), a previous study showed that at a rumen pH of 7 the VFA and ammonia-N concentrations were decreased and a higher acetate propionate ratio was observed. However, at a pH of 5.5 the opposite is true. At pH 5.5, when cinnamon oil and cinnamaldehyde were added to the diet, the acetate: propionate ratio decreased, VFA concentrations increased, and ammonia-N concentrations decreased which indicates that it could be used in high concentrate diets with a positive effect on animal performance. Plant extracts could increase total volatile fatty acid (VFA) production and it is believed to be at the site of attachment of bacterial cells to the substrate (Hart et al., 2007). The inefficiency of N utilisation of ruminants is of concern as the extra N is excreted in the urine and faeces. Essential oils are thought to have the potential to improve the usage of N in ruminants (Benchaar et al., 2009).

2.2.3 Effects on animal performance

The phytonutrient that was evaluated in this study is *Capsicum Oleoresin* (capsicum). Capsicum is part of the terpenoid group and is found in hot peppers. Capsicum was chosen for this study due to the possible effects of plant extracts on animal performance. Also, capsicum could have a positive effect on the immune system by increasing the viability of macrophages as well as the secretion of TNF- α , IL-1 β from macrophages (Liu et al., 2011). According to a study by Lui et al.

(2012) in which capsicum, among other plant extracts, was fed to weaner piglets capsicum showed a possible benefit in preventing pathogenic infection and maintaining normal intestinal function through stimulating the immune response of macrophages. Capsicum could increase small peptides and amino acids by enhancing the small peptide concentration through increased peptidolysis and deamination, although there are contradictory results based on this finding (Calsamiglia et al., 2007a). Capsicum supplementation increased DM and water intake of cattle by up to 12% (Fandino et al., 2007). As seen in the research of Rodríguez-Prado et al. (2012) using Holstein cattle, capsicum could also lead to a more consistent DMI throughout the day which is favourable for maintaining a more stable rumen environment with a constant supply of nutrients to rumen microbes. A study by Hernández et al. (2009) showed similar results in finishing cattle. Feed was consumed evenly over the course of the day indicating the possibility that capsicum could regulate DMI. This can lead to a decrease in the magnitude of fluctuations in rumen pH (Rodríguez-Prado et al., 2012). The same study also showed an increase in VFA concentrations, which corresponds with the increase in DMI. A meta-analysis done by Khiaosa-ard & Zebeli (2014) as to the effect of essential oils on the performance of dairy cattle, beef cattle, and sheep showed an increase in propionate production and a decrease in acetate production, thus not affecting the total VFA produced. This effect was found to be most significant, especially in beef cattle, at a dosage of 0.25 g/kg DM diet fed. A higher inclusion level was found to decrease total VFA production. Based on this result, the inclusion level of capsicum was determined to be 0.25 g/kg DM feed in the current study. Also found in the meta-analysis was the decrease in methane production in a similar way to monensin by reducing the precursors for methane production by inhibiting gram-positive bacteria which could increase the available hydrogen for propionate production. In a recent study by Haasbroek et al. (2014), feedlot cattle were fed the same diet with only the feed additive that was different between the diets. The treatments were a positive control, an essential oil blend, and Acid Buf. They found that the essential oil blend (capsicum, cinnamaldehyde, and eugenol) could potentially increase dry matter intake and ADG, leading to better growth performance.

2.3 Calcified marine algae (AB)

2.3.1 Introduction

Due to the high demand for animal products in the dairy and feedlot industry, animals are fed high concentrate diets in order to stimulate maximum intake. The small amount of effective fibre in high concentrate diets generally causes less rumination which reduces the buffering action of bicarbonate in saliva, and clearly plays a significant role in the regulation of rumen pH. Normal rumen pH is around 6.2, and as animals consume feed the pH generally drops due to the production of VFA by bacteria in the rumen. If the pH drops too low (below pH 5.5) then certain metabolic disorders could be encountered such as sub-acute rumen acidosis (SARA) (O'Grady et al., 2007) or have the consequence of a decrease in DMI and inconsistent intake. To counter this decrease in pH, ruminants produce saliva during the mastication and rumination process which contains Na-bicarbonate, thus acting as a buffer. Sometimes this buffering capacity can be overwhelmed and metabolic disturbances can occur. To avoid this, a buffer can be added to the feed to contribute to increased buffering capacity.

2.3.2 Mode of action

Commercially, the most commonly used buffer is sodium-bicarbonate. In a study by Cruywagen et al. (2015) it was reported that Na-bicarbonate is effective in increasing the pH in the rumen; however, only for a short period of time. A control group in the same study showed a pH below 5.5 for 13h of the day; however, the addition of sodium bicarbonate reduced the time to 8.7h of the day. In a study by Calitz (2009) on lactating dairy cows it was found that calcified marine algae have a significantly higher buffering capacity compared to sodium bicarbonate in terms of the amount of acid required to reduce the pH by one unit. The buffer evaluated in this study was Acid Buf (AB), manufactured from calcified marine algae (*Lithothamnium calcareum*) gained from seaweed off the Irish coast. The seaweed is washed, milled, and dried to produce the powder used in the end product. Acid Buf has a lattice structure consisting of aragonite, vaterite, and calcite which, if compared to the stable calcite structure of limestone, shows a much higher reactivity for acids. It is composed of 30% calcium, 5.5% magnesium, and other trace minerals in small quantities.

Acid Buf is a compound that will slowly be released in the rumen and by doing so increases the pH in the rumen after feeding. This could result in an improvement in feed digestion in the rumen due to a more constant pH. The honeycomb structure of AB does not absorb acid. Its high surface area-weight ratio creates greater exposure for a chemical acid neutralisation reaction to occur. The structure is solubilised in the rumen as the acidity is neutralised; soluble calcium and magnesium are released as a consequence (Cruywagen et al., 2015).

2.3.3 Effects on animal performance

Cruywagen et al. (2004) found that AB had a positive influence in the rumen and that milk yield, milk fat, 4% FCM, and milk protein content were improved. The number of hours per day that the ruminal pH was below 5.5 was reduced from 13 to 4 hours by adding AB to the diet (Cruywagen et al., 2015). Acid Buf proved to buffer optimally at a pH of 5.6 to 6.5 (Calitz, 2009).

The effect of rumen buffers on animal performance has varying results, according to studies performed by Nicholson & Cunningham (1961) and Lassiter & Alligood (1967). In the study done by Nicholson & Cunningham (1961) they fed feedlot cattle between 2 and 4% sodium bicarbonate and found an improvement in feed intake and gain. In the study by Lassiter & Alligood (1967) they did not observe an improvement in feed intake or gain when feeding sodium bicarbonate at 3% inclusion. In another study by Huntington et al. (1977), supplemented sodium bicarbonate and sodium bentonite at 2 and 4% each showed an improved average daily gain and intake over the feeding period. The 4% level of both sodium bicarbonate and sodium bentonite resulted in a lower response than the 2% level indicating that it was excessive at 4% inclusion. Acid Buf has been shown to contribute to a more constant pH, limiting the drop in pH after feeding when nutrient concentration is highest in the rumen. The improvement in the rumen environment could result in an improvement in the VFA profile favouring propionic acid and therefore less variation in the supply of glucose to the animal (Cruywagen et al., 2004). The higher propionic acid levels favour higher milk production and milk protein production. Fibrolytic bacteria have shown a decrease in activity at a rumen pH below 5.8, and amolytic bacteria have shown a decrease in activity at a rumen pH above 6.1. In results reported by Cruywagen et al. (2015) AB assisted in reducing the fluctuation of rumen pH around 5.5

to 5.8. This could be favourable to both the amolytic bacteria and fibrolytic bacteria. The study has also shown an increase in the total VFA concentration due to a shorter period of rumen pH below 5.5, compared to the ionophore treatment.

2.4 Objectives

The objectives of this trial were to determine (1) to what extent ionophores (monensin) can either be replaced by other natural feed additive alternatives such as buffers (AB) or plant extracts (capsicum) and (2) to what extend combinations of these additives will affect feedlot performance. The hypothesis investigated is that calcified marine algae and/or capsicum can be used as an alternative to monensin due to similar feedlot performance.

Chapter 3

Materials and Methods

3.1 Facilities

A pilot trial and an experimental trial were conducted at a well-managed commercial sheep feedlot in the Gauteng province of South Africa. The average daily temperatures were 17.8°C in June to 26.7°C in January, and the night temperatures were 1.6°C in June to 15°C in January. The annual rainfall was on average 570 mm. The pen size was 10 x 20 m holding 65 animals per pen and allowing 3.1 m² per animal. The feed bunk allowance per animal was 6.5 cm and fresh water was available ad libitum.

3.2 Pilot trial

The pilot trial was conducted at the feedlot over a period of two weeks before the experimental trial to determine at what level capsicum might have a negative effect on DMI. This was done due to the strong chilli flavour of the product. Eight pens, each containing 65 male lambs, were allocated to a control and three different capsicum inclusion level treatments. The three levels were 10, 25, and 40 g/ton final feed (\pm 88% DM). Monensin at a level of 18 ppm was included in all four experimental treatment diets. There were therefore two pens per capsicum level and 130 lambs allocated to each treatment level. Daily feed intake, expressed as a percentage of body weight and average daily gain, was measured for a period of two weeks. Once the three levels were evaluated, a final decision was made on the inclusion levels of capsicum for the final experimental trial. Starting weight of animals entering the feedlot was used to calculate intake as a percentage of body weight. Animals were also weighed at the end of the two-week pilot trial to determine ADG.

3.3 Experimental trial

3.3.1 Treatments

The trial commenced during March 2014 and was completed by Aug 2015. Due to limited space in the facilities, limited funds, risk of mortalities, and the fact that it is standard practice in most South African feedlots to add monensin to the diets there was no negative control in this study. The experimental treatments were as follows:

1. **Positive control (Mon):** Lambs were fed according to standard practices that included monensin (18 ppm) in the diet in order to have a standard against which the other treatments could be measured.
2. **Calcified marine algae (AB):** Monensin replaced with AB (5 kg/ton).
3. **Capsicum (Caps):** Monensin was replaced with capsicum (25 g/ton).
4. **Monensin/Capsicum combination (Mon + Caps):** Monensin (18 ppm) and capsicum (25 g/ton) combination.

5. **Monensin/calcified marine algae combination (Mon + AB):** Monensin (18 ppm) and AB (5 kg/ton) combination.
6. **Calcified marine algae/capsicum combination (AB + Caps):** Monensin was replaced with AB (5 kg/ton) and capsicum (25 g/ton) combination.

Six pens, each containing 65 lambs, were used per treatment. Stocking density of 3.1 m² per lamb was used in the trial. A total of 2327 lambs completed the trial. All the lambs were processed according to the normal processing procedures of the feedlot after which the lambs were randomly allocated to the different treatments. Lambs were fed for a maximum of 70 days during the trial period. Animals were individually weighed on days 1 and 10 (when the lambs changed over from starter diet to grower diet) and were weighed again at 21 days when the diet was changed over from grower to finisher, as well as on the day of slaughter. Animals were not slaughtered at the same time but were removed and slaughtered when they were deemed ready to be marketed at the correct meat classification grade. This was done to avoid financial losses to the feedlot.

3.3.2 Animals

Lambs, mainly mutton Merinos, were randomly allocated to one of six treatments. Finding all-male lambs proved to be difficult and it was therefore decided to divide lambs into groups based on a ratio of 60 males to 40 females. It resulted in 1870 rams and 457 ewes in the trial. It was ensured that the ratio of male to female lambs per treatment remained the same so that each treatment would have the same male and female repeats. Average starting weights of lambs were 30.9 kg ± 1.8 kg. Underweight (<25 kg)/overweight (>35 kg) lambs were not included in the trial. The lambs were vaccinated for pulpy kidney, malignant oedema, black quarter, tetanus, pasteurellosis, and botulism; they were also dewormed and received ear implants.

Table 3.1 Different coloured ear tags were used to distinguish between different treatments

Treatment	Colour
Monensin	Yellow
AB	Green
Capsicum	Red
Capsicum + Monensin	Pink
AB + Monensin	Blue
Capsicum + AB	Orange

Lambs were sheared at day 10 and individual live weights were determined before shearing as per standard operating procedure of the feedlot. Not shearing the lambs could have had a negative effect on their production performance. Lambs remained in their designated pens for the duration of the trial.

Lambs were regarded as market ready once they reached ±47 kg live weight and a predetermined body condition. All animals that were identified as market ready were weighed on the Thursday of each week for slaughter. The lambs that were ready for marketing were slaughtered the following Monday and weighed the same morning before being transported to the abattoir.

3.3.3 Feeding and feed analysis

The standard feedlot diets were fed during the trial and formulated according to the nutrient requirements of a 30 kg Merino lamb using the Agricultural Modelling and Training Systems (AMTS) formulation programme (Tylutki et al., 2008). The ingredient and nutrient composition of the diets are shown in Table 3.2 and fulfil the requirements as set by the NRC (2001).

Three different types of feedlot diets were fed during the trial: starter, grower, and finisher.

Table 3.2 Ingredient and nutrient composition (%) of the three lamb feedlot diets

	Starter	Grower	Finisher
Ingredients			
Hominy Chop	14.0	15.0	15.0
Brewers grain	16.0	14.0	13.0
Milled maize	29.0	34.0	39.0
Molasses meal	15.0	15.0	15.0
Milled Lucerne	20.0	16.0	12.0
Premix ¹	6.0	6.0	6.0
Nutrient composition			
Dry matter (DM)	88.5	88.4	88.3
Metabolisable energy (MJ/ Kg DM) ²	9.8	10.0	10.2
Crude protein (CP)	18.0	17.4	16.8
Acid detergent fibre (ADF) g/kg	145.8	132.2	118.6
Neutral detergent fibre (NDF) g/kg	232.9	218.2	203.5
Ether extract (EE)	3.9	3.9	3.9
Starch	24.9	27.9	31.0
Calcium (Ca)	0.4	0.3	0.3
Phosphate (P)	0.3	0.3	0.3
Ash	4.7	4.3	3.9

¹Contains different feed additives according to experimental protocol; Standard feedlot premix used. Supplied per kg of dietary DM: 15 mg of CU, 65 mg of Zn, 28 mg of Mn, 0.7 mg of I, 0.2 mg of Co, 0.3 mg of Se, 6000IU of Vit A, 600 IU of Vit D and 47 IU of Vit E.

²ME = 0.82 x (GE x IVOMD) (Robinson et al., 2004)

Feed samples were taken from each feed (starter/grower/finisher) on days 3, 10, 35, and 50. Six subsamples were taken, mixed, and divided into quarters. A sample from each quarter was taken, pooled, and stored in a refrigerator for later analysis. Feeds were analysed for DM by drying in a forced-air oven at 60°C for a 24-hour period. After the drying process, samples were ground through a Wiley mill with a 1-mm screen and analysed for DM at 105°C for 24 h, and for ash as prescribed by AOAC (2000). Crude protein (CP) was analysed according to AOAC (2000) procedure nr 989.06, and ether extract (EE) according to AOAC (2000) procedure nr 920, 39. In vitro organic matter digestion (IVOMD) was analysed using the procedure described by Tilly & Terry (1963); and gross energy (GE), neutral detergent fibre (NDF), and acid detergent fibre (ADF) were determined (Van Soest et al., 1991). Neutral detergent fibre was determined using the Ankom fibre analyser with the addition of sodium sulphate and amylase. Ash was analysed using the AOAC procedure nr 942.05. Calcium (Ca) was calculated using the wet ash procedure as described in AOAC (2000) nr 934.40. Phosphorus (P) was analysed using procedure nr 964.06 described in AOAC (2000). The

metabolisable energy (ME) was calculated using a South African feed database on the total mix ration (Van der Merwe & Smith, 1991). Starch concentrations were determined according to McRae & Armstrong (1968).

Monensin was included at 18 ppm per ton. Capsicum was included at a level of 25 g commercial product/ton as was determined during the pilot trial. The commercial product (Xtract 7035®, Pancosma) consists of 20% capsicum oleoresin and 80% carrier. The buffer (Acid Buf®, Celtic Sea Minerals) was included at 5 kg/ton final feed and formulated into the ration due to the calcium (30%) and magnesium (5%) levels which influence the premix composition.

The standard feeding and feed bunk management practices performed at the feedlot were continued during the trial. Lambs were fed three to four times daily to minimise sorting of feed, which was not pelleted but in meal form. If the number of feedings per day were less, it could have led to a decrease in production performance due to sorting of the feed. The feed bunk management practices at the feedlot included feed bunk scoring in the morning before feeding by weighing the feed refusals, after which the feed allocation was determined for that specific day. Feed and fresh drinking water were available ad libitum. The starter diet was fed from days 1 to 10 and the grower diet from day 11 until the animals reached a live weight of \pm 35 kg. Finisher diet was fed from 35 kg live weight until slaughter. The diets were not supplemented with any β -agonist. The feed additives were mixed into the premix/HPC and then added to the final diet.

3.3.4 Health

The animals were observed daily for any signs of morbidity. These signs included a hanging head, separation from the rest of the group, not consuming feed, which can be judged by the hollow space behind the last rib, and also observation for diarrhoea as an indication of digestive disturbances. Animals that were pulled due to respiratory and other non-digestive reasons were removed from the trial and not returned to their original pens after treatment. Animals pulled for metabolic disturbances were treated and then returned to their respective pens directly after treatment. This ensured that the reduction in production due to respiratory and other diseases not related to metabolic disturbances did not impact production performance as there is currently no proof that either AB or capsicum has any direct effect on lung health.

When an animal was removed from the trial due to non-digestive reasons, the animal was weighed on the day of removal and total days in trial were recorded. All morbidities, mortalities, and health treatments were recorded.

3.3.5 Rumen sampling

Rumen samples were collected during the trial. Six animals per treatment were randomly selected at the start of the trial. Five ml rumen fluid samples were taken by means of rumenocentesis (Nordlund et al., 1994) on days 1, 3, 13, and three days after the lambs had reached 35 kg live weight, on average. Reasons for the selected sampling days were as follows: day 1 to evaluate the rumen pH which had not yet adapted to a high grain based diet as was the case in this trial. Day 3 was selected to determine if the rumen had adapted to the starter diet. Animals changed to the grower diet on day 10. Day 13 was selected to see if the rumens were able to adapt to the grower diet. The

diet was changed from grower to finisher diet once the animals reached 35 kg live weight. Three days after the change of diet, rumen samples were collected again to determine if the rumens were able to adapt to the grower diet.

3.3.5.1 Rumenocentesis sampling procedure

The lamb was restrained by two trained assistants in order to keep the animal on its feet. The left flank was sterilised, using an alcohol-based disinfectant. A 16-gauge needle was inserted into the left flank (caudal to the xiphoid process and left of the ventral midline). The needle was passed through the skin and into the rumen. Rumen fluid was aspirated using a 10 ml sterile syringe (Hanie, 2006). Rumen fluid pH was measured within 1 minute after sampling. The rumen sample was mixed with orthophosphoric acid (De Jong & Berschauer, 1983) to stop microbial activity in the sample at a ratio of 1 orthophosphoric acid to 5 rumen fluid, and stored at -20°C. A total of 144 samples were collected. Sampling time was always started from 13:00 and taken as quickly and accurately as possible.

3.3.6 Slaughter and rumen scoring

A commercial sheep abattoir was used for all the slaughtering. Rumen scoring was performed at the abattoir. The following procedure was used for the rumen scoring done at the abattoir: the tag numbers as well as the colour was noted in the order the lambs entered the slaughter line. The rumen was removed from the dead lamb and tagged with a number by making a small slit in the rumen wall. The rumen was washed by hand in a basin according to the standard procedure of the abattoir, and then spread open over a table and examined for stars and lesions. The rumen tag number was recorded and reconciled with the slaughter line order to correlate the correct rumen to the correct lamb. Scoring for the stars and lesions were given from none (1) to severe (5). The size was classified as small (<3 cm), medium (3–5 cm) and large (>5 cm).

3.3.7 Parameters measured

- The individual live weights were determined on days 1, 10 (during wool shearing), 21, 35, 50, and at end weight. Animals were weighed at the same time of the day and compared to the previous weighings of that pen.
- Average dry matter intake (DMI) per pen/day. Feed refusals were recorded daily.
- Health problems were dealt with according to the same standard operating procedure (SOP) implemented per feedlot. Digestive related pulls were treated and returned to their respective pens. Non-digestive pulls were treated and removed from the trial.
- All animals that had died during the trial period were subjected to a post-mortem.
- Meat classification was done during the slaughtering process (all animals) according to SAMIC specifications (SAMIC, 2006).
- Rumen scoring was done during the slaughtering process including number and size of stars and lesions (\pm 75% of animals/treatment).
- Dressing percentage.

- Cold carcass mass (CCM).
- Feed conversion ratio (FCR).
- Average daily gain (ADG).

3.3.8 Statistical analyses

Data was analysed using a one-way analysis of variance which was applied to all production data (Snedecor & Cochran, 1980). The pens were regarded as the experimental units for all parameters except rumen, lung, and health parameters. Animals were randomly allocated to the different treatments. In addition, Linear mixed model repeated measurements analysis was used to analyse DMI and BW as dependant variables specifying pen, day, and the pen-x-day interaction as random effects. The day, treatment, and day-x-treatment interactions were specified as fixed effects. Predicted means were separated using Fisher's protected least significance difference test (LSD) at the 5% level of significance. In all tests significance was declared at $P < 0.05$ and tendencies at $P < 0.10$. Row by column chi-square testing was applied to all categorical (health and rumen) data. Data were analysed using the statistical programme GenStat® (Payne et al., 2011).

Chapter 4

Results and discussion

4.1 Pilot trial

The performance results of lambs in the pilot trial are presented in Table 4.1. Due to the low number of experimental units (two pens/treatment), no statistical data analyses were done. The ADG was numerically lower in the 10 g/ton treatment, compared to the other treatments. Dry matter intake of the treatments was similar, differing by only 78 g between the highest and lowest treatment group intakes. The FCR for the 25 g/ton treatment was numerically better compared to the other treatments, leading to the conclusion that the 25 g/ton was the preferred inclusion level to be used in the experimental trial. This was also discussed and confirmed with the manufacturers of capsicum (Pancosma S.A.).

Table 4.1 Pilot trial – effect of different levels of capsicum on DMI of feedlot lambs

	Control	10g/ton ¹	25 g/ton ¹	40 g/ton ¹
Starting weight (kg)	39.70	38.81	38.60	38.99
End Weight (kg)	44.06	42.88	43.01	43.49
Mid mass (kg)	41.88	40.85	40.81	41.24
DMI/day (kg)	1.674	1.665	1.646	1.724
Intake ² (% of Mid mass)	4.00	4.08	4.03	4.18
ADG (kg/day)	0.273	0.254	0.276	0.281
FCR ² (kg feed/kg gain)	6.142	6.550	5.971	6.137

¹Inclusion level of the commercial capsicum product consisting of 20% capsicum oleoresin and 80% carrier

²Dry matter basis (88% DM)

4.2 Experimental trial

The growth performance results of sheep in the experimental trial are presented in Table 4.2. Only days on feed (DOF) were different between some of the treatments ($P < 0.05$). No other differences regarding growth performance were observed between the treatments ($P > 0.05$).

Lambs in the Mon group had the shortest days on feed and the Caps + AB group had the longest days on feed, compared to the other treatments. However, ADG of the different treatments were not significantly different between treatments ($P > 0.05$).

Table 4.2 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on production performance of feedlot lambs

Parameter	Mon	AB	Caps	Mon + Caps	Mon + AB	Caps + AB	P-value
Start weight (kg)	30.90	31.25	31.04	31.07	31.14	30.40	0.25
End weight (kg)	47.11	47.96	47.41	47.96	48.76	47.74	0.32
ADG (1- slaughter) (kg)	0.298	0.289	0.293	0.287	0.302	0.289	0.67
Gain (day 1-35)(kg)	12.06	11.65	11.99	11.94	11.82	11.52	0.98
Gain (day 1-50)(kg)	16.16	16.14	16.40	16.97	17.29	16.97	0.25
Gain (day 1- slaughter)(kg)	16.41	16.8	16.72	17.12	17.74	17.66	0.37
Days on feed (day)	56.8 ^a	60.5 ^c	58.6 ^b	60.7 ^c	60.4 ^c	63.2 ^d	<0.001
DMI (kg/d)	1.564	1.617	1.591	1.584	1.576	1.579	0.87
FCR (DMI/kg gain)	5.519	5.915	5.625	5.499	5.483	5.549	0.41
Carcass weight (kg)	22.77	22.99	22.90	23.09	23.21	22.97	0.88
Dressing %	48.21	47.92	48.01	47.99	47.58	47.95	0.85

^{abcd} means in a row without a similar superscript differ ($p < 0.05$)

4.2.1 Average daily gain

Animals were evaluated by weighting the lambs to determine if they had reached marketing weight (± 47 kg live weight). They were then removed from the pen and final live weights were determined. A difference was found in the days on feed (DOF), but no difference in the ADG, start, and end weights between the treatments was found ($P > 0.05$). Days on feed is a product of the start and end weights as well as the ADG. As there were no statistical differences in these three factors and only in DOF it could be assumed that the difference between the DOF days of the treatment groups could be contributed to other factors and not only the treatment. The most likely explanation for the differences in the DOF could be due to the management practice at the feedlot of only slaughtering every seven days and not every day. This could have contributed to less variation in the DOF data. This scenario indicates the challenges that can occur when a scientific trial is conducted under commercial conditions. Starting weights and ADG of the lambs were comparable to the South African industry norm and were also similar to results from other sheep feedlot studies (Eckerman et al., 2013; Nockels et al., 1978). A study by Joyner et al. (1979) on the effect of monensin on ADG and feed efficiency reported similar starting and final weights as in this study. Nockels et al. (1978) also performed a study on the optimum level of monensin in feedlot lamb diets, and the starting and final weights are in line with those in this study. Due to the lack of feedlot lamb data, dairy and beef data will also be referenced. In a study by Mcknight et al. (1979), Holstein steers were fed similar diets in which the level of monensin inclusion was the only difference. They found the best results in the 33g/ton range although not significantly better than 22 g/ton. The weight constraints in this study are therefore comparable to other studies and the South African industry norm.

The average daily gain of 0.293 kg/day in this study was lower compared to historical data from the feedlot where the trial was conducted. This could be due to difference in the lamb starting weight or environmental factors such as the weather or wind speed which could influence daily

intake affecting ADG (Mader et al., 2006). It could be speculated that if the ADG was higher in the range of 0.350 kg/day or more, as was the case in a study conducted by Yeaman et al. (2013), the rumen environment could have been challenged more. In the study by Price et al. (2009) it was reported that feedlot lambs of similar starting and end weights were fed ionophores, as was done in this study. Although they concluded that the effect of ionophores was negligible in feedlot lamb finisher diets, they reported an ADG of between 0.310 and 0.340 kg/day, which was higher than in this current study. In that case the Caps and AB could prove more effective as the animals' own and natural ability to deal with the extra acidity would be overwhelmed. A study performed in Texas by Sluiter et al. (2007) with similar-sized lambs reported ADG comparable to that of the current study. A study performed by Ferreira et al. (2011) on feedlot lambs also observed an ADG of 0.276 kg to 0.287 kg/day. In their study, Dunn et al. (1979) also found a comparable ADG to that of the current study. In Figure 4.1 the ADG of the different treatments have been plotted. The relatively high ADG on days 1 to 10 can be explained by gut fill as the lambs were weighed on an empty stomach at processing (day 1). Thereafter the ADG of all the treatments stabilised in a narrow range.

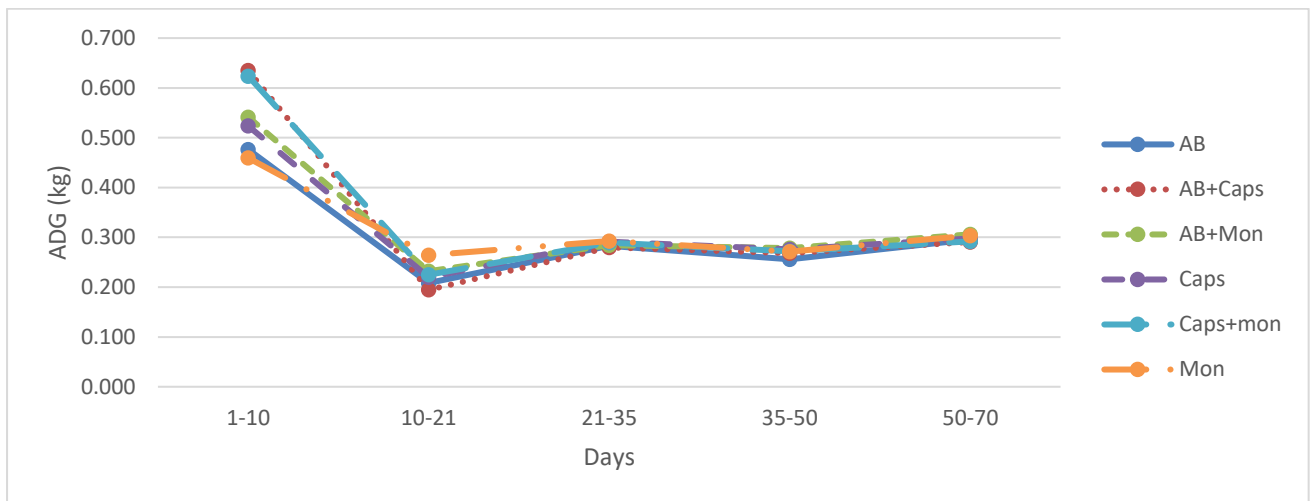


Figure 4.1 The effect of monensin, calcified marine algae, and Acid Buf supplemented in different combinations on the performance of feedlot lambs

4.2.2 Total gain

Total gain in all the treatments in the first 10 days were not different from each other ($P > 0.05$); however, there was a numerically lower gain in the Mon group of 3.77 kg vs the average of the other treatments of 4.36 kg. The remaining time from day 10 until slaughter the weight gain between the treatments no difference was observed. The lower gain in the Mon group could possibly be due to the lambs taking longer to adjust to the diet versus the other treatments when they adjusted more rapidly to the final ration. In a study by Haasbroek et al. (2014), they did not find the Mon treatment to decrease performance and showed that the monensin, essential oil, and AB performed similar in terms of the gain over the trial period. Joyner et al. (1979) also observed a decrease in DMI for the adaptation phase when monensin was fed to ruminants. This is supported by the results in this study, as shown in Figure 4.2. The DMI of the Mon group decreased from day 3 until day 6 after which it returned to normal, similar to the other treatments. The lower intake would have caused a

lower rate of gain. The capsicum or AB in combination with monensin has shown potential to alleviate the possible negative effects of monensin on feed intake during the adaptation period (Duffield et al., 2012).

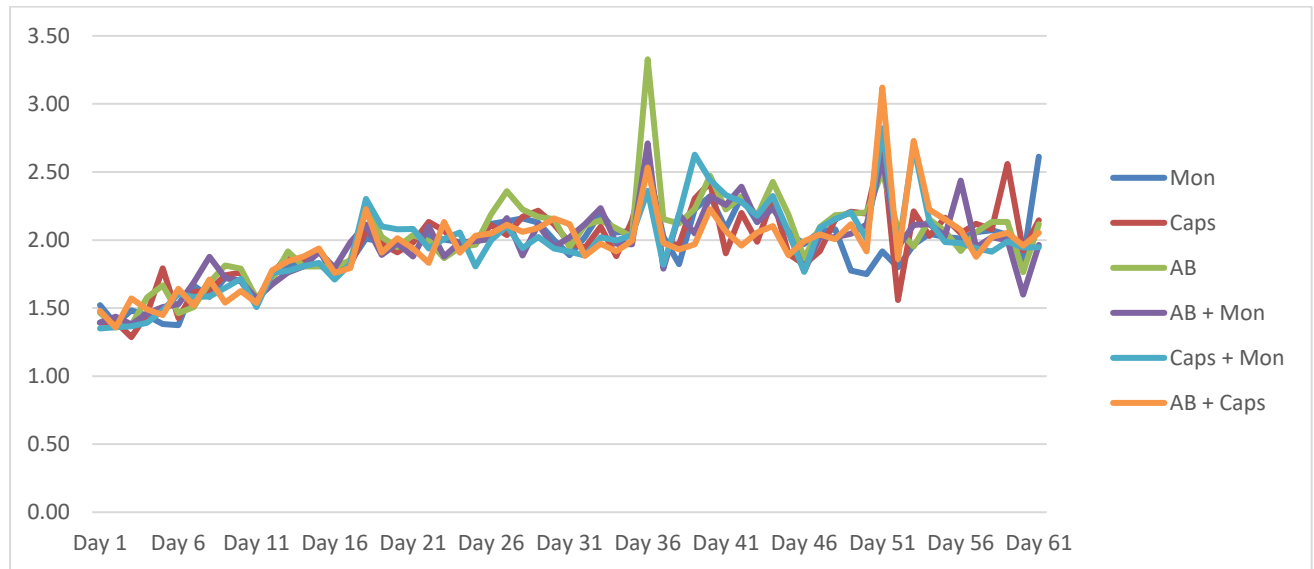


Figure 4.2 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on DMI of feedlot lambs over the total trail period

4.2.3 Dry matter intake and feed conversion ratio

Dry matter intake and FCR results are presented in Table 4.2. There were no differences between the different treatments (also see Figure 4.2) ($P > 0.05$). In this study the animals were fed three times daily. This could have masked the effect of capsicum oleoresin which, as mentioned in the discussion above, could assist in regulating intake of the animal during the day (Pritchard & Knutsen, 1995). Dry matter intake measured in this study is comparable to that in other studies. Cammack et al. (2005) reported a DMI of 1.5 kg/lamb/day, and Bickell et al. (2014) observed similar daily DMI. From the results of this study it can be concluded that replacing Mon with AB, Caps or combinations thereof will not impact the daily feed intake. A study by Huntington et al. (1977) on the effect of adding sodium bentonite or sodium bicarbonate to feedlot diets on the performance of lambs reported FCR of between 5.9 and 6.7 over the experimental period. This is slightly higher compared to the treatments in this study. This could be due to better formulated diets or better raw materials compared to the study by Huntington et al. (1977). In a study by Dunn et al. (1979) in which sodium bentonite and sodium bicarbonate were added to feedlot lamb diets a similar FCR was found in the range of 5.2 to 5.4. The University of Colorado recommends FCR for lambs of 4.9 to 5.5 kg feed/kg gain (Stanton & LeValley, 2014). In a study by Joyner et al. (1979) where the effect of monensin had been evaluated in terms of feedlot lamb, ADG and feed conversion also showed similar FCR of an average of 6 to 6.4. The current study indicates the same range although slightly better.

From these studies it can be concluded that the effect of replacing monensin with either AB or Caps or a combination thereof will not have a negative impact on the performance of the lambs in the feedlot.

4.2.4 Dressing percentage

Dressing percentage was consistent throughout the different treatments with no differences found ($P>0.05$). In the study by Van Emon et al. (2013) the dressing percentage of the lambs were in the range of 50%; however, these lambs were larger at slaughter (80 kg final weight) than the lambs used in this trial. Eckerman et al. (2013) also reported on a study to determine the effect of zeranol on lamb growth, and the dressing percentage was also 50%. However, lambs were culled at 65 kg instead of the 45 kg as in this study; thus, the dressing percentage can be expected to be lower in this study due to a smaller carcass. From these two studies it will be fair to assume that the dressing percentage in this study was within the normal range, which indicates that replacing monensin with either AB or Caps will not decrease the slaughter percentage and will therefore not impact the profitability of the feedlot.

As illustrated in Figure 4.2, the gain subsequent to all the treatments follows the same pattern. It is also clear that the curve starts to plateau at the end as the sheep approach slaughter weight (Figure 4.3). This is likely to be due to the lambs reaching a higher level of lipid synthesis versus muscle synthesis. There is a correlation between higher body weight and a greater deposit of fat (McDonald et al., 2002). In order to maintain a lean carcass, the lambs had to be slaughtered at this point to avoid penalties on carcass grading. Slaughtering at this weight resulted in a carcass in the range of 22 to 24 kg, as shown Table 4.2. More days on feed would have resulted in bigger carcasses but this is not what the market demands and therefore the lambs were slaughtered at 45 to 47 kg. The finisher diet fed to the lambs from 35 kg upwards was also formulated with higher energy density than the grower ration. The lower protein in the ration and high energy density will cause an increase in propionic acid production and an increase in the rate of fat synthesis which is an energy inefficient process (McDonald et al., 2002). This will contribute to the plateau in gain seen in the graph as muscle growth slowed down.

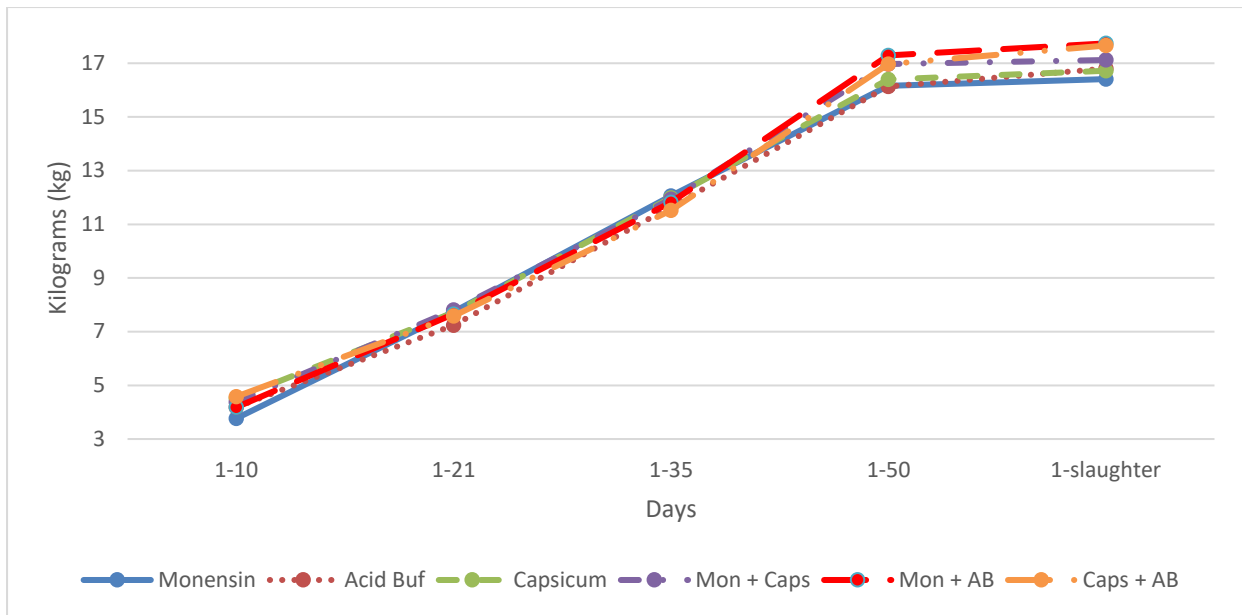


Figure 4.3 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on the weight gain of feedlot lambs for different periods

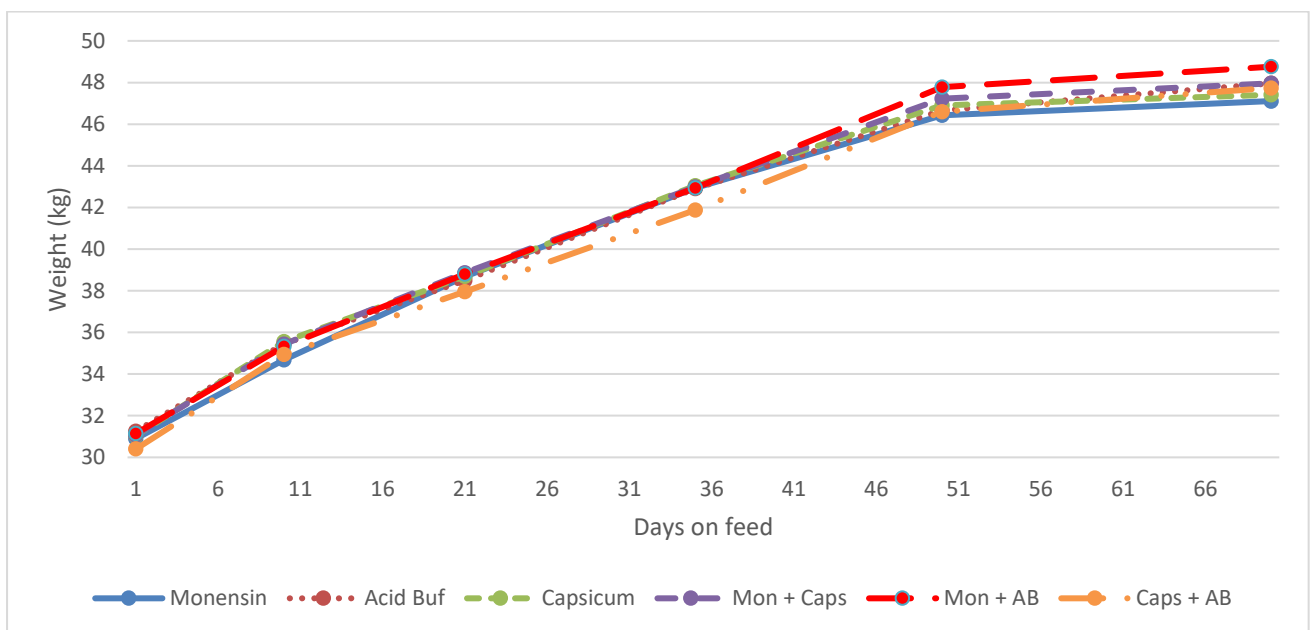


Figure 4.4 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on the live weight change of feedlot lambs over the total feeding period

In this study, no significant synergistic, additive, or complementary effects were observed.

4.2.5 Health

Mortalities and morbidities (both digestive and other) data are presented in Table 4.3. There were no differences between treatments ($P>0.05$). There were only three digestive mortalities in the trial and these were from different treatments. The other mortalities were all respiratory-related. The

incidences of both the morbidities and mortalities were within the industry norm. The percentages presented in Table 4.3 were calculated by dividing the total number of mortalities by the total number of lambs per treatment. Morbidities were calculated in a similar way but were also split into the number of treatment pulls.

Table 4.3 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on morbidity and mortality

	Monensin (%)	AB (%)	Capsicum (%)	Mon + Caps (%)	Mon + AB (%)	Caps + AB (%)	P-value
Mortalities	1.0	1.5	1.6	1.3	1.0	1.6	0.963
Morbidities							
1 st pull	4.6	7.0	4.9	5.9	5.0	4.7	0.671
2 nd pull	0.5	0.3	0.5	0.5	0.5	0.5	0.994
3 rd pull	0.3	0.0	0.3	0.0	0.3	0.0	0.701

4.2.6 Rumen health and pH

Of the rumen score data collected at slaughtering, shown in Table 4.4, only medium stars were different between treatments ($P < 0.05$). The AB treatment presented the highest number of medium stars and Mon/Mon + AB treatments the lowest. Overall, the other rumen health parameters indicated no differences ($P > 0.05$). These rumen lesions are mainly caused by subclinical acidosis which is common in a feedlot situation. Subclinical acidosis can be caused by an array of factors, for example variation in intake, feed bunk management and ad libitum vs restrictive feeding. (Schwartzkopf-Genswein et al., 2002). According to a study by Haasbroek et al. (2014), there was a significant reduction in the amount of rumen lesions found in the Xtract treatment, which consisted of a combination of three different essential oils (eugenol, capsicum oleoresin, and cinnamaldehyde), compared to monensin and AB treatments indicating that capsicum could also have a positive effect on the rumen health of the animals. In this study, however, capsicum did not affect health. Rumen lesions were observed most commonly on the ventral floor of the ventral sac, and lesions were occasionally found on the ventral floor of the caudo-dorsal blind sac and on the pillar between the anterior and posterior sac. Similar lesions were observed by Rezac et al. (2014). They found that small stars and lesions do not significantly impact the ADG of the animals; however, large stars and large lesions do have an impact on the ADG. The number of large stars and lesions in this study was relatively low; it is therefore unlikely that the rumen damage affected ADG. This could change if the animals are fed higher starch diets leading to a greater challenge for the animals to maintain rumen health. Although to determine this, another trial will have to be conducted. There is an accepted range of rumen damage in the feedlot industry due to it being an indication that the ration has the correct energy density and the lambs are being fed for optimum performance, which is close to the results seen in this study. A study by Rezac et al. (2014) also found rumen damage at 24% in cattle. Stars would indicate rumen wall damage during the adaptation phase, and lesions are more indicative of finisher phase rumen damage (Rezac et al., 2014).

Normal rumen pH of animals fed a high starch diet is usually in a range of 5.6 to 6.2 (Schwartzkopf-Genswein et al., 2002). High starch diets can result in high VFA and lactic acid levels

in the rumen, decreasing rumen pH levels. If the pH of the rumen drops below 5.8 then the total acid load inhibits the growth of some bacteria and subclinical acidosis could develop (Schwartzkopf-Genswein et al., 2002). As shown in Figure 4.5, at arrival on day 1 there was a large variation in the pH between treatments. The differences in initial rumen pH between individual animals highlighted the physiological variation between animals. As the animals started to consume the different experimental treatments, it is clear from the rumen pH measured on day 3 and specifically on day 13 that the different additives probably assisted in stabilising the rumen pH. The differences on day 3, when the lambs had been on the starter ration for 3 days, could still be due to the rumen adapting to the new grain-based diet ($P < 0.05$). It has been shown that rumen microbes can take up to 14 days to adjust to a new ration due to the changes in the microbial population (Fernando et al., 2010). Differences in the pH change from days 13 to 30 were observed ($P < 0.05$) (Table 4.5). Although different, all pH levels measured were above 5.8, thus no animals experienced subclinical rumen acidosis. This correlates with the low percentage of damaged rumens (Table 4.4). This could be an indication that the capsicum and AB affected the rumen in such a way that the end result in terms of rumen pH is similar to that of monensin. In a study by Castillo-Lopez et al. (2014) on fistulated feedlot steers, they found that pH decreases over the time of the feeding period when feeding monensin diets. This was also the case in the current study; however, the Caps and AB did not show the same effect and pH was numerically higher at the end of the feeding period. This could indicate that the additives assist in rumen pH regulation. In a study by De Jong et al. (1983) when rumen fistulated sheep were fed diets containing varying levels of monensin, they found that the rumen pH of the lambs fed 20 g/ton was 6.50, which is consistent with the results of this study. Although there were differences ($P < 0.05$) regarding medium stars between the treatments with Mon and Mon + AB lower than the other treatments, it is difficult to explain as Mon + Caps has significantly higher medium stars, compared to Mon or Mon + AB. Therefore, one cannot conclude that monensin had a significant effect on the incidence of medium stars in feedlot lambs.

Table 4.4 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on rumen health

Parameter	Class	Mon	AB	Caps	Mon + Caps	Mon + AB	Caps + AB	P- value
Rumen score (Overall)	Damaged (%)	12.9	14.7	18.1	18.8	15.8	14	0.258
	Healthy (%)	87.1	85.3	81.9	81.2	84.2	86	
Small stars	No (%)	97.2	93.4	94.3	93.7	93.5	94	0.273
	Yes (%)	2.8	6.6	5.7	6.3	6.5	6	
Medium stars	No (%)	99.1	94.7	96.8	96.6	99.1	96.8	0.005
	Yes (%)	0.9 ^a	5.3 ^b	3.2 ^b	3.4 ^b	0.9 ^a	3.2 ^b	
Large stars	No (%)	98.5	98.4	98.1	98.1	97.8	99.4	0.724
	Yes (%)	1.5	1.6	1.9	1.9	2.2	0.6	
Small lesions	No (%)	99.1	98.7	99	97.5	99.1	99.4	0.303
	Yes (%)	0.9	1.3	1	2.5	0.9	0.6	
Medium Lesions	No (%)	97.8	97.5	97.1	98.4	96.9	97.5	0.855
	Yes (%)	2.2	2.5	2.9	1.6	3.1	2.5	
Large lesions	No (%)	95.1	97.2	94.6	95.3	95.7	97.8	0.266
	Yes (%)	4.9	2.8	5.4	4.7	4.3	2.2	

^{abcd} means in a row without a similar superscript differ (p<0.05)

Table 4.5 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on rumen pH of feedlot lambs

	Mon	AB	Caps	Mon + Caps	Mon + AB	Caps + AB	P- value
Rumen pH Day 1	6.16 ^{ab}	5.83 ^b	5.77 ^b	6.48 ^a	5.62 ^b	5.73 ^b	0.035
Rumen pH Day 3	6.07 ^{ab}	5.85 ^b	5.88 ^b	6.52 ^a	5.67 ^b	5.58 ^b	0.026
Rumen pH Day 13	6.10	5.91	6.03	6.16	5.92	6.11	0.813
Rumen pH Day 30	5.95	5.95	6.09	6.35	5.88	6.05	0.379
Rumen pH change (D3 - D1)	-0.09	0.02	0.11	0.04	0.05	-0.15	0.098
Rumen pH change (D13 - D3)	0.03	0.05	0.15	-0.36	0.25	0.53	0.234
Rumen pH change (D30 - D13)	-0.15 ^b	0.04 ^b	0.06 ^b	0.19 ^a	-0.04 ^{bc}	-0.06 ^c	0.002

^{abcd} means in a row without a similar superscript differ (p<0.05)

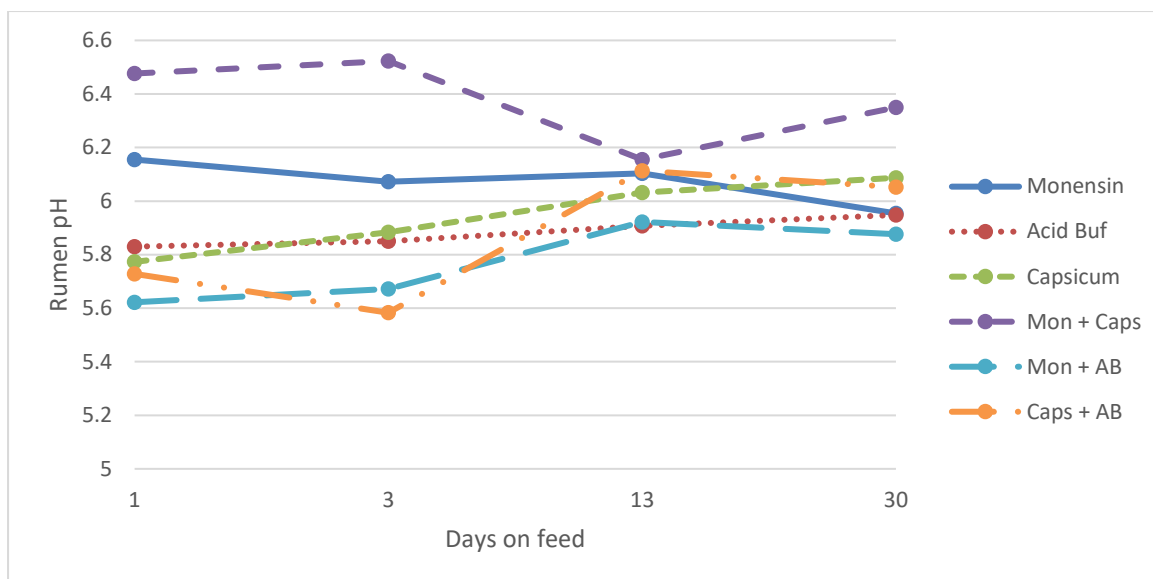


Figure 4.5 The effect of monensin, calcified marine algae and capsicum supplemented in different combinations on rumen pH of feedlot lambs

Due to the similarity in production performance parameters between treatments, it was decided not to analyse the rumen samples for VFA profile.

4.2.7 Economics

Due to the low profit margin on lambs in the feedlot all costs have to be carefully considered to be as low as possible for most benefit to the feedlot. One of the advantages of monensin is that the daily cost per animal is low. Natural alternatives are more expensive compared to ionophores with no added incentive for its use. In table 4.6 below the inclusion level of each of the products has been added for treatments 1 through 3. As treatments 4 to 6 are combinations of the products, it is not relevant to compare the cost. Total amount of feed consumed per animal varied between 88.8 kg and 97.8 kg (Table 4.6). It is clear that monensin at R0.02 per animal per day compared to Caps at R0.05 and AB at R0.10 is still more cost effective. If an incentive is offered to feedlot using natural products it will become more economically viable to feed either Caps or AB.

Table 4.6 Economic calculation comparing the cost effectiveness of the feed additives used in this study

	Inclusion level (g/kg)	Price (R/kg)	Unit price (R/g)	DMI (kg/day)	Average DOF	Inclusion in feed (g)	Daily cost (R/animal/day)	Total cost (R/animal)
Rumensin	0.09	112.00	0.11	1.564	59	1.60	0.02	0.93
Xtract 7035	0.025	1,342.07	1.34	1.591	59	2.33	0.05	3.15
Acid Buf	5	11.98	0.01	1.617	59	489.14	0.10	5.71

Chapter 5

Conclusion

Results suggest that monensin can be replaced by natural alternatives without any significant effect on the production, health, and efficiency of feed utilisation in typical South African sheep feedlot rations. However, the cost of the natural alternatives is higher compared to monensin and will require an improved performance relative to Mon to render it viable to a commercial feedlot. No synergistic effects were observed when the feed additives were fed in combination. If a situation develops in future that ionophore antibiotics are no longer allowed in ruminant diets, such as in Europe for instance, capsicum and AB present excellent alternatives that provide the same production advantages as monensin to aid feedlot operations to be competitive.

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