The use of an oregano oil extract as feed-additive for Jersey cows grazing on ryegrass pasture in spring

By

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Declaration

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Abstract

Title:	The use of an oregano oil extract as feed-additive for Jersey cows grazing on ryegrass pasture in spring
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Monensin was approved in the 1970's as a feed additive to ruminant diets. Since then, many studies on the effects of Monensin were done. Its mode of action includes the improvement of feed conversion by altering rumen fermentation. This alteration results in a change in the rumen microbial population. Some processes that benefit from the manipulation of rumen microbial population are volatile fatty acid production, peptide degradation and amino acid deamination.

The use of ionophores as an antibacterial product in animal feeds were banned by the European union (EU) in 2006 because of chemical residues found in the edible product making it potentially unhealthy for human consumption and it is also socially unacceptable. Thus, alternative sources need to be identified to help improve the rumen microbial population. Such an alternative could be plant based EO. Oregano (*Origanum vulgare*) is a natural anti-bacterial compound affecting a variety of gram positive and gram negative bacteria. It has been reported to improve the overall health and production of lactating dairy cows by enhancing rumen fermentation.

The aim of this study was to determine the effect of an essential oil extracted from oregano on production and rumen fermentation of Jersey cows grazing ryegrass pasture during spring. Effects were determined on milk yield, milk composition, live weight body condition, rumen pH, Ammonia-nitrogen (NH_3-N) and volatile fatty acid (VFA) composition, organic material (OM) and neutral detergent fibre (NDF) digestibility of pasture in the rumen.

Fifty four early lactation Jersey cows were blocked, according to days in milk (DIM), 4% fat corrected milk (FCM) and lactation number. Cows within blocks were randomly allocated to one of the three treatments. The three treatments were as follows: Control (CON; maize based concentrate with no feed additives), an ionophore treatment, (MON; a maize based concentrate with monensin provided a daily dose at 300 mg per cow), and an essential oil treatment (EO; a

maize based concentrate with oregano extract provided at a daily dose of 1.15 g per cow. Cows received 6 kg of concentrate in the milking parlour and were allocated 10 kg dry matter (DM) of ryegrass pasture, divided into two grazing periods after each milking. Before milking, cows were separated into their respective treatment groups for milking and the consumption of their specific concentrate treatments. Milk yield was recorded on a daily basis. Composite milk samples were collected per cow on a bi-weekly basis. Live weight and body condition score (BCS) were determined before and after the study. Six rumen cannulated cows were used in the rumen study. Two cows were randomly allocated to each of the three treatments in a 3 x 3 Latin square design (three treatments and three periods) thus all the cows were subjected to all three treatments over the experimental period. Ruminal pH, volatile fatty acids (VFA) concentration, ruminal ammonia-nitrogen (NH_3-N), and *in sacco* degradability were determined.

The daily average milk yield and milk fat content did not differ among treatments ($P > 0.05$) and were 20.5, 20.3 and 20.4 kg per cow and 4.5, 4.5 and 4.6 % for cows receiving the CON, MON and EO concentrates respectively. Milk protein and milk lactose content increased ($P < 0.05$) for the two additive treatments in comparison to control and were 3.39^{b}, 3.55^{a} and 3.60^{a} % for milk protein and 4.50^{b}, 4.80^{a} and 4.80^{a} % for milk lactose where cows received the CON, MON and EO treatments, respectively.

Ruminal pH values did not differ among treatments, however, the average overall pH over the 24 hour profile was higher for the two additive treatments. There were no differences in total volatile fatty acid concentrations among the three treatments. With regards to individual VFA, propionate was decreased in the MON treatment when compared to the CON treatment. The ruminal ammonia nitrogen concentration did not differ among treatments. There were no differences in DM and NDF degradability (DMd and NDFd) on the 6 h incubation period but monensin increased the DMd at 30 h incubation and both monensin and oregano increased NDFd after 30 h incubation.

To conclude the use of monensin and oregano oil extract have shown to be beneficial with regards to increasing the milk protein and milk lactose content as well as the NDFd. The average overall pH from the pH profile resulted in the two additive treatments being higher when compared to the control treatment. This could be beneficial to rumen fermentation and have a positive effect on the microbial population. As monensin and oregano oil extract showed similar results, oregano oil extract can be considered as an alternative natural feed additive to monensin.

Uittreksel

Title:	Die gebruik van ‘n oregano olie-ekstrak as voerbymiddel vir Jerseykoeie wat raaigras gedurende die lente bewei.
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Monensin is in die 1970's goedgekeur as 'n voerbymiddel in herkouerdiëte. Sedertdien het navorsing met betrekking tot die invloed van monensin vinnig toegeneem. Die werking van monensin sluit die verbetering van voeromsetting in deur die verbetering van rumenfermentasie. Die verandering in rumenfermentasie het ‘n invloed op die rumenmikrobiese bevolking. Sommige prosesse wat voordeel trek uit die manipulasie van die rumenmikrobiese bevolking is onder andere vlugtige vetsuurproduksie, peptiedafbraak en aminosuurdeaminering.

Die gebruik van ionofore as 'n antibakteriese produk in veevoere is gedurende 2006 deur die Europese Unie verbied as gevolg van chemiese residue wat in die eetbare produk gevind is. Die residue maak dit moontlik ongesond vir menslike verbruik en dit is ook nie sosiaal aanvaarbaar nie. Alternatiewe produkte wat help om die rumenmikrobiese bevolking te verbeter en manipuleer moet geïdentifiseer word. Een alternatiewe produk is plantgebasseerde essensiële olies. Origanum (*Origanum vulgare*) is 'n natuurlike anti-bakteriële produk wat 'n verskeidenheid van gram-positiewe en gram-negatiewe bakterieë in die rumen beïnvloed. Daar is gevind dat die algemene gesondheid en produksie van 'n lakterende melkkoeie verbeter deur die verbetering van rumen fermentasie.

Die doel van hierdie studie was om die invloed van 'n essensiele olie-ekstrak uit oreganum op die produksie en rumenfermentasie van Jerseykoeie wat raaigras gedurende die lente bewei, te bepaal. Effekte is ondersoek op melkproduksie, melksamestelling, lewende massa, liggaamskondisie, rumen pH, ammoniak stikstof konsentrasie (NH3-N) en vlugtige vetsuursamestelling (VFA) asook ruminale droeë materiaal (DMd)- en vesel-verteerbaarheid (NDFd) van die weiding.

Vier en vyftig Jersey koeie in vroeë laktasie is geblok volgens dae in melk (DIM), 4% vet gekorigeerde melk (VGM) en laktasie nommer. Koeie binne blokke is ewekansig aan een van die drie behandelings toegeken. Die drie behandelings was as volg: Kontrole (CON, mielie-gebaseerde konsentraat met geen voer-bymiddel nie), ‘n ionofoor-behandeling (MON; mielie-gebaseerde konsentraat met monensin teen 300 mg / koei per dag), essensiële olie-behandeling (EO; mielie-gebaseerde konsentraat met oreganum ekstrak teen 1.15 g / koei per dag). Koeie het 3 kg konsentraat in die melkstal ontvang met elke melking en 10 kg droeë material (DM) raaigras-weiding is per koei toegeken. Weidingsessies is verdeel in twee periodes, naamlik na elke melking. Voor melking is die koeie geskei om te verseker dat hulle die regte behandeling in die melkstal ontvang. Melkproduksie is daagliks aangeteken. Saamgestelde melk monsters is tweeweekliks per koei versamel. Lewende massa (LW) en liggaamskondisie (BCS) is aan die begin en teen die einde van die studie bepaal. Ses rumen-gekannuleerde koeie is in die rumen studie gebruik. Twee koeie is toegeken aan elk van die drie behandelings in 'n 3 x 3 Latynse vierkant ontwerp (drie behandelings en drie periodes) en al die koeie het gevolglik al drie behandelings gedurende die eksperimentele periode ontvang. Ruminale pH, VFA konsentrasie, ruminale NH3-N, en *in sacco* degradeerbaarhede; DMd en NDFd is bepaal.

Die daaglikse gemiddelde melkproduksie en melkvetinhoud het nie beduidend tussen behandelings verskil nie ($P > 0.05$) en was 20.5, 20.3 en 20.4 kg/koei per dag en 4.5, 4.5 en 4.6% vir koeie op die drie behandelings (CON, MON, EO), onderskeidelik. Melk proteïen en melk laktose het aansienlik toegeneem ($P < 0.05$) vir die twee behandelings met die supplemente in vergelyking met die CON behandeling met waardes van 3.4, 3.6 en 3.6% vir melk proteïen en 4.5, 4.8 en 4.8% vir koeie op die CON, MON en EO behandelings, onderskeidelik. Die rumen pH oor 24 uur het nie verkil tussen die drie behandelings nie en die totale VFA konsentrasie het ook nie verskil nie. Die propionaatkonsentrasie het wel afgeneem op die MON behandeling in vergelyking met die CON. Die rumen NH3-N het nie tussen die drie behandelings verskil nie. Die DMd en NDFd verteerbaarhede van die raaigras in die rumen het nie verskil na ‘n 6 h inkubasieperiode nie. Na 30 h inkubasie het monensin ‘n hoër DM verteerbaarheid getoon en in beide die MON en EO-behandelings het die NDF-verteerbaarheid verhoog wanneer dit vergelyk word met die CON.
Om saam te vat; die gebruik van monensin en oregano olie ekstrak het getoon dat dit voordelig kan wees met betrekking tot die verhoging van die melk proteïen en melk laktose inhoud, sowel as die NDFd. Die gemiddelde pH van die pH profiel het gelei tot hoër gemiddeldes vir die twee voerbyvoegsel behandelings in vergelyking met die kontrole behandeling. Dit kan voordelig wees om rumen fermentasie te verbeter en dus ‘n positiewe uitwerking te hê op die mikrobiese bevolking. Monensin en oregano olie ekstrak het soortgelyke resultate getoon en daarom kan oregano olie ekstrak beskou word as 'n alternatiewe natuurlike voerbyvoegsel.

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Abbreviations

AA	Amino acids
ADF	Acid detergent fibre
AM	Morning
BCS	Body condition score
BUN	Blood urea nitrogen
cm	Centimeter
CP	Crude protein
DIM	Days in milk
dL	Deciliter
° C	Degree Celsius
DMI	Dry matter intake
DM	Dry matter
ECM	Energy corrected milk
EE	Ether extract
eNDF	Effective neutral detergent fibre
EO	EO
EU	European Union
FCM	Fat corrected milk
g	Gram
GE	Gross energy
ha	Hectare
IVOMD	In vitro organic matter digestibility
kg	Kilogram
LW	Live weight
ME	Metabolisable energy
MJ	Mega Joules
Mmol	Milli-mol
MUN	Milk urea nitrogen
NDF	Neutral detergent fibre
NEFA	Non-esterified fatty acids
NH3-N	Ammonia nitrogen
NPN	Non protein nitrogen
NSC	Non-structural carbohydrates
NRC	National Research Council

OM	Organic matter
PM	Afternoon
%	Percentage
SCC	Somatic cell count
SD	Standard deviation
SEM	Standard error of the mean
TMR	Total mixed ration
R	South African Rand
RPM	Rising plate meter
RUP	Rumen undegradable protein
VFA	Volatile fatty acids

Chapter 1 Introduction

Through the years of livestock production, one of the main goals with regards to ruminant nutrition was being able to manipulate the ruminal microbial ecosystem to improve the efficiency of converting animal feeds to edible animal products, fit for human consumption (Kilic *et al.,* 2011). This includes products such as milk, meat and eggs. This manipulation was made possible by the use of different feed additives such as antibiotics, ionophores, methane inhibitors, yeasts and enzymes (Patra, 2011). Modulating rumen fermentation can lead to enhanced growth, increased milk yield, improvement of daily feed intake, as well as to improved feed efficiency (Patra, 2011). Antibiotics are used at non-therapeutic levels and are commonly included in the diet to increase feed efficiency and to prevent diseases. The use of antibiotics in this manner has, however, been criticized because of the emergence of multi-drug resistant bacteria that may pose a risk to human health (Benchaar *et al.,* 2007). Residues of the chemicals in milk and meat make it unfit for human use. Toxicity problems also resulted for the host ruminant, negatively affecting the microbial population (Patra, 2011). Due to these findings and the consumer wanting to be healthier, the EU has banned the use of ionophore antibiotics as feed additives since January 2006 (Regulation 1831/2003/EC) (Nogueira, 2009). This ban has led to nutritionists and microbiologists becoming more interested in bioactive plant factors that can modify the rumen fermentation processes in a natural way (Kilic *et al.,* 2011).

According to Shaver (2010), EO (EO) have been widely used as an alternative to monensin. The use of EO is becoming more popular in animal nutrition as a natural alternative to antibiotics (Kilic *et al.,* 2011). Plants are a natural part of the herbivore diet and these plants contain bioactive compounds which include EO, tannins and saponins. These compounds have antimicrobial properties, making them a natural antibiotic which can improve the feed utilization and health of ruminants (Patra, 2011). For the purpose of this study, we specifically wanted to look at the use of EOEO in dairy cattle nutrition.

In this trial we used an essential oil extract from *Origanum vulgaris*. This EO is the most used because of its strong antibacterial properties (Tekippe *et al*., 2011; Hristov *et al*., 2013). According to (Sivropoulou *et al*., 1996), the primary active components in oregano EO are carvacrol (75-80%), thymol (1-3%), p-cymene (8%) and γ-terpinene (2%). Oregano possesses a number of beneficial properties for use in dairy cattle nutrition. These properties include being a broad spectrum bacteria killer, maintaining intestinal stability, have an appetite stimulating effect and present a form of protection against methane gas production (Logeman, 2013).

Chapter 2 Literature review

2.1 Introduction

A total mixed ration (TMR) is a mixed balanced diet given to the cows. The diet is formulated in such a way so that all the requirements of the cows are met and the productivity of the cows is increased. The biggest disadvantage of a TMR system is that it is labour intensive. Cows need to be grouped with regards to production and narrow profit margins can lead to low profitability (Schroeder & Park, 2010). Dairy production from pasture is a common practise used in the Southern Cape (Botha *et al*., 2008b). Producing milk from pasture is more cost effective because pasture is the cheapest source of forage available to dairy cows. An increase in pasture intake may lead to an increase in the profit margin (Clark & Kanneganti, 1998). However, pasture alone is not adequate in providing sufficient nutrients and minerals to high producing dairy cows and therefore these requirements need to be met by supplementing the cows with a dairy concentrate (Kolver & Muller, 1998). Energy is the first limiting factor in milk production and should be the most important factor to be met followed by protein (Kolver & Muller, 1998). A concentrate supplement should thus be fed to balance the nutritional intake of the cows. Concentrate supplements need to be specifically formulated according to the actual needs, whether it be a pasture based herd or a TMR system (Kolver, 2003).

Feed additives are often included when concentrates or total mixed rations are formulated for dairy cows (Hutjens, 2011). The type of feed additive and dose is based on the feeding system used. Antibiotic ionophores such as monensin are used in growing cattle diets as a feed additive to manipulate rumen fermentation (McGuffey *et al.,* 2001). In recent years the use of monensin was approved to be used in dairy cattle diets for improved milk production and immune response. However, the use of antibiotics has been banned by the European Union (EU) in 2006 and is thus not being used in Europe but is still used in America, New Zealand, Australia and South Africa. This ban has led nutritionists and rumen microbiologists to identify alternative safe feed additives with the same effect as antibiotics (Frankic *et al.,* 2009). Such an alternative is plant based EO. In this review kikuyu/ryegrass pastures will be discussed. In addition, the EO, Oregano, will be compared to the use and mode of action of antibiotics.

2.2 Pasture based systems

2.2.1 Kikuyu-ryegrass pastures

Kikuyu (*Pennistetum clandestinum)* -based pastures over-sown with ryegrass (*Lolium multiflorum)* is a very popular and widely used forage source for dairy farms in the Southern Cape (Botha *et al.,* 2008b). Kikuyu is a C4 grass species (Dickinson *et al.*, 2004) and is reliant on irrigation and nitrogen fertilisation for high production and it also tolerates heavy grazing, making it a suitable pasture for dairy cattle (Van der Colf, 2011). Kikuyu is highly productive during the summer (November to February) but in the winter kikuyu goes into a dormant phase. The forage quality of kikuyu is low, and consequently milk production per cow is also low (Marais, 2001).

The over-sowing of kikuyu with an annual ryegrass is incorporated in the Southern Cape region (Botha, 2003). The seasonal dry matter (DM) production is increased and the quality and nutritive value of the pasture is improved (Botha, 2003). Kikuyu/ryegrass pasture is high producing (60 to 70 kg DM ha^{-1}) during the spring (September to October), palatable, highly digestible and has a good nutritive value (Botha *et al.,* 2008b). Combining these two species ensures that the fodder flow is maintained and that there is always sufficient grazing available throughout the year (Botha, 2003).

There are however nutritional limitations of the pasture which need to be attended to (Stockdale, 2000; Peyraud & Delaby, 2001). These limitations include, 1) insufficient rumen undegradable protein (RUP) regardless of a high crude protein content (CP), 2) low fermentable carbohydrate content, 3) a low fibre content in young pastures and 4) low levels of various minerals including calcium, phosphorous, magnesium, sulphur and zinc (Muller, 2003). Dairy concentrates are thus formulated in such a way as to supplement the pasture where it lacks nutrients, i.e., energy, protein and minerals (Muller, 2003; Steyn, 2012).

2.2.2 General information on grasses

2.2.2.1 Kikuyu

Kikuyu is a perennial tropical grass and uses a C4 photosynthetic pathway. It is very robust, creeps extensively by rhizomes (below soil) and stolons (above soil), forming a very dense sod (Dickinson *et al.*, 2004). Kikuyu is adapted to 700 to 750 mm of rain but does favour a higher annual rainfall and misty areas. Kikuyu is sensitive to hot dry conditions but under irrigation performs well. It can grow in most soils but prefers a soil with high fertility, especially to nitrogen content (Donaldson, 2001). Kikuyu can withstand severe grazing and is a popular pasture to use during the summer and autumn months as it is most active during this time of year (Botha *et al.*, 2008b). In the Southern Cape, during late winter and spring (August and September), kikuyu goes

into a dormant phase and becomes inactive (Botha, 2003).

2.2.2.2 Ryegrass

Italian Ryegrass, *Lolium multiflorum,* is a highly productive and nutritious annual grass favouring cool seasons (Donaldson, 2001). It has a higher growth rate during the winter (June to August) compared to the summer (October to February). In comparison to kikuyu, annual ryegrass has higher metabolisable energy (ME) content (*ca* 1 MJ/kg DM) (Fulkerson *et al.*, 2006). Annual ryegrass is therefore a suitable candidate to be incorporated into kikuyu pastures (Van Wyngaard, 2013). During the autumn and spring it is regarded as an excellent pasture for grazing dairy cows (Donaldson, 2001). Italian ryegrass is consequently a major contributor to the fodder flow systems in the Southern Cape during mid-July to September (Thom & Prestidge, 1996). Annual ryegrasses require a rainfall of 900 mm during the growing season or under irrigation need 25 mm per week. During the hot summer months, when rainfall is low, irrigation is thus important for growth (Donaldson, 2001).

2.2.3 Production potential of the kikuyu/ryegrass pasture

The growth rate of kikuyu pasture is high at 67.0 kg DM ha/day (Botha *et al*., 2008a) during the summer and autumn season, enabling the pasture to support high stocking rates (Van der Colf, 2011). Kikuyu has a low forage quality during winter and spring in comparison to other temperate species such as ryegrass (Van der Colf, 2011). In a study on the strategic incorporation of different C3 grasses such as annual and perennial ryegrass done by Van der Colf (2011), it was found that the highest seasonal DM yield of a kikuyu based pasture over-sown with Italian ryegrass occurred during spring (August to October). The daily growth rate of the pasture during spring was 60 to 70 kg DM/ha. In the winter (April to August) the production was much lower; with a daily growth rate of 15 kg DM/ha (Dickinson *et al.,* 2004). For the pasture to reach genetic potential and optimal production the environment needs to be favourable for photosynthesis and growth (Booysen, 1966). The rate of photosynthesis is determined by a few limiting factors. These factors include, light intensity, carbon dioxide concentration, temperature, water availability and leaf size. During winter months the light intensity and the temperature is lower. These conditions lead to a lower photosynthesis rate resulting in limited growth and a decrease in production potential (Tainton, 2000). As the pasture grows older, the amount of leaf mass increases, limiting the rate of photosynthesis because of the shadowing effect of the leaves on the roots. Extending the grazing cycle of the pasture will have a negative impact on the production potential of the pasture and will lead to leaf tillers dying and will lead to the decay of pasture (Tainton, 2000).

2.2.4 Establishment of ryegrass

For the southern Cape region, which is a frost free, winter rainfall area, the establishing time for ryegrass is during the autumn (mid-February to mid-April) (Donaldson, 2001). Care must be taken not to sow the seedlings too early in the warmer summer months as it will lead to a low establishment success of the seedlings. If sown in mid-March, the pasture will be available for first grazing mid-May (Dickinson *et al.,* 2004). Correcting the soil according to soil analyses prior to establishing the ryegrass into a well prepared seed bed could lead to optimal seedling establishment resulting in high production with a permanent irrigation system in place (Botha *et al.,* 2008a).

2.2.5 Grazing Management

If sown in autumn, the pasture will be available for grazing 6 to 10 weeks after establishment. Grazing can commence when the pasture reaches a height of 20 cm and grazing should be ceased when a stubble height of 5 to 6 cm is reached. This entails that the pasture will be optimally utilized without grazing being wasted and ensuring the dairy cows have sufficient intake (Donaldson, 2001). During winter, the growth rate of kikuyu is at its lowest because it becomes dormant and growing ceases (Van der Colf, 2011). Ryegrass pasture is usually optimally utilized by rotational strip grazing. The pasture is divided by an electric fence and a new grazing portion is made available after each milking session. A grazing cycle of 24 to 28 days is recommended to enable a sufficient regrowth period (Donaldson, 2001). Good management of such a pasture should be implemented in order for optimal use of the pasture and to ensure enough fodder is available to the dairy cows. When annual ryegrass is sown into kikuyu pastures the seasonal fodder availability is changed and the DM production during spring is increased (Botha *et al.,* 2008a). In a study done by Botha *et al.* (2008b) it was found that in comparison to a kikuyu and a kikuyu-clover pasture, the kikuyu-ryegrass pasture delivered more uniform seasonal fodder availability. Variation in grazing capacity and milk production is therefore reduced.

2.2.6 Estimating pasture intake

Estimating the pasture intake of the grazing cows is a useful management tool and can be used as an aid to fodder flow planning. Direct and indirect methods can be used to determine pasture intake (Van Wyngaard, 2013). Direct methods involve the cutting, collecting, drying and weighing samples from the pasture and then estimating the yield. It is a more precise method to estimate pasture yield but it is time consuming and invasive of the pasture making it less practical for routine yield estimations. Many indirect methods have been developed in order to determine the pasture yield. The most used methods are the pasture ruler and rising plate meter. Both these

methods need to be calibrated first for the specific pasture and precision is important. Once calibrated these two methods are very helpful and an easy tool for estimating pasture yield (Hall & Deak, 2007).The rising plate meter is a measuring instrument based on the Ellinbank pasture meter developed and designed by the Dairy Research Institute, Australia. The rising plate meter (RPM) relies on both plant height and density. The combined measurement refers to the "bulk density". It manually records the height of the pasture at 5 mm increments (Sanderson *et al.*, 2001). The pasture yield can then be estimated by the use of a calibration equation. The difference between pre-and post-grazing heights are calculated and the average height is correlated with forage bulk density and then converted to yield using a calibration equation (Hall & Deak, 2007).

2.2.7 Energy supplementation and substitution rate

Pasture-based dairy systems are commonly used in the Southern Cape. These systems result in lower-cost feeding with a high milk output per unit of land (Bargo *et al*., 2003). Pasture-only systems however have a low DM and energy intake and therefore supplementation should be used to ensure sufficient energy intake. Milk production would be sustained and may increase which will optimize the productivity of the cow as well as profit (Fales *et al*., 1995). High quality pastures are not sufficient enough in providing all the required nutrients to the producing dairy cows thus supplementation is needed to meet all the requirements (Dixon & Stockdale, 1999). Feeding a supplement, however, leads to a decreased intake of pasture, a phenomenon referred to as substitution rate (Grainger & Mathews, 1989; Faverdin *et al*., 1991; Stockdale, 2000; Sairanen *et al*., 2006). The substitution of pasture with the concentrate is undesirable and leads to pasture not being fully utilized (Faverdin *et al*., 1991). The cows substitutes the supplement for part of the pasture they would have grazed (Stockdale, 2000). The rate of substitution is positively correlated to the amount of supplement fed to the dairy cow (Stockdale, 2000). The type of supplement used has an effect on the substitution rate and animal performance (Stockdale, 2000). Dairy supplement diets usually contain high levels of grain to supplement the energy needs of the producing cow. However, there are two major factors limiting the use of grain in dairy diets; a reduction in milk fat content and the low fibre values of the high quality forage usually grazed (Dixon & Stockdale, 1999). The reduction in milk fat is caused by the excessive intake of readily fermentable carbohydrates (RFC) which shifts the rumen VFA production towards propionate and decreases the acetate to propionate ratio (Seymour *et al*., 2005).

2.3 Feed additives used in dairy nutrition

2.3.1 Introduction

Feed additives are non-nutritive compounds usually administered to the animal through the diet or through the water supply (Adesogan, 2008; Hutjens, 2011). The use of feed additives may cause a desired animal response in a non-nutrient role. Sought after effects include a shift in rumen pH, weight gain and growth, a modification of metabolic pathways positively influencing production, (Hutjens, 2011) enhancement of rumen development in younger ruminants, a reduction in methanogenesis increasing the efficiency of energy utilization, improving the efficiency of nitrogen utilization and improving overall rumen digestibility (Adesogan, 2008).

Several feed additives are currently used in dairy cow rations. Additives are not a fixed requirement, however, they may have a positive effect on the rumen environment and in turn, on animal production. Therefore the producer should consider the use of feed additives as part of his/her diet formulation. Hutjens (2011) summarized the following additives used commonly in dairy diets:

- Antibiotic ionophores facilitates in selective transportation of ions across bacterial walls which in turn shifts rumen fermentation and microbial selection leading to an increase in feed efficiency for the lactating cow and a reduction in ketosis and displaced abomasums in transition cows (Eastridge, 2006; Adesogan, 2008).
- Protected choline is involved with fat transport and acts as a methyl donor used to minimize the formation of fatty liver and increases fat mobilization. It might have beneficial properties with regards to a decrease in ketosis for cows after parturition (Sales *et al*., 2010).
- Enzymes are provided that aid in improving nutrient digestibility and availability specifically fibre digestion. It can also detoxify harmful metabolites (Adesogan *et al*., 2007).
- EO are complex secondary metabolites extracted from various plants and have strong antimicrobial properties. It may increase effectivity of rumen fermentation by reducing protein deamination, increasing propionate production, increasing feed efficiency and/or improve the hydrogen status in the rumen (Benchaar *et al*., 2006; Patra, 2011).
- Magnesium oxide acts as an alkalizer by increasing the rumen pH. It may increase the uptake of blood metabolites by the mammary gland which can raise the milk fat content (De Ondarza, 2003; Hutjens, 2011).
- Niacin affects the coenzyme systems in biological reactions. In early lactating cows, the energy balance may be increased, ketosis is controlled and the rumen protozoa are stimulated. It may be beneficial to use in diets for high producing lactating cows that are in a negative energy balance (Hutjens, 2011).

- Probiotics also known as direct-fed microbes destroy all the unfavourable organisms in the rumen by producing metabolic compounds. *Aspergillus Oryzae* (AO) and Yeast cultures (YC) are two of the most common probiotics. AO and YC contain fibrolytic enzyme activities that stimulate bacteria responsible for fibre digestion. This may stabilize the rumen pH by reducing lactic acid and can reduce the effects of stressful conditions (Chiquette, 1995; Eastridge, 2006; Adesogan, 2008).
- Rumen buffer/Sodium Bicarbonate is added to increase the DMI of the dairy cow. It may help in stabilizing the rumen pH (Hutjens, 2011).
- Yucca extract binds ammonia to the glycofraction extract of the *Yucca shidigera* plant, reducing the urea nitrogen in plasma and milk. This function improves nitrogen efficiency in ruminant animals (Hutjens, 2011).

Of all the feed additives available, antibiotic ionophores and EO have shown to have the highest anti-microbial properties and may have the greatest potential for improved rumen fermentation which might increase production in lactating dairy cows and will be discussed in more detail.

2.3.2 Antibiotic Ionophores

Ionophores are classified as an antibiotic and are organic compounds mainly, naturally produced from the bacteria strain, *Streptomyces* spp. Since the first approval of the antibiotic ionophores in 1971, it has been used in the poultry industry to control coccidiosis. In later years the use of antibiotic ionophores became more common in many sectors of the poultry and cattle industries (McGuffey *et al.,* 2001; Hutjens, 2012). The main use for antibiotic ionophores was to manipulate rumen fermentation. By manipulating rumen fermentation, the efficiency of feed utilization was improved, as well as weight gain in growing cattle (Ipharraguerre & Clark, 2003). With further research on the use of antibiotic ionophores, the benefits on the biological actions in cattle were classified into three areas of effects, which are (Bergen & Bates, 1984):

1) Increased efficiency of energy metabolism of rumen bacteria (and/or) the animal.
2) Improved nitrogen metabolism of rumen bacteria (and/or) the animal.
3) Retardation of digestive disorders resulting from abnormal rumen fermentation.

Antibiotic ionophores enable selective transportation of ions across the outer cell membrane of bacteria (Adesogan, 2008). The less complex membrane structures of gram-positive bacteria cells are thus more susceptible to the transportation of ions made possible by antibiotic ionophores. Gram-negative bacteria have a much more complex double membrane making it

more difficult for antibiotic ionophores to effectively inhibit the growth of these cells (McGuffey *et al.,* 2001).

All of these actions offer nutritional and metabolic advantages to the animal that is supplemented with the ionophore compared to an un-supplemented animal. These advantages are then used by the animal to increase production and improve efficiency (McGuffey *et al.,* 2001).

2.3.3 Essential oils

Plants form a natural part of the diet of herbivores. They produce quite a variety of organic compounds that are derived from secondary metabolism present in the plant. This secondary metabolism does not seem to have an effect on the growth and development of the plants (Balandrin *et al*, 1985). Products produced from this secondary metabolism were thought to be waste products from primary metabolism but it was found that they are actually responsible for the odour and colour of plants and spices (Gershenzon & Croteau, 1991; Benchaar *et al*., 2008). They have important ecological functions such as serving as chemical messengers between the plant and its environment and they often exhibit anti-microbial properties, protecting the plants from various bacteria, yeasts and moulds (Gershenzon & Croteau, 1991). The secondary metabolites found in plants are difficult to classify because of their metabolic pathways that differ and mode of action which overlap (Calsamiglia *et al*., 2007). However, they can be classified into three groups: saponins, tannins, and EO (Patra, 2011). Saponins and tannins have been extensively researched over the years. However, the effects on ruminal fermentation and mode of action of EO have only recently been researched and tested. Consequently, information is quite limited (Calsamiglia *et al.,* 2007).

EO are naturally occurring aromatic volatile compounds that can be extracted from plants by different distillation methods, mainly steam distillation (Benchaar *et al.,* 2006). These oils can be extracted from various parts of the plant. The EO extracted from the different parts can vary in composition and chemical structure (Benchaar *et al.,* 2008). The term “essential” is derived from the word “essence”, meaning smell or taste which provides the specific flavours and odours to the many plants (Nogueira, 2009).These oils are secondary metabolites found in plants and chemically they are not true oils but rather usually made up of terpenoids, such as (Figure 1) monoterpenes, sesquiterpenes and phenylpropanoids (Patra, 2011). Essential oils has been identified with a number of beneficial features including antimicrobial properties, effective against gram-positive and gram-negative bacteria, as well as other micro-organisms such as protozoa and fungi (Benchaar *et al.,* 2006). These properties have led to nutritionists and rumen microbiologists being more interested in the use of EO as rumen modifiers with regards to rumen fermentation and its different processes, such as volatile fatty acid production, protein degradation and metabolism, inhibition of methane gas production and efficient feed utilization (Patra, 2011). Essential oils are natural

products making them safe to use in animal feeds. The edible products are safe for human consumption (Benchaar *et al.,* 2008).

Essential oil molecules are lipophilic, enabling them to interact with the cell membrane of gram positive bacteria which can be toxic causing death of the bacteria cells. Gram negative bacteria cells are encapsulated (Griffin *et al*., 1999) making it harder for the EO molecules to penetrate the bacteria cell. Some EO molecules however, are small enough to penetrate through the capsule and gain access to the inner membrane of the gram negative bacteria, damaging the cell (Jouany & Morgavi, 2007). It is also able to coagulate the cytoplasmic material inside the cell causing decreased cell function or result in cell death (Burt, 2004). Effective doses vary between different EO because of the large differences in chemical composition (Jouany & Morgavi, 2007).

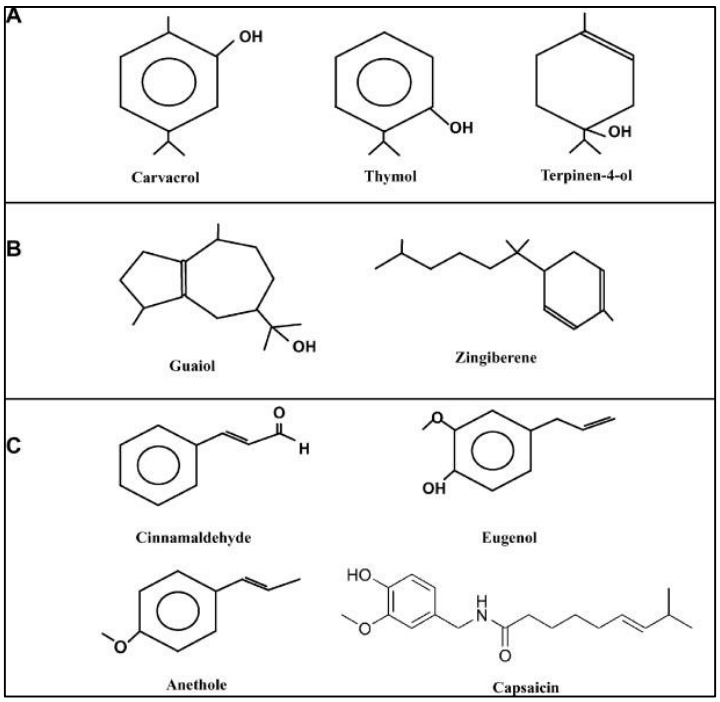

Figure 1 The chemical structures of the main Monoterpenoid (A), sesquiterpenoid (B), and phenylpropanoid (C), components of essential oils

2.4 Monensin

Monensin is a carboxylic polyether ionophore which is naturally produced by a strain of *Streptomyces cinnamonensis* (Duffield *et al.,* 2008a; Łowicki & Huczynski, 2013). Monensin is used throughout the cattle industry for therapeutic and production purposes (Varga, 2004). Monensin has been used in lactating dairy cow diets in several countries such as Australia, New Zealand, Mexico, Argentina, Brazil, Chile, Uruguay and South Africa (Muller *et al.,* 2006). In 2004, the use of Monensin was approved by the FDA (US Food drug and administration) for the use in lactating dairy diets as well as dry cow diets. The use of monensin in diets is strictly controlled by a specific dosage range. The labelling guidelines allow for a daily inclusion rate of 185 mg to 660 mg per lactating dairy cow, and for dry cows a rate of 115 mg to 410 mg per cow (Hutjens, 2012). Monensin is also used as a growth promoter in growing beef cattle. By improving the efficiency of feed conversion, the rate of gain is increased with the same feed intake or by maintaining the rate of gain at a lower feed intake (McDonald *et al.,* 2011).

Rumensin® is a product of Elanco Animal Health with the chemical name; Monensin sodium and is given to animals as a sodium salt (Varga, 2004). Monensin is 20 % active in Rumensin® and is provided orally through addition to the diet (Duffield *et al.,* 2008a).

2.4.1 Mode of action

Antibiotic ionophores, specifically monensin, interfere with the transport of ions across the membranes of bacterial cells, leading to energy loss inside the cell, eventually resulting in bacterial cell death (Duffield *et al.,* 2008a). Monensin selectively inhibits gram-positive bacteria and is unable to penetrate gram negative bacteria (Figure 2), due to the more complex nature of the bacterial cell walls (Adesogan, 2008). The infiltration of the outer cell membrane of gram positive bacteria by monensin causes a rapid and repeated efflux of intracellular K+ from the cell to its outside environment. In response an influx of extracellular protons (Na+ and H+) occurs. Acidity of the cell increases as the K+ concentration is depleted, inhibiting protein synthesis. The ATPase pumps are stimulated to pump out the excess protons present in the cell, draining the cell of its energy reserves and decreasing bacterial growth. Eventually cell death occurs because of the high cytoplasmic acidity in the bacterial cell (McGuffey *et al*., 2001; Adesogan, 2008; Łowicki & Huczyński, 2013). Monensin affects the ruminal microbe population. Van der Merwe *et al*. (2001) indicated in a study that monensin had toxic effects on the ruminal protozoa. The species of protozoa determined the magnitude of the defaunating effects of monensin and reduced the rumen protozoa with 30 to 100 % suggesting that monensin had selective toxicity. This change in rumen microbal population affects ruminant metabolism as well as the fermentation of feed (Duffield *et al.,* 2008a). Both energy and nitrogen metabolism efficiency is increased, and the risk of bloat and

lactic acidosis is decreased (Duffield *et al.,* 2008b). The use of monensin also leads to a shift in the molar proportions of VFA production, increasing propionate and decreasing acetate and butyrate production (Muller *et al*., 2006). The shift in ruminal VFA production corresponds with a reduction in methane losses which in turn increases energy efficiency (Van Der Werf *et al.,* 1997).

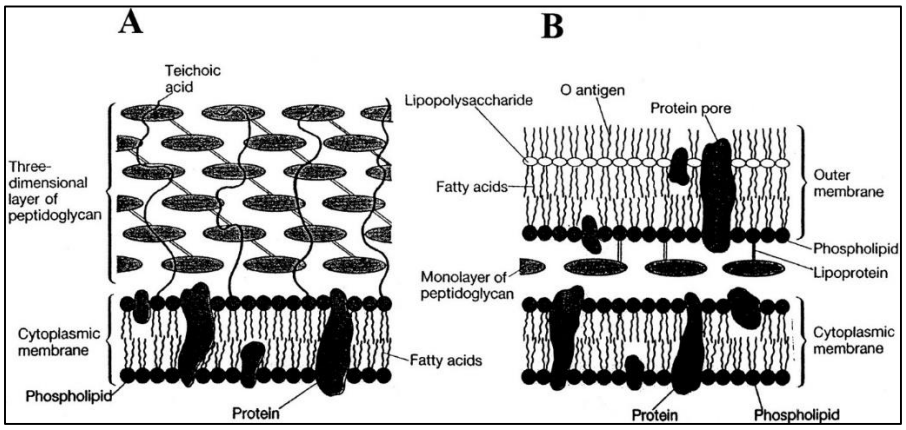

Figure 2 A- Gram positive bacterial cell wall B - Gram negative bacterial cell membrane (Volk, 1992)

2.4.2 Effects of monensin supplementation

2.4.2.1 Milk production potential and composition

The use of monensin in the diet of lactating cows has been shown to have a positive effect on milk production and milk composition (McGuffey *et al*., 2001; Ipharraguerre & Clark, 2003). Monensin has an effect on the molar proportions of VFA in the rumen. A shift toward propionate production results in an increase in the energy value of feeds and this may result in higher milk production (McGuffey *et al*., 2001; Muller *et al*., 2006). Monensin decreases the milk fat content by decreasing the ruminal production of acetate and butyrate and by inhibiting the ruminal biohydrogenation of long chain fatty acids (Fellner *et al.,* 1997). Milk fat synthesis is also inhibited by the enhancement of trans-10, cis-12 conjugated linoleic acid supply to the mammary gland (Baumgard *et al.,* 2000).

Duffield *et al.* (2008b) analysed a total of 37 papers containing 70 trials and concluded that monensin increased milk production and milk production efficiency. They have found that the dry matter intake (DMI) has decreased as well as the milk fat content and milk protein content. An increase in milk production in conjunction with no change or a decrease in DMI was found when monensin was added to dairy cow diets (Van der Werf *et al*., 1997; Phipps *et al*., 2000). These findings may suggest that the use of monensin can improve the efficiency of feed utilization (Osborne *et al*., 2004).

Beckett *et al.* (1998) and Ruiz *et al.* (2001) added monensin at daily dosage rates of 320 mg per cow and 350 mg per cow, respectively to the diet. The overall outcome was an increase in mean daily milk production of 0.75 L per cow and 1.85 kg per day respectively, in comparison to a control diet. Ramanzin *et al.* (1997) and Broderick (2004) found that the addition of monensin at a daily inclusion of 300 mg/d and 244 mg/d, respectively, had no effect on milk production.

Beckett *et al*. (1998) found that the milk fat content did not decrease compared to the control diet. However, Ruiz *et al.* (2001) found a decrease of milk fat and protein content. It decreased by 0.12, 0.06 percentage points, respectively. Sauer *et al*. (1998) and Benchaar *et al*. (2006) fed monensin at a daily inclusion rate of 24 ppm and 350 mg/d, respectively, reported a reduction in milk fat content, and no change in protein content.

Muller *et al.* (2006) reviewed 14 research papers and concluded that supplementing monensin to grazing dairy cows had the same milk yield response as to dairy cows fed monensin in confinement. In comparison to control diets, the grazing cows on monensin supplement showed an increase in the daily milk yield average of 0.9 kg per cow. The milk fat and milk protein content decreased with 0.1% and 0.05 %, respectively.

Ramanzin *et al*. (1997) and van der Merwe *et al.* (2001) observed that adding monensin to the diet did not cause any effect on milk fat and protein content in comparison to a control diet.

A response in milk production is independent of the stage of lactation but not the type of diet fed to the lactating cows. The depression of milk fat is independent of the administered dose, stage of lactation, BCS and the type of diet (Ipharraguerre & Clark, 2003).

Table 1 A summary of the effect of monensin on milk production and milk composition in various studies

Author	Herd	Type of monensin[1]	kg Conc/d	Nutritive Value of concentrate DM	Type of pasture	Nutritive value of pasture[2] DM	Effect on Milk production	Effect on Milk composition[3]
Hayes *et al*., (1996)	Three New Zealand dairy herds	CRC	N/A	N/A	Spring pasture	CP- 160-200g/kg ME- 10.5-11.9MJ/kg	Increased	MF-No effect MP-No effect
Beckett *et al*., (1998)	12 Australian Dairy herds	CRC 300mg/d	N/A	N/A	Spring pasture	N/A	Increased by 0.75L/d	MF-No effect MP-No effect
Ruiz *et al*., 2001	Holstein	Monensin premix 350mg/day	6.4kg/d	CP- 86 g/kg	Fresh cut Orchard grass, Ad Lib	CP-16.4g/kg	Increased by 1.85 kg	MF-No effect MP-No effect
Van der Merwe *et al*., (2001)	Holstein	Monensin premix 300mg/day	10kg/d	CP- 120g/kg ME-11.5MJ/kg	Kikuyu/clover	CP-200-250g/kg	No effect	MF-No effect MP-No effect
Gallardo *et al*., (2005)	Holstein	CRC 335mg/d	TMR	CP- 162 g/kg	Lucerne mix	CP-282g/kg	Increased by 1.1kg/d	MF- Reduced MP- No effect
Grainger *et al*., (2008)	Holstein	CRC 320mg/d	5kg/d	CP-129g/kg	Ryegrass	CP- 212g/kg	No effect	MF-No effect MP-No effect

[1] - CRC - Controlled release capsule
[2] - CP - Crude protein, ME - Metabolisable Energy
[3] - MF - Milk fat content, MP - Milk protein content

2.4.2.2 Body condition score and body weight

The use of body condition scoring (BCS) is a helpful management tool enabling the producer to determine the energy status of the dairy herd (Kellog, n.d.). The subcutaneous fat cover can be determined by assigning a score to the amount of fat observed over certain skeletal parts of the cow's body (Kellog, n.d.). Evaluating fatness or thinness in cows is done according to a five-point scale (Heinrichs & Ishler, n.d.).

It has been found that cows supplemented with monensin showed an increase in BCS, body weight and ADG (Van der Merwe *et al*., 2001; Duffield *et al*., 2008b). Improved BCS and body weight could be attributed to the improved efficiency of energy metabolism when monensin is supplemented (Duffield *et al*., 2008b). An interaction between treatment and BCS shows significance when comparing three levels of BCS: thin (< 3.25), fair (3.25 to 3.75) and fat (> 3.75) and the effect of monensin supplementation. When cows are classified as thin, supplementation had no significant effects on BCS, but fair and fat classified cows showed an increase milk

production and BCS (Ipharraguerre & Clark, 2003). An increase in weight gain during early lactation may lead to increased fertility rates and higher conception rates in the dairy herd (Van der Merwe *et al*., 2001).

The dosage rate of monensin influences the extent to which BCS is improved when cows are supplemented. In a study by Van der Werf *et al.* (1997), different dosage levels of monensin and its effects on Holstein and Jersey cows was investigated. It was found that BW gain was increased for the treatment cows on a daily high dose (450 mg/cow) of monensin. The BCS consistently also increased for the cows on the same treatment.

2.4.2.3 Rumen health and functionality

2.4.2.3.1 Rumen pH

Readily fermentable carbohydrates form part of the concentrate supplements fed to dairy cows (Bargo *et al.*, 2003). The carbohydrates are rapidly fermented in the rumen and leads to a decrease in rumen pH (Carruthers & Neil, 1997; Bargo *et al*., 2003; Sayers *et al*., 2003). The pH of the rumen is highly variable and influences both the microbial population and volatile fatty acid production (Shriver *et al*., 1986). Cellulolytic bacteria function optimally at a rumen pH of 6.2 to 6.8 (Shriver *et al*., 1986). Once the rumen pH drops below 6.0, cellulolytic and methanogenic bacteria activity and productivity is reduced. A more acidic environment of pH 5.2 to 6.0 is optimal for starch digesting bacteria (Ishler *et al.*, 1996). Protozoal activity can be greatly reduced if the rumen pH drops below 5.5. All these processes should be accommodated by maintaining a rumen pH of 5.8 to 6.4 through feeding practices (Ishler *et al*., 1996; De Ondarza, 2000).

A common digestive disorder, acidosis, generally affects high-producing dairy cows (Nocek, 1997). Sub-acute acidosis has a ruminal pH ranging between 5.2 and 5.6 (Owens *et al*., 1998). The low ruminal pH is caused by an increase in the consumption of rapidly fermentable carbohydrates and deficient levels of physically effective fibre (NRC, 2001). High lactic acid levels in the rumen causes a ruminal pH below 5.2 and results in acute acidosis (Owens *et al*., 1998). Excessive VFA production may be a more important contributor to acidosis (McGuffey *et al*., 2001). Lactic acid producing strains of bacteria, such as *Streptococcus bovis*, can be affected by ionophores (Varga, 2004). Various strains of lactic acid producing bacteria can be inhibited by the addition of monensin, thus decreasing levels of lactic acid and reducing the occurrence of acidosis (Dennis & Nagaraja, 1981). A more stable rumen pH can be obtained when the lactic acid production is reduced (Varga, 2004).

Bloat is a commonly found problem in ruminant animals that are fed high grain diets or graze legume pastures (McGuffey *et al*., 2001). An excess production of foam occurs in the rumen (Bartley *et al*., 1983). An entrapment of gas in the reticulo-rumen and failure of the gas escaping

causes acute abdominal enlargement (McGuffey *et al*., 2001). In severe cases and left untreated, bloat can cause death. Monensin can be effective in controlling legume bloat by inhibiting the bacteria producing mucous which, entraps the gas and reduces the methane and CO_2 production and the viscosity of rumen fluid (Varga, 2004).

In studies done by Nagaraja *et al*. (1981, 1982) monensin and lasalocid were intraruminally infused and they found that the control fed cattle showed clinical signs of acidosis where the treatment cows showed mild signs of diarrhoea. Whereas the treatment cows also had a higher rumen pH compared to the control cows. In a rumen fluid sampling analysis, a colony count of *S.bovis* and *Lactobacillus* (lactate producing, gram positive bacteria) were done and lower levels were found in the ionophore treated cows. Plazier *et al.* (2000), McGuffey *et al.* (2001) and Benchaar *et al.* (2006) found that the rumen pH of monensin treated cows have increased in comparison to a control diet. When feeding a daily low level monensin supplementation (250 mg/cow), Broderick (2004) found no effect on ruminal pH. The drastic change in the diet of transition cows may cause acidosis. Adding monensin to the pre-partum diet and continuing into the post-partum diet may help to reduce acidosis (McGuffey *et al*., 2001).

2.4.2.3.2 Rumen Volatile fatty acids

Carbohydrates can be divided into structural and non-structural carbohydrates. The structural carbohydrates include the NDF component of the diet and non-structural includes the sugar and starches (Grant, 1991). These carbohydrates undergo rumen microbial fermentation and produce volatile fatty acids (VFA). The VFA contribute to almost 80% of the energy needs of the animal. The primary VFA include acetate, propionate, butyrate and iso-butyrate. If the forage: concentrate ratio is decreased the acetate: propionate ratio is decreased. When the cellulose and hemi-cellulose ratio is increased the acetate: propionate ratio will increase. This means forage is important for VFA production (Ishler *et al*., 1996).

Acetate makes up 50 to 60% of the total VFA concentration present in the rumen. It is predominant in high forage diets and aids in maintaining milk fat content. The level of acetate decreases when the diet contains high levels of concentrates and low forage, or when the diet contains heat treated starch and when the oil intake is high. Acetate enters the blood at the highest quantity (Bergman, 1990; Ishler *et al*., 1996). Propionate makes up 18 to 20% of the total VFA concentration. It is highest in high grain diets. It provides energy through the conversion to blood glucose in the liver. Propionate is used in the production of milk lactose. Energy produced from propionate is also used for live weight gain and is a more efficient energy source which produces fewer gasses (Bergman, 1990; Ishler *et al*., 1996). Twelve to 18% of the total VFA concentration is made up of butyrate. It provides energy through the rumen wall by being converted to ketones during the absorption through the rumen wall. The ketone bodies are an energy source for fatty

acid synthesis, skeletal muscles and other body issues. Butyrate is an important energy source that contributes to milk synthesis for early lactating cows that are often in a negative energy balance (Ørskov, 1986; McDonald *et al*., 2011).

Monensin causes a shift in rumen VFA production. The change in molar proportion of the various VFA is due to the defaunation caused by monensin (Van der Merwe *et al*., 2001) changing the bacterial composition in the rumen. Propionate production is increased and butyrate and acetate production decreased. The increase in propionate production can be partially explained by the replacement of gram-positive bacteria with more gram-negative bacteria (Russel, 1987). Diets low in fibre and high in concentrate/grain often have depressing effects on milk production. A low fibre diet causes a decrease in the molar proportion of acetate and increased proportion of propionate. Ionophores mimic the dietary effects of a low fibre, high concentrate diet and therefore may also reduce the milk fat content (Davis and Brown, 1970; Muller *et al*., 2006).

Ruiz *et al.* (2001) and Broderick (2004) reported that the acetate:propionate ratio had decreased when monensin was added to the diet of dairy cows. This ratio decrease was caused by the increase in propionate and decrease in acetate. When cattle are fed 50:50 forage: concentrate diet, the propionate-to-acetate ratio is higher than compared to cattle fed 70:30 forage: concentrate diets (Ruiz *et al*., 2001). Ionophores can inhibit methanogenesis by lowering the availability of hydrogen and formate, the two primary substrates needed for methanogens. Bacteria which produce these substrates are much more sensitive to ionophores leading to limited function of the methanogens and thus decreasing methane production. (McGuffey *et al.,* 2001) Methane gas is reduced making it a more environmentally friendly product (Muller *et al.,* 2006).

2.4.2.3.3 Rumen ammonia nitrogen

Rumen microbes use free ruminal ammonia nitrogen (NH_3-N) for protein synthesis and are utilized for microbial growth and fermentation of feeds for energy (Hoover, 1986). The minimum concentration of ruminal NH_3-N required for maximum microbial synthesis as identified by Satter and Slyter (1974) is 5 mg/dl. According to a review of ten studies by Bargo *et al.* (2003), when cows grazed pasture and were given a concentrate supplementation the optimal range for ruminal NH_3-N is from 8.5 to 30 mg/dl and a mean ruminal NH_3-N of 18.3 mg/dl. Maintaining it in this range may improve the synthesis of microbial protein, digestibility and feed intake (McDonald *et al*., 2011).

During early research the effects of ionophores on nitrogen metabolism in the rumen were noted. Some of the research reported that monensin reduced the rumen ammonia concentration. *In vitro* research with monensin on nitrogen metabolism done by Van Nevel and Demeyer (1977) reported a reduction in protein degradation, ammonia accumulation and microbial nitrogen. Hyper ammonia producing bacteria are gram-positive, which are more sensitive to the effects of

monensin, therefore increasing the post ruminal supply of protein and peptides (Hobson and Stewart, 1997). Furthermore, peptidolysis and amino acid deamination is reduced by the use of ionophores, consequently increasing the post ruminal supply of proteins and peptides further (Adesogan, 2008; Hutjens, 2012). In an *in vivo* experiment done by Ruiz *et al.* (2001), it was demonstrated that a decrease in ruminal ammonia occurred with the addition of monensin to a typical dairy cow diet. It was concluded that monensin may spare amino acids from wasteful rumen degradation thus supplying more protein post-ruminaly, potentially increasing milk protein content and the efficiency of N utilization in dairy cows grazing pasture.

2.4.2.4 Digestibility

Plazier *et al.* (2000) reported that the apparent digestibility of NDF as well as ADF increased if cows were fed a high forage diet supplemented with monensin. In the same study when the cows were on a high concentrate diet with monensin supplementation, there were no effects on fibre digestion. In studies done by Ali-Haïmoud *et al.* (1995) and Benchaar *et al.* (2006) the apparent digestibility of starch, was unaffected by monensin supplementation. Benchaar *et al.* (2006) found an increase in the apparent digestibility of CP for cows supplemented with monensin in comparison to cows recieving diets not supplemented with monensin. Plazier *et al.* (2000) reported an increase in the N digestibility of early lactating cows on a high concentrate diet supplemented with monensin whereas Ali-Haïmoud *et al.* (1995) found no effect on N digestibility. On a high forage diet, Plazier *et al.* (2000) reported no effects on N digestibility when supplemented with monensin.

2.5 Oregano

Since the ban on the usage of antibiotic ionophores in animal feeds in 2006 by the EU the focus has moved to the use of more natural products, such as plants and specifically their EO. Oregano (*Origanum Vulgare*) is a medicinal plant used centuries ago by the ancient Greek civilisation. It was used for various types of illnesses as well as for the treatment of sores and aching muscles (Nolte, n.d.).

Oregano is a common species of *Origanum*, a genus of the mint family (*Lamiaceae*). Naturally it grows in warm-temperate western and south western Eurasia and regions in the Mediterranean. It is a perennial herb that grows from 20 to 80 cm tall and has opposite leaves with a length of 1 to 4 cm and purple flowers 3 to 4 mm in length. This herb prefers a soil pH range of between 6.0 (mildly acidic) and 9.0 (strongly alkaline) (Meyers, 2005).

DOSTO® Concentrate 500 is a pure natural, essential oregano oil (flavouring compound) product of DOSTOFARM® (Westerstede, Northern Germany), which is a fine powder and not

water-soluble. It has a light cream colour and is strongly aromatic. It contains 500 000 mg/kg natural oregano oil.

2.5.1 Active ingredients

Oregano oil consists mostly of carvacrol (62 to 68%) and thymol (1 to 3%). Oregano is part of the terponoid group which is the more abundant group of metabolites found in EO and are derived from an isoprenoid structure. Terponoids can further be divided into monoterpenoid and sesquiterpenoid families to which most of the EO belong. Carvacrol and thymol are monoterpenoid phenolic structures and have an oxygenated cyclic hydrocarbon structure, as seen in Figure 1, making them highly active against bacteria. Specifically in carvacrol and thymol, the hydroxyl group and the dislocated electrons allow for the interaction with water through hydrogen bridges as the main active site, making them particularly active against microorganisms (Calsamiglia *et al.,* 2007).

2.5.2 Mode of action

Both terpenoids and phenylpropanoids have cyclic hydrocarbons with a hydrophobic nature enabling them to interact with the cell membrane of gram positive bacteria (Griffin *et al*., 1999). This structure enables the EO to interact with cell membranes and accumulate in the lipidic bilayer of bacteria (Figure 2). The spaces between the chains of fatty acids are thus occupied causing conformational changes in the membrane structure (Sikkema *et al*., 1994). It results in fluidification and expansion of the bacterial cell (Griffin *et al*., 1999). The stability of the membrane is compromised and thus leads to the leakage of ions across the cell membrane which in turn leads to a loss in ionic gradient across the transmembrane (Griffin *et al*., 1999; Ultee *et al*., 1999; Cox *et al*., 2001). Large amounts of energy is required by the bacterial cell to counteract the loss of ions by means of an ionic pump which can cause the cell to survive, but because of the high energy demand by the pumps, bacterial cell growth is reduced (Calsamiglia *et al.,* 2007).

Gram positive bacterial membranes are most easily affected by the mechanism of interaction within cell walls, by the hydrophobic compounds of EO. The membrane is also less complicated in comparison to those of gram negative bacteria. The reduction in gram positive bacteria leads to a reduction in products such as methane, ammonia, and acetate produced by the bacteria (Lee *et al.*, 2003). Gram negative bacteria thus increase as well as the products they produce i.e., propionate and butyrate (Lee *et al*., 2003).

Gram negative bacteria have a much more complex membrane structure, making it somewhat difficult for EO to penetrate the membrane (Cox *et al*., 2001). The external cell wall (outer membrane) is hydrophilic and thus does not allow the entrance of lipophilic substances such as most EO and monensin (Cimanga *et al*., 2002). These substances are not able to penetrate the

external cell wall of gram negative bacteria. However, the external membrane of gram negative bacteria is not completely impermeable to hydrophobic substances (Griffin *et al*., 1999; Dorman & Deans, 2000). The aromatic hydrocarbon structure and low molecular weight of carvacrol and thymol molecules enable them to interact with the lipid bilayer of cells by interacting with water (through hydrogen bridges) to cross the cell wall slowly and by diffusion through the polysaccharide or through the membrane proteins (Helander *et al*., 1998).

Helander *et al.* (1998) reported that carvacrol and thymol have the capacity to disintegrate the outer membrane of gram negative bacteria. They observed the release of membrane polysaccharides and the increased permeability of the cytoplasmic membrane. This mode of action thus make them effective modulators of gram positive and gram negative bacterial cell walls which can reduce their function or lead to cell death. However, these properties make them less selective against specific microbial populations making it more difficult to modify rumen fermentation (Calsamiglia *et al.,* 2007).

2.5.3 Factors affected by oregano supplementation

2.5.3.1 Milk production potential and composition

In vivo studies with regards to the effects of EO on milk production and composition are limited. Results of the few studies are inconsistent because of the different types of EO and dosage levels that were tested (Patra, 2011). Several studies done with a mixed EO product have yielded the same results. No changes in DMI, milk production or milk composition (Hosoda *et al*., 2005; Yang *et al*., 2007; Benchaar *et al*., 2006; Benchaar *et al*., 2007; Spanghero *et al*., 2009; Tassoul and Shaver, 2009). Kung *et al.* (2008) fed an EO complex (CRINA) and found an increase in milk yield but no other changes in milk components when compared to a control diet. In a study done by Santos *et al.* (2010), an essential oil complex fed to the cows impacted the production of high producing cows by enhancing fat synthesis and increasing the milk fat content. The increase in milk fat content may be due to the enhanced acetate production in the rumen or due to the change in the acetate: propionate ratio.

Limited information is available on milk production and the *in vivo* effects of oregano supplementation. In an experiment done by Tekippe *et al.* (2011), they found that milk yield was not affected by supplementation of dried *Origanum vulgare* leaves fed at a daily rate of 500 g to the diet of lactating dairy cows. The composition of the milk was also not affected by the supplementation. However there was a trend for improved milk fat content and milk fat yield while the 3.5% FCM yield was increased compared to the control diet. In a similar experiment done by Hristov *et al.* (2013), the milk yield was unaffected by the supplementation of dried *Origanum vulgare* leaves at daily inclusion rates of 0 g, 250 g 500 g or 750 g. The milk composition and milk

fatty acids were also unaffected. Oliveira *et al.* (2014) found that the inclusion of oregano did not increase milk production but milk composition parameters were not determined.

2.5.3.2 Body Condition Score and body weight

Data on the use of EO and its effect on BCS are quite limited. In a study done by Benchaar *et al.* (2006), they compared the effects of EO and monensin added to the diet of dairy cows. It was concluded that with regards to body weight that the cows fed EO in their diet had shown an increase in average daily gain when compared to no EO supplementation (body weight gain 0.44 vs. 0.15 kg/d respectively). Tassoul and Shaver (2009), found no significant differences for body condition score (BCS) and live weight (LW) when comparing EO supplementation with a control diet. Santos *et al.* (2010) found a positive response for BCS when fed a control diet, but observed a negative response in dairy cows fed an EO diet (0.063 vs. -0.016 units/28 days, respectively).

2.5.3.3 Rumen health and functionality

2.5.3.3.1 Rumen pH

In vitro studies have been done with regards to EO and the effect on ruminal pH. Busquet *et al.* (2006) and Benchaar *et al.* (2007) observed an increase in ruminal pH when carvacrol was added to *in vitro* 24-hour batch culture incubations at a dosage of 3000 mg/L, compared to a control with no EO additive. The pH values for carvacrol and control were 6.01 vs 5.58 respectively. Lower levels of carvacrol had a lower, to no effect on ruminal pH. Soltan *et al.* (2011) found that EO had no effect on the ruminal pH of *in vitro* 24-hour batch culture incubations when compared to a control culture. Jahani-Azizabadi *et al.* (2011) noted an increase of the final pH of a 24-hour batch culture containing oregano, relative to a control medium.

In vivo studies have shown that the ruminal pH was increased by the addition of EO to the diets of lactating dairy cows (Benchaar *et al.,* 2003 and Benchaar *et al.,* 2006). These findings might be helpful in order to maintain the ruminal pH when high grain diets are being fed (Benchaar *et al.,* 2006). Yang *et al.* (2011) reported that the ruminal pH did not differ among treatments of a control and EO supplementation diet. Diets containing *Origanum vulgare* leaves fed to lactating dairy cows did not affect the ruminal pH in comparison to a control diet (Tekippe *et al.,* 2011; Hristov *et al*., 2013).

2.5.3.3.2 Rumen volatile fatty acids

Benchaar *et al.* (2003); Newbold *et al.* (2004) and Benchaar *et al.* (2006) have reported no change in ruminal total VFA concentrations as well as the molar proportions of individual VFA when various EO were tested in *n vitro* batch cultures. In a study done by Castillejos *et al.* (2005) where a specific blend of essential oil compounds (BEO, CRINA® RUMINANTS) were tested *in vitro* it was reported, that the total VFA concentrations had increased with no change in molar proportions of individual VFA in comparison to a control. A few EO were tested *in vitro* at 5 different doses (0, 0.3, 3, 30 and 300 mg/l) by Cardozo *et al.* (2006). Among these oils was oregano. The pH of the *in vitro* batch culture was maintained at either 7.0 or 5.5. For oregano at a supplementation rate of 30mg/l the lower pH yielded an increase in propionate production and a decrease in total VFA, branched-chain VFA, acetate proportion and acetate: propionate ratio. However, at an increased dosage rate (300 mg/L) the overall result was a decrease in VFA concentration for all the EO tested. An *in vitro* study by Busquet *et al*. (2006), reported that carvacrol, at an inclusion rate of 300 mg/L, increased the molar proportions of butyrate and decreased propionate and acetate. A contradictory response was noted when the dose was increased to 3000 mg/L. The molar proportions of propionate increased, butyrate decreased and acetate was not affected. The results from an *in vitro* study by Soltan *et al.* (2011) showed that the molar proportions of butyrate increased with no effect on acetate and propionate proportions when carvacrol were added to a 24-hour batch culture at doses of 5 and 10 µl 75 ml of culture fluid. However at a higher dose of 20 µl 75 ml of culture fluid, the individual and total VFA decreased. A challenge arises to determine the correct dose rate for the use of different EO to have favourable effects on rumen metabolism and influencing the VFA concentrations.

The addition of *Origanum vulgare* leaves to a TMR diet of lactating dairy cows had no effect on the concentrations of the total and individual VFA. However a trend existed for increased acetate: propionate ratio (Tekippe, *et al.,* 2011; Hristov *et al.*, 2013).

2.5.3.3.3 Rumen ammonia nitrogen

The metabolism and utilization of dietary protein plays a vital role in dairy nutrition. If the cows are able to reduce the amount of waste products resulting from protein breakdown, less protein will need to be fed and thus the feed costs can potentially be decreased. It may also result in fewer waste products from such as methane gas being released into the environment (Tekippe, 2010). EO can be beneficial in dairy cow diets with regards to more optimal use of dietary protein (Benchaar *et al*., 2008). Hyper ammonia producing (HAP) bacteria make up about 1% of the rumen bacterial population but they possess a very high deamination activity (Russel *et al*., 1988). EO are able to inhibit HAP bacteria which can decrease the ammonia concentration and deaminase

activities in the rumen.

Work done by Borchers (1965) with EO and its effects on rumen fermentation resulted in a decrease in ammonia N concentrations and an accumulation of amino acids, *in vitro*, when 1 g of thymol was added to an *in vitro* batch culture. In a later study by Broderick and Balthorp (1979), results showed that thymol inhibited the deamination of amino acids. The deamination of amino acids plays a major role in protein metabolism because it is the primary step by which dietary amino acids can be lost by being turned into ammonia in the rumen (Tekippe, 2010). When protein is not efficiently utilized by the rumen microbes, nitrogen accumulates in the form of ammonia, which is an undesirable waste product from protein breakdown (Tekippe, 2010).

McIntosh *et al.* (2003) observed a 9% decrease in the rate of amino acid (AA) deamination in an *in vitro* 48 hour batch culture, treated with an EO mixture. The rate of ammonia production could be decreased in the rumen, which may benefit the efficiency of protein utilization (Wallace *et al*., 2002). An *in vitro* study with a mixture of EO by Newbold *et al.* (2004) reported a reduction of 25% in bacterial deaminative activities. Castillejos *et al.* (2006) found that supplementing a blend of EO to an *in vitro* culture had no effect on ruminal NH_3-N concentrations between treatments. Busquet *et al.* (2006) found that carvacrol and oregano oil at a dose rate of 3000 mg/L substrate decreased the NH_3-N concentrations. Jahani-Azizabadi *et al.* (2011) used an *in vitro* batch culture to test what effects various EO will have on the NH_3-N concentration. They reported that in contrast to the control, the NH3-N concentration was reduced with the addition of several EO in individual batch culture mediums. Carvacrol and thymol have the ability to accumulate amino acid nitrogen and thus decrease the ammonia nitrogen concentration (Jahani-Azizabadi *et al.,* 2011).

The effects of EO *in vitro* have shown to be positive but *in vivo* studies are quite limited. Benchaar *et al.* (2006, 2007) found that supplementing lactating dairy cows with an EO blend at 0.75 and 2 g/day had no effect on ruminal NH_3-N concentrations. Yang *et al.* (2007) found that garlic fed at 5 g/d to lactating dairy cows had no effect on ruminal microbial protein synthesis or NH_3-N.

2.5.3.3.4 Digestibility

Several *in vitro* and *in vivo* studies with EO have shown an alteration of the breakdown of plant cell walls, nutrients available to the animal and the digestion rate (Tekippe, 2010). With the addition of EO, care must be taken that the nutrient digestibility isn't negatively affected (Tekippe, 2010).

Only a few *in vivo* studies have tested the use of EO in dairy cow diets. Several *in vitro* studies have been done but *in vivo* studies and information regarding the nutrient digestibility in the rumen are limited (Santos *et al.,* 2010). A mode of action of EO is to have an effect on the bacterial colonisation pattern specifically targeting starch rich substrates in the rumen (Patra, 2011). A

second mode of action is the inhibition of HAP bacteria which are involved in the deamination of amino acids (Patra, 2011).

Kung *et al.* (2008) found no effect on the NDF digestibility of feeds when treated with a mixture of EO. In several studies (Meyer *et al.,* 2009; Malecky *et al.,* 2009; Santos *et al.,* 2010) the digestibility of feeds were not affected with the addition of EO to the diet. Yang *et al.* (2007) did a study on Holstein cows receiving 40: 60 forage: barley-based concentrate diet. They reported that ruminal digestibility of DM were higher (13%) for juniper berry EO (2 g/d) compared to a control diet. However the experimental treatments did not affect the total tract digestibility of DM, organic matter, fibre and starch. A suggestion was made that the increase in ruminal digestibility was due to the increase in ruminal digestion of dietary protein. Benchaar *et al.* (2007) evaluated the addition of an essential oil blend and type of diet on nutrient digestibility and found there were no effects on the total tract DM, CP, NDF or ADF digestibility between diet treatments. Benchaar *et al.* (2008) observed when adding cinnamaldehyde at 1 g/day to dairy diets that there were no difference in the total tract digestibility of DM, OM, CP, NDF and ADF.

Castillejos *et al.* (2006), added thymol to continuous culture fermenters at doses of 5, 50 and 500 mg/L. A decrease in digestibility of DM, NDF and ADF was found at the highest dose rate. The lower levels had no negative effect on DM, NDF and ADF digestibility. This means that the effect on digestibility is dose dependent.

Generally the addition of EO and their active compounds may cause a slight increase in nutrient digestibility or have no effect. By increasing the digestibility of feeds *in vivo*, efficient use of nutrients by dairy cows will be improved (Tekippe, 2010).

Chapter 3 Problem statement

Antibiotics are used at non-therapeutic levels to modulate rumen fermentation. This may lead to enhanced growth, increased milk yield, improvement of daily feed intake as well as improve the feed efficiency and prevent disease. The use of antibiotics in this manner has, however, been criticized because of the emergence of multi-drug resistant bacteria that may pose a risk to human health. Residues of the chemicals in milk and meat make it unfit for human use and can be potentially toxic for the host animal, negatively affecting the microbial population. Due to these findings and the consumer wanting to be healthier, the EU has banned the use of antibiotic ionophores as feed additives since January 2006. This ban has led to nutritionists and microbiologists becoming more interested in bioactive plant factors that can modify the rumen fermentation processes in a natural way. Bioactive plant factors such as EO are becoming more popular in animal nutrition as a natural alternative to antibiotics. Plants used as a forage source, are a natural part of the herbivore diet and thus make it safe for use in animal diets. EO have antimicrobial properties making them a natural antibiotic which can improve the feed utilization and health of ruminants. The aim of this study was to determine the effect of an oregano oil extract (Dosto 500) on milk production, milk composition and rumen fermentation of cows grazing ryegrass pasture in spring and to establish whether oregano EO could replace monensin in pasture based dairy cow diets.

Chapter 4 Materials and methods

4.1 Location and duration of study

The study was performed on the Outeniqua Research Farm situated near George in the Western Cape province of South Africa. The farm is situated at 33° 58’ 702” S and 22° 25’ 222” E at an altitude of 204 m above sea level. The climate of the George area is temperate consisting of moderate temperatures. During the time that this study was conducted, temperatures ranged from a minimum of 11 °C and a maximum of 21 °C on average. The annual rainfall for this region over a 45 year period is 731.45 mm (ARC, 2011). The soil of the specific paddock used consisted of two distinct soil forms. In the northern part of the paddock the soil consists of Escourt form and the southern part that has a slightly downward slope consists of the Witfontein form (Soil Classification Working Group, 1991).

The study was performed from 15 September 2014 to 21 November 2014. The whole study ran over 67 days, including an adaption period of 14 days (starting on 15 September 2014) and the data collection started on 29 September 2014. The shortened adaption period was because of time constraints and changes that occurred (Kikuyu starts to grow and ryegrass begin to seed) in the pasture from the middle to the end of November that might have influenced the results.

4.2 Paddock design and pasture parameters

4.2.1 Design of the kikuyu/ryegrass pasture paddock

A permanent irrigated kikuyu/Italian ryegrass pasture of an estimated size of 8.55 ha was divided into 39 strips by using electrically charged poly wire. During the study, kikuyu was in a dormant phase (Botha, 2003) and thus the camp mostly consisted of Italian ryegrass. The separate strips had a length of 150 m and width of 15 m. As indicated in Figure 3, strips 35 to 39 were slightly smaller than the rest of the strips. The poly wire had nine irrigation heads, spaced evenly down from end to end. This resulted in 10 x 15 m spaces within each strip, except for strips 35 to 39. The green stars indicate the location of water troughs. The numbers on the left hand side indicate the strip numbers.

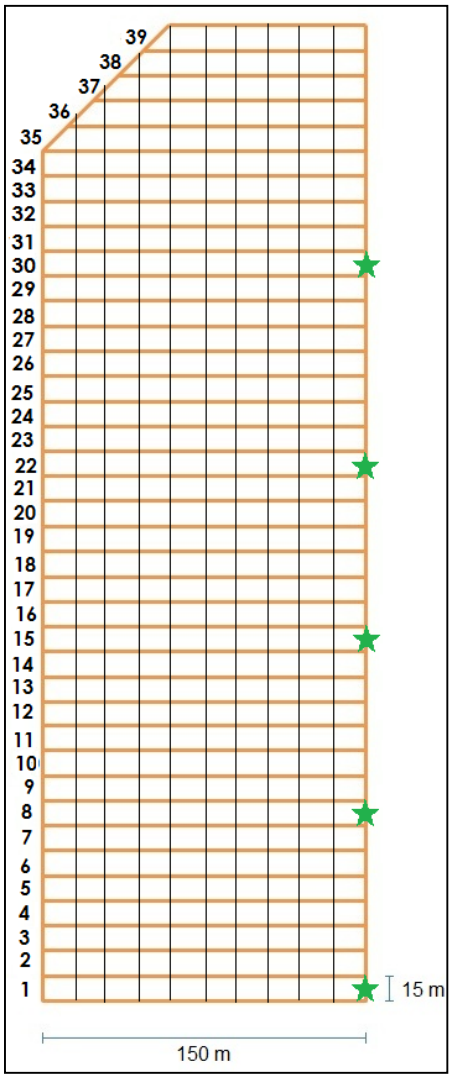

Figure 3 Diagramatic presentation of the 8.55 ha kikuyu/ryegrass pasture used during the study

4.2.2 Establishment of the pasture

The 8.55 ha paddock consisted of an established kikuyu (*Pennisetum clandestinum*) pasture. An annual ryegrass, Italian ryegrass (*Lolium multiflorum* Lam. var. *italicum* cv Jeanne), was over-sown into the established kikuyu pasture. Before the kikuyu pasture was oversown with ryegrass it was grazed down to a stubble height of 50 mm (RPM reading of 10). The pasture was then mulched to ground level (1.6 m Nobili with 24 blades; Botha, 2003). The Italian ryegrass was established into the mulched kikuyu pasture at a seeding density of 25 kg/ha during May 2014. A direct drill with no-till planter (2.4 m Aitchison 3116C Seedmatic with 16 rows) was used to establish the seeds. The pasture was then rolled over with a 2.33 m Cambridge type light land roller (Botha, 2003).

4.2.3 Pasture management

The pasture was strip grazed from West to East. The Jersey cows were allowed a dry matter intake (DMI) of 10 kg per day on the pasture. By measuring the height of the pasture before and after each grazing, the DMI of the cows could be determined. The height was measured by the use of a rising plate meter (RPM). A RPM reading of 10 (stubble height = 50 mm) after grazing was maintained for optimal pasture utilization. After each milking session the cows received a new strip to graze. The pasture was grazed in a 28-day grazing cycle. Using RPM readings before grazing on of each grazing strip and calculating pasture yield by means of a regression equation, pasture was allocated to cows using an electrical fence. After grazing, pasture was fertilized with 42 kg N/ha applied as limestone ammonium nitrate.

Figure 4 The Jersey cows used in the trial, grazing kikuyu/ryegrass pasture

4.2.4 Pasture yield estimation

The RPM was used to determine the estimated DM yield per area. The disk meter has an area of 0.98m^2. A total of 100 RPM readings were taken in a zig-zag pattern, before grazing to obtain a representative height of the grazing available. After grazing, the pasture strip was measured again with the RPM in a similar way. A seasonal regression was determined by cutting grass samples every second week, from the beginning of the production study. Nine samples of varying heights (three low, three medium and three high) were taken at each sampling. The height of the grass was determined by the RPM. A metal ring with the same diameter as the RPM was placed over the RPM and dropped onto the grass. All the grass inside the ring was cut at a height of 30 mm above ground and placed into brown paper bags. The cut samples were weighed wet, placed in a dry oven for 72 hours at 60 °C and weighed again. The DM content and thus the DM yield could be determined. A linear model was used to calculate the seasonal regression. The linear model correlates the RPM reading with the pasture DM yield.

The following equation was used, Y = (a x H) + b

Y - Dry matter yield (kg DM/ha)

a - Gradient

H - Recorded height on RPM

b - intercept value

The regression equation used was Y = 83.093*H – 588.78

Figure 5 Using a RPM to measure the height of the pasture and to determine the estimated DM yield per area by means of a regression equation

4.2.5 Pasture allocation

Pasture yield was estimated by using the RPM and pasture was allocated at a daily allowance of 10 kg DM/cow. The RPM height after grazing was set at above 30 mm, divided into two grazing periods after each milking. Pasture was managed to obtain a post-grazing height of 10 to 12 on the RPM. All pasture strips were measured before and after grazing by taking 100 readings with the RPM.

4.2.6 Pasture quality

Weekly pasture samples were taken. Three representative samples of the pasture at an area of 0.098 m^2 each and stubble height of 30 mm (RPM reading of 6), were cut a day before grazing. The cut samples were placed in brown paper bags taken to the laboratory and weighed (Sartorius BP8100, weighing accurately to 0.1 g). It was then dried at 60°C for 72 hours (Botha, 2003) and weighed again to determine the dry matter content of the pasture. Samples were stored for later analysis at the Stellenbosch University laboratory.

4.2.7 Animal welfare

Ethical clearance was obtained through the Western Cape Department of Agriculture and a DECRA approval number was issued: R14/102.

4.3 Production Study

4.3.1 Experimental design

Fifty-four early lactation Jersey cows of the Outeniqua Research farm were used in this study. A completely randomised block design was used to allocate the cows to the three different treatments. The 54 cows were divided into 18 blocks. The blocks were defined according to milk yield (averaged three weeks before start of the study), days in milk (20 to 120 DIM), lactation number and on their 4% FCM during three previous weeks. By using the Gaines formula we were able to calculate the 4% FCM, where 4% FCM = [0.4 x kg milk] + [15 x kg milk fat] (Gaines, 1928). Each block consisted of 3 comparable cows and cows within blocks were randomly allocated (Random number function, Microsoft Excel, 2010) to one of the three treatments which resulted in three groups with similar parameters as seen in Table 2.

The three treatments were as follows:

- Control (CON), maize based concentrate with no feed additives
- Ionophore (MON), maize based concentrate with daily dosage of monensin (300 mg per cow). The product used was Rumensin®, a product of Elanco Animal Health.
- Essential oil (EO), maize based concentrate with a daily inclusion rate of oregano oil extract at 1.15g per cow. The product was Dosto 500 concentrate, a dry powdered product of DOSTOFARM.

Table 2 The pre-trial mean and standard deviation for milk yield, 4% FCM, DIM, lactation number, milk fat (%) and milk protein (%) of the three treatment concentrate groups (n = 18)

Parameter[1]	Treatment[2]		
	Control	Monensin	Oregano
Milk yield (kg)	20.5 ± 1.59	20.5 ± 1.97	20.5 ± 1.91
4% FCM (kg)	23.5 ± 1.97	23.5 ± 2.27	23.3 ± 2.31
DIM	79.2 ± 35.60	84.4 ± 26.20	95.1 ± 33.83
Lactation no.	4.7 ± 2.42	3.2 ± 1.35	3.9 ± 1.86
Milk fat (%)	5.0 ± 0.24	5.0 ± 0.38	4.9 ± 0.26
Milk protein (%)	3.6 ± 0.15	3.6 ± 0.18	3.7 ± 0.15

1 - FCM- Fat corrected milk; DIM- Days in milk
2 - Control – Concentrate containing no feed additive; Monensin – concentrate containing 300 mg monensin/cow/d; Oregano – concentrate containing 1.15 g of oregano/cow/d fed a treatment concentrate at 6 kg/d (as-is)

The cows received different coloured tags, attached to a light metal chain and secured with a cable tie. Each colour represented a different treatment. The CON group received blue tags, the MON group had red tags and the EO group had yellow tags. These tags were used to identify cows on each treatment. Cows grazed as one group and were separated into the different treatment groups before milking to facilitate concentrate feeding in the milking parlour.

Figure 6 Coloured tags, attached to a light metal chain, assigned to the Jerseys cows in the three different groups, to facilitate grouping prior to milking

The concentrate formulation was done by Professor Robin Meeske and Mr Bernard van der Merwe from NOVA feeds. The additives were mixed into three different premix packs (6 kg/ton) as shown in Table 4 by Camelus Feeds (11 Jones Street, Oudtshoorn) and sent to NOVA's feed mill in George (Nova feeds George, Industrial Area, George, Western Cape, South Africa). The different concentrates were mixed pelleted and placed into 3 different coloured 50 kg bags. The ingredient composition of the dairy concentrate fed to the three treatment groups is presented in Table 3. The premix composition used in the three different concentrate treatments is presented in Table 4.

Table 3 Ingredient composition of experimental dairy concentrates fed to the three different treatment groups at 6 kg/cow/day in the milking parlour during milking sessions

Ingredient	Concentrate
	g/kg DM
Maize meal	716
Hominy Chop	150
Molasses syrup	50
Soja oil cake	50
Feed Lime	15
MonoCaP	5
Salt	5
MgO	3
Premix*	6
ME MJ/kg	12.5
CP (g/kg)	109.2
Ca (g/kg)	8.4
P (g/kg)	3.8

*Three different premixes were included in the three concentrate treatments

Table 4 The composition of the premix added to the three different concentrate treatments

Ingredient	Premix		
g/ton or as stated	Control	Monensin[1]	Oregano[2]
Vitamin A (IU)	9 000 000	9 000 000	9 000 000
Vitamin D3 (IU)	600 000	600 000	600 000
Vitamin E (IU)	12 000	12 000	12000
Cobalt	1.2	1.2	1.2
Copper	30	30	30
Iron	90	90	90
Iodine	2.3	2.3	2.3
Magnesium	300	300	300
Manganese	120	120	120
Selenium	0.45	0.45	0.45
Zinc	150	150	150
Maize meal carrier	4 220	3 972	4 030
Additive	0	250	192

[1]-Monensin is 20% active in Rumensin thus a daily inclusion of 1500 mg Rumensin per cow = 300 mg monensin/cow/d
[2]-Oregano oil extract (Dosto500) fed at a daily inclusion of 1.15 g per cow

4.3.2 Milking and feeding program

The distance covered by the cows from the camp to the parlour was about 800 m. They were led to the parlour and back to camp as one group. Before milking (at 05:30 and 13:30) cows were separated into their respective treatment groups by separating the three coloured groups (Figure 6) for milking and the consumption of their individual concentrate treatments. The milking

system used on the farm is a twenty point Dairy Master swing over milking machine which has weigh-all electronic milk metres (Total pipeline Industries, 33 Van Riebeeck Street, Heidelberg, 6665). Before the cows entered the parlour the individually weighed 3 kg feed bags were emptied into the evenly spaced feed troughs. Once everything was ready in the parlour, the first two groups could enter. CON and MON groups entered the parlour first followed by EO group. The milking procedure ensured that udder health was maintained and to care for the overall well-being of the cows. The troughs were checked to ensure that cows consumed all their concentrates before they were allowed to move out of the parlour. Each cow received 6 kg (as-is) of one of the three concentrate treatments. They were offered 3 kg in the morning and 3 kg in the afternoon. The 3 kg bags was weighed out accurately (Bizerba FC.15 scale with 0.1 g accuracy) in plastic bags and stored in the feed room marked feed bags for easy identification of the three treatments. After milking, the cows were taken back down to the pasture as a group. Individual milk yield was recorded electronically for every milking using the Dairy Master Milk meters and computer software.

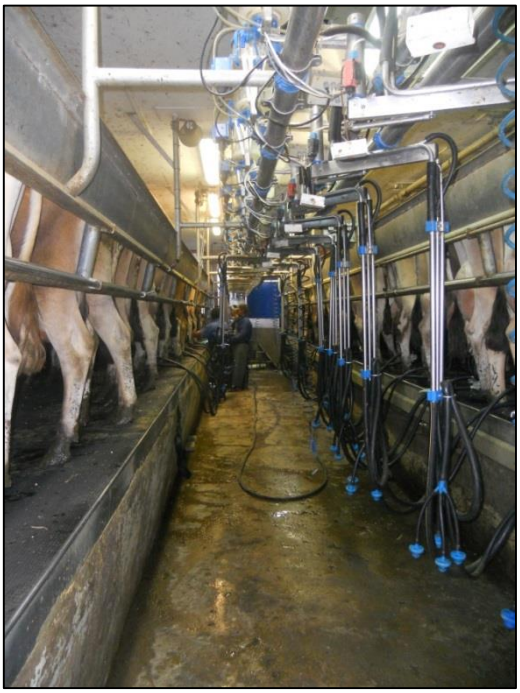

Figure 7 The twenty-point swing-over milking system of Dairy Master used in the parlour with weigh-all electronic milk meters

4.3.3 Data Collection

4.3.3.1 Live weight and Body Condition Score (BCS)

Cows were weighed on a Tru-Test EziWeigh version 1.0 scale (0.5 kg accuracy, Auckland New Zealand) and body condition scored (Scale 1 to 5) over two consecutive days at the commencement and completion of the study (before afternoon milking). The BCS was subjectively done by Pieter Cronje (Jersey herd manager at Outeniqua research farm) on a scoring system with a one to five point scale (Wildman *et al.,* 1982). The scoring was done by focusing on the hindquarters and the palpation of the back. The cows were weighed and body scored again at the end of the study. The mean BW and BCS were calculated for each treatment group at the commencement and end of the production study. These two means were used to determine the change in BW and BCS over the whole production study.

4.3.3.2 Pasture and concentrate samples

Pasture and feed samples were collected once a week and dried at 60 °C for 72 hours. The concentrate samples were pooled for every two weeks and stored for later proximate analysis. Pasture samples were taken by placing a metal ring of 0.98 m^2 and 30 mm height over a piece of pasture. Grass inside the ring was then cut to stubble height. This was done three times per sampling. Grass was placed into brown paper bags and taken to the laboratory. A blank brown paper bag of same size was weighed on the scale (Sartorius BP8100, weighing accurately to 0.1 g). Scale was tared and the three bags were weighed “wet”. Weights were recorded. After the drying process the blank bag was weighed again and scale tared. The dried bags were weighed again and weights were recorded. Representative concentrate samples of each treatment were taken every week. Concentrate samples were placed into brown paper bags weighed and dried to determine the DM content following the same procedure that was followed for the pasture samples.

4.3.3.3 Milk yield and milk samples

The daily milk yield of each cow was recorded by the Dairy Master Milk meter. A composite milk sample was collected per cow on a biweekly basis and sent for analysis (fat, protein, lactose, milk urea nitrogen (MUN) and somatic cell count (SCC) to Deltamune (Oudtshoorn Laboratory, BOKOMO Premises, 15 – 17 Rademeyer Street, Oudtshoorn 6625). A 24 ml representative milk sample was obtained by dividing the sampling into two collection periods. One ml of milk sample was collected for every one hour between milking intervals. The hour difference between morning and afternoon milking was 8 hours and afternoon to morning milking was 16 hours. This resulted in a sampling volume of 16 ml at PM milking and the next AM milking 8 ml to make up a pooled 24

hour representative milk sample. The bottles used in the sampling procedure were tilted three times to mix the milk and then the correct volume was placed into smaller sampling bottles. The milk samples were preserved by using potassium dichromate ($K_2Cr_2O_3$) tablets in the smaller sampling bottles. These samples were then transported after the morning milking to Deltamune where milk component analyses were performed.

4.4 Rumen study

4.4.1 Experimental design

Six ruminaly cannulated cows were used in the rumen study. Two cows were randomly allocated to each of the three treatments in a 3 x 3 Latin square design (three treatments and three periods) as shown in Table 5.

Table 5 Experimental design of the 3 x 3 Latin square rumen study with three treatments and three periods

Parameter	Control	Monensin	Oregano
Period 1	Cow 1 and 2	Cow 3 and 4	Cow 5 and 6
Period 2	Cow 5 and 6	Cow 1 and 2	Cow 3 and 4
Period 3	Cow 3 and 4	Cow 5 and 6	Cow 1 and 2

Cows were subjected to a 20 day adaptation period, followed by a 7 day measurement period. After the different rumen measurements were complete the cows were rearranged among treatments and the same adaptation and sampling procedures were followed until the end of the trial.

4.4.2 Data collection

4.4.2.1 pH profile of the rumen

Rumen pH was measured using the Tru Track data indwelling logger system (TruTrack Data Logger, www.intech.co.nz). A watertight rubber hose and fittings were used to attach the logger and electrode to the rumen cannula in place reducing logger malfunction and cow discomfort. The probe was emerged into the rumen content. The capsule was custom made to fit into a Bar Diamond #1C rumen cannula. Loggers were calibrated one day before they were inserted into the cows using the Omnilog Data Management Program (Version 1.64) with buffer solutions of pH 4, 7 and 9. Loggers were started, placed in a bucket with distilled water for 12 hours, inserted into the rumen at 06:30 after morning milk and left in the cow for 72 hours (day 1 to 4 of measurement), recording pH at 10 minute intervals. At the end of 72-hour period the pH-loggers were removed and placed back into a bucket of clean water for three hours still logging.

After 3 hours the loggers were connected to the Omnilog Data Management Program via the computer, the loggers were stopped and all the data collected were transferred to an Excel file. The data was later processed by determining the average pH values for 30-minute intervals over the three 24-hour periods that the recordings were taken. During the second and third period the same loggers were assigned to the same cow as in the first period to rule out any variation in external factors.

Figure 8 Indwelling pH logger being calibrated using the OmniLog Data Management Program to measure the rumen pH over a 72-hour period

4.4.2.2 Sampling of rumen fluid

Rumen samples were collected by inserting a tube through the lid of the rumen cannula, and by using a hand operated suction pump, a negative vacuum was formed and rumen liquor could be extracted to a bottle connected to the end of the tube. These samples were extracted to determine the concentration of VFA and ruminal NH_3-N. Around 100 ml per cow was extracted. The sampling was done at 06:00, 14:00 and 22:00. Rumen pH was measured immediately, using a handheld pH meter (WTW pH340i pH meter/data logger attached with a WTW Sentix 41 pH electrode). After the measurement of the pH, the bottles were tightly sealed to avoid oxygen exposure. The bottles were then taken to a laboratory on site pending filtration. The rumen liquor was strained through 4 layers of cheese cloth to remove any solid particles. The remaining fluid was then transferred into two separate bottles, one bottle for the analysis of VFA and the other for analysis of rumen NH3-N. The samples were immediately frozen pending further analysis at the Stellenbosch University Laboratory.

Figure 9 Using a customised hand pump to extract rumen fluid samples via the cannula plug

4.4.2.3 In sacco study

An in sacco study was carried out to determine the digestibility of ryegrass pasture. The method as described by Cruywagen (2006) was used. Ryegrass was cut at a height of 50 mm when 1.2 ton DM of grass was available. The samples were weighed and dried in the oven for at 60 °C for 72 hours. After drying the grass samples were weighed again to determine DM content. The samples were cut into 5 to 10 mm pieces (Taweel et al., 2004). Before preparation, the nylon bags were placed in the oven at 60°C for 24 hours. The “dry” weight of the bags was recorded. Approximately 5 g of ryegrass was weighed accurately (± 0.001 g; Sartorius L420P scale) and placed into nylon bags (10 cm X 20 cm, 53 µm pore size; Bar diamond Inc, P.O. Box 60, Parma, Idaho, USA). Nylon stockings were used in the incubation study. A marble was inserted as a weight into a nylon stocking leg and secured in place with a knot. This prevented the stocking to float to the top of the rumen. Two bags, separated by a knot were inserted into one leg of the stocking. Another leg of the stocking contained three bags separated by knots. The two stockings were then connected to a cannula plug with a ring attached to it using the catcher technique (Cruywagen, 2006). At an incubation time of 6 hours the leg containing two bags was taken out. The other stocking leg containing the three bags was incubated for 30 hours. After the 30 hour incubation time the stocking and plug was removed and the normal cannula plug was replaced. Two nylon bags were prepared as blanks during each run to determine substrate/nutrient losses at time 0. These blank bags were frozen and washed with the other bags to determine the soluble fraction of the sample. After removal from the rumen the bags were washed under running tap water and frozen pending further processing. Once the trial was completed all the bags were thawed in a bucket filled with clean cold water. Once thawed, the bags were washed in a Defy

Twin tub washing machine (3 x 3 min) and rinsed. After each cycle water was drained and replaced with clean water. The bags were placed on a dry rack to remove excess moisture. Once almost dry the bags were dried at 60 ˚C for 24 hours. Bags were weighed and the bag contents for each cow were pooled for each incubation time to determine the DM and NDF content. Samples were stored in plastic bottles, pending analysis at the Stellenbosch University Laboratory.

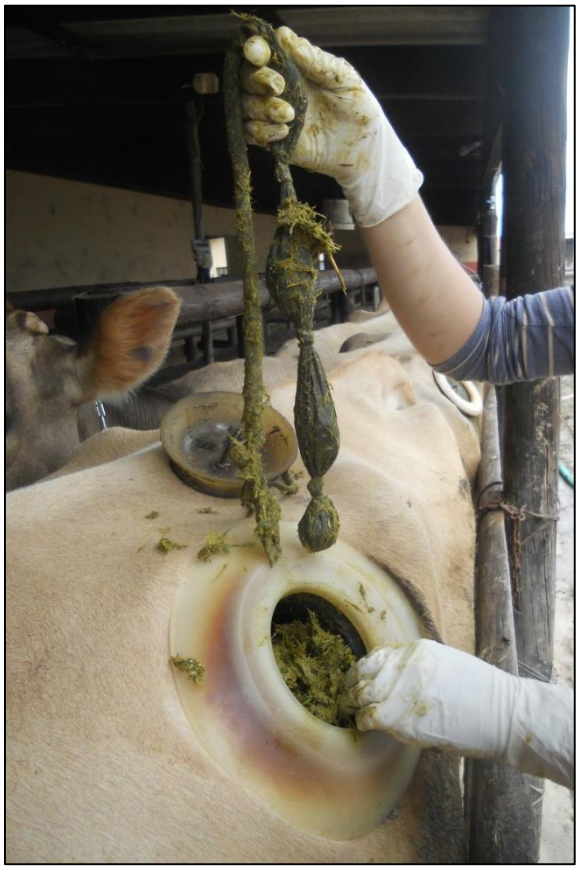

Figure 10 Removing the stocking containing nylon bags after incubation period of 6 or 30 hours

4.5 Analytical procedures

4.5.1 Pasture and concentrate samples

The dried pasture and concentrate samples was weighed and stored for later analysis. The samples were analysed for; DM, OM, GE, IVOMD, CP, NDF, ADF, Ether extract, Ca, P, Mg.

All the concentrate and pasture samples were analysed in duplicate for DM (AOAC, 2002; method 934.01), ash (AOAC, 2002; method 942.05), CP (AOAC, 2002; method 990.03; using the Leco N analyser, model FP 528), NDF (Mertens, 2002), ADF (Raffrenato and Van Amburgh, 2011), EE (AOAC, 2002; method 920.39), GE (MC 1000 Modular Calorimeter, Energy Instrumentation, Sandton, South Africa, 2146), and IVOMD (Buys *et al.,* 1996). Calcium (Ca) (sample preparation: AOAC, 2000: procedure 935.13; sample analyses: Giron, 1973) and phosphorous (P) (AOAC, 2000: procedure 965.17).

The ME of the pasture and the treatment concentrate was calculated using the following equations: 0.82 x GE x (IVOMD% ÷ 100) and 0.84 x GE x (IVOMD% ÷ 100), respectively (Robinson *et al*., 2004).

4.5.2 Milk samples

The milk samples were analysed for milk fat, protein, lactose, somatic cell count (SCC) MUN and pH. The milk fat, protein, lactose and MUN were analysed with the FOSS CombiFoss FT+ milk analyser (FOSS, Foss Allé, DK-3400 Hilleroed, Denmark) by means of the mid-infrared spectroscopy and the SCC was analysed with flow cytometry. The method is SANAS ISO17025 accredited for all the parameters reported.

4.5.3 Rumen fluid samples

4.5.3.1 VFA Analysis

Before the samples could be analysed for VFA concentration it first had to go through a "clean-up" procedure. The samples were thawed and 1.5 ml of rumen fluid was pipetted into 2 mL centrifuge tubes. The tubes were placed in a centrifuge and spun at 12000 x g for 10 min. Six hundred microliter of supernatant was transferred in duplicate, into 2 mL centrifuge tubes. Two reagents were prepared, a calcium hydroxide solution (CHS) and a cupric sulphate solution (CSR). Six hundred microliter of CHS was added to each tube. Three hundred microliter of CSR was then added to each tube. The tubes were capped, vortexed to mix thoroughly and then frozen overnight at -10˚C. The tubes was thawed centrifuged again for 10 min at 12000 x g. A 1000 µL aliquot of supernatant was transferred to 2 mL centrifuge tubes containing 28 µL concentrated sulphuric acid. The tubes were capped, vortexed and frozen again overnight at -10˚C. The samples were thawed and 500 uL of diethyl ether was added. The tubes were centrifuged for at 12000 x g for 10 minutes and at 4 °C. The supernatant was transferred to vials and analysed on a gas chromatograph. (Modified from Siegfried *et al*., 1984)

4.5.3.2 Rumen ammonia nitrogen concentration

The samples were thawed and 2 mL of rumen fluid was transferred to 2 mL centrifuge tubes. The tubes were centrifuged and spun for 5 min at 6000 x g. Fifty microliter of the supernatant was transferred in duplicate to test tubes. To each test tube 2.5 mL of phenol reagent was added and then mixed. Then 2 mL of hypochlorite reagent was added and mixed. The tubes were placed in a rack and incubated in a water bath at 95 °C for 5 min. The tubes were then placed in an ice bath for 5 to 7 min to cool down. The samples were transferred to spectrophotometer vials. The spectrophotometer was zeroed using blanks (0mM = 0.1 N HCl) and the absorbance of the samples were measured at λ = 630 nm. A regression equation was calculated using standard solutions and then used to calculate the ammonia concentration (Broderick and Kang, 1980).

4.5.4 *In sacco* study

The residue of the nylon bags was used to determine the concentration of NDF after the two different incubation times. The content of the two 6 hour bags and that of the three 30 hour bags was separately pooled for each cow and treatment. The pooled residue was ground through a 1 mm sieve before further analysis. Samples were then analysed for NDF as described in 4.5.1.

4.6 Statistical analyses

4.6.1 Production study

Measurements were analysed by analysis of variance (ANOVA) for a randomised block design (RBD) consisting of three treatments in 18 blocks, to test for differences between the three treatment effects. The residuals were acceptably normal with homogeneous treatment variances, except in the case of SCC, which were then log (base 10) transformed.

For measurements, such as milk production, weight and FCM, that were taken at the start and the end of the trial, covariance analysis was used to test for significant (linear) relationships between the before and after measurements and then for differences between treatment effects. If the relationship was not significant, then ANOVA was used to test for differences between treatment effects on the end-of-study measurements. Treatment means were compared using Tukey's least significant difference (LSD) test at the 5% level of significance (Snedecor & Cochran, 1980). Data were analysed using the statistical program GenStat® (Payne, 2014).

The model used is described by the following equation:

$$Y_{ij} = \mu + T_i + B_j + e_{ij}$$

Where Y_{ij} = Variable studied during the period

μ = overall mean of the population

T_i = effect of the i^{th} treatment

B_j = effect of j^{th} block

E_{ij} = random error associated with each Y

4.6.2 Rumen Study

pH profile over 24h

Linear mixed model analysis, also known as REML analysis was applied to the pH profile data over 24h to model the correlation in a repeated measurements analysis (Payne, 2014). The fixed effects were specified as hour, treatment and the hour by treatment interaction, while the random effects were specified as the square, square by period and square by period by hour interaction. Means were compared using Tukey's least significant difference (LSD) test at the 5% level of significance.

Rumen VFA and NH3-N

The ruminal VFA and NH3-N data were analysed as a replicated 3 x 3 Latin square testing for differences between treatment effects. Treatment means were compared using Tukey's least significant difference (LSD) test at the 5% level of significance. Data were analysed using the statistical program GenStat® (Payne, 2014).

Chapter 5 Results and discussion

5.1 Nutrient composition of concentrates

The chemical composition of the three concentrate treatments fed to the dairy cows is shown in Table 6.

Table 6 Chemical nutrient composition of the three concentrate treatments (6 kg fed as is)

Nutrient[1] (g/kg) or as stated	Treatment concentrate[2]		
	Control	Monensin	Oregano
DM	900 ± 0.2	903 ± 0.2	904 ± 0.1
OM	950 ± 2.7	955 ± 6.2	950 ± 3.1
IVOMD (%)	92.2 ± 1.26	92.5 ± 0.89	92.5 ± 0.47
ME (MJ/kg)	13.3 ± 0.17	13.2 ± 0.22	13.2 ± 0.17
CP	122 ± 3.6	120 ± 1.1	122 ± 2.4
NDF	105 ± 2.5	98 ± 2.9	98 ± 5.3
EE	44.2 ± 3.05	42.6 ± 1.25	47.3 ± 1.71
Ca	7.90 ± 0.141	7.78 ± 0.286	7.70 ± 0.235
P	4.33 ± 0.043	4.33 ± 0.109	4.40 ± 0.071
Mg	3.08 ± 0.083	3.13 ± 0.110	3.10 ± 0.071
Ca:P ratio	1.8:1 ± 0.02	1.8:1 ± 0.02	1.8:1 ± 0.04

1 - DM- dry matter; OM – organic matter; IVOMD – in vitro organic matter digestibility; ME - metabolisable energy (calculated); CP – crude protein; NDF – neutral detergent fibre; ADF – acid detergent fibre; EE – ether extract; Ca – calcium; P – phosphorous; Mg – Magnesium; Ca:P - ratio between calcium and phosphorus
2 - Control – Concentrate containing no feed additive; Monensin – concentrate containing 300 mg monensin/cow/d; Oregano – concentrate containing 1,15 g of oregano/cow/d fed a treatment concentrate at 6kg/d (as-is)

The results correspond well to the estimated formulation made by NOVA feeds (Table 3) for the concentrate treatments. The DM value of the concentrate treatments were 900 g/kg. This indicates that the feeds were mixed and stored correctly and did not contain excess moisture which might influence the DMI of the treatment concentrate. It corresponds with other studies where a treatment concentrate was fed where the DM was 908 g/kg and 898 g/kg respectively (Bargo *et al*., 2002; Van Wyngaard, 2013). The OM value of 950 g/kg indicates that the concentrate treatments had a high organic content and a low level of ash. It is indicative of the fact that the feed were stored correctly and that the concentrates were clean and not contaminated by dust. The OM value in the current study agrees with to that of Bargo *et al.* (2002) and Gallardo *et al.* (2005) with a value of 916 g/kg and 959 g/kg respectively, in a supplemented concentrate fed to dairy cows on pasture. The IVOMD% of the three concentrate treatments was high suggesting the concentrates were highly digestible. The ME value obtained from the chemical analysis of the concentrate treatments are higher than the value formulated for by NOVA Feeds (13.3 MJ/kg vs. 12.5 MJ/kg). Therefore, the ME content was more than sufficient to maintain milk production. The CP value of the feed was higher than formulated for (121 g/kg vs. 109 g/kg) but the CP content was similar for the three treatments. The CP content is comparable with the values (122 g/kg) reported by Van

Wyngaard (2013) when a concentrate was fed to dairy cows on pasture. The NDF values were similar among the concentrate treatments. The treatment concentrate contained a high level of maize decreasing the NDF value and thus increasing the digestibility of the concentrate. The ether extract values of the concentrate treatments were lower than 50 g/kg. Care must be taken to ensure the total diet of the grazing dairy cows is not too high in fat. This will have a negative impact on the rumen fermentation process by reducing productivity of fermentation. The calcium content of ryegrass pasture tends to be lower than that required by lactating cows (NRC, 2001) and therefore a higher than normal level of calcium was supplemented. The phosphorous level was adequate to meet the requirements of lactating dairy cows (NRC, 2001). Ryegrass pasture tends to have a magnesium deficiency and therefore magnesium was supplemented in the concentrate treatments. A disorder common in dairy cattle grazing pasture is grass tetany or hypomagnesaemia (Elliott, 2009). The main cause of this disorder is a magnesium deficiency in the pasture. It affects the blood calcium and magnesium levels of the dairy cows and leads to restricted muscle movement. This may cause the death of an animal. This can be prevented by supplementing magnesium in the concentrate.

As expected, the composition of the three concentrate treatments did not differ from each other, with the only difference being the additive.

5.2 Pasture quality

5.2.1 Pasture nutrient composition

The mean nutrient composition of the kikuyu/ryegrass pasture grazed over the 8 week study period is shown in Table 7.

Table 7 Mean and standard deviation of the nutrient composition of the kikuyu/ryegrass pasture (n = 8) grazed by the Jersey cows during the eight week study period

Nutrient[1] g/kg DM or as stated	Kikuyu/ryegrass pasture
DM	135 ± 1.7
OM	881 ± 7.1
IVOMD %	82.2 ± 2.67
ME (MJ/kg)	11.2 ± 0.19
CP	246 ± 10.5
NDF	494 ± 25.9
ADF	255 ± 13.1
EE	49.2 ± 3.09
Ca	4.10 ± 0.402
P	4.13 ± 0.35
Mg	3.38 ± 0.35
Ca:P ratio	1:1 ± 0.19

DM- dry matter; OM – organic matter; IVOMD – in vitro organic matter digestibility; metabolisable energy (calculated); CP – crude protein;NDF – neutral detergent fibre; ADF – acid detergent fibre; EE – ether extract; Ca – calcium; P – phosphorous; Mg – Magnesium; Ca:P - ratio between calcium and phosphorus

The ryegrass pasture in the current study had a DM value of 135 g/kg, which concurs with the DM values of 137 g/kg and 128 g/kg, for the ryegrass pasture reported by Steyn (2012) and Van Wyngaard (2013), respectively. The OM value of the pasture is quite high suggesting there was not too much inorganic material present in the pasture. The IVOMD% of the pasture (82.2%) was high, which is typical of a high quality ryegrass pasture during spring (Bargo *et al*., 2003). The ME of the pasture is comparable to previous studies done on this paddock (Van Wyngaard, 2013). The CP value of 246 g/kg DM is quite high suggesting a high quality pasture was available to the cows (Bargo *et al.*, 2003). The CP value of the current study is higher than expected when compared to other studies on ryegrass pastures. Meijs (1986), Sayers *et al*. (2003) and Meeske *et al*. (2006) found the CP to be 218.5 g/kg, 238.8 g/kg and 207 g/kg DM, respectively. However, Lingnau (2011) reported a higher CP value of 259 g/kg DM on the ryegrass pasture. The NDF value of the ryegrass pasture (494 g/kg) agrees with that of Lingnau (2011) and Van Wyngaard (2013) who reported it at 541 g/kg and 493 g/kg, respectively. According to the NRC (2001) the NDF value of ryegrass pasture averages at 450 g/kg. This indicates that the NDF was within the range as stated by the NRC (2001). A lower NDF value is desired, as this would have a positive effect on DMI (Ball *et al.,* 2001) As the pasture matures, the NDF value will increase because the cell wall content have increased leading to a more bulk fill of the diet and limiting DMI (Ball *et al*., 2001). Acid detergent fibre (ADF) is highly correlated with the digestibility of the plant cell walls. The more mature the plant the higher the ADF and less digestible it will be. The energy digestibility is negatively correlated to the ADF. A low ADF is preferred because that means a higher net energy will be available. The ADF value of 255 g/kg correlates well to the value as stated by the NRC (2001), 250g/kg. This value also agrees to that reported by Lingnau (2011), 261 g/kg and Van

Wyngaard (2013), 301 g/kg. The ether extract value of 49 g/kg for the ryegrass pasture indicates that the crude fat was lower than 50g/kg. A value higher than 50 g/kg crude fat will have a negative effect on the efficiency of rumen fermentation (Wheeler, 1993). The Ca to P ratio was 1:1 in the pasture and therefore Ca was supplemented in the treatment concentrate.

5.2.2 Regression

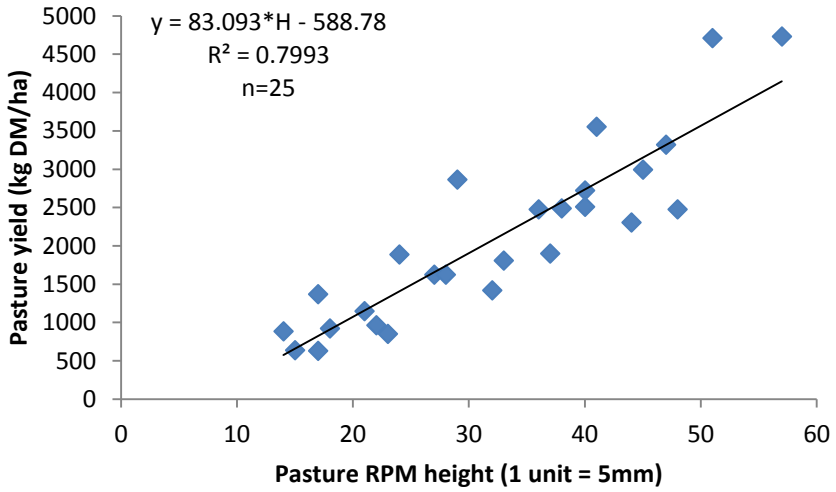

Figure 11 The seasonal regression correlating the rising plate meter (RPM) reading with the kikuyu/ryegrass pasture yield (kg DM/ha) used throughout the study

During the course of the study a seasonal regression was determined for the kikuyu/ryegrass pasture and it generated the following equation: y = 83.093*H - 588.78, where 'Y' = available DM herbage (kg/ha) and 'H' = RPM height reading. Figure 11 shows the calculated seasonal regression. The equation was only used after the conclusion of the study, and was used as a single regression for pre- and post-grazing estimations.

5.2.3 Pasture DMI

The pasture yield, allowance and estimated pasture intake are shown in Table 8. Using the RPM the pre- and post-grazing heights could be determined. Using the regression equation of Y = 83.093*H – 588.78, the pasture yield could be determined.

Table 8 Mean and standard deviation of the pre- and post-grazing rising plate meter readings (n=89), pasture yields, pasture allowance and pasture intake determined using a single calculated regression (Y = 83.093*H – 588.78)

Parameter[1]	Pasture Values
Pre-grazing	
RPM reading	29.5 ± 4.5
Pasture yield (kg DM/ha)	1861 ± 371
Pasture allowance (kg DM/cow/day)	9.3 ± 1.7
Post-grazing	
RPM reading	11.3 ± 1.6
Pasture yield (kg DM/ha)	348 ± 132
Pasture removed (kg DM/ha)	1517.2 ± 347.3
Pasture intake (kg DM/cow/day)	7.2 ± 1.4

1-RPM – Rising plate meter; DM – Dry matter
± - Mean and standard deviation

As specified by Stockdale (2000), the post-grazing height on the RPM was within range of an RPM reading of 10 to 12 RPM. This indicates well utilized pasture and ensures optimal pasture regrowth and quality. Even though the RPM is inaccurate in the determination of pasture DMI (Reeves *et al*., 1996), it is a helpful management tool to allocate pasture to the cows in a correct way. By accurately measuring the pre- and post-grazing heights of the pasture, it will ensure the pasture is optimally utilized and grazed down correctly. The paddock used in the current study (Figure 3) was grazed three times throughout the duration of the study. The cows were allocated a new strip of grazing after each milking session which resulted in short grazing periods. According to Smith *et al.* (2005) this increases the reliability of the RPM. The pasture allowance was set at 10 kg DM/cow/day (Section 4.2.3). As seen in Table 8 the average pasture allowance was 9.3 kg DM/cow/day, which is close to the estimated 10 kg DM/cow/day. The under-estimation may be attributed to the use of a generalised linear seasonal regression on the specific pasture calculated by Van der Colf (2011) to determine the pasture yield and to allocate the pasture for each grazing session. However, the values presented in Table 8 were calculated using my own regression equation (Figure 11) which was calculated by taking regression samples throughout the study. According to this linear regression equation a bit less pasture was allocated to the cows, which resulted in a lower intake than initially estimated for. The difference between pasture allocation and actual pasture intake are attributed to the height of 3 cm above ground (6 RPM) for the cut regression samples. The cows were allowed to graze to a height of 5 cm (10 to 12 RPM). The lower cut samples are done to give a leeway if cows do graze lower than 5 cm above ground. The potential pasture intake could also be lower because of the cows trampling and contaminating the grazing. The lower pasture intake may have had an effect on the milk production and rumen

parameters. These results are discussed later in this chapter. The R^2-value of 80% for the regression indicates that 20% of the pasture DM yield was not declared by the regression equation. This suggests that the regression underestimated the DM yield of the pasture and that the use of the RPM is not accurate enough when estimating pasture DM yield. However the after-grazing height shows that the pasture allowance was not a problem. In a previous study done on the same pasture by Van Wyngaard (2013), the R^2 value was 75 %.

5.3 Production Study

5.3.1 Milk production and milk composition

The results from the milk production study are presented in Table 9. There were no differences in milk yield among treatments. The three treatments had no effect on 4% FCM, milk fat content, MUN, and SCC. The two feed additives used resulted in a higher milk protein content and a higher milk lactose content compared to the control treatment.

Table 9 Mean milk yield, 4% fat corrected milk (FCM), milk components (fat, protein, lactose, milk urea nitrogen (MUN), somatic cell count (SCC) of cows receiving the three different treatment concnetrates fed at 6 kg/cow/d (as is) and grazing ryegrass pasture in spring

Parameter[1]	Treatment concentrate[2]			SEM[3]	P-value
	Control	Monensin	Oregano		
Milk yield (kg/cow/d)	20.5	20.3	20.4	0.48	0.963
4% FCM (kg/cow/d)	22	22	22	0.4	0.686
Milk fat (g/kg)	45.2	44.7	45.6	0.12	0.88
Milk Protein (g/kg)	33.9[b]	35.5[a]	36.0[a]	0.055	0.003
Milk Lactose (g/kg)	45.2[b]	47.9[a]	48.3[a]	0.053	<0.001
MUN (mg/dL)	12.8	13.0	13.1	0.34	0.821
SCC (x 1000/ml)	216	212	210	0.083	0.861

1 - FCM-fat corrected milk; MUN – milk urea nitrogen; SCC- somatic cell count

2 - Control – Concentrate containing no feed additive; Monensin – concentrate containing 300mg monensin/cow/d; Oregano – concentrate containing 1,15 g of oregano/cow/d fed a treatment concentrate at 6kg/d (as-is)

3 - SEM – standard error of mean

a,b means in the same row with different superscripts differ (P<0.05)

According to literature, milk production and composition may differ according to the dose of feed additive, to the mode of feed additive supplement, to the stage of lactation, to the level of production and the type of diet (TMR vs Pasture supplemented with concentrates) (Gandra *et al.*, 2010).

5.3.1.1 Milk production

The milk yield and 4% FCM (Table 9) did not differ ($P > 0.05$) among treatments. These results agree with a study done by Van der Merwe *et al.* (2001) where Holstein cows allowed to graze a kikuyu/clover pasture were fed a concentrate containing monensin at a daily inclusion rate

of 300 mg monensin per cow at 10 kg/day (as - is). In the current study cows were fed a concentrate at 6 kg per day (as – is) and, as found by Van der Merwe *et al*. (2001), supplementation of monensin did not have an effect on milk production. The Kikuyu/clover pasture used by Van der Merwe *et al.* (2001), had a CP value of 200 to 250 g/kg which compare to the kikuyu/ryegrass pasture CP value of 246 g/kg in the current study.

The current study also agrees with a study done by Grainger *et al*. (2008) where Holstein-Friesian cows received a monensin controlled-release capsule as a bolus releasing 320 mg/d of monensin. The cows grazed a ryegrass pasture with a lower CP value of 212 g/kg, but received a cracked barley grain concentrate at a daily rate equivalent to that of the current study. In the study by Grainger and co-authors (2008) supplementation of monensin did not have an effect on milk production.

Supplementation of monensin, however, showed an increase in milk production in a few studies. Beckett *et al.* (1998), Ruiz *et al.* (2001) and Gallardo *et al.* (2005) reported an increase in milk production where cows were fed monensin in a concentrate and had access to fresh forage as compared to a control concentrate. This increase in milk production can be the result of monensin improving the milk production efficiency of pasture based dairy cows. In a meta-analysis done by Duffield *et al.* (2008b) the authors concluded that pasture-based diets and the addition of monensin to the concentrate had a positive effect on milk production by increasing milk production efficiency.

With regards to the *in vivo* effect of EO in dairy cows there is limited information available. However, in previous production studies the overall outcome of EO additives was inconsistent in regards to the production potential of dairy cows (Patra, 2011). The addition of 1g/d cinnamaldehyde had no effect on milk production of supplemented cows fed a TMR (Benchaar *et al*., 2008). Milk yield was unaffected in two separate experiments where cows were supplemented with garlic (5g/d), juniper berry (2g/d) (Yang *et al*., 2007) and peppermint (20g/kg of DM) (Hosoda *et al*., 2005). In an experiment done by Tekippe *et al.* (2011), it was reported that milk yield was unaffected by supplementation of dried *Origanum vulgare* leaves at a daily inclusion rate of 500g per cow to the TMR diet of lactating dairy cows. In a similar study done by Hristov *et al.* (2013), the milk yield was unaffected by supplementation of oregano leaves. The form in which the oregano is given may contribute to added effects, but in the current study, a concentrated form of Oregano was used and did not affect the milk production.

5.3.1.2 Milk fat content

The milk fat content (Table 9) did not differ among treatments ($P > 0.05$). The milk fat content ranged between 4.4 to 4.6% and this falls within the herd average of 4.5% for the Outeniqua Jersey dairy herd and concur to results by Van Wyngaard (2013).

Previous work states that a decrease in milk fat content can be expected when using ionophores in the dairy ration (Ipharraguerre & Clark, 2003). Monensin decreases the milk fat content by decreasing the ruminal production of acetate and butyrate and by inhibiting the ruminal biohydrogenation of long chain fatty acids (Fellner *et al.,* 1997; Overton *et al.*, 2006). In studies done by Beckett *et al.* (1998) and Ruiz *et al.* (2001) no differences in milk fat content were observed when monensin was added to the diet (Table 1). In a grazing study by Grainger *et al.* (2008) it was reported that the milk fat content was not affected by monensin supplementation as a controlled-release capsule (CRC). In studies done by Phipps *et al*. (2000) and Broderick (2004) the milk fat content decreased when monensin was supplemented in lactating cows. An increase in milk yield but a parallel decrease in milk fat content found by Phipps *et al.* (2000) might suggest a dilution effect which contributes to changes in milk constituents.

An increase in milk fat content were reported by Santos *et al*. (2009) when an essential oil blend was supplemented to cows on a TMR diet. In another study by Santos *et al.* (2010), the milk fat content and milk fat yield increased when an EO mixture containing, eugonol, geranyl acetate and coriander were fed to dairy cows on a TMR diet.

Other studies done with a blend of EOs (CRINA) by Kung *et al*. (2008), Benchaar *et al.* (2006) and Benchaar *et al*. (2007) found no effect on milk fat content when compared to a control TMR fed to dairy cows. In studies done by Benchaar *et al.* (2008) and Benchaar & Chouinard (2009) on specific EOs such as 1 g/d cinnamaldehyde, 50 mg/kg DMI of eugonol no effects were reported on milk fat content when compared to a control diet. No effect on milk fat content were reported by Yang *et al.* (2007) and Hosoda *et al.* (2005) when dairy cows were supplemented with 2 g/d garlic or 2 g/d juniper berry and 20 g/kg DM peppermint, respectively. Tekippe *et al.* (2011) and Hristov *et al.* (2013) did studies using oregano leaves and found no difference in milk fat content when compared to a control TMR.

5.3.1.3 Milk protein content

Milk protein content does not readily respond to protein levels in concentrate supplements (Bargo *et al.,* 2003) and thus no differences were expected. However, the milk protein content of cows fed the two feed additives in the current study showed an increase ($P < 0.05$) in comparison to the control treatment (Table 9). Various factors affect milk composition. These factors include: genetics, stage of lactation, number of lactations, milk production potential, environment, disease

(i.e. mastitis) and nutrition (Grant, 2007). In three studies using monensin as a feed additive (Hayes *et al*., 1996; Green *et al*., 1999; Phipps *et al*., 2000), significant decreases were reported for milk protein content (Ipharraguerre & Clark, 2003). These three studies also recorded a significant increase in milk production suggesting that the decrease in milk protein content may be because of a dilution effect. In studies done by Vasquez (2012) and Abdi *et al.* (2013), it was found that there were no effects to the milk protein content of cows on a TMR diet treated with monensin. In a grazing study done by Grainger *et al.* (2008), monensin was supplemented to lactating dairy cows as a CRC releasing 240 mg/d of monensin. The cows were supplemented with 5 kg of concentrate and results showed that there was no effect on milk protein content when compared to unsupplemented cows.

In several studies by Benchaar *et al.* (2006; 2007; 2008) the addition of an EO blend (CRINA), cinnamaldehyde or eugonol to the TMR diets of dairy cows it was reported that the milk protein content were unaffected when compared to cows on a control diet. No effect on milk protein content were reported by Yang *et al.* (2008) when cows were fed 2 g/d of garlic or juniper berry. Hosoda *et al.* (2005) also reported no change in milk protein content when lactating dairy cows were supplemented with 20 g/kg DM peppermint. Spanghero *et al*. (2009) reported that EO supplementation (40 and 80 g/cow/d) to rations had a positive effect on the milk protein content of lactating Holstein cows.

Tassoul and Shaver (2009) reported that the milk protein content reduced by 0.15% units in comparison to a control when lactating cows were supplemented an EO complex. The milk protein content of lactating dairy goats were increased with EO additives in a study by Kholif *et al.* (2012). They concluded that the increase in milk protein content may be as a result of improvement of ruminal microbial protein synthesis.

In a study by Van Wyngaard (2013) an average milk protein content of 3.5% was recorded for Jersey cows grazing a kikuyu/ryegrass pasture in spring. The milk protein content obtained during the current study was within the range of the recorded milk protein content by Van Wyngaard (2013) as well as the average milk protein content level for the Outeniqua Jersey herd, grazing ryegrass pasture in spring. The increase in milk protein content can be beneficial to the producer as it increases the economic value of the milk. The milk protein content can be increased by dietary manipulation. An increase in concentrate supplement fed to dairy cows may increase the milk protein content (Kennelly & Glimm, 1998; Bargo *et al*., 2002; Sayers *et al*., 2003). An increase in carbohydrate sources to the rumen micro-organisms optimises the synthesis of microbial protein (Bargo *et al*., 2002).

Amino acids from the microbial protein are digested and absorbed through the small intestine, providing the necessary components needed for milk protein synthesis (Schwab *et al*., 2008). Other factors that may increase milk protein content include; the genetic merit of the individual cow, parity (1st lactation, lower milk protein content), stage of lactation (later in lactation, higher milk protein content), and the breed of cow (Jersey cows: 3.8 to 3.9% breed average) (Grant, 2007).

5.3.1.4 Milk lactose content

The milk lactose content was higher ($P < 0.001$) in the two feed additive treatments compared to the control treatment (Table 9). This is contrary to other experiments where no increases found in the lactose content. The lactose component in milk ranges between 4.7 and 4.8% (Gibson, 1989; NRC, 2001). In the current study both feed additives in comparison to control treatment, increased the lactose to the optimal range.

Thomas *et al.* (2005) did a study where monensin was added to a TMR diet. There were no differences found for milk components in comparison to a control diet. In a comparison study between 350 mg/d monensin and 2 g/d EO supplemented to lactating dairy cows by Benchaar *et al*. (2006) it was reported that the two additives did not have an effect on the milk lactose content when compared to an unsupplemented diet. In a grazing study done by Grainger *et al*. (2008), a monensin CRC was used to determine the effects on milk production and composition. They reported that there were no effects on the milk lactose content of lactating dairy cows. Cant *et al.* (1997) found no difference in the lactose content among two treatment diets. The one diet contained fish oil and the other diet monensin (INCLUSION). Vasquez (2012) tested the effect of monensin fed at the pre- and postpartum stage and concluded that if monensin was fed through the dry period as well as postpartum it did increase the milk lactose yield ($P = 0.03$).

Limited studies have reported the effect of EO and the milk lactose content. Benchaar *et al*. (2006) reported that the daily supplementation of monensin (350 mg/d) and EO (2 g/d) in a TMR diet to lactating dairy cows, there were no significant effects on milk lactose content.

The change in milk lactose content can be ascribed to the health of the udder and the milk SCC levels (Welper & Freeman, 1992). Somatic cell count has a high correlation with fat and proteins and a negative correlation with lactose and amount of milk (Rajćević *et al*., 2003). An increase in SCC may decrease the milk lactose content. Pirisi *et al.* (2000) and Rajćević *et al.* (2003) both reported that when the SCC levels were > 500 000 cells/mL of milk, the milk lactose content decreased. At a level of 100 000 to 250 000 cells/mL of milk, the lactose content was higher when compared to the lactose content at the higher SCC levels. In the current study the milk lactose increased for the two feed additive treatment concentrate, the SCC levels did not differ significantly among treatments. It is within the range stated by Gibson (1989) for the average

lactose content of 4.7% for Jersey cows.

Improved energy availability might contribute to the higher milk lactose content. In an unpublished study done by Van Wyngaard (2015) lactating Jersey cows grazed a ryegrass pasture and three groups were fed, no concentrate, 4 kg concentrate and 8 kg concentrate per cow on a daily basis, respectively. The lactose content differed numerically between the no concentrate and the 8 kg concentrate treatments with the milk lactose content averaged 4.3 and 4.6 respectively. This indicates that the concentrate, which provides energy to the cows, may have a contribution to the energy status of the cow and have a positive effect on the milk lactose content.

5.3.1.5 Milk urea nitrogen

The MUN levels of the milk (Table 9) did not differ among the three treatments ($P > 0.05$). Under typical TMR situations Kohn (2007) reported MUN concentrations of between 8 to 12 mg/dl. This means adequate amounts of protein was available to the cows in the current study. According to Bargo *et al.* (2003) the MUN values for cows on pasture-based systems are higher when compared to cows on a TMR system. An average MUN value of 19 mg/dl was previously reported in various grazing studies where cows were supplemented (Khalili & Sairanen, 2000; Bargo *et al*., 2002; Delahoy *et al*., 2003). In the current study a MUN concentration of 13 mg/dl were obtained for all three treatments and is in agreement to the results from various pasture based studies where cows were fed a concentrate supplement (Khalili & Sairanen, 2000; Meeske, *et al*., 2009; Van Wyngaard, 2013). This indicates that MUN was in the acceptable range for pasture-based systems.

5.3.1.6 Somatic cell count

There was no difference (Table 9) in the SCC ($P > 0.05$) among treatments. The SCC values recorded in the current study are lower than the legal requirement level (< 500 000 cells per mL milk) for human consumption (De Villiers *et al.*, 2000). The low SCC values show that the udders were in a healthy condition. Milk production may decrease when the SCC rises above 500 000 cells/mL of milk (Raubertas & Shook, 1982; Eberhart *et al*., 1982). An infection in the mammary gland causes damage to the milk secreting cells and reduces their capacity for producing milk (Campbell, n.d.).

5.3.2 Live weight and body condition score

All three groups had increased LW and BCS but there were no differences among treatments with regards to LW and BCS or the change in LW and BCS (Table 10).

Table 10 Effect of the three concentrate treatments fed at 6 kg/cow/d (as is), on body weight and body condition score of Jersey cows grazing kikuyu/ryegrass pasture in spring

Parameter[1]	Treatment concentrate[2]				
	Control	Monensin	Oregano	SEM[3]	P-value
LW before (kg)	391	406	406	6.9	0.22
LW after (kg)	398	409	407	6.5	0.433
LW change (kg)	6.56	2.42	1.08	1.89	0.119
BCS before	2.17	2.17	2.15	0.034	0.946
BCS after	2.25	2.29	2.31	0.027	0.315
BCS change	0.083	0.125	0.153	0.034	0.358

1 – LW - Live weight, BCS - Body condition score
2 - Control – Concentrate containing no feed additive; Monensin – concentrate containing 300mg monensin/cow/d; Oregano – concentrate containing 1,15 g of oregano/cow/d fed a treatment concentrate at 6 kg/d (as-is)
3 - SEM – standard error of mean

The current study was over a short study period and therefore no significant effect on LW and BCS was expected. Gandra *et al.* (2010), found no effect on live weight, change in live weight, body condition score, and change of body condition score ($P > 0.05$) with two different levels of monensin was added to the concentrate diets of lactating cows. These results from Gandra *et al.* (2010) differ from Duffield *et al*. (2008b), who reported that supplementation with sodium monensin increased the BCS by 0.03 points, and a 0.06 kg/day increase in live weight of lactating cows. The differences can be explained by the differences in phase of lactation, and milk yield level in the evaluated animals among the present study and the ones cited by Duffield *et al*. (2008). In the current study a numerical change in BCS is 0.04 and 0.08 more for the MON and EO treatment groups in comparison to the CON treatment. These agree with the results of Duffield *et al*. (2008b).

5.4 Rumen study

5.4.1 Rumen pH profiles

The rumen pH recorded with the indwelling pH loggers are presented in Figure 12. The ruminal pH over a 24 hour period did not differ among the three treatments.

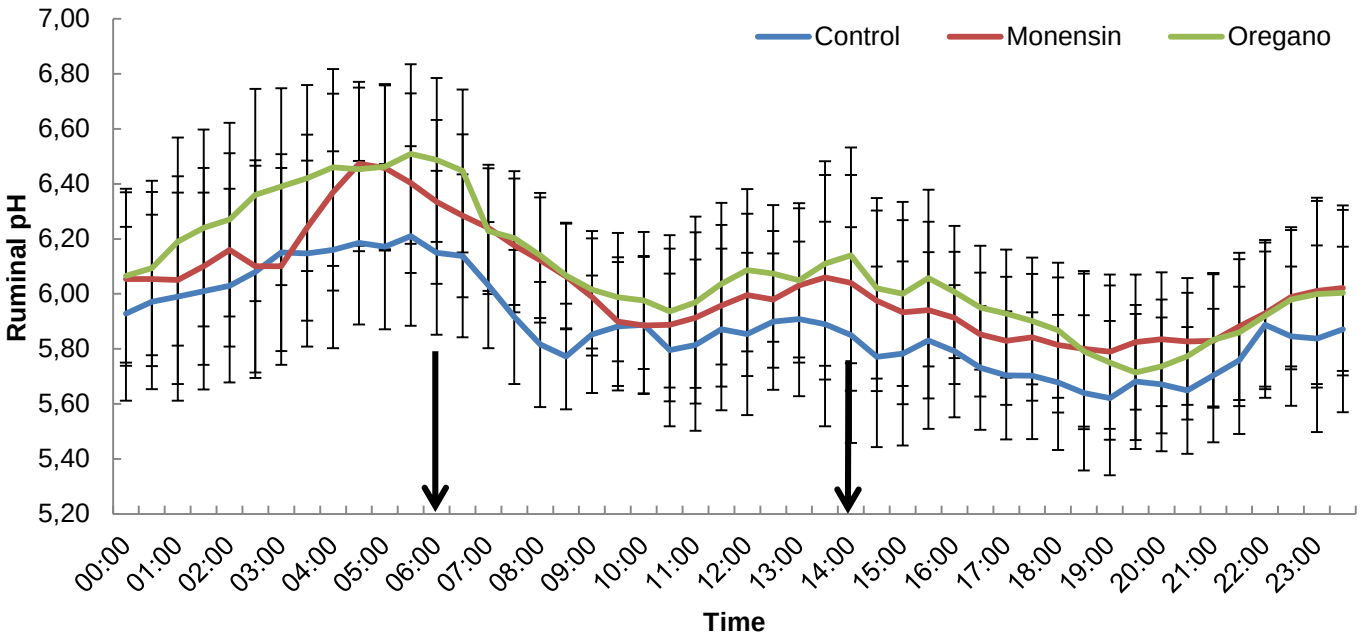

Figure 12 The mean diurnal rumen pH (indwelling loggers) over a 24 hour period of Jersey cows (n = 6) fed a treatment concentrate at 6 kg/cow/day (as is), which included no feed additive or monensin or oregano, respectively, grazing a kikuyu/ryegrass pasture in spring. Arrows indicate feeding times. Error bars indicate SEM.

There were no differences among treatments for the rumen pH recorded for the three concentrate treatments (Figure 12). A distinctive drop in pH can be seen for the three treatments at 6:00 and 14:00 which corresponds with the time of feeding in the milking parlour. The drop in pH post-feeding is a result of readily fermentable carbohydrates present in the concentrate treatments being rapidly degraded (Dixon & Stockdale, 1999). The highest pH was reached in the early mornings (05:00 to 06:00) before morning feeding and the lowest pH at 18:00 to 20:00 in the evenings. This agrees with Bargo *et al.* (2002), who reported that the ruminal pH is at its highest before the feeding of concentrate and is at its lowest 2 to 5 hours after the consumption of a concentrate. The highest pH values were a result of the rumen having enough time to stabilise after the PM feeding of the concentrate treatments. The two cyclic drops in rumen pH indicate normal diurnal pH fluctuation (Mould *et al*., 1983; Hoover, 1986). These drops in pH last only for about two hours and don't have negative effects on microbial activity. At a pH lower than 6.0 the growth of certain bacterial species ceases, which results in a reduction in fibre digestion (Hoover,

1986). The pH values in the current study are within the normal range (5.8 to 6.4) for optimal rumen health and function (Kolver & Muller, 1998).

Nagaraja *et al.* (1981; 1982) did a couple of studies testing the ruminal infusion of monensin and lasalocid and it was found that the treatment cows had a higher rumen pH compared to the control cows. In a rumen fluid sampling analysis by Nagaraja *et al* (1981; 1982), a colony count of *S.bovis* and *Lactobacillus* (lactate producing, gram positive bacteria) were done and lower levels were found in the ionophore treated cows. This suggests that acidosis can be reduced by inhibiting lactic acid producing bacteria which decreases the ruminal pH. Several studies found that the rumen pH of monensin treated cows have increased in comparison to a control diet (Plazier *et al.,* 2000; McGuffey *et al.,* 2001; Benchaar *et al.,* 2006). Ramanzin *et al.* (1997) found no change in ruminal pH when control fed cows was compared to monensin supplemented cows. Broderick (2004) found no effect on ruminal pH when feeding a low level monensin supplementation of 250 mg/cow/d. Ruiz *et al.* (2001) reported no effect on the ruminal pH of dairy cows supplemented with monensin and fed fresh forage.

An *in vivo* study by Benchaar *et al.* (2006), found that EO increased the ruminal pH when compared to a control cow diet. They concluded that these findings might be useful in high grain diets to reduce the risk of developing acidosis. In a later *in vitro* study by Benchaar *et al.* (2007), the addition of carvacrol to a 24- hour batch culture increased the ruminal pH when compared to a control batch. Busquet *et al.* (2006) reported in an *in vitro* batch culture incubation with carvacrol (300 and 3000 mg/L) that the final pH increased when compared to a control incubation. Yang *et al*. (2007) reported no effect on ruminal pH when dairy cows were fed garlic or juniper berry EO. The ruminal pH did not differ when lactating cows were supplemented oregano leaves in a TMR in comparison to a control diet (Tekippe *et al.*, 2011; Hristov *et al*., 2013). Results from several EO studies are inconsistent and more research needs to be done with regards to the effects on ruminal pH. There are, however, some positive results which can be useful with regard to the use in dairy cow diets and the effect on rumen fermentation.

Table 11 presents the pH values measured at three different time intervals for the cows on three different concentrate treatments. There were no differences in pH at the three time intervals or the daily average among the three concentrate treatments.

Table 11 The mean ruminal pH values from a handheld pH meter measured at three different time intervals of cows (n = 6) on the three concentrate treatments fed at 6 kg/cow/day (as is), which included no feed additive or monensin or oregano, respectively, grazing a kikuyu/ryegrass pasture in spring.

Time	Treatment concentrate[1]			SEM[2]	P-value
	Control	Monensin	Oregano		
06:00	6.40	6.35	6.53	0.233	0.209
14:00	6.01	6.02	6.11	0.045	0.266
22:00	5.77	5.83	5.75	0.0697	0.686
Mean (Handheld)	6.06	6.07	6.13	0.029	0.228
Mean (pH Loggers)	5.89[b]	6.03[a]	6.08[a]	0.1261	<0.001

1 Control – Concentrate containing no feed additive; Monensin – concentrate containing 300 mg monensin/cow/d; Oregano – concentrate containing 1,15 g of oregano/cow/d, treatment concentrate fed at 6 kg/day (as-is)
2 SEM – standard error of mean

These results in Table 11 agree with the results from the indwelling pH loggers (Figure 8) by following the same trend in pH. The values from the handheld pH, however, noticeably differed from the pH values of the logging system when compared at the three time points. Various reasons contribute to these differences. One reason may be that rumen fluid samples were not taken from the same place as where the loggers were positioned and another reason may be that the samples were exposed to air when the pH was measured with the handheld meter. The pH varies at different locations inside the rumen and during the day (Colman *et al*., 2010). At 22:00 the low pH values can be seen in Table 11 which coincides with Figure 8 showing the low pH values at around that time. There were differences in the average pH of the pH logging system where monensin and oregano resulted in a higher average pH when compared to the control treatment. The overall mean over the 24 hour pH profile measured by the indwelling pH loggers, did differ among treatments. MON and EO treatments had a higher mean pH when compared to the CON treatment. The higher overall pH may have beneficial effects on rumen fermentation and microbial population. Fibre degrading microbes will be able to work optimally at a higher pH (Hoover, 1986) and have a positive effect on the dry matter degradability (DMd) and neutral detergent fibre degradability (NDFd).

Table 12 Mean time (hours) that the rumen pH were below a specific pH (6.4 to 5.6) of cows (n = 6) fed the three concentrate treatments at 6 kg a day and grazing ryegrass pasture in spring

pH	Treatment[1]			SEM[2]	P-value
	Control	Monensin	Oregano		
< 6.4	23	18	18	3.62	0.601
< 6.2	21	15	15	3.63	0.498
< 6.0	17	12	11	3.5	0.529
< 5.8	10	9	8	2.29	0.767
< 5.6	3	5	3	1.121	0.430

1 Control – Concentrate containing no feed additive; Monensin – concentrate containing 300 mg monensin/cow/d; Oregano – concentrate containing 1,15 g of oregano/cow/d, treatment concentrate fed at 6 kg/day (as-is)
2 SEM – standard error of mean

There were no differences among the three treatments with regards to the time the rumen pH were below a specific pH value. The rumen pH was below 5.6 for a short period of time. A longer time spent below a pH 5.6 could have led to a detrimental effect on rumen fermentation and decreased microbial activity (Hoover, 1986; Shriver *et al*., 1986). Overall, the rumen pH was above 6.0 throughout the day and these are favourable conditions for rumen fermentation.

5.4.2 Rumen fluid sampling

5.4.2.1 Volatile fatty acid profile

The daily mean ruminal concentrations (mM) of VFA during the rumen study are presented in Table 13. According to Bargo *et al*. (2003) the mean total VFA concentration usually ranges between 90.3 to 151.4 mM. The total VFA concentration in the current study is well within range suggested by Bargo *et al.* (2003). Table 13 represents the daily mean concentrations of ruminal VFA measured from the ruminal fluid samples. The rumen fluid samples were taken at three different time intervals. The mean ruminal VFA concentrations measured at the three different time intervals for the three concentrate treatments are presented in Table 14.

Table 13 Daily mean of ruminal volatile fatty acids (VFA) concentrations, measured in the ruminal fluid of cows (n=6) on the three concentrate treatments fed at 6kg/cow/day (as is), which included no feed additive or monensin or oregano, respectively, grazing a kikuyu/ryegrass pasture in spring.

Parameter[1]	Treatment Concentrate[2]			SEM[3]	P-Value
	Control	Monensin	Oregano		
Total VFA	117	113	118	1.65	0.116
Acetate (mM)	70.7	68.4	70.9	1.34	0.408
Propionate (mM)	24.5^{a}	22.1^{b}	23.2^{ab}	0.46	0.027
Butyrate (mM)	18.0	18.3	19.8	0.63	0.162
Valerate (mM)	1.45^{a}	1.31^{b}	1.48^{a}	0.028	0.013
Iso-butyrate (mM)	1.09	1.10	1.18	0.040	0.249
Iso-valerate (mM)	1.33	1.32	1.44	0.072	0.498
Acetate:Propionate ratio	2.94	3.14	3.09	0.097	0.387
Total VFA molar %					
Acetate %	60.5	60.8	60.1	0.58	0.694
Propionate %	23.4^{a}	21.1^{b}	22.2^{ab}	0.44	0.027
Butyrate %	17.2	17.5	18.9	0.60	0.162
Valerate %	1.38^{a}	1.25^{b}	1.41^{a}	0.027	0.012
Iso-butyrate %	1.04	1.05	1.13	0.039	0.24
Iso-valerate %	1.27	1.26	1.37	0.0675	0.474

1 VFA- Volatile fatty acid
2 Control – Concentrate containing no feed additive; Monensin – concentrate containing 300 mg monensin/cow/d; Oregano – concentrate containing 1,15 g of oregano/cow/d, treatment concentrate fed at 6 kg/day (as-is)
3 SEM – standard error of mean
a,b means in the same row with different superscripts differ (P<0.05)

Table 14 The mean ruminal concentrations (mM) of volatile fatty acids measured at three different time intervals for cows (n = 6) on the three concentrate treatments fed at 6 kg/cow per day (as is), which included no feed additive or monensin or oregano, respectively.

Rumen parameter	Time	Treatment concentrate			SEM	P-Value
		Control	Monensin	Oregano		
Total VFA	06:00	112.8	100.8	107.4	5.03	0.31
	14:00	107.6^{b}	119.9^{a}	129.1^{a}	2.81	0.005
	22:00	130.8	116.9	117.6	4.71	0.14
Acetate	06:00	68.2	61.1	65.7	2.97	0.302
	14:00	65.7^{b}	72.7^{ab}	77.7^{a}	2.13	0.021
	22:00	78.2	71.5	69.3	3.19	0.201
Propionate	06:00	21.92	18.67	19.43	1.172	0.203
	14:00	22.23	23.75	24.99	0.813	0.133
	22:00	29.47^{a}	23.9^{b}	25.23^{ab}	0.998	0.018
Butyrate	06:00	18.64	17.35	18.68	1.186	0.683
	14:00	16.39^{b}	19.91^{a}	22.16^{a}	0.57	0.001
	22:00	18.9	17.65	18.69	0.679	0.432
Valerate	06:00	1.357	1.122	1.156	0.0893	0.213
	14:00	1.215^{b}	1.343^{b}	1.542^{a}	0.1033	<0.001
	22:00	1.756	1.46	1.73	0.0868	0.095
Iso-butyrate	06:00	1.17	1.145	1.15	0.0631	0.955
	14:00	0.961^{b}	1.07^{ab}	1.247^{a}	0.0528	0.023
	22:00	1.132	1.079	1.155	0.048	0.554
Iso-valerate	06:00	1.517	1.421	1.369	0.1227	0.702
	14:00	1.085^{b}	1.19^{ab}	1.445^{a}	0.0788	0.043
	22:00	1.398	1.34	1.491	0.0864	0.503
Acetate:Propionate ratio	06:00	3.145	3.291	3.391	0.5232	0.545
	14:00	3.015	3.117	3.12	0.1097	0.755
	22:00	2.672^{b}	3.017^{a}	2.754^{b}	0.0554	0.011

1 VFA- Volatile fatty acid
2 Control – Concentrate containing no feed additive; Monensin – concentrate containing 300 mg monensin/cow/d; Oregano – concentrate containing 1,15 g of oregano/cow/d, treatment concentrate fed at 6 kg/day (as-is) 3 SEM – standard error of mean
a,b means in the same row with different superscripts differ (P<0.05)

The molar proportions of the three main VFA's normally vary between 50 and 60% for acetate, 18 and 20% for propionate and 12 and 18% for butyrate (McDonald *et al*., 2011). In the current study, the molar proportions of the VFA were within those ranges. The total VFA concentrations (117, 113 and 118 mM, for CON, MON and EO treatments, respectively) agree to those reported by Benchaar *et al.* (2006) who supplemented monensin and EO to the diet of dairy cattle. Studies done by Ruiz *et al.* (2001), Callaway *et al.* (2003) and Benchaar *et al*. (2006) reported that ionophore supplementation had no effect on the total VFA concentration. However, a study done by Ponce *et al.* (2012) reported that ionophore supplemented dairy cows resulted in a decreased concentration of the total VFA.

In the current study, a slight numerical decrease in total VFA concentration for monensin can be seen when compared to the control diet. However, the total VFA concentration for the monensin and oregano treatments was higher ($P < 0.05$) than that of the control treatment at 14:00 (Table 14). For the other two time intervals there were no differences in total VFA concentrations. An *in vivo* study by Benchaar *et al.* (2006) it was reported that the addition of EO did not have effect on the total VFA concentration. These results concur to previously reported studies by Benchaar *et al.* (2003) and Newbold *et al. (*2004). Castillejos *et al.* (2006) supplemented thymol and eugonol at four different dosage levels to an *in vitro* batch culture study. At the higher dosage levels of 500 and 5000 mg/L the total VFA concentration have decreased. The same findings were reported by Busquet *et al.* (2006) where increasing levels of carvacrol reduced the total VFA concentration in a batch culture study. A too high level of EO can have detrimental effects on the VFA concentration and therefore an optimal dosage range needs to be established. Similar to the current study, Tekippe *et al.* (2011) and Hristov *et al.* (2013) have reported no effects to the total VFA concentration when cows were supplemented oregano leaves in a TMR diet and compared to a control diet.

Acetate contributes to milk fat production and is predominant in high forage diets (Ishler *et al*., 1996). In the current study there were no differences in acetate concentration among treatments and this corresponds to the milk fat content (Table 9) that did not differ among the three concentrate treatments. However, the acetate concentration was higher ($P < 0.05$) for the EO treatment in comparison to the CON treatment at 14:00 (Table 14). The MON treatment did not differ from the EO or CON treatment at 14:00. The acetate concentration did not differ among treatments at the other two time intervals measured. Benchaar *et al.* (2006) did an *in vivo* study on the effects of monensin and EO on VFA concentration and found no difference in acetate concentration for either of the two treatments when compared to the control diet. Ruiz *et al.* (2001) reported no change in acetate concentration when a monensin treatment was compared to a control diet. Castillejos *et al.* (2006) supplemented thymol and eugonol at four different dosage levels to an *in vitro* batch culture. At a dosage level of 5 mg/L the acetate concentration decreased. The supplementation of carvacrol at 300 mg/L decreased the acetate concentration of a rumen batch culture (Busquet *et al.*, 2006). In the same study, oregano oil had no effects on the acetate concentration.

Propionate is a contributor to milk production (Ishler *et al.*, 1996). Monensin has been reported to increase the propionate concentration in the rumen (Richardson *et al*., 1978) and therefore an increase was expected on the MON treatment of the current study. However, the results in Table 13 show that there was a decrease ($P < 0.05$) in propionate when compared to the CON treatment. This decrease in propionate was not anticipated and cannot be readily explained. The EO treatment, however, did not differ from the MON or CON treatments. Conversely, the

propionate concentration for MON treatment were lower ($P < 0.05$) when compared to the CON treatment at 22:00 (Table 14). The EO treatment did not differ from the other two treatments at 22:00. The propionate concentration did not differ among treatments for the other two measured time intervals. The milk production results also concur with the propionate results. These results in the current study on propionate differ statistically but it did not have a biological significant effect on milk production. Numerous studies have reported that ionophore supplementation such as monensin to batch cultures has increased the propionate concentration and reduced the production of methane (McGuffey *et al*., 2001). However, Ruiz *et al.* (2001) and Benchaar *et al*. (2006) found no effects on the propionate concentration with monensin supplementation. The effects of monensin and EO on propionate can be ascribed to the dosage levels as well as the type of diet. The propionate concentration have increased at a dosage level of 3000 mg/L for both carvacrol and oregano oil as reported by Busquet *et al.* (2006).

Butyrate is a contributor to milk yield. The butyrate concentration in the current study did not differ among the three concentrate treatments and this coincides with no difference milk yield (Table 9) that did not differ among the three concentrate treatments. However, the butyrate concentration for the MON and EO treatments was higher ($P < 0.05$) than that of the CON treatment at 14:00 (Table 14). For the other two time intervals there were no differences in butyrate concentrations. According to the literature, monensin supplementation had no effect on the butyrate concentration when supplemented to dairy cows (Ruiz *et al.*, 2001; Benchaar *et al.*, 2006). Yang *et al.* (2007) reported that the supplementation of garlic or juniper berry extracts had no effect on the butyrate concentration. An *in vitro* study by Busquet *et al.* (2006), it was reported that carvacrol at a dosage of 300 mg/L increased the butyrate concentration but at a dosage level of 3000 mg/L the butyrate concentration decreased. In the same study, oregano oil also at a dosage level of 300 mg/L, increased the butyrate concentration.

The valerate concentration differed ($P < 0.02$) between the MON and CON treatments in the current study. The valerate concentration for MON was lower when compared to the CON and EO treatments. The valerate concentration was higher for the EO treatment when compared to the MON and CON treatment at 14:00. The treatments did not differ at the other two time intervals. The daily mean valerate concentration however did differ statistically (Table 13). These values agree with the findings of Bargo *et al.* (2002) who studied the effects of concentrate supplementation to grazing dairy cows.

The forage: concentrate ratio has an effect on the acetate: propionate ratio. When the forage: concentrate ratio is increased, the acetate: propionate ratio usually also increases. This means that forage is important for VFA production (Ishler *et al*., 1996). In the current study, the acetate: propionate ratios did not differ among treatments and were within the same range of 2.9 to 3.5 as reported by Van Wyngaard (2013). However, the acetate: propionate ratio was higher ($P <$

0.05) for the MON treatment when compared to the CON treatment at 22:00 (Table 14). This corresponds with the lower propionate concentration for MON at 22:00 when compared to the CON treatment. The ratio did not differ among treatments for the other two time intervals. Benchaar *et al.* (2006) reported no effect on the acetate: propionate ratio when monensin was supplemented in the diet of dairy cattle. This agrees with a study done by Ali-Haïmoud *et al*. (1995) who also reported no effect in actetate: propionate ratio for monensin supplemented cows. Sauer *et al.* (1998); Ruiz *et al*. (2001) and Broderick (2004) reported a decrease in acetate: propionate ratio for dairy cows supplemented with monensin. The differences between studies can be attributed to the different dosage levels of monensin in the diets as well as the type of diet (Benchaar *et al*., 2006). No effects to the acetate: propionate ratios have been reported by Benchaar *et al.* (2006; 2007) and Yang *et al.* (2007), when EO were supplemented to dairy cows. Castillejos *et al.* (2006) reported an increase in acetate: propionate ratio when thymol was supplemented at 500 mg/L.

5.4.2.2 Rumen ammonia nitrogen profile

The mean rumen ammonia concentrations (mg/dL) measured at three different time intervals are presented in Table 15. The rumen ammonia concentrations did not differ among treatments or between the three time intervals.

Table 15 Mean rumen ammonia nitrogen concentration (mg/dL) measured at three different time intervals (n = 6) within the rumen of dairy cows, on the three concentrate treatments fed at 6kg/cow/day (as is), which included no feed additive or monensin or oregano, respectively, grazing a kikuyu/ryegrass pasture in spring.

Time	Treatment concentrate[1]			SEM[2]	P-value
	Control	Monensin	Oregano		
06:00	14.2	13.6	14.5	1.47	0.923
14:00	11.4	13.4	10.5	0.88	0.136
22:00	14.7	14.6	13.8	0.68	0.595
Mean	13.4	13.85	12.9	0.70	0.647

1 Control – Concentrate containing no feed additive; Monensin – concentrate containing 300 mg monensin/cow/d; Oregano – concentrate containing 1,15 g of oregano/cow/d, treatment concentrate fed at 6 kg/day (as-is)
2 SEM – standard error of mean

The minimum level of rumen NH_3-N needed for microbial fermentation is between 1 to 6 mg/dl (Satter & Slyter, 1974; Hoover, 1986). The rumen ammonia concentration can go up to 80 mg/dl and will still not inhibit rumen micro-organism activity (Satter & Slyter, 1974). The daily mean rumen ammonia nitrogen concentration falls within the suggested range (8.7 to 32.2 mg/dl) of Bargo *et al*. (2003). This suggests that the N from the pasture was efficiently utilized in the rumen. The rumen ammonia concentration in the current study was sufficient to maintain rumen activity and microbial fermentation.

Free ruminal NH_3 are used by rumen microbes (especially fibre digesting bacteria) for protein synthesis and are utilized for microbial growth and fermentation of feeds for energy (Hoover, 1986). Maintaining the NH_3-N concentration in this range may improve the synthesis of microbial protein, digestibility and feed intake (McDonald *et al*., 2011).

In an *in vivo* experiment by Ruiz *et al.* (2001), they reported that monensin supplementation decreased the ruminal ammonia concentration when compared to a control treatment. A conclusion was made that monensin may spare amino acids from wasteful rumen degradation thus supplying more protein post-ruminaly, potentially increasing milk protein content and the efficiency of N utilization in dairy cows grazing pasture. This agrees with the results in the current study where the milk protein content of cows in the monensin concentrate treatment increased when compared to the control treatment. The effects of EO *in vitro* have shown to be positive but *in vivo* studies are quite limited. Benchaar *et al.* (2006, 2007) reported that no effects were found on ruminal ammonia N concentrations where lactating dairy cows were supplemented with an EO blend at 0.75 and 2 g/day. Yang *et al.* (2007) found that garlic fed at 5 g/d to lactating dairy cows had no effect on ruminal microbial protein synthesis or ammonia N concentration. The results in the current study agree with those of Benchaar *et al.* (2006, 2007) and Yang *et al.* (2007).

5.4.3 *In sacco* bag study

The *in sacco* DM and NDF disappearance of ryegrass pasture of cows on the three concentrate treatments are presented in Table 16.

Table 16 The *In sacco* dry matter disappearance and neutral detergent fibre disappearance of the available kikuyu/ryegrass pasture at two incubation periods (n = 6) within the rumen of dairy cows, fed the three different concentrates at 6 kg/cow per day (as is), which included no feed additive or monensin or oregano, respectively, grazing a kikuyu/ryegrass pasture in spring.

Parameter[1]	Incubation period (h)	Treatment concentrate[2]			SEM[3]	P-value
		Control	Monensin	Oregano		
DMd (%)	6	40.6	40.9	41.2	0.84	0.862
	30	82.5^{b}	84.7^{a}	84.4^{ab}	0.46	0.029
NDFd (%)	6	14.5	14.3	15.4	1.42	0.838
	30	74.8^{b}	78.1^{a}	78.1^{a}	0.76	0.035

1 – DMd- dry matter disappearance; NDFd – neutral detergent fibre disappearance
2 - Control – Concentrate containing no feed additive; Monensin – concentrate containing 300 mg monensin/cow/d; Oregano – concentrate containing 1,15 g of oregano/cow/d
3 - SEM – standard error of mean
a,b means in the same row with different superscripts differ (P<0.05)

5.4.3.1 Dry matter disappearance

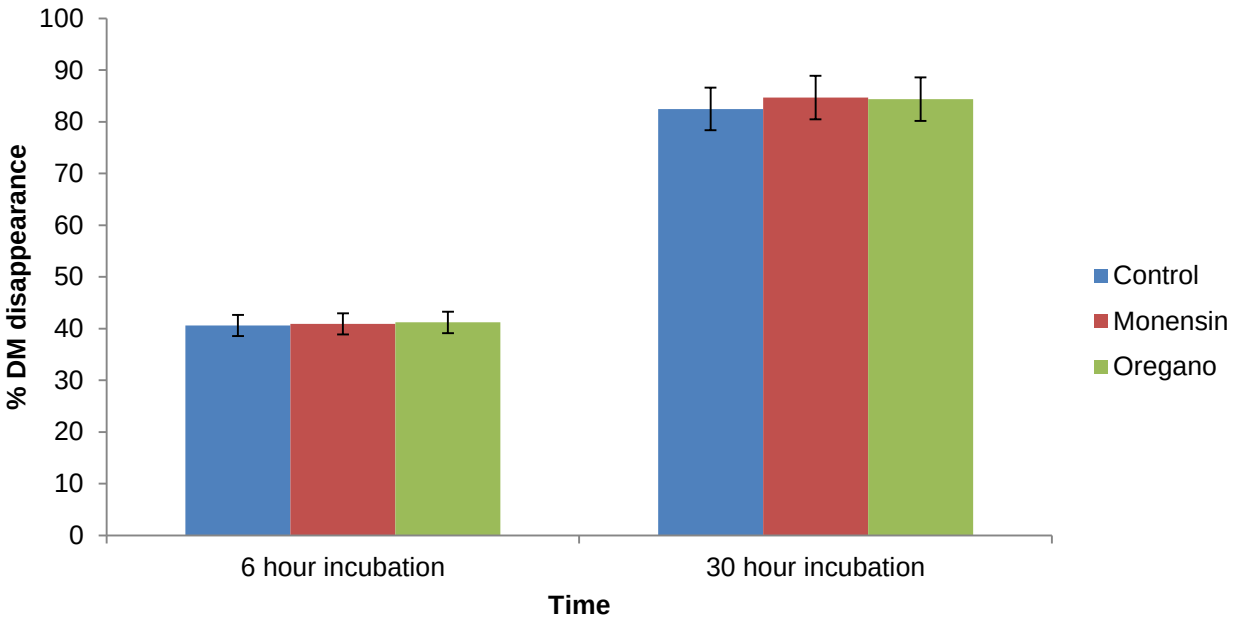

Figure 13 Mean percentage Dry matter disappearance (DMd) of the pasture at an incubation time of 6 and 30 hours within the rumen of the dairy cows (n = 6) for the three different treatments fed at 6 kg/cow per day (as is), which included no feed additive or monensin or oregano, respectively, grazing a kikuyu/ryegrass pasture in spring (error bars indicate SEM).

The *in sacco* dry matter digestibility of the pasture samples incubated in the rumen for 6 and 30 hours is presented in Figure 13. There were no differences (Table 16) among treatments after 6 hours of incubation ($P > 0.05$). The DM digestibility for the 30 hour incubation increased ($P < 0.05$) for both feed additives as compared to the CON treatment. The high DMd values for the additive treatments after the 30 hour incubation correspond to the high IVOMD% value (82.2%) of the ryegrass pasture presented in Table 7.

The higher DMd values coincide with the higher milk protein values obtained during the production study (Table 9). The increase in digestibility of the pasture suggests that more nutrients were available for microbial protein synthesis and therefore had a positive effect on milk protein content. Yang *et al.* (2007) reported that ruminal digestibility of DM was higher (13%) for a juniper berry EO (2 g/d) compared to a control diet where Holstein cows received a 40% forage, 60% barley-based concentrate diet. However, the experimental treatments did not affect the total tract digestibility of DM, OM, fibre and starch. A suggestion was made that the increase in ruminal digestibility was due to the increase in ruminal digestion of dietary protein. Benchaar *et al.* (2007) and Benchaar *et al.* (2008) reported that the addition of an EO blend to a TMR had no effects on the total tract DM digestibility.

5.4.3.2 Neutral detergent fibre disappearance

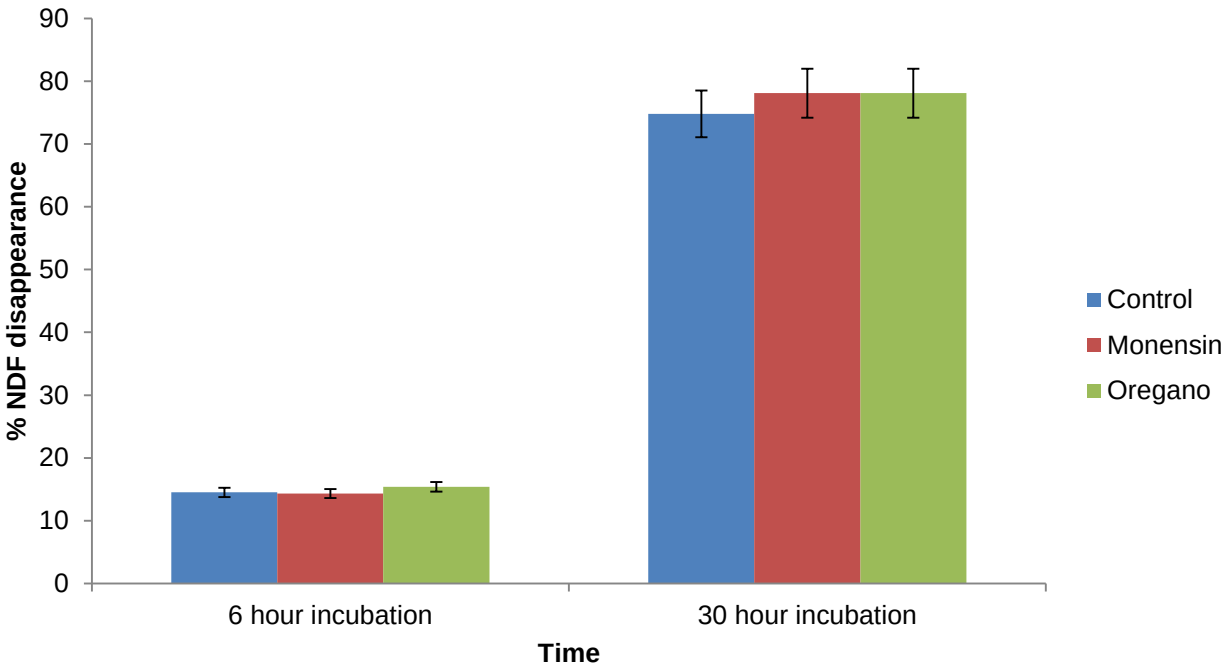

Figure 14 Mean percentage of neutral detergent fibre disappearance (NDFd) of pasture incubated at 6 and 30 hours within the rumen of the dairy cows (n = 6) for the three different treatments fed at 6 kg/cow/day (as is), which included no feed additive or monensin or oregano, respectively, grazing a kikuyu/ryegrass pasture in spring (error bars indicate SEM).

The NDF disappearance of pasture samples incubated for 6 and 30 hours within the rumen of the dairy cows is presented in Figure 14. After 6 hour incubation there was no difference ($P > 0.05$) among the three treatments for NDF digestibility. The NDF digestibility after 30 hours of incubation showed an increase ($P < 0.05$) for both feed additives in comparison to the CON treatment. The high NDFd values in the current study were expected because the pasture was of high quality (Table 7). The average rumen pH profile of the cows in the current study was also higher for both the additive concentrate treatments which could be a result of the higher NDFd. An increase in fibre degradability has potential to affect milk production positively. The higher overall average pH probably resulted in a higher activity of fibre degrading microbes which resulted in the higher fibre disappearance values. More fibre was likely utilized by the cow in the rumen and these could have led to the increased milk protein and lactose contents. A possibility of increased energy availability from the feeds could also have had the positive effect on milk composition.

Plaizier *et al.* (2000) reported that when a high forage diet is supplemented with monensin the apparent digestibility of NDF as well as ADF increased. However on a high concentrate diet with monensin supplementation, there were no effects on fibre digestion. Ali-Haïmoud *et al.* (1995) and Benchaar *et al.* (2006) reported that the apparent digestibility of starch, was unaffected by monensin supplementation. Kung *et al.* (2008) reported in a study with a mixture of EO there were

no effects on the NDF digestibility of feeds. In several studies (Malecky *et al.,* 2009; Santos *et al.,* 2010) the digestibility of feeds were not affected with the addition of EO to the diet.

Benchaar *et al.* (2007) evaluated NDF and ADF digestibilities when a TMR diet was supplemented with an EO blend and found no effect on either NDF or ADF digestibility between diet treatments. Benchaar *et al.* (2008) reported that, when adding cinnamaldehyde at 1 g/day to dairy diets, there were no difference in the total tract digestibilities of NDF and ADF.

Generally, the addition of EO and their active compounds may cause a slight increase in nutrient digestibility or have no effect. By increasing the digestibility of feeds *in vivo*, it would improve the efficient use of nutrients by dairy cows (Tekippe, 2010).

Chapter 6 Conclusion

The addition of monensin or oregano essential oil extract to a concentrate supplement for Jersey cows grazing ryegrass pasture in spring did not affect the milk yield or 4% FCM. The milk fat content did not differ among treatments and agreed with the herd average for the Outeniqua Jersey herd. However, monensin and oregano oil extract treatments increased the milk protein and milk lactose content when compared to the control treatment. This increase may be beneficial to the farmer as it may have a positive influence on the milk price as farmers are paid on milk quantity as well quality. A possibility of improved energy availability of the feed could be a contributor to the increase in milk quality. The MUN levels did not differ among treatments. The average MUN value of 13 mg/dL suggests that the cows had a sufficient protein intake and that it is within the acceptable range for pasture based systems. The somatic cell count (mean of 213 000 cells/mL) did not differ among treatments and are well below 500 000 cells per mL of milk which make it fit for human consumption and suggests that the udders were in a healthy condition. There were no differences in LW and BCS among the three treatments. Cows in all three treatment groups gained weight and had an improved condition score at the end of the study.

The rumen pH profile over a 24 hour period did not differ among treatments. The profile followed a normal diurnal pH fluctuation. Two distinctive drops in pH were seen after the concentrates had been consumed and the highest pH was before the morning feeding. The overall mean pH measured by the pH logging system, however, differed among the three treatments. Monensin and oregano had a higher mean pH when compared to the control treatment. There were no differences among treatments regarding time spent below certain critical pH values. The rumen pH was below 5.6 for a short period of time. A longer time spent below a pH 5.6 could have led to a detrimental effect on rumen fermentation. Overall, the rumen pH was above 6.0 throughout the day and these are favourable conditions for rumen fermentation. The total VFA concentration did not differ among treatments and agreed with the range suggested by Bargo *et al*. (2003). In the current study there were no differences in acetate concentration among treatments and this corresponds to the milk fat content that did not differ among the three concentrate treatments. The propionate concentration was lower on the monensin treatment when compared to the control treatment. This decrease in propionate was not expected, but the results concur with the milk production, that did not differ, as propionate has an effect on milk production. Butyrate is a contributor to milk yield. The butyrate concentration in the current study did not differ among the three treatments, which is in line with the milk yield that did not differ among treatments. The rumen ammonia nitrogen concentrations also did not differ among treatments measured at the three time intervals. The concentration falls well within the range as suggested by Bargo *et al*. (2003). The dry matter degradability did not differ among treatments at the 6 hour incubation period

but monensin increased the dry matter degradability after 30 hours of incubation when compared to the control. Oregano did not differ from monensin or the control treatment. The neutral detergent fibre degradability did not differ among treatments at the 6 hour incubation period. After the 30 hour incubation period, the neutral detergent fibre degradability increased in the monensin and oregano treatments when compared to the control treatment. An increase in fibre digestion could have led to a higher energy availability of the feeds and therefore have an effect on the milk composition (increase in milk protein and lactose content).

To conclude, the use of monensin and oregano oil extract have shown to be beneficial with regards to increasing the milk composition as well as the NDFd. The average overall pH from the pH profile resulted in the two additive treatments being higher when compared to the control treatment. This could be beneficial to rumen fermentation and have a positive effect on the microbial population. As monensin and oregano oil extract showed similar results, oregano oil extract can be considered as an alternative natural feed additive to monensin.

Chapter 7 Economic evaluation

The daily and monthly income of the three concentrate treatments were calculated and compared among treatments (Table 17). The daily and monthly income only represents the margin above feed costs and does not take other expenses with regards to the daily and monthly farm operation into consideration.

The calculations were made on a herd size of 300 Jersey cows, which is the average herd size in the Southern Cape of South Africa. The milk production and milk composition of the three treatment groups are represented in Table 17 as well as the milk price for each treatment. The milk price was received from Nestlé for September to November 2014 and it is evident that the milk composition had an effect on the milk price. An increase in milk protein content resulted in an increased milk price. The cost of the treatment concentrate was obtained from NOVA feeds and the pasture costs were obtained from the Outeniqua Research farm for September 2014.

The economical evaluation of the current study demonstrated that the two feed additives resulted in an increase in milk price because of the increase in milk composition. As shown in Table 17 the daily additional income per cow was R1.50 for the monensin treatment and R3.20 for the oregano treatment. If the use of the two additives is to be considered, care must be taken that the price of the feed additives does not exceed R 1.50 for monensin and R3.20 for the oregano treatment in order to ensure a profit when using these feed additives. It is up to the producer/feed company to calculate the costs involved when an additive is to be considered and make a decision based on the cost evaluation.

Table 17 Profit as calculated for margin above feed costs for all three treatments and the effect of additve on income

Parameter	Treatment concentrate		
	Control	Monensin	Oregano
Milk yield (kg/cow per day)	20.5	20.3	20.4
Milk fat (g/100g)	4.52	4.47	4.56
Milk protein (g/100g)	3.39	3.55	3.6
Milk lactose (g/100g)	4.52	4.79	4.83
Milk price (R*/L)	4.65	4.77	4.83
Milk income (R/cow per day)	95.30	96.83	98.53
Milk income (R/herd**per day)	28 598	29 049	29 560
Feed price (R/t)	3 740	3 740	3 740
Feed additive price (R/t)	0	0	0
Feed price (R/cow per day)	22.44	22.44	22.44
Feed price (R/herd per day)	6 731	6 731	6 731
Pasture price (R/kg)	1.20	1.20	1.20
Pasture price (R/cow per day)	12	12	12
Pasture price (R/herd per day)	3 600	3 600	3 600
Total feed input cost (R/cow per day)	34.44	34.44	34.44
Total feed input cost (R/herd per day)	10 330.56	10 330.56	10 330.56
Margin over feed cost (R/cow per day)	60.9	62.4	64.1
Margin over feed cost (R/herd per day)	18 267	18 719	19 229
Margin over feed cost (R/herd per month)	548 008	561 562	576 871
Additional income (R/cow per day)	0.0	1.50	3.20
Additional income (R/herd per day)	0.0	451.80	962.10

* - South African currency, rand

** - Herd = 300 cows which is the average herd size in the Southern Cape of South Africa.

Chapter 8 Critical evaluation

Milk yield: Care must be taken to regularly service the milk meters in order to increase the accuracy of milk yield. This is time consuming and requires some maintenance but is a necessity on any dairy farm.

Time lapse between morning and afternoon milk: The time lapse between the morning and afternoon milk session was 8 hours and the afternoon to next morning milk session was 16 hours. Ideally the time lapse should be spread evenly between milking sessions to ensure better udder health and less production stress as well as spreading feeding times evenly will result in a more even utilization of nutrients. It is, however, unpractical with regards to labour and time constraints in a public sector environment to spread out the milking sessions further.

Rumen fluid sampling times: Three time intervals (6:00, 14:00, and 22:00) were used for the sampling of rumen fluid. Four sampling periods spread evenly over a 24 hour period may have yielded more accurate rumen parameter results and could have enabled a trend to be established with regards to the ruminal VFA and ammonia nitrogen concentration. However the purpose of the study was only to determine the effect of the two feed additives on the VFA and ammonia nitrogen concentrations. Sampling times could be taken before and after consumption of treatment concentrate to yield a more accurate effect on ruminal parameters.

Rumen fluid sampling area: The sampling technique used to extract rumen fluid involved collecting samples from various regions in the rumen. This may cause a sampling error because according to literature the pH, VFA and ammonia nitrogen levels differ between the various regions in the rumen. A more standardised technique should be developed to extract the rumen fluid and yield a more homogenous rumen sample.

The pH logging system: The calibration of the indwelling pH loggers was a long and time consuming process but overall the system was functional and reliable pH data could be used for the study.

Comparing the rumen study results to the effect on production: The rumen study results from six cows were used to explain the milk production and composition results for the 54 lactating cows. VFA has an effect on milk production and milk composition. Looking at the VFA results from the 6 rumen cannulated may be an indication to the actual results obtained in the production study.

Length of adaption period: The adaption period was 14 days long. An adaption period of 3 weeks is considered optimal in order for the rumen environment to fully adapt to the feed as well as feed additive. However, due to the seasonality of the pastures, the adaption period had to be shortened.

Dosage rate of the feed additive: An optimal dosage rate has yet to be determined, especially for the oregano treatment. The dosage rate used of 1,15 g/cow/d was a suggested rate given by the company.

Pelleting of feed: There was a concern that the pelleting process may have an effect on the oregano additive. The inclusion rate was therefore increased from 1 g to 1,15 g/cow/day to reduce any changes. However there were significant results for the oregano treatment suggesting that the oregano did have an effect on production and ruminal parameters.

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