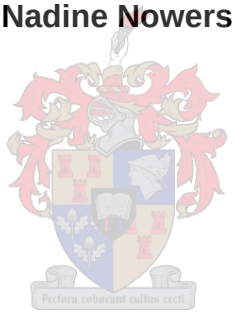
The effect of an oregano oil extract in a lactating dairy cow diet on production responses of Holstein cows

by

Nadine Nowers



Thesis presented in partial fulfilment of the requirements for the degree of Master of Science in the Faculty of Animal Science at Stellenbosch University

Supervisor: Prof. C. W. Cruywagen

March 2016

DECLARATION

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Date: March 2016

ABSTRACT

Title: The effect of an oregano oil extract in a lactating dairy cow diet on production responses of Holstein cows

Name: N. Nowers

Supervisor: Prof. C. W. Cruywagen

Institution: Department of Animal Sciences, Stellenbosch University

Degree: MScAgric

Forty Holstein cows, 178 ± 17 (SE) DIM and weighing 624 ± 9 (SE) kg, were used in a lactation trial of 60 days to determine the effect of oregano essential oil on milk production and milk composition. The cows were ranked according to milk yield, DIM and lactation number and each consecutive pair formed a block. Treatments were allocated randomly to each of the 20 blocks. An essential oil product (Dosto Concentrate 500; DOS), was evaluated against a placebo control (CON) treatment. Cows were housed in a semi-open free-stall barn with sand beds and had free access to fresh water. All cows received a basal diet consisting of lucerne hay (53% NDF and 11% CP) that was offered *ad libitum* and 28 kg/day of a semi-complete lactation feed, offered twice daily at 07:30 and 16:00. All refused feed were weighed back weekly to determine intake per group. Treatments (DOS and CON) only differed in terms of a maize based supplement that, in the case of the DOS treatment, every 300 g maize supplement portion, contained 0.5 g of Dosto Concentrate 500. The cows were milked twice daily at 06:00 and 15:30 and the supplements were offered individually to cows in the milking parlour during each milking. Milk yield, milk composition and cow weights were recorded daily via the Afikim system. Milk samples were also collected during weeks three and eight for composition analysis at the Elsenburg Dairy Laboratory. Data collected over time were subjected to a repeated measurements ANOVA, while mean values were analysed according to a main effects ANOVA with treatment and block as main effects. All data were analysed with the aid of Statistica 64 version 12 and significance was declared at $P < 0.05$. Regarding all 40 cows, treatment had no effect ($P > 0.05$) on milk yield or milk composition over the entire period. However, in the CON treatment, the lactose content was higher ($P < 0.05$) during the first two weeks and the milk protein content was higher ($P < 0.05$) from week four to eight. When data of the ten top milk producing cows per treatment were analysed separately, the fat content and milk fat yield were higher ($P < 0.05$) for the DOS treatment during the first three weeks of the trial and lactose was higher ($P < 0.05$) for the CON treatment in the first week. Mean milk yield of the ten top milk producing cows per treatment did not differ ($P > 0.05$) and was 37.9 kg for the DOS treatment and 37.3 kg for the CON treatment. Mean fat content and fat yield was higher ($P < 0.05$) in the DOS treatment (37.1 g/kg and 1.41 kg/day) than in the CON treatment (33.8 g/kg and 1.26 kg/day). The higher fat content also resulted in a higher ($P < 0.05$) energy corrected milk yield of cows in the DOS treatment than in the CON treatment (38.8 and 36.6 kg/day, respectively). With regards to feed intake, the CON group consumed on average 17.3 kg more roughage per week than the DOS group. After a two

month period 12 milk samples were collected from each group and was sent to be evaluated in terms of microbiological quality and sensory characteristics. The microbiological quality of the milk samples was evaluated by using petrifilm plates for total aerobic counts (TAC) and coliform counts (CC). Based on the microbiological analysis, all the milk samples were considered suitable for human consumption (< 200 000 cfu/ml). The treatment group differed ($P \leq 0.001$) from the control group in terms of aroma and flavour. No oregano flavour was detected and the difference in aroma and flavour was probably due to the difference in fat content. It was concluded that oregano essential oil in dairy cow diets stimulated milk fat production and increased energy corrected milk yield in high milk producing dairy cows. Oregano essential oil had no adverse effect on milk aroma and flavour.

UITTREKSEL

Titel: Die invloed van ‘n oregano olie-ekstrak in ‘n lakterende suiwelkoei dieet op melkproduksie van Holstein-koeie

Naam: N. Nowers

Studieleier: Prof. C. W. Cruywagen

Instansie: Departement Veekundige Wetenskappe, Universiteit Stellenbosch

Graad: MScAgric

Veertig Holsteinkoeie, 178 ± 17 (SF) DIM en met ‘n massa van 624 ± 9 (SF) kg, is in ‘n laktasiestudie van 60 dae gebruik om die invloed van oreganum essensiële-olie op melkproduksie en melksamestelling te bepaal. Koeie is gerangskik volgens melkproduksie, DIM en laktasienommer en elke opeenvolgende paar het ‘n blok gevorm. ‘n Produk van ‘n essensiële olie (Dosto Concentrate 500; DOS) is geëvalueer teenoor ‘n placebo-kontrolebehandeling (KON). Koeie is in ‘n gedeeltelike oop, vrystaande stal met ‘n sandvloer gehuisves en hulle het vrye toegang tot water gehad. Alle koeie het ‘n basiese diet ontvang wat bestaan het uit lusernhooi (53% NVV en 11% RP) wat ad lib voorsien is, plus 28 kg/dag van ‘n semivolledige rantsoen wat daagliks in twee porsies om 07:30 en 16:00 verskaf is. Die voerinmame per groep is bepaal deur die onbenutte voer weekliks terug te weeg. Die behandelings (DOS en KON) het slegs verskil in terme van die mieliegebasseerde supplement, waar in die geval van die DOS behandeling, elke 300 g mieliesupplement 0.5 g Dosto Concentrate 500 bevat het. Koeie is tweekeer per dag teen 06:00 en 15:30 gemelk en die supplement is individueel per koei in die melkstal tydens elke melking voorsien. Melkopbrengs, melksamestelling en koeimassas is daagliks bereken deur middel van die Afikim-sisteem. Melkmonsters is gedurende week drie en week agt geneem en deur die Elsenburg Suiwellaboratorium ontleed vir melksamestelling. Data wat oor tyd versamel is, is met behulp van ‘n herhaalde-waarnemings ANOVA ontleed, terwyl gemiddelde waardes volgens ‘n hoofeffek ANOVA ontleed is met behandeling en blok as hoofeffekte. Alle data is met behulp van Statistica 64 (weergawe 12) ontleed. Betekenisvolheid is teen $P < 0.05$ verklaar. Behandeling het geen invloed ($P > 0.05$) op melkopbrengs of melksamestelling oor die totale periode gehad nie. In die KON behandeling is egter gevind dat die laktose-inhoud hoër was ($P < 0.05$) gedurende die eerste twee weke van die studie en dat melkproteïeninhoud hoër was ($P < 0.05$) vanaf week vier tot week agt. Wanneer die data van die top tien melkproduserende koeie afsonderlik ontleed is, is gevind dat die vetinhoud en melkvetopbrengs gedurende die eerste drie weke van die studie hoër ($P < 0.05$) was vir die DOS behandeling en dat laktosevlakke van die KON behandeling hoër ($P < 0.05$) was gedurende die eerste week. Die gemiddelde melkopbrengs van die tien top koeie per behandeling het nie betekenisvol verskil nie en was 37.9 kg vir die DOS behandeling en 37.3 kg vir die KON behandeling. Die gemiddelde vetinhoud en vetopbrengs was hoër ($P < 0.05$) in die DOS behandeling (37.1 g/kg en 1.41 kg/dag) as in die KON behandeling (33.8 g/kg en 1.26 kg/dag). Die hoër vetinhoud het ‘n hoër energie-gekorrigeerde melkopbrengs vir koeie in

die DOS behandeling teenoor die KON behandeling tot gevolg gehad (38.8 kg/dag en 36.6 kg/dag onderskeidelik). Die KON groep het ten opsigte van voerinname, 17.3 kg meer ruvoer per week verbruik. Twaalf melkmonsters per behandeling is in die agtste week van die studie geneem vir mikrobiologiese kwaliteit en sintuiglike evaluering. Die mikrobiologiese kwaliteit van die melk is geëvalueer deur gebruik te maak van petrifilmplaatjies vir totale aerobiese tellings (TAT) en coliform-tellings (CT). Na aanleiding van die mikrobiologiese ontledings was al die melkmonsters geskik vir menslike gebruik (< 200 000 cfu/ml). Die behandelingsgroep het ten opsigte van aroma en geur van die kontrolegroep verskil ($P \leq 0.001$), maar geen oreganogeur is waargeneem nie. Die verskille ten opsigte van aroma en geur was waarskynlik as gevolg van die verskil in vetinhoud. Die gevolgtrekking vanuit die studie was dat oreganum essensiële-olie die melkvetproduksie gestimuleer het en tot verhoogde energie-gekorrigeerde melkopbrengs in hoë-produserende melkkoeie gelei het. Oregano essensiële-olie het geen nadelige invloed op melk aroma en geur nie.

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List of Abbreviations

AA - Amino acids
AC - Aerobic counts
AD - Acid detergent
ADF - Acid detergent fibre
ADL - Acid detergent lignin
BCS - Body condition score
BPW - Buffered peptone water
Ca - Calcium
CC - Coliform counts
C/ CON - Control group
Cfu - Colony forming units
CF - Crude fibre
CP - Crude protein
CRC - Controlled release capsules
CTAB - cetyl trimethylammonium bromide
DIM - Days in milk
DM - Dry matter
DOS/ T - Dosto treatment group
ECM - Energy corrected milk
EE - Ether Extract
EO - Essential oils
EU - Europe
FCM - Fat corrected milk
GE - Gross energy
HCl - Hydrochloric acid
H_2O - Water
H_2SO_4 - Sulphuric acid
IOP - Ionophores
K - Potassium
MEO - Mixture of essential oils
MFC - Milk fat content
Mg - Magnesium
MO - Monensin
MPC - Milk protein content
MUN - Milk urea nitrogen
N - Nitrogen
NaOH - Nitrogen hydroxide
$Na_2B_4O_7$ - Sodium borate decahydrate

Na_2HPO_4 - Disodium phosphate anhydrous

Na_2SO_3 - Anhydrous sodium sulphite

ND - Neutral detergent

NDF - Neutral detergent fibre

NE - Net energy

P - Phosphorous

PUFA - Polyunsaturated fatty acid

RBD - Randomised block design

RSA - Republic of South Africa

SE - Standard error

SEM - Standard error mean

SFA - Saturated fatty acids

SPC - Standard plate count

TAC - Total aerobic counts

TCC - Triphenyl tetrazolium chloride

TMC - Total microbial counts

TMR - Total mixed ration

UFA - Unsaturated fatty acids

VFA - Volatile fatty acid

VRB - Violet red bile

CHAPTER 1

Introduction

Antibiotic ionophores, such as monensin and lacalocid, have proven to be effective in the reduction of protein and energy losses in the rumen and they can improve the feed efficiency of individual cows. They are widely used to manipulate the rumen microbial population to increase propionate production that results in increased milk production and aids in the prevention of ketosis. However, the use of antibiotics in livestock, has become a concern to the public, due to the development of drug resistant bacteria (Calsamiglia et al., 2007). Since ionophores are classified as antibiotics, the use is banned in the EU and some other countries. There is also an increase in consumer resistance in the RSA against the use of antibiotics in animal feeds.

Therefore essential oils are being investigated by producers as alternative feed additives to ionophores for their dairy cows to improve the feed efficiency and the overall health of the animals (Yang et al., 2007). Several studies have been done on the supplementation of essential oils using in vitro methods such as batch cultures and continuous cultures. Many of these studies have demonstrated that the active components of essential oils could favourably modify rumen metabolism (Yang et al., 2007). In order to make a decision on whether one should use essential oils as alternatives to ionophores, it is important to first define what ionophores are, how they work and most importantly what favourable properties they have that are currently of importance to dairy farmers. The use of essential oils should then be evaluated and upon comparing the benefits of ionophores with those gained from the supplementation of essential oils, one can develop a personal preference towards the one or the other.

Essential oils, such as oregano oil, may present this alternative as it has been shown that they can positively affect microbial taxa to shift fermentation end products towards propionate and an increased efficiency of energy utilization (Hristov et al., 2013). Recent studies have found that in some cases, the supplement of dairy cow diets with *Origanum vulgare* L. leaves resulted in higher milk production, better feed efficiency and lowered the methane gas production in the rumen (Hristov et al., 2013). When the use of oregano to naturally increase the efficiency in dairy farming was investigated, the following benefits were noted: Oregano is a natural broad-spectrum bacteria killer, it improves intestinal stability and has an appetite stimulating effect.

The suppliers of the organic feed additive, Dosto Concentrate 500, claim that the additive may aid in the conservation of the total mixed ration (TMR), improve feed intake, increase the production of saliva, production of milk with a higher quality and may improve the health status of the dairy herd. The aim of the present study was to investigate the effect of an essential oil extract from *Origanum vulgare* L. in the diet of lactating dairy cows on feed intake, milk yield, milk composition and the sensory characteristics of milk.

CHAPTER 2

Literature Review

2.1 Ionophores

2.1.1 What are ionophores?

Ionophores (IOP) are feed additives given to cattle to assist with increasing their feed efficiency and rate of gain. Ionophores can be classified as carboxylic polyether antibiotics and are produced through fermentation products by various species of *Streptomyces* bacteria (Ipharraguerre et al., 2003). In many areas of the cattle and poultry industries IOP's are now used extensively. In 1971, the IOP monensin was approved for the use in broiler diets in order to manage the outbreak of coccidiosis, caused by intestinal parasites (McGuffey et al., 2001). The use of monensin in cattle diets was approved in 1975 by the Food and Drug Administration, as scientific data indicated that meat and milk produced from animals that were given these additives were deemed safe for human consumption (Ipharraguerre et al., 2003). There are two main types of IOP's that are widely used all over the world, but particularly in South Africa:

- Monensin - marketed under the brand name *Rumensin*
- Lasalocid - marketed under the brand name *Bovatec*

2.1.2 Mode of action of ionophores

Improvements observed in animal productivity, due to the inclusion of ionophores (IOP's) in dairy cow diets, are due to the disruption of biological membranes of certain bacteria. Ionophores are lipophilic compounds that are toxic to various bacteria, fungi and protozoa. Their lipophilic nature enables them to penetrate into the bacterial membranes and alter the movement of ions from and into the cell (Ipharraguerre et al., 2003). This forces the bacteria to waste energy by having to pump ions across their cell membranes in order to maintain an equilibrium state. The susceptible bacteria will eventually die out and this leads to a change in the rumen population composition. The bacteria mentioned above can be categorised as gram negative (starch fermenting bacteria) or gram positive bacteria (fibre fermenting bacteria). Gram negative bacteria have a two layered cell wall with a high lipid content, their cell walls have a low resistance to physical disruptions and they are more resistant to antibiotics than gram positive bacteria. On the other hand gram positive bacteria have a single layered cell wall with a low lipid content, their cell walls have a high resistance to physical disruptions. Unlike gram negative bacteria, gram positive bacteria are more susceptible to antibiotics.

The rumen is an anaerobic ecosystem where various species of bacteria ferment ingested feed to produce volatile fatty acids (VFA's) and bacterial protein that are major sources of nutrients for the cow. However, 12% of dietary carbon and energy can be converted to ammonia, heat and methane. Except for ammonia that can partly be used by the ruminant, the others are fermentation end products that are unusable to the animal and

can represent a loss of feed energy and protein from the cow into the environment (Ipharraguerre et al., 2003). The two most abundant VFA's in the rumen are acetic acid and propionic acid. Propionic acid is more readily changed into glucose by cattle, than the other VFA's, as it has the highest ability to be utilized from feed energy for productive purposes. The ratio between acetic and propionic acid influences the efficiency with which VFA's are utilized for energy and a decrease in the A:P ratio increases the proportion of the gross energy (GE) that would be available to the animal. Therefore a major benefit of feeding IOP's to lactating dairy cows is the potential to alter the A:P ratio towards more propionate (Ipharraguerre et al., 2003). Due to the changes in ruminal fermentation caused by IOP's, the feed efficiency and overall health of dairy cows can be improved. Ionophores cause feed intake to decrease slightly but the average daily gain remains unchanged or may increase (Ipharraguerre et al., 2003). The IOP's lasalocid and monensin are approved for the use in dairy cows as it possibly attributes to an increase in milk production (McGuffey et al., 2001).

2.1.3 Ionophores and the control of metabolic disorders

Conditions such as acidosis, bloat and ketosis that occur in animals could be linked to disturbances in rumen fermentation. When feeding ionophores, the conditions can possibly be reduced because of its antimicrobial activities (McGuffey et al., 2001).

Bloat

In cattle, there are two main types of bloat that can occur. One type occurs when feedlot type rations are fed (grain bloat). The second type occurs in cattle, grazing legumes (McGuffey et al., 2001). A surplus production of foam in the rumen results in both bloat types. In severe instances, bloat often leads to death within a few hours after ingesting the bloat causing meal. Monensin and lasalocid have been included in the diets of cattle when grain fed, as well as on legume grazing. Studies on the effects of these two ionophores (IOP's) on preventing and controlling bloat have shown that lasalocid is more effective in managing grain bloat than monensin (McGuffey et al., 2001). Low inclusion levels of lasalocid prevented grain-induced bloat, when it was supplemented before introducing high grain diets. Grazing animals are at a high risk for legume bloat in some areas, where lucerne and clover are predominant or the only forages for dairy cattle. The incidence of legume bloat may be controlled by supplementing monensin as a controlled-release capsule to dairy cattle (McGuffey et al., 2001).

Acidosis

The consumption of rapidly fermentable carbohydrates places the dairy cow at risk for acidosis. Acidosis is generally linked to the build-up of lactic acid production, resulting in that the bacteria that normally use lactic acid cannot keep up with production. However, excessive volatile fatty acid (VFA) production may be a more significant contributor to chronic acidosis problems (McGuffey et al., 2001). Lactic acid is about ten times stronger than the other rumen acids and causes the rumen pH to decrease. As the pH drops below 6.0 fibre

digestion is depressed. Ionophores have the potential to control the acidosis by two mechanisms. The first mechanism is through the IOP's effects on lactic acid producing bacteria (McGuffey et al., 2001). Major strains of bacteria, that cause lactic acid in the rumen, are inhibited by the supplementation of monensin and lasalocid. The eating dynamics of cattle, that are fed diets containing ionophores, are changed. This being the second mechanism by which ionophores may have an impact on acidosis (McGuffey et al., 2001).Monensin reduces day-to-day variability in intake by individually fed cattle. By including monensin in the diets the variance in feed intake can be reduced and cattle tend to consume smaller but more frequent meals.

Ketosis

Ketosis is a metabolic process that occurs in cattle when the energy demands (high milk production) exceed energy intake and results in a negative energy balance. Ketonic cows often have low blood glucose concentrations. Stored fats are then broken down for energy, resulting in a build-up of acids called ketones within the body. All dairy cows in early lactation (first six weeks) are at risk of ketosis. Clinical ketosis arises in nearly 5% of dairy cows (McGuffey et al., 2001). The clinical signs of this disease include the loss of appetite, loss of body weight, decreased milk production, increased ketones in the blood and a fatty liver. These signs are not evident during subclinical ketosis. Monensin has been fed to dairy cows in controlled studies. In order to evaluate the effect of monensin on subclinical ketosis, it was supplemented to dairy cows in controlled studies. An increase in the serum glucose levels were observed in cows given monensin controlled released capsules (CRC), by 15% (McGuffey et al., 2001). The controlled studies confirmed that subclinical ketosis can be reduced in cows receiving a monensin capsule. The cows fed monensin CRC showed a reduction in positive milk ketone tests (McGuffey et al., 2001).

2.1.4 Effect of inclusion of ionophores in lactating dairy cow diets

In prior studies to determine what effect the inclusion of ionophores (IOP's) has on lactating dairy cows, the following results were found: Table 2.1 shows the effect of lasalocid and monensin on the lactation performance of dairy cows.

Table 2.1 Effect of lasalocid and monensin on lactation performance of dairy cows (McGuffey et al., 2001).

Item	**Trials (no.)**	**Control**	**IOP**	***P* <**	**Item**	**Trials (no.)**	**Control**	**IOP**	***P* <**
Lasalocid					**Monensin**				
Milk, L/d	6	28.7	28.3	NS	Milk, L/d	11	27.5	28.8	0.01
Fat, %	6	3.67	3.51	NS	Fat, %	11	3.98	3.78	0.01
Fat, kg/d	6	1.037	0.958	0. 01	Fat, kg/d	9	1.037	1.032	NS
Protein, %	6	3.02	2.99	NS	Protein, %	11	3.25	3.20	0.05
Protein, kg/d	6	0.858	0.837	NS	Protein, kg/d	9	0.846	0.872	0.01
DMI, kg/d	6	19.4	18.6	NS	DMI, kg/d	6	21.7	21.2	NS

As seen in Table 2.1 and from results found in various studies: The administration of IOP's to lactating cows had no effect on dry matter intake (DMI). Results from an Italian study suggested that when cows are fed on high-grain diets the administration of IOP's is more likely to result in a larger depression of DMI than when fed high-forage diets (Ipharraguerre et al., 2003). Administering IOP's to dairy cows either does not affect or increase milk production. The fat yield of milk was significantly depressed from cows that had been administered lasalocid and the milk fat content decreased when the cows were administered the monensin premix. In most studies the milk protein content of IOP- treated cows was lower than that of the controlled cows, however milk production was significantly increased, suggesting that the reduction in milk protein content could be due to a dilution effect (Ipharraguerre et al., 2003).

2.2 Essential oils

2.2.1 What are essential oils?

Essential oils (EO) are volatile aromatic compounds that have an oily appearance and they are extracted from plants (Tassoul et al., 2009). The meaning of "essential", derived from "essence", is to taste or smell. This relates to these essential oils in providing specific flavours and odours to many plants (Nogueira, 2009). These plant essential oils display a wide range of antimicrobial activities that enable them to alter ruminal fermentation and improve the production performance in lactating dairy cattle, when used as dietary supplements (Tassoul et al., 2009). Since the ban of feed-grade antibiotics and ionophores in Europe in 2006, EO have been widely used as an alternative to monensin. Much like monensin sodium, essential oils potentially impact the rumen by inhibiting deamination and methanogenesis, resulting in a decreased production of acetate, ammonia and methane, while increasing propionate and butyrate production (Karnezos, 2010). Essential oils also have a similar effect to monensin, in the way that they may affect the cell membranes of gram negative bacteria. Some essential oils can work within the cell of gram positive bacteria (Paulson, 2008). Essential oils can be given in different forms – oil, powder, pellet, and encapsulation. The form in which EO are administered to animals may affect how it works in the rumen. It is not yet clear how much effect the type of diet or class and stage of production of the animals has on key responses.

2.2.2 Mode of action of essential oils

Essential oils are comprised of numerous components. It is most likely that their antimicrobial properties are not due to a precise mode of action, but can rather be contributed to numerous targets in the bacterial cell (Benchaar et al., 2008). Essential oils interact with several cellular components and they have the ability to modify a reaction at their targets. Therefore numerous cellular targets can be modified by these components. Essential oils utilise their antibacterial properties by interacting with the processes that are linked with the bacterial cell membrane, such as electron transport, ion gradient, protein translocation and phosphorylation (Benchaar et al., 2008). Due to their hydrophobic nature, essential oils show a high affinity towards lipids of bacterial cell membranes. Gram-positive bacteria appear to be more susceptible to the antibacterial activities

of the essential oils, than the gram-negative bacteria. Gram-negative bacteria have an outer layer that surrounds their cell wall and this outer layer acts as a permeability barrier, which limits the access of hydrophobic compounds to the cell (Benchaar et al., 2008). However, the active essential oil compounds carvacrol and thymol inhibit the growth of gram-negative bacteria successfully by disrupting the outer layer of the cell wall. They are able to penetrate the inner membrane of gram-negative bacteria, due to their small molecular weight (Benchaar et al., 2008).

2.2.3 Brief discussion of active components of essential oils

The reduction of acetate and methane production, the increase of propionate production and the modification of proteolysis, peptidolysis or deamination within the rumen are enabled by the properties of oils such as thymol, eugenol and cinnamaldehyde (Calsamiglia et al., 2007). It is important to know the supplementation levels of these and other essential oils in order to avoid fermentative depression in the rumen that may occur at high dosages (Spanghero et al., 2008). The type of essential oil, as well as the blend of oil will have an effect on the results seen from the supplementation of these oils. The effects of essential oils are highly dependent on the acidity in the rumen, the rumen microbial population as well as the adaptation period of the bacteria to the essential oils (Spanghero et al., 2008).

Thymol

Thymol is a monoterpene which has strong antimicrobial properties against a broad variety of bacteria. It is also known to be one of the well-researched active components of essential oils. Thyme and oregano oils contain large quantities of thymol (Calsamiglia et al., 2007). In studies where they have included the use of thymol, it was found that in some instances the accumulation of amino acids (AA) and a reduction in ammonia nitrogen (N) concentration occurred. Evans et al. (2000) conducted a study of this oil and they reached a conclusion that the energy metabolism of microorganisms are affected by thymol, particularly that of *Streptococcus bovis* and *Selenomonas ruminantium*. Methane and lactate concentrations were reduced by thymol and the overall nutrient digestion as well as the volatile fatty acid (VFA) production was reduced at higher levels of supplementation. This is a clear indication that microbial metabolism was inhibited. A reduction in the uptake of glucose, as well as the loss of integrity of the cell membrane, may possibly have created this effect. At lower dosages thymol has very little to no effect on in vitro rumen microbial fermentation. According to many in vitro studies, the effects of thymol are pH and diet dependent. At a high pH (6.5) thymol increased the acetate-to-propionate ratio and when thymol was supplemented to a ruminal fluid with a lower pH (5.5), a decrease in the acetate-to-propionate ratio was observed (Calsamiglia et al., 2007). As the pH decreased, there was an increase in the antibacterial effect of the essential oil of thyme. During the supplementing of thyme, it is essential that the ruminal conditions subjected to the use of this additive, must be defined, in order for the rumen microbial fermentation altered into the best desired direction (Calsamiglia et al., 2007).

Eugenol

Eugenol is a phenol with a broad-spectrum antibacterial activity which makes it an effective enemy against gram-positive and gram-negative bacteria. It is also one of the main components in cinnamon and clove bud oils. Davidson et al. (2000), had conducted a study with the inclusion of eugenol in the dairy cow's diet. The resulted showed lower magnitudes of acetate and branched-chain volatile fatty acid (VFA) as well as a higher proportion of propionate. The nitrogen (N) metabolism had been affected in terms of an increase in the peptide N and causing the amino acid (AA) N concentrations to be increased numerically (Busquet et al., 2006). The fermentation profile suggested that when eugenol is supplemented at optimal doses, the energy efficiency and utilisation of proteins in the rumen are improved (Davidson et al., 2000). It appears that the N utilization in the rumen of lactating cows, as well as the VFA production, may be improved by the inclusion of eugenol (Calsamiglia et al., 2007).

Cinnamaldehyde

Cinnamaldehyde is a phenylpropanoid with antibacterial properties and is the main active component of cinnamon oil. Studies done by Cordozo (2004), suggested that cinnamon oil modified the nitrogen (N) metabolism of microorganisms in the rumen, by inhibiting peptidolysis. Cinnamaldehyde was found to decrease the total volatile fatty acid (VFA) and ammonia concentrations. A test was conducted to observe the effect of low doses of cinnamaldehyde in a long term continuous culture fermentation. Results indicated that the proportion of butyrate was increased and the molar proportion of acetate, numerically decreased by the doses of cinnamaldehyde (Cordozo, 2004). At higher dosages the proportion of acetate was reduced and the molar proportions of butyrate and propionate were increased. Most of the studies that have been conducted indicated that the supplementation of cinnamaldehyde has no effect on N metabolism. In contrast to other components of plant essential oils, the membrane stability is not affected by cinnamaldehyde and the interaction with proteins found deeper within the cell may be related to its mode of action. The effects obtained when supplementing cinnamaldehyde are also diet and pH dependent. At pH 7.0 cinnamaldehyde resulted in an increased acetate-to-propionate ratio and a lower total VFA concentration (Calsamiglia et al., 2007). At pH 5.5 the total VFA concentration increased and the acetate-to-propionate ratio and the ammonia N concentration, decreased. The antimicrobial effect increases as the pH decreases from 7.0 to 5.5 (Calsamiglia et al., 2007).

2.2.4 Effect of essential oils on milk production and milk composition

Possible uses of plant essential oils as dietary supplements to dairy cows have been investigated in numerous studies over the past years. However, these investigations that were conducted were mostly laboratory based and were of a short term nature. Work done on the effect of essential oils on the milk production of dairy cows is very limited (Benchaar et al., 2006). Benchaar observed no changes in dry matter intake (DMI), milk production and milk components when cows were given either 750 mg or 2 g of a specific mixture of essential

oils (MEO). Yang et al. (2007) observed that the addition of garlic and juniper berry oils, had no effect on milk production, milk composition and DMI, when supplemented into dairy cow diets.

Tassoul et al. (2009) completed a trial that involved cows pre-calving to 15 weeks into lactation. Twenty cows were used as a control group and 20 others were given 1.2 g of an essential oil product, CRINA. The CRINA is made from a blend of several plant oils. There was no observed benefit to the cows that received the supplement prepartum. There was an improvement noted for fat corrected milk and dry matter intake, the longer the cows received the oils. This trial was discontinued after 100 days. An adaptation time should be allowed for the oils in the rumen before the full benefits can be achieved (Paulson, 2008). Another trial with the use of CRINA had previously been done on 33 dairy cows, with older cows that were in mid-lactation. Supplemented cows showed a slight improvement in milk production, increased components and increased fat corrected milk yields (Paulson, 2008).

A field trial involving 170 cows, also supplementing CRINA, had been conducted by Nogueira (2009). Similar results were seen between treated and control cows, but the treated cows showed a slightly higher increase in milk production and more fat corrected milk (Nogueira, 2009). The effects of the addition of essential oils and ionophores (monensin) on digestion, milk production, milk composition and ruminal fermentation in dairy cows were evaluated by Benchaar et al. (2006), within a trial that they had conducted. Table 2.2 compares the effect of essential oils (EO) and monensin (MO) on the milk production and milk composition of dairy cows.

Table 2.2 Effect of essential oils (EO) and monensin (MO) on the milk production and milk composition of dairy cows (Benchaar et al., 2006).

	-EO		+EO			Contrast [1]*P*		
Item	**-MO**	**+MO**	**-MO**	**+MO**	**SEM**	**EO**	**MO**	**Inter**
Milk yield, kg/d	34.4	34.1	32.1	34.0	0.9	0.19	0.38	0.23
4% FCM*, kg/d	35.0	32.9	31.7	33.7	0.8	0.22	0.76	0.06
Milk composition, %								
Fat	4.07	3.77	4.00	3.91	0.07	0.61	0.03	0.18
Protein	3.58	3.51	3.62	3.46	0.06	0.93	0.11	0.51
Lactose	4.68	4.63	4.59	4.66	0.04	0.40	0.78	0.18
Total solids	13.0	12.6	12.9	12.8	0.1	0.96	0.04	0.19
Milk urea N, mM	11.8	13.0	12.3	12.2	0.3	0.64	0.06	0.05
Milk yield, kg/d								
Fat	1.41	1.28	1.34	1.34	0.03	0.26	0.28	0.03
Protein	1.23	1.18	1.15	1.17	0.05	0.37	0.75	0.49
Lactose	1.61	1.59	1.48	1.58	0.05	0.20	0.40	0.21
Total solids	4.49	4.30	4.12	4.34	0.12	0.22	0.91	0.13

*4% FCM= 0.4 (kilograms of milk) + 15.0 (kilograms of fat).

[1]*P*-value for factorial contrasts: essential oils (+EO vs –EO), monensin (+MO vs –MO), and the interaction between EO and MO (Inter).

As seen in Table 2.2, the addition of essential oils (EO) did not have an effect on the milk production and milk composition. The milk urea N and fat concentrations in milk were respectively higher and lower for cows supplemented with monensin (MO) than compared with cows on a diet without MO supplementation (Benchaar et al., 2006). Milk protein and lactose concentrations were unaffected by EO and MO addition. The milk fat concentration was decreased with MO supplementation, but the supplementation of monensin had no effect on the milk protein content. Monensin supplementation tendended to increase the milk urea N concentration, but this effect was not seen when supplemented in combination with EO. Besides the interaction between EO and MO for fat yield, the supplementation of these additives to the diet did not change the yields and milk components significantly (Benchaar et al., 2006). Dosage amount is certainly important, as it appears one could feed too little or too much to get a desirable result. This is not only driven by feeding rate, but also the purity of the product being used. Effective doses may vary between different EO because of the large differences in chemical composition. It is important to determine the correct dose rate for the use of EO in order to have favourable effects on rumen metabolism and the influence on the VFA concentration. Castillejos et al. (2006) added thymol to culture fermenters at doses of 5, 50 and 500 mg/L. At the highest dose, a decrease in the digestibility of DM, NDF and ADF was found. At the lower dose levels, no changes were found on DM, NDF and ADF digestibility. This suggests that the effect of digestibility may be dose dependent. In a study done by Spanghero et al. (2009), the impact of a blend of EO, micro-encapsulated and fed at increasing levels in diets of high yielding cows, was investigated. Results captured from the study are shown in Table 2.3.

Table 2.3 Effect of increasing essential oil (EO) dose level on milk production and composition (Spanghero et al., 2009).

	EO dose level, g/cow/d				***P*[2]**	
	0	**40**	**80**	**120**	**L**	**Q**
Milk yield, kg/d	31.39	30.70	31.16	31.18	0.92	0.40
Milk composition:						
Fat, g/kg	36.8	38.2	37.0	37.1	0.87	0.15
Protein, g/kg	31.7	32.0	32.3	31.4	0.70	0.06
Lactose, g/kg	49.5	49.8	49.8	49.6	0.69	0.17
Urea, mg/100 ml	31.3	32.0	31.7	31.3	0.95	0.70
Somatic cells, 1000/ml	39	39	41	42	0.61	0.90
Milk component yields, kg/d						
Fat	1.16	1.17	1.15	1.16	0.81	0.66
Protein	0.99	0.98	1.01	0.98	0.89	0.55
Lactose	1.55	1.53	1.55	1.55	0.98	0.63
Milk energy:						
Content, MJ/kg	2.99	3.05	3.01	3.00	0.78	0.05
Yield, MJ/d	93.8	93.6	93.8	93.5	0.85	0.95

[2]Probability levels of the linear (L) and quadratic (Q) effects of increasing EO dose.

From Table 2.3 the following deductions can be made: At intermediate essential oil dosages, the milk protein content was unaffected. The milk fat yield and milk fat content was not influenced. The milk energy content was increased, but only at the two intermediate levels of feeding, due to a combination of similar tendencies (protein) and numerical (fat) effects in the milk components that were used to calculate it. However, there was no effect on milk yield, milk component yield or milk energy output (Spanghero et al., 2009).

2.2.5 Essential oils as dietary supplements for dairy cattle

Different blends of essential oils (EO) are supplemented to dairy cows to enhance pre-fresh, fresh-cow and early lactation intakes. The supplementation of these oils tend to have a positive influence on dry matter intake (DMI) and rumen bacterial flora helps minimize the negative energy balance in transition cows and EO can also help avoid the occurrences of metabolic and infectious disease challenges that make up the fresh-cow complex (Karnezos, 2010). Research has shown so far that the use of essential oils for ruminant production can be divided into the following categories:

- Stimulation of rumen fermentations
- Inhibition of methanogenesis
- Modification of the production of and profile of the ruminal volatile fatty acid (VFA), nitrogen metabolism or both

All of the above are important in ruminal nutrition. The inhibition of methanogenesis, reduces the impact that methane has as a greenhouse gas and it provides the animal with more energy. By modifying the ruminal VFA profile, the amount of propionate will be increased and the amounts of lactate and methane will be reduced without the reduction of the total production of VFA. The same accounts for nitrogen metabolism, some EO inhibit the degradation of proteins in the rumen, potentially improving the amount and the quality of amino acids available for milk production (Nogueira, 2009).

2.3 Oregano

2.3.1 What is oregano?

Origanum vulgare L. extracts originate from Portugal and they are strong candidates to replace synthetic chemicals used by the health industry (Teixeira et al., 2013). Along with human consumption, animal foodstuff and ornamental uses; aromatic plants are especially suitable for multifunctional sustainable crop models (De Falco et al., 2013). A large number of these aromatic species belong to the family *Lamiaceae*. Oregano is a hardy, bushy perennial herb that grows up to 90 cm high, with an erect hairy stem, dark green oval leaves and a profusion of pink flowers clustered in heads at the top of the branches. Oregano oil is extracted from the dried, flowering tops of the herb by steam distillation. Oregano oil has a powerful, spicy, camphor-like aroma, is pale yellow in colour and medium to watery in viscosity.

In folk medicine, *Origanum vulgare* is used to treat respiratory disorders, rheumatoid arthritis and urinary tract disorders (Teixeira et al., 2013). There are also some reports regarding the anti-mutagenic and anti-carcinogenic effect of oregano; representing an alternative for the potential treatment and/or prevention of certain chronic ailments, like cancer (Arcila-Lozano et al., 2004).

Some of the properties of this plant's extracts are currently being studied due to the growing interest for substituting synthetic additives commonly found in foods (Arcila-Lozano et al., 2004). Oregano has a good antioxidant capacity and also presents antimicrobial activity against pathogenic microorganisms like *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus epidermidis*, among others. These are all characteristics of interest for the food industry because they may enhance the safety and stability of foods (Arcila-Lozano et al., 2004).

Every year more studies are performed to determine the effect of including oregano in animal feeds. However, to date very few studies have been done *in vivo*. With the increased numbers of consumers resisting to purchase antibiotic supplemented animal products, the need for alternative feed supplements is growing. It is thus important to implement studies, regarding oregano, to determine the possible benefits that it could have on the production parameters of both monogastric and ruminant animals.

2.3.2 Composition of oregano oil

Oregano is probably one of the most widely used aromatic plants, whose essential oils are particularly rich in mono- and sesquiterpenes (De Falco et al., 2013). The main chemical components are carvacrol, thymol, p-cymene and linalool as shown in Figure 2.1. The oregano composition depends on the specie, climate, altitude and time of recollection and the stage of growth (Arcila-Lozano et al., 2004).

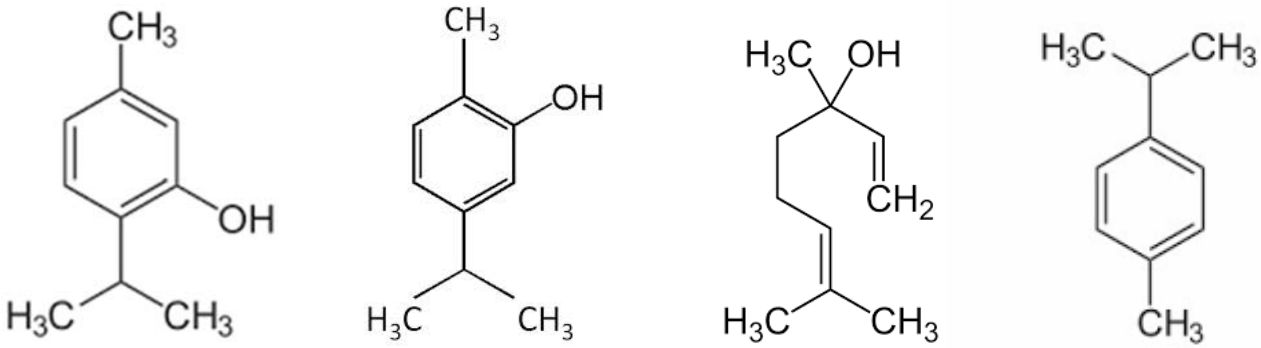


Figure 2.1 The main active chemical components of oregano oil.

Oregano oil that is obtained from *Origanum onites* consists of 24-53% carvacrol, 2-24% thymol, 4-8% p-cymene and 0.1-14% linalool (Toncer et al., 2009). Biological activities of oregano depend mainly on

carvacrol and thymol. Nutrients like vitamins A, C and E, calcium, magnesium, zinc, iron, potassium, manganese, copper, boron and niacin are also found in oregano oil.

2.3.3 Mode of action of oregano oil

The compounds in oregano oil work together to provide the antimicrobial effects this oil is so well-known for. Carvacrol is its most important component and is responsible for many of its health benefits. Carvacrol has powerful antimicrobial properties, and has been shown to help break through the outer cell membranes that help protect bacteria from your immune system.

Antibacterial properties

Cavacrol, thymol and p-cymene are the main components in oregano oil, responsible for its antibacterial properties. Cavacrol and thymol are terpenoids, which means that they interact with the cell membranes of microorganisms. They diffuse the lipid layer of the cell membrane and cause pores between the fatty acids, due to their hydrophobic properties (Ropapharm Int., Ropadairy). Alterations in the conformation of membranes, changes the permeability of those cell membranes for cations H^+ and K^+ (Ropapharm Int., Ropadairy). Inhibition of essential processes such as ATP synthesis, due to the alteration of the ion concentrations, results in bacterial death. The antibacterial properties of oregano oil effects both gram positive and gram negative bacteria.

Anti-oxidative properties

Due to its active components, oregano oil has strong anti-oxidative properties. These properties include; protecting feed material while in storage and protects feed in the digestive tract from oxidation. Furthermore the active components reduce free radicals, which are occurred phagocytosis action of macrophages, protecting the intestine villi from pre-oxidation (Ropapharm Int., Ropadairy). As a result of the bilateral protection, the absorption of nutrients increases.

Fungicidal properties

Cavacrol and thymol are responsible for the fungicidal effect of oregano oil. The mode of action of these compounds make oregano oil a suitable feed additive, as it will protect feed material from yeast and mold during storage.

2.3.4 Effect of oregano oil on DMI and BW of dairy cows

A common hypothesis regarding dry matter intake (DMI) is that oregano would reduce DMI in ruminants, thus improving the feed efficiency of the dairy herd. Hristov et al. (2013) executed a 20-day trial, feeding eight

cannulated cows different levels of oregano. Dry matter intake and feed efficiency for the control diet versus the diets with different levels of oregano can be seen in Table 2.4.

Table 2.4 Dry matter intake and feed efficiency of cows fed control or *Origanum vulgare* L. (OV) -supplemented diets. Adapted from Hristov et al. (2013).

	*Diet					Contrast, [1]*P*		
Item	**Control**	**LOR**	**MOR**	**HOR**	**SEM**	**OR-Con**	**L**	**Q**
DMI, kg/d	28.3	28.3	27.5	26.6	1.81	0.18	0.014	0.37
Feed efficiency, kg/kg	1.46	1.59	1.6	1.63	0.195	< 0.001	0.001	0.15

*Control = 0 g of OR leaves/d; LOR = 250 g of OR leaves/d; MOR = 500 g of OR leaves/d; HOR = 750 g of OR leaves/d

[1]OR-Con = OR leaves-supplemented diets versus control; L = Linear and Q = quadratic responses to OR supplementation

Hristov et al. (2013) found that the oregano supplementation linearly decreased (P = 0.014) DMI of the cows. The decrease in DMI, however, did not have an effect on milk yield. Feed efficiency was greater (P < 0.001) for OR compared with the control and increased linearly (P = 0.001) with increased OR supplementation rates. It can thus be hypothesised that OR, perhaps due to its strong, objectionable odour of its EO compounds will likely reduce DMI in ruminants (Hristov et al., 2013). If thus reduction in DMI does not result in decreased production, feed efficiency may be improved.

A 21 day crossover trial using eight primi-parous and eight multiparous cows was executed by Tekippe et al. (2011). A control diet versus a diet with oregano leaves were used for this trial. Table 2.5 shows the results for the DMI as well as BW.

Table 2.5 Dry matter intake and body weight of cows fed control or *Origanum vulgare* L. (OV) -supplemented diets. Adapted from Tekippe et al. (2011).

Item	**Control**	***OV**	**SEM**	***P* - value**
DMI, kg/d	26.7	26.0	3.01	0.24
Feed efficiency	1.66	1.72	0.066	0.11
Cow BW, kg	640	640	46.2	0.97

*OV = 500 g of OV/d.

These results showed that the intake of dry matter (DM) as well as body weight (BW) was not effected by feeding oregano leaves. Feeding oregano leaves resulted also had no effect on feed efficiency.

De Oliveira et al. (2014), executed a trial investigating the economic performance of dairy cows when fed different levels of oregano. Table 2.6 shows the results in terms for DMI and BW from this trial.

Table 2.6 Intake and body weight obtained with the addition of oregano to the diet. Adapted from De Oliveira et al. (2014).

	Oregano inclusion level %				
Item	**0**	**0.8**	**1.6**	**2.4**	**CV (%)**
DMI, kg/d	14.89	14.87	15.41	15.97	5.93
*BW, kg	3.06	4.14	3.94	3.97	-

*BW = body weight variation

A positive linear effect for dry matter intake (DMI) was found, which led to an increase of 0.471 kg for each percent unit of oregano added to the diet. With the increase in DMI it was expected that the production would increase, however this did not occur. Although an upward trend was visualised with the variation in BW in relation to the control diet, no influence was presented by the inclusion of oregano.

Cavacrol – based phytogenic feed additives are primarily marketed for monogastric species. However when reading through literature based on trials involving herb feeding to monogastric animals: Muhl et al. (2007) trials with pigs and Bravo et al. (2011) - trials with poultry, both these trials resulted in no effect on intake. Lee et al. (2003) found when feeding cavacrol to female broiler chickens, there was a decrease in DMI, but no effect was found on feed efficiency.

2.3.5 Effect of oregano oil on milk yield and milk composition of lactating dairy cows

Hristov et al. (2013) investigated the effect of *Origanum vulgare* L. leaves on the rumen fermentation, production and the milk fatty acid composition in lactating dairy cows. Table 2.7 shows the results for the milk yield and milk composition for Hristov's trial.

Hristov et al. (2013) found that the milk fat content presented a tendency for a quadratic increase in milk fat content when the supplementation rate of oregano was increased. The 3.5% fat corrected milk (FCM) feed efficiency showed a tendency to increase linearly with increased oregano supplementation levels. The milk urea nitrogen (MUN) concentration decreased for the oregano diets in comparison to the control diet. No significant effects were found on the 3.5% FCM, as well as no significant effects were found on milk yield and milk fat yield. Hristov's trial was short term based and it was recommended that these results be followed up by long term period trials.

Table 2.7 Production parameters of dairy cows fed control or *Origanum vulgare* L. leaves-supplemented diets. Adapted from Hristov et al. (2013).

	*Diet					Contrast, [1]*P*		
Item	**Control**	**LOR**	**MOR**	**HOR**	**SEM**	**OR-Con**	**L**	**Q**
Milk, kg/d	43.4	45.2	44.1	43.4	7.44	0.41	0.76	0.13
Milk fat, %	3.26	3.25	3.11	3.57	0.169	0.69	0.14	0.06
Fat yield, kg/d	1.37	1.45	1.33	1.49	0.171	0.50	0.44	0.53
MUN, mg/100 ml	9.3	8.5	7.9	8.2	0.59	0.04	0.07	0.21
3.5% FCM, kg/d	41.2	43.2	40.6	42.9	5.92	0.58	0.86	0.89
3.5% FCM feed efficiency, kg/d	1.45	1.51	1.48	1.61	0.128	0.16	0.07	0.55

*Control = 0 g of OR leaves/d; LOR = 250 g of OR leaves/d; MOR = 500 g of OR leaves/d; HOR = 750 g of OR leaves

[1]OR-Con = OR leaves-supplemented diets versus control; L = Linear and Q = quadratic responses to OR supplementation

Tekippe et al. (2011) investigated rumen fermentation and production effects of dairy cows when fed *Origanum vulgare* L. leaves. Milk yield and milk composition results from Tekippe's trial are shown Table 2.8.

Table 2.8 Production parameters of cows fed control or *Origanum vulgare* L. (OV) - supplemented diets. Adapted from Tekippe et al. (2011).

Item	**Control**	***OV**	**SEM**	***P* - value**
Milk, kg/d	43.6	44.1	3.58	0.61
Milk fat, %	3.12	3.29	0.281	0.02
Milk fat yield, kg/d	1.37	1.45	0.210	0.01
3.5% FCM, kg/d	41.0	42.0	4.83	0.09
3.5% FCM feed efficiency	1.45	1.64	0.034	0.001
Milk NE efficiency	64.4	68.0	1.26	0.004

*OV = 500 g of OV/d.

The results in Table 2.8 show that there was an increase in the milk fat content as well as an increase in the milk fat yield from the cows fed the oregano diet. The Milk NE efficiency also increased on the oregano diet. The 3.5% FCM feed efficiency increased when compared to the control diet.

Manipulating the long chain polyunsaturated fatty acids (PUFA) concentrations in milk, particularly n – 3 and n – 6 FA, can lead to important health benefits in humans. A two week study feeding herbs and clovers in combination with a TMR to dairy cows, was carried out by Petersen et al. (2011) to investigate the effect of herb feeding on n – 3 and n – 6 fatty acids in cow milk. The feeding of different types of herbs resulted in an increased concentration of n – 3 and n – 6 fatty acids in cow milk. Chilliard et al. (2001) reported that the n – 3 and n – 6 FA concentrations in milk can be increased by manipulating the diet. The greatest impact on milk fat concentrations can be made by including herbage in dairy cow diets.

2.3.6 Effects of Oregano oil versus the effects of Ionophores on lactating dairy cows

In order to distinguish if oregano oil can be used as a replacement for the use of ionophores as feed additives in the dairy cow industry, it is beneficial to compare the effects that the two additives may have on dairy cows. In Table 2.9 the benefits of using oregano oil are compared to the benefits attained when feeding ionophores.

Table 2.9 A summary of the effects of feeding oregano oil vs ionophores to dairy cows.

Oregano Oil	**Ionophores**
Conservation of total mixed ration	Aids in the prevention of ketosis
Lower methane gas production in the rumen	Improves energy metabolism
Better feed intake	Reduce the voluntary feed intake
Better feed efficiency	Improve feed efficiency
Possible increase in milk production	Possible increase in milk production
Better milk quality	May alter milk components
Better health status	General health improvement

As seen in Table 2.9, the benefits attained when feeding oregano oil compared to ionophores are similar. It thus seems that oregano oil would be able to be used as a replacement feed additive to ionophores. However, further research is needed to evaluate the magnitute of the effects in the above mentioned traits.

2.4 Factors that affect milk composition

Nutritional changes in the feed rations can alter the fat concentration as well as the milk protein concentration. Fat concentrations are the most susceptible to dietary changes and can vary over a range of nearly 3.0 percentage units (Varga et al., 2010). This response however, is inconsistent and often related to the amount and type of fat being fed. Excessive amounts of dietary fat have shown to decrease milk protein production. Fat substitution for ruminal available carbohydrate may depress microbial protein synthesis and thus decrease the amount of amino acids available at the udder (Varga et al., 2010). Some nutritionists recommend adding 1% unit more undegradable protein for every 3% added fat in the ration (Varga et al., 2010). The growth of some groups of rumen bacteria can be affected by unsaturated fatty acids (UFA), and UFA can potentially inhibit fat synthesis in the mammary gland (Lock et al., 2012). Saturated fatty acids (SFA), on the other hand are considered to be inert in the rumen. Relling et al. (2007) performed a trial investigating the effect of feeding

rumen-inert fats differing in their degree of saturation on dry matter intake (DMI) and milk production amongst other parameters. Results for Relling's trial are displayed in Table 2.10.

Table 2.10 Dry matter intake and milk yield, fat content and fat yield in mid-lactation dairy cows fed rumen-inert fats. Adapted from Relling et al. (2007).

	*Diet				
Item	**Control**	**SFA**	**MUFA**	**PUFA**	***P***
DMI, kg/d	23.8	23.1	22.1	22.0	0.12
Milk yield, kg/d	36.9	37.3	35.8	34.8	0.44
Fat, %	3.37	3.86	3.32	2.61	0.03
Fat yield, g/d	1,249	1,436	1,184	911	0.02

*SFA= Saturated fatty acids; MUFA = Mono-unsaturated fatty acids; PUFA = Poly-unsaturated fatty acids

Feeding different inert fats did not influence DMI. No effect was found on the milk yield. An increase in fat content and fat yield was found, when increased levels of SFA were fed in comparison to MUFA and PUFA. Including more SFA into dairy diets could possibly increase the fat content and fat content in the milk.
Dietary protein supplements typically increase milk protein secretion but have variable effects on milk protein content (Lock et al., 2012). Milk protein concentrations can change with approximately 0.6 percentage units (Lock et al., 2012). Formulation models and feeding management of lactating cattle should focus on reducing the excess protein in the diet, to improve the efficiency of use of feed protein. Other solid constituents in milk as well as lactose and minerals, do not respond greatly to dietary manipulations (Varga et al., 2010). Many non-nutritional factors can also influence the milk components such as; genetics and environment, milk production levels, stages of lactation, disease, season, facilities, cow comfort and the age of the cow.

Genetics and environment

Using traditional breeding techniques can change the milk composition, however these changes are minimal and are only achieved after several years. Heritability estimates for milk composition are high at 0.5 and relatively low for yield at 0.25 (Varga et al., 2010). Although most producers are focused on breeding for greater milk yields per cow, attention should be given to component yields. Genetics should be directed towards increasing fat, protein and non-fat solid yields. With that said yields for fat, protein, non-fat solids and total solids are highly and positively correlated to milk yield (Varga et al., 2010). Selection programs should thus emphasize on milk yield, increasing fat and protein yields at the same time.

Feed intake and peak milk production

Maximising feed intake can shift the cow's energy balance from a negative to a positive state during early lactation. Increasing feed intake can increase milk protein by 0.2 to 0.3 units (Varga et al., 2010). A slow increase in feed intake postpartum could lengthen the days to peak production. Fat cows have shown to have depressed appetites at calving, resulting to delays to peak milk yield. Body condition scores (BCS) play an important role in feed intake, as cows with a BCS over 3.75 at calving, can reduce dry matter intake (DMI) with 1.5 - 2% for every 0.25 BCS above 3.75 (Varga et al., 2010). Therefore managing the feed intake of dairy cows pre- and postpartum could influence the days to peak milk production.

Season

Research has shown that milk fat and milk protein percentages are higher during autumn and winter and lowest during spring and summer (Varga et al., 2010). This variation could be related to a change in the feed available as well as weather conditions. High humidity decreases dry matter intake and respectively decreases energy intake, which leads to a reduction in milk components (Varga et al., 2010).

Disease

A reduction in fat and casein and an increase in whey content in milk, can be found when cows have mastitis. Cows giving milk with elevated somatic cell counts (> 500, 000 somatic cells/ml), have longer coagulation time and forms weaker curds, than milk from healthy cows (Varga et al., 2010).

Stage of lactation

During early lactation the concentrations of milk fat and protein are at their highest and at their lowest during peak lactation (Varga et al., 2010). The reasoning for this, is that with greater milk yields, the component percentages are generally decreased, but the component yields remain unchanged or will increase.

Equipment

When cows are not completely milked out, the milk fat can be reduced. Faulty equipment such as improper vacuums, improper cooling and milk freezing can also contribute to a reduction in milk fat (Varga et al., 2010).

Age

Age does not really effect the milk fat content, however with age the milk protein content will gradually decrease. A survey of Holstein DHIA lactation records, indicated that milk protein content will typically decrease with 0.1 to 0.15 units over five lactations or 0.02 to 0.05 units per lactation (Varga et al., 2010).

2.5 Sensory attributes of milk

2.5.1 Microbiological analysis using Petrifilm

Microbiology involves working with organisms that, when viewed under normal circumstances, are too small to see. Special techniques, methods and apparatus are usually required to examine these organisms. Agar gel substrates are often used to grow microorganisms. For the current trial an alternative growth substrate, petrifilm 3M plates (Merck Biolab, South Africa), were used. For the milk bacteria analysis, petrifilm plates were used for total aerobic counts (TAC) and coliform counts (CC), with emphasis on *E.coli*. After the microbiological results were obtained, the milk samples were cleared for sensory analysis.

A petrifilm plate is a thin, sample ready, dehydrated version of the conventional petri dish agar plate (Ball, 2008). Petrifilm is used globally in the food industry to monitor quality and to audit cleaning and sterilisation processes (Ball, 2008). Pertrifilms are widely used because of their cost-effectiveness, convenience and simplicity (Silva et al., 2005). Lazar et al. (2010) found that, when using petrifilm the thermostating period was reduced in comparison to other classic methods. Another advantage of using petrifilm over agar plates is that media preparation is unnecessary and it saves both labour and time (de Sousa et al., 2005). Ten petrifilms take up the same space as a single petri dish agar plate. Only a few disadvantages have been noted when using petrifilm, such as: petrifilms are more sensitive in detection and could possibly lead to an increased risk of false positive results (Silva et al., 2005) and overcrowded plates decrease the visibility of colonies in the centre of the plate and many small colonies are seen on the edges (de Sousa et al., 2005). When this occurs, further dilution of the samples are required.

Petrifilms are available with a number of different mediums for counts of aerobic bacteria, moulds and yeasts, coliforms and *Escherichia coli* (*E.coli*) (Harrigan, 1998). For aerobic bacteria an aerobic count (AC) plate is used. The AC plate contains standard method nutrients, a cold water gelling agent and triphenyl tetrazolium chloride (TCC) which is an indicator that colours bacterial colonies red (Ball, 2008). Coliform count (CC) plates are used for coliform bacteria. Ball (2008) describes coliforms to be members of the *enterobacteriacea* family which ferment lactose to produce gas and they are indicators of faecal contamination, particularly in dairy products. The CC plate contains violet red bile (VRB) lactose ingredients, a cold water gelling agent and TCC, turning the coliform colonies red. The E.coli/Coliform (EC) plate is the same as the CC plate, except that it contains a BCIG chromogenic indicator.

2.5.2 Sensory evaluation of milk samples

There have been several studies done evaluating the flavour of cow's milk, when essential oils (EO) are included into the animals diet, however very few studies have been done to investigate the effect of dried herbs on the sensory characteristics of milk (Lacerda et al., 2014). Milk flavour can be affected by the diet of the dairy cow, especially when fed the addition of herbs (Larsen et al., 2013). A herb-based diet contains an

increased content of volatiles which may affect the sensory properties of milk. When changing the cows' diet it is important to investigate how these changes will influence the milk produced.

As defined by the Institute of Food Technology, sensory evaluation is a scientific method that is used to analyse, evoke, interpret and measure responses to products as perceived through the senses of sight, smell, touch, taste and hearing (Kemp et al., 2009). Kemp et al. (2009) emphasises the importance on how only the most appropriate individuals should be recruited, screened and selected to take part in the sensory tests: they should be given the necessary tools to complete the test effectively and they should receive positive and constructive feedback on their performance. Discrimination tests should be used when the sensory specialist wants to determine whether two samples differ from one another (Lawless et al., 1998).

There are numerous different discrimination tests available, they include triangle tests, duo-trio tests, paired comparison tests and *n*-alternative forced-choice tests. In a triangle test, three samples are presented simultaneously to the panellists. Two samples are from the same formulation and one is from a different formulation. Panellists must indicate, by using a score sheet, which one of the three is the odd sample. The null hypothesis for the triangle test states that the long-run probability (P_t) of making a correct selection, when there is no perceptible difference between the samples is one in three (H_o: $P_t = ⅓$) (Lawless et al., 1998). The alternative hypothesis states that the probability that the underlying population will make the correct decision when they perceive a difference between the samples will be larger than one in three (H_a: Pt >⅓) (Lawless et al., 1998). Triangle tests can be useful as there is no conflict in the data, the analysis is easy to execute and accurate. However there are some disadvantages to using triangle tests, such as: data accuracy can be corrupted, due to panellists being able to see each other's reactions. Silence and separation of panellist by booth-like partitions may decrease the occurrence of data corruption.

Essential oils (EO) are natural plant products that can be acquired through steam distillation of the plant material. EO mainly consist of volatile compounds such as monoterpenes and sesquiterpenes and they have strong flavours and smells (Simon, 1990). In an experiment to determine the composition of mono-and sesquiterpenes in milk, Viallon et al. (2000), found that the composition in milk was directly related to the composition of the same components that were found in that particular animal feed. Terpenes are transferred rapidly into milk, however levels in the milk decrease excessively when the cows are taken off of the EO diet.

In a trial by Larsen et al. (2013), different ways were investigated to transfer volatile components to milk by adding EO of oregano and caraway to the dairy cows' diets. The results from Larsen et al. (2013) indicated that the composition of volatile compounds that were found in the milk, did not directly reflect the volatile composition of the feed. Larsen et al. (2013) suggested that the above stated may most likely be because of the fact that terpenes are metabolised by the cow. Sensory evaluation results in this trial also indicated that when high levels of terpenes were measured in the milk, the milk had more of a fresh and less stale flavour. Larsen et al. (2013) suggests that when moderate amounts of herbs are used animal feeds, the flavour of the milk may be improved.

Frost et al. (2001) performed a trial, investigating the effect of various factors and combinations on the sensory properties and the perceived fattiness of milk and compared them to the actual fat content (0.1%, 1.3%, 3.5% fat) milk used. The results from this trial indicated that there were larger sensory differences between the milk with 0.1% fat and 1.3% fat, than 1.3% and 3.5% fat leading to the assumption that fat does not affect the sensory properties of milk in a linear fashion (Frost et al., 2001).

Lacerda et al. (2014) investigated the effect of including different levels of dry oregano in the feed of dairy cows on the quality of the milk as well as the sensory characteristics of pasteurized milk. This trial results indicated that the diet did not have an effect on the sensory characteristics of the milk and Lacerda et al. (2014) suggested that oregano could be supplemented without causing perceptible sensory changes, noticeable to consumers.

The aim of the present study was to investigate how the inclusion of the essential oil, *Oreganum vulgare* L., into the current diet of dairy cows, will affect the sensory properties (aroma and flavour) of milk. After a two month feeding period, with the inclusion of oregano oil, raw milk samples were collected, bottled and sent to the Department of Food Science's laboratory (Stellenbosch University) for sensory evaluation. The evaluation was done by a panel of trained assessors by means of a triangle test. Evaluating foods by using simple consumer orientated descriptive terms, provides more applicable and sensitive data for product development and quality assessment, opposed to traditional tests which focus only on the defects of products (Claassen et al., 1992).

2.6 Conclusion

Ionophores are widely used in ruminant diets, due to positive effects on feed efficiency, nitrogen and energy utilization. However, the rising concern about the use of antibiotics for animal production have caused some producers to look towards alternative feed additives. The growth in the use of essential oils will possibly be driven by the public's desire for food produced with natural methods. Only a limited amount of data with controlled studies with dairy cows are available to recommend which oil or combination of oils will work best with different diets. The actions and mechanisms of these oils are not yet fully understood nor is it known whether the wrong or inappropriate use of essential oils could affect the results on milk production or composition. The effects of essential oils seems to be diet and pH dependent with certain oils working better with a particular diet. Most studies done with essential oils have only been carried out in-vitro in a controlled lab setting. Every new opportunity for improving production, efficiency, sustainability or profitability deserves consideration. Essential oils appear to have the potential to replace ionophores and antibiotics in ruminant nutrition, but much research is still needed before specific recommendations can be made for specific conditions. Oregano seems to have several favourable properties and many of these properties may contribute to better milk yields and milk quality. However, the effects of feeding oregano to dairy cows, have not yet been discovered completely and that opens the door to many research opportunities in the future to better understand the effects that natural herbs such as oregano may have on dairy cow production.

2.7 References

Arcila-Lozano, C. C., Loarca-Piña, G., Lecona-Uribe, S. & González de Mejía, E., 2004. Oregano: properties, composition and biological activity. Arch. Latinoam. Nutr. 54: 100-110.

Ball, P.J., 2008. Using Petrifilm in Student Microbiology Studies [Online]. Available: http://file.southernbiological.com/Assets/WorkshopNotes/WorkshopNotes_Petrifilm.pdf [2015, May 12].

Benchaar, C., Calsamiglia, S., Chaves, A., Fraser, G., Colombatto, D., McAllister, T. & Beauchemin, K., 2008. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol. 145: 209-228.

Benchaar, C., Petit, H. V., Berthiaume, R., Whyte, T. D. & Chouinard, P. Y., 2006. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production, and milk composition in dairy cows. J. Dairy Sci. 89: 4352-4364.

Bravo, D., Utterback, P. & Parsons, C. M., 2011. Evolution of a mixture of cavacrol, cinnemalddehyde, and capsicum oleoresin for improving growth performance and metabolizable energy in broiler chick fed corn and soybean meal. J. Appl. Poult. Res. 20: 115-120.

Busquet, M., Calsamiglia, S., Ferret, A. & Kamel, C., 2006. Plant extracts affect in vitro rumen microbial fermentation. J. Dairy Sci. 89: 761-771.

Calsamiglia, S., Busquet, M., Cardozo, P. W., Castillejos L. & Ferret, A., 2007. Invited review: Essential oils as modifiers of rumen microbial fermentation. J. Dairy Sci. 90: 2580-2595.

Castillejos, L.S., Calsamiglia, S., Ferret, A. & Losa, R., 2006. Effects of a specific blend of essential oil compounds and the type of diet on rumen microbial fermentation and nutrient flow from a continuous culture system. Anim. Feed Sci. Technol. 119:29-41.

Chilliard, Y., Ferley, A. & Doreau, M., 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livest. Prod. Sci. 70: 31-48.

Claassen, M. & Lawless, H., 1992. Comparison of Descriptive Terminology Systems for the Sensory Evaluation of Fluid Milk. J. Food Sci. 57: 596-600.

Cordozo, P. W., Calsamiglia, S., Ferret, A. & Kamel, C., 2004. Effects of natural plant extracts on protein degradation and fermentation profiles in continuous culture. J. Anim. Sci. 82: 3230-3236.

Davidson, P. M. & Naidu, A. S., 2000. Phyto-phenols. Pages 265-293 in Natural Food Antimicrobial Systems. Naidu, A. S., ed. CRC Press, Boca Raton, FL.

De Falco, E., Mancini, E., Roscigno, G., Mignola, E., Taglialatela-Scafati, O. & Senatore, F., 2013. Chemical Composition and Biological Activity of Essential Oils of *Origanum vulgare* L. *subsp. vulgare* L. under Different Growth Conditions. Molecules 18: 14948-14960.

De Oliveira, J. S. O., de Silva, F. F., Costa, L. T., da Silva, G. M., de Almeida Meneses, M. & de Oliveira Rodrigues, E.S., 2014. Economic performance of dairy cows fed diets with different levels of oregano. Acta. Sci. Anim. Sci. 36: 393-398.

De Sousa, G.B., Tamagnini, L.M., Gonzalez, R.D. & Budde, C.E., 2005. Evaluation of Petrifim™ method for enumerating aerobic bacteria in Crottin goat's cheese. J. Apple. Microbiol. 37: 214-216.

Evans, J. D. & Martin, S. A., 2000. Effects of thymol on ruminal microorganisms. Curr. Microbiol. 41: 336-340.

Frost, M.B., Dijksterhuis, G. & Martens, M., 2001. Sensory perception of fat in milk. Food Quality and Preference 12: 327-336.

Harrigan, W.F., 1998. Laboratory methods in food microbiology. Great Britain: WBC book manufactures.

Hristov, A. N., Lee, C., Cassidy, T., Heyler, K., Tekkippe, J. A., Varga, G. A., Corl, B. & Brandt, R.C., 2013. Effect of *Origanum vulgare* L. leaves on the rumen fermentation, production and milk fatty acid composition in lactating dairy cows. J. Dairy Sci. 96: 1189-12012.

Ipharraguerre, I. R. & Clark, J. H., 2003. Usefulness of ionophores for lactating dairy cows: A review. Anim. Feed Sci. Technol. 106: 39-57.

Karnezoz, P., 2010. How essential oils can fit into U.S. Dairy Rations. Nutritional plus, July: 7.

Kemp, S.E., Hollowood, T. & Hort, J., 2009. Sensory Evaluation A Practical Handbook. United Kingdom: Wiley-Blackwell publishing.

Lacerda, E.C.Q., Bauer, L.C., Oliveira, J.S., Silva, F.F., Carvalho, S.A., Macedo, M.S., de Souza, N.E. & Simionato, J.I., 2014. Effect of the dietary inclusion of dried oregano (*Origanum vulgare* L.) on the characteristics of milk from Holstein x Zebu cows. J. Anim. Sci.192: 101-105.

Larsen, M. K., Lejonklev, J., Petersen, M.A., Mortensen, G., Kidmose, U. & Weisbjerg, M.R., 2013. Transfer of essential oil volatiles from feed to milk. Submitted for publication in proceedings of the 10th wartburg symposium.

Lawless, H.T. & Heymann, H., 1998. Sensory Evaluation of Food: Principles and Practices. New York. Springer Science.

Lazar, C., Rotar, A.M., Apostu, S. & Buzgau, G., 2010. Comparitive Studies Concerning Microbial Quality of Ecological and Conventional Milk. UASVM. 67: 266-272.

Lee, K. W., Everts, H., Kappert, H. J., Yeom, K. H. & Beynen, A. C., 2003.Dietry cavacrol lowers body weight gain but improves feed conversion in female broiler chickens. J. Appl. Poult. Res. 12: 394-399.

Lock, A. L. & Van Amburgh, M. E., 2012. Feeding for Milk Components. In WCDS Advances in Dairy Technology 24: 265-277.

McGuffey, R. K., Richardson, L. F. & Wilkinson, J. I. D., 2001. Ionophores for dairy cattle: Current status and future outlook. J. Dairy Sci. 84: 194-203.

Muhl, A. & Liebert, F., 2007. Growth and parameters of microflora in intestinal and faecal samples of piglets due to the application of a phytogenic feed additive. J. Anim. Physiol. Anim. Nutr. (Berl.) 91: 411-418.

Nogueira, P., 2009. Essential oils in Dairy Cow diets. Dairy Briefs, September: 8.

Paulson, J., 2008. Essential oils as dietary supplements for dairy cattle. Dairy Star. 2 August: 2

Petersen, M.B., Soegaard, K. & Jensen, S.K., 2011. Herb feeding increases n – 3 and n – 6 fatty acids in cow milk. Livestock Sci. 141: 90-94.

Relling, A. E. & Reynolds, C. K., 2007. Feeding rumen-inert fats differing in their degree of saturation decreases intake and increases plasma concentrations of gut peptides in lactating dairy cows. J. Dairy Sci. 90: 1506-1515.

Ropapharm International, Ropadairy for dairy cows, viewed 9 July 2015, http://www.ropapharm.com/upload/pdf/Booklet_Ropapharm_Ropadairy.pdf

Silva, B.O., Caraviello, D.Z., Rodrigues, A.C. & Ruegg, P.L., 2005. Evaluation of Petrifilm for the Isolation of Staphylococcus aureus from Milk Samples. J. Dairy Sci. 88: 300-306.

Simon, J. E., 1990. Essential oils and culinary herbs. Pages 472-483 in Advances in New Crops. J. Janick & J. E. Simon, ed Timber Press, Portland, OR.

Spanghero, M., Robinson, P. H., Zanfi, C. & Fabbro, E., 2009. Effect of increasing doses of a microencapsulated blend of essential oils on performance of lactating primiparous dairy cows. Anim. Feed Sci. Technol. 153: 153-157.

Spanghero, M., Zanfi, C., Fabbro, E., Scicutella, N. & Camellini, C., 2008. Effects of a blend of essential oils on some end products of in vitro rumen fermentation. Anim. Feed Sci. Technol. 145: 364-374.

Tassoul, M. D. & Shaver, R. D., 2009. Effect of a mixture of supplemental dietary plant essential oils on performance of periparturient and early lactation dairy cows. J. Dairy Sci. 92: 1734-1740.

Teixeira, B., Marques, A., Ramos, C., Serrano, C., Matos, O., Neng, N.R., Nogueira, J.M., Saraiva, J.A. & Nunes, M.L., 2013. Chemical composition and bioactivity of different oregano (*Origanum vulgare*) extracts and essential oil. J. Food Sci. Agric. 93: 2707-2714.

Tekippe, J. A., Hristov, A. N., Heyler, K. S., Cassidy, T. W., Zheljazkov, V. D., Ferreira, J. F. S., Karnati, S. K. & Varga G. A., 2011. Rumen fermentation and production effects of *Origanum vulgare* L. leaves in lactating dairy cattle. J. Dairy Sci. 94: 5065-5079.

Toncer, O., Karaman, S., Kizil, S. & Diraz, E., 2009. Changes in Essential Oil Composition of Oregano (*Origanum onites* L.) due to Diurnal Variations at Different Development Stages. Not. Bot. Hort. Agrobot. Cluj. 37: 177-181.

Varga, G. A. & Ishler, V. A., 2010. Managing Nutrition for Optimal Milk Components [Online]. Available: http://www.extention.org/pages/11300/managing-nutrition-for-optimal-milk-components [2015, May 19].

Viallon, C., Martin, B., Verdier-Metz, I., Pradel, P., Garel, J. P., Coulon, J. B. & Berdague, J. L., 2000. Transfer of mono terpenes and sesquiterpenes from forages into milk fat. Lait 80: 635-641.

Yang, W. Z., Benchaar, C., Ametaj, B. N., Chaves, A. V., He, M. L. & McAllister, T. A., 2007. Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. J. Dairy Sci. 90: 5671-5681.

CHAPTER 3

Materials and Methods of Milk Production study

The production trial was conducted over a 2 month period, from 5 August 2014 to 7 October 2014. Ethical clearance was approved (SU-ACUD15-00071) by the Research Ethics Committee: Animal Care and Use.

3.1 Animals and Housing

The animals involved in this trial were healthy lactating Holstein dairy cows, held on the Welgevallen experimental farm in Stellenbosch (see Appendix B). These cows had no specific problems of feed intake, lameness or somatic cell counts. In total, forty high producing cows were used in this trial. The dairy cows were housed in a free-stall barn, in the same way that they are housed normally and shown in Figure 3.1 below.

Figure 3.1 Free-stall barn housing two groups consisting of twenty cows each.

The only difference was, for the duration of the trial, the barn was divided in two by using gates to keep the two experimental groups apart as shown in Figure 3.2 below.

Figure 3.2 Housing allowed for groups to be separated by two gates for the duration of the trial.

Each group had similar and unlimited access to feed, clean water and sand sleeping beds. As shown in Figure 3.3, the cemented walking areas between the feed troughs and sleeping beds, were cleaned thoroughly twice daily by a water flushing system. Sleeping beds were cleaned twice daily and the sand was replaced weekly. The cows are part of the normal Maties Milk dairy and were managed and monitored with, and in the same way, as the rest of the herd.

Figure 3.3 Sleeping- and walking areas were cleaned twice daily with replacement of bedding weekly.

3.2 Diets and Treatments

The cows were allocated to treatments using a randomized block design in order to eliminate variation and to allow for valid comparisons to be made. The forty cows were allocated in two groups (see Appendix B). Twenty cows for a Control group and twenty cows for the Treatment group. In order to avoid accidental mixing of the groups, the control group were marked with a single strip of green insulation tape, wrapped loosely around the narrow part of their tails and the treatment group were marked in the same manner but with red tape. The cows were randomly allocated to groups, according to: days in milk, daily milk production, milk protein/fat content and lactation number, so that each group had equal distribution of cows with above characteristics.

All the cows received a basal diet (no pasture) that consisted of Lucerne hay (provided *ad libitum*) and a pelleted semi-complete feed. Six bales of lucerne hay (average weight of 26 kg/bale) were offered for *ad lib.* access and the semi-complete pellets were provided at 560 kg/group per day. Cows were fed twice daily at 7:30 and 16:00. Treatments (discussed later) differed in terms of a supplement that either contained an oregano oil extract (DOS) or a placebo (CON).

The cows were allocated to their groups a week prior to the start of the trial and were made accustomed to receiving the supplement, by feeding 6 kg (300 g x 20 cows) of the supplement to each group in addition to their concentrate feed and roughage in their feeding troughs as shown in Figure 3.4 a and b.

Figure 3.4 a

Figure 3.4 b

Figure 3.4 a and b Adaptive feeding of supplement with concentrate and roughage in housing feeding troughs.

Once the trial started, each cow in the DOS treatment received 1 g of Dosto Concentrate 500 (oregano oil extract) per day. This was the recommended dose given by the supplier of the Dosto Concentrate 500 product. The supplements were mixed by Afgri Feeds and were fed in the milking parlour. The DOS supplement was mixed such that 300 g of the supplement would deliver 0.5 g of Dosto 500 per milking, thus a total of 1 g per day (600 g of supplement). Cows were milked twice daily, at 6:00 and at 15:30. The control cows received 600 g of the CON supplement per day. Bags that contained 300 g of supplement each were weighed individually and distributed by hand in the milking parlour feeding troughs to each cow, where ten cows could be milked at a time. The CON group were milked first to avoid possible carry-over of Dosto 500. After each group of ten cows had been milked, the refusals of the supplements (if any) were collected in separate bags, to be weighed. The refusals were fed to the cows again with their normal diet where they were housed, mainly distributing to cows whom did not consume much in the parlour. In this way it was ensured that each cow consumed the required daily amount. The feeding troughs were cleaned thoroughly after each milking session.

3.3 Data Collection

Milk production was automatically recorded with the aid of the Afikim system in the dairy. By using this system the following could be measured: Daily milk production per cow at each milking session. Milk protein, fat and lactose contents, as well as somatic cell counts, were also recorded daily at each milking via the AfiLab system. Furthermore milk samples were taken monthly and sent to the Elsenburg Dairy Laboratory for the same analyses and also for milk urea nitrogen which is not determined with the AfiLab system. A sensory evaluation was also done (see Chapter 5) at the end of the trial to evaluate the sensory characteristics of the milk.

To calculate the energy corrected milk (ECM) yield the following equation was used:

$$\text{ECM (kg/d)} = (0.3246 \text{ x milk yield}) + (12.86 \text{ x milk fat yield}) + (7.04 \text{ x milk protein yield})$$

Feed intake (distribution and refusals) was recorded weekly, collectively per group. The intake of the supplement was recorded after every feeding session in the milking parlour. Any sanitary events and treatments were registered. Body weight after milking, as determined with the AfiWeigh system, and body condition score (BCS), as estimated by dairy manager, were recorded at the beginning and at the end of the trial. Body condition score was determined on a five point scale (NRC, 2001) where 1 = severely underweight (emaciated) and 5 = severely overweight.

3.4 Chemical Analyses

3.4.1 Feed concentrate, supplements and roughage

Moisture and Dry Matter (DM)

AOAC (2002) Official Method 934.041: Determination of moisture contents of feed.

The moisture and DM content of the feed samples were determined by drying the sample above 100 ˚C for 24 h and measuring the water loss.

Procedure:

Clean labelled porcelain crucibles were dried for at least two hours in a drying oven at 100 ˚C. The crucibles were then placed in a desiccator and allowed to cool for 30 min. The weight of each crucible was then recorded accurately with a four decimal scale. With the crucible on the scale, the scale was zeroed and 2 g of the sample weighed into the crucible and the weight recorded to the fourth decimal. The weighing procedure was repeated for all 14 feed samples. The crucibles containing the feed samples were placed in the oven at 100 ˚C and dried for 24 hours. The crucibles were transferred to a desiccator and allowed to cool down for at least 30 min, after which the weight of the dried samples were recorded.

% Moisture = [(dry crucible mass + sample mass – mass of dry sample in crucible) / (sample mass)] x 100
DM = 100 – % Moisture

Ash

AOAC (2002) Official Method 942.05: Ash.

Ash is determined by burning the sample in a furnace to remove any organic matter. The residue is inorganic matter and represents the total mineral content, but it does not give information on specific minerals.

Procedure:

The same procedure was used as to determine moisture content. The crucibles containing the dried feed samples were placed in a temperature controlled furnace at 500 ˚C for six hours. After six hours, the furnace was switched off to cool down for at least two hours. The crucibles were transferred to a desiccator for 30 min to cool down to room temperature and was then weighed and recorded to the fourth decimal.

% Ash = [(mass of crucible and ash – mass of empty dry crucible) / sample mass] x 100
%Organic matter = 100 – % Ash

Crude Fibre (CF)

AOAC (2002) Method 962.09: Crude Fibre in animal feed.

Crude Fibre is measured by boiling the sample in acid and then in alkali. The residue represents the less readily digestible carbohydrates, mostly cellulose and some lignin.

Reagents:

0.128 M H_2SO_4 (sulphuric acid) = 6.96 ml 98% H_2SO_4 made up to 1 L distilled H_2O
0.313 M NaOH (nitrogen hydroxide) = 12.5 g NaOH made up to 1 L distilled H_2O

Procedure:

1. Oven dried glass crucibles were placed on the scale, the scale was zeroed and approximately 1 g of the feed sample was weighed into each crucible.

2. The crucibles were then placed into the extraction unit (Fibretec/Dosifibre apparatus).

3. By flushing the tubes with H_2O, any leakages could be detected.

4. The valves were then closed and the water taps were opened to allow cooling.

5. 150 ml H_2SO_4 solution was poured into each tube.

6. The protective plate was placed in front of the heating unit and the temperature was set to 100 ˚C.

7. The solution was brought to boiling point and the temperature was reduced to 65 ˚C, so that the samples could cook slightly for 30 min.

8. The heat was then turned off and the solution was filtered by using the vacuum setting.

9. The samples were then washed off three times with boiling distilled water.

10. All the valves were then closed and 150 ml NaOH solution was poured into each tube.

11. The temperature was set at 100 ˚C just until the boiling point was reached and the temperature was then reduced to 65 ˚C for 30 min.

12. The heat was then turned off and the crucibles were filtered.

13. The samples were flushed three times with boiling distilled water.

14. The crucibles were removed and placed into the drying oven over night.

15. The crucibles were placed in a desiccator for 30 min to cool down before being weighed.

16. The crucibles were placed in the temperature controlled furnace at 500 ˚C for six hours.

17. The furnace was turned off and left to cool for two hours. When cooled the crucibles were placed in a desiccator for 30 min. The crucibles were then weighed individually.

% Crude Fibre = [(Weight of crucible and sample after drying – weight of crucible and sample after ashing) / sample mass] x 100

Crude Protein (CP)

AOAC (2002) Method 990.03: Crude Protein in animal feed.

Using the LECO FP 528 machine (Dumas method) the total nitrogen content in the feed can be measured. The CP value contains both true protein (which contains amino acids) and non-protein nitrogen compounds.

Procedure:

The LECO FP 528 was always left switched on and the oven was set at 850 ˚C.

1. Blanks (gas) were analysed until a plateau was reached. Three to six blanks were analysed to set the blank standard.

2. When samples have a protein content less than 20%, Alfalfa is used as the calibration standard. Three alfalfa standards, weighed into tin foil cups at 0.10 g each, were analysed. The % nitrogen for alfalfa should fall in the range of 3.96 to 4.04.

3. By using small tweezers, an empty tin foil cup was placed on the scale and zeroed. The feed sample was then weighed to approximately 0.1000 g (no less) in the tin foil cup. Weights and sample codes were then typed into the computer software program. The foil cup was closed and folded over into a small teardrop shape and then placed into the carousel sample tray, at the corresponding number for the recorded weight. Once the first sample was placed in carousel, the analysis process could begin and the sample was combusted and within 3 min the nitrogen percentage was obtained.

% Crude Protein = % N x 6.25

Crude Fat: Ether-Extract (EE)

AOAC (2002) Method 920.39: Crude Fat in animal feed.

Ether extract is determined by treating the sample with the sample ether in order to remove any lipid compounds.

Reagents: Diethyl ether

Procedure:

1. Oven dried aluminium beakers were weighed and the weights were recorded.

2. Extraction thimbles were placed on the scale and the scale was zeroed. Approximately 2 g samples were weighed into the thimbles.

3. A small piece of cotton wool was placed on top of the feed sample in the thimble to avoid any loss of the sample.

4. Each beaker was filled with 50 ml diethyl ether.

5. The water taps were turned on before switching on the heating element.

6. The oil bath was switched on as well as the extraction fan.

7. Once the oil bath's ready light went on, the thimbles were placed into the extraction tubes with the corresponding beakers placed underneath.

8. The extraction tubes were lowered and it was important to ensure that the tubes were fitted securely to the beakers.

9. The thimbles were lowered into the ether, the taps were turned to the boiling function, and the samples were left to cook for 15 min.

10. The thimbles were raised for 30 min, while on the rinsing cycle.

11. The taps were closed in order for the ether to be collected. Samples were allowed to boil for 15 min.

12. The beakers were removed and placed in a drying oven for 30 min, so that all the ether could evaporate.

13. The beakers were transferred to a desiccator to cool for 30 min.

14. The beakers were then weighed.

Crude fat or Ether Extract % = [(mass of beaker and fat – mass of beaker) / mass of sample] x 100

Crude Fat: Acid Hydrolysis

AOAC (2002) Method 954.02: Crude Fat in animal feed.

Crude fat is determined by acid hydrolysis with Hydrochloric acid (HCl), followed by the extraction of hydrolysed lipid materials with mixed ethers. The ether is then evaporated and the residue is heated to a constant weight and expressed as % crude fat.

Reagents:
Diethyl ether
Petroleum ether 40 – 60 ˚C
Ethanol
HCl 38%

Procedure:

1. Clean glass cups were placed in the drying oven at 100 ˚C overnight.

2. The cups were placed in the desiccator for 30 min to allow for cooling and were then weighed.

3. About 2 g of the feed samples were weighed accurately to 0.001 g and transferred to test tubes.

4. 2 ml Ethanol was added to each sample in the test tubes.

5. 10 ml HCl was then added to each test tube.

6. The test tubes were placed into a water bath to boil for 30 min.

7. The test tubes were taken out of the water bath and left to cool for about 30 min until room temperature was reached.

8. The boiled samples were poured into separating funnels and rinsed with 10 ml ethanol.

9. A volume of 25 ml of diethyl ether was added to each funnel, and each funnel was twirled for 1 min. A volume of 25 ml of petroleum ether was then added to each funnel and once again each funnel was twirled for 1 min. The upper portion in of fat residue in each funnels was then poured into the fat cups and the cups were placed on a sand bath so that the ether could evaporate.

10. A volume of 15 ml of diethyl ether was added to each funnel and each funnel was shaken for 1 min. Again 25 ml of petroleum ether was added to each funnel and each funnel was shaken for 1 min. The upper portion of fat residue in each funnel was then carefully decanted poured into the fat cups and the cups were placed on a sand bath so that the ether could evaporate.

11. Once again a volume of 15 ml of diethyl ether was added to each funnel and each funnel was shaken for 1 min. Again 25 ml of petroleum ether was added to each funnel and each funnel was shaken for 1 min. The upper portion of fat residue in each funnel was then carefully decanted poured into the fat cups and the cups were placed on a sand bath so that the ether could evaporate.

12. The cups were transferred to the desiccator to cool for about 30 min.

13. The cups were weighed accurately

Fat % = [(mass of fat cup plus fat – mass of fat cup) / mass of sample] x 100

NDF: Neutral detergent fibre

ANKOM Technology 11/14 method 6.

NDF is determined by boiling the feed sample in a detergent solution with a pH of 7.0. The soluble portion (sugars, starch, pectins, lipids, soluble carbohydrate and protein is removed and the insoluble NDF fraction remains. The NDF contains cellulose, hemicellulose, lignin, silica and any heat damaged proteins. The NDF also provide information that can be used to estimate the potential intake of forage. Forages with a high NDF content can be considered to be lower in quality. When the NDF content of forages is high, it may result in lower intake and vice versa.

Reagents:
Neutral detergent solution: (for 1 L)

- To 200 ml distilled H_2O add 18.61g EDTA ($Na_2EDTA.2\ H_2O$) and 6.81 g sodium borate decahydrate ($Na_2B_4O_7$ - 10 H_2O). Heat and stir until dissolved.
- To 100 ml distilled H_2O add 4.56 g of disodium phosphate anhydrous (Na_2HPO_4). Heat and stir until dissolved.
- To 500 ml distilled H_2O add 30 g Sodium lauryl sulphate. Stir to dissolve then add 10 ml of triethylene glycol.
- Add EDTA-borate and phosphate solutions to Na Sodium sulphate solution. Stir well and adjust volume if needed.

Procedure:

1. Oven dried F57 filter bags (W_1) were hot weighed.

2. The scale was zeroed and 0.5 g of the sample (W_2) was weighed directly into the bag.

3. Blank filter bags were weighed and sealed so that a blank bag correction (C_1) could be made.

4. All the bags were sealed 4 mm from the edge using a heat sealer.

5. The bags were placed on the bag suspender trays (3 bags per tray) and the suspender was lowered into the fibre analyser vessel. The bag suspender weight was placed on top to keep it submerged.

6. An amount of 0.5 g (±0.1) of anhydrous sodium sulphite (Na_2SO_3) was added to the vessel.

7. A minimum of 1500 ml ND solution was poured into the vessel, the lid was sealed and the agitator and heat were turned on. The solution was left to boil for 60 min.

8. The heat and agitator were turned off and the draining valves were opened slowly and carefully to drain the solution.

9. The valves were closed and then 2000 ml of boiling water was poured into the vessel.

10. The agitator was turned on and the samples were allowed to rinse for 5 min.

11. The rinsing procedure was repeated two more times. After rinsing, the samples were taken out of the vessel and pressed lightly to remove excess water.

12. The samples were then placed into the drying oven for 24 hours. Samples were then hot weighed (W_3).

NDF % (as received basis) = ((W3 – (W1 x C1)) x 100) / W2

Minerals

ALASA, 1998.

Phosphorous (P), potassium (K), calcium (Ca) and magnesium (Mg) were analysed for by the Institute for Plant Sciences at Elsenburg.

3.4.2 Additional analyses on roughage

ADF: Acid detergent fibre

ANKOM Technology 8/06 Method 5

The ADF content is determined by boiling the feed sample in an acid detergent solution. The soluble portion is removed and the insoluble ADF fraction remains. The ADF contains cellulose and lignin. The ADF is used to get an estimation of the digestibility and energy value of the forage. High levels of ADF means that the forage is less digestible and would have a lower energy value.

Reagents:

Acid detergent solution: Add 20 g cetyl trimethylammonium bromide (CTAB) to 1 L 1.00 N H_2SO_4.

Procedure:

1. Oven dried Ankom F57 filter bags (W_1) were hot weighed.

2. The scale was zeroed and 0.5 g of the sample (W_2) was weighed directly into the bag.

3. Blank filter bags were weighed and sealed so that a blank bag correction (C_1) could be made.

4. All the bags were sealed 4 mm from the edge using a heat sealer.

5. The bags were placed on the bag suspender trays (3 bags per tray), and the suspender was inserted into the fibre analyser vessel. The bag suspender weight was placed on top to keep it submerged.

6. A minimum of 1500 ml of the AD solution was poured into vessel. The bag suspender must be covered by the solution.

7. The lid was sealed and the agitator and heat were turned on. The solution was left to boil for 60 min.

8. The heat and agitator were turned off and the draining valves were opened slowly and carefully to drain the solution.

9. The valves were closed and then 2000 ml of boiling water was poured into the vessel.

10. The agitator was turned on and the samples were allowed to rinse for 5 min.

11. The rinsing procedure was repeated two more times. After rinsing, the samples were taken out of the vessel and pressed lightly to remove excess water.

12. The samples were then placed into the drying oven for 24 hours. Samples were then hot weighed (W_3).

ADF % (as received basis) = $((W_3 – (W_1 \times C_1)) \times 100) / W_2$

3.5 Statistical Analyses

Data collected over time were subjected to a repeated measurements ANOVA, while mean values were analysed according to a main effects ANOVA with treatment and block as main effects. Data collected from this trial period were analysed using the program Statistica 64 version 12. Significance was declared at $P < 0.05$. Production results from all the cows, as well as production results from the top ten producing cows from each group were statistically analysed.

3.6 References

ALASA, 1998. Handbook of feeds and plant analysis. Method 6.1.1 – Dry Ashing. Palic, D. (Ed).

AOAC International, 2002. Official methods of analysis (17th edition), Association of Official Analytical Chemists Inc. Arlington, Virginia, USA.

CHAPTER 4

ABSTRACT

The effect of an oregano oil extract on milk production parameters in Holstein cows

Forty Holstein cows, 178 ± 17 (SE) DIM and weighing 624 ± 9 (SE) kg, were used in a lactation trial of 60 days to determine the effect of oregano essential oil on milk production, milk composition and feed intake. The cows were ranked according to milk yield, DIM and lactation number and each consecutive pair formed a block. Treatments were allocated randomly to each of the 20 blocks. An essential oil product (Dosto Concentrate 500), (DOS) was evaluated against a placebo control (CON) treatment. Cows were housed in a semi-open free-stall barn with sand beds and had free access to fresh water. All cows received a basic diet consisting of lucerne hay (53% NDF and 11% CP) that was offered *ad libitum* and 28 kg/day of a semi-complete lactation feed, offered twice daily at 07:30 and 16:00. The feed refusals were weighed back weekly to determine intake per group. Treatments (DOS and CON) only differed in terms of a maize based supplement. In the case of the DOS treatment, each 300 g maize supplement portion contained 0.5 g of Dosto Concentrate 500. The cows were milked twice daily at 06:00 and 15:30 and the supplements were offered individually to cows in the milking parlour during each milking. Milk yield, milk composition and cow weights were recorded automatically on a daily basis via the Afikim system. Milk samples were also collected during weeks 3 and 8 for composition analysis at the Elsenburg Dairy Laboratory. Data collected over time were subjected to a repeated measurements ANOVA, while mean values were analysed according to a main effects ANOVA with treatment and block as main effects. All data were analysed with the aid of Statistica 64 version 12 and significance was declared at $P < 0.05$. Treatment had no effect on milk yield or milk composition over the entire period. However, in the CON treatment, the lactose content was higher ($P < 0.05$) during the first two weeks and the milk protein content was higher ($P < 0.05$) during weeks four to eight. When data of the top ten milk producing cows per treatment were analysed separately, the fat content and milk fat yield were higher ($P < 0.05$) for the DOS treatment during the first three weeks of the trial and lactose was higher ($P < 0.05$) for the CON treatment in the first week. Mean milk yield of the top ten milk producing cows per treatment did not differ between treatments and was 37.9 kg for the DOS treatment and 37.3 kg for the CON treatment. Mean fat content and fat yield was higher ($P < 0.05$) in the DOS treatment (37.1 g/kg and 1.41 kg/day) than in the CON treatment (33.8 g/kg and 1.26 kg/day). The higher fat content also resulted in a higher ($P < 0.05$) energy corrected milk yield of cows in the DOS treatment than in the CON treatment (38.8 and 36.6 kg/day, respectively). With regards to feed intake, the CON group consumed on average 17.3 kg more roughage per week than the DOS group. It was concluded that oregano essential oil stimulated fat production and increased energy corrected milk yield in high milk producing dairy cows.

Key words: feed additives, ionophores, essential oils, milk components.

4.1 Introduction

The use of ionophores, such as monensin and lacalocid, are widely used to manipulate the rumen microbial population. This increases propionate production that results in increased milk production and aids in the prevention of ketosis. However, since ionophores are classified as antibiotics, they are banned in Europe and some other countries. There is also an increase in consumer resistance in the RSA against the use of antibiotics in animal feeds. There is thus a need to find natural alternatives to ionophores to be used in animal feeds. Essential oils, such as oregano oil, may present this alternative as it has been shown that they can positively affect microbial taxa to shift fermentation end products towards propionate and an increased efficiency of energy utilization. The aim of the present study was to investigate the effect of an essential oil extract from *Origanum vulgare* L. in the diet of lactating dairy cows on feed intake, milk yield and milk composition.

4.2 Materials and Methods

For detailed materials and methods for the production trial please refer back to Chapter 3.

Animals and Housing

Forty Holstein cows, 178 ± 17 (SE) DIM and weighing 624 ± 9 (SE) kg, were used in a lactation trial of 60 days. The cows were ranked according to milk yield, DIM and lactation number. Twenty cows were allocated per treatment group. Cows were housed in a semi-open free-stall barn with sand beds and had free access to fresh water. For the duration of the trial, the barn was divided in two by using gates to keep the two experimental groups apart.

Feeding and Treatments

The same standard diet fed offered twice daily at 07:30 and 16:00: Semi-complete lactation cubes at 560 kg/group per day and 6 lucerne bales/group per day. The control (CON) treatment was a maize based supplement without the addition of oregano oil extract (Dosto Concentrate 500). The Dosto (DOS) treatment was a maize based supplement with the addition of oregano oil extract (Dosto Concentrate 500), which was added at 0.5 g per 300 g of the maize based supplement. The dosage level was recommended by the supplier of the Dosto Concentrate 500 product. Cows received the respective supplements individually in two portions of 300 g in the milking parlour at 06:00 and 15:30.

Feed refusals

Feed refusals were weighed back weekly to determine intake per group. The treatment supplement refusals were collected for each cow directly after milking, weighed back individually. These refusals were fed to the

cows again with their normal diet where they were housed, mainly distributing to the cows that did not consume much in the parlour.

Data Collection and Statistical Analyses

Milk yield, milk composition and cow weights were recorded daily via the Afikim system. All data were analysed with the aid of Statistica 64 version 12 and significance was declared at $P < 0.05$. Data collected over time were subjected to a repeated measurements ANOVA. Average milk production and milk component values were analysed according to a main effects ANOVA with treatment and block as main effects. Because cows were housed in groups, individual feed intake could not be measured. Group feed intake was measured weekly, but not analysed statistically. Data were analysed of all the cows and then again of the top ten producing cows in each treatment group.

4.3 Results and Discussion

4.3.1 Chemical analyses of feedstuffs

Duplicate samples of each feed source (Lucerne hay, semi-complete feed and the supplements) were taken (twice) and analysed for chemical- and mineral composition. The results are presented in Table 4.1 and Table 4.2.

Table 4.1 Chemical composition of the semi-complete feed, treatment supplements and lucerne hay.

Item	Semi-Complete Cubes	Dosto Supplement[1]	Control supplement	Lucerne
DM, %	87.8	88.4	88.2	91.2
Fat, % (ether extract)	3.1	4.3	4.0	1.4
Fat, % (acid hydrolysis)	3.6	4.7	4.7	2.0
Moisture, %	12.2	11.6	11.8	8.8
Ash, %	6.0	4.1	4.8	6.7
Crude Fibre, %	11.7	5.2	4.9	42.0
Crude Protein, %	15.7	17.0	16.5	10.6
NDF[2], %	22.9	15.1	15.3	53.4
ADF[2], %	-	-	-	44.2

[1]Dosto 500: the commercial oregano essential oil product that was used in the trial.

[2]DM- Dry matter, NDF- Neutral detergent fibre, ADF- Acid detergent fibre, ADL- Acid detergent lignin.

Table 4.2 Mineral composition of the semi-complete feed, treatment supplements and lucerne hay.

Item	Semi-Complete Cubes	Dosto Supplement[1]	Control supplement	Lucerne
Phosphorous, %	0.365	0.445	0.440	0.200
Calcium, %	1.095	0.750	1.530	0.880
Magnesium, %	0.270	0.265	0.240	0.215
Potassium ,%	1.305	0.660	0.660	2.430

[1]Dosto 500: the commercial oregano essential oil product that was used in the trial.

No ingredient changes were made to the semi-complete feed and the lucerne bales fed to the cows, were purchased from the same supplier during the two month trial period. However, the quality of the lucerne from one batch to the other differed in terms of weight and leafiness. Feed quality can affect milk production, feed costs and animal health. To compensate for changes in quality, the amount of lucerne bales offered to the cows, was adjusted accordingly and where possible bales were specifically chosen for the trial cows. The chemical composition of the Dosto (DOS) supplement was generally similar to that of the control (CON) supplement. The only difference between the two supplements was that the Ca content of the CON supplement was twice as high as that of the DOS supplement.

Production responses will be discussed in two parts. Section 4.3.2 will cover the results of all 40 experimental cows, while the results of the top 10 cows in each treatment group will be discussed in Section 4.3.3.

4.3.2 Production response of all the trial cows

With reference to plant additives, the dose of the feed additive and the mode of action of the feed additive can influence milk production and milk composition (de Oliveira et al., 2014). However, non-nutritional factors, such as the stage of lactation, and the level of production can also influence the milk production and milk composition (Varga et al., 2010).

The milk production parameters and mean body weight of all the experimental cows are presented in Table 4.3. There was no difference in milk yield between the treatments. The milk components and yields for fat and protein, respectively, did not differ between treatments. Milk lactose content and energy corrected milk (ECM) yield also did not differ between treatments. Cows were housed in groups, therefore individual dry matter intake (DMI) could not be determined. Dry matter intake was recorded per treatment group weekly. At the beginning of the trial the control (CON) group already consumed more roughage than the Dosto (DOS) group, thus the intake results could not be statistically analysed. The mean DMI/group per week was 845 kg for the CON group and 824 kg for the DOS group. Over the entire trial period the CON group consumed on average 17.3 kg more roughage per week compared to the DOS group. With regards to the semi-complete lactation pellets, both the

CON and the DOS group consumed the entire feeding amount of 3920 kg concentrate per week (no refusals from either group).

Table 4.3 Mean milk production and composition and mean body weight of all the cows in the treatment- and control groups during the 9 weeks trial.

Item	Control supplement	Dosto supplement	SEM	*P* – value
Milk, kg/d	32.2	32.3	1.716	0.940
Milk fat, %	3.61	3.69	0.0922	0.529
Milk fat yield, kg/d	1.145	1.194	0.0531	0.515
Milk protein, %	3.25	3.19	0.0359	0.243
Milk protein yield, kg/d	1.039	1.026	0.0502	0.857
Milk lactose, %	4.76	4.72	0.0198	0.158
*ECM, kg/d	32.5	33.1	1.547	0.778
Body weight, kg	632	646	13.83	0.493

*ECM- Energy corrected milk

Milk Yield

Over time there was no interaction between the treatments (P = 0.834).There was no difference (P = 0.932) found for the main effects, thus the treatments did not differ from one another. Figure 4.1 presents the mean milk yield for both groups over time.

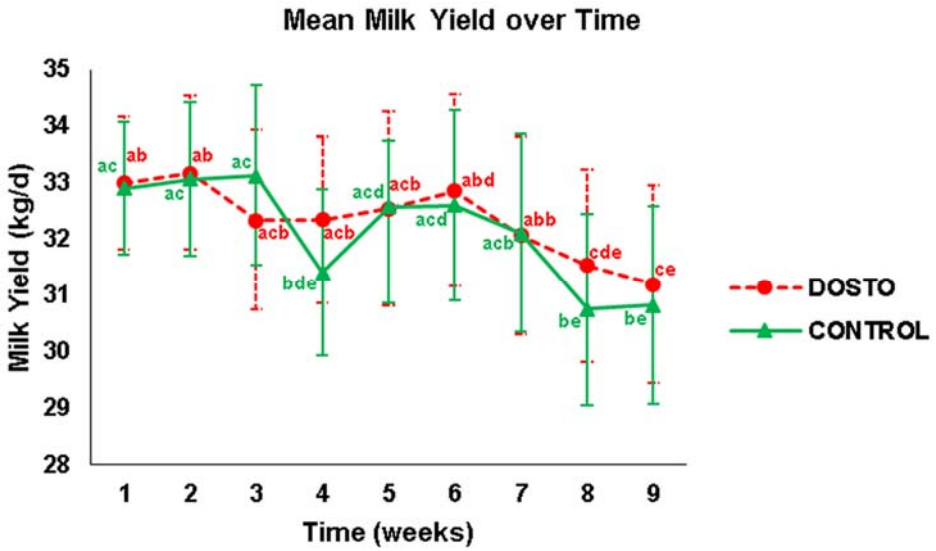


Figure 4.1 Mean milk yield of all the trial cows.

*Means with different superscripts differed (P < 0.05) between treatments and over time.

The milk yield remained fairly constant for the DOS group over the first seven weeks, but a decline in yield was observed during Weeks 8 and 9. In the CON group, milk yield declined during Weeks 4 and again during Weeks, 8 and 9. In both treatment groups, milk yield declined towards the end of the trial (Weeks 8 and 9) as lactation progressed. Results from this trial support the results found by several authors claiming that feeding oregano had no significant effect on milk production (Hristov et al., 2013; Tekippe et al., 2011).

Milk Fat Content and Milk Fat Yield

Interaction between treatments occurred (P = 0.019) over time. However no differences were found between the treatments. In contrast, Tekippe et al. (2011) found that, when feeding oregano, the milk fat content as well as the milk fat yield increased significantly in comparison to the control group. On the other hand Hristov et al. (2013) also reported no difference in milk fat content and milk fat yield between treatments. Results for milk fat content over time observed in the current study are shown in Figure 4.2.

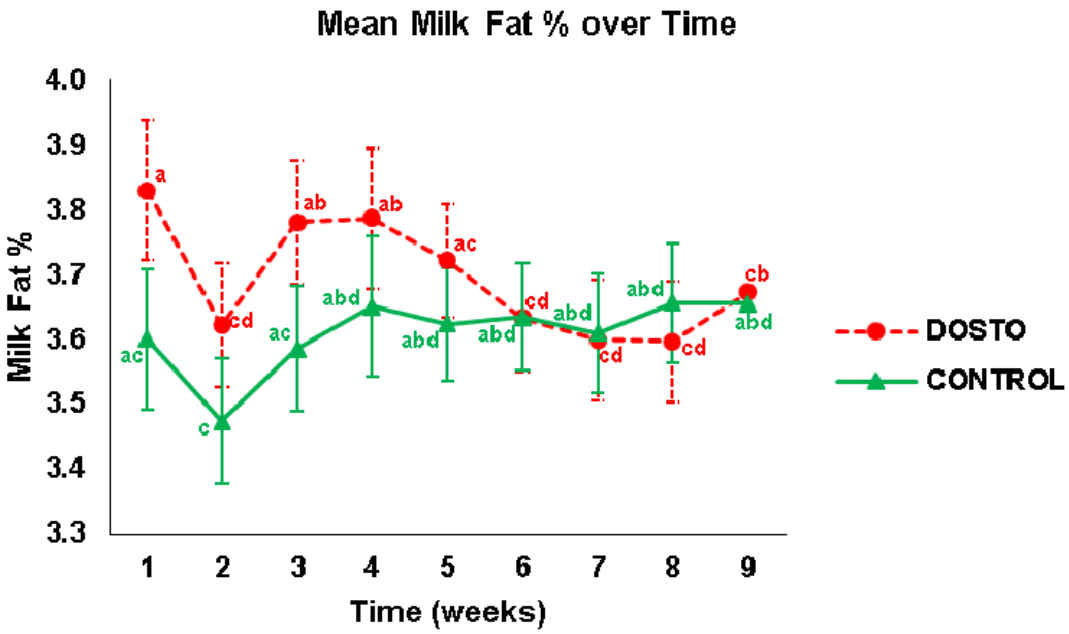


Figure 4.2 Mean milk fat content of all the trial cows.

*Means with different superscripts differed (P < 0.05) between treatments and over time.

The milk fat content of the DOS group decreased during Week 2, but recovered in the following week. From Weeks 6 to 8, a decrease in milk fat content was observed, followed by a slight increase in Week 9. Except for a sudden drop in milk fat content during Week 2 (as was also observed in the DOS treatment) the milk fat content of the CON cows did not change significantly over time.

Regarding milk fat yield over time, there was no interaction between the treatments (P = 0.084). There was no difference (P = 0.502) for the main effects, thus at no point in time, did the treatments differ from one another. The mean milk fat yield is presented in Figure 4.3.

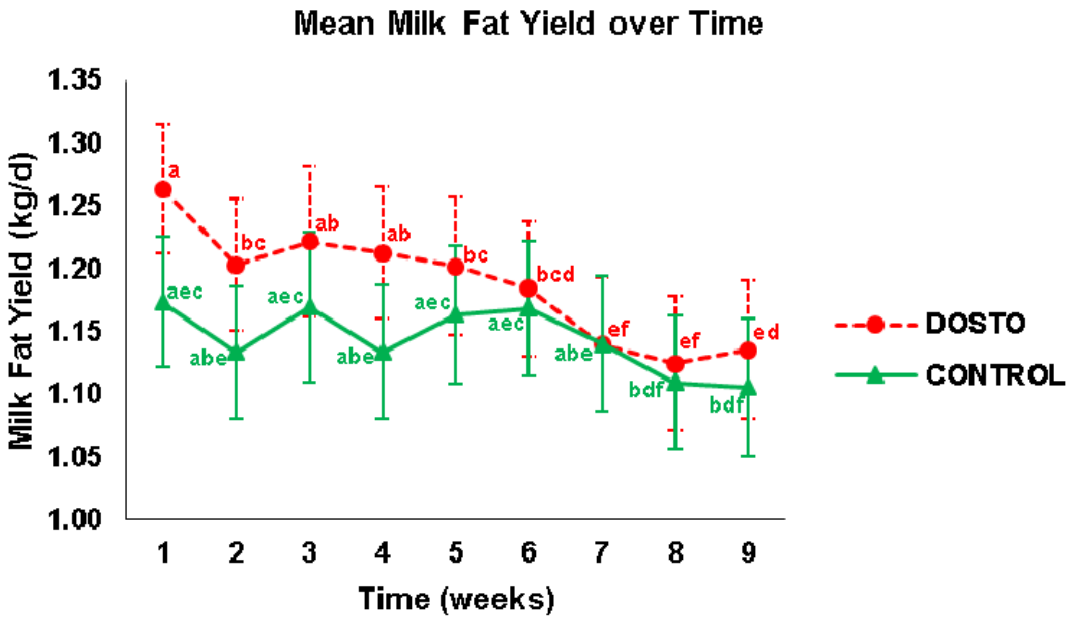


Figure 4.3 Mean milk fat yield of all the trial cows.

*Means with different superscripts differed (P < 0.05) between treatments and over time.

The milk fat yield of the DOS group decreased during Weeks 2, and again during Weeks 5 to 6 and Weeks 7 to 9. This was likely a combined effect of milk fat content and milk yield that also decreased towards the end of the trial period. In the CON group, the milk fat yield also declined towards the end of the trial period, in this case presumably only as a result of the decline in milk yield.

Milk Protein Content and Milk Protein Yield

Interaction between treatments (P = 0.002) occurred over time. The mean milk protein content is presented in Figure 4.4.

The milk protein content (MPC) of the DOS group was higher in Week 1in comparison to the MPC of the CON group. However, during Weeks 4 and 5, and weeks 7 and 8, the MPC of the CON group was higher than the MPC of the DOS group. This is in contrast with other authors that found no difference in the protein composition and protein yield when cows were fed oregano (Hristov et al., 2013; Tekippe et al., 2011). The MPC of the DOS group decreased during Week 3. Towards the end of the trial the MPC of the DOS group did not differ from Week 1. The MPC of the CON group increased during Weeks 4 to 9. In the CON group, the MPC was higher towards the end of the trial, than in Week 1.

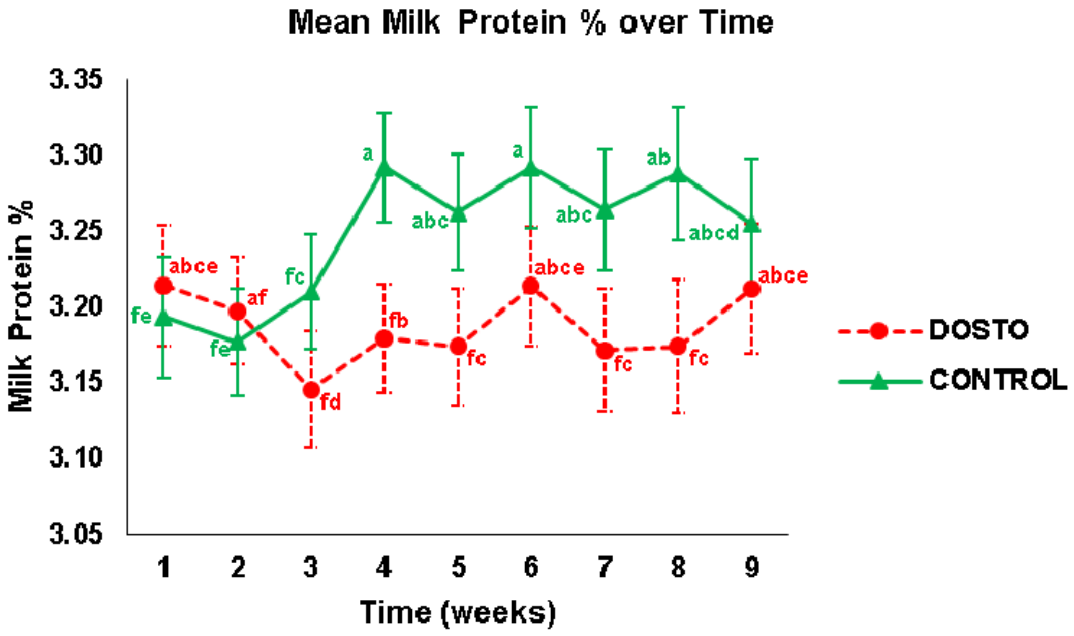


Figure 4.4 Mean milk protein content of all the trial cows.

* Means with different superscripts differed ($P < 0.05$) between treatments and over time.

When looking at the milk protein yield over time, there was no interaction between the treatments ($P = 0.456$). There was no difference ($P = 0.837$) for the main effects, thus at no given time, were any differences found between the treatments. The mean milk protein yield is presented in Figure 4.5.

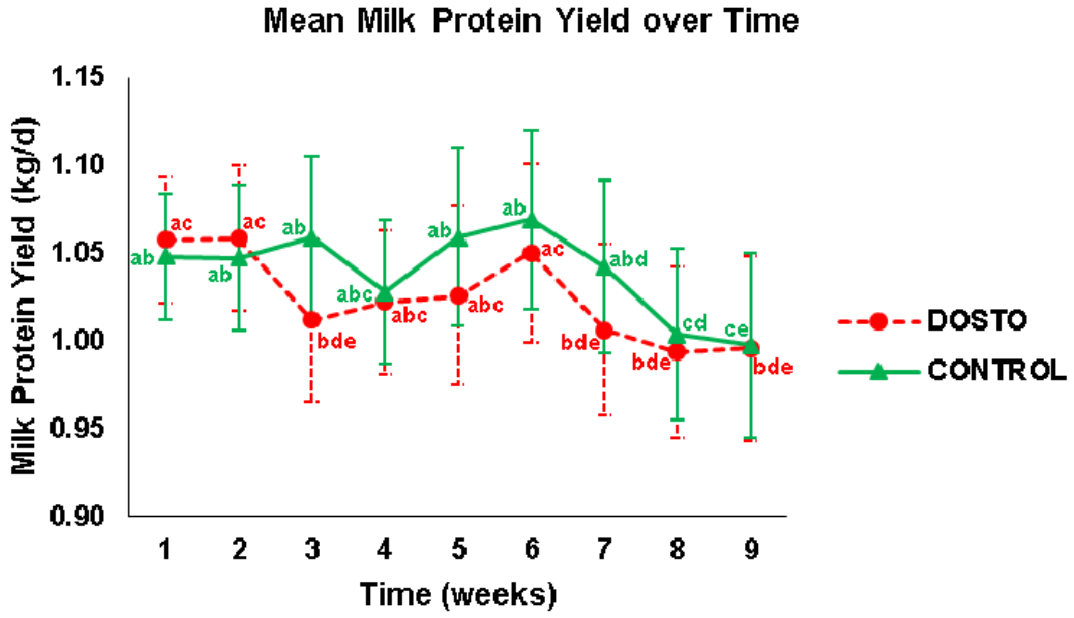


Figure 4.5 Mean milk protein yield of all the trial cows.

*Means with different superscripts differed ($P < 0.05$) between treatments and over time.

The milk protein yield of the DOS group declined during Week 3 and Weeks 7 to 9. The milk protein yield of the CON group declined during Weeks 8 and 9. The milk protein yield of both treatment groups decreased towards the end of the trial, compared to Week 1.

Milk Lactose Content

Interaction between the treatments ($P < 0.001$) occurred over time. The mean lactose content is presented in Figure 4.6. The lactose content of the CON group was higher than the lactose content of the DOS group during Weeks 1 and 2. The lactose content of the CON group declined during Weeks 2 to 9.The lactose content of the DOS group only declined during Week 9. The lactose content for both the treatment groups declined towards the end of the trial, compared to Week 1. A possible explanation for the decline in milk lactose content for the CON group could be due to the fact that the lactose content in dairy cow milk is generally higher during the first couple of weeks of lactation and will decline as lactation progresses

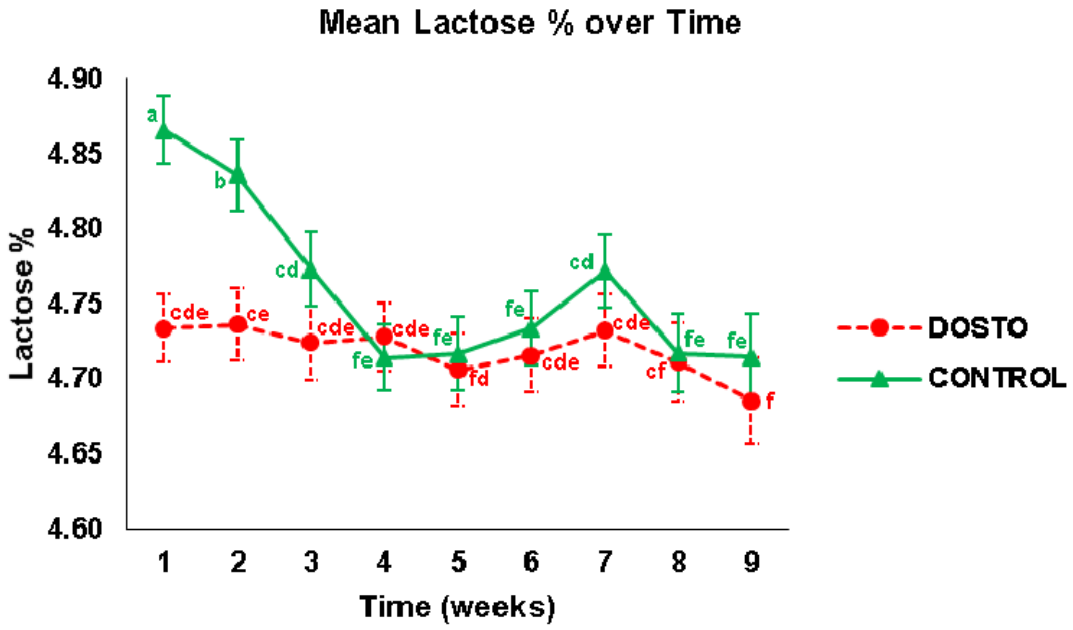


Figure 4.6 Mean lactose content of all the trial cows.

*Means with different superscripts differed ($P < 0.05$) between treatments and over time.

Energy Corrected Milk Yield

No interaction ($P = 0.604$) occurred over time between the treatments. There was no difference ($P = 0.758$) for the main effects, thus the treatments did not differ from one another. The mean ECM yield over time is presented in Figure 4.7.

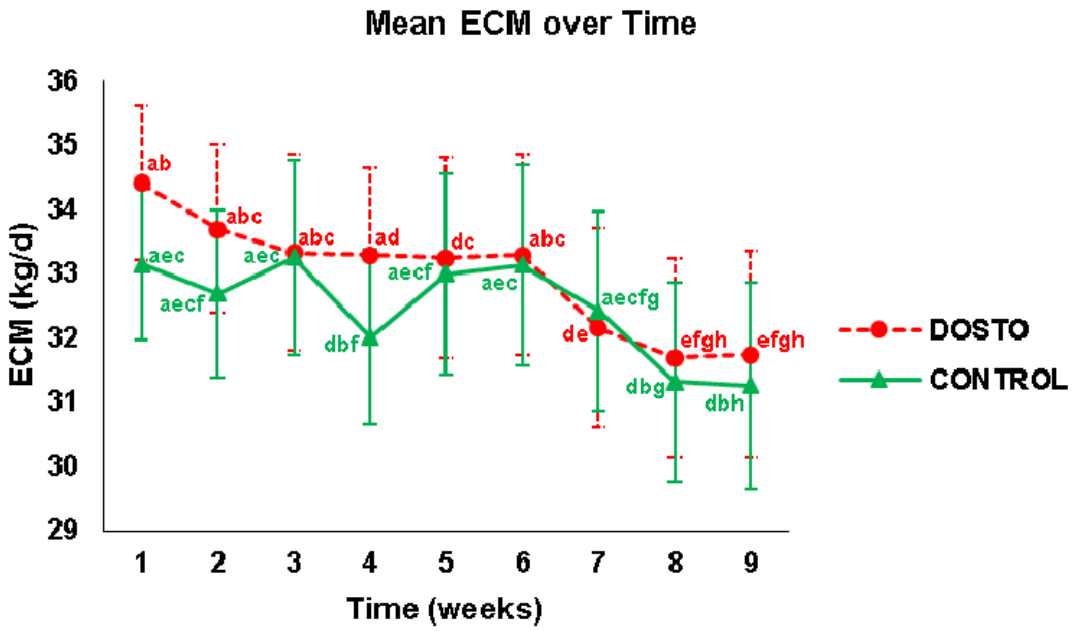


Figure 4.7 Mean energy corrected milk (ECM) yield of all the trial cows.
*****Means with different superscripts differed ($P < 0.05$) between treatments and over time.

The ECM of the DOS group decreased during Week 5 and again during Weeks 7 to 9. The ECM yield of the CON group decreased during Week 4 and again during Weeks 8 and 9. The ECM yield of both treatment groups decreased towards the end of the trial, compared to Week 1.

Body Weight and Body Condition Scores

Interaction between treatments occurred ($P = 0.034$) over time. However, no differences were found between the treatments. The initial mean body weight of the DOS group was slightly, but not significantly, higher than that of the CON group. Cows were blocked according to milk yield and DIM when they were initially allocated to the treatment groups and it happened that the DOS group had a higher mean initial BW. However, the changes in BW during the trial followed the same general pattern for both groups. Changes in body weight of the experimental cows over the nine week trial period are shown in Figure 4.8. The cow body weights of the DOS group increased during Weeks 6 to 9. Body weight of the CON group increased during Weeks 5 to 9. The mean body weight of the cows in both treatment groups increased towards the end of the trial, compared to Week 1.

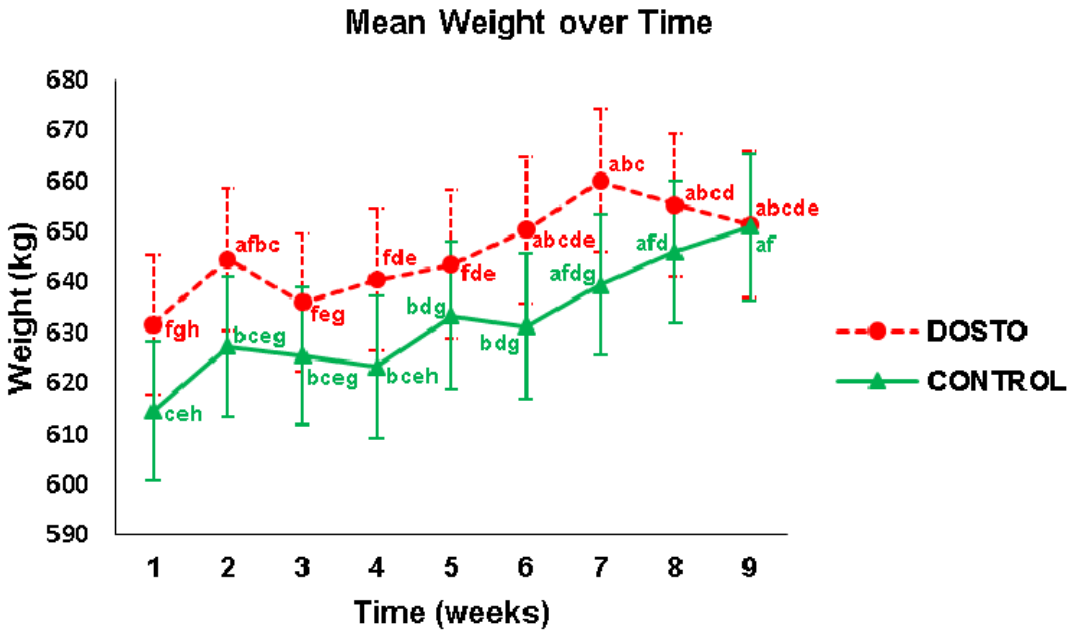


Figure 4.8 Mean body weight change of all the trial cows over time.

*Values with different superscripts differ ($P < 0.05$) between treatments over time.

In previous research on this subject, most of the authors indicated that the variation in BW presented no influences by the inclusion of oregano and that the oregano fed group showed an upward trend in relation to the control group (de Oliveira et al., 2014; Tekippe et al., 2011).

With regards to the change in body condition score (BCS) as of the onset of the trial to the BCS measured at the end (Week 9) of the trial, no interaction ($P = 0.418$) occurred between the treatments. There was no differences ($P = 0.0736$) between the main effects, CON and DOS. Table 4.4 presents the mean BCS and SD of the treatment groups.

Table 4.4 Mean BCS pre- and post-trial for the CON and DOS treatments.

Treatment	Trial	Mean	SD	*P*
CON	Pre	2.675	0.0469	0.418
DOS	Pre	2.537	0.0469	0.418
CON	Post	2.962	0.0509	0.418
DOS	Post	2.887	0.0509	0.418

Furthermore, we evaluated the BCS changes of the individual cows, within each treatment group. The mean BCS of the cows in both groups improved ($P< 0.0001$) when scores were allocated before the start of the trial and then again during the last week of the trial period. Because both groups showed an improvement in BCS, the increase could not be justified as a result of feeding oregano. Both groups possibly benefitted by receiving supplements in addition to their original diet.

4.3.3 Production response of the ten top producing cows in each treatment group

Data of the ten top producing cows in each treatment group (see Appendix C), selected according to mean milk yield obtained during the trial period, were statistically analysed. The selection of these cows was not related to days in milk (DIM).The milk production parameters and mean body weights are presented in Table 4.5.

There were no differences in mean daily milk yield between the treatments. The milk protein and lactose contents and protein yield did not differ between treatments. Milk fat content and milk fat yield was, however, higher ($P < 0.05$) for cows in the Dosto (DOS) treatment than for cows in the control (CON) treatment. There was a strong tendency ($P = 0.055$) for energy corrected milk yield to be higher in the DOS treatment. Treatment had no effect on final body weights.

Table 4.5 Mean milk production and composition, as well as body weight of the ten top producing cows for both the treatment- and control group.

Item	Control supplement	Dosto supplement	SEM	*P* - value
Milk, kg/d	37.27	37.88	0.599	0.494
Milk fat, %	3.38	3.71	0.101	0.0441
Milk fat yield, kg/d	1.255	1.406	0.0389	0.0231
Milk protein, %	3.18	3.14	0.0504	0.637
Milk protein yield, kg/d	1.183	1.188	0.0216	0.874
Milk lactose, %	4.75	4.71	0.0300	0.405
*ECM, kg/d	36.6	38.8	0.705	0.0549
Body weight, kg	615	645	20.35	0.317

*ECM- Energy corrected milk

Milk Yield

Over time, there was no interaction ($P = 0.994$) between the treatments. There was no difference ($P = 0.799$) for the main effects, therefore the treatments did not differ from one another regarding milk yield. Figure 4.9 presents the mean milk yield over time.

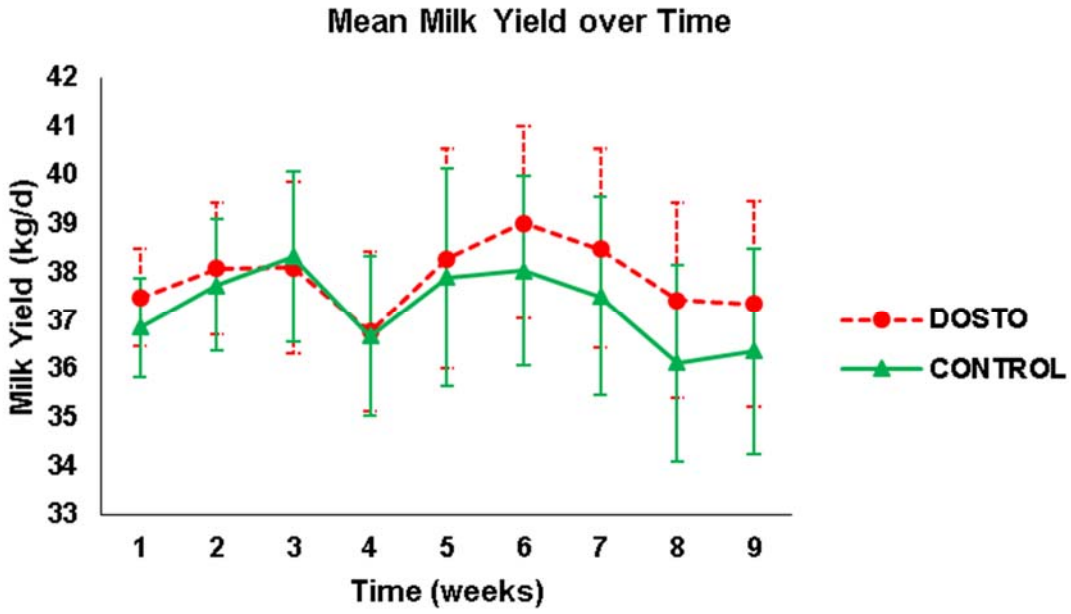


Figure 4.9 Mean milk yield of the ten top producing cows.

Limited information is available with regards to the *in vivo* effects of essential oils (EO) in dairy cow diets. However, available literature presented similar results with regards to milk production, compared to the current study. In a similar study, the milk yield was unaffected by the supplementation of *Origanum vulgare*. L leaf material to Holstein cows during a 20-day experimental period (Hristov et al., 2013). Milk yield was unaffected in a trial carried out by de Oliveria et al. (2014), where the supplementation of different levels of oregano in addition to a sugar cane based diet was investigated. Results from the current study also support the results found by Tekippe et al. (2011), where a lactating trial was conducted in order to study the influence of supplementing *Origanum vulgare*. L in addition to a TMR diet, on the production responses of dairy cows. The results from Tekippe et al. (2011) showed that there was no influence on milk yields when supplementing diets with oregano. Other plant-based EO additives also proved to have no influence on milk production. In a study by Yang et al. (2007), in addition to a TMR consisting of 40% forage and 60% barley-based concentrate, cow diets were supplemented with garlic (5g/day) and juniper berry (2g/day). Supplementing the TMR diets with garlic and juniper berry had no effect on milk yield (Yang et al., 2007). Milk yield and milk composition was not affected by the addition of cinnamaldehyde and eugenol to TMR diets of dairy cows (Tager and Krause, 2011).

Milk Fat Content and Milk Fat Yield

Interaction occurred between treatments (P = 0.048) over time. The mean milk fat content over time is presented in Figure 4.10. The impact of nutrition and nutritional changes in the ration can readily alter milk fat concentrations.

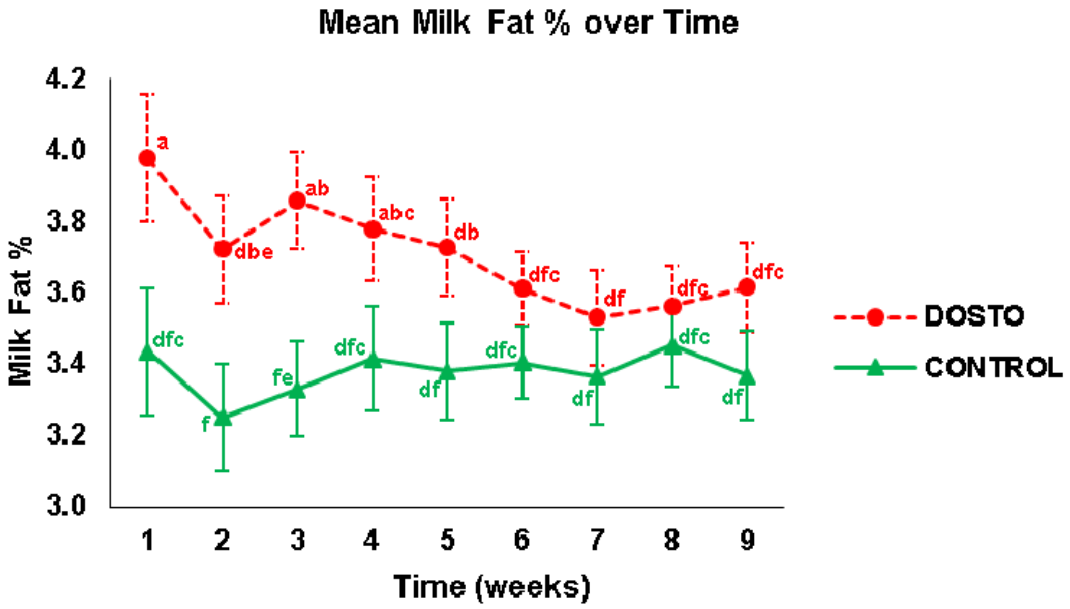


Figure 4.10 Mean milk fat content of the ten top producing cows.

*Means with different superscripts differed ($P < 0.05$) between treatments and over time.

The milk fat content (MFC) of the DOS group was higher in comparison to the MFC of the CON group during Weeks 1 to 3. The MFC of the DOS group decreased during Week 2 and again during Weeks 5 to 7. Towards the end of the trial the MFC of the DOS group was lower than at the beginning of the trial, but started to increase during the last two weeks. The decrease in MFC from Weeks 4 to 6 agreed with the increase in milk yield observed during that time. As milk yield started to decrease from Week 6, an increase in MFC followed from Week 7. The MFC varied less in the CON treatment.

Yang et al. (2007) found no effect on the milk fat content, when supplementing TMR diets with garlic and juniper berry. The results from Yang et al. (2007) suggest that the addition of EO from garlic and juniper berry has minimal beneficial effects on dairy cow production. The EO did, however, show a potential to improve the feed digestibility in the rumen. Feeding a mixture of essential oils to dairy cows had no effect on the milk fat concentrations and the milk fat yields (Benchaar et al., 2007).

In contrast to others, but supporting the results from the current study, an increase in milk fat content was reported by Santos et al. (2009) when an essential oil blend was supplemented to cows on a TMR diet. Milk fat content and milk fat yield increased when an EO mixture containing eugenol, geranyl acetate and coriander were fed to high producing dairy cows on a TMR diet (Santos et al., 2010). In a trial conducted by Hristov et al. (2013) no differences in milk fat content was observed. On the other hand, Tekippe et al. (2011) reported that feeding oregano at increased levels of supplementation resulted in an increase in milk fat content and in milk fat yield.

In the current study, over time no interaction (P = 0.101) occurred between treatments for milk fat yield. The mean milk fat yield is presented in Figure 4.11.

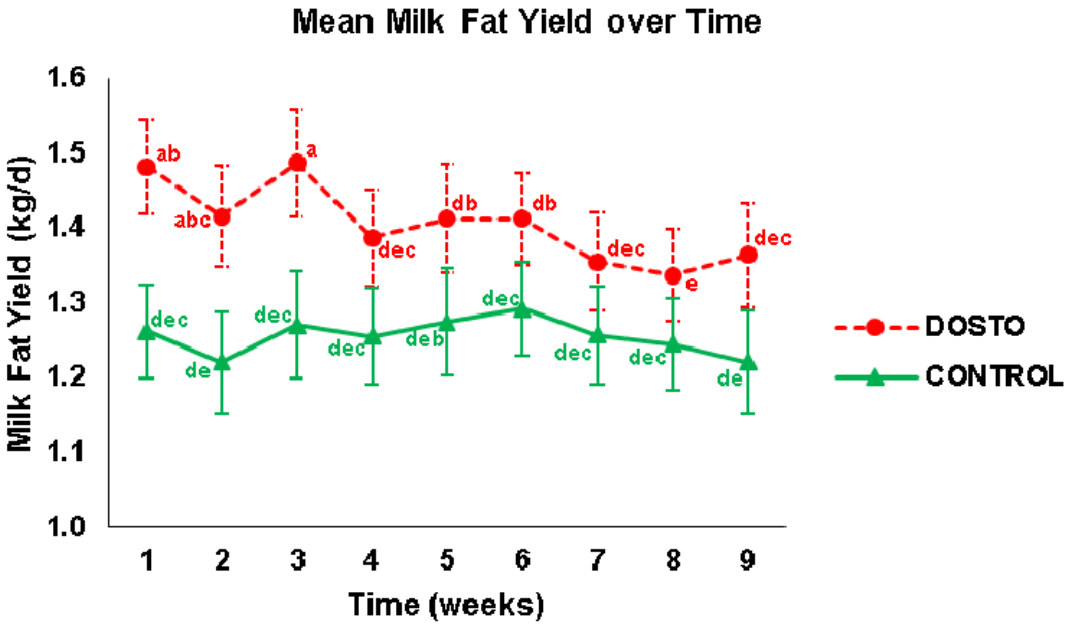


Figure 4.11 Mean milk fat yield of the ten top producing cows.

*Means with different superscripts differed (P < 0.05) between treatments and over time.

The milk fat yield of the DOS group was higher in comparison to the milk fat yield of the CON group, during Weeks 1 to 3. The milk fat yield of the DOS group decreased during Week 4 and again during weeks 7 to 9. Towards the end of the trial the milk fat yield of the DOS group had declined, compared to Week 1. Little variation in milk fat yield of the CON group occurred during the trial.

The increase in milk fat content and milk fat yield observed in this study may be a due to an energetic shift away from body condition gain, suggesting that oregano oil may have enhanced the production of acetate and/or the ratio of acetate to propionate production in the rumen (Santos et al., 2010).

Milk Protein Content and Milk Protein Yield

Interaction occurred (P = 0.037) between treatments over time. However, there were no differences between the treatments. The mean protein content over time is presented in Figure 4.12. Milk protein is not readily changed by nutritional changes. The blend of amino acids in the rumen-degradable protein will impact protein production.

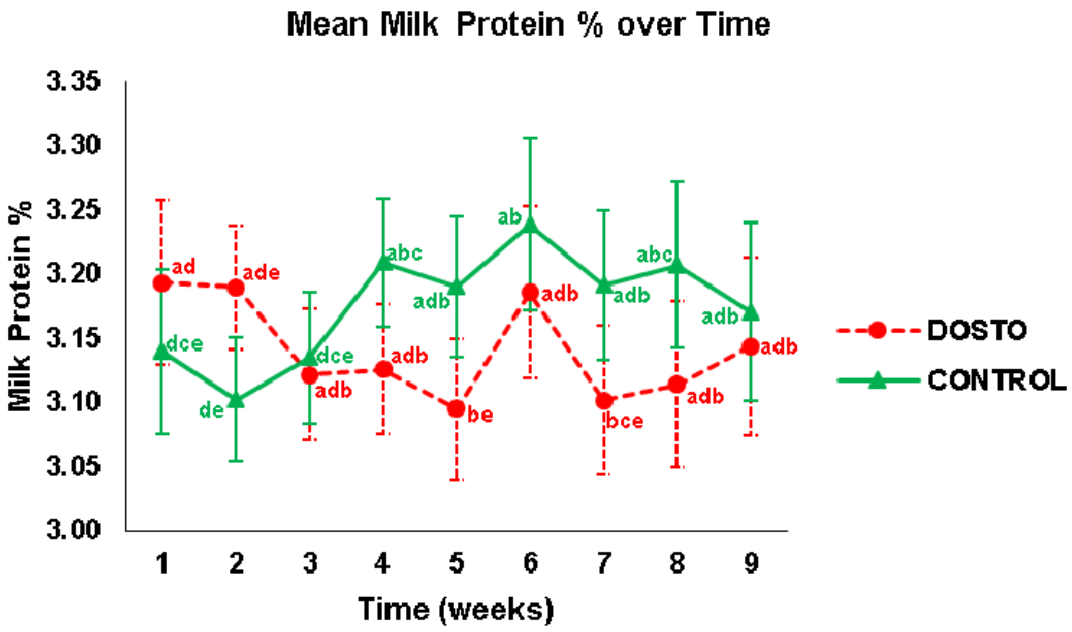


Figure 4.12 Mean milk protein content of the ten top producing cows.

*Means with different superscripts differed ($P < 0.05$) between treatments and over time.

The milk protein content of the DOS group decreased during Weeks 5 and 7. The milk protein content of the CON group increased during Week 6. No difference in the milk protein content was found towards the end of the trial, in both treatment groups, compared to Week 1.

No effect on milk protein content was reported by Yang et al. (2007). On the other hand, Spangero et al. (2009) reported that supplementing a commercial blend of micro-encapsulated EO at increasing doses to high yielding dairy cows, had a positive effect on the milk protein content. In contrast, Tassoul and Shaver (2009) reported that the milk protein content reduced by 0.15% units, in comparison to a control diet when lactating dairy cow diets were supplemented with an EO complex. In several studies by Benchaar et al (2006; 2008) the addition of an EO blend, cinnamaldehyde and/or eugenol to the TMR diets of dairy cows, milk protein content was unaffected.

In more recent studies, Tekippe et al. (2011) and Hristov et al. (2013) found that supplementing dairy cow diets with *Origanum vulgare*. L leaf material, did not affect the milk protein content nor did it influence the milk protein yields.

In the current study, overtime no interaction ($P = 0.720$) occurred for milk protein yield. There was no difference ($P = 0.944$) for the main effects, therefore the treatments did not differ from one another. The mean milk protein yield is presented in Figure 4.13.

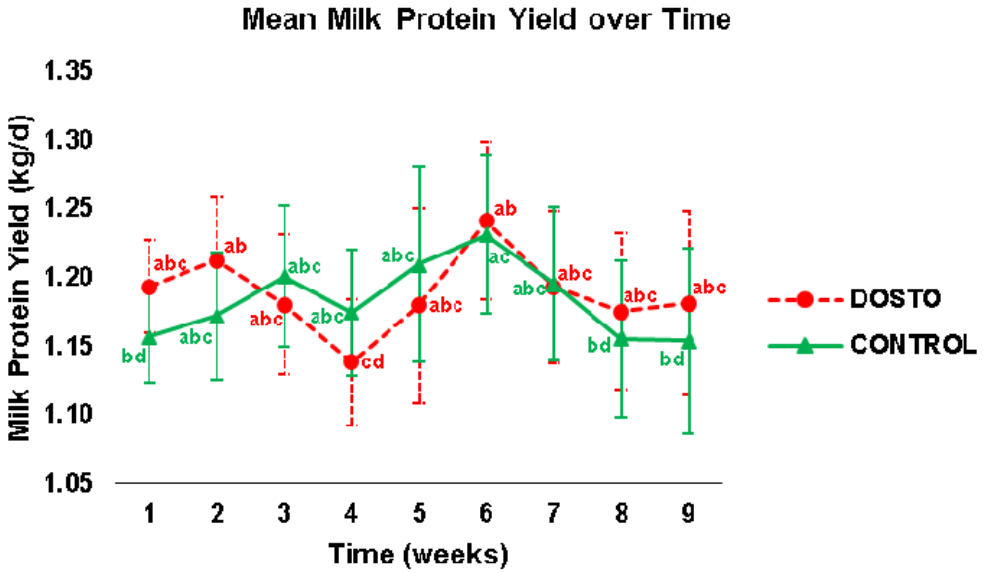


Figure 4.13 Mean milk protein yield of the ten top producing cows.

*Means with different superscripts differed ($P < 0.05$) between treatments and over time.

No variation in the milk protein yield occurred during the trial for the DOS group. The milk protein yield of the CON group increased during Week 6. No differences in the milk protein yield was found towards the end of the trial, in both treatment groups, compared to Week 1.

Lactose Content

In the current study, Interaction occurred ($P < 0.001$) between treatments over time. The mean lactose content over time is presented in Figure 4.14.

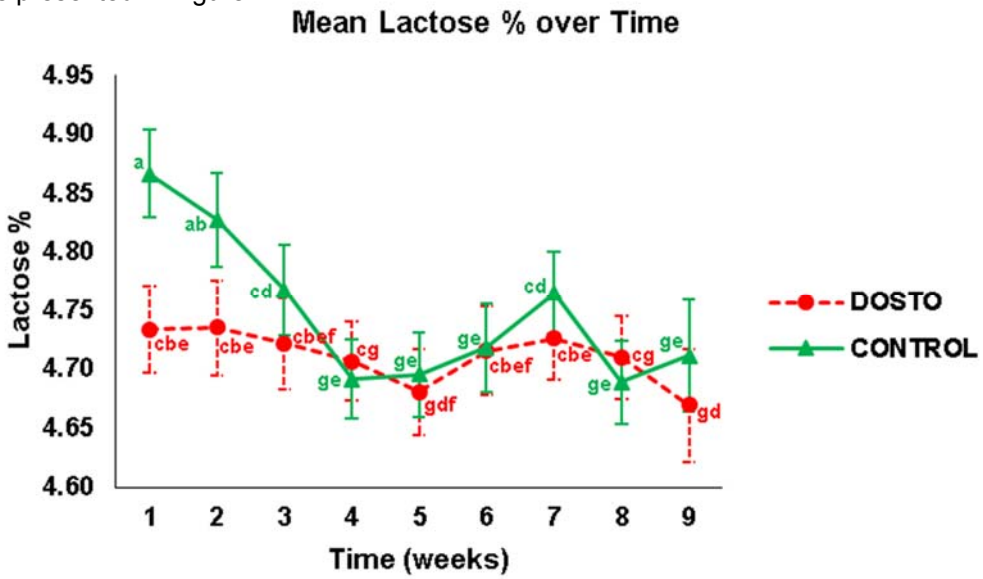


Figure 4.14 Mean lactose content of the ten top producing cows.

*Means with different superscripts differed ($P < 0.05$) between treatments and over time.

The lactose content of the CON group was higher in comparison to the lactose content of the DOS group, during Week 1. The lactose content of the CON group decreased significantly during Weeks 3 to 9. The lactose content of the DOS group decreased during Weeks 5 and 9. In both groups, the lactose content was lower towards the end of the trial, compared to Week 1.

Benchaar et al. (2006) reported that the supplementation of EO at 2g/day in a TMR diet to dairy cows, had no effect on the milk lactose content. Spangero et al. (2009) found no effect on the milk lactose content. When supplementing a TMR diet with cinnamaldehyde and eugenol, the milk lactose content was unaffected (Tager and Krause, 2010). Supplementing dairy cow diets with garlic and juniper berries, proved to have no effect on the milk lactose content (Yang et al., 2007). Tekippe et al. (2011) and Hristov et al. (2013), found that supplementing dairy cow diets with dried *Origanum vulgare*. L leave material had no effect on the milk lactose content.

In contrast to the above mentioned, a study was carried out to investigate the effect of the inclusion of dried *Origanum vulgare*. L to crossbred Holstein X Zebu cow diets on milk characteristics (Lacerda et al., 2014). The results from Lacerda et al. (2014) showed a reduction in the milk lactose content. This result was unexpected as lactose is more stable than the other nutrients in milk and is less susceptible to dietary changes.

Energy Corrected Milk Yield

Over time no interaction (P = 0.880) occurred between the treatments. There was no difference (P= 0.337) found for the main effects, therefore the treatments did not differ from one another. The mean ECM yield over time is presented in Figure 4.15.

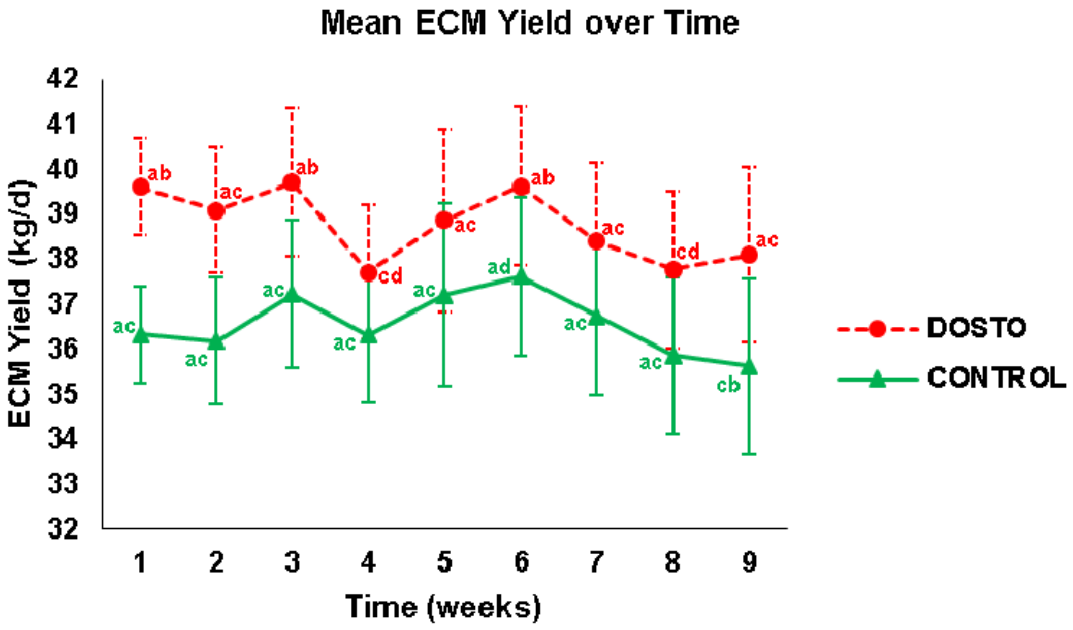


Figure 4.15 Mean energy corrected milk (ECM) yield of the ten top producing cows.

***Means with different superscripts differed (P < 0.05) between treatments and over time.

The ECM yield of the DOS group decreased during Weeks 4 and 8. No variation in the ECM yield occurred during the trial, for the CON group. No difference in ECM yield for both treatment groups towards the end of the trial, compared to Week 1. The graphs of ECM over time followed the same pattern for both groups, but there was a strong tendency (P = 0.055) for the mean ECM to be higher for the DOS group than for the CON group (Table 4.4).

In a study by Schieder et al. (2015), 55 Holstein cows received a partly mixed ration with the addition of a phytogenic feed additive (includes herbs, spices, EO and plant extracts).Their results showed a significantly higher milk yield compared to the control diet. The increase in milk yield and enhanced quality of the milk solids resulted in a superior amount of ECM (33.01 vs 31.89 kg/d: P > 0.05) compared to the control diet.

Body weight

In the current study, over time no interaction (P = 0.331) occurred between the treatments. There were no differences (P = 0.311) found for the main effects, therefore the treatments did not differ from one another. Figure 4.16 presents the mean body weight over time.

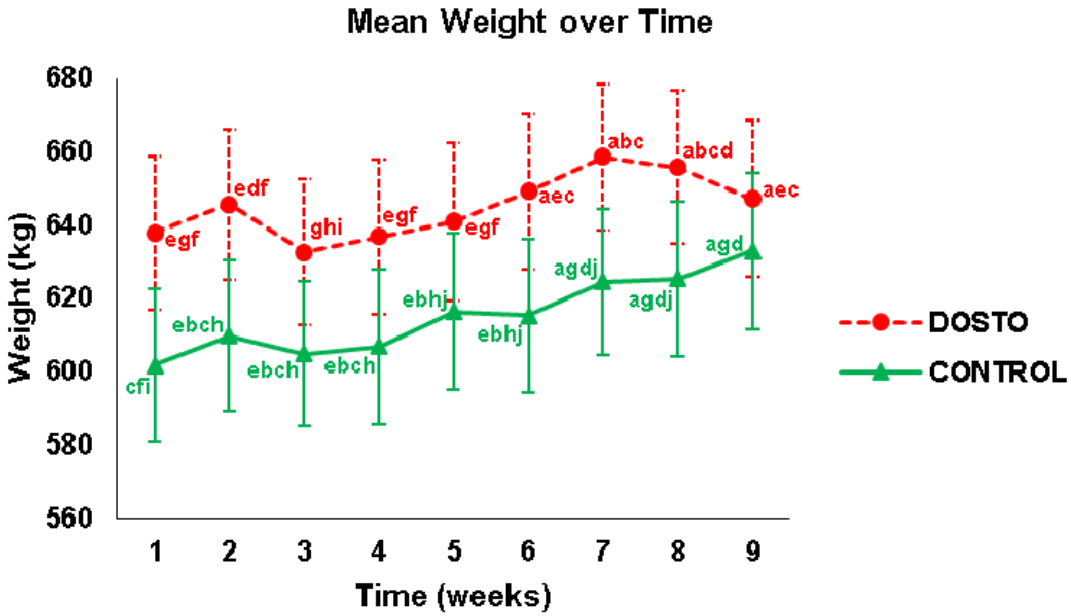


Figure 4.16 Mean body weight of the ten top producing cows.

*Means with different superscripts differed (P < 0.05) between treatments and over time.

The cow body weights increased during Weeks 7 and 8 in the DOS group and during Weeks 5 to 9 in the CON group. Body weights of the CON group increased towards the end of the trial, compared to Week 1, whereas weights of the DOS group did not differ towards the end of the trial.

Tekippe et al. (2011) found that the body weight of dairy cows was unaffected when their diets were supplemented with dried *Origanum vulgare*. L leaf material. In another study, the variation in body weight

presented no influences by the inclusion of oregano to their diets, but showed an upward trend in relation to the control diet (de Oliveira et al., 2014).

4.4 Conclusion

The addition of oregano essential oil to dairy cow diets stimulated fat production and increased energy corrected milk yield in high producing dairy cows. The significant effect on milk fat content and milk fat yield in the current study may be attributed to slight shifts in microbial fermentation in the rumen. The acetate to propionate ratio may have been increased due to the oregano treatment, but this is something that would have to be confirmed in future research.

Future studies should look at extending the trial period. Furthermore, separate trials could be done in different seasons to evaluate the influence of external environmental factors. Rumen fermentation and total tract digestibility trials could be conducted in order to investigate fatty acid production.

4.5 References

Benchaar, C., Calsamiglia, S., Chaves, A., Fraser, G., Colombatto, D., McAllister, T. & Beauchemin K., 2008. A review of plant-derived essential oils in ruminant nutrition and production. Anim. Feed Sci. Technol. 145: 209-228.

Benchaar, C., Petit, H. V., Berthiaume, R., Whyte, T. D. & Chouinard, P. Y., 2006. Effects of addition of essential oils and monensin premix on digestion, ruminal fermentation, milk production, and milk composition in dairy cows. J. Dairy Sci. 89: 4352-4364.

De Oliveira, J. S. O., de Silva, F. F., Costa, L. T., da Silva, G. M., de Almeida Meneses, M. & de Oliveira Rodrigues, E.S., 2014. Economic performance of dairy cows fed diets with different levels of oregano. Acta. Sci. Anim. Sci. 36: 393-398.

Hristov, A. N., Lee, C., Cassidy, T., Heyler, K., Tekkippe, J. A., Varga, G. A., Corl, B. & Brandt, R.C., 2013. Effect of *Origanum vulgare* L. leaves on the rumen fermentation, production and milk fatty acid composition in lactating dairy cows. J. Dairy Sci. 96: 1189-12012.

Lacerda, E.C.Q., Bauer, L.C., Oliveira, J.S., Silva, F.F., Carvalho, S.A., Macedo, M.S., de Souza, N.E. & Simionato, J.I., 2014. Effect of the dietary inclusion of dried oregano (*Origanum vulgare* L.) on the characteristics of milk from Holstein x Zebu cows. J. Anim. Sci.192: 101-105.

Santos, M.B., Robinson, P.H. & Williams, P., 2009. Effects of feeding an essential oil complex on whole tract nutrient digestion and productive performance of lactating dairy cows. J. Dairy Sci. 87 (Suppl. 1):375.

Santos, M. B., Robinson, P. H., Williams, P. & Losa, R., 2010. Effects of addition of an essential oil complex to the diet of lactating dairy cows on whole tract digestion of nutrients and productive performance. J. Anim. Feed Sci. Technol. 157: 64-71.

Schieder, C., Boczonadi, A. & Miller, B., 2015. Effect of a phytogenic feed additive on the feed intake and milk production in dairy cows. J. Anim. Sci. 98: 820.

Spanghero, M., Robinson, P. H., Zanfi, C. & Fabbro, E., 2009. Effect of increasing doses of a microencapsulated blend of essential oils on performance of lactating primiparous dairy cows. J. Anim. Feed Sci. Technol. 153: 153-157.

Tagar, L. R. & Krause, K. M., 2011. Effects of essential oils on rumen fermentation, milk production, and feeding behaviour in lactating dairy cows. J. Dairy Sci. 94: 2455-2464.

Tassoul, M. D. & Shaver, R. D., 2009. Effect of a mixture of supplemental dietary plant essential oils on performance of periparturient and early lactation dairy cows. J. Dairy Sci. 92: 1734-1740.

Tekippe, J. A., Hristov, A. N., Heyler, K. S., Cassidy, T. W., Zheljazkov, V. D., Ferreira, J. F. S., Karnati, S. K. & Varga G. A., 2011. Rumen fermentation and production effects of *Origanum vulgare L*. leaves in lactating dairy cattle. J. Dairy Sci. 94: 5065-5079.

Varga, G. A. & Ishler, V. A., 2010. Managing Nutrition for Optimal Milk Components [Online]. Available: http://www.extention.org/pages/11300/managing-nutrition-for-optimal-milk-components [2015, May 19].

Yang, W. Z., Benchaar, C., Ametaj, B. N., Chaves, A. V., He, M. L. & McAllister, T. A., 2007. Effects of garlic and juniper berry essential oils on ruminal fermentation and on the site and extent of digestion in lactating cows. J. Dairy Sci. 90: 5671-5681

CHAPTER 5

ABSTRACT

Effect of Oregano Essential Oil on the Sensory Characteristics of milk

The objective of this study was to evaluate the effects of the inclusion of oregano essential oils, as a feed additive into dairy cow diets, on the sensory characteristics of milk. Forty Holstein cows were divided into two groups of twenty, a control group (receiving maize meal without oregano) and a treatment group (receiving maize meal with oregano oil). The cows were fed their usual total mixed ration (TMR) and lucerne *ad libitum*, twice daily after milking. During milking 300 g of the premixed additive feed, containing 0.5 g oregano (treatment group), were fed to the cows in the milking parlour. After a two month period 12 milk samples were collected from each group and was sent to be evaluated. The microbiological quality of the milk samples was evaluated by using petrifilm plates for total aerobic counts (TAC) and coliform counts (CC). Based on the microbiological analysis, all the milk samples were considered suitable for consumption (< 200 000 cfu/ml). The treatment group differed ($P \leq 0.001$) from the control group in terms of aroma and flavour. It was concluded that oregano essential oil had no adverse effect on milk aroma and flavour.

Key words: aroma, dairy, bacterial counts, flavour, ruminants.

5.1 Introduction

A production study was carried out, feeding an oregano oil supplement to a group of twenty lactating Holstein cows. The overall milk production and milk production parameters were measured, as discussed in previous chapters. In general when dairy cows feed on strong flavoured feedstuffs, the flavour is often carried over into the milk. Some consumers may have a distaste for drinking milk that has a tainted flavour or aroma. Oregano has a prominent herb-like aroma and flavour and it was thus important to carry out a sensory evaluation, in order to determine whether or not this aroma and flavour would be carried over to the milk.

Ideally, milk that has to undergo any form of sensory analysis whereby the tests are carried out by humans, should be pasteurised. Pasteurisation has two main objectives: to kill pathogens that may occur in milk and to increase shelf life (Raw Milk vs. Pasteurized Milk, 2014). Milk samples that were collected to undergo a sensory analysis in this study, were bottled individually directly after the cows had been milked and were thus not pasteurised. Before sensory evaluation the milk therefore had to undergo a microbiological test to determine its suitability for human ingestion. Total microbial counts (TMC) were done on all the samples and they were also analysed for the presence of coliforms and/or *Escherichia coli* (*E.coli*) colonies.

5.2 Materials and Methods

Towards the end of the milk production trial, representative milk samples (1 L per sample) were collected randomly from 12 cows per treatment group. The milk samples were bottled and stored at -18 °C pending microbiological analysis.

5.2.1 Microbiological analysis of unpasteurised milk samples

Frozen milk samples were thawed over a period of 24 hours at 7 °C. Testing of the samples were done over two days, where six samples per treatment were analysed daily. Figure 5.1 shows that milk samples were diluted using 10 ml of rooibos extract in 90 ml of sterilized buffered peptone water (BPW), by using a sterile pipette each time. The mixture was thoroughly homogenized. The samples containing the BPW were used in a series of dilutions (10^{-1}, 10^{-2} and 10^{-3}), in which 1ml of the dilution was transferred to 9 ml of a sterile 25% Ringers solution. Using a fresh sterile pipette, 1ml of the highest dilution was transferred to a petrifilm plate. Using the same pipette, 1 ml of the second highest dilution was transferred to a petrifilm plate and this was repeated for the third highest dilution. Petrifilm 3M plates (Merck Biolab, South Africa) were used for total aerobic counts (TAC) and coliform/*E.coli* counts (CC). The TAC plates were incubated at 30 °C for 18-24 hours and the CC plates were incubated at 37 °C for 18-24 hours. The plates were then enumerated according to the number of colonies found on the plate and these results were recorded.

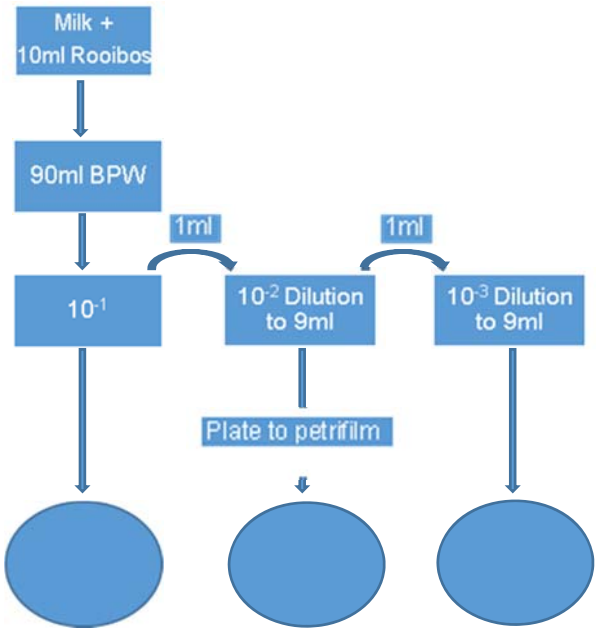


Figure 5.1 Dilution series of milk used on petrifilm plates.

The colony forming units (cfu) per ml of milk can be calculated by multiplying the plate count by the dilution factor of that particular plate.

Example: If the 1 in 100 dilution plate had 12 colonies, the count for the undiluted milk would then be

$$\mathbf{12 \times 100 = 1200\ } \boldsymbol{cfu/ml}$$

5.2.2 Sensory evaluation of aroma and flavour of dairy milk samples

Experimental Design

A randomised block design (RBD) was used to evaluate two different treatments, a treatment (T) group and a control (C) group of samples. Each group had 12 samples. The RBD consisted of eight panellists, testing three samples during each session, resulting in a 3X8 = 24 block design. Standard trained panellists, with 100 hrs of training, were used for the evaluating purpose. The panellists were seated at individual sensory booths, which were light- and temperature – controlled (21 °C). Four sensory evaluating sessions were carried out on the milk samples. Three bottles of milk from both treatment groups were used per session. From each bottle eight samples were used so that each panellist could test one sample from every bottle:

Sample 1-8 bottle 1

Sample 9-16 bottle 2

Sample 17-24 bottle 3

The milk samples were evaluated by making use of a triangle test, also known as a type of discrimination test.

Triangle Test

The milk samples were served cold, from the fridge, in wine tasting glasses, that were covered with a lid. Milk samples were served in a completely randomised order and were marked with random three-digit codes as generated by the Compusense five sensory software program (Compusense®, Guelph, Canada). Panellists were supplied with filtered water and apple slices between samples to cleanse their palate.

The panellists were presented with three milk samples and they were told that two of the samples were the same and one sample was different. They were then asked to remove the lids from the glasses and assess the samples from left to right, in the order presented and then to determine the sample that is different/odd. Panellists were asked to use the palate cleansers between each sample. In addition to selecting the odd sample, panellists were asked to give a reason/comment on why they have made that particular selection.

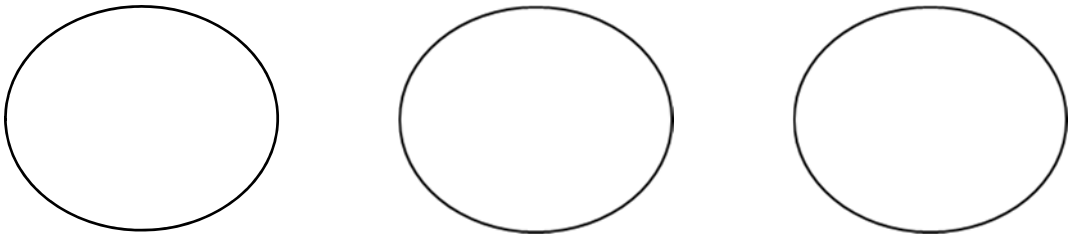
There are six possible orders of sample presentation:

CCT	TTC
CTC	TCT
TCC	CTT

For this trial, aroma and flavour were evaluated to determine whether or not the treatment had an effect on the sensory components of milk. Aroma and flavour were tested for in different sessions and on a new set of samples. The design and triangle test were carried out in exactly the same manner. The only difference was, that for the flavour test, the milk samples were tasted whereas the samples for aroma were not. In Figure 5.2 below is an example of the answer sheet that was given to judges.

Questionnaire for a Discrimination Test (Triangle Test)

Testing of Milk: Date



285 **001** **964**

TRIANGLE TEST: AROMA / FLAVOUR	
NAME OF JUDGE: _____	**JUDGE NO:** _____

INSTRUCTIONS

- You have received three cooled samples. Two of these samples are the same and one is different. **Circle the number of the sample that is different (ODD)**
- Please taste samples from left to right
- Rinse your mouth with water between samples

Figure 5.2 Questionnaire for a discrimination test.

5.3 Results and Discussion

5.3.1 Bacterial Counts

The first batch of samples that were microbiologically analysed produced the microbial counts shown in Table 5.1.

Table 5.1 Total counts cfu/ml for Samples 1-6 from Treatment (T) -and Control (C) groups.

C1-5300	T1-12800
C2-5700	T2-12400
C3-4200	T3-13500
C4-3000	T4-13100
C5-5300	T5-14400
C6-5800	T6-14900

Coliforms were only present in samples: T1 (50 cfu/ml) and T6 (10 cfu/ml). None of the above samples tested positive for *E.coli*.

Shown in Table 5.2 below are the results for the second batch of microbiologically analysed samples.

Table 5.2 Total counts cfu/ml for Samples 7-12 from Treatment (T) -and Control (C) groups.

C7-3900	T7-12100
C8-4300	T8-14000
C9-5800	T9-12800
C10-3400	T10-13400
C11-4300	T11-14300
C12-4100	T12-14100

Coliforms were only present in samples: T10 (10 cfu/ml) and T12 (80 cfu/ml). None of the above samples tested positive for *E.coli*.

According to law R0853 of 2004, pasteurised milk or raw milk for consumption must have a standard plate count (SPC) of less than 50 000 cfu per ml before leaving the factory and raw milk for further processing must have a SPC of less than 200 000 cfu per ml (Gouws, 2015). If the bacterial counts exceed the limit, the milk will not last until it's used by date or will not be fit for human consumption. A count of 25 - 250 bacterial colonies is ideal when quantifying growth on AC petrifilm plates (Gouws, 2015). Using multiple dilutions to inoculate plates, the chances of having at least one plate that falls within this range increases.

The results that were obtained from the microbiological analysis, revealed that all of the milk samples had microbial counts that fell well within the regulated boundaries for raw milk for further processing. In conjunction

with the acceptable microbial counts, the fact that no samples tested positive for *E.coli*, deemed the milk safe to be used for the sensory analysis.

5.3.2 Triangle Test

Aroma

A list of comments for some of the correctly identified odd samples, were tabulated as shown in Table 5.3 below. From all the comments only nine answers suggested that the panellist chose the correct answer due to the herb-like or plant-like aroma, however some of the panellists made a comment that the odd sample was herby even though, in those cases the odd sample belonged to the control group. Four comments suggested, that when the odd sample was from the control group, the milk aroma was less cow/barn-like and this coincides with eight comments that were made on some of the odd samples that were from the treatment group, that were described as having a barn-like or natural cow-like aroma.

Table 5.3 Comments for some of the correctly identified odd samples for aroma.

Registration Code & Station Number	Session	Sample - Set	Sample	Comment
5	1	13	1	Low in herbs
5	2	5	1	Slightly herby
5	2	13	1	Herby
7	2	15	1	Less Barn-like or cow-like aroma compared to 221 and 608
9	2	24	1	Slightly herbaceous aroma, less milky aroma
7	3	15	1	Not barn-like or cow-like aroma as detected in other two samples
7	3	23	1	Other two samples more barn-like or cow-like aroma compared to 120
7	4	15	1	Slight barn-like aroma, but less prominent compared to previous sessions
1	1	9	2	Slightly herby
3	1	11	2	Plant-like
4	1	12	2	Slight herb-like aroma
5	1	21	2	Low in herby smell
7	2	7	2	Barn-like or cow-like aroma, less creamy compared to 421 and 217
7	2	15	2	Barn-like aroma\Barn-like aroma
5	2	21	2	More herby
3	3	3	2	Dairy-like
7	3	7	2	Barn-like or cow-like aroma

Results represented in Table 5.4, indicates that out of a possible 96 samples, 53 samples were correctly identified as being the odd sample.

Table 5.4 Number of correctly identified odd samples for the aroma test.

Test 1 Samples	C=Sample 1 T=Sample 2
Incorrect	43
*Correct	53
Total	96
Confidence	1.000
Significance (p-value)	0.000

*Correct = Odd Sample Selected

The minimum numbers of correct responses to reject the null hypothesis of 'no difference' at selected significance levels with a total number of assessors 'n' is indicated in Table 5.5 below.

Table 5.5 Number of correct answers necessary to establish level of significance (see Appendix A).

No. of Judgments	10%	* 5%	** 1%	*** 0.1%
96	39	41	44	48

H_o Treatment does not have an effect on the aroma of milk.
H_a Treatment does have an effect on the aroma of milk.

The outcome of 53 correct answers indicates that the treatment group differed ($P \leq 0.001$) from the control group in terms of aroma. We can thus conclude that treatment had an effect on the aroma of milk; however, this was not due to a prominent herb-like aroma, but possibly due to a slight difference in the milk fat content between the groups.

Flavour

A list of comments for some of the correctly identified odd samples, were tabulated as shown in Table 5.6 From all of the comments only one answer suggested that the panellist chose the odd sample due to the sample having a herb-like flavour. Five comments suggested that the odd treatment sample was less sweet, and on the other hand five comments suggested that the odd treatment sample was sweeter.

Table 5.6 Comments for some of the correctly identified odd samples for flavour.

Registration Code &Station Number	Session	Sample -Set	Sample	Comment
7	1	15	1	Lower in creamy flavour
7	3	15	1	Lower dairy/creamy aroma compared to other samples
7	4	15	1	Less creamy
7	4	23	1	Less creamy
2	1	2	2	Less sweet
7	1	7	2	Less creamy compared to the other samples
9	1	8	2	More watery, less creamy
2	1	10	2	Less herb or just more natural
3	1	19	2	Sweet
3	2	3	2	Dairy creamy sweet
7	2	7	2	Sweeter compared to other two samples
2	2	10	2	Less sweet
5	2	13	2	More richer milk (more body)
1	2	17	2	Sweet
5	2	21	2	Sweet and has more body
7	3	7	2	Cow-like or barn-like flavour
1	3	9	2	Less sweet
9	3	16	2	More creamy
1	3	17	2	Less sweet
3	3	19	2	Creamy dairy
3	4	3	2	Not so sweet as the others
7	4	7	2	Creamier

Results represented in Table 5.7 below, shows that out of a possible 96 samples, 50 samples were correctly identified as being the odd sample.

Table 5.7 Number of correctly identified odd samples for the flavour test.

Test 2 Samples	C=Sample 1 T=Sample 2
Incorrect	46
*Correct	50
Total	96
Confidence	1.000
Significance (p-value)	0.000

*Correct = Odd Sample Selected

H_o Treatment does not have an effect on the flavour of milk
H_a Treatment does have an effect on the flavour of milk

The outcome of 50 correct answers indicates that the treatment group differed ($P \leq 0.001$) from the control group in terms of flavour. We can thus conclude that the treatment does have an effect on the flavour of milk, however this was not due to a prominent herb-like flavour, but possibly due to a slight difference in the milk fat/or lactose content between the groups. In contrast, a sensory test executed by 48 panellists, resulted in 26 correct responses, however 31 correct answers were required to establish significance at $\alpha = 0.05$ (Tekippe et al., 2011). Therefore the sensory results from Tekippe's trial showed that the cow's milk, from cows that were fed oregano, did not differ from the control fed cows. However the short trial period and small panellist size could have influenced the results (Tekippe et al., 2011).

5.4 Conclusion

The inclusion of oregano oil in the diet of dairy cows, influenced the sensory characteristics, aroma and flavour, of milk. The reason for the control and treatments groups to have differed from one another was not due to a prominent herb like aroma or flavour, but possibly due to their respective fat contents. Further studies can be done to investigate whether or not the process of pasteurization could influence the sensory attributes of milk. The measurement of the volatile components in the different feeds could determine the influence they have on the milk composition and thus possibly influence the sensory attributes of the milk. The triangle test is a robust test that cannot quantify differences, therefore different sensory evaluating techniques can be investigated in order to quantify the differences. It was concluded that the pertinent oregano aroma of the experimental feed was not transferred to the milk.

5.5 References

Raw Milk vs. Pasteurised Milk. 2014. Armchair Science Journal, February.

Tekippe, J.A., Hristov, A.N., Heyler, K.S., Cassidy, T.W., Zheljazkov, V.D., Ferreira, J.F.S., Karnati, S.K. & Varga, G.A., 2011. Rumen fermentation and production effects of *Origanum vulgare* L. leaves in lactating dairy cattle. J. Dairy Sci. 94: 5065-5079.

CHAPTER 6

General Conclusion

During the production study, forty lactating Holstein cows were grouped into two groups of twenty. The groups were housed in the same semi-open free stall barn, only separated by gates. Both groups received the same forage and semi-complete feed and were fed the same amounts thereof. In addition to their regular diet, the treatment group received a maize-meal based supplement with the inclusion of the oregano oil extract, at a single dose, whereas the control group received the maize-meal supplement without the oregano oil extract. When looking at the milk production results of all the cows, there were no differences between treatments for milk yield. There were no differences in the milk fat content and milk fat yields between the groups. The milk protein content was higher at the beginning of the trial for the Dosto (DOS) group in relation to the control (CON) group however, towards the end of the trial the milk protein content was higher for the CON group in comparison to the DOS group. No differences were found for milk protein yields between the groups. Lactose content was higher for the CON group during the first couple of weeks, in relation to the DOS group. Energy corrected milk (ECM) yields also did not differ between treatments. Body weight presented no differences between the groups. The results from the ten top milk producing cows presented no differences between treatments for milk yield, milk protein content and milk protein yield. The milk fat content as well as the milk fat yield, of the DOS group was, however, higher than that of the CON group during the first couple of weeks. The lactose content was higher during the first week for the CON group in comparison to the DOS group. There was a strong tendency ($P = 0.055$) for mean ECM to be higher in the DOS group than in the CON group. No differences were observed between treatments for body weight. After a two month period 12 milk samples were collected from each group and was sent to be evaluated. The microbiological quality of the milk samples was evaluated by using petrifilm plates for total aerobic counts (TAC) and coliform counts (CC). Based on the microbiological analysis, all the milk samples were considered suitable for consumption (< 200 000 cfu/ml). The treatment group differed ($P \leq 0.001$) from the control group in terms of aroma and flavour.

In conclusion the addition of oregano essential oil in dairy cow diets stimulated milk fat production and increased energy corrected milk yield in high milk producing dairy cows. Oregano essential oil had no adverse effect on milk aroma and flavour.

CHAPTER 7

Critical Evaluation

Seasonal change: The trial started in winter and ended in spring. During the winter months, problems occurred with severe rain and muddy conditions. However, the chilled conditions seemed to have had a positive influence on the appetite of the cows, as they consumed the supplements more efficiently during this time. During the spring period the temperatures soared and the comfort of the cows during milking times were affected. This period also brought back flies, which caused irritability amongst the cows. The combination of heat and flies influenced the efficiency in the consumption of the supplements and may have influenced the milk production.

Housing facilities: Although the groups were housed in two groups in the same housing structure and were separated by gates, it would have been more beneficial if the cows could be allocated to individual pens. This would allow for recording individual feed intake and it would have ensured that the cows received the same amount of feed, whereas in the current study, the dominant cows got to the feed first.

Form of supplementation: For this trial the supplement was fed in a meal form. The cows firstly were not accustomed to being fed in the milking parlour, during milking. Secondly, they were not used to this form of feed. Because the cows were used to a pelleted feed, it would be beneficial to feed the supplement in a pellet form to ensure intake.

Additional supplement testing: Do to the demand of the supplier of the Dosto Concentrate 500 product, a maize based supplement was used as the control treatment. In addition to the control and Dosto treatments, it would have been beneficial to have tested the effect of an antibiotic feed additive, such as monensin, in comparison to the oregano additive and the placebo. However, funds and the number cows available for the trial were limited.

Appendix A

Critical values table for triangle test

Table A.1 Minimum numbers of correct responses to reject the null hypothesis of 'no difference' at selected significance levels with a total number of assessors 'n'.

n	Significance (%)						n	Significance (%)					
	30	**20**	**10**	**5**	**1**	**0.1**		**30**	**20**	**10**	**5**	**1**	**0.1**
5	3	4	4	4	5	-	**33**	13	14	15	17	18	21
6	3	4	5	5	6	-	**34**	13	15	16	17	19	21
7	4	4	5	5	6	7	**35**	13	15	16	17	19	22
8	4	5	5	6	7	8	**36**	14	15	17	18	20	22
9	4	5	6	6	7	8	**42**	17	18	19	20	22	25
10	5	6	6	7	8	9	**48**	19	20	21	22	25	27
11	5	6	7	7	8	10	**54**	21	22	23	25	27	30
12	5	6	7	8	9	10	**60**	23	24	26	27	30	33
13	6	7	8	8	9	11	**66**	25	26	28	29	32	35
14	6	7	8	9	10	11	**72**	27	28	30	32	34	38
15	6	8	8	9	10	12	**78**	29	30	32	34	37	40
16	7	8	9	9	11	12	**84**	31	33	35	36	39	43
17	7	8	9	10	11	13	**90**	33	35	37	38	42	45
18	7	9	10	10	12	13	**96**	35	37	39	41	44	48
19	8	9	10	11	12	14	**102**	37	39	41	43	46	50
20	8	9	10	11	13	14	**108**	40	41	43	45	49	53
21	8	10	11	12	13	15	**114**	42	43	45	47	51	55
22	9	10	11	12	14	15	**120**	44	45	48	50	53	57
23	9	11	12	12	14	16	**126**	46	47	50	52	56	60
24	10	11	12	13	15	16	**132**	48	50	52	54	58	62
25	10	11	12	13	15	17	**138**	50	52	54	56	60	64
26	10	12	13	14	15	17	**144**	52	54	56	58	62	67
27	11	12	13	14	16	18	**150**	54	56	58	61	65	69
28	11	12	14	15	16	18	**156**	56	58	61	63	67	72
29	11	13	14	15	17	19	**162**	58	60	63	65	69	74
30	12	13	14	15	17	19	**168**	60	62	65	67	71	76
31	12	14	15	16	18	20	**174**	62	64	67	69	47	79
32	12	14	15	16	18	20	**180**	64	66	69	71	76	81

APPENDIX B

Cows used during production study

Table B.1 Cows grouped in the control group, had green bands around their tails and were grouped according to milk yield, DIM and lactation number.

Index	Cow Number	Calving Date	Days in milk	Lactation Number	Milk Yield (kg/day)
1	8	2014/07/04	32	2	31.3
5	123	2014/05/13	84	3	38.0
11	701	2014/07/26	10	5	36.3
17	823	2013/12/27	221	4	31.6
19	907	2013/12/19	229	3	29.3
20	913	2013/12/02	246	2	39.4
21	918	2013/11/18	260	2	31.0
22	1002	2013/12/24	224	2	30.0
23	1008	2013/10/08	301	2	27.7
24	1009	2013/12/28	220	2	28.7
25	1013	2014/06/03	63	2	40.9
27	1020	2013/11/13	265	2	26.2
29	1033	2013/12/12	236	2	25.5
31	1104	2014/05/09	88	2	35.7
32	1105	2014/03/18	140	2	33.3
35	1108	2013/10/28	281	1	29.5
36	1114	2013/11/04	274	1	32.4
37	1120	2013/10/28	281	1	25.3
38	550418	2014/04/25	102	7	33.7
39	551015	2014/06/24	42	3	38.2

Table B.2 Cows grouped in the treatment group, had red bands around their tails and were grouped according to milk yield, DIM and lactation number.

Index	Cow Number	Calving Date	Days in milk	Lactation Number	Milk Yield (kg/day)
2	22	2013/12/08	240	2	28.5
3	71	2013/12/02	246	4	30.9
4	98	2013/08/03	367	4	26.5
6	382	2014/05/23	74	6	29.9
7	415	2014/06/22	44	7	37.5
8	524	2014/02/14	172	5	36.0
10	618	2014/08/03	4	6	--
13	801	2013/12/06	242	3	26.0
14	802	2014/07/29	7	5	30.7
15	813	2014/01/30	187	4	40.1
16	822	2014/06/23	43	4	37.3
18	826	2013/08/18	352	3	32.9
26	1014	2014/07/06	30	1	27.9
28	1029	2014/02/02	184	2	35.4
30	1037	2013/10/27	282	2	28.8
33	1106	2013/09/16	323	1	32.8
34	1107	2013/11/29	249	1	24.2
1	1122	2014/02/07	179	1	23.8
1	550509	2014/08/18	7	4	38.5
40	551032	2013/11/05	273	2	33.8

APPENDIX C

Top producing cows

Table C.1 Ten top producing cows from the control group.

Index	Cow Number	Calving Date	Days in milk	Lactation Number	AVE Milk Yield During Trial (kg/day)
1	8	2014/07/04	32	2	33.7
5	123	2014/05/13	84	3	37.7
11	701	2014/07/26	10	5	49.4
20	913	2013/12/02	246	2	36.8
25	1013	2014/06/03	63	2	40.4
31	1104	2014/05/09	88	2	33.4
32	1105	2014/03/18	140	2	31.9
36	1114	2013/11/04	274	1	33.9
38	550418	2014/04/25	102	7	37.3
39	551015	2014/06/24	42	3	38.2

Table C.2 Ten top producing cows from the treatment group.

Index	Cow Number	Calving Date	Days in milk	Lactation Number	Milk Yield (kg/day)
7	415	2014/06/22	44	7	40.4
8	524	2014/02/14	172	5	34.0
10	618	2014/08/03	4	6	44.4
14	802	2014/07/29	7	5	46.8
15	813	2014/01/30	187	4	43.6
16	822	2014/06/23	43	4	33.0
28	1029	2014/02/02	184	2	32.9
33	1106	2013/09/16	323	1	33.3
1	550509	2014/08/18	7	4	37.2
40	551032	2013/11/05	273	2	33.3