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**Effects of palm oil supplementation and  
fibrolytic enzymes in high forage diets on growth  
and carcass characteristics of lambs**

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By

Lené van der Walt

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Supervisor: Professor E.C. Webb

Co-supervisor: Dr A Hassen

“Nothing can stop God’s plan for your life” Isaiah 14:27

## **Declaration:**

I declare that this dissertation, which I hereby submit in partial fulfilment of the requirements for the degree MSc (Agric) Animal Science: Production Management in the Department of Animal and Wildlife Sciences at the University of Pretoria is my own work and has not been previously submitted by me for a degree at this or any other tertiary institution. I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by the University of Pretoria will not infringe any third-party rights.

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Lené van der Walt

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## Abstract

This study formed part of a greater departmental research project on methane mitigation strategies in livestock, fed poor quality feeds or kept under extensive grazing conditions. Globally, livestock represents a large source of methane (CH<sub>4</sub>) from anthropogenic activities, mostly from enteric fermentation by ruminants. The aim of this project was to study the effects of dietary methane mitigation interventions on growth and carcass characteristics, due to changes in the composition of adipose tissues in livestock. Feasible methane mitigation technologies can only be adopted once the effect on the entire production cycle has been evaluated, including the potential adverse effects of such technologies on product composition and quality. Although previous research has focused on the manipulation of carcass composition by dietary oil and fat supplementation, the novelty of the present study is to investigate the possible consequences of supplementing fibrolytic enzymes and palm oil on the fatty acid composition and the related effects on carcass and meat quality of livestock. In this study 40 South African Mutton Merino (SAMM) ram lambs were stratified according to their initial body weight and randomly assigned to one of the following four treatment groups with ten lambs as a sampling unit for each treatment. The lambs were fed high-forage based total mixed rations (TMR) that consisted of a TMR diet supplemented with Megalac as bypass oil (C); TMR supplemented with 3% palm oil (PO); TMR with cellulase and xylanase enzymes (1:1) (ET) and TMR supplemented with 3% palm oil and cellulase and xylanase enzyme (O\*E), for approximately 120 days. After approximately 100 days and target weight of ca. 42kg, all lambs were slaughtered at a commercial abattoir, carcasses were electrically stimulated (21V, 60Hz, 120s) and chilled at 4°C for 24 hours. Hot carcass weight (HCW), cold carcass weight (CCW) and pH of carcasses were recorded, followed by collection of three rib-cut samples to determine carcass composition and fatty acid profiles of subcutaneous and intramuscular adipose tissue. Lambs fed treatment ET and C gained 20g more per day than those in the PO and O\*E treatments. Lambs in treatment PO took 8 days longer to reach target slaughter weight. HCW and CCW were higher in treatments C and ET than in treatments PO and O\*E. The average HCW 19.24±1.5kg was within the industry norm. Dressing percentages in this study were in line with industry averages of 45%, even though treatments PO and O\*E had a ± 3% lower dressing percentage compared to other treatment groups. Supplementation of palm oil

increased meat % of carcass composition by more than 4%. A lower percentage carcass fat was observed in treatment PO and O\*E. The proportion of fatty acids from this study was similar across all four treatments groups; numerically these values were so small, even negligible in some cases. Interaction effects were detected between oil and enzyme treatments for SFA's, MUFA's and the UFA's in subcutaneous adipose tissue. Oil treatment groups in the present study differed from the other treatment groups and generally reduced the PUFA concentration. Palm oil supplementation affected the subcutaneous fatty acid profiles of lambs, which include C14:0, C16:0, C17:0, C18:0, C18:2n6c, C20:3n6 and C20:4n6. A general decrease in SFA concentration was observed for oil treatments in all of the SFA where oil supplementation had a significant effect, except for stearic acid (C18:0) where PO treatment increased the fatty acid concentration by almost 3%. Palm oil supplementation decreased linoleic acid (C18:2n6c) concentration, but tended to increase the EPA (C20:3n6) in subcutaneous tissue. Similar effects were observed in fatty acids of intramuscular adipose tissue. Fatty acid concentration for treatments PO and O\*E were lower in concentration of myristic acid (C14:0) and palmitic (C:16) fatty acid. Palm oil supplementation had a negative effect in stearic acid (C18:0) and arachidic acid (C20:0) and increased the fatty acid concentrations compared to treatment C and ET. Interestingly ET treatment had an increasing effect on margaric (C17:0) fatty acid concentration compared to treatment PO and O\*E. Overall where oil treatment groups were of significance a reduction in fatty acid concentrations was observed in PO treatment. A decrease in fatty acid concentration was observed in PO treatment that were highly significant in linoleic acid (C18:2n6) and  $\alpha$ -linolenic acid (C18:3n3). If methane mitigation strategies through dietary interventions are successful in lowering the carbon footprint it can be concluded that although palm oil and/or enzyme treatment groups showed significant effects in some of the evaluated parameters, mentioned above, numerically these values were minor, and probably negligible. Although lambs in treatment groups supplemented with oil took a few days longer to reach target weight, differences between treatment groups for all other parameters, ADG, HCW, CCW, pH at 24 hours and D% (dressing percentage) were small. With no negative effects on carcass characteristics or fatty acids consumer resistance should not be at risk.

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## Abbreviations

ADG	Average daily gain
C	Carbon
C: N	Carbon to nitrogen ratio
ca.	Approximately / (circa)
CH <sub>4</sub>	Methane
CLA	Conjugated linoleic acid
DM	Dry matter
DMI	Dry matter intake
FA	Fatty acid
FAO	Food and Agriculture Organization
H <sup>+</sup>	Hydrogen cation
LCFA	Long chain fatty acid
MUFA	Monounsaturated fatty acids
N	Nitrogen
n-3	Omega-3 fatty acid
n-6	Omega-6 fatty acid
n-9	Omega-9 fatty acid
P: S	Polyunsaturated to saturated fatty acid ratio
PUFA	Polyunsaturated fatty acids
SAMM	South African Mutton Merino
SCFA	Short chain fatty acids
SFA	Saturated fatty acids
VLCPUFA	Very long chain polyunsaturated fatty acid

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## Chapter 1

### Introduction and motivation

#### 1.1 Project theme

An Investigation of the consequences of enteric methane mitigation technologies on product quality and the physiological effects of such strategies on fat composition and content.

#### 1.2 Project title

Effects of enteric methane mitigation strategies on growth and carcass characteristics of lambs fed high-forage diets.

#### 1.3 General introduction

This study formed part of a greater departmental research project on methane mitigation strategies in livestock through dietary supplementation of fibrolytic enzymes and oil aimed to improve the utilization of poor quality feeds by livestock under extensive grazing conditions. This project was divided into two phases.

Phase one was completed by another student where the project aims were to investigate the effect of dietary treatments on feed intake, nutrient digestibility and efficiency of utilization high fibre diets. In this study methane production and emission per lamb was expected to reduce due to increased nutrient digestion and rumen fermentation associated to dietary supplementation of fibrolytic enzymes or oil or a combination of both. The results of this project were reported separately.

Although there are published research on manipulation of carcass composition with oils and fats, this was not the primary focus of this investigation. The focus of the present study, phase two, was to specifically investigate the possible consequences of these mitigation technologies, i.e. dietary supplementation of fibrolytic enzymes and oil, on product quality and its physiological effects on fat composition and content. It is crucial to study the effects of the methane mitigation strategies on the extrinsic and intrinsic aspects of carcass and meat quality of sheep to ensure an acceptable product quality.

In order to study mitigation technologies, it is important to assess on a whole farm basis to consider the whole production cycle, including potential adverse effects on product quality.

#### **1.4 Aim**

The aim of this research was to study the effects of methane mitigation strategies through dietary interventions on product quality. This was done by supplementing sheep on a high-forage diet with fibrolytic enzymes (cellulase and xylanase) and / or palm oil.

#### **1.5 Motivation**

Methane mitigation strategies focused on improved utilization of high fibre diets resulting in lowered methane production per animal product under extensive production systems. In addition, a shift in rumen fermentation due to fibrolytic enzymes and addition of palm oil may affect muscle and fat metabolism. This inspires the novelty of this research to investigate the possible consequences of these mitigation technologies on fatty acid synthesis and the related effects on carcass and meat quality in livestock.

Production systems affect both extrinsic and intrinsic aspects of carcass and meat quality in livestock. Extrinsic aspects that are of interest include growth, efficiency, average daily gain and carcass weight. While intrinsic factors of animal products that could be affected include dressing percentage, carcass composition (bone, muscle and fat content), carcass fat content and colour.

Appearance, taste and nutritional value are two important quality attributes of meat and no methane mitigation strategy will be adopted if there are adverse effects on product quality (Webb & O'Neill, 2008).

## Chapter 2

### Literature review

#### 2.1 Introduction

Roughgarden (1979) mentioned that the significant growth in human population, higher income, longer life expectancy along with changing food preferences, are rapidly increasing the demand for livestock products, while globalization is boosting trade in livestock inputs and products. Rosegrant (2001) reported that the world has experienced an unprecedented escalation in population growth during the past century, with a billion-people added every decade during the last three decades alone. The world's population is expected to grow from six billion people in 2000 to 7.5 billion people in 2020. According to the United Nations, the world population reached seven billion on October 31, 2011, after the USA Census Bureau estimated that the 7 billion mark should only have been reached on March 12, 2016. (<<http://www.worldometers.info/world-population/>>. Accessed 2016 December 20.) Thus, the population is already increasing at a much faster rate than was anticipated.

It was estimated that the global livestock production has to double by 2050 to meet the rising global population (Ilea, 2009). This too can be an underestimated value and with current data it can be speculated that livestock production has to almost triple to meet future demands. In South Africa, economic and environmental conditions significantly influence production systems and could lead to shifts from an extensive production system to a more intensive production system before marketing.

Sheep are kept and bred in large grazing systems in South Africa. The majority are fattened and marketed directly from these grazing systems while small proportions are generally fattened for short periods in feedlots to ensure efficient production and meet market requirements.

This proposes a few challenges for methane mitigation strategies. One challenge is to develop ways to deliver methane mitigating technologies to livestock in extensive grazing conditions with limited labour and human interaction. A related challenge is to develop cost effective strategies that will not have negative effects on ruminant production for different diets. The basic principle is to increase the

digestibility of feedstuffs, either by modifying feed or by manipulating the digestive process.

National Research Council (U.S.A) (2001) the committee on animal nutrition mentioned that the public is concerned about how diet impacts health and risk for disease. Reports in the news and social media often confuse consumers because it is sometimes difficult for media reporters and writers to evaluate scientific and clinical studies; thus, important messages may be disregarded, misinterpreted, or downplayed.

One of the first nutrients altered by the food industry was fat, because of its implications in cardiovascular disease, stroke, and cancer. Food scientists and human nutritionists introduced low-fat foods, which led to a decline in saturated fat intake. The composition of beef, pork, and poultry products has been altered through genetic selection and through the identification of nutrition requirements by the National Research Council's Committee on Animal Nutrition (National Research Council (U.S.A) Committee on Animal Nutrition, 2001).

Farmers are unlikely to adopt new measures unless they are cost effective and there is a positive economic impact on animal production.

## 2.2 Enteric methane production from sheep

Globally enteric methane (CH<sub>4</sub>) emissions from livestock can be regarded as the single most significant source of greenhouse gas emissions from human-related activities (Cottle *et al.*, 2011).

Several reviews of enteric CH<sub>4</sub> production and mitigation options have been published (Johnson & Johnson, 1995; Boadi *et al.*, 2004; Monteny *et al.*, 2006; Beauchemin *et al.*, 2008; De Klein & Ekard, 2008; McAllister & Newbold 2008; Rowlinson *et al.*, 2008; Buddle *et al.*, 2011; Eckard *et al.*, 2010; Hegarty *et al.*, 2010; Martin *et al.*, 2010; Shibata & Terada, 2010; Cottle *et al.*, 2011).

Methane is generated prolifically in the digestive tracts of ruminant livestock. Ruminants can convert otherwise unusable plant materials into nutritious food and fibre in the rumen which produces CH<sub>4</sub> as a by-product of microbial fermentation of fibrous feeds (breakdown of cellulose and other macromolecules) by enteric fermentation. Both the farmer and the environment would benefit substantially from methane mitigation strategies to reduce enteric CH<sub>4</sub> production resulting in reduced emissions, improved feed efficiency and system efficiency.

Many factors influence enteric CH<sub>4</sub> emissions, which varies among animal species and among individuals of the same species. These factors include level of feed intake, type of carbohydrate in the diet, feed processing, addition of lipids or ionophores to the diet, alterations in the rumen microflora, energy intake and several other animal and diet factors like quantity and quality of feed, animal body weight and age (Johnson & Johnson, 1995).

Manipulation of these factors can reduce CH<sub>4</sub> emissions. Many techniques exist to quantify CH<sub>4</sub> emissions from individual or groups of livestock. Enclosure techniques are precise but require trained animals and may limit movement. Isotopic and non-isotopic tracer techniques may also be used effectively. Prediction equations based on fermentation balance or feed characteristics have been used to estimate CH<sub>4</sub> production. These equations are useful, but the assumptions and conditions that must be met for each equation limit their ability to accurately predict CH<sub>4</sub> production. Methane production from groups of animals can be measured by mass balance, micrometeorological, or tracer methods. These techniques can measure CH<sub>4</sub> emissions from animals in either indoor or outdoor enclosures. Use of these techniques and knowledge of the factors that impact CH<sub>4</sub> production can result in the development of mitigation strategies to reduce CH<sub>4</sub> losses. Implementation of these



strategies should result in enhanced animal productivity and decreased contributions to the atmospheric CH<sub>4</sub> budget (Johnson & Johnson, 1995).

The particular practices a livestock producer uses to improve production will depend on the circumstances of his or her operation, including the goals to be achieved and the natural, financial, and labour resources available.

### 2.3 Methane mitigation strategies in livestock

A number of technologies exist to reduce CH<sub>4</sub> from enteric fermentation. Methane emissions can be reduced through management strategies that improve productivity and efficiency of livestock production and result in lower emissions per unit of milk or meat produced. The basic principle is to increase the digestibility of feedstuffs, either by modifying feed or by manipulating the digestive process. Nutritional management strategies promise to have the greatest short-term impact (Cottle *et al.*, 2011). Most technologies to control CH<sub>4</sub> production have not proved cost effective. Mitigation strategies aimed at reducing populations of methanogens usually involve inhibition of methanogens and include alternatives of removal of hydrogen ions so that fermentation is not impeded. Greater efficiency means that a larger portion of the energy in the animals' feed is directed toward production (milk, meat and draught power), so that CH<sub>4</sub> emissions per unit product are reduced (Keogh & Cottle, 2009, Cottle *et al.*, 2011).

United States Environmental Protection Agency US-EPA (2005) listed a series of management measures that could improve a livestock operation's production efficiency and reduce greenhouse gas emissions in numerous ways: Through management strategies, grazing management or pasture quality can be improved to increase animal productivity, which can reduce the amount of CH<sub>4</sub> emitted per unit of animal product. Nutritional strategies through rumen manipulation include: modifications of the rumen microbial population (e.g. by vaccines, probiotics and defaunation) or gastrointestinal tract environment (feeding grain, fats, oils, antibiotics and tannins); replacing roughage with concentrate; inhibiting hydrogen-producing reactions or promoting alternative reactions which accept hydrogen cations (H<sup>+</sup>) during re-oxidation of reducing equivalents (Hegarty, 1999).

Another technical option is to increase the level of starch or rapidly fermentable carbohydrates in the diet, so as to reduce excess hydrogen and subsequent CH<sub>4</sub> formation. Improving the animal itself through genetics and reproductive efficiency which leads to increased productivity can be accomplished through breeding. (Steinfeld *et al.*, 2006).

Manure with a high nitrogen content will emit greater levels of CH<sub>4</sub> than manure with lower nitrogen contents. Hence increasing the carbon to nitrogen ratio (C: N) in feeds can reduce emissions by improved manure management (Monteny *et al.*, 2006).

More advanced technologies that are also being studied are reduction of hydrogen production by stimulating acetogenic bacteria, defaunation eliminating certain protozoa from the rumen and vaccination to reduce methanogens (Monteny *et al.*, 2006).

Despite extensive research to identify nutritional strategies that reduce enteric CH<sub>4</sub> production, on-farm adaption is expected to be slow and mainly limited to measures that improve feed efficiency, such as lipid supplementation.

## 2.4 Effect of supplemented palm oil on growth and carcass characteristics

The type and amount of fat in the ration considerably affect the amount, distribution and composition of body fat, which in turn largely determines carcass and meat quality (Solomon *et al.*, 1991; Nürnberg *et al.*, 1998; Bas & Morand-Fehr, 2000; Castro *et al.*, 2005).

Palm oil contains a high percentage of saturated fatty acids (SFA), mainly palmitic acid (16:0) plus myristic acid (14:0) (Manso *et al.*, 2009). A study was done on twenty-seven lambs to investigate the effects of the inclusion of 4% hydrogenated palm oil (HPO) or sunflower oil (SFO) in the concentrate on animal performance, carcass and meat quality and fat characteristics and fatty acid composition. Neither HPO nor SFO affected any of the carcass characteristics studied, meat pH and meat and fat colour ( $p < 0.05$ ). Over the last decade, fat supplementation became a common practice to increase the energy density of the diet for ruminants (Bauman *et al.*, 2001), with palm oil supplements being the most used, since it does not have the negative effects on rumen fermentation as unsaturated oils (Jenkins, 1993). Lamb fat is characterized by a high SFA content, and low a polyunsaturated fatty acid (PUFA) content (Enser *et al.*, 1996), due to the bio-hydrogenation of unsaturated fatty acids (UFA) by rumen microflora (Doreau & Ferlay, 1994).

Inclusion of lipids in the diet has the potential to reduce CH<sub>4</sub> production (Beauchemin *et al.*, 2008; Moate *et al.*, 2011). This mitigation strategy is of questionable value as a small reduction in CH<sub>4</sub> production means the cost to benefit ratio is low. There is an increasing body of literature to indicate that supplementation of diet with lipids that are not protected from gastric digestion reduces enteric CH<sub>4</sub> emissions. Generally, it is recommended that total fat should not exceed 6-7% of dietary dry matter (DM) otherwise a depression in dry matter intake (DMI) may occur. Dietary lipid supplementation reduces CH<sub>4</sub> emissions by decreasing ruminal organic matter fermentation, the activity of methanogens and protozoal numbers, and for lipids rich in UFAs, through hydrogenation of fatty acids (Johnson & Johnson, 1995). With LCFA sources, CH<sub>4</sub> emissions are partly lowered through reduced fibre digestion (McGinn *et al.*, 2004; Beauchemin *et al.*, 2007) and decreased DMI (Jordan *et al.*, 2006; Beauchemin & McGinn, 2006) The inclusion of palm oil at 3% in the diet did not affect ( $p < 0.05$ ) growth rate or carcass weight (Karami *et al.*, 2013).

Dutta *et al.* (2008) suggested that including palm oil in the diet could improve average daily gain (ADG) and animal performance. Previous research has shown that adding fats to the diet for fattening lambs did not cause reduced animal performance or modification in carcass characteristics (Haddad & Younis, 2004).

The effects of supplemental palm oil on nutrient utilization, feeding economics and carcass characteristics in post-weaned lambs under feedlot condition were observed by Dutta *et al.* (2008) and results indicated that addition of 5% palm oil in a concentrate mixture improved growth performance and feed conversion efficiency of weaned lambs keeping the quality of meat unaffected (Dutta *et al.*, 2008).

Lough *et al.* (1993) recorded greater ADG and feed efficiency in lambs fed a high-forage diet supplemented with palm oil (10.7%). Ruminant CH<sub>4</sub> production was reduced by 34% with palm kernel oil, thus lambs had more energy for growth with a decreased loss of energy from the rumen (Musselman, 2000). Supplements containing lipids high in PUFA (omega-3 fatty acids) or supplements with lower lipid content but at rates sufficient for lipid to exceed 5% of total DMI may lead to a decrease in the extent of fibre digestion (Doreau *et al.*, 1997). This is mainly due to modified ruminal microbial eco-system (Doreau & Chilliard, 1996). Addition of palm oil in the concentrate mixture increases the energy density of feed and supplies some PUFA to the fast-growing lambs as well. Dressing percentage (D%) is affected by stomach content and skin (wool) when live weights were recorded, and is known to increase with animal weight and degree of fatness (Kirton *et al.*, 1995).

Action is required if, as predicted, global meat production is projected to more than double from 229 million tonnes in 1999/2001 to 465 million tonnes in 2050. The U.S. Food and Agriculture Organization estimates that agricultural CH<sub>4</sub> output could increase by 60 percent by 2030 (Steinfeld *et al.*, 2006). The strategy used to decrease CH<sub>4</sub> by including palm oil is favourable, but its adoption will be challenged by the high cost of oil, and its direct competition with human food.

Taste and nutritional value are two important quality attributes of meat and no CH<sub>4</sub> mitigation strategy will be adopted if there are adverse effects on meat quality.

## 2.5 Fatty acids

Fat is an unpopular constituent of meat for consumers, being considered unhealthy. Modern recommendations range from excluding fats all together, to a moderate consumption of fats due to its essential role in the body.

Besides a lower total fat intake, human nutritionists are recommending a higher intake of polyunsaturated fatty acids (PUFA), and especially of n-3 fatty acids at the expense of n-6 fatty acids (Department of Health, 1994).

In most Western-type diets SFA content should be reduced and the PUFA content increased (World Health Organization (WHO), 2003).

The emphasis has shifted away from fat quantity to fat quality. This approach may adversely affect future meat consumption by consumers who are becoming increasingly critical about the food they eat (Laaksonen *et al.*, 2005; Ohlund *et al.*, 2007).

Fatty acid composition in meat has long been studied but still receives a lot of attention in research because of its implications in human health. Mutton meat is characterized as having a high fat content thus seen to be a major source of fat in the diet. Being high in SFA and having a low polyunsaturated: saturated ratio (P:S), it has been implicated in diseases associated with modern life which include various cancers and especially coronary heart disease (Milicevic D., 2014). For this reason, ways to improve the P:S during meat production is required (Enser *et al.*, 1996) This has led to studies on ways to manipulate the fatty acid composition of meat. Research anticipated that the most effective strategy of improving fatty acid composition is through dietary manipulation. Field *et al.*, (1978) demonstrated that diet can influence fatty acid composition in ram lambs and that diet was an important consideration in the production of ram lambs for meat.

Western diets tend to contain 11–30 times more omega-six (n-6) fats than the omega-3 (n-3) fats, which has been hypothesized as a significant factor for the rising rate of inflammatory disorders and obesity (Palmquist, 1994; Simopoulos, 1999; Calder, 2004). Reducing the levels of n-6 or increasing the n-3 fats in diets will lead to muscles with more favourable n-6 to n-3 ratios (Givens *et al.*, 2006) which may help to improve consumer health (Wood & Enser, 1997). The addition of lipids to ruminant feed is a tool used when an increase in the energy density of the diet is desired without increasing the proportion of grains as a source of energy, since grains represent a

more expensive fraction of the diet (Silva *et al.*, 2011). The major sources of lipids with SFA's include palm oil, palm kernel oil, tallow and lard.

The association between animal fat and cardiovascular disease has been studied and dietary fat has also been hypothesized to increase the risk of colorectal cancer (Lin J.,2004). However, a pooled analysis of results from thirteen case-control studies reported no association between dietary fat intake and colorectal cancer risk (Lin *et al.*, 2004; Webb & O'Neill, 2008).

Fat and LCFA, whether in adipose tissue or muscle, contribute to important aspects of meat quality and are central to the nutritional and sensory values of meat (Webb & O'Neill, 2008).

There are no inter-conversions between the n-6 and n-3 fatty acid family, but the presence of one of them may suppress the conversion of the other. In ruminants n-6 and n-3 containing dietary fats are hydrolysed by rumen microorganisms and hydrogenated to mainly stearic acid. During some feeding conditions, the dietary linoleic (C18:2n6) and  $\alpha$ -linolenic (C18:3n6) acid are only partially hydrogenated, whereby oleic acid and trans-, odd chain-, branched chain-, and conjugated fatty acids are formed (National Research Council (U.S.A) Committee on Animal Nutrition, 2001).

In the fat and fatty acid profile of lean meat from the most commonly used food producing animals it is seen that SFA's amount to 33–46%, MUFAs to 26–47%, and PUFA's to 10–38% of total fatty acids. Of the n-6 fatty acids C18:2n-6 is the most abundant in ovine tissue (Webb & O'Neill, 2008).

### 2.5.1 Dietary essential fatty acids and non-essential fatty acids

The n-3 and n-6 fatty acids function as carriers of the fat-soluble vitamins A, D, E, and K and play a crucial role in the immune response of both man and animal.

Linoleic (C18:2n6) and  $\alpha$ -linolenic acid (C18:3n3) are essential fatty acids with 18-carbon molecules, which cannot be synthesized in the mammalian organism, and therefore must be supplied in the diet of animals and man. These fatty acids are precursors for the longer chain, higher PUFA's of the n-6 and n-3 fatty acid families, which are formed in the tissues by chain elongation and desaturation processes (Mohrhauer & Holman, 1963).

According to Smith (2007), the two most important 20-carbon essential fatty acids are arachidonic acid (C20:4n6), which is formed by desaturation and elongation of linoleic acid, and eicosapentaenoic acid (C20:5n3, EPA) which is formed by desaturation and elongation of  $\alpha$ -linolenic acid (C18:3n6).

Non-essential fatty acids (NEFA) are those fatty acids that are not necessarily provided for by the diet only but which the animal can synthesize from acetyl CoA with various co-factors. The major NEFAs are glycerophospholipids and sphingolipids which are the 16- and 18-carbon SFA's and n-9 MUFAs (Smith, 2007).



### 2.5.2 Composition of fatty acids in tissues

Fatty acid composition determines the firmness of adipose tissue and the oxidative stability of muscle, which in turn affects flavour and muscle colour (Wood et al., 2004).

Since the turn of the century nutritionists have focussed on the type of PUFA and the balance in the diet between n-3 PUFA's formed from  $\alpha$ -linolenic acid (C18:3) and n-6 PUFA formed from linoleic acid (C18:2) (Williams, 2000). The n-6:n-3 of PUFA is also a risk factor in cancers and coronary heart disease, especially the formation of blood clots leading to a heart attack (Enser, 2001). The recommendation is for a ratio of less than four. As with the P:S, meat can be manipulated towards a more favourable n-6:n-3. The increasing awareness of the need for diets to contain higher levels of n-3 PUFA has focused on the importance of meat as a natural supplier of these to the diet. The n-6:n-3 PUFA is particularly beneficial (low) in ruminant meat, especially from animals that have consumed grass which contains high levels of C18:3. Ruminants also naturally produce conjugated linoleic acid (CLA) which may have a range of nutritional benefits in the diet (Enser, 2001). Fatty acids are involved in various "technological" aspects of meat quality. Groups of fat cells containing solidified fat with a high melting point appear whiter than when liquid fat with a lower melting point is present, so fat colour is another aspect of quality affected by fatty acids (Wood et al., 2002). The ability of UFAs, especially those with more than two double bonds, to rapidly oxidise, is important in regulating the shelf life of meat (rancidity and colour deterioration) (Wood et al., 2004).

### 2.5.3 Manipulation of fatty acids composition in sheep

Several reviews have been published covering studies describing manipulation of fatty acid composition of meat (Nürnberg *et al.*, 1998; Demeyer & Doreau, 1999; Jakobsen, 1999; Wood *et al.*, 2004), but paying less attention to long chain PUFA. In most studies, an increased n-3 content in the intramuscular fat was accompanied with a decreased n-6 deposition, mainly due to a lower n-6 dietary supply between treatments. This resulted in a more favourable n-6: n-3. Within dietary PUFA, it is recommended that the intake of fatty acid of the n-6 series (e.g. linoleic acid; C18:2n6) should remain constant whilst that of the n-3 series (e.g.  $\alpha$ -linolenic acid; C18:3n3) and in particular the very-long-chain n-3 fatty acid associated with a reduction in coronary heart disease, eicosapentaenoic acid [EPA (C20:5n3)] and docosahexaenoic acid [DHA (C22:6n3)] should be increased (Department of Health, 1994).

The presence of micro-organisms in the rumen makes fatty acid composition in beef and sheep more difficult to manipulate through a diet than in monogastric species. Nevertheless, there are some clear effects of diet on tissue fatty acid composition in ruminants due to extensive microbial biohydrogenation.

Grass is a naturally rich source of C18:3n-3. Other factors that may influence the fatty acid content and composition of grass include variety and ensiling conditions such as wilting, and additive use (Dewhurst & King, 1998). Drying and storage of forages also appears to decrease C18:3n-3 and increase C16:0, especially under conditions of high humidity (Noble, 1981). Total UFA in subcutaneous fat were higher in diets that contained > 72% maize: roughage. Total PUFA followed the same trends; higher amounts were present in diets that were high in maize. Total UFA were higher in fat from heavier lambs, but total PUFA decreased as weight increased (Field *et al.*, 1978)

Sinclair, 2007 reported that despite fresh forage being a particularly rich source of C18:3n-3 and vegetable oils being high in 18:2n-6 and 18:3n-3. The process of biohydrogenation in the rumen generally results in proportionally less than 0.1 of these essential dietary fatty acids reaching the small intestine. Increases in muscle content of  $\alpha$ -linolenic fatty acid (C18:3n3) of 1–2-fold have been achieved by supplementation with oil, or oilseeds, whilst increases of 1–3-fold have been obtained from grazing grass compared to concentrates, but in general the polyunsaturated to saturated fatty

acid ratio (P:S) in mutton meat has remained low at approximately 0.2–0.3. Substantial improvements in the P:S ratio of up to 0.57 and increases in muscle and adipose tissue levels of C18:3n3 ( $\alpha$ -linolenic fatty acid) of up to 4 g/100 g fatty acid can be obtained, but rely on protecting dietary PUFA from bio-hydrogenation. Additionally, increasing tissue supply of  $\alpha$ -linolenic fatty acid (C18:3n3) will result in only a small improvement in muscle concentration of the nutritionally beneficial eicosapentaenoic (C20:5n3) and docosahexaenoic / DHA (C22:6n3), with meaningful increases relying on a dietary supply of these very-long-chain PUFA production of a fatty acid profile in mutton meat that is higher in PUFA, particularly the advantageous very-long-chain PUFA, and with flavour and eating characteristics that meet specific market preferences, is a suitable area for research.

Despite the apparent negative impact of ruminal metabolism on muscle fatty acid content, this process is often incomplete and several of the intermediaries can have positive effects on human health (e.g. trans-11 C18:1 and CLA) some of which have been shown to have metabolic and health-promoting properties (Ip *et al.*, 1999; Bauman *et al.*, 2001).

The ingestion of C18 PUFA's may affect a shift in bio-hydrogenation of these fatty acids so that larger proportions flow through to the duodenum in unesterified form (Duncan *et al.*, 1974; Mayes & Orskov, 1974; Orskov *et al.*, 1974; Orskov *et al.*, 1979; Casey & Van Niekerk, 1985; Casey *et al.*, 1988; Webb, 1992.) Lipids ingested in the food and synthesised in the rumen by micro-organisms are absorbed mainly in the duodenum and are transported in the plasma by specialised proteins, referred to as lipoproteins, to various sites in the body (Webb, 1992). When the diet is supplemented with esterified fatty acids, elevated concentrations of triglyceride in the digesta pass into the duodenum. The amount of lipid entering the duodenum is normally considerably larger than that ingested in the diet as Sutton *et al.* (1970) conveyed the number of fatty acids entering the duodenum ingested either on a high roughage and high concentrate diet to be respectively 40% and 104 % higher. It is obvious that microbial synthesis is responsible for these elevated concentrations of fatty acids in the duodenum.

#### 2.5.4 Factors influencing fatty acids composition in sheep

Wood et al. 2008, stated that the full range of PUFA is detected in sheep adipose tissue and muscle demonstrated in Table 1. Linoleic acid (C18:2n6) is a major ingredient of feeds for all species. It is deposited in muscle phospholipid at a high level where it and its long chain products e.g. arachidonic acid (C20:4n6) compete well for insertion into phospholipid molecules. In all species, the proportion of C18:2n6 (linoleic acid) declines in muscle as fat deposition increases. The main reason is that phospholipid, where linoleic acid (C18:2n6) is located, declines as a proportion of muscle lipid and the proportion of neutral lipid, with its higher content of saturated and MUFAs, increases. Oleic acid (C18:1cis9), formed from stearic acid (C18:0) by the enzyme stearoyl Co-A desaturase, is a major component of neutral lipid and in ruminants the same enzyme forms CLA. Like linoleic acid (C18:2n6),  $\alpha$ -linolenic acid (C18:3n3) is an essential fatty acid and is important to ruminants since it is the major fatty acid in grass. Greater bio-hydrogenation of C18:3n3 ( $\alpha$ -linolenic acid) and a long rumen transit time for forage diets also limits the amount available for tissue uptake compared with C18:2n6 from concentrate diets. A positive feature of grass feeding is that levels of the nutritionally important long chain n-3 PUFA are increased i.e. EPA (20:5n3) and DHA (22:6n3). Future research should focus on increasing n-3 PUFA proportions in lean carcasses and the use of biodiverse pastures and conservation processes which retain the benefits of fresh leafy grass offer opportunities to achieve this. The varying fatty acid compositions of adipose tissue and muscle have profound effects on meat quality.

**Table 1** Fatty acid composition (g/100g fatty acid) and content (g/100g total fatty acids in subcutaneous adipose tissue and muscle) of loin chops in sheep (*Enser et al.*, 1996)

Fatty acid methyl ester	Fatty acid content of fat (g/100g)	Fatty acid content of muscle (g/100g)
14:0	4.1	3.3
16:0	21.9	22.2
16:1 cis	2.4	2.2
18:0	22.6	18.1
18:1 cis - 9	28.7	32.5
18:2n-6	1.3	2.7
18:3n-3	1	1.37
20:4n-6	ND	0.64
20:5n-3	ND	0.45
n-6:n-3	1.4	1.3
P:S	0.09	0.15
Total	70.6	4.9

Scollan *et al.*, 2006 specified in his research that a major trans fatty acid is C18:1 trans vaccenic which is a bio-hydrogenation product of C18:2n6. This fatty acid is converted to CLA (C18:2cis-9, trans-11) in adipose tissue by the action of stearoyl Co-A desaturase, the same enzyme responsible for the production of C18:1cis-9 from C18:0. Like C18:1cis-9, both C18:1 trans vaccenic and CLA are at higher proportions in neutral lipid than phospholipid and higher in adipose tissue than muscle. Conjugated linoleic acid is also produced in the rumen but synthesis from C18:1 trans vaccenic in tissues is quantitatively the most important contributor to tissue levels.

Conjugated linoleic acid has health benefits in the human diet although meat from ruminants makes only a small contribution towards nutritionally significant levels. The total amount of SFA and UFA was unaffected in intramuscular fat. The n-3 fatty acids were included at the expense of oleic acid and n-6 fatty acids. Experiments with lambs indicated the same results as seen in Table 2 (Nürnberg *et al.*, 1996). The intramuscular fat quality of longissimus muscle in lambs and steers fed on pasture was

better for human nutrition because of the high proportion of n-3 fatty acids. (Nürnberg *et al.*, 1996).

**Table 2** Influence of feeding regime on fatty acid composition (% of total fatty acids) of longissimus muscle in lambs (Nürnberg *et al.*, 1996)

	Permanent on pasture (n=20)	Pasture and indoors finished (n=20)	Significant effect
Live weight (kg)	38.2	43.7	+
C18:2	5.8	8.0	+
C18:3	2.0	1.2	+
C20:4	2.7	2.4	-
C20:5	1.7	0.7	+
C22:6	1.2	0.9	-
n-3 fatty acids	4.9	2.8	+
n-6 fatty acids	8.5	10.4	+

Greater concentrations of fatty acids accumulated with increasing slaughter weight. It was concluded by Webb (1994) that the amount of fat and the concentrations of fatty acid in adipocytes depend directly on the live weight and maturity of ruminants, while the profile of fatty acid deposited depend primarily on the diet.

Factors such as sex, live weight, breed type, feeding regimen, hormones, dietary fat and age have been related to fatty acid composition. Although diet does not change body fat composition in ruminants as much as in monogastric animals there is evidence that some diet induced changes in fat composition do occur (Cramer *et al.*, 1967; Ziegler *et al.*, 1967; Miller *et al.*, 1967). Since organoleptic characteristics are influenced by feed, slaughter weight and sex, it seems logical that there would be a relationship between fatty acid composition and organoleptic characteristics.

## 2.6 Enzymes

The increasing economic pressures on livestock producers demand more efficient utilization of low-grade feedstuffs. One increasingly popular approach to this problem is to supplement animal diets with fibrolytic enzymes in an attempt to aid the digestion and absorption of poorly available nutrients, or to remove anti-nutritional factors from the diet (Walsh *et al.*, 1993).

Flachowsky 2011, specified that all animals use enzymes to digest feed. These are either produced by the animal itself, or by microbes naturally present in the gut. Ruminant feed enzyme additives, primarily xylanases and cellulases, are concentrated extracts resulting from bacterial or fungal fermentations that have specific enzymatic activities. Improvements in animal performance due to the use of enzyme additives can be attributed mainly to improvements in ruminal fibre digestion resulting in increased digestible energy intake. Animal responses are greatest when fibre digestion is compromised and when energy is the first limiting nutrient in the diet (Flachowsky, 2011).

It is interesting that the enzyme in mammalian systems are only able to insert double bonds between the carboxyl group and the first double bond already present in the fatty acids, while plant enzyme systems can only insert new double bonds between the last double bond and the terminal portion of the fatty acid (Christie, 1982).

Enzyme additives currently used in ruminant diets are concentrated fermentation products containing fibre-digesting enzymes such as cellulases and hemi-cellulases. There is increasing evidence to suggest that when properly formulated enzymes can improve ruminal fibre digestion and the productivity of ruminants (Beauchemin *et al.*, 2003). Enzymes that improve fibre degradation typically lowered the acetate-to-propionate ratio in rumen fluid (Eun & Beauchemin, 2007) which is thought to be the primary mechanism whereby CH<sub>4</sub> production is decreased. While it is premature to recommend the use of feed enzymes for CH<sub>4</sub> abatement, research suggested that the potential of enzymes additives for CH<sub>4</sub> abatement warrants further research (Beauchemin *et al.*, 2008).

The inclusion of cellulose and xylanase in ruminant feed has proven to bring about substantial improvement in fermentation of organic matter, neutral detergent fibre, acid detergent fibre and rumen fermentation parameters (i.e. ammonia-nitrogen [N], volatile fatty acids, digestibility of feed and animal performance (Krueger *et al.*, 2008; Azzaz *et al.*, 2012). There is limited information available with regard to

associated CH<sub>4</sub> production. In a few studies where CH<sub>4</sub> production was of interest, various results were reported; decreased CH<sub>4</sub> production (Eun & Beauchemin, 2007) or increased CH<sub>4</sub> production (Chung *et al.*, 2012) while no effect on methane production has also been reported (McGinn *et al.*, 2004). In a recent study, it was also recorded that addition of cellulose and xylanase enzymes increased CH<sub>4</sub> production due to shifts in VFA profiles, which favoured acetate production, and increased fermentation resulted in increased H<sup>+</sup> due to acetate shifted fermentation (Gemedá & Hassen, 2015).

It is my opinion that in intensive livestock production systems feed is the biggest single cost and if not digested efficiently there is an extra cost to both producer and environment. Supplementing feeds with specific enzymes can improve the nutritional value of feed ingredients, increase the efficiency of digestion and assist in breakdown of anti-nutritional factors e.g. fibre and phytate, resulting in improved animal performance (Gemedá B.S, 2015).



## Chapter 3

### Materials and Methods

#### 3.1 Introduction

A study was conducted on South African Mutton Merino lambs at the University of Pretoria's experimental farm at the small stock section from October 2014 until mid-February 2015. This formed part of a greater departmental research project to improve the utilization of poor quality feeds while simultaneously mitigate the excess emission of CH<sub>4</sub> from ruminant livestock, using sheep as an animal model fed a high-forage diet. In particular, the focus of this research was to specifically study the possible consequences of these mitigation technologies on physiological effects and product quality.

#### 3.2 Ethical approval

This research conformed to the laboratory animal ethical code of research of the University of Pretoria

The research protocol of the current study was approved by the Animal Ethics Committee of the University of Pretoria (Project number: EC084-14) (refer to Ethical approval certificate in Appendix A

### 3.3 Different phases of the research project

#### **Phase 1:** First 60 days of the trial formed part of another MSc trial

This phase of the trial was part of a parallel MSc project and results were reported separately, phase two followed where lambs were fed the same diets till target weight was reached. Phase one consisted of three parts. The first part was the measurement of intake and faecal excretion to determine the digestibility of the feed. The second part included the measurement of the amount of CH<sub>4</sub> production from the sheep. The digestibility and CH<sub>4</sub> production was measured simultaneously while in the open-circuit respiration chambers. For this purpose, lambs were randomly selected from each of the four treatments and placed in an open-circuit respiration chamber. The sheep were fitted with a faecal bag that was emptied once a day before feeding. The third part was the growth performance of lambs (weight gain and ADG, FCR was calculated as kg feed consumed/kg body weight gain). The experiments were conducted using a completely randomized design

#### **Phase 2:** Last 60 days of trial (focus of current dissertation)

From day 60 of the trial the lambs were fed to a target slaughter weight of 42kg continuing in same manner as previous 60 days to keep the trial as uniform as possible. After reaching slaughter weight the lambs were transported to a commercial abattoir where they were slaughtered and post mortem sampling was done. Lambs were slaughtered in three consecutive groups on day 90,100 and finally day 112 of the trial.

This dissertation focused only on phase two of the research project.

### 3.4 Specific objectives

Specific objectives of this study was to examine the effect of fibrolytic enzyme and oil supplementation on the following carcass and meat characteristics:

- Growth (ADG)
- Dressing percentage
- Carcass composition (changes in bone, muscle and fat relationship)
- Fatty acid composition of meat
- Meat colour and subcutaneous fat colour

### 3.5 Hypothesis

HA: Fibrolytic enzymes and / or bypass oil supplementation has a significant effect on carcass fat content and composition

H0: Fibrolytic enzymes and / or bypass oil supplementation has no significant effect on carcass fat content and composition

### 3.6 Experimental design

In September 2014, 40 SAMM ram lambs (3 months old) were selected on a farm in the district the Free State province for the trial. All lambs were vaccinated with one-shot ultra and Prodose yellow. Details of treatments throughout the trail are given in table 6. Lambs were kept on veld and fed additional creep feed until they were transferred to the experimental farm on 13 and 14 October 2014. The lambs were weighed on arrival, tagged and dosed with anthelmintic treatment (4ml Eradiworm that contains levamisole HCl 37,5 mg/ml; praziquantel 18,8 mg/ml to control various worms per lamb). The average body weight on arrival was  $17.79 \pm 1.61$ kg.

The lambs were then stratified according to their body weight, blocked and randomly assigned to 1 of the 4 treatment groups with 10 lambs as a sampling unit in each treatment group as outlined and presented in Table 5.

The treatment groups were as follows:

**Control treatment (C)**: only the TMR with bypass oil (megalac)

**Oil treatment (PO)**: TMR with 3% palm oil supplementation

**Enzyme treatment (ET)**: TMR with 10.4  $\mu$ l enzyme per 0.5g feed (Cellulase and Xylanase combination in a ratio of 1:1).

**Oil & enzyme treatment (O\*E)**: TMR with 3% palm oil supplementation and 10.4  $\mu$ l enzyme supplementation per 0.5g feed (Cellulase and xylanase combination in a ratio of 1:1)

Enzymes were measured and mixed into the feed by hand 24 hours prior to feeding. Palm oil was selected based on the *in vitro* data of a parallel MSc project. Palm oil was mixed into the diets by hand before feeding each morning.

The lambs were fed diets *ad libitum* for ca. 120 days, after being adapted to the control and treatment diets for 10 days. Weighed quantities of the feed was offered to lambs once daily at 07:00 and the residue of the previous day's offer, if any, was weighed back the next day and discarded before fresh feed was offered. Fresh water was available at all times. The pens were cleaned once every week, including the feed and water troughs. Sheep were weighed once a week every Friday morning around seven o'clock starting on the 24<sup>th</sup> of October after the adaption period.

A high-forage based total mixed ration (TMR), was formulated to fulfil in the maintenance and growth requirements of growing lambs. This diet was used as the control diet and as a basis to formulate four different treatments that were evaluated during the growth trial. The basal diet was formulated for a 20kg Merino lamb, gaining 200g each day. Composition of TMR is given in Table 3.

**Table 3** Composition of the total mixed ration to be fed to the lambs (provided from parallel MSc. projects lab data)

Raw material	Composition (%)
Maize meal	8
Maize bran	5
Eragrostis curvula hay	30
Soyabean oil cake	13
Feed lime	0.2
Mineral premix	0.2
Lucern hay	20
Hominy chop	10
Sunflower oil cake	12.8
MCP	0.8
Parameter	Composition
Dry matter (%)	88.7
Ash (%)	6.9
Crude protein (%)	13.3
Fat (%)	5.8
Starch (g/kg)	236.1
NDF (g/kg)	308.6
ME (MJ/kg DM)	10.1
Ca (%)	0.9
P (%)	0.3

Each treatment had four pens with two sheep per pen divided into five blocks. Each pen received one of the four treatments. Thus, the pens were considered as the experimental units and the two sheep in each pen is the observational unit. All the ram lambs were used in the 120-day growth trial. Intake was measured and calculated by subtracting the refusal/orts from amount of feed offered the day before. A random hand sample was taken from the amount of feed given (kg) each day as a retention sample for feed analysis later.

Lambs were housed in 20 covered pens, which were 3.2 x 2.2 meters and randomly allocated two lambs per pen according to treatment and block.

	Pen 3		Pen 2	
	Pen 4		Pen 1	
	Pen 5			
	Pen 6			
	Pen 7			
	Pen 8			
	Pen 9			
	Pen 10			
	Pen 11			
	Pen 12			
	Pen 13			
	Pen 14			
	Pen 15			
	Pen 16			
	Pen 17			
	Pen 18			
	Pen 19			
	Pen 20			

Figure 1 Experimental layout of pens

**Table 4** Schematic representation of adaption phase

Day	Treatment 1
1&2	500g TMR + ad libitum roughage
3&4	1000g TMR + ad libitum roughage
5&6	1500g TMR + ad libitum roughage
7&8	2000g TMR + ad libitum roughage
9&10	ad libitum TMR
Day	Treatment 2
1&2	485g TMR + 15 g palm oil + ad libitum roughage
3&4	970g TMR + 30 g palm oil + ad libitum roughage
5&6	1455g TMR + 45 g palm oil + ad libitum roughage
7&8	1940g TMR + 60 g palm oil + ad libitum roughage
9&10	ad libitum TMR
Day	Treatment 3
1&2	500g TMR + 10,4 ml enzyme + ad libitum roughage
3&4	1000g TMR + 20,8 ml enzyme + ad libitum roughage
5&6	1500g TMR + 31,2 ml enzyme + ad libitum roughage
7&8	1940g TMR + 41,6 ml enzyme + ad libitum roughage
9&10	ad libitum TMR
Day	Treatment 4
1&2	485 g TMR + 15 g palm oil + 10,4ml enzymes + ad libitum roughage
3&4	970g TMR + 30 g palm oil + 20,8 ml enzyme + ad libitum roughage
5&6	1455g TMR + 45 g palm oil + 31,2 ml enzyme + ad libitum roughage
7&8	1940g TMR + 60 g palm oil + 41,6 ml enzyme + ad libitum roughage
9&10	ad libitum TMR

**Table 5** Schematic representation of experimental design and blocking

Pen	Treatment	Block	Sheep no
1	1	1	41
	1	1	40
2	2	1	11
	2	1	28
3	3	1	25
	3	1	16
4	4	1	12
	4	1	37
5	1	2	38
	1	2	36
6	2	2	29
	2	2	18
7	3	2	1
	3	2	4
8	4	2	26
	4	2	39
9	1	3	21
	1	3	3
10	2	3	6
	2	3	35
11	3	3	27
	3	3	13
12	4	3	30
	4	3	8
13	1	4	24
	1	4	15
14	2	4	5
	2	4	14
15	3	4	10
	3	4	20
16	4	4	32
	4	4	22
17	2	5	31
	2	5	19
18	3	5	23
	3	5	7
19	4	5	42
	4	5	34
20	1	5	9
	1	5	33



### 3.7 Animal health

**Table 6** Treatments throughout the trial

Product name	Indications
Multivax P Plus (Sheep of all ages 2 ml subcutaneously with the dosage repeated after an interval of four to six weeks)	For the active immunisation of sheep against pulpy kidney, malignant oedema, blackquarter, tetanus and pasteurellosis caused by the above listed organisms. In particular the vaccine is recommended as an aid in the prevention of pneumonic and septicaemic pasteurellosis in lambs.
Eradiworm (Sheep and goats: 2 ml/10 kg body mass.)	Roundworm and tapeworm remedy for sheep, goats, cattle and ostriches.
Zolvix (1mL per 10kg body weight (corresponding to 2.5mg monepantel per kg)	ZOLVIX is for the treatment and control of AAD-sensitive strains of gastrointestinal roundworms, including macrocyclic lactone, benzimidazole, levamisole and morantel-resistant strains in sheep.
Sulfatrim (3-5ml)	Sulfatrim® Is a broad-spectrum antimicrobial bactericidal antibiotic injectable solution that contains sulfamethoxazole (part of the sulfonamide group) and trimethoprim (a diaminopyridine). Sulfatrim® is recommended for treatment of infections of the gastrointestinal tract, urogenital system, respiratory tract and septicemias caused by Gram positive, Gram negative bacteria and protozoa. The use is recommended in Dairy Cattle, sheep, goats, swine, dogs, cats and barnyard fowl.

**Table 6** Treatments throughout the trial continued

Product name	Indications
Prodose® Green (Use only as directed. Shake before use.)	<p>Indications: Roundworm, milk tapeworm and liver fluke.</p> <p>Prodose yellow LA contains Closantel 7,5 % m/v for liver fluke, conical fluke, nasal bot and roundworm remedy for sheep and goats with residual efficacy against reinfestations of wireworm and hookworm.</p>
One-shot ultra	<p>for vaccination of healthy sheep as an aid in preventing blackleg caused by Clostridium chauvoei, malignant oedema caused by Clostridium septicum; black disease caused by Clostridium novyi; gasgangrene caused by Clostridium sordellii; and enterotoxaemia and enteritis caused by Clostridium perfringens type B, C and D, and pneumonic pasteurellosis caused by Mannheimia (Pasteurella) haemolytica type A1, One-Shot Ultra™ 7 consists of killed, standardised cultures of Clostridium chauvoei, Clostridium septicum, Clostridium novyi, Clostridium sordellii and Clostridium perfringens type C and D, immunity against Clostridium perfringens type B is provided by the beta toxoid of type C and the epsilon toxoid of type D and inactivated whole cultures of M. haemolytica propagated to increase the production of leukotoxin and capsular and cell-associated antigens, with a special, water-soluble adjuvant (Stimugen™)</p>

### 3.8 Slaughter details:

All 40 lambs were slaughtered at random at a target weight of ca. 42 kg at a commercial abattoir using standard South African techniques as described by Cloete *et al.* (2004). Live weight was determined 24 h prior to slaughtering on empty body weight before being transported for 30 minutes via truck to the Agricultural Research Council commercial abattoir.



**Figure 2** Illustration of internal organ evaluation post slaughter

Slaughtering method and sampling procedures:

Carcasses were electrically stimulated (21V, 60Hz, 120s) and chilled at 4°C for 24 hours. Recordings made for each carcass included: hot carcass weight (HCW) directly after slaughter; cold carcass weight (CCW) was taken after being chilled at 4°C for 24 hours. Carcasses were classified using the South African Carcass Classification System that has been in use since June 1992 (Agricultural Product Standards Act, 1990 (Act no.119 of 1990)) and classifies lamb, mutton, beef and goat carcasses based on a set of predefined characteristics. Back fat depth was measured approximately 5g samples of subcutaneous adipose tissue collected from the left side of each carcass at the point over the 8-9<sup>th</sup> rib, 25 mm from the midline and was stored in sealed polyethylene bags at -20°C for subsequent fatty acid analysis (Webb *et al.*, 1994).

## Fatty acid analysis

Lipid extraction procedure as discussed by Webb and Casey (1995): Lipids were extracted in duplicate by means of a modification (Ways and Hanahan, 1964) of the chloroform: methanol (2:1, v/v) method (Folch et al., 1957). Butylated hydroxytoluene (2,6 DI-tert-BUTYL-P-CRESOL) was included as an antioxidant. Methyl esters of the fatty acid component of the neutral triglycerides were prepared according to the NaOH/ methanol method (AOAC, 1975). These esters were separated on a polar phase SP2330 column (2 m X 3 mm, packed with Silar 10C coated on Gas Chrom Q) fitted to a Shimadzu Tracera gas chromatograph with a barrier ionization discharge detector. Profiles of the cis-trans fatty acids from subcutaneous adipose tissue were obtained from fat samples that were treated with n-hexane at 35°C for 24 h, after which the fatty acids were esterified according to the method of Van Wijngaarden (1967). The cis-trans fatty acids isomers were then separated on a SP2560 fused silica capillary column (100 mX 0.2 mm) fitted to a Varian 3700 gas chromatograph (Webb et al., 1994a). Standards for the fatty acids were obtained from Nu-Chek-Prep. Inc. (Elysim, MI, USA). Fatty acids were expressed in both normalised (molar proportion) and gravimetric (milligrams per gram of fresh tissue) formats (Slover and Lanza, 1979; Huerta-Leidenz N.O, 1993).

To determine carcass composition a three-rib sample (ribs 8-9-10) was cut from the left side of each carcass, the ventral extremity of the sample being on a line drawn from the pubic symphysis to the middle of the first rib, to obtain an estimate total carcass composition (Casey *et al.*, 1988).

The pH was measured at the same location by means of a temperature compensating Orion pH meter. The instrument was calibrated using pH4-standard (pH=4) and pH7-standard (pH=7). The meat probe was rinsed with deionised water between samples, measured just above 13<sup>th</sup> rib at 45 min, 24 h post mortem (McGeehin *et al.*, 2001). Fat colour was analysed using coded (numerical value given) colour palettes of white and yellow and scored, measured at the 13<sup>th</sup> rib.



**Figure 3** Carcasses post slaughter (left); measuring of pH at 24 hours post slaughter

### **3.8 Statistical analysis**

Data was recorded in Microsoft Excel, checked for duplicates and correctness. Data analysis was conducted by means of Multi factor analysis of variance (MANOVA) using the GLM procedure in SPSS (2015) with all analyses performed at a significance level of  $p < 0.05$ . Results are presented as LSMeans  $\pm$  standard deviation (SD.).

## Chapter 4

### Results and Discussion

#### 4.1 Carcass and growth results

**Table 7** Summary statistics of combined methane mitigating treatment effects on carcass growth results of the control and methane mitigation diets on average treatment groups

Parameter	LS mean (kg) [ $\bar{x} \pm SD$ .]	Significance (P=f)
Days on feed	98.0 $\pm$ 9.17	0.016
ADG (kg)	0.256 $\pm$ 0.034	0.182
Slaughter weight (kg)	42.7 $\pm$ 2.28	0.437
Total weight gain (kg)	24.9 $\pm$ 2.44	0.437
Hot carcass weight (HCW) (kg)	19.24 $\pm$ 1.518	0.035
Cold carcass weight (CCW) (kg)	18.75 $\pm$ 1.482	0.043
pH @ 24 hours	5.56 $\pm$ 0.154	0.097
Dressing %	45.0 $\pm$ 2.20	0.001
Fat %	29.25 $\pm$ 4.350	0.18
Meat %	49.89 $\pm$ 4.802	0.039
Bone %	20.86 $\pm$ 3.671	0.218

**Table 8** Effects of different oil and enzyme treatment groups on carcass characteristics of sheep

<i>Parameter</i>	<i>LS mean (kg)</i> <i>[ x ± SD.]</i>				<i>Significance</i> <i>(P=f)</i>		
	<i>C</i>	<i>O</i>	<i>E</i>	<i>O*E</i>	<i>O</i>	<i>E</i>	<i>O*E</i>
<i>Days on Feed</i>	94.20 ± 7.51	101.80 ± 9.68	93.20 ± 7.32	102.80 ± 8.80	<b>0.00</b>	0.94	0.69
<i>ADG (kg)</i>	0.27 ± 0.03	0.25 ± 0.04	0.27 ± 0.03	0.24 ± 0.03	0.05	0.49	0.40
<i>Slaughter Weight (kg)</i>	42.77 ± 2.02	43.43 ± 2.77	42.72 ± 2.14	41.90 ± 2.19	0.86	0.26	0.23
<i>Total Weight Gain (kg)</i>	25.01 ± 1.31	25.67 ± 3.41	24.88 ± 2.77	24.09 ± 1.81	0.86	0.26	0.23
<i>HCW (kg)</i>	19.75 ± 1.54	18.97 ± 1.61	19.96 ± 1.18	18.29 ± 1.30	<b>0.00</b>	0.64	0.27
<i>CCW (kg)</i>	19.22 ± 1.49	18.48 ± 1.61	19.45 ± 1.14	17.85 ± 1.29	<b>0.00</b>	0.70	0.28
<i>pH @ 24 Hours</i>	5.60 ± 0.15	5.47 ± 0.12	5.56 ± 0.10	5.62 ± 0.20	0.45	0.17	<b>0.04</b>
<i>Dressing %</i>	46.13 ± 1.94	43.66 ± 1.96	46.72 ± 1.40	43.64 ± 1.65	<b>0.00</b>	0.57	0.57
<i>Fat %</i>	30.55 ± 2.74	27.62 ± 3.75	30.94 ± 5.81	27.88 ± 4.08	<b>0.03</b>	0.74	0.94
<i>Meat %</i>	47.01 ± 4.89	53.40 ± 2.64	48.11 ± 4.67	51.04 ± 4.40	<b>0.00</b>	0.66	0.29
<i>Bone %</i>	22.44 ± 4.58	18.99 ± 2.87	20.95 ± 3.70	21.08 ± 2.97	0.15	0.82	0.12

C= Control; O= Palm oil supplemented treatment group; E= Enzyme supplemented treatment group; O\*E= Combination of palm oil and enzyme supplemented treatment group



#### 4.1.1 Days on feed and average daily gain (ADG)

Results obtained from the MANOVA in this study are summarized and discussed in tables and figures to present the trends and significant effects where necessary.

On arrival, the average weight of the 40 lambs were  $17.79 \pm 1.61$  kg. Lambs were stratified according to weight from a minimum weight of 14.4 kg to a maximum weight of 20.60 kg, and randomly assigned to one of four treatment groups (allocated by means of number from one to four).

The initial live weights were included as a co-variant in GLM to correct for small differences at the start of the trial.

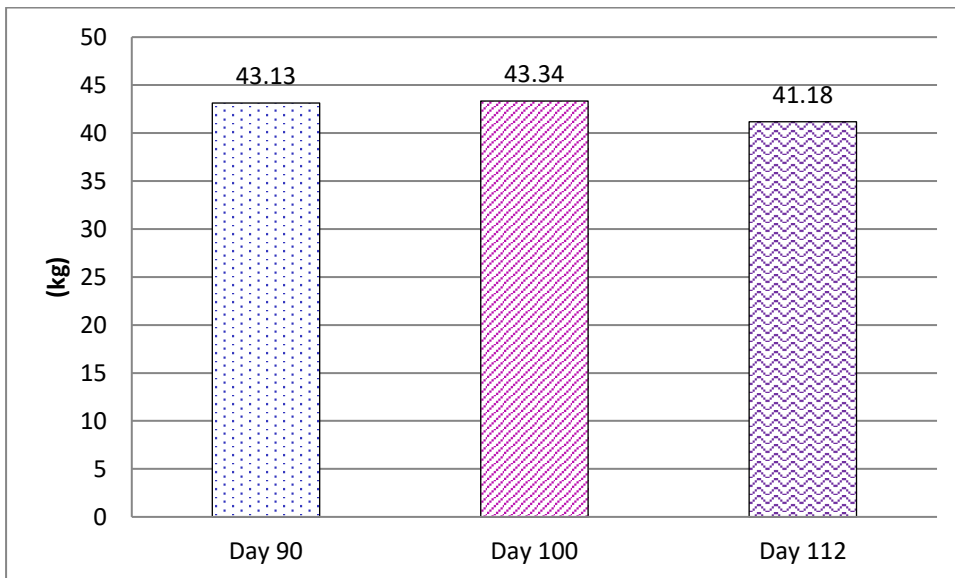
All 40 lambs had been slaughtered after approximately 100 days at a mean slaughter weight of  $42.70 \pm 2.28$  kg which is close to the norm of 43 kg slaughter weight in feedlots, depending on breed (Sheridan, 2003).

Results from this study as presented in Table 7 and Table 8 from treatment with three percent palm oil supplementation (PO), from here on referred to as oil treatment group or oil supplementation, agree with that of Castro *et al.* (2005) who reported no significant differences for ADG and DMI when lambs received up to six percent of supplemented fat. There was a trend ( $p < 0.1$ ) for treatment PO to lower the ADG of lambs, with an overall mean value of  $0.256 \pm 0.034$  kg/day (Table 7). Lambs that were not supplemented with oil (ET) gained ca. 20 g more per day. This decrease in ADG subsequent in PO treatment should be taken into account when considering the implementation of palm oil supplementation as a methane mitigation strategy and weighed against return on investment. If this methane mitigation strategy has a significant effect in reducing excess methane produced, feedlots will also have to take into account that lambs in treatment PO, took on average 8 days ( $p < 0.05$ ) longer to reach target weight for slaughter (Table 8).

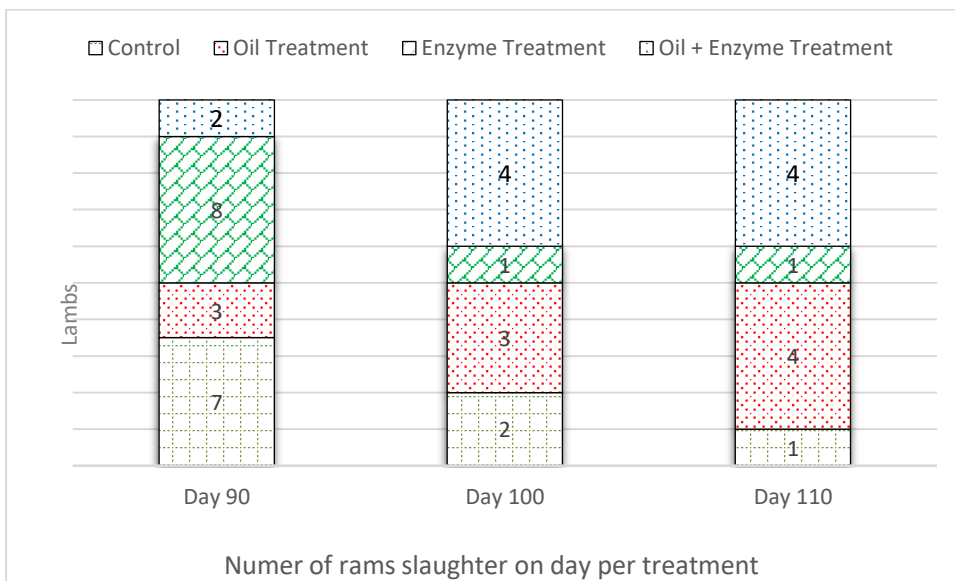
In terms of meat colour, changes were not expected, since it is usually associated to differences in fat content, carcass fatness or ultimate pH (Priolo *et al.*, 2001), and no differences were observed for these parameters. Likewise, no differences were observed in fat colour.



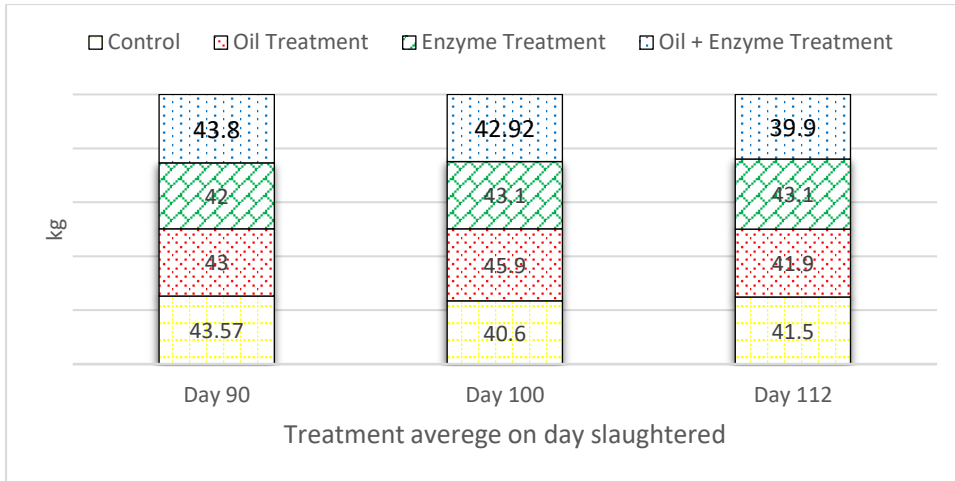
### 4.1.2 Slaughter weight and total weight gain



**Figure 4** Average slaughter weight of all treatment groups, on 3 different slaughter occasions.



**Figure 5** Number of sheep slaughtered per treatment group on three different slaughter occasions



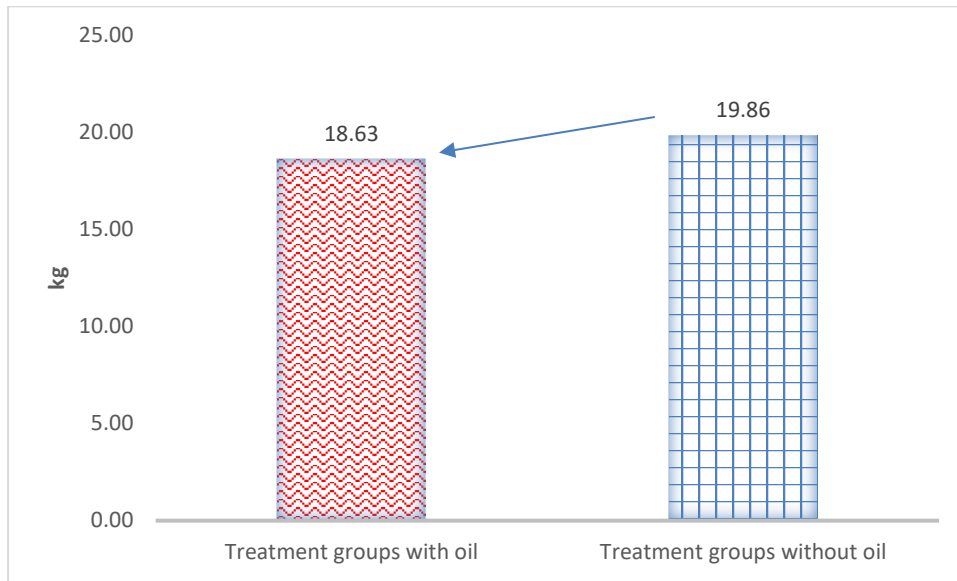
**Figure 6** Average slaughter weight per treatment group on three different occasions

Lambs were slaughtered in three groups on different occasions approximately ten days apart (Figure 4). This was due to the constraint that lambs had to be slaughtered in groups at an arranged time that made it practical and financially feasible for the abattoir. In the statistical procedures, the effect of slaughter day was included as a random factor. Results indicated that it was not significant for slaughter day effect, so it was excluded in subsequent analyses. Lambs gained  $24.9 \pm 2.43$  kg on average after being fed for the specified feeding period (Table 7). As expected not all of the treatment groups reached the specified slaughter weight at the same time and thus a slight numerical difference between average slaughter weights is seen (Figure 5&6), but there was no difference ( $p < 0.05$ ) between treatments groups for slaughter weight and total weight gain (Table 7)

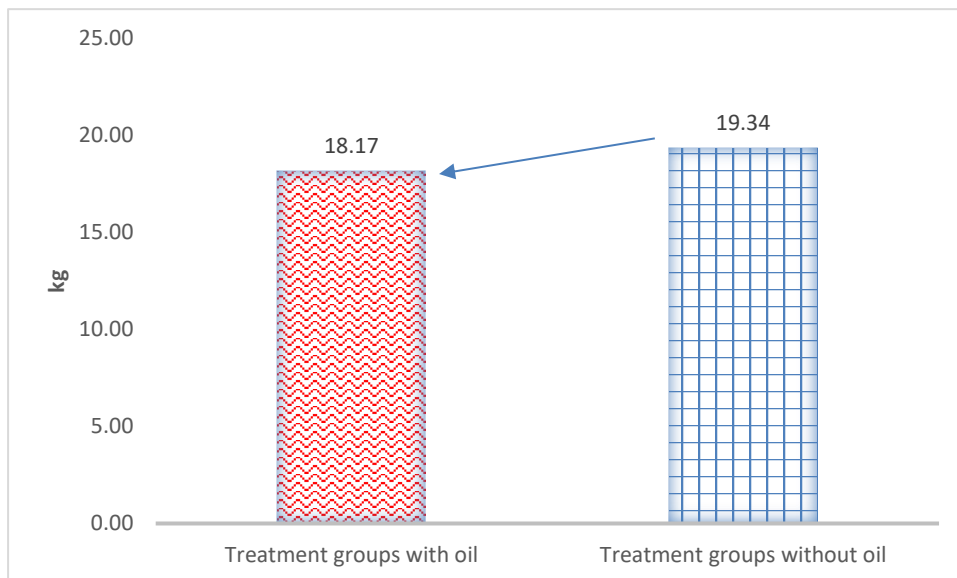
The average slaughter weight of three different slaughter groups are given in Figure 5. The slaughter weight of the final group of ten sheep was less than 42kg on day 112. This was a result of the predetermined dates set for slaughter, which caused four of the ten sheep to be below target weight, mostly from treatment O\*E (Figure 5&6).

Indicated in Figure 6 is the number of lambs slaughtered per treatment group on three different occasions. Figure 5 illustrated that treatment ET reached the target weight earlier ( $p < 0.05$ ) than treatment PO. About 75% of the sheep slaughtered in the first group were from the control treatment group C and ET; it can be speculated that palm oil supplementation may have an effect on growth rate possibly due to lower NDF digestibility from oil supplemented but further research is needed to confirm this.

### 4.1.3 Hot Carcass weight and cold carcass weight (kg)



**Figure 7** Effect of palm oil supplementation on hot carcass weight (kg)



**Figure 8** Effect of palm oil supplementation on cold carcass weight

\*Arrow indicates significant difference ( $p < 0.05$ )

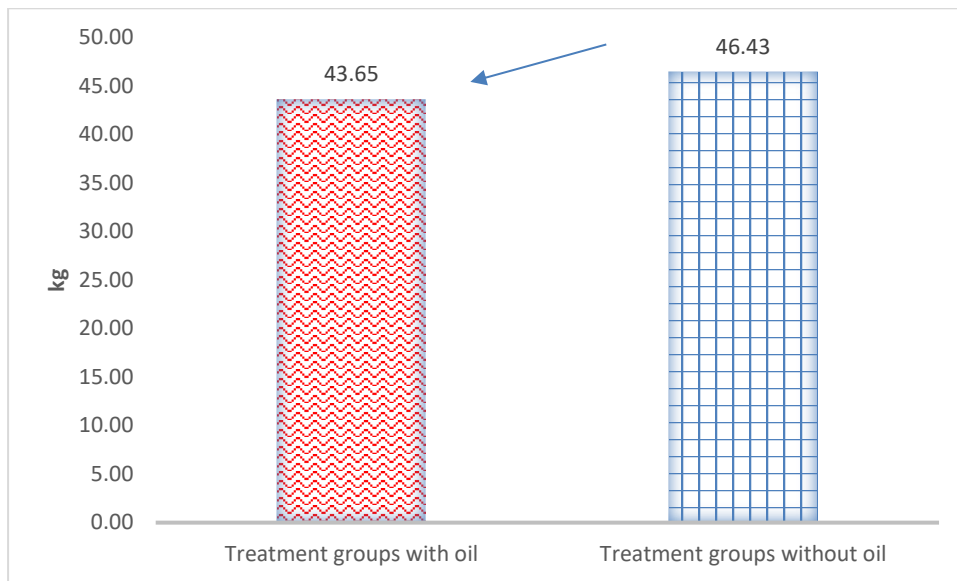
The carcass price information report provided by the Red Meat Association (<http://rvav.co.za/priceinformationreport>) in February 2015 specified that the average HCW reported around week one, two and three of slaughter period for A2 carcasses were 19.67kg, 18.87kg, 18.51kg respectively per week and 22.51kg, 20.00kg and 23.54kg for A3 carcasses respectively per week. Classification of slaughtered lambs for this study with average HCW of around 19kg was assigned to class A2, and average of 21.95kg was assigned to class A3. Most carcasses were classed A2, except for two that were classed A3.

Both HCW and CCW were affected by dietary treatments supplemented with oil ( $p < 0.05$ ) (Figure 7&8). Carcass weights for Treatments C and ET were higher ( $p < 0.05$ ) than PO and O\*E. (Table 8) The average HCW  $19.24 \pm 1.5$ kg still falls well within the industry norm compared to carcass weights reported in price information report provided by the Red Meat Association. Even though oil supplementation generally decreased HCW, causing more than a kilogram difference between treatment groups, all treatment groups still fall in the same class of carcass classification. The inclusion of palm oil at 3% in the diet did not affect growth rate or carcass weight ( $p > 0.05$ ) in a study done by Karami *et al.* (2003). These reported results do not concur with results in the present study.

#### **4.1.4 pH at 24 hours post mortem**

The main effects of oil and enzyme supplementation was not significant for pH at 24-hour post mortem. However, there was an interaction effect ( $p < 0.05$ ) between oil and enzyme treatments (Table 8). The TMR with 3% palm oil (PO) decreased pH at 24 hours in the absence of enzyme supplementation, and slightly increased ( $p > 0.05$ ) pH at 24 hours in the presence of enzyme supplementation. The TMR with 10.4  $\mu$ l enzyme per 0.5g feed (ET) decreased in the absence of oil supplementation for pH at 24 hours, and increased pH in the presence of oil supplemented diets. Although differences were seen, it is not of major concern in term of carcass quality as overall average pH of  $5.56 \pm 0.15$  is still within normal pH range of 5.4-5.8 expected after 24 hours. Cloete *et al.* (2012) measured a pH of  $5.54 \pm 0.69$  for SAMM reared on pasture, and Webb *et al.* (1995) recorded a pH of 5.46 for SAMM fed maize-based diets ad lib., which demonstrates different pH profiles of sheep fed in different ways. Normal carcass pH profiles indicate acceptable conversion of muscle to meat. This suggests that carcass and meat quality of treated animals (lambs) were not affected significantly by a methane mitigating strategy of supplementing palm oil and/or fibrolytic enzymes. Consequently, no adverse effects on consumer acceptance of the meat is expected.

#### 4.1.5 Dressing percentage



**Figure 9** Effect of palm oil supplementation on dressing percentage.

\*Arrow indicates significant difference ( $p < 0.05$ )

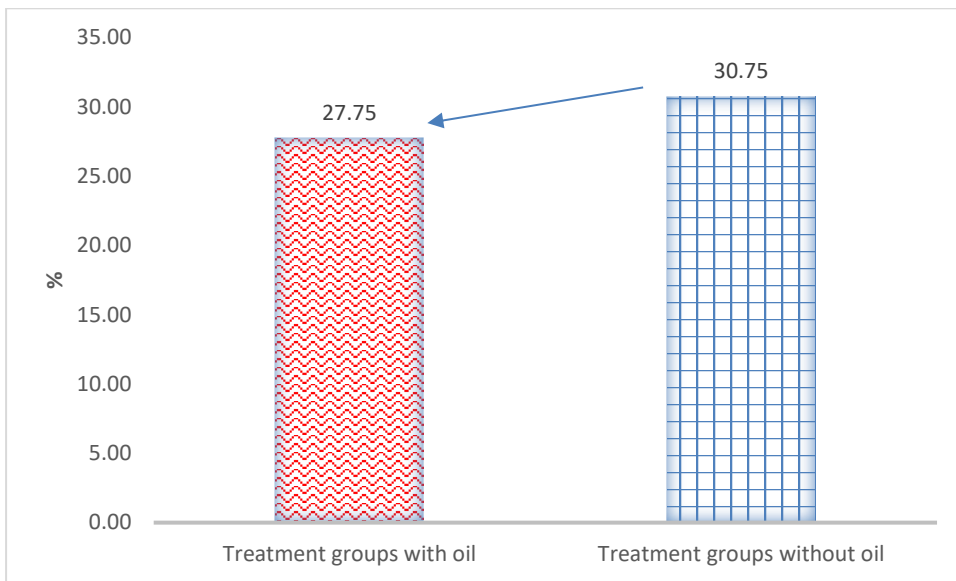
Dressing percentage (D %) reflects the proportion of a live weight that results in carcass weight. It is the portion of hot carcass weight (HCW) relative to pre-slaughter weight expressed as a percentage:

$$\text{Dressing Percentage} = \frac{\text{Hot Carcass Weight}}{\text{Live Weight}} \times 100$$

This parameter is of particular importance to producers selling on basis of HCW as the live weight prior to dispatch combined with D% enables them to more accurately target the carcass specifications of a price network.

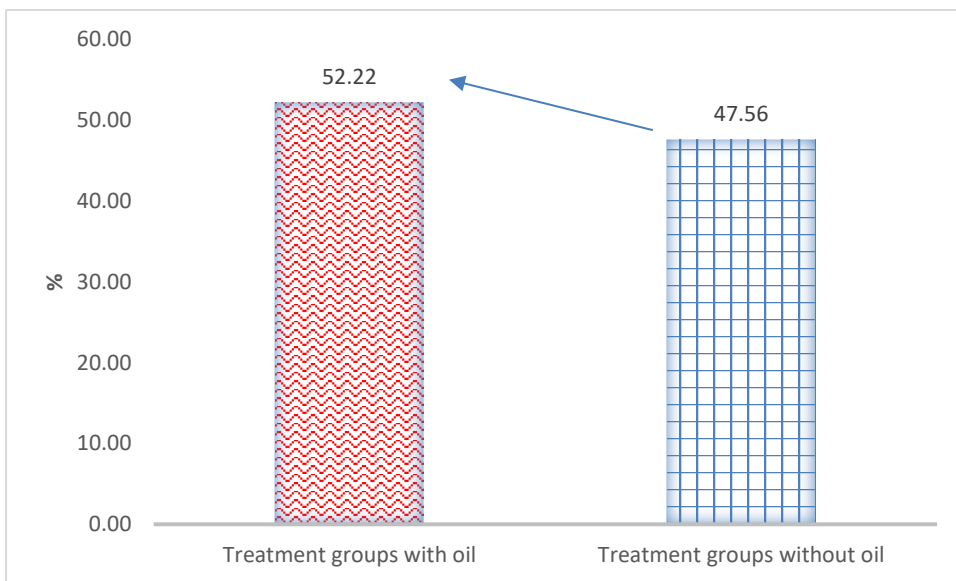
Dressing percentages in this study are in line with industry averages of around 45%, even though PO treatment had ca. three percent lower ( $p < 0.05$ ) D% compared to ET treatment (Figure 9). Dressing percentage of  $43.7 \pm 0.7\%$  was reported in a study by Cloete *et al.* (2012) from SAMM reared on pasture. Slightly higher dressing percentages of  $45.04 \pm 2.20\%$  were recorded in this study for total mixed rations fed to lambs.

#### 4.1.6 Carcass composition (fat, meat and bone percentage)



**Figure 10** Effect of palm oil supplementation on carcass fat percentage

\*Arrow indicates significant difference ( $p < 0.05$ )



**Figure 11** Effect of palm oil supplementation on carcass meat percentage

\*Arrow indicates significant difference ( $p < 0.05$ )

Carcass composition between overall treatment averages only differed for carcass meat percentage (Table 7). Supplementation of palm oil increased ( $p < 0.05$ ) carcass meat percentage by more than 4% (Figure 11). As expected palm oil supplementation significantly affected carcass fat %. Interestingly Treatment PO had a lower percentage carcass fat, than lambs on Treatment C and ET (Table 8).

Growth and development of the animals is the basis of meat production whereas amount and site of fat in the carcass influences its quality (Mahgoub & Lu, 1998).

According to Lawrie & Ledward (2006), the entire development process takes place in a shorter timespan for early maturing breeds than for late maturing breeds, indicating that the muscle:bone of a late maturing should be lower than that of an early maturing breed at the same chronological age, until physiological maturity is reached by both. Furthermore, bone development takes preference over muscle and fat development (Lawrie & Ledward, 2006). The percentage of bone in carcass will be higher at birth than at any other given time and the percentage of fat the lowest (Berg & Butterfield, 1968). As development continues the ratio of bone to carcass weight decreases while muscle ratio increases, and thus the muscle to bone ratio increases with increase in carcass weight. Muscle tissue has the highest growth rate between birth and maturity (Berg & Butterfield, 1968). As maturity is reached the percentage of muscle to carcass weight will start to decrease as the percentage of fat starts to increase (fattening phase) (Berg & Butterfield, 1968). Thus it can be deduced that differences in the physical composition of certain cuts will be present, as influenced by age and degree of fatness (Lawrie & Ledward, 2006). Carcass results from this study for SAMM are presented in Table 7.

Shorland (1953) was one of the first researches to relate dietary fat to growth and body fat composition. The author postulated that (i) the amount and composition of dietary fat, (ii) the rate of growth of fatty tissues, (iii) differential distribution of dietary fatty acids between various fatty tissues and (iv) differences in animal species could influence the composition of animal depot fats.

## 4.2 Adipose tissue fatty acid results

A complete Multi factor ANOVA was done using the GLM procedure including an enzyme and oil interaction. An overall summary of adipose fatty acid profiles is presented in Table 9 and Table 10 for subcutaneous and intra muscular adipose tissue respectfully, followed a discussion of separate groups that were of significance.

The effects of adding fat to the diets for ruminants depend not only on the type of fat (Doreau & Chilliard, 1997) but also on the amount added. Likewise, it has been proposed that the digestive processes in the hindgut compensated for the possible reduction in digestion in the rumen resulting in a limited effect on whole tract digestion of fat (Sutton et al., 1983). Changing the fatty acid composition of subcutaneous adipose tissue using palm oil produced a firmer fat compared to soybean oil (Teye *et al.*, 2006) which also influenced lipid melting point.

The proportion of fatty acids in this study was similar across all four treatments groups even though differences were detected; numerically these values were so small, even negligible in some cases. The intramuscular adipose tissue had a higher fatty acid content than subcutaneous adipose tissue, but the fatty acid composition of the two were broadly similar, Wood et al. (2008). If one compares results in this study with previous research done, (Field et al., 1978; Webb, 1992; Lough et al., 1993; Fischer et al., 2000; Castro et al., 2005; Dutta et al., 2008; Manso et al., 2009;) similar differences were detected between different fatty acids, it could be assumed that there will be no alternative effect on taste of products (Webb & Oneil, 2008).

Differences in adipose tissue fatty acid composition were attributed to type of diet (Bulent *et al.*, 2013). The type of diet also affected energy intake and resulted in heavier lambs with fatter carcasses. Thus, changes in age and carcass fatness may have perplexed the effects of ration on the fatty acid composition of tissues (Daniel *et al.*, 2004).



**Table 9** Effects of different oil and enzyme treatment groups on subcutaneous fatty acid composition of sheep

Subcutaneous fatty acids	LS mean (Molar %) [ $\bar{x} \pm SD.$ ]					Significance ( $P=f$ )		
	Overall	C	O	E	O*E	O	E	O*E
Saturated fatty acids	60.18±2.57	59.73±1.93	61.04±3.16	61.23±1.90	58.79±2.52	0.487	0.638	<b>0.025</b>
Monounsaturated fatty acids	35.02±2.36	34.92±2.35	34.63±2.82	33.91±1.59	36.51±1.96	0.121	0.553	0.056
Polyunsaturated fatty acids	4.78±0.83	5.35±0.73	4.34±0.79	4.86±0.82	4.64±0.76	<b>0.021</b>	0.723	0.124
Ratio of unsaturated/saturated fatty acids	0.66±0.07	0.68±0.06	0.64±0.09	0.64±0.05	0.70±0.07	0.440	0.669	<b>0.030</b>
Saturated fatty acids	Overall	C	O	E	O*E	O	E	O*E
C8:0	0.04±0.02	0.041±0.03	0.04±0.02	0.03±0.01	0.04±0.02	0.285	0.473	0.174
C10:0	0.24±0.04	0.24±0.043	0.24±0.03	0.24±0.03	0.25±0.04	0.657	0.517	0.487
C12:0	0.19±0.40	0.13±0.03	0.36±0.76	0.12±0.04	0.12±0.06	0.369	0.330	0.379
C14:0	3.13±0.29	3.28±0.34	3.00±0.29	3.19±0.27	3.06±0.22	<b>0.028</b>	0.887	0.454
C16:0	27.25±1.54	28.20±1.29	26.52±1.29	28.48±1.02	26.02±0.99	<b>0.000</b>	0.781	0.303
C17:0	1.58±0.32	1.61±0.34	1.47±0.28	1.76±0.42	1.48±0.18	<b>0.049</b>	0.412	0.490
C18:0	27.77±2.65	26.24±2.51	29.42±3.26	27.40±2.09	27.82±1.82	<b>0.034</b>	0.788	0.097
Unsaturated fatty acids	Overall	C	O	E	O*E	O	E	O*E
C16:1	2.29±0.50	2.29±0.36	2.40±0.49	2.03±0.30	2.41±0.62	0.145	0.442	0.405
C18:1n9c	32.20±2.06	32.09±2.42	31.63±2.36	31.58±1.53	33.43±1.49	0.291	0.321	0.086
C18:2n6c	3.56±0.60	4.00±0.60	3.19±0.50	3.83±0.43	3.27±0.45	<b>0.000</b>	0.790	0.446
C20:3n6	0.13±0.11	0.12±0.06	0.11±0.11	0.06±0.05	0.20±0.13	<b>0.052</b>	0.591	<b>0.024</b>
C22:1n9	0.24±0.28	0.21±0.25	0.30±0.35	0.09±0.11	0.32±0.31	0.076	0.619	0.430
C20:4n6	0.59±0.19	0.65±0.21	0.55±0.21	0.51±0.19	0.66±0.77	0.659	0.809	<b>0.040</b>

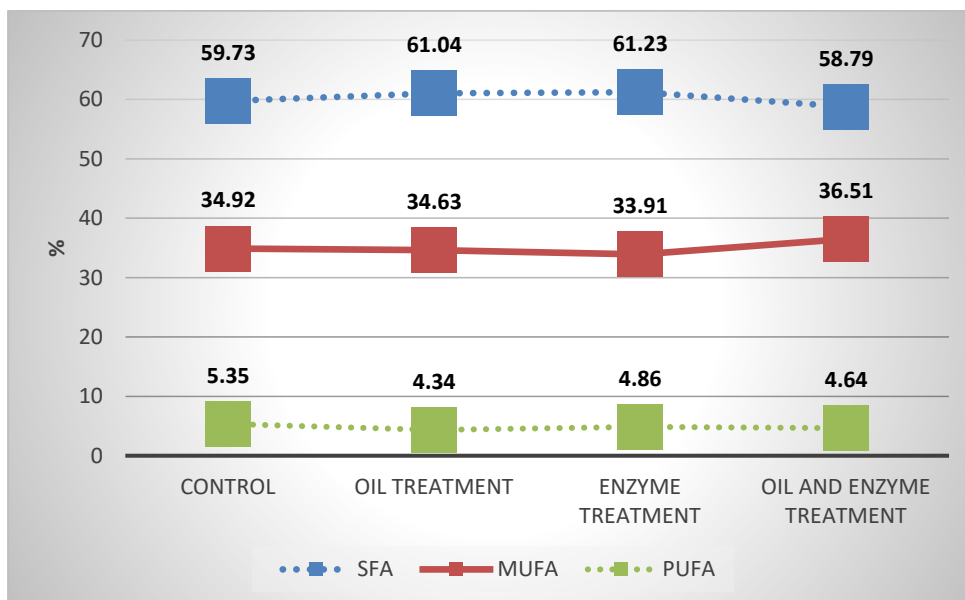
C = Control; O = Palm oil supplemented treatment group; E = Enzyme supplemented treatment group; O\*E = Combination of palm oil and enzyme supplemented treatment group

#### 4.2.4 Subcutaneous fatty acids results summary

Collectively the desirable fatty acids which mainly included stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2),  $\alpha$ -linolenic acid (C18:3), EPA (C20:5) and DHA (C22:6) in beef and lamb/mutton, ranged between 63-71%. The average of this study was 63.5% which was well within the norm.

The composition of LCFAs (C8:0 to C20:4n6) was summarized in Table 9 where oleic acid (C18:1n9c) was the most abundant (32.20%) fatty acid, which coincided with other research where oleic acid was also the major fatty acid in mutton meat (Demirel *et al.* 2004).

Values of fatty acid composition agree with those from Castro *et al.* (2005). The most abundant fatty acid in this study being oleic (C18:1 cis-9), followed by palmitic (C16:0) and stearic (C18:0) acids. Previous research has shown that the effects of palm oil supplements on fatty acid composition were more likely to be evident in internal depots such as omental and mesenteric fat (Castro *et al.*, 2005). As reported by Manso (2009), palm oil supplementation, inclusion of 4% hydrogenated palm oil did not affect any carcass characteristics studied, including meat pH and meat and fat colour. These results were similar with results in this study.

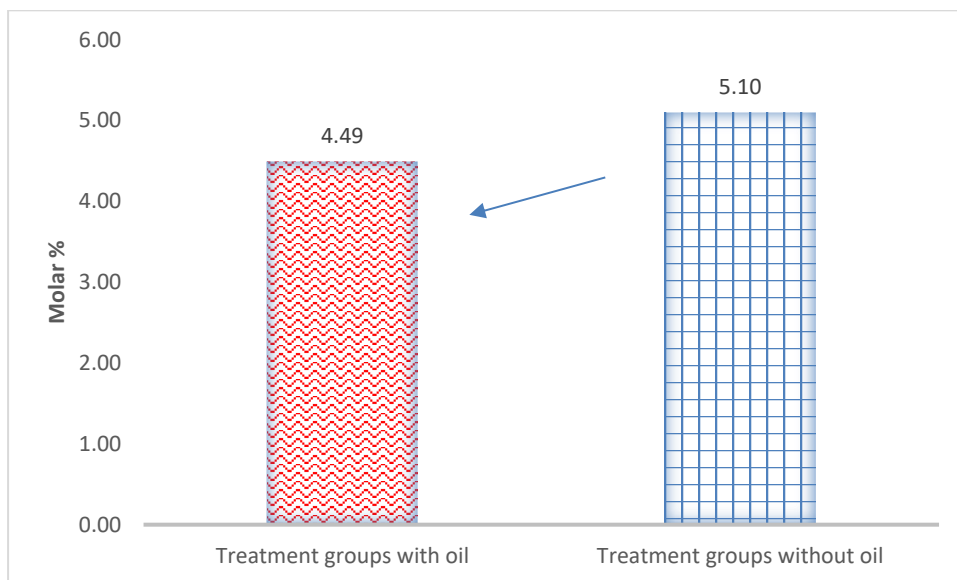


**Figure 12** Overall fatty acid proportions of subcutaneous adipose tissue (\*\*dotted line illustrates differences)

From the results of the subcutaneous fatty acid composition presented in Table 9, interaction effects ( $p < 0.05$ ) between oil and enzyme treatment groups were observed on components for saturated fatty acid (SFA), monounsaturated fatty acid

(MUFA) and unsaturated to saturated fatty acid ratio (UFA:SFA) respectfully. The interaction effect observed in SFA caused the concentration of SFA to increase ( $p < 0.05$ ) in PO treatments and ET treatments, but decreased ( $p < 0.05$ ) in O\*E treatment. Solomon *et al.* (1992) reported a corresponding increase in saturated fatty acid levels in subcutaneous and intramuscular adipose tissue. Thus, it can be speculated that the interaction effect between oil and enzyme suppresses the SFA's in the treatment group supplemented with both oil and enzymes.

Monounsaturated fatty acids (MUFA) has a tendency ( $p < 0.1$ ) for an interaction effect between oil and enzyme, concentrations of MUFA were slightly lower in PO treatment group, but increased in O\*E treatment. The level of MUFA decreased in the ET treatment group. It can also be speculated that there was a synergistic interaction effect in O\*E treatment due to the overall increase in amount of MUFA compared to other treatment groups, but further investigation is needed. Similar results were reported for the treatment group supplemented with oil displayed a lower MUFA concentration than that of lambs in the control treatment group.



**Figure 13** Effect of palm oil supplementation on polyunsaturated fatty acids in subcutaneous tissue.

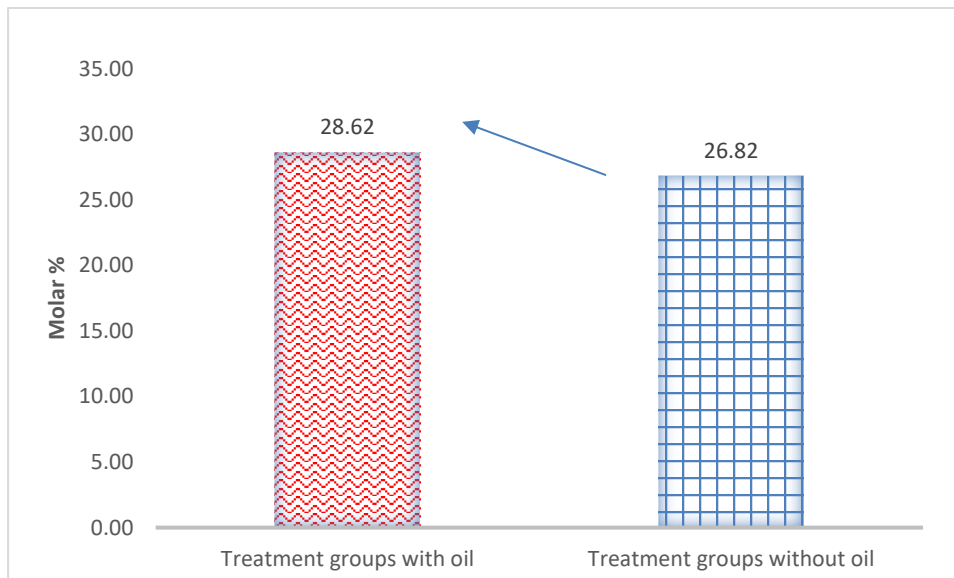
\*Arrow indicates significant difference ( $p < 0.05$ )

There was not an interaction effect for PUFA, however palm oil supplementation in the present study differed ( $p < 0.05$ ) from the other treatment groups and generally reduced the PUFA concentration (Figure 13).

These results coincide with those published by Castro *et al.* (2005). Solomon *et al.* (1992) also confirmed a similar decrease in PUFA subcutaneous adipose tissue.

Treatment O\*E outperformed ( $p < 0.05$ ) the other treatment groups in UFA:SFA. Concentration of fatty acid decreased in treatment PO, likewise treatment ET also decreased UFA:SFA concentration.

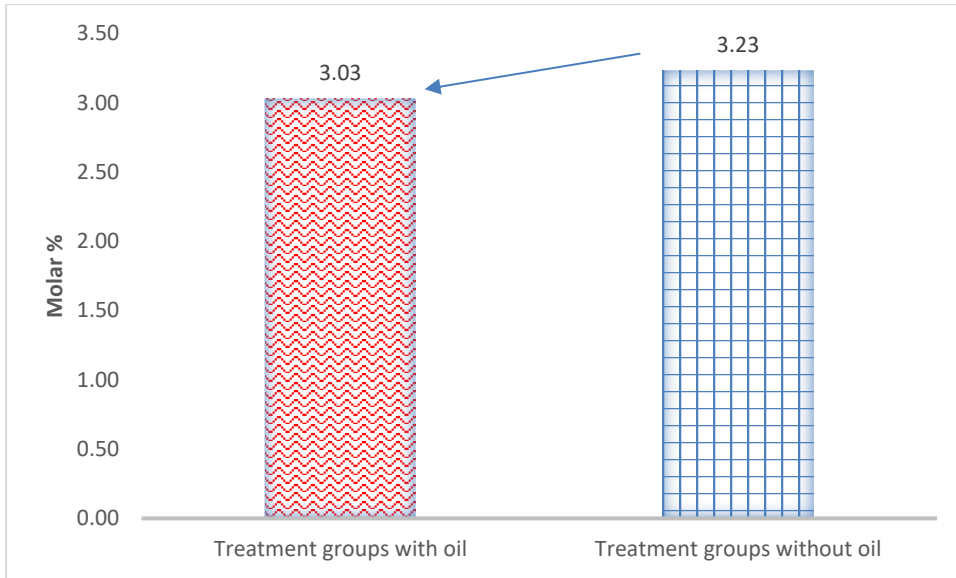
#### 4.2.4.1 Subcutaneous saturated fatty acids



**Figure 14** Effect of palm oil supplementation on stearic acid (C18:0) in subcutaneous tissue.

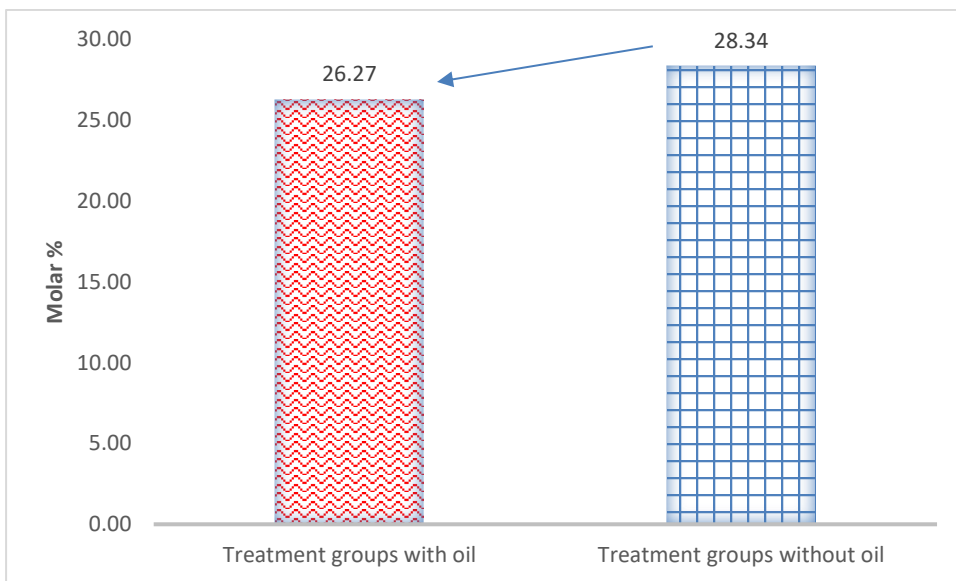
\*Arrow indicates significant difference ( $p < 0.05$ )

In all of the SFA's where oil supplementation had a significant effect, a general reduction in SFA concentration was observed for treatment PO, except for stearic acid (C18:0) where oil treatments generally increased ( $p < 0.05$ ) the fatty acid concentration by almost 3% illustrated in Figure 14. There was a trend observed for an interaction effect between oil and enzyme ( $p < 0.1$ ) where treatment PO increased C18:0 fatty acid concentration in subcutaneous adipose tissue.



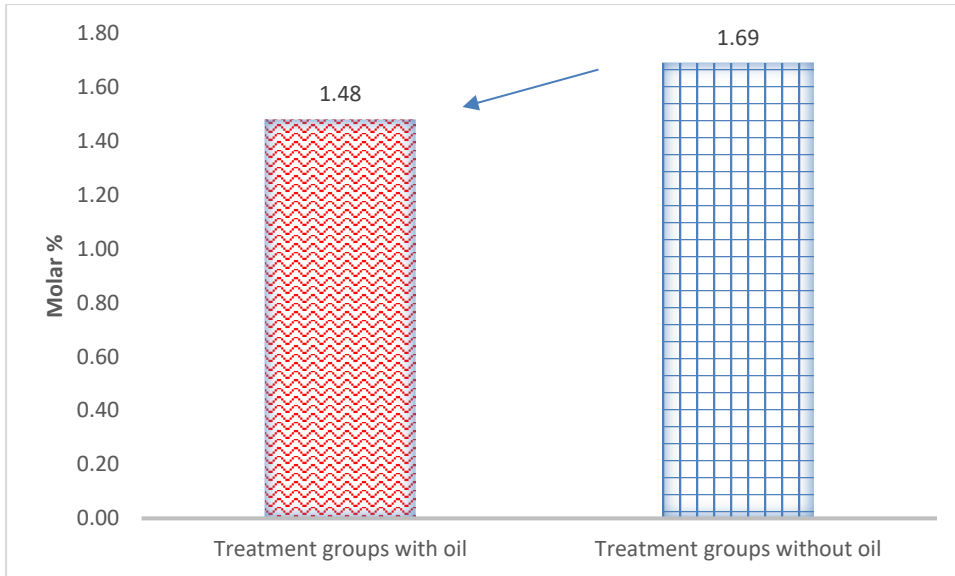
**Figure 15** Effect of palm oil supplementation on C14:0 in subcutaneous tissue

\*Arrow indicates significant difference ( $p < 0.05$ )



**Figure 16** Effect of palm oil supplementation on C16:0 in subcutaneous tissue

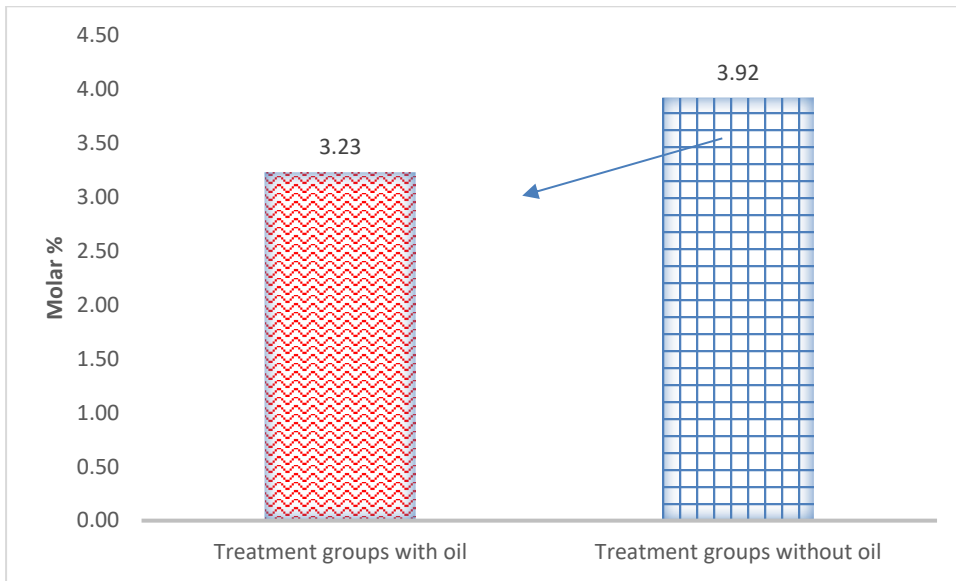
\*Arrow indicates significant difference ( $p < 0.05$ )



**Figure 17** Effect of palm oil supplementation on C17:0 in subcutaneous tissue  
\*Arrow indicates significant difference ( $p < 0.05$ )

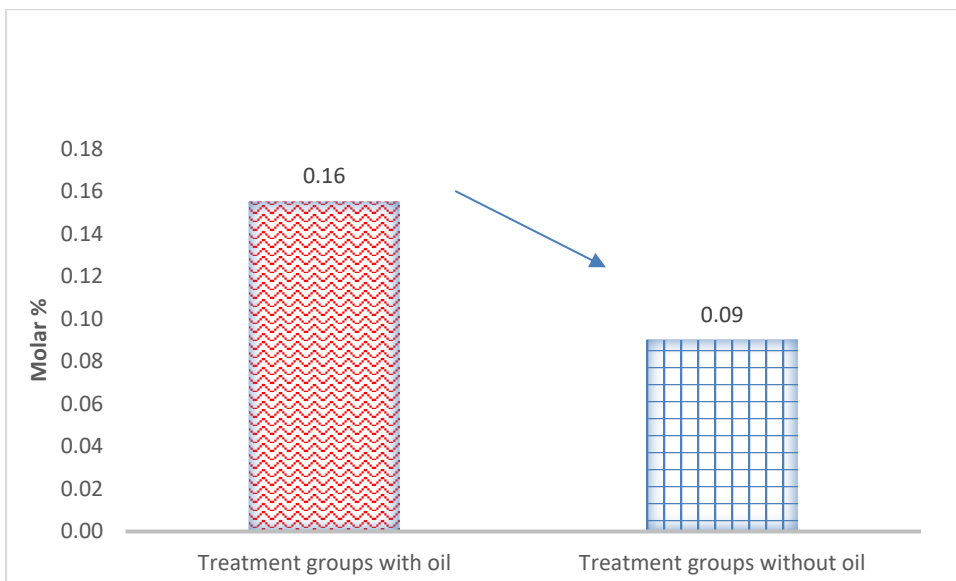
Partida et al. (2007) also reported that the ratio of palmitic (C16:0) and stearic (C18:0) acids was significantly affected by a palm oil dietary supplement. Where significant differences were observed for fatty acid myristic acid (C14:0), palmitic acid (C16:0), margaric acid (C17:0), palm oil supplementation generally resulted in a reduction of fatty acid concentrations illustrated in Figure 15, 16 and 17 compared to treatment ET and C

#### 4.2.4.2 Subcutaneous unsaturated fatty acids



**Figure 18** Effect of palm oil supplementation on C18:2n6 in subcutaneous tissue

\*Arrow indicates significant difference ( $p < 0.05$ )



**Figure 19** Effect of palm oil supplementation on C20:3n6 in subcutaneous tissue

\*Arrow indicates significant difference ( $p < 0.05$ )

The results of n-6 unsaturated fatty acids agree with the literature. In most studies, increased n-3 concentration in the intramuscular fat was accompanied with a decreased n-6 deposition, mainly due to a lower n-6 dietary supply between treatments. In this study palm oil supplementation decreased ( $p < 0.05$ ) linoleic acid (C18:2n6) fatty acid concentration, and increased ( $p < 0.05$ ) the EPA (C20:3n6) fatty acid concentration in subcutaneous tissue. An interaction effect ( $p < 0.05$ ) was also observed for EPA (C20:3n6) the values were so small it can be overlooked.

The interaction effect detected in arachidonic acid (C20:4n6), decreased ( $p < 0.05$ ) the concentration of fatty acid, also treatment ET decreased ( $p < 0.05$ ) the concentration of fatty acids. A slight increase ( $p < 0.05$ ) in the concentration of arachidonic (C20:4n6) fatty acid concentration in treatment O\*E was detected.

A reduction ( $p < 0.05$ ) of linoleic acid (C18:2n6) seen in treatment PO could be of concern as this is an essential fatty acid that cannot be synthesized de novo, but the mean deviation is so small that it is negligible.

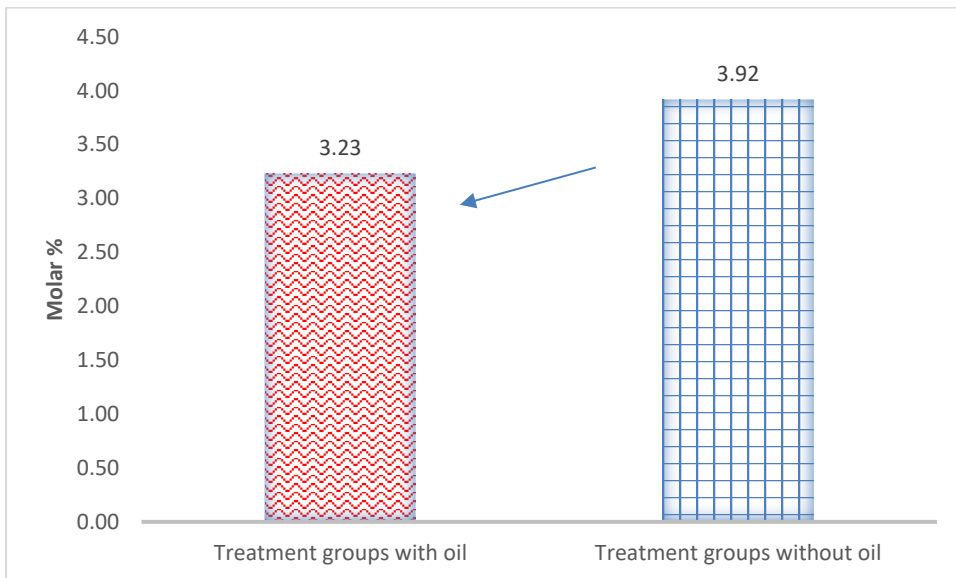


**Table 10** Effects of different oil and enzyme treatment groups on intramuscular fatty acid composition of sheep

Intramuscular adipose tissue fatty acids	LS mean (Molar %) [ $\bar{x} \pm SD.$ ]					Significance ( $P=f$ )		
	Overall	C	O	E	O*E	O	E	O*E
Saturated Fatty acids	63.61±2.91	63.31±2.38	64.13±2.20	63.12±4.11	63.86±2.95	0.419	0.806	0.967
Monounsaturated Fatty acids	33.20±2.77	33.17±2.19	33.17±2.19	33.32±3.83	33.13±2.97	0.919	0.951	0.919
Polyunsaturated Fatty acids	3.12±0.58	3.50±0.45	2.57±0.42	3.50±0.46	2.89±0.42	<b>0.000</b>	0.258	0.265
Ratio of unsaturated/ saturated fatty acids	0.57±0.08	0.58±0.06	0.56±0.05	0.59±0.11	0.57±0.07	<b>0.368</b>	0.735	<b>0.993</b>
Saturated fatty acids	Overall	C	O	E	O*E	O	E	O*E
C10.0 F	0.21±0.12	0.25±0.17	0.16±0.09	0.21±0.13	0.22±0.09	0.280	0.760	0.197
C12.0 F	0.34±0.47	0.32±0.22	0.18±0.12	0.22±0.11	0.60±0.88	0.106	0.222	0.504
C14.0 F	3.41±0.70	3.73±0.50	3.11±0.94	3.64±0.63	3.18±0.48	<b>0.014</b>	0.966	0.705
C16.0 F	29.75±1.91	31.20±1.29	29.03±1.46	30.42±2.02	28.35±1.52	<b>0.000</b>	0.157	0.922
C17.0 F	1.46±0.34	1.40±0.37	1.31±0.37	1.61±0.32	1.54±0.25	0.445	<b>0.045</b>	0.948
C18.0 F	28.30±3.97	26.28±3.44	30.24±3.90	26.52±3.92	30.15±3.12	<b>0.002</b>	0.948	0.889
C20.0 F	0.16±0.05	0.15±0.05	0.17±0.05	0.14±0.05	0.20±0.03	<b>0.006</b>	0.469	0.291
Unsaturated fatty acids	Overall	C	O	E	O*E	O	E	O*E
C16.1 F	2.09±0.60	2.00±0.87	1.99±0.50	2.11±0.56	2.29±0.41	0.685	0.292	0.602
C18.1n9t F	3.20±1.29	3.03±0.95	3.02±0.65	4.12±1.98	2.63±0.78	<b>0.057</b>	0.363	<b>0.062</b>
C18.1n9c F	27.38±2.15	27.59±1.35	27.68±2.15	26.59±2.03	27.64±2.91	0.414	0.454	0.493
C18.2n6c F	2.60±0.54	2.90±0.46	2.14±0.41	2.96±0.47	2.39±0.38	<b>0.000</b>	0.269	0.509
C18.3n6F	0.18±0.05	0.17±0.05	0.18±0.05	0.18±0.05	0.18±0.05	0.858	0.909	0.656
C18.3n3 F	0.21±0.05	0.25±0.06	0.18±0.05	0.23±0.04	0.20±0.05	<b>0.002</b>	0.936	0.204
C20.4n6 F	0.08±0.03	0.09±0.04	0.07±0.03	0.08±0.03	0.07±0.03	<b>0.060</b>	0.272	0.371

C= Control; O= Palm oil supplemented treatment group; E= Enzyme supplemented treatment group; O\*E= Combination of palm oil and enzyme supplemented treatment group

#### 4.2.5 Fatty acid concentration of intramuscular adipose tissue samples

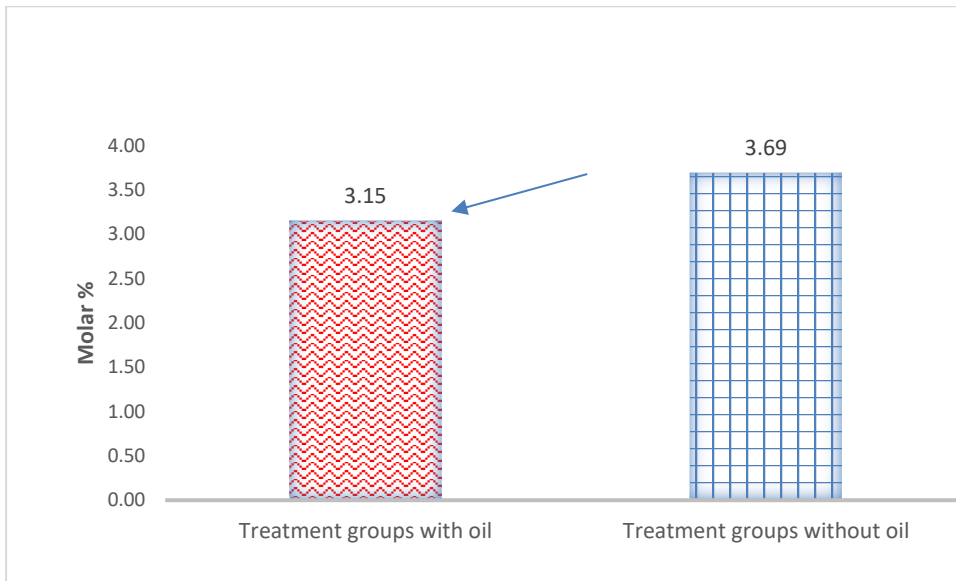


**Figure 20** Effect of palm oil supplementation on polyunsaturated fatty acids in intramuscular adipose tissue.

\*Arrow indicates significant difference ( $p < 0.05$ )

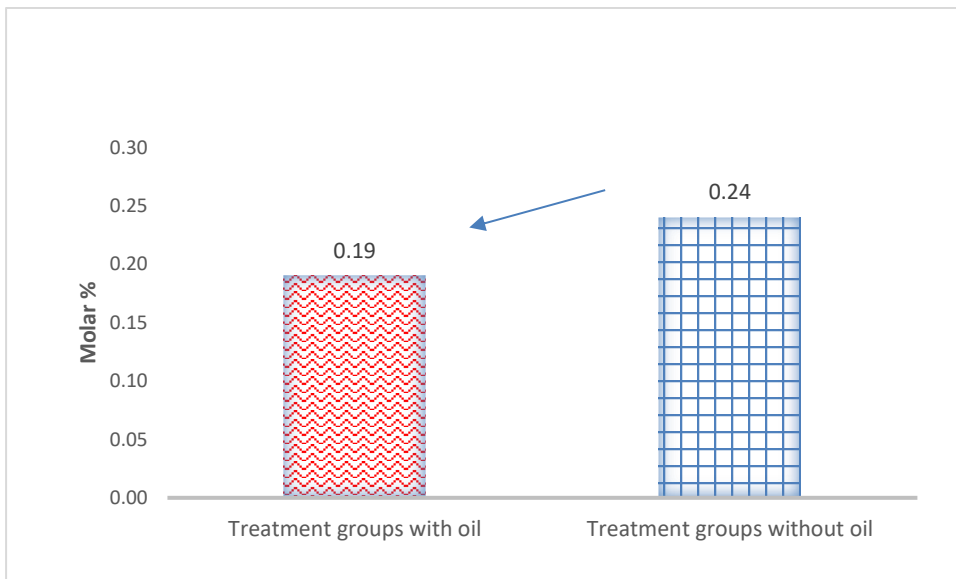
Treatment PO in the present study decreased ( $p < 0.05$ ) the overall concentration of PUFA in intramuscular adipose tissue compared to treatment C and ET. No differences were observed for overall saturated and monounsaturated fatty acids of intramuscular adipose tissue.

#### 4.2.5.1 Intramuscular adipose tissue saturated fatty acids



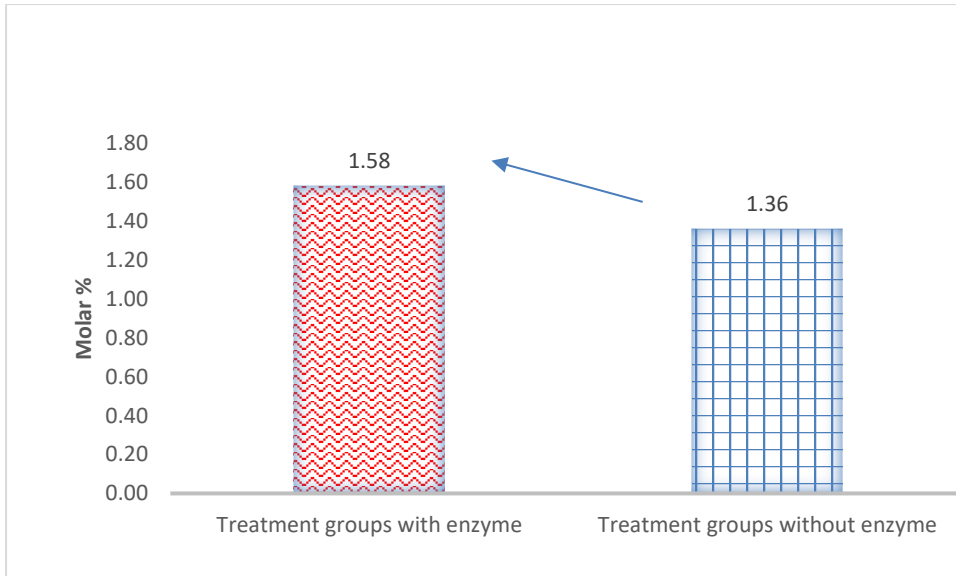
**Figure 21** Effect of palm oil supplementation on C14:0 in intramuscular tissue

\*Arrow indicates significant difference ( $p < 0.05$ )



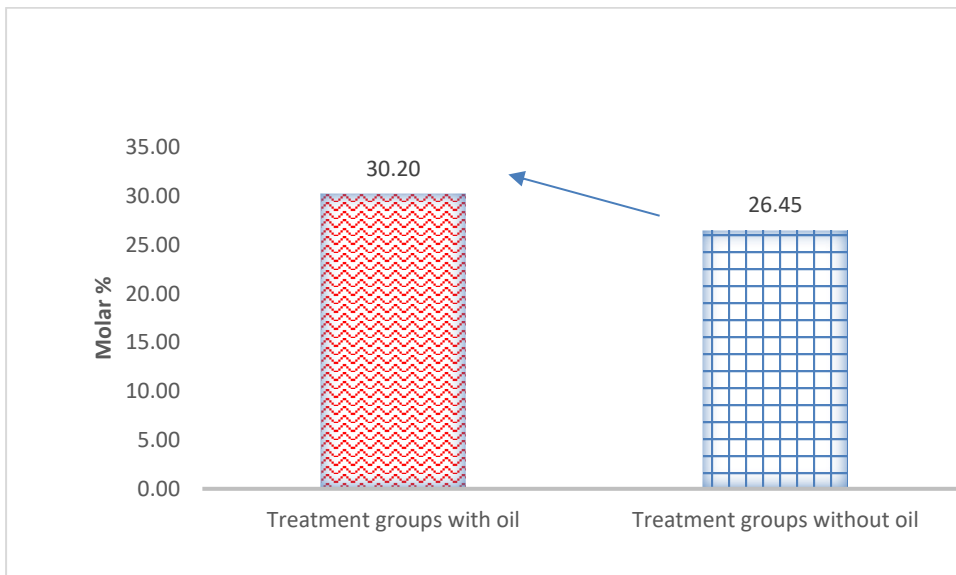
**Figure 22** Effect of palm oil supplementation on C16:0 in intramuscular tissue

\*Arrow indicates significant difference ( $p < 0.05$ )



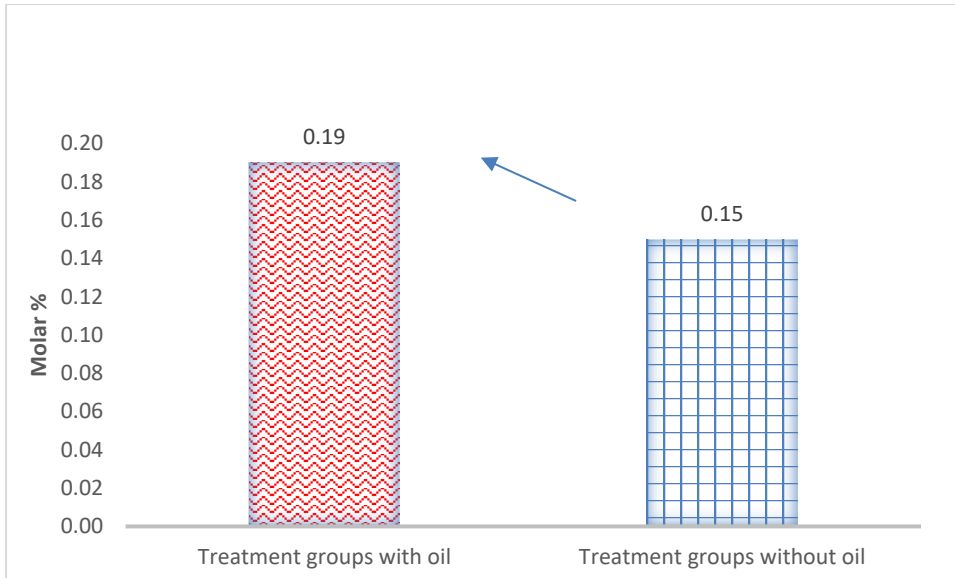
**Figure 23** Effect of palm oil supplementation on C17:0 in intramuscular tissue

\*Arrow indicates significant difference ( $p < 0.05$ )



**Figure 24** Effect of palm oil supplementation on C18:0 in intramuscular tissue

\*Arrow indicates significant difference ( $p < 0.05$ )



**Figure 25** Effect of palm oil supplementation on C20:0 in intramuscular tissue

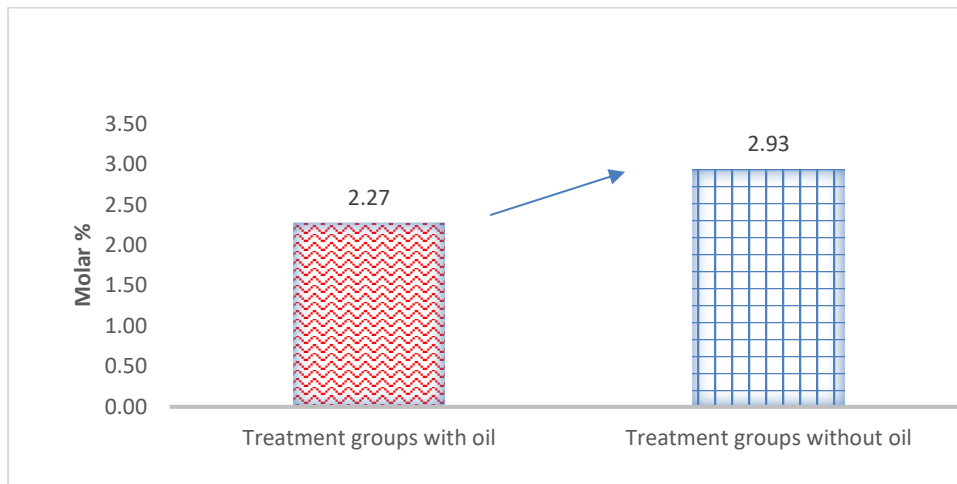
\*Arrow indicates significant difference ( $p < 0.05$ )

No interaction effects were observed for the saturated fatty acids of intramuscular adipose tissue. Fatty acid concentration for treatment PO was generally lower ( $p < 0.05$ ) in concentration of myristic acid (C14:0) and palmitic (C:16) fatty acid (Figure 21 & 22). Palm oil supplementation had the opposite effect in stearic acid (C18:0) and arachidic (C20:0) fatty acid concentrations and increased ( $p < 0.05$ ) the fatty acid concentrations compared to treatment groups without oil supplementation illustrated in Figure 24 & 25.

Interestingly treatment ET had a significant increase ( $p < 0.05$ ) effect on margaric (C17:0) fatty acid concentration compared to treatment groups without enzyme supplemented (Figure 23).

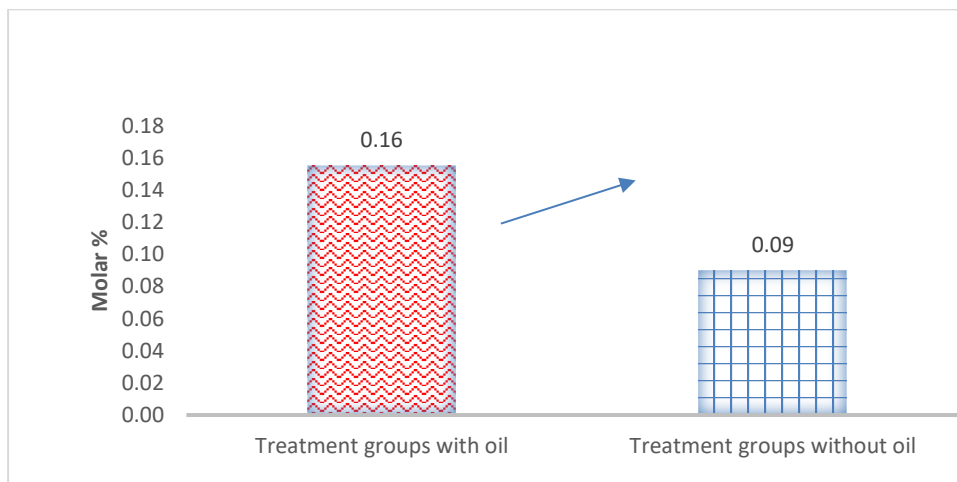
Overall where oil treatment groups were of significance a reduction in fatty acid concentrations was observed in oil supplemented treatment groups.

#### 4.2.5.2 Intramuscular adipose tissue of unsaturated fatty acids



**Figure 26** Effect of palm oil supplementation on C18:2n6 in intramuscular tissue

\*Arrow indicates significant difference ( $p < 0.05$ )



**Figure 27** Effect of palm oil supplementation on C18:3n3 in intramuscular tissue

\*Arrow indicates significant difference ( $p < 0.05$ )

An overall decrease ( $p < 0.05$ ) in fatty acid concentration was observed in treatment PO in linoleic acid (C18:2n6) and  $\alpha$ -linolenic acid (C18:3n3) (Figure 26 & 27). Oil treatment also tended ( $p < 0.1$ ) to lower the fatty acid concentration of oleic acid (C18:1n9t) and arachidonic acid (C20:4n6).

## Chapter 5

### Conclusion and recommendations

From the results in this study HA hypothesis can be accepted. HA: Fibrolytic enzymes and / or bypass oil supplementation has a significant effect on carcass fat content and composition.

Palm oil supplementation had no major effects on carcass characteristics that were of interest in this study, namely: growth, dressing percentage, carcass and fatty acid composition and carcass meat and fat colour. There was a trend for PO to lower ADG, this will be a factor to take into account when considering the implementation of palm oil supplementation as a methane mitigation strategy, and weighed against return on investment. If research is published that palm oil and enzyme supplementation used as a methane mitigation strategy has a significant effect in reducing excess methane produced, feedlots will also have to take into account that lambs in PO treatment took on average 8 days ( $p < 0.05$ ) longer to reach target weight for slaughter.

In terms of meat colour, no differences were observed, since it is usually associated to differences in fat content, carcass fatness or ultimate pH, and no differences were observed for these parameters.

The HCW and CCW were lower in treatment PO. Carcasses classed A2 and A3 is preferred by South-African consumers, and consumer resistance against the implementation of palm oil as a methane mitigation strategy should thus be unlikely.

Dressing percentages in this study are in line with industry averages of around 45%, even though PO had a lower dressing percentage compared to treatment C and ET. Carcass composition between overall treatment averages only differed for meat %. The PO treatment increased meat % of the carcass. As expected palm oil supplementation significantly affected carcass fat %, interestingly treatments PO and O\*E had a lower percentage carcass fat, than lambs on treatments C and ET.

The proportion of fatty acids from this study were similar across all four treatments groups even though significant differences were detected. Numerically these values were so small, even negligible in some cases. Interaction effects were detected between oil and enzyme treatments for SFA's, MUFAs and UFA:SFA in subcutaneous adipose tissue numerically there is only a minor difference between

treatment groups. Oil treatment groups in the present study differed from the other treatment groups and generally reduced the PUFA concentration.

Treatment with palm oil supplementation significantly affected the subcutaneous fatty acid profiles of lambs, which include C14:0, C16:0, C17:0, C18:0, C18:2n6c, C20:3n6 and C20:4n6. In all of the SFA's where oil supplementation had a significant effect, a general reduction in SFA concentration was observed for oil treatments, except for stearic acid (C18:0) where treatment PO generally increased the fatty acid concentration. Palm oil supplementation decreased ( $p < 0.05$ ) linoleic acid (C18:2n6c) fatty acid concentration, but tended to increase the EPA (C20:3n6) fatty acid concentration in subcutaneous tissue.

More or less the same significant effects were noticed in intra muscular tissue results where palm oil supplementation also affected C20:0 and C18:3n3 together with other fatty acids mentioned above. Fatty acid concentrations for treatment PO were generally lower ( $p < 0.05$ ) in concentration of myristic acid (C14:0) and palmitic (C:16) fatty acid. Palm oil supplementation had the opposite effect in stearic acid (C18:0) and arachidic (C20:0) fatty acid concentrations and increased ( $p < 0.05$ ) the fatty acid concentrations significantly compared to treatments ET and C.

Interestingly treatment ET had a significant increase effect on margaric (C17:0) fatty acid concentration compared to treatment OP.

Overall where oil treatment groups were of significance a reduction in in fatty acid concentrations was observed in treatment PO.

If methane mitigation strategy through dietary interventions are successful in lowering the carbon footprint it can be concluded that although palm oil and/or enzyme treatment groups showed significant effects in some of the evaluated parameters, numerically these values were minor, and probably negligible. Although lambs in treatment groups supplemented with oil took a few days longer to reach target weight, differences between treatment groups for all other parameters, ADG, HCW, CCW, pH @ 24 hours and D% were small. With no negative effects on carcass characteristics observed consumer resistance should not be expected.



## 5.1 Critical review and recommendations

Methane mitigating research is generally challenging since the capacity of the methane chambers are rather limiting. Despite these limitations, the current study had sufficient lambs per treatment to accurately assess the effects of methane mitigating strategy of supplementing palm oil and or fibrolytic enzymes on carcass composition and quality. To accommodate this limitation of 10 lambs per treatment group, we sourced as similarly as possible lambs and used a completely randomized design to sort them into treatment groups. Ideally 20 lambs per treatment group with more replicates would have been more statistically relevant and accurate. In future studies male and female lambs, of different breeds can be recommended, with higher concentrations or different of oil and enzymes can be researched.

There were a few areas open for improvement for future experiments.

A recommendation would be to pellet feed to improve consistency (Figure 5).



**Figure 28** Different diets were not mixed unanimously



Figure 29 Feed troughs weren't uniform and some wires holding troughs up against the fence broke from time to time spilling feed.

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## APPENDIX A: AEC APPROVAL FORM



UNIVERSITEIT VAN PRETORIA  
UNIVERSITY OF PRETORIA  
YUNIBESITHI YA PRETORIA

### Animal Ethics Committee

PROJECT TITLE	Effect of fibrolytic enzymes and bypass oil supplementation on the performance and carcass characteristics of sheep fed a high forage diet
PROJECT NUMBER	EC084-14
RESEARCHER/PRINCIPAL INVESTIGATOR	Ms L van der Walt

STUDENT NUMBER (where applicable)	290 62 595
DISSERTATION/THESIS SUBMITTED FOR	MSc (Agric Animal Science)

ANIMAL SPECIES	Sheep	
NUMBER OF ANIMALS	40	
Approval period to use animals for research/testing purposes	October 2014- March 2015	
SUPERVISOR	Prof. E Webb	

**KINDLY NOTE:**

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

<b>APPROVED</b>	Date	27 October 2014
CHAIRMAN: UP Animal Ethics Committee	Signature	



## Animal Ethics Committee

PROJECT TITLE	Effect of oil type and addition of fibrolytic enzymes on fibre digestion, enteric methane production and performance of sheep fed a high forage diet
PROJECT NUMBER	EC035-14
RESEARCHER/PRINCIPAL INVESTIGATOR	M Booysc

STUDENT NUMBER (where applicable)	280 422 48
DISSERTATION/THESIS SUBMITTED FOR	MSc

ANIMAL SPECIES	Sheep	
NUMBER OF ANIMALS	32	
Approval period to use animals for research/testing purposes		May – December 2014
SUPERVISOR	Dr. A Hassen	

**KINDLY NOTE:**

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

<b>APPROVED</b>	Date	28 April 2014
CHAIRMAN: UP Animal Ethics Committee	Signature	