Harper Adams University

Enhancing the eating quality of concentrate fed lambs

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PhD

April-2019

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A thesis submitted to the Harper Adams University in accordance with the requirements for the award of the degree of Doctor of Philosophy

April-2019

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Abstract

Two studies were carried out to investigate the effect of dietary concentrate carbohydrate and fat source, and vitamin E level on animal performance, carcass composition and meat quality of concentrate fed lambs. In the first study, forty Suffolk cross Texel ewe lambs were blocked by live weight (LW) into four treatments (ten lambs /treatment): Grazed grass (G), barley based concentrate (B), dried grass based concentrate (DG) and sugar beet based concentrate (SB). The three concentrate diets were formulated to provide a similar level of crude protein, ether extract and an effective rumen degradable protein/fermentable metabolisable energy ratio >10.0 g/MJ. Diets DG and SB provided a similar water soluble carbohydrate content, but different proportions of neutral detergent fibre, whereas, in diet B the energy was supplied mainly as starch. Diet B contained Megalac© (rich in saturated fatty acids), whereas diets DG and SB contained linseed oil (high in C18:3*n*-3). Diet B was formulated to contain 60 mg vitamin E (α-tocopherol-acetate)/kg DM, and diets DG and SB to contain 250 mg vitamin E (α-tocopherol-acetate)/kg DM. Lamb performance on diet G was lower than that of those fed the concentrate diets. Concentrate carbohydrate source, fat source and vitamin E concentration did not affect animal performance, carcass composition or carcass measurements. Lambs fed diets DG or SB had a similar muscle C18:3*n*-3, C20:5*n*-3, cis-9, trans-11 CLA, *n*-6: *n*-3, C18:2*n*-6: C18:3*n*-3 and vitamin E content to those finished on grass. Lambs fed on either the grass or concentrate diets had similar lipid stability and sensory evaluation characteristics. In the second study, forty Suffolk cross Texel wether lambs were blocked and allocated by live weight to one of four treatments: Grazed grass (FG) or one of three iso-energetic and iso-nitrogenous diets, based on barley that contained Megalac© with 250 mg vitamin E (α- tocopherol-acetate)/kg DM (BML), or linseed oil at two levels of vitamin E 250 (BLL) and 500 (BLH) mg/kg DM. Lambs fed the concentrate diets had a higher live weight gain than those finished on grass. Concentrate fat source and vitamin E level did not affect lamb performance, although lambs finished on BLH tended to have a lower feed conversion ratio compared to those fed diet BLL. Compared to lambs fed BML, the C18:3*n*-3, C20:5*n*-3 and C22:6*n*-3 content of *longissimus dorsi* muscle from lambs fed diets BLL or BLH were increased, although, cis-9, trans-11 CLA was low compared to lambs fed diet FG or those diet DG (experiment 1). Muscle derived from lambs finished on BML or BLH had an enhanced shelf life (colour and lipid stability) compared to those fed BLL or FG. Neither grass nor concentrate diets affected the sensory attributes of lambs as perceived by consumers. Overall, the meat quality of concentrate fed lambs can be improved by inclusion of linseed oil with supra- nutritional vitamin E.

Acknowledgements

I would like to thank the following people, without those assistance, this study would not have been done.

I am indebted to the director of the study Dr Robert Wilkinson and supervisors Professor Liam Sinclair and Dr Karim Farag for their continuous support and guidance throughout the study.

I would also like to thank the technician staff at sheep unit (Giles Vince and Carrie Gauld) abattoir (Euro quality lambs) (Rizvan Khalid and Asif Rao) and laboratory staff (Dr Victoria Talbot, Mr Amjad Ali and Kevin Jones) for their assistance in animal caring, slaughtering and laboratory work. Further thanks go to the HRP student Thomas Kearns and MSc. student Cathal Cassidy.

I should like to thank Professor Ian Richardson and Katharine Gaye Hallett at the University of Bristol for their assistance in vitamin E determination.

Special thanks go to the PhD community at HAU who have helped me directly or indirectly with a special thanks to my good friends Araz Abbi, Hawar Zebari, Ahmed Kafi, Dr Sherwan Mostafa and Dr Jamal Khurshid for their help during this study.

I am very grateful to the Regional Food Academy department staff mainly Dr Wilatsana Porsi and Claire Hutchinson for their assistance during sensory evaluation.

The financial support for this study was provided by the higher committee for education development (HCED) in Iraq and is gratefully acknowledged.

Heartfelt thanks to my parents, brothers, sisters and my wife Awaz, sons; Ava and Pawan for their patience and encouragement. Special thanks go to Mr Mosa Zawity and Sulaiman Zawity for the financial and emotional support over the past four years.

Author’s declaration

This thesis has been composed by myself. All sources of information are shown in the text and listed in the references. This thesis has not been presented in any previous application for a degree.

Reyzan Hamo

A part of this study has previously appeared

Hamo RA, Sinclair LA, Farag KW, Wilkinson RG 2018. Enhancing the eating quality of concentrate fed lambs. Proceeding of the British society of animal science (annual meeting), Dublin, p 175.

List of abbreviations

α-TTP	α-Tocopherol Transfer Protein
BHB	β-Hydroxybutyrate
CHO	Carbohydrate
CLA	Conjugated Linoleic Acid
CMb	Carboxymyoglobin
CP	Crude Protein
DHA	Docosahexaenoic Acid
DLWG	Daily live Weight Gain
DM	Dry Matter
DMb	Deoxymyoglobin
EE	Ether Extract
EPA	Eicosapentaenoic Acid
FA	Fatty Acid
FCR	Feed Conversion Ratio
GE	Gross Energy
IMF	Intramuscular Fat
LD	*Longissimus Dorsi*
LT	*Longissimus Thorasic*
LW	Live weight
MB	Myoglobin
MDA	Malonaldehyde
ME	Metabolisable Energy
MMb	Metmyoglobin
MUFA	Monounsaturated FA
NDF	Neutral Detergent Fibre
NEFA	Non-Esterified Fatty Acid
OM	Organic Matter
OMb	Oxymyoglobin
PUFA	Polyunsaturated Fatty Acid
ROS	Reactive Oxygen Species
Se	Selenium
SFA	Saturated Fatty Acid
SM	*Semimembranosus* Muscle
TBA	Thiobarbituric Acid
TBARS	Thiobarbituric Acid Reactive Substances
VFA	Volatile Fatty Acid
WSC	Water Soluble Carbohydrate

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Chapter 1

1.0. Literature review

1.1. Introduction

In the UK, consumers prefer grass finished lamb over concentrate finished lamb (Wood, 2005) due to better animal welfare, a relatively short supply chain, and eating quality of this type of meat (Sañudo *et al*., 1998; Fisher *et al*., 2000 and Font i Furnols *et al*., 2009). The majority of British lambs are finished on grass but a significant proportion fail to reach their target weight before the end of the grass growing season, and so are finished on concentrate, or alternative fodder crops (EBLEX, 2014). In addition to these, a proportion of lambs are reared intensively to finish out of season, in spring, when lamb supply is low and market price high (AHDB, 2018). This variation in lamb finishing system means that both the chemical composition and eating quality of lamb fluctuates throughout the year. Manipulation of the diet of concentrate finished lamb provides an opportunity to enhance its eating quality and produce a more consistent market product throughout the year.

There are several factors that have a significant effect on the performance, carcass composition and organoleptic properties of lambs (Solomon *et al*., 1980). However, diet has the greatest influence on its eating and flavour quality (Fisher *et al*., 2000; Wood *et al*., 2004 and Nute *et al*., 2007). Elements of the diet, such as carbohydrate source, polyunsaturated fatty acid (PUFA) composition, antioxidant content (e.g. vitamin E and carotenoids), branch chain fatty acid and skatole concentration have a significant effect on the eating and flavour characteristics of lamb (Sinclair, 2007; Wood *et al*., 2008; Santé-Lhoutellier *et al*., 2008). Meat from grass finished lamb is characterized by having a high concentration of *n*-3 PUFA which have been associated with improved eating and flavour characteristics (Fisher *et al*., 2000), as well as reducing the risk of coronary heart disease (Department of Health, 1994). In contrast, meat from concentrate finished lamb has a high content of saturated FAs, branch chain FAs, and C18:2*n*-6 (Young *et al*., 2003; Nuernberg *et al*., 2005). Branched chain FAs are responsible for the characteristics of lamb, and are formed from propionate produced during the fermentation of starch in the rumen (Young *et al*., 1997). Nute *et al*. (2007) reported that lambs fed concentrate diets had a higher content of C18:2*n-6* than those fed grass and that this was negatively correlated with normal lamb flavour. The variation in meat flavours is caused by fatty acids (FAs) that produce volatile, lipid oxidation products and odours during cooking (Wood *et al*., 2003).

Sheep meat has been criticised for its FA composition (Aurousseau *et al*., 2004), as low ratio of polyunsaturated FA (PUFA) to saturated FA (P:S ratio) increases the risk of cardiovascular diseases (Nieto and Ros, 2012). It is recommended that the P:S ratio in the human diet should be >0.4 (Department of Health, 1994). Previous work has demonstrated that the FA profile in sheep meat can be manipulated to better meet the requirements of the human diet using different sources of FAs such as linseed oil, fish oil, marine algae and protected linseed and soybean (Cooper *et al*., 2004; Demirel *et al*., 2003 and Wachira *et al*., 2002). However, little attention has been directed at manipulating the FA profile with respect to improving the eating and flavour quality, or to producing a FA profile similar to that of grass finished lambs.

It has been reported that vitamin E affects meat quality by acting as an antioxidant and reducing colour deterioration and PUFA oxidation (Wood *et al*., 2003; Macit *et al*., 2003). A high content of unsaturated FAs has been associated with reduced shelf life, and it has been shown that the higher the unsaturated FA content of meat, the higher vitamin E required to prevent lipid oxidation (Young *et al*., 1997; Ponnampalam *et al*., 2012). The intensity of redness (saturation or chroma), declines more rapidly as the display period progresses in the muscle of lambs fed concentrate diets, and it reaches the end of its shelf life sooner compared to those fed forages (Kasapidou *et al*., 2012; Baldi *et al*., 2016). Lambs finished on concentrate diets tend to have lower tissue vitamin E concentrations than those fed fresh grass (Whittington *et al*., 2006). This might influence the shelf life, flavour and eating quality of lamb finished on different diet types, although few studies have investigated the effect of vitamin E supplementation with high dietary PUFA in growing lamb diets.

The hypothesis to be tested in the current study were:

1- Can the chemical composition of grass finished lamb be replicated by using different concentrate carbohydrate and fat sources, and vitamin E levels?

2- Can the shelf life and eating quality of lamb meat finished on concentrate diets be improved by using different dietary fat sources and vitamin E levels?

1.2. Structure of the UK sheep industry

The sheep population in the UK has decreased by 3% from 34.8 million in 2017 to 33.8 million in 2018 (DEFRA, 2018). The UK sheep flock consists of approximately 16.3 million breeding ewes (DEFRA, 2018) which produced a total of 299,000 tonnes of meat in 2018, consumption was 298,000 tonnes making the UK 100% self-sufficient in sheep meat (AHDB, 2018a). The UK is currently the world's third largest exporter and second largest importer of sheep meat, exporting a total of 96,000 tonnes, which goes to the EU (96%). However, 96,000 tonnes are also imported (79% from New Zealand) (DEFRA, 2018). The sheep industry is an important part of the UK's agricultural industry and is worth £1 billion to the UK's economy (IBIS, 2018). However, sheep meat is the smallest sector of the UK's red meat industry (13.77 %), but it commands the highest price per kg (AHDB, 2016).

1.2.1. Stratification of the UK sheep industry

Lamb production systems vary between countries according to the climate and feed availability during the year. Some countries such as the UK, Ireland, New Zealand and Australia have suitable conditions to grow forage and finish most of their lambs on grass (Ponnampalam *et al.*, 2010; Montossi *et al.*, 2013). In contrast, a large number of countries such as the USA, Spain and most countries in the Middle East do not have a suitable climate for growing forages. Therefore their lamb production system is based on an intensive feedlot system (cereal-based diets) (Sañudo *et al.*, 2006).

In the UK, sheep production systems are unique to different regions, and the term stratification has been given. This system is well designed and divided into three tiers: hill, upland and lowland depending on the different types of breed, environments and habitats (Figure 1.1).

The first tier is hill breeds which are found in Scotland, Northern England and Wales. There are specific breeds such as Welsh mountain, Scottish Black face and Swaledale that are maintained as purebred breeding flocks in hill areas under harsh condition and tend to have one lamb per season. Male and surplus female lambs are transferred to upland/lowland to be fattened. Ewes that have lambed several times are also transferred to uplands, the second tier of the system, and crossed with long wool crossing rams such as Border Leicester and Blue Faced Leicester to produce Mule and half-bred ewes. Male crossbred and female of second crossbred are sent to slaughter. The first cross ewe lambs of upland are transferred to lowland areas, the third tier, where they are crossed with terminal sire breeds such as Texel, Suffolk to produce a crossbreed slaughter lambs (AHDB, 2017; NSA, 2017).

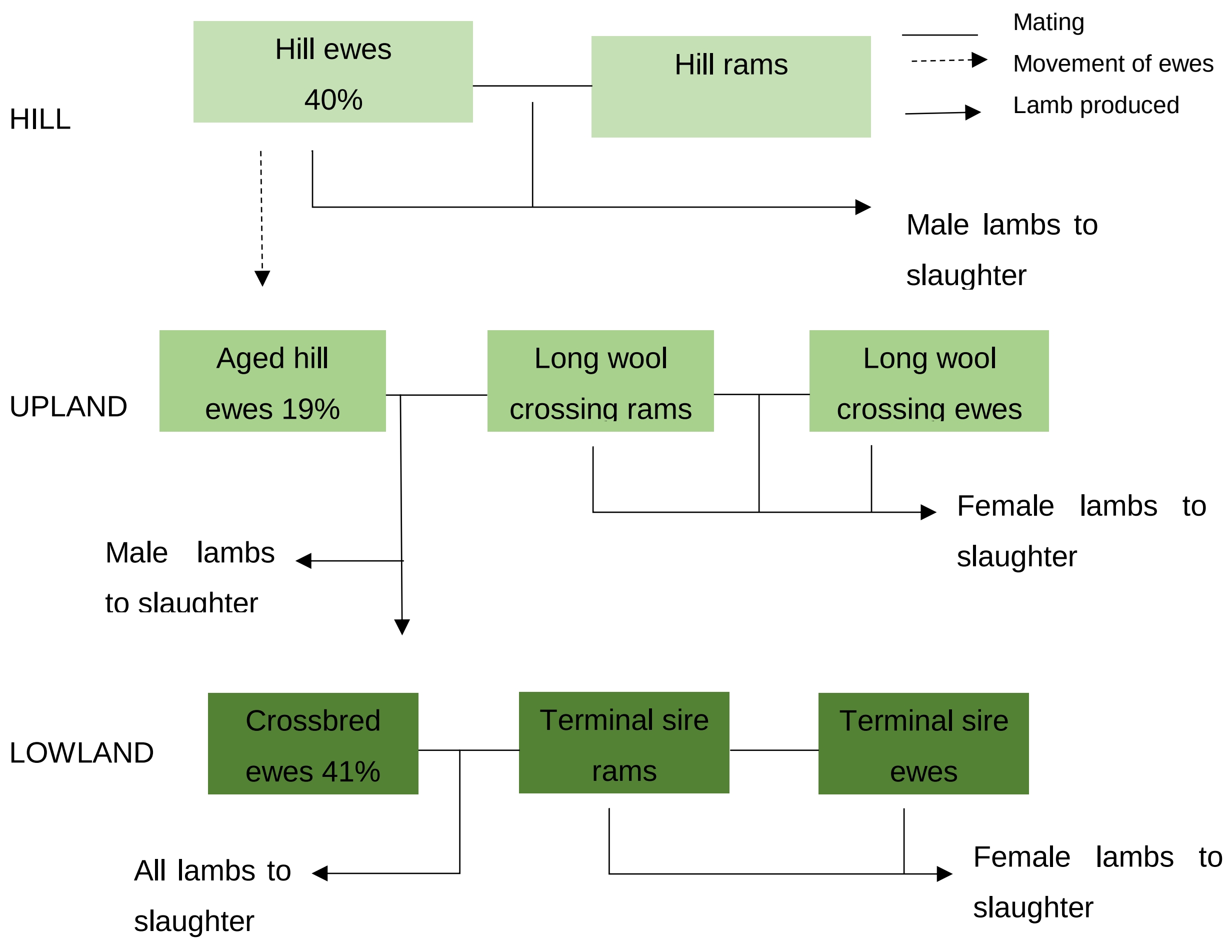


Figure 1. 1. UK Stratified sheep system (HCC, 2004; AHDB Beef and lamb, 2018).

Accordingly, lamb production system in the UK is divided into three systems; early weaning/early slaughter which relates to lowland sheep production, late weaning/early slaughter and late weaning/late slaughter relate to hill and upland sheep production with different physical performance targets for each system are shown in (Table 1.1).

1.2.2. Early weaning/early slaughter

Early lamb production: is when lambs are born in late of December or early of January. The aim is to market when the price is high during Easter (Figure 1.2). As grass growth is poor at this time of the year, they receive a large amount of concentrates.

1.2.3. Late weaning/early slaughter

Mid-season lamb production or spring lambing: is where the lambs are born between February and April and market lambs throughout the summer months. This is the most practised system in the UK, which maximises the use of grass and minimise the use of concentrate diets before the grass quality starts to decline. However, only 80% of the lambs achieves their target slaughter weight by grazing on grass. The remainder (20%) lambs are sold as store lambs.

1.2.4. Late weaning/late slaughter

Store lamb production or late lambing: is where the lambs are born in late of April and May. The aim is to finish them in the late of the season or early of the following year. These lambs require concentrate diets either if the lambs kept indoors on forage and concentrated feeding or outdoors on grass when the quality and quantity is not sufficient to finish on it.

Therefore, concentrate finishing is a component of all lamb production systems and it has a major role in early and late lambing production systems (Table 1.1).

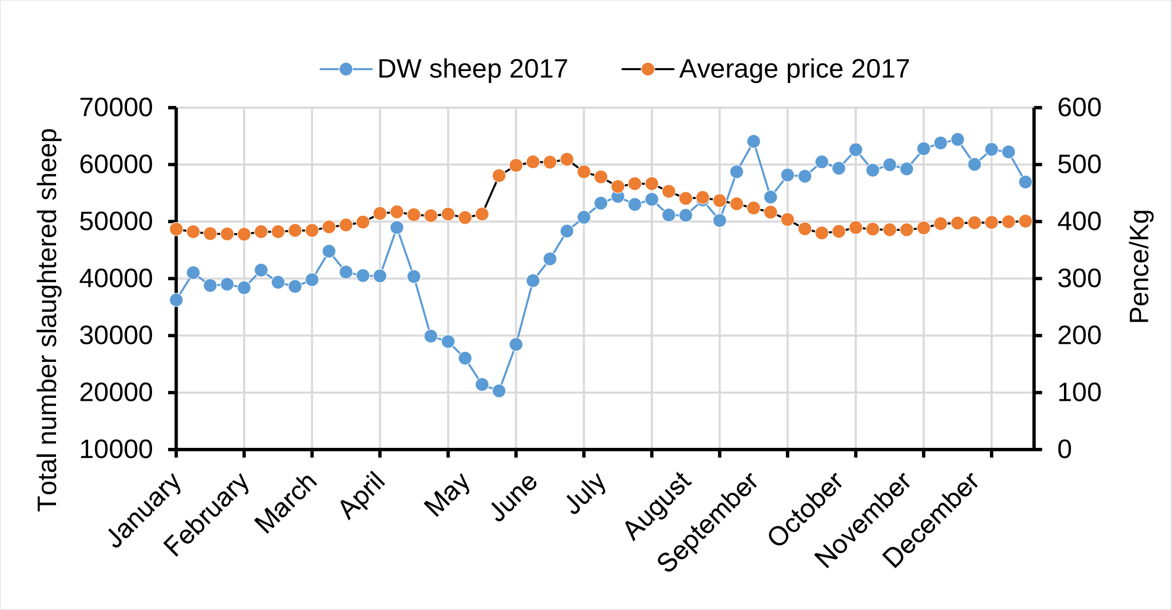


Figure 1. 2. Total number of deadweight sheep (DW) and average price in 2017 (AHDB, 2018a).

Table 1. 1. Physical performance targets for different lamb production systems in the UK (AHDB, 2016a).

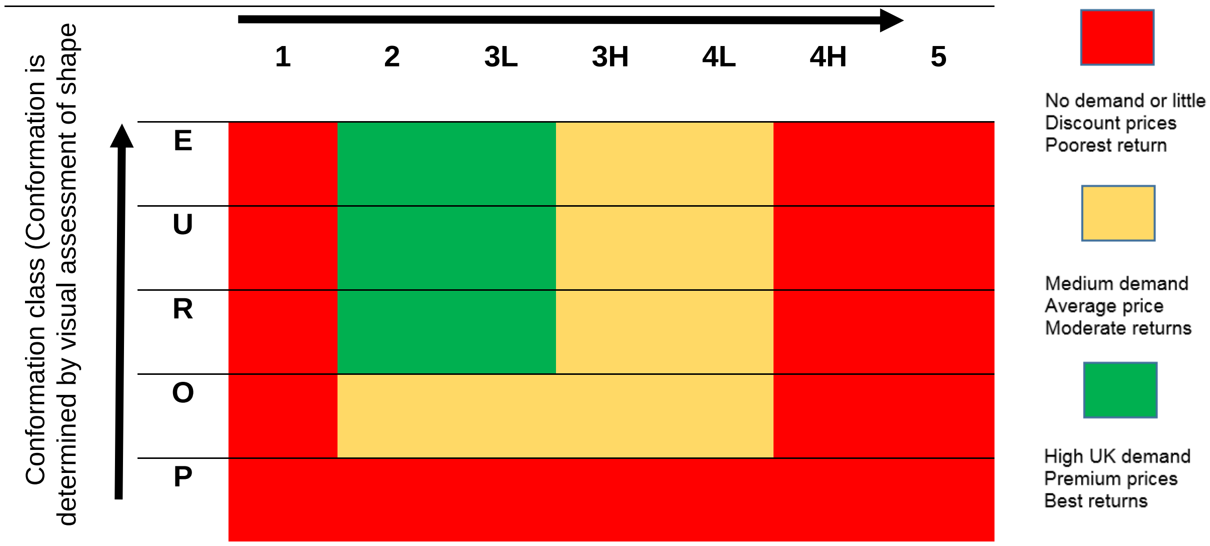
Finishing system	**Early weaning/ early slaughter**	**Late weaning/ early slaughter**	**Late weaning/ late slaughter**
Weaning age (days)	115	118	118
Slaughter age (days)	160	163	258
Slaughter LW (kg)	42.3	41.4	42.3
Carcass weight (kg)	19.7	19.1	19.7
Killing out %	47%	46%	47%
Daily LW gain (g)	240	210	110
Creep feed/lamb (kg/lamb)	11	2	9

1.3. Carcass composition and eating quality of meat

1.3.1. Whole body and carcass composition

Market research has shown that the majority of consumers prefer lean and tender meat which is safer to eat and from a trusted source (AHDB, 2018b). In addition, consumers are looking for a consistent product that gives them a satisfying eating experience. Lamb products are considered tasty, but consumers perceive that lamb is expensive and fatty. The current carcass classification system in the UK and Europe is EUROP (where E = excellent to P = poor) for carcass conformation and numerical assessment for fatness (where 1 very lean to 5 very fat), class 3 and 4 subdivided into L (leaner) and H (fatter). Combining conformation and fatness scores determine the market requirement for each type of carcass score (Table 1.2). Most farmers aim to produce animals within the green colour scores where the demand and price are highest (Figure 1.3). However, the percentage of UK lambs falling within target specification is 59.3% (R3L) and outside the target are 24.7% (too fat) and 15.7% (poor conformation) (AHDB, 2018b). Carcasses with better conformation score produce a greater amount of saleable meat, while fat score has the greatest effect on the saleable meat, with fatter carcasses yielding less meat to sell. A comparison of two carcasses of different conformation and fat class U3L and R4H is a good example. The U3L carcass has increased the retail value compared to R4H that also has additional costs during rearing lamb (Table 1.3).

Fat class (Fat is determined by a visual appraisal of external fat over)



	1	2	3L	3H	4L	4H	5
E	🟥	🟩	🟩	🟨	🟨	🟥	🟥
U	🟥	🟩	🟩	🟨	🟨	🟥	🟥
R	🟥	🟩	🟩	🟨	🟨	🟥	🟥
O	🟥	🟨	🟨	🟨	🟨	🟥	🟥
P	🟥	🟥	🟥	🟥	🟥	🟥	🟥

🟥 No demand or little
Discount prices
Poorest return

🟨 Medium demand
Average price
Moderate returns

🟩 High UK demand
Premium prices
Best returns

Figure 1. 3. EUROP system for conformation and carcass fatness score.

Table 1. 2. Different markets require different typical target class (AHDB, 2018b).

Main market	**Carcass weight (kg)**	**Conformation**	**Fat**
Butchers	16-25	E, U, R	2, 3L, 3H
Supermarket	16-21	E, U, R	2, 3L, possibly 3H
Exports	9-21	E, U, R	2, 3L

Table 1. 3. Comparison of meat yield (kg) from two different carcass classes (AHDB, 2016b).

	Carcass weight	**Shoulder**	**Leg**	**Chops**	**Chump**	**Neck**	**Trim and fat**	**Total meat**
U3L	19.00	4.14	4.76	2.86	1.44	2.06	3.76	15.24
R4H	19.00	3.56	4.40	2.86	1.02	2.40	4.77	14.24

Regular handling and weighing lambs are an essential practice for sheep farmers to avoid over fat, poor conformation, overweight and enhancing the killing out percentage. Fortnightly weighing and handling lambs post weaning required to optimise target carcass weight, fat cover and killing out percentage, which depends on age, diets type, gender and breed (Table 1.4).

Table 1. 4. Estimating killing out % of lambs at different ages (Mchugh, 2008).

Lamb age		**Killing out %**
Preweaning	**Postweaning**	
10-13 weeks	-----	50%
14 Weeks	-----	48%
-----	Late summer	45%
-----	Autumn/winter	43%

1.3.2. Shelf life (colour and lipid oxidation)

Consumers consider meat colour to be one of the most important characteristics because discolouration can be used as an indicator of a spoiled product (Cierach and Niedźwiedź, 2014). Consumers prefer bright red, fresh meat, which is linked to the presence of oxygenated myoglobin (Dikeman and Devine, 2014). Any change in colour may affect consumer preference and reduce sale as a result of surface discolouration, about 15 % of beef retail is discounted which contributes to an annual revenue loss of $1 billion (Smith *et* *al.*, 2000). In order to recuperate lost profit, improvements in meat colour are required which depends on the knowledge of myoglobin and its chemical states (Li and Liu, 2012). Meat colour or meat discolouration is influenced by both biological and technological factors (Sañudo *et al*., 1998b) The most important biological factors are myoglobin content, which varies according to species, breed, sex, muscle type, age and pH. The technological factors include animal diets, temperature, light and packaging techniques.

Myoglobin (Mb) is a sarcoplasmic protein that is responsible for the fresh meat pigment (Listrat *et al.*, 2016). The chemical states and amount of myoglobin determine about 90 % of the colour of the meat surface. The molecules of myoglobin consist of a globular single chain protein and central haem iron that plays a major role in meat colour characteristics. The chemical elements that bind to the haem group and its redox state determine the colour characteristics, the reduced form ferrous (Fe^{2+}) or the oxidised form ferric (Fe^{3+}) state of iron. In fresh meat, myoglobin is usually present in any of the four-redox states that differ in colour (Suman and Joseph, 2013) (Figure 1.4). These are deoxymyoglobin (DMb), oxymyoglobin (OMb), carboxymyoglobin (CMb) and metmyoglobin (MMb). DMb, OMb and CMb exist in the form of ferrous states. Meat in the state of DMb has a purplish red colour while the meat of both OMb and CMb redox states have a bright cherry red colour (Suman and Joseph, 2013). To distinguish between these two red colours by eyes is impossible (Cornforth *et al.*, 2008). The haem iron of myoglobin is occupied by oxygen in the case of OMb, and CO in the case of CMb and gives an attractive bright cherry red colour. With time, the 3 forms of ferrous (Fe^{2+}) are oxidised to their ferric state (Fe^{3+}) in Mb, which produces a brown colour (Mancini and Hunt, 2005).

The colour of the meat can be evaluated visually or instrumentally. A visual colour appraisal is done by a trained panel or untrained consumers who then express their opinion on the meat colour. Instrumentally, meat colour can be evaluated by measuring the quantity of meat pigment using a spectrophotometer (Krzywicki, 1979). The commonly used method is the CIELAB system to evaluate meat colour, which was proposed in 1976 by C.I.E. (Commission Internationale de l`Eclairage) based on three-dimensional space where L* is lightness, a* is redness and b* is yellowness. The results of the CIE system have been closely related to the proportion of OMb and MMb in the meat (MacDougall, 1982). Colour quantification in the CIELAB system uses a recognised measurement geometry to obtain a reflectance spectrum on the meat (500 nm – 600 nm) and calculate CIE (L*, a*, b*) values for a specific illuminant (D65 illuminant) (Krzywicki, 1982). The co-ordinate L* denotes luminosity, a* redness and b* yellowness. The co-ordinate a* and b* can be used to calculate saturation $(a^{*2} + b^{*2})^{½}$ and Hue (tan-1 (b*/a*) x 180/π) (CIE, 1976). Colour saturation is usually expressed as Chroma and refers to the purity or the intensity of colour, or the stability of red colour over time. Hue refers to browning colour. The saturation value of 18 is defined to be the limit value for the bright red colour (shelf life). This is based on the definition of saturation value in term of MMb percentage for beef when the value ≥ 20 is bright red, 18 is dull, 14 is brown and < 12 is grey to green (MacDougall, 1982).

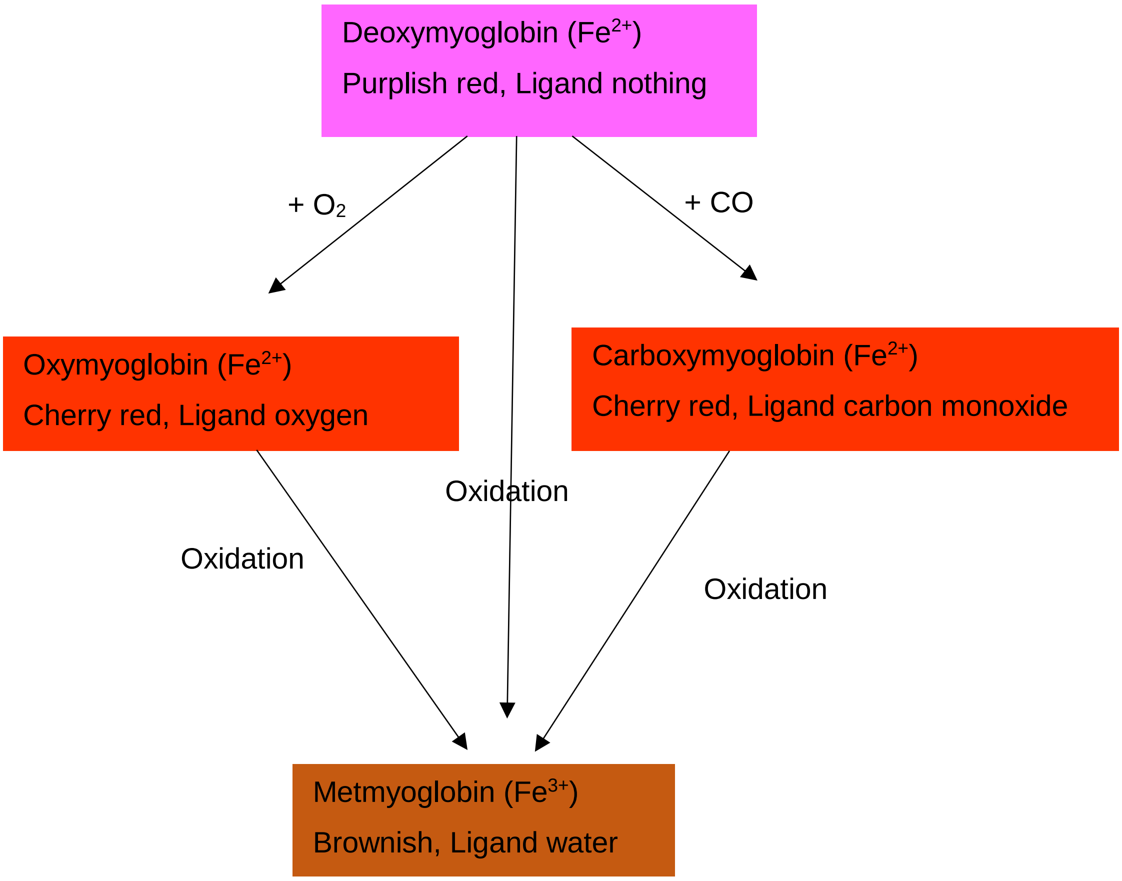


Figure 1. 4. Colour characteristics of fresh meat according to myoglobin states (adapted from Suman and Joseph, 2013).

Lipid oxidation is one of the most important factors that influence the consumer perception of meat (Min and Ahn, 2005) through its effect on colour, aroma and flavour and consequently nutritional value (Guyon *et al.*, 2016). In addition, some toxic compounds produced during the lipid oxidation reaction such as malonaldehyde contribute to the deteriorative process in humans (Kurt, 1999). One of the secondary products of lipid oxidation is aldehyde that directly reacts with protein and modifies the organoleptic properties of meat (Mottram, 1998). Lipid oxidation is influenced by many factors such as animal species, breed, diet and post-slaughter process (Morrissey *et al.*, 1998).

Lipid oxidation or autoxidation is a result of free radical chain reactions in three simultaneous phases (initiation, propagation and termination) (Hamilton *et al.*, 1997). Initiation is the first step of lipid oxidation when a hydrogen atom is removed from a methylene carbon in FAs (RH) by reactive oxygen species (OH• and HOO•) and produces a lipid radical (R•). This process becomes faster and easier when the number of a double bond in FA increases, which is why PUFA are more susceptible to oxidation (Halliwell and Chirico, 1993).

Initiation: $$RH + \text{Initiator } (OH\bullet \text{ and } HOO\bullet) \longrightarrow R\bullet + H_2O \qquad (1)$$

Propagation involves a reaction between a radical FA (R•) and oxygen to produce a peroxy radical (ROO•).

Propagation: $R\bullet + O_2 \longrightarrow ROO\bullet$ (antioxidants acts here) (2)

Unstable peroxyl-FA radicals (ROO•) then react either with another free FA or with itself to produce different FA radicals and cyclic peroxides and the process is continued (chain reaction).

$$ROO\bullet + RH \longrightarrow ROOH + R\bullet \qquad (3)$$

In addition, lipid hydroperoxides (ROOH) undergo a break down to produce hydroxyl radical (HO•) and lipo radical (RO•) by catalysis with Fe^{2+}.

$$ROOH + Fe^{2+} \longrightarrow RO\bullet + HO^{-} + Fe^{3+} \qquad (4)$$

$$Fe^{3+} + ROOH \longrightarrow ROO\bullet + Fe^{2+} + H^{+} \qquad (5)$$

The alkyl radical or lipo radical (RO•) may also become involved in further free radical reaction or may undergo breakdown to produce off flavour compounds (Mottram, 1998).

Termination involves the radical reaction stopping when two radicals react and produce a non-radical species.

Termination: $ROO\bullet + ROO\bullet \longrightarrow ROO\text{-}OOR$ (6)

$$ROO\bullet + R\bullet \longrightarrow ROOR + O_2 \qquad (7)$$

Lipid oxidation produces a wide rand of byproduct compounds, including off flavour compounds such as ketones, aldehydes, alcohols, acids, esters, furans and cyclic ketones. Most of these compounds have an intense odour and mainly contribute to the overall odour and flavour of the meat (Guyon *et al.*, 2016) (Figure 1.5).

The second phase of lipid oxidation is likely to occur directly pre-slaughter and certainly during the early stage post-slaughter (Morrissey *et al.*, 1998). During the conversion of muscle to meat, the biochemical changes provide conditions such that the highly unsaturated FAs in cell membranes are no longer able to control the oxidative process because the balance between antioxidants and pro-oxidants capacity disrupts and favours oxidation (Kerry and Ledward, 2009). The rate and extent of oxidation in phase two is likely to depend on the degree of tissue damage in live animals. Some events and techniques post slaughter, such as early post-mortem pH reduction, carcass temperature and electrical stimulation disrupt cell and cellular organelles and release catalytic metal ions (Morrissey, 1994).

The third phase of lipid oxidation is the most significant phase that occurs during handling, processing, storage and cooking. During this phase, high molecular weight sources of iron such as haemoglobin, myoglobin and ferritin release iron that reacts with low molecular weight compounds such as amino acids, nucleotides and phosphates to form chelates (Min and Ahn, 2005). These chelates in biological tissues act as catalysts for lipid oxidation (Halliwell and Chirico, 1993). In addition, some of the high molecular weight compounds such as haemoglobin and myoglobin can also directly catalyse lipid oxidation (Monahan *et al.*, 1993).

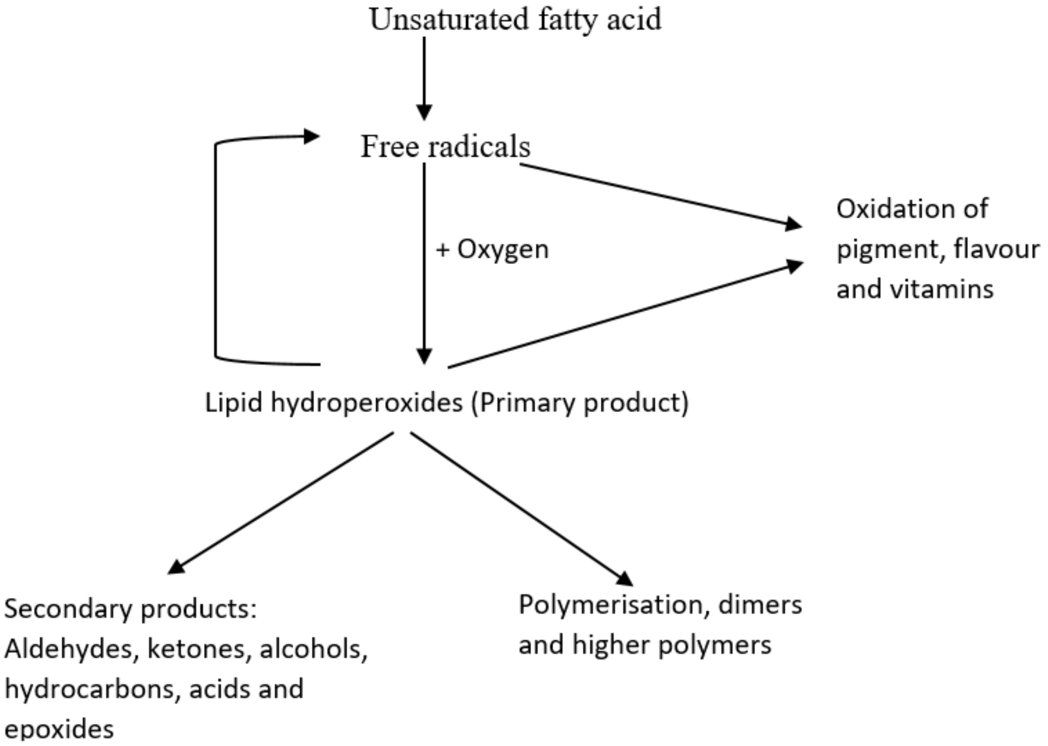


Figure 1. 5. Mechanism of overall lipid oxidation.

The end product of lipid oxidation is an aldehyde, so oxidative changes can be quantified by measuring these secondary by-products (Guyon *et al*., 2016). The most widely used method for measuring lipid oxidation in foods is the thiobarbituric acid TBA- essay, due to its simplicity and the fact that it results highly correlated to sensory evaluation scores (Tarladgis *et al*., 1960; Gray and Pearson, 1985). Data obtained from this test is usually expressed as malonaldehyde (mg/kg tissue). The principle behind this test is the reaction between two molecules of TBA and one molecule of malonaldehyde to form a pink malonaldehyde-TBA complex that can be quantified by using spectrophotometer at 532nm (Guyon *et al*., 2016).

A threshold of off flavour that is generally accepted and detected by sensory evaluation is above 0.5 mg MDA/kg meat (Tarladgis *et al.*, 1960). However, Wood *et al.* (2008), suggested that the upper limit for TBARS 0.5 mg MDA/kg meat which was based on pork and may not be appropriate for lamb or beef because the natural level of lipid oxidation is higher.

1.3.3. Eating quality

Meat quality includes eating characteristics such as flavour and tenderness and nutritional values that depend on the chemical composition of muscles such as FA content and lean to fat ratio (Santé-Lhoutellier and Pospiech, 2015). One of the important aspects of eating quality is flavour, which is produced from the cooked meat. Once the meat cooks, the reactions between carbohydrates and proteins, and their products start to produce a meat flavour (Mottram, 1998). In addition, the end products of lipid degradation such as aldehydes, alcohols and ketones have a role to play in the development of this meat flavour (Melton, 1999).

Flavour has been considered one of the most important factors which influence consumer purchasing decision (Reicks *et al*., 2011). The combination of two sensations of the taste and odour are responsible for the meat flavour, although juiciness and mouthfeel also influence flavour perception (Farmer, 1994). The four taste sensations (sweet, sour, salt and bitter) can be distinguished by the receptors in the mouth, while receptors can recognise thousands of smells in the human nose. Low molecular weight volatile substances are responsible for the odour, while larger molecular weight and water-soluble compounds are mainly responsible for the taste (Farmer, 1994).

The flavour of meat products can be measured using people (either trained or untrained panellists) and is currently not possible to measure instrumentally (Wood and Richardson, 2004). However, the flavour precursor compounds in the meat can be measured to understand flavour development by instruments such as gas chromatography (GC) and high-performance liquid chromatography (HPLC), but these are not considered as a substitute to trained or untrained panellists (Wood and Richardson, 2004; Dikeman and Devine, 2014).

There are thousands of volatile compounds that can stimulate the odour sensation, including aliphatic and aromatic compounds that generally contain a heteroatom (N, S, O). This gives a precise electronic configuration that is distinguishable by receptors in the human nose. Unpleasant odours can be produced from the meat and food by enzymatic action. This can be due to microbiological spoilage or the animal's metabolism (Jelen, 2012). In addition, off flavour can also arise either from external contamination or from autoxidation of fats (Hui *et al*., 2001). However, the main sources of odour compounds in meat are the chemical reactions, which occur during heating. These chemical compounds responsible for odour have no nutritional value but are produced from compounds in meat that do have nutritional value (Farmer, 1994; Mottram, 1998) (Table 1.5). More than 800 volatile compounds have been identified in the aroma of cooked beef (Farmer, 1994) and 271 compounds in lamb or mutton (Mottram, 1998). However, only a small number of those compounds play a major role in the aroma of cooked meat, depending on their concentration and threshold of detection by the human nose (Khan *et al*., 2015).

Table 1. 5. Odour precursors and their compounds in cooked meat (Mottram, 1998).

Odour Precursors	**Compound**	**Mechanism**
n-6 fatty acids	Trans-2-nonenal	Thermal oxidation
n-6 fatty acids	Trans,trans-2-4decadienal	Thermal oxidation
n-6 fatty acids	1-Octe*n*-3-one	Thermal oxidation
Proline	2-Acetyle-1-pyrroline	Maillard reaction
Methionine	Methional	Strecker degradation
Phenylalanine	Phenyl acetaldehyde	Strecker degradation
Cysteine and ribose or Thiamin	2-Methyl-3-furanthiol	Maillard reaction
Cysteine and ribose or Thiamin	Bis 2-methyl-3-fury1 disulphide	Thermal degradation
β-Carotene	β-Ionone	Oxidative degradation

Meat flavour (taste) precursors are generally comprised of non-volatile compounds and water-soluble compounds that are responsible for the taste or sensory properties (Bailey, 1994). These compounds include peptides, some amino acids and hypoxanthine (bitter), acids (sour) some amino acids and sugar (sweet) and sodium salts and inorganic salts (salty) (Farmer, 1994; Khan *et al*., 2015). Large molecular weight fibrillar and sarcoplasmic proteins are considered less important (Kerry and Kerry, 2011). The reaction between amino acids and sugars are heat-induced reaction called Maillard reactions (Mottram, 1998). The intermediates of these reactions are converted to meat flavour compounds by oxidation, condensation, decarboxylation and cyclization (Min *et al*., 1989). The heteroatoms (N, S, O) including furanones, furans, pyrazines, thiazoles, thiophenes and dicarbonyl compounds are particularly important to meat flavour (Farmer, 1994). Related reactions such as Strecker degradation produce other important compounds such as aldehydes, ammonia and hydrogen sulphide which influence the formation of meat flavour compounds (Bailey, 1983).

The required temperature to break down amino acids and sugars to give volatile compounds are much higher than those required to produce Maillard products (Shahidi *et al*., 1986). During cooking, some sugars are degraded into furanones and furfurals, while amino acids are relatively stable and are unlikely to undergo pyrolysis except at the surface during grilled or roasted meat (Shahidi *et al*., 1986; Mottram, 1998). Sulphur containing amino acids (such as cysteine) and ribose are the most important compounds for meaty flavour (Mottram, 1998). In addition, fat (FAs) also plays an important role in meat flavour development, as they act as a solvent for other flavour compounds (Wood and Richardson 2004). It is well documented that undesirable flavours and aromas are produced during prolonged storage of meat due to the oxidation of fat within meat (Wood *et al*., 1999). Oxidation and thermal degradation of FAs occur at a much lower temperature than the Maillard reaction and produce a variety of compounds that are responsible for the species-specific flavours (Young *et al*., 1997). Approximately half of the volatile compounds produced during cooking are lipid-derived, although most of these may not be important as they have high odour thresholds. However, some of them, especially aldehydes, alcohols and ketones are important as they have low odour thresholds (Melton, 1999). The reactions between lipid degradation products and Maillard reaction products produce a further range of flavour compounds, and some of the Maillard reaction products are also inhibited such as pyrazines. Lipid oxidation products are increased as unsaturated FAs increase and generated free radicals catalyse the oxidation of less unsaturated FAs. It has been confirmed that phospholipids containing unsaturated FAs are more important than triacylglycerols containing saturated FAs in meat flavour development (Mottram and Edwards, 1983; Wood and Richardson, 2004).

Tenderness is the most complex parameter of eating quality (Maltin *et al*., 2003; Warner *et al*., 2010). At first, it seemed like a simple “tender” or “tough”, but it was eventually found that describing tenderness is quite difficult (Warner *et al*., 2010). Tender, hard, tough, soft, mushy, string, firm, ease of fragmentation or chewy descriptors are used to describe tenderness (Kerth, 2013). Therefore, all of these textural traits make mechanically or objectively tenderness measurement difficult as the machines cannot take into consideration all of those descriptors (Danso *et al*., 2017). The best comprehensive method for measuring tenderness is subjectively by humans (Kerth, 2013).

The resistance to tooth pressure when biting meat is the most common way to express tenderness or how much force is required to bite a piece of meat (Tornberg, 1996). Resistance to tooth pressure (initial tenderness) and ease of fragmentation (sustained tenderness) are the most common measurements used in freshly cooked meat. Tenderness is divided into three categories; protein tenderness, connective tissue and background effect (Kerth, 2013). Amount of myofibrillar protein degradation and sarcomere contractile state determine protein tenderness, while amount and type of three layers of collagen; epimysium (around muscle), perimysium (muscle bundles) and endomysium (muscle fibres) determine connective tissue status (Tornberg, 1996; Listrat *et al*., 2016). Flavour and juiciness also indirectly determine a background effect of tenderness assessment (Juárez *et al*., 2012). The most common and accepted object measure tenderness is a shear force.

Objectively, the Warner-Bratzler instrument is the most widely used instrument in evaluating meat tenderness by shearing a well-defined piece of meat with the shearing action being perpendicular to the longitudinal orientation of muscle fibre (Honikel, 1998).

1.3.4. Nutritional indices

Polyunsaturated FAs to saturated FAs (P:S) and omega-6 to omega-3 (*n*-6: *n*-3) ratios are considered to be an important index for human health. Givens *et al*. (2006) presented the P:S and *n*-6: *n*-3 ratios in meats of three main species in the UK; lamb, beef and pork (Table 1.6). This shows that in general lamb and beef have low P:S ratio due to the biohydrogination process in the rumen, while pork has a desirable P:S ratio (>0.4) according to the Department of Health (1994). Ruminants have a favourable *n*-6: *n*-3 ratio (<4.0) as recommended by the Department of Health (1994) mainly due to the presence of C18:3 in grasses but it is high in pork due to the feeding pig with high concetrate diets (rich in *n*-6).

Table 1. 6. Relative fatty acid ratios for human health (Givens et al., 2006) and recommended in meat (Department of Health, 1994).

	Lamb	**Beef**	**Pork**	**Recommended**
P:S ratio	0.093	0.076	0.41	> 0.4
n-6: *n*-3 ratio	1.36	2.09	7.35	< 4.0

1.4. Factors affecting the chemical composition and eating quality of lamb

1.4.1. Animal factors

1.4.1.1. Age and stage of maturity

Body form and body composition change dramatically and continually during growth (Irshad *et al*., 2013). Growth and development intensity of various tissues can also vary (Figure 1.6). As an animal matures, it undergoes physiological and biochemical changes. This includes an increase in the ratio of muscle to bone, followed by a decrease in muscle growth rate and an increase in the ratio of fat to muscle (Lawrie and Ledward, 2006). However, the mature weight and rate of maturation differ between breeds and sexes, such that at a similar chronological age different breeds and sexes exhibit different physiological ages (Lambe *et al*., 2007; Irshad *et al*., 2013; De Lima Jùnior *et al*., 2016).

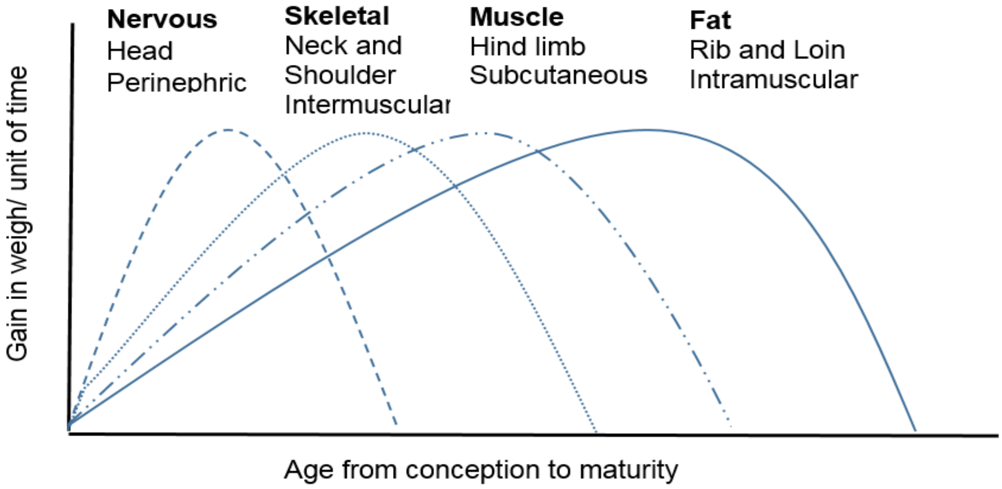


Figure 1. 6. The rate of increase of different portions of the body (adapted from Lawrie and Ledward, 2006).

Age and weight of animals at slaughter are interlinked, and most studies analyse these two factors together. For animals from the same genetic base at the time of slaughter, a greater weight implies greater age, unless feed is restricted (Doreau and Chilliard, 1997; Polidori *et al*., 2017; Budimir *et al*., 2018). Age and slaughter weight influence on the consumer acceptability in many countries (Font i Furnols *et al*., 2006). Therefore, special attention is required for the procedure in the study of meat quality. The quality of sheep meat at different weights and ages relates to changes in the chemical and physical characteristic of the meat coinciding with growth and development (Santos-Silva *et al*., 2002; Polidori *et al*., 2017).

Polidori *et al*. (2017) evaluated the chemical composition and physical characteristics of *longissimus dorsi* (LD) muscle from lambs at two ages (2 and 5 months) and found an increase in fat, protein, conjugated linoleic acid (CLA), iron, manganese and collagen and a reduction in lightness values with increasing slaughter age. Also, in a study on the effect of two slaughter weight (24 and 30 kg) on LD muscle colour, Santos-Silva *et al*. (2002) found a darker meat and a higher redness value with increasing slaughter weight.

Regarding shear force, meat from older animals tends to be tougher compared to younger animals. Garcia *et al*. (2005) evaluated shear force from lambs of different genotypes slaughtered at two ages (150 and 300 days) and observed that the meat from lambs slaughtered at 150 days was more tender compared to those slaughtered at 300 days. Shear force measurements are also affected by lamb weight (Sañudo *et al*., 1996). The tenderness of lamb meat at different slaughter weights (12 and 20 kg) was evaluated, and meat from lambs slaughtered at 12 kg was found to be more tender and juicy than meat from lambs slaughtered at 20 kg (Juárez *et al*., 2009).

The effect of slaughter weight on the FA composition of sheep is controversial. Results from Díaz *et al*. (2003) showed that there was no effect of different lamb slaughter weight (10, 12 and 14 kg) on the FA composition of LD muscle. However, Santos-Silva *et al*. (2002) showed an increase in total FA, palmitic acid, monoenoic acids and a reduction in polyunsaturated FAs when slaughter weight increased from 24 to 30 kg. Tejeda *et al*. (2008) reported that increasing slaughter weight increased the percentage of C12:0, C14:0 in LD muscle, C18:3*n*-3 and C20:2*n*-6 in *semimembranosus muscle* (SM) muscle. In a recent study on the effect of slaughter age on a FA composition of lambs slaughtered at different ages, Polidori *et al*. (2017) found that the C18:2 cis 9, trans 11 and the saturated FA content in the LD of lambs slaughtered at 5 months was higher than that of lambs slaughtered at 2 months. Wood *et al*. (2008) reviewed the effect of age on the FA composition ruminant meat and reported an increase in the proportion of monounsaturated FAs and CLA, a reduction in saturated FAs, and a constant level of α-linolenic acid in the adipose tissue of older animals.

The impact of animal age and slaughter weight on physical and chemical properties of meat quality can also affect the eating quality of meat, Hopkins and Mortimer, (2014) reported that meat from sheep over two years old was tougher compared to sheep one year old when assessed by a trained test panel. Related work (Thompson *et al*., 2005) evaluated the impact of lamb (6 months) and mutton (48 months) on tenderness and found that meat from the lamb was more tender than mutton meat when assessed by consumer panellists. The impact of slaughter weight on meat tenderness has also studied. For example, Juárez *et al*. (2009) evaluated the effect of two Spanish breeds (dairy breed and meat bread) and slaughter weight (12 kg and 20 kg) on meat traits, meat from both breeds at a slaughter weight of 12 kg was more tender than that of lambs slaughtered at 20 kg when assessed by a trained taste panel. However, Tejeda *et al*. (2008) did not find a significant difference in sensory quality traits of LD muscles from light lambs (26 kg) compared to heavier lambs (29 kg).

Increasing the slaughter weight of young lambs have been found to provide more intense odour and lamb flavour (Arsenos *et al*., 2002; Martínez-Cerezo *et al*., 2005 and Teixeira, *et al*., 2005). There is a general contention that, as animal age increases the intensity of flavour also increases (Young *et al*., 1997), although the results are controversial (Hopkins and Mortimer, 2014). Butler-Hogg and Francombe (1985) demonstrated that meat from a lamb at 43 weeks of age was more flavoursome than meat from a lamb at 16 weeks of age and suggested this may be due to a higher muscle concentration of fat. In contrast, Pethick *et al*. (2005) found that there was no effect of increasing slaughter age on the liking of the flavour when the cuts were trimmed from subcutaneous and intermuscular fat. Gkarane *et al*. (2017) reported that the slaughter age (196, 242, 293, 344 and 385 days old) of two sheep breeds had no effect on off flavours, and the age effects on sensory attributes were quadratic but pointed out that lambs were slaughtered at different period of the year.

1.4.1.2. Breed and sex

Breed is a complex factor, and it is difficult to assess its effect on meat quality due to variations in carcass weight, age, degree of maturity etc. However, it has a large effect on carcass characteristics, although the comparison is complicated by differences in genetic improvement programmes between countries (Sañudo *et al*., 2008). The effect of breed or genotype in lambs varies and depends upon which factors are being compared. Lambe *et al*. (2008) found variation in tenderness and ultimate pH between Scottish BF and Texel. The number of muscle fibres can explain changes in these parameters (Karamichou *et al*., 2006). Breed also affects chemical composition of meat such as DM, crude protein, ash, collagen, myoglobin and intramuscular fat (Juárez *et al*., 2009; De Lima Jùnior *et al*., 2016). Breeds of different frame size at the same carcass weight have different fat content, with breeds of smaller frame size being physiologically older and fatter than those of larger frame size (Marino *et al*., 2008). Arvizu *et al*. (2011) showed breeds such as Dorper have a greater amount of intramuscular fat at the same age than Rambouillet, which is not specialised for meat production. Also, Fisher *et al*. (2000) reported that the Suffolk breed has a heavier carcass weight and a higher amount of subcutaneous and intermuscular fat than either the Soay or Welsh mountain breeds. Breeds can also affect the FA composition of meat. According to Fisher *et al*. (2000); Demirel *et al*. (2006) and Marino *et al*. (2008) there is a significant variation in the level of mono, polyunsaturated FAs and saturated FAs in muscle, this was attributed to different rates of intramuscular deposition and membrane phospholipid proportion.

Genotype or breed also affects various sensory attributes of sheep meat, especially juiciness. There is a strong relationship between the degree of marbling (intramuscular fat) and juiciness (De Lima Jùnior *et al*., 2016). Cloete *et al*. (2012) observed that lower scores for juiciness and succulence were associated with lower levels of intramuscular fat in Merino sheep when compared to dual purpose Dohne Merino sheep. It has also been noted that meat from the Merino genotype has lower juiciness (Hopkins *et al*., 2011).

The effect of sex on meat quality traits in sheep has been extensively studied by comparing castrated with non-castrated and female lambs. Production advantages (faster growth and leaner carcass) can be achieved by castrating males or retaining entire males (Hopkins and Mortimer, 2014), although, the influence on the eating quality is less clear. Corbett *et al*. (1973) found that sex (wether, cryptorchid or ewe) lightweight crossbred lambs did not affect muscle pH and shear force. However, when older lambs (20 months) were compared with same age ewes, they found that the entire lambs (ram) have a higher pH and shear force value in the LD (Cloete *et al*., 2012). In a review by Hopkins *et al*. (2001), castrated lambs had a higher LD pH than wether or ewe lambs, and 19% had more than the critical pH value of 5.8, but this did not effect colour. In contrast, LD muscle from wether lambs was lighter in colour than ewe lambs but is unlikely to be detected by the consumer (Hopkins and Mortimer, 2014) and further studies reported no such differences between sexes (Hopkins *et al*., 2007).

Shear force is an important trait, and the early work of Corbett *et al*. (1973) showed no effect of sex on the shear force when LD and SM muscle shear force was measured, this could be because lambs were young. However, Channon *et al*., (2003) showed that there was an interaction between age and shear force, and reported that there was no difference in the shear force of either LD or SM muscles between wethers and cryptorchids at age 8 months, but after that, the shear force value of cryptorchids was higher. Also, Cloete *et al*. (2012) confirmed that in older animals the shear force of LD muscle from rams increased by 9% compared to ewes. Likewise, older ram lambs when slaughtered at the same age, were less tender than wether lambs, although trained panellists could not detect the difference (Young *et al*., 2006).

Sex effect on meat composition especially its effect on fat deposition has been studied extensively. Ewe lamb meat is often higher in fat content than wether or ram (Peña *et al*., 2005; Pérez *et al*., 2007; Rodríguez *et al*., 2008). When Texel male and female lambs were evaluated for muscle and fat deposition, it was observed that females were fatter and had less muscle than males when adjusted to the same carcass weight (Johnson *et al*., 2005). Recently, Pannier *et al*. (2014) reported that despite the carcass weight correction, the intramuscular fat (IMF) in female lambs were significantly higher than male lambs. Also, Craigie *et al*. (2012) reported female lambs had a higher level of IMF than male lambs, although rams were used instead of castrated lambs.

Potentially, the effects of sex on muscle FA concentrations is more important. Lower levels of polyunsaturated FAs in LD muscle of wether lambs was recorded when compared to rams (Solomon *et al*., 1990). Similarly, Facciolongo *et al*. (2018) found significantly higher LD muscle polyunsaturated FAs in male lambs compared to female lambs when lambs were slaughtered at the same age (100 days). Interestingly, higher long chain FAs (EPA+DHA) in LD muscle of female were recorded, and it was proposed that as female lambs reach their reproductive stage, they need more long chain FAs for producing series-3 eicosanoids that are linked with the process of ovulation, conception and pregnancy (Ponnampalam *et al*., 2014). The work of Johnson *et al*. (2005) found by contrast that there was no significant difference in these long chain FAs between male and female lambs.

Ram or entire male lambs tend to have a more off flavour or abnormal flavour, which accumulate with age (Gkarane *et al*., 2017) which can be associated with sexual hormone development. Several earlier studies showed no difference in the eating quality between entire male lambs, wether and ewe lambs (Butler-Hogg *et al*., 1984; Cloete *et al*., 2012). However, Sutherland and Ames, (1996) found abnormal flavour from ram lambs by the age of 30 weeks when compared to wether lambs, and concluded that ram lambs should be finished by 20-24 weeks of age. In a recent study using an untrained consumer assessment of LD and SM muscle of terminal sire lambs, Pannier and Gardner *et al*. (2014) concluded that female lambs had a better eating quality score than male lambs.

1.4.1.3. Rate of gain

Different growth rates (high, moderate, low and compensatory growth) can cause a modification in fatness and consequently on meat quality (Sañudo *et al*., 1998a; Hopkins *et al*., 2007b). In general, feed restriction produces leaner carcasses and less fat within carcasses of equal weight (Murphy *et al*., 1994; Sañudo *et al*., 1998b; Hopkins *et al*., 2007a). The efficiency of lean production is increased, and fat accumulation is decreased by food reduction (restriction). Murphy *et al*. (1994) studied the effect of restricted fed concentrate diets (100, 85 and 70% of *ad libitum*) on performance and carcass composition, lean tissue was increased and fat accumulation reduced with restricted feeding. It was also reported that carcasses from 100 to 85% of restriction reduced more fat than 85 to 70% of the restriction (Murphy *et al*., 1994). Reduction of fat varies according to: 1) age of animal, younger animals mobilise more lean than fat from the carcass (Sañudo *et al*., 1998b; Polidori *et al*., 2017). 2) the rate of weight loss, sheep that lost weight slowly contained less fat while sheep that lost weight rapidly contained more fat than normally grown sheep (Kabbali *et al*., 1992). 3) breed, breeds from desert areas are adapted to move reserves at a minimum biological cost. 4) constant weight, when sheep weight remains constant fat content increases, lean tissue decreases and bone length increases (Bennett *et al*., 1991). The effect of compensatory growth on fat content is controversial. This may be related to the energy level, length of the recovery period and restriction period (duration and severity). Sheep recovering a short refed period sheep would have less fat when compared to normally fed sheep (Afonso and Thompson, 1996), while lambs slaughtered a long time after the end of the initial regrowth period, may be similar or fatter than normally fed lambs (Kabbali *et al*., 1992). A greater effect of compensatory growth is found in the redistribution of fat within carcass than the amount of fat (Chestnutt, 1994). Hopkins *et al*. (2005) evaluated meat and eating quality of lambs fed either a low or a high plane of nutrition, and reported that low plane lambs produced tougher loins and topsides based on shear force. However, the plane of nutrition did not affect any eating quality attributes as assessed by the consumer (Hopkins *et al*., 2005). Same authors in 2007a reported that there was an effect of growth path on shear force value with the lowest value for the early weaned and restricted lambs, which was related to the higher activity of protease enzymes at post slaughter.

1.4.2. Dietary factors

1.4.2.1. Human health and consumer perception

In recent years many researchers have associated red meat consumption with the two main chronic diseases; cardiovascular disease and colon cancer (Cross *et al*., 2007; Kontogianni *et al*., 2008; McAfee *et al*., 2010). The main red meat constituents that have been linked to these conditions are fat content, FA compositions and possibly carcinogenic compounds such as heterocyclic amines that are formed during cooking (Bingham *et al*., 2002). Most dietary unsaturated FAs (>90%) in ruminants are hydrogenated to saturated FAs in the rumen (Sinclair, 2007; Wachira *et al*., 2000). Major saturated FAs within beef and lamb meat (C14:0 myristic acid, C16:0 palmitic acid and C18:0 stearic acid) are significantly associated with the coronary heart disease (CHD) (Hu *et al*., 1999). Myristic and palmitic acids have comparable effects on both LDL and HDL cholesterol, but overall have little effect on the total cholesterol: HDL cholesterol ratio (Micha and Mozaffarian, 2010). However, others argue that C18:0 stearic acid has little effect on raising cholesterol concentrations in human (McAfee *et al*., 2010; Wyness *et al*., 2011). Stearic acid, compared with other SFA, has been shown to lower plasma LDL cholesterol levels, and have no effect on HDL cholesterol (Hunter *et al*., 2010). Therefore, even though stearic acid is a SFA, it does not appear to adversely affect CVD risk, possibly because it is desaturated in part to oleate (18:1n-9) during metabolism (Briggs *et al*., 2017). The predominant monounsaturated FA (MUFA) in red meat is C18:1 oleic acid (30 - 40% of fat in meat) (Realini *et al*., 2004; Baldi *et al*., 2019). Red meat contains PUFA that are known as essential FAs because they cannot be synthesised by the human body. These PUFAs include linoleic (*n*-6) and α-linoleic acid (*n*-3), however, their concentration in red meat is low but contributes substantially to human intakes (Williamson *et al*., 2005). Red meat also contains low amounts of long chain *n*-3 PUFA including EPA, DPA and DHA that have been beneficially linked to heart health (Enser *et al*., 1998). Despite the low concentrations of these long chain *n*-3 PUFA in red meat, they contribute to intake, as there are few rich sources of these *n*-3 PUFA a part from fish oil (Williamson *et al*., 2005). Ruminant meat also contains a small amount of a naturally occurring FA called conjugated linoleic acid which is produced from incomplete biohydrogenation of unsaturated FAs in the rumen (Harfoot and Hazlewood, 1997; Buccioni *et al*., 2012). The CLAs (cis-9, trans11 and trans-10, cis-12) have received attention as being anti-carcinogenic, anti-atherogenic and anti-inflammatory (Kritchevsky, 2000; Kelley *et al*., 2007; Kennedy *et al*., 2010). Red meat is considered a main constituent of the diet to nutrient intakes as it is a rich in high quality protein, B vitamins, haem iron, selenium and zinc with higher absorption or bioavailability when compared to alternative food sources (Robinson, 2001; Williamson *et al*., 2005).

Consumption of ≥285 g/d red meat has increased the plasma concentration of total cholesterol, low-density lipoprotein (LDL) and triglycerides compared to vegans, vegetarians, or moderate and low meat consumptions (Li *et al*., 1999). In a study by Wagemakers *et al*. (2009), it was found that there was no relationship between moderate meat consumption (18-61 g/d) and cholesterol concentration in blood. The earlier recommendation of the Committee on Medical Aspects of Food Policy (COMA) 1991 was that intakes of red and processed meat should not increase, and intakes higher than 140 g/d should be reduced. In 1997, the World Cancer Research Fund (WCRF) report reduced recommended red and processed meat intakes to 80 g/d. The most recent report (WCRF, 2007) recommended that red meat intake should be 71 g/d or 500 g/week, and processed meat should be avoided.

Red meat consumption per capita in the UK over 20 years has decreased compared to the previous years by three fifths (Bourlakis and Weightman, 2007). This is associated with the recommendations of the various food agencies to reduce red meat consumption and eat less fat to avoid or reduce cancer and cardiovascular diseases. In addition, other factors have also contributed to the reduction of red meat consumption, such as lifestyle and price (Robinson, 2001).

Fat content and fat composition (e.g. saturated fat) of red meat are the main issues for consumers (Cross *et al*., 2007). Recently, advances in animal production and butchery techniques have resulted in a leaner meat and a reduction in fat content (Robinson, 2001). This means the lean red meat is much lower in fat content (4-10g/100g) than what consumers think (Higgs, 2000). The fat composition of red meat has also varied depending on the proportion of lean and fat content. Lean meat is described to have lower SFA (< 2 g/ 100 g of meat) and higher PUFA (Williamson *et al*., 2005). For beef trimmed offcuts, total fat content and SFA is equal to or less than of poultry or fish meat while some researchers have found, that there are no benefits to consume white meat in term of lipoprotein concentration in blood (Wolmarans *et al*., 1999; Beauchesne-Rondeau *et al*., 2003)

1.4.2.2. Grass vs concentrate

Lamb production systems differ between countries and depend mainly on feed availability and pasture growth cycle. Production systems including both extensive and intensive systems are determined indirectly by environmental conditions (De Brito *et al*., 2016). Lambs fed on concentrate diets generally grow faster and have higher daily gain than lambs finished on pasture (Priolo *et al*., 2002; Borton *et al*., 2005; Armero *et al*., 2015). This has contributed to the consistency of the supplied nutrients to animals finished on concentrated diets (De Brito *et al*., 2017). However, pasture type and nutrient availability determine animal performance when finished on grass; lambs finished on lucerne pasture had a similar growth rate compared to those finished on a commercial concentrate based diet (Díaz *et al*., 2002). Lambs raised on lucerne, exhibited a higher growth rate and better carcass traits than those finished on annual ryegrass and cereal supplements (Burnett *et al*., 2012; De Brito *et al*., 2017). Also, Nuernberg *et al*. (2008) found growth rate and daily gain were higher for lambs fed a pasture diet compared to those on a concentrated diet. It is of interest that lambs fed on concentrate diets have higher carcass weights and carcass yields when compared to those finished on grass even at the same growth rate and slaughter weight. This can mainly be attributed to the higher dry matter intake, and larger digestive system of lambs finished on pasture (Priolo *et al*., 2002).

It has been reported that dietary fat sources can affect the chemical composition of meat especially fat profile (Wood *et al*., 2003; Sinclair, 2007). The FA composition of forage (grass) and concentrate (cereal based) diets are different and lead to different FA compositions in tissues. Sheep fed concentrate diets produced tissues higher in branched chain FAs particularly 4-methyloctanoic acid which is derived from propionate which tends to be higher when water soluble carbohydrate content of diets is high (Young *et al*., 2003).

Grass finished ruminants produce meat products with higher content of *n*-3 PUFA and lower SFA compared to those finished on concentrate diets, which improve the P:S and *n*-6: *n*-3 ratios (Wood and Enser, 1997; Nuernberg *et al*., 2005; Kasapidou *et al*., 2012). Lamb raised on grass compared with concentrates results in an increase in C16:0, C18:3*n*-3, and a decrease in C18:0, C18: 1*n*-9t, C18: 2*n*-6 concentrations in muscle (Wood and Enser, 1997; Nuernberg *et al*., 2008; Lind *et al*., 2009). Despite the change in concentrations of C18:3*n*-3 and C18:2*n*-6 from finishing lambs on grass, long chain PUFAs (C20:4*n*-6, C22:6*n*-3, C20:5*n*-3) are often less affected (Nuernberg *et al*., 2008). Fisher *et al*. (2000) also found that long chain PUFAs were higher in SM muscle of Suffolk lambs fed grass compared to those fed on concentrate diets. Lambs finished on pasture or diets, rich in forage enhance fibrolytic microorganism growth (Palmquist *et al*., 2005). These microorganisms are mainly responsible for the biohydrogenation process in the rumen and increase C18:1 trans 11 production which is the precursor for most of the cis 9, trans 11 CLA in muscle (Sinclair, 2007; De Brito *et al*., 2017).

The results of research on the effect of pasture versus concentrate feeding on meat colour have confirmed that diet has affects, both subjectively (meat brightness) and objectively (meat lightness) (Alessandro *et al*., 2001). Ruminant meat is darker when finished on pasture than when finished on concentrate diets (Priolo *et al*., 2001; Webb and Erasmus 2013). However, some studies have not found a difference between finishing system during the early post-mortem period (Díaz *et al*., 2002; Howes *et al*., 2015).

The oxidative stability of meat is determined by its antioxidant and pro-oxidant content (Zervas and Tsiplakou, 2011). The presence of more unsaturated FA, especially those with two or more double bond (PUFA), makes the meat more susceptible to oxidation and colour deterioration, with subsequent effects on sensory characteristics (Renerre 2000; Wood *et al*., 2003). Oxidation of linoelic acid (C18:2*n*-6) is 10 times faster than that of oleic acid (C18:1*n*-9), and for linolenic acid (C18:3*n*-3) is 20 to 30 times faster than that of oleic acid (C18:1*n*-9) (Li and Liu, 2012). Because of this, it has been suggested that the differences between saturated to unsaturated FAs in the meat could alter its shelf life (oxidative stability and colour) (Wood *et al*., 2003). Vatansever *et al*. (2000) showed that shelf life was highly affected by the diet concentration of highly oxidisable *n*-3 PUFA, where greater colour deterioration and lipid oxidation was related to a higher proportion of *n*-3 PUFA within the meat (Santé-Lhoutellier *et al*., 2008).

Pasture or grass based diets have been shown to have a high proportion of *n*-3 PUFA, α-tocopherol and other antioxidants (Wood *et al*., 2003; Li and Liu, 2012), but not consistently. Lambs finished on pasture have been shown to have a longer shelf life compared to those finished on concentrate diets (Baldi *et al*., 2016). This is possibly related to the stage of maturity of the pasture or grass, which varies in the level of *n*-3 PUFA, α-tocopherol and other antioxidants (Elgersma *et al*., 2003; Lindqvist *et al*., 2014). Luciano *et al*. (2009) found that shelf life (colour and lipid oxidation) of both raw meat and minced cooked meat of lambs fed fresh vetch (harvested daily) was improved compared to lambs fed concentrate diets. In the study of Santé-Lhoutellier *et al*. (2008), lipid oxidation of meat from lambs fed pasture diet was lower compared to those finished on concentrate diet. However, the diet types did not affect colour parameters of the meat despite the high vitamin E contents in meat from lambs finished on pasture. It was suggested that the formation of metmyoglobin during oxidation of myoglobin was assisted by some other factors (Li and Liu, 2012).

Flavour is considered the most important characteristics after tenderness that affects consumer preference (Thompson *et al*., 2005). Lambs finished on forage are characterised as having a stronger lamb flavour (Sañudo *et al*., 1998; Fisher *et al*., 2000). Both Fisher *et* *al*. (2000), and Sañudo *et al*. (2000) reported that when lambs from different breeds and finished under different production system were assessed for eating quality by panellists, forage fed lambs, which had higher levels of PUFA 18:3*n*-3 were preferred by the British panellists, Whereas, the Spanish panellists preferred concentrate fed lambs, which had higher levels of PUFA 18:2*n*-6. It was suggested that the previous experience of panellists or testers determined which kinds of meat were preferred. Priolo *et al*. (2002) reported that lambs finished on concentrates had a less intense flavour and a lower livery off flavour than those finished on grass pasture. In addition, more off-odour and off-flavours have been found in loin chops from lamb finished on ryegrass than those finished on concentrates (Borton *et al*., 2005). Moreover, further studies have reported that a combination of forage and cereal finishing rations are acceptable to British consumers (Font i Furnols *et al*., 2009). However, British consumers have been shown to preferentially purchase grass fed lambs (Font i Furnols *et al*., 2011). Legumes (lucerne and white clover), maize silage, brassicas and weeds have also been found to transmit off flavour to lamb (Channon *et al*., 2003). To restore normal lamb flavour grazing grass for 7 days has been suggested to be sufficient (Park *et al*., 1972). Additionally, Priolo *et al*. (2001) reported that pasture types and plant composition could modify meat flavour.

The juiciness and tenderness of meat can be affected by its fat content (Warriss, 2000). Some evidence shows that lower concentrate feeding levels and consequently lower energy diets produce more juicy and tender meat than grass fed lambs (Solomon and Lynch, 1988). Similarly, there is other research that indicates high energy diets produce more tender meat (Sañudo *et al*., 1998).

1.4.2.3. Seasonal variation in lamb quality

Separation of age, weight and seasonal effects on lamb quality is very difficult. Generally, older/heavier lambs are tougher; lambs born in the spring and slaughtered in autumn/winter are tougher than those slaughtered during the summer (EBLEX, 2011). Miranda-de la Lama *et al*. (2012) found meat from lambs slaughtered in summer was more juicy and less lamb odour intensity than meat from lambs slaughtered during the winter, although, there was no difference between season on overall liking flavour. It has also been found that overall liking for lambs fed either on a concentrate or grass silage based diet and slaughtered in March is reduced compared to those lambs slaughtered in November (Phillips and Wheeler, 2008).

1.5. Dietary manipulation of the eating quality of concentrate fed lamb

1.5.1. Effect of carbohydrate (CHO)

1.5.1.1. CHO chemistry, dietary sources and digestion

Carbohydrates are a neutral compounds that contain carbon, hydrogen and oxygen with the formula $(CH_2O)_n$, where n is three or more. Carbohydrates are classified into simple sugars and non-sugars (McDonald *et al*., 2011). Simple sugars are subdivided according to the number of carbon atoms presents in the molecule, into monosaccharides and oligosaccharides (Whistler and Smart, 1953). Monosaccharides include trioses, tetroses, pentoses, hexoses and heptoses, whereas oligosaccharadies include disaccharides, trisaccharides and tetrasaccharides (Mussatto and Mancilha, 2007). Non- sugars are divided into polysaccharides (glycans) and complex carbohydrates. Polysaccharides or glycans are polymeric carbohydrate molecules consisting of a long chain of monosaccharide units bonded together by the glyosidic bonds (Mussatto and Mancilha, 2007). These are divided into two groups, homoglycans that have only one unit of monosaccharide (starch, cellulose, glycogen), and the heteroglycans which contain more than one type of monosaccharide unit (pectic substances and hemicellulose) (Cui, 2005). The complex carbohydrates include those compounds that contain CHO and non-carbohydrate molecules (lipids and proteins). These types include glycolipids and glycoproteins which have structural and biological importance (Whistler and Smart, 1953).

The primary carbohydrate sources of ruminants are fibrous feeds which contain a variable amount of cellulose, hemicellulose, starch and water soluble carbohydrates (WSC) (Mussatto and Mancilha, 2007). Young pastures contain approximately 400 g/kg DM of cellulose and hemicellulose and 200 g/kg DM of water soluble carbohydrate (WSC), whereas, mature pasture, straw and hay, contain a higher content of cellulose and hemicellulose and a lower content of WSC (Coleman *et al*., 2002). The proportion of lignin in ruminant diets varies between 20 to 120 g/kg DM (Coleman *et al*., 2002). Its concentration limits digestibility of diets as it is indigestible by rumen microbes. The rumen microorganisms mainly *Fibrobacter succinogenes, Ruminococcus flavefaciens,* and *R. albus,* and also anaerobic fungi attack carbohydrates (Hungate *et al*., 1997). In general, >90% of digestible CHOs are digested in the rumen, and 10% are digested in the small and large intestine (Nocek *et al*., 1991). Digestion of CHOs in ruminants is divided into two stages (Niwiska, 2012); first is the breakdown of complex CHOs into simple sugars by extra-cellular microbial enzymes, with the second stage being digestion and metabolism of simple sugars which is similar in many aspects to the metabolism of CHO by the animal itself (Niwiska, 2012) (Figure 1.7).

Cellulose is hydrolysed into cellobiose by β-1, 4-glucosidases, which is then converted either to glucose-6-phosphate by the action of phosphorylase or to glucose. Starch is first converted to maltose and isomaltose by amylases, then both of them are converted to either glucose or glucose-6-phosphate by maltases, maltose phosphorylases or 1, 6-glucosidases.

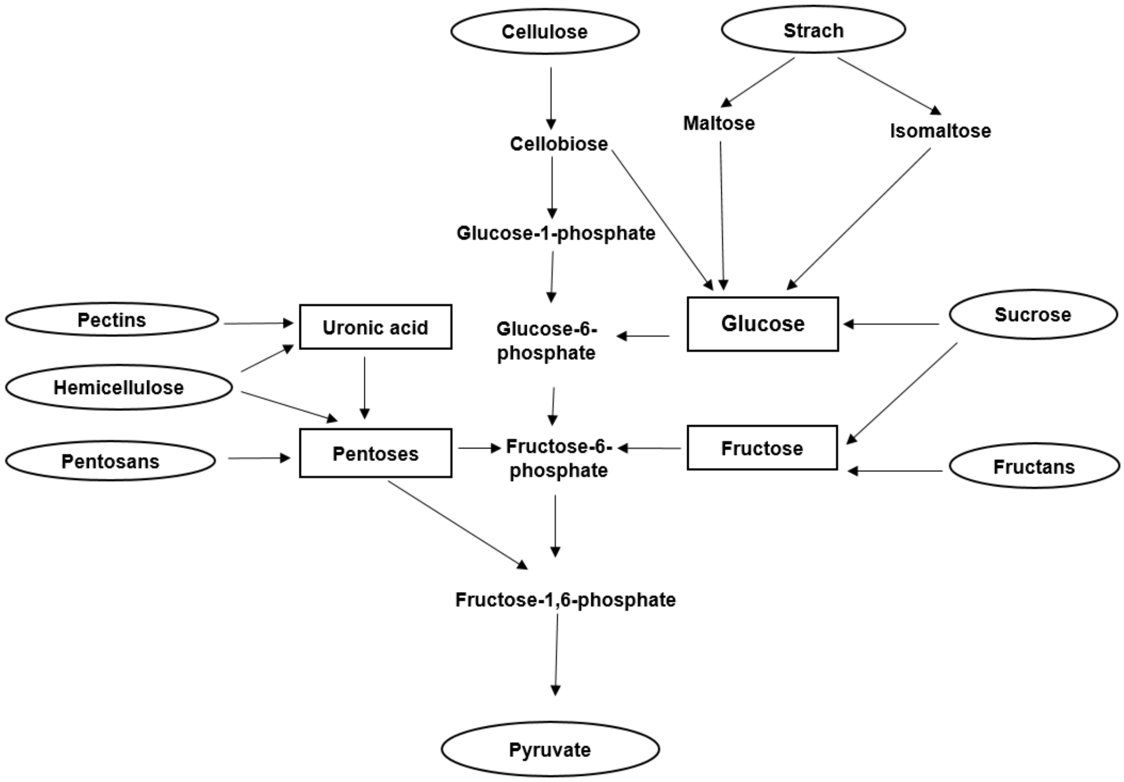


Figure 1. 7. Conversion of CHOs into pyruvate (Baldwin, 1995).

Fructose is produced from the digestion of sucrose and fructans by the enzymes attacking 2, 1 and 2, 6 linkage of fructans. Pentoses are mainly produced by enzymes attacking β-1, 4 linkages of hemicellulose. Pectins and pentosans are also converted indirectly into pentoses by involved enzymes pectinesterase and polygalacturonidases (Whistler and Smart, 1953).

The intermediate product of the first stage of CHO digestion is pyruvate which is a precursor of the endproducts of rumen CHO digestion which are; acetate, propionate, butyrate, carbon dioxide and methane (Figure 1.8). Small quantities of additional VFAs are also produced such as valerate, isobutyrate, 2-methyl butyrate and 3-methyl butyrate by deamination of proline, valine, isoleucine and leucine, respectively (Nafikov and Beitz, 2007).

Acetate is produced from pyruvate through a pathway of acetyl phosphate with methane and carbon dioxide also being produced. Propionate is produced from pyruvate through two alternative pathways. First, through lactate when the proportion of concentrate in the diet is high or through a second pathway, where succinate is involved when the diet is rich in fibrous forage (Bergman, 1990). Lactate produced from the first pathway may accumulate in the rumen when the diet is too high in concentrates and can lead to acidosis (Harmon *et al*., 1985). The volatile fatty acids (VFAs) produced from microbial fermentation in the rumen are absorbed through the rumen wall.

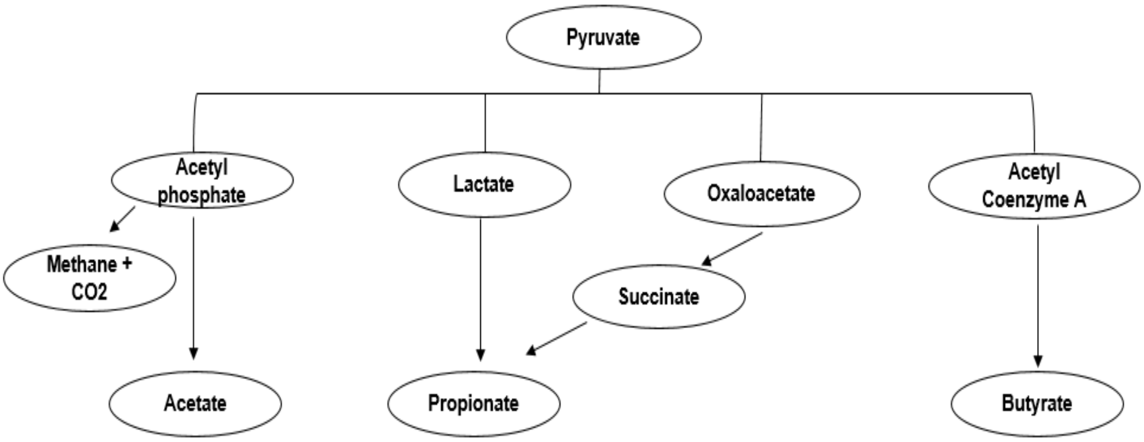


Figure 1. 8. Conversion of pyruvate into volatile fatty acids (Dijkstra et al., 1993).

The relative proportions of VFAs very according to the source of CHOs. Generally, the proportions of acetate: propionate: butyrate ratios derived from hexoses are 65: 21: 14, respectively (McDonald *et al*., 2011). However, these ratios differ from the actual ratios as some amino acids are also fermented in the rumen (Suárez *et al*., 2006). The total concentration of VFAs in the rumen varies according to the ruminant's diet and time of feeding as the absorption of individual VFAs is different (Doreau *et al*., 1997). In general, the relative molar proportion of acetic: propionic ratio is 70: 20 for ruminants fed mature herbage. This ratio is reduced to 60: 30 when the ruminant fed on a less mature herbage and especially with diets high in concentrates (60%) (McDonald *et al*., 2011; Dijkstra *et al*., 2012).

1.5.1.2. Effects on meat quality

It has been found that feeding sheep with different sources of carbohydrates can modify the chemical composition and eating quality of meat (Fraser and Rowarth, 1996; Díaz *et al*., 2002). Olfaz *et al*. (2005) studied lambs fed on control diet (60% commercial concentrate + 40% grass hay) or a mixture of 40% and 60% sugar beet pulp that was partially substituted with grass hay. It was reported, that the inclusion of 60% SBP significantly reduced stearic, oleic and arachidonic acids, but increased the palmitic and linoleic acid content of LD muscle compared to the control diet (Olfaz *et al*., 2005). It was also found that the ultimate pH and cooking loss decreased and lightness increased compared to the control diet (Olfaz *et al*., 2005). However, dietary inclusion of SBP did not affect sensory attributes reported by a trained taste panel.

Oliveira *et al*. (2017) evaluated the effect of different starch levels (mid 35 % and high 50% DM) and rumen degradable starch (mid 70% and high 80%) on the chemical composition of lamb meat. Meat from lambs fed on high starch had a lower shear force value compared to mid degradability starch (Oliveira *et al*., 2017). The total lipid content of meat was not affected by treatment, however, saturated FA and cis MUFA increased, and trans MUFA decreased in lambs fed on the mid starch diet (Oliveira *et al*., 2017).

Pre slaughter muscle glycogen stores have been recognised to be crucial for meat quality characteristics (Immonen *et al*., 2000). Lambs fed on pasture and concentrate have different levels of glycogen (Santé-Lhoutellier *et al*., 2008). A high ultimate pH is primarily found in undernourished animals as these animals are unable to store sufficient glycogen reserves in muscles (Pethick *et al*., 1999). Ruminants fed on pastures, which are typically low in starch and rich in fibre, and where the ratio of acetate: propionate is high tend to have a lower muscle glycogen content than those raised on concentrate diets which are rich in starch, and where the ratio of acetate: propionate is low (Martin *et al*., 2004; De Brito *et al*., 2017a). Propionate is a glycogenic VFA, therefore, pasture finished animals generally have a higher ultimate pH than concentrate finished animals, although usually within the normal range (Priolo *et al*., 2002). There is a high correlation between muscle ultimate pH and meat colour (Calnan *et al*., 2016). Feeding ruminants with a high level of digestible carbohydrate sources or sugars for a few weeks or days pre slaughter has been shown to increase glycogen stores in muscle and reduce ultimate pH (Andersen *et al*., 2005).

Sheep fed on concentrate diets (rich in starch) tends to have increased levels of branch chain FAs, especially 4-methyloctanoic acid in muscle (Sinclair, 2007). This FA is related to the pastoral flavour and species flavour characteristics of sheep meat, that also tends to be higher in rams than castrates (Young *et al*., 2003). This is caused by changes in the ruminal fermentation patterns that result in an increase in propionate and the oxidative deamination of branched chain amino acids (Young *et al*., 2003).

In an experiment investigating the effect of replacing cereal concentrates with dried citrus pulp (24% and 35%) on the shelf life of lamb meat, Inserra *et al*. (2014) reported no treatment effects on ultimate pH and lightness, but redness, yellowness, chroma and lipid oxidation values all reduced after 4 days of ageing in vacuum pack. This was attributed to the presence of a high content of phenolic compounds in dried citrus pulp rather than the effect of carbohydrate sources. In addition, Caparra *et al*. (2007), reported that the inclusion of dried citrus pulp (30% and 45%) did not affect the chemical analysis of meat.

1.5.2. Effect of lipid source

1.5.2.1. Lipid chemistry, dietary sources and digestion

Lipids are a generic name for a variety of fatty substances that occur naturally in the animal body and are important in body functions (Riediger *et al*., 2009). Generally, body fat is classified into structural and stored fats. Structural fats form an integral part of cell membranes in all tissues and organs such as phospholipid and glycolipids (Carruthers and Melchior, 1986), whereas the storage fats provide an energy reservoir and mostly exists in adipose tissue such as triacylglycerol (Klaus, 2004). The FA in structural lipids are high in PUFA whereas the storage lipids are generally high in saturated FAs (Hulbert *et al*., 2014). Also, lipids are classified into complex lipids (phospholipids and triglycerides) (Figure 1.9) and simple lipids (cholesterol).

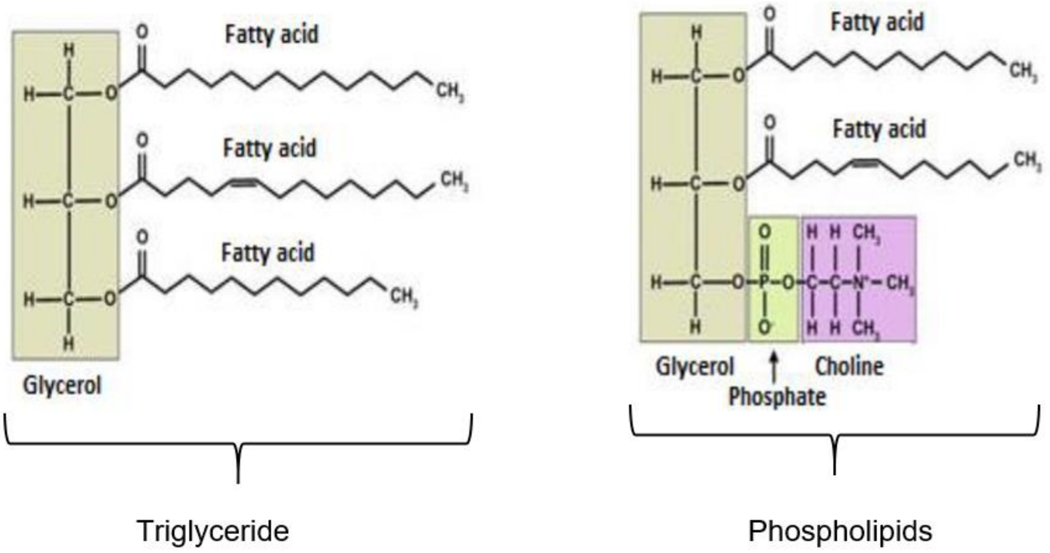


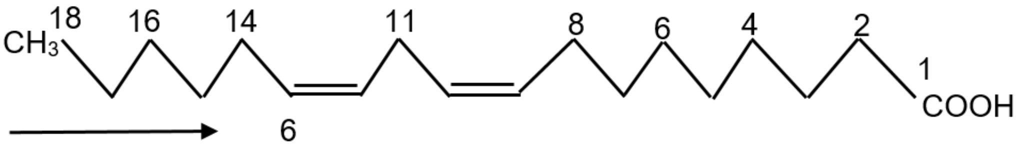
Figure 1. 9. Structure of triglyceride and phospholipid (adapted from Gurr *et al*., 2002)

The main components of complex lipids are FA, which consists of a hydrocarbon chain length of variable length with a methyl group at one end and the carboxyl group at the other (Lobb and Health, 2007). There are different types of FAs according to the nature of the bond between the carbon atoms (Table 1.7) (Sikorski and Kołakowska, 2003).

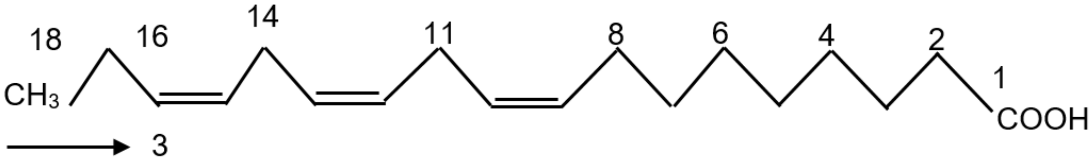
Table 1. 7. Common, systematic and carbon numbers of different fatty acids.

Common name	**Systematic name**	**Abbreviation**
Caprylic acid	Octanoic acid	C8:0
Capric acid	Decanoic acid	C10:0
Undecanoic acid	Undecanoic acid	C11:0
Lauric acid	Dodecanoic acid	C12:0
Tridecanoic acid	Tridecanoic acid	C13:0
Myristic acid	Tetradecanoic acid	C14:0
Myristoleic acid	9-tetradecenoic *acid*	C14:1
Palmitic acid	Hexadecanoic acid	C16:0
Palmitoleic acid	Hexadecenoic acid	C16:1
Heptadecanoic acid	Heptadecanoic acid	C17:0
Heptadecenoic	Cis-10-Heptadecenoic	C17:1
Stearic acid	Octadecanoic acid	C18:0
Vaccenic acid	Trans-11-Octadecenoic *acid*	C18:1*n*-11t
Oleic acid	Cis-9-Octadecenoic acid	C18:1*n*-9c
Elaidic acid	Trans-9-Octadecenoic acid	C18:1*n*-9t
Linoliec acid	Cis-9,12-Octadecadienois acid	C18:2*n*-6c
Linolelaidic acid	Trans-9,12-Octadecadienois acid	C18:2*n*-6t
Rumenic acid (CL)	Cis-9,trans-11 or trans-10,cis-12-Conjugated linoleic acid	C18:2*n*-6
α-Linolenic acid	Cis-9,12,15-Octadecatrienoic acid	C18:3*n*-3
Arachidic acid	Eicosanoid acid	C20:0
Gadoliec acid	Cis-11-Eicosenoic acid	C20:1*n*-9t
Arachidonic acid	Cis-5,8,11,14-Eicosatetraenic acid	C20:4*n*-6c
Behenic acid	Docosanoic acid	C22:0
Eicosapentenoic acid (EPA)	Cis-5,8,11,14,17-Eicosapentaenoic acid	C20:5*n*-3
Docosapentaenoic acid (DPA)	Cis-7,10,13,16,19-Docosapentaenoic Acid	C22:5*n*-3
Docosahexenoic acid (DHA)	Cis-4,7,10,13,16,19-Docosahexenoic acid	C22:6*n*-3
Tricosanoic acid	Tricosanoic acid	C23:0
Lignoceric acid	Tetracosanoic acid	C24:0

In the carbon chain, a single bond between pairs of carbon is called a saturated bond. The FAs with one double bond are called monounsaturated FA, and those with more than two double bonds are called polyunsaturated FAs (Davidson and Cantrill, 1985). Unsaturated FAs are also classified according to the location of the first double bond in the hydrocarbon chain in relation to the terminal methyl group (CH_3) of FA molecule; this is known “n” classification or omega (Lawson, 1995). When the location of the first double bond is either carbon 3 or 6 away from the methyl group end of the FAs, is termed *n*-3 or *n*-6 (Figure 1.10).



Linoleic acid, C18:2n-6 (9, 12 Octadecadienoic)



Linolenic acid, C18:3n-3 (9, 12, 15 Octadecadienoic)

Figure 1. 10. Structure of n-3 and n-6 polyunsaturated FAs (adapted from Abedi and Sahari, 2014).

The source of FA in ruminant diets might be plants. Many plants contain a high concentration of FAs within leaves, grain or seeds (Givens *et al*., 2000). Animal by products provides both essential (linoleic, linolenic and arachidonic acids) and non-essential FAs (palmitic and stearic acids) (Palmquist and Jenkins, 1980). The FA composition of different fat sources varies as shown in Table 1.8. Grass is the predominant source of FA in the diet of grazing ruminants. Fresh grass is characterised by a high content of C18:3*n*-3 (0.50-0.75 g/g of the total FAs) (Dewhurst and King, 1998), mostly concentrated in the chloroplast (Sargent, 1997). However, the concentration of C18:3*n*-3 varies between species and stage of maturity (Dewhurst *et al*., 2001). In concentrate diets, plant seed oils are the main sources of FAs. For instance, linseed, soybean, palm oil, cottonseed and sunflower, each of these are characterised by the existence of a high content of specific FA (Table 1.8), While fish oil and marine algae are high in long chain PUFA (C20:5*n*-3 and C22:6*n*-3).

Table 1. 8. Fatty acid composition (g/kg of total fatty acids) of various fat sources.

	C16:0	**C18:0**	**C18:1*n*-9**	**C18:2 *n*-6**	**C18:3 *n*-3**	**C20:5 *n*-3**	**C22:6 *n*-3**
Grass[1]	208	32.9	NA[4]	140	492		
Linseed oil[2]	60	30	170	134	553		
Soybean[2]	92-122	36-54	177-255	505-568	75		
Palm oil[2]	440	40	366	91	2		
Fish oil (tuna)[2]	10-19	1-4	9-13	16	8	69	197
Grass silage[2]	170	26	33	184	587		
Maize silage[2]	210	36	292	348	37		
Cottonseed[3]	230	24	175	523	20		
Sunflower[3]	63	43	203	649	<10		

[1]French *et al*. (2000); [2]Givens *et al*. (2001); [3]Sauvant *et al*. (2004); [4]NA= data not available;

Many researchers have reviewed lipid metabolism in ruminants (Harfoot, 1978; Sinclair, 2007; Boccioni *et al*., 2012). Microbial processes in the rumen modify the FA composition of diets before they reach the small intestine where they are absorbed. Lipolysis is an initial step in ruminant lipid metabolism and a prerequisite for biohydrogenation, thus any small quantities of PUFA reaching the small intestine could be due to a reduction in lipolysis. This may determine the rate of biohydrogenation in the rumen (Buccioni *et al*., 2012). Free FAs and associated compound are released when microbial enzymes such as lipase, phospholipase and galactosidase hydrolyse ester bonds within dietary lipids to produce free FAs and glycerol (Doreau and Chilliard, 1997; Boccioni *et al*., 2012) (Figure 1.11). The numbers and activity of microorganisms that are capable of hydrolysing ester bonds are highly specified (Fay *et al*., 1990). Various bacterial strains of *Butyrivibrio fibrisolvens* and *Anaerovibrio lipolytica* can hydrolyse the ester bond, but *B. fibrisolvens lipase* hydrolyses phospholipids, *A. lipolytica* hydrolyses only tri- and di-glycerides, and their rates of hydrolysis differ. Recently, it has been reported that different species of rumen bacteria belonging to the *Clostridium*, *Propionibacterium*, *Staphylococcus*, and *Selenomonas* genera and *Pseudomonas aeruginosa* strain have lipolytic activity (Unni *et al*., 2016; Enjalbert *et al*., 2017). Lipase activity also occurs in ciliatae protozoa, but not in fungi (Dehority, 2003). Free FA may also arise from hydrolysis of plant galactolipids and phospholipids catalysed by several bacterial *galactosidases* and *phospholipases* such as *phospholipase A and phospholipase C,* which are produced by rumen microbes (Jenkins, 1993).

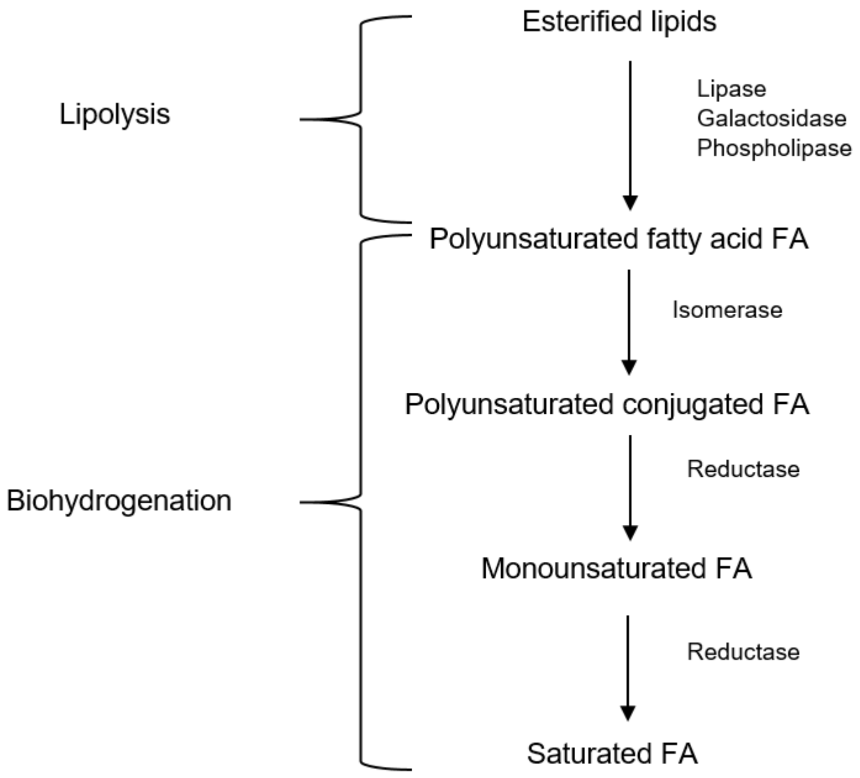


Figure 1. 11. Lipolysis and biohydrogenation (adapted from Buccioni et al., 2012)

After lipid hydrolysis, the free carboxyl group of free FAs are a prerequisite for biohydrogenation to form FA having a high degree of saturation (Harfoot and Hazelwood 1997). The reason behind this process is unknown. However, it has been suggested that this might be a mechanism for rumen microorganisms to protect themselves from the toxic effects of unsaturated FAs (Palmquist and Jenkins, 1980). After FAs are hydrolysed, a trans-11 unsaturated double bond is produced when the cis-12 double bonds of C18:3*n*-3 and C18:2*n*-6 are converted by a process called isomerisation (Buccioni *et al*., 2012). Following the isomerisation reaction, cis-9 and trans-11 bonds are hydrogenated by a reductase enzyme to produce vaccenic acid (C18:1 11 trans), then to stearic acid (C18:0) which is the predominant product of biohydrogenation (Harfoot and Hazlewood, 1997) (Figure 1.12). There are two main groups of hydrogenating bacteria (Harfoot and Hazelwood, 1997). Group A bacteria, which include *Butyrivibrio* (mainly B. *fibrosolvens*), *Micrococcus, Ruminococcus* and *Lactobacillus*, act on C18:2 *n*-6 to produce trans-11 C18:1 (vaccenic acid) (Lourenco *et al*., 2010; Hussain *et al*., 2016). Whereas, groups A and B act on C18:3 *n*-3 to produce trans-11 C18:1 and CLA. Group B bacteria, which include *Fusocillus spp* and B. *proteoclasticum*, then hydrogenate cis and trans 11 C18:1 to C18:0 stearic acid) (Vasta *et al*, 2019). However, the amount of vaccenic acid that is hydrogenated is affected by the type and concentration of dietary PUFA (Wachira *et al*., 2002; Sinclair, 2007). The literature also suggests that the contribution of protozoa to biohydrogenation is related to the ingested of bacteria (Devillard *et al*., 2006). Devillard *et al*. (2004) observed that the CLA and vaccenic acid content of rumen protozoal cells were higher than that in bacteria, which suggests that protozoa may also be a major pool of CLA and vaccenic acid in the rumen. This was confirmed by using real time PCR to quantify the contribution of protozoa to duodenal FA flow, finding that protozoa contribute 400 g/kg of vaccenic acid, 300-360 g/kg of cis-9 trans 11 and 400 g/kg of trans-10, cis-12 of CLA leaving the rumen (Yáñez-Ruiz *et al*., 2007). The simplest explanation is that protozoa do not form CLA and VA, but that they are very efficient in incorporating intermediates of bacterial BH.

Hydrogenation of PUFA is mostly complete with 70-90% of saturated FA being saturated in the rumen (Chilliard, 1993). However, some PUFA escape biohydrogenation and reaches the small intestine with their original structure (Jenkins, 1993). To increase the concentration of long chain PUFA in the ruminant meat many methods have been developed such as lipid encapsulation, FA saponification and inclusion of fish oil (Wachira *et al*., 2002; Capper, 2005 and Wood *et al*., 2008).

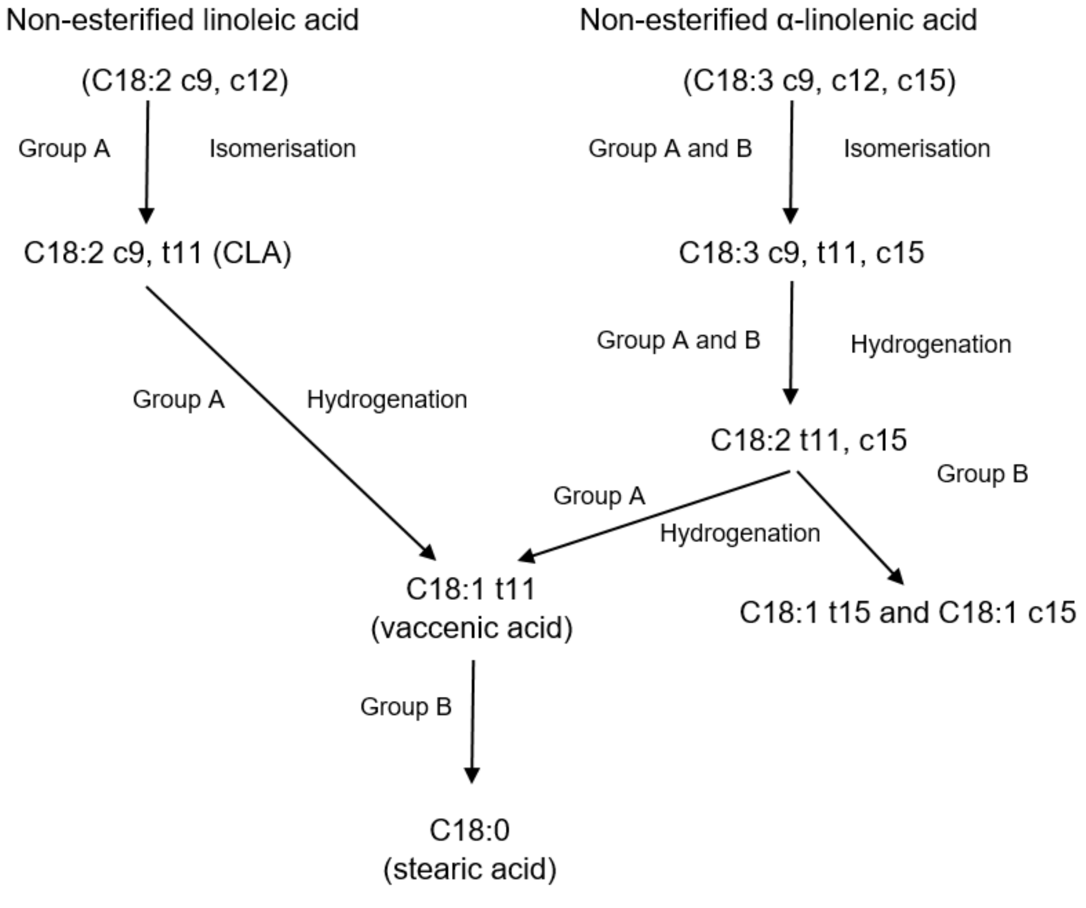


Figure 1. 12. Biohydrogenation of linoleic and linolenic acid pathway in the rumen (adapted from Harfoot and Hazlewood, 1997).

1.5.2.2. Effects on carcass chemical composition

Animal production system and diet both influence the fat and FA composition of meat (Webb and O’Neill, 2008). Meat quality is also influenced by fat and FAs (Wood *et al*., 2008). There are different sources of FAs in ruminant diets which can be used to manipulate the FA profile of meat such as linseed oil, fish oil, marine algae, protected linseed and forage (e.g. grass) (Wachira *et al*., 2003; Demirel *et al*., 2003; Cooper *et al*., 2004). The composition of FA varies in each dietary fat and leads to changes in the FA composition in tissues (Wood *et al*., 2008; Watkins *et al*., 2013). As previously mentioned, as a result of bio-hydrogenation the proportion of saturated FA in ruminant tissues is high (Enser *et al*., 1998; Wood 2008). However, various factors such as level of feeding are known to influence rumen outflow rate. At high levels of feeding rumen outflow rate is relatively high compared to lower levels of feeding. This is likely to reduce the effects of biohydrogenation and increase PUFA to the small intestine (Wachira *et al*., 2000). Following absorption, this is available for incorporation into animal tissues (Wood *et al*., 2008).

The effect of forage (grass and silage) and concentrate diets on the FA composition of sheep meat have been investigated. Palmitic acid, oleic acid, linoleic acid and linolenic acid are the major FA in the grass (Channon *et al*., 2003). Leaf chloroplast is characterised as containing a high proportion of these FAs (Sauvant *et al*., 2004). It has been reported that grass fed sheep had a significantly lower proportion of saturated FAs such as palmitic acid and stearic acid, and had a higher proportion of PUFA, which increases the proportion of P:S ratio (Fisher *et al*., 2000; Wood *et al*., 2003; Sinclair, 2007). Nuernberg *et al*. (2008) reported that grass feeding increases linolenic acid (C18:3*n*-3), conjugated linoleic acids and in general PUFA in lamb meat which lead to an increase P:S ratio and an important reduction in *n*-6: *n*-3 ratio.

As a result of the saturated nature of the ruminant products, research has focussed on methods of protecting PUFA sources from microbial biohydrogenation in the rumen. Feeding linseed oil and whole oilseeds (such as linseed, rapeseed and sunflower) instead of extracted oil seeds offers some protection. For example, when lambs were fed whole rapeseed, saturated FAs were reduced significantly in muscle tissues compared to lambs fed rapeseed meal diet (Solomon *et al*., 1991; Jenkins, 1993). Also, Wachira *et al*. (2002) reported that when lambs were fed a diet containing linseed oil (rich in 18:3*n*-3), the proportion of α- linolenic acid (18:3*n*-3) doubled in both the LD and adipose tissue, while conjugated linoleic acid increased only in lean tissue. Furthermore, fish oil (rich in EPA and DHA) and marine algae (rich in PUFA) have been used to enhance the nutritional quality of ruminant meat (Cooper *et al*., 2004; Raes *et al*., 2004; Urrutia *et al*., 2016). The study of Cooper *et al*. (2004) reported that when feeding lambs either marine algae with fish oil, or marine algae with protected linseed oil, increased DHA (22:6*n*-3) and EPA (20:6*n*-3) in LD muscle and adipose tissue; as a result, favourable changes were observed in P:S ratio and in *n*-6:*n*-3 ratio.

1.5.2.3. Effects on shelf life and eating quality

The FA profile of muscle influences several aspects of meat quality including colour, lipid oxidation and flavour (Wood *et al*., 2004). An increase in unsaturated FAs can lead to a reduction in shelf life due to an increase in lipid and colour oxidation (Nute *et al*., 2007). The PUFAs are a key role in flavour development and are mostly incorporated in the phospholipid fraction (Mottram, 1998). These FAs are oxidised during storage, processing and cooking, the interaction between lipid oxidation products and Maillard reaction compounds can alter meat flavour (De Brito *et al*., 2017). A study by Nute *et al*. (2007) examined the effect of different oil sources; linseed oil (rich in C18: 3*n*-3), fish oil and protected lipid supplement (3:1 ratio of C18:2*n*-6 to C18:3*n*-3), fish oil/marine algae (rich in long chain PUFA) and combination of protected lipid supplement and marine algae on colour, lipid stability and eating quality of lamb. The highest TBAR values were reported in muscle from the group fed fish oil/marine algae (6.2 mg/kg), while, the lowest values were found in the group fed linseed oil (1.2 mg/kg), all other groups had values above 2.0 mg/kg. The colour also deteriorated in the same order with the highest being fish oil/marine algae and the lowest being the linseed oil group. All groups, except that given linseed oil, had low taste panel scores for lamb flavour and high scores for abnormal lamb flavour (Nute *et al*., 2007). The FA profile was significantly influenced by the dietary oil sources with the linseed oil group had the highest proportion of C18:3*n*-3, protected lipid supplement had the highest C18:2*n*-6, while a combination group (fish oil/marine) had the highest muscle C22:6*n*-3 content. The flavour scores were correlated to the FAs profile of LD phospholipid, negative correlations were found between lamb flavour and C18:2*n*-6 and long chain PUFAs (Table 1.9).

Table 1. 9. Correlation between flavour scores and fatty acids (%) in phospholipid fraction in LD muscle.

Proportion %	**Lamb flavour**	**Abnormal flavour**
C18:2*n*-6	-0.25	0.11
C18:3*n*-3	0.51	-0.49
C20:5*n*-3	-0.13	0.24
C22:6*n*-3	-0.28	0.32

1.5.3. Effect of vitamin E and selenium

1.5.3.1. Vitamin E chemistry, dietary sources, digestion and requirement

Vitamin E is a common name for eight molecules which are soluble in lipid, having chroman ring and 12 carbon side chain with 4 methyl groups. α- tocopherol has a saturated side chain whereas, tocotrienol has three double bonds on the side chain (Ballet *et al*., 2000). Both of these compounds act as antioxidants to different levels by protecting cell membrane from lipid peroxidation (Decker *et al*., 2000). There are four tocopherols which differ in the methyl group homologues on the chromanol ring (α-, β- γ- and δ-) and four tocotrienols (α-, β- γ- and δ-) (Machlin, 1980). The difference between tocopherols and tocotrienols is due to the unsaturation of the side chain in tocotrienols (Figure 1.13). Tocopherols are considered to have the highest biological activity, especially α-tocopherol which is the most active form in controlling oxidative processes (Mcdowell, 2000). The d-α-tocopheryl-acetate and dl- α-tocopheryl acetate are the two most common commercially available forms of α-tocopherol (Pryor, 1996). The d-α-tocopheryl-acetate is produced from vegetable oil by extraction of natural tocopherol and then acetylated, but dl- α-tocopheryl acetate is artificially produced (Hidiroglou *et al*., 1988)

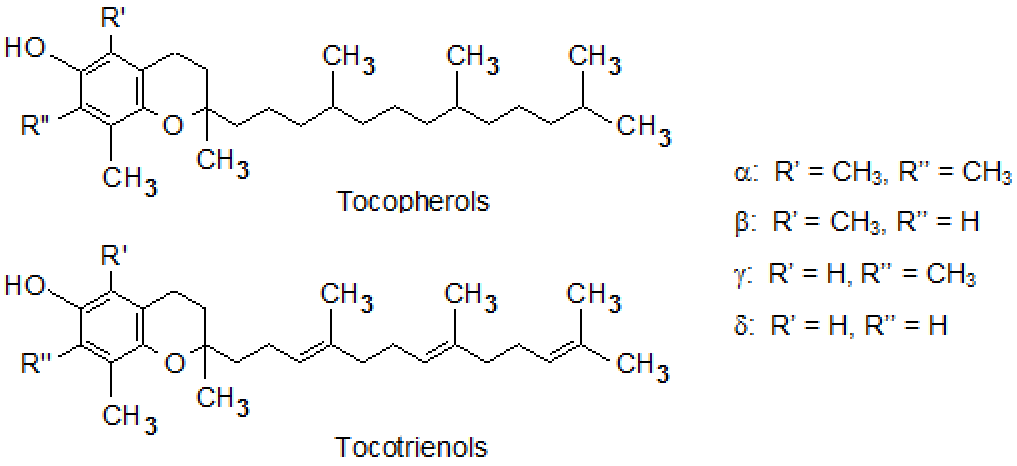


Figure 1. 13. The chemical structure of Vitamin E (tocopherol and tocotrienol) (adapted from Machlin, 1980).

Vitamin E has been found in almost all food which is used by man (Kurt, 1999). The concentration and distribution of vitamin E in food vary and are influenced by animal and plant species (Ballet *et al*., 2000). In addition, stage of plant maturity, harvesting time, processing and storage time all affect both the quantity and availability of vitamin E (Ballet *et al*., 2000). As vitamin E is a fat-soluble vitamin, it is mainly found in oilseeds (Faustman *et al*., 1998) such as soybean (110 mg/100 g) and maize oil (15 mg/100 g), whereas forages contain less (alfalfa contain only 5 mg/100 g). The level of α- tocopherols in different vegetable oil and feedstuff are shown in Table 1.10.

Table 1. 10. The α- tocopherol contents of various vegetable oils and feedstuff.

Oil	**α- tocopherol (mg/100g)**
Wheat germ[1]	119
Sunflower[1]	49
Cottonseed[1]	44
Perennial ryegrass[1]	31.3-36.2
Linseed[1]	0.49
Feedstuff	**α- tocopherol (mg/kg DM)**
Grass and legumes (Green forage)[2]	161 (9-400)[5]
Dehydrated Lucerne[2]	125 (28-238)[5]
Grass and legume hays[2]	61 (10-211)[5]
Red clover +perennial ryegrass[3]	33
Birdsfoot + timothy[3]	86
Barley[4]	18.5
Alfalfa, dehydrated[4]	135.5
Grass, dehydrated[4]	122.5

[1]Kurt, 1999; [2]Ballet *et al.*, 2000; [3]Lindqvist *et al.*, 2014; [4]Sauvant *et al.*, 2004 ; [5]Range

Vitamin E digestion occurs in the small intestine lumen and is similar to that of dietary fat being facilitated by the presence of pancreatic lipase and bile to form micelles prior to enterocyte uptake (Gagné *et al*., 2009) (Figure 1.14). These micelles can solubilise hydrophobic substances and diffuse into the glycocalyx (unstirred water layer) layer to approach the brush border membrane of the intestinal epithelial cells (enterocyte) (Kayden and Traber, 1993). Vitamin E absorption can occur by passive diffusion through the enterocyte membrane, although, there are three membrane proteins associated with cholesterol absorption that may also be involved with the absorption of tocopherol. These membrane proteins are intracellular cholesterol transporter 1, scavenger receptor class B type I and CD36 molecule (Reboul, 2017). Once vitamin E is absorbed, it is incorporated into triglyceride-rich chylomicrons (as alcohol) before being released into the lymph system and general circulation (Mcdowell, 2000). Once triglyceride-rich chylomicrons enter the circulation system, they are hydrolysed by endothelium-bound lipoprotein lipase (LPL) to produce chylomicron remnants (Gagné *et al*., 2009). Some vitamin E and free FAs are released and transferred into the peripheral tissues. Also, chylomicron remnants (carrying vitamin E) are then taken up by hepatic endocytosis through receptors; low density lipoprotein (LDL)-cholesterol receptor and LDL receptor-related protein (Gagné *et al*., 2009).

A cytoplasmic hepatic protein α-tocopherol transfer protein (α-TTP) has a specific affinity to α-tocopherol which limits the absorption of other forms of vitamin E (Gagné *et al*., 2009). The excess α-tocopherol and other forms of vitamin E (β, γ and δ) are excreted into the bile or in urine (1%) after being metabolised by side chain degradation to from carboxyethyle-hydroxychroman (α-CEHC) which is a main metabolite of tocopherol (Gagné *et al*., 2009). The function of α-TTP is also to transfer vitamin E that has been taken up by hepatic cells into the plasma via very low density lipoprotein (VLDL). The VLDL are hydrolysed by LPL into high density lipoprotein (HLP) - cholesterol particles and intermediate density lipoprotein (IDL). Approximately 55% of VLDL and IDL that are formed are taken up by the liver, whereas, the remaining 45% is catabolised into LDL then delivered into other peripheral tissues (Hidiroglou *et al*., 1992; Gagné *et al*., 2009).

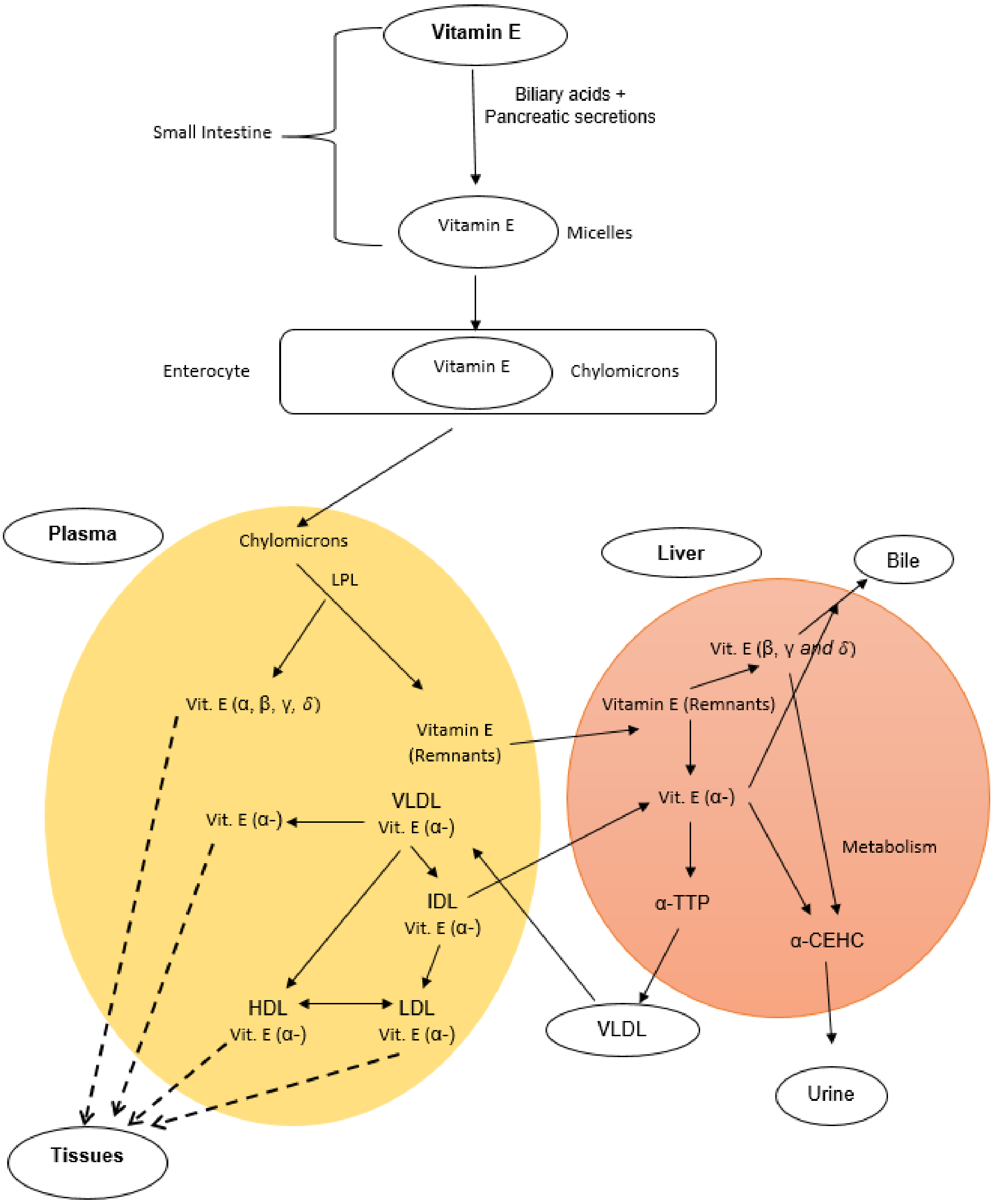


Figure 1. 14. Metabolism of vitamin E (adapted from Gagné et al., 2009).

In addition to the presence of vitamin E in plasma, it is also found in most tissues, but mainly in the liver, adipose tissue and muscle in the form of α-tocopherol isoform which is mostly concentrated in cell fractions rich in membranes such as mitochondria and microsomes (Hidiroglou *et al*., 1992; Arnold *et al*., 1993).

Animals are unable to synthesise vitamin E; thus dietary sources are required to fulfil their requirements, and continuous intake is required to maintain its concentration throughout the body (Kerry and Ledward, 2009). In ruminants, it has been found that there is no pre-intestinal absorption of vitamin E. Ingested vitamin E is not destroyed by rumen microorganisms (Leedle *et al*., 1993). Moreover, a stabilised form of vitamin E (dl-alpha-tocopheryl acetate) is widely used with no degradation reported (Chikunya *et al*., 2004). As mentioned before vitamin E digestion is similar to fat digestion, thus fat in the diet is required for vitamin E absorption effectively (Jeanes *et al*., 2004). There is an antagonistic relationship between vitamin E absorption and unsaturated FAs, high level of polyunsaturated FAs (rich in linoleic acid) in the diet can negatively affect the absorption of vitamin E (Hidiroglou *et al*., 1992).

Until recently, the minimal vitamin E requirement in order to avoid deficiency in sheep was 15 mg/kg DM (NRC, 1985) for 20 kg lambs, and 20 mg/kg DM for heavier lambs, pregnant and lactating ewes. These values assume an adequate supply of Se. The vitamin E status of animals is commonly assessed by plasma and serum vitamin E concentration (Hidiroglou *et al*., 1992). A plasma α-tocopherol concentration of < 2 µg/ml has been considered deficient as animals show clinical signs of white muscle disease (NRC, 2007) . To maintain plasma α-tocopherol concentration ≥ 2 µg/ml, 5.3 mg of dietary vitamin E/kg of live body weight (BW) is required for most lamb production situations (NRC, 2007). In addition, if the goal is to extend shelf life or enhance immune response, 10 mg/kg BW vitamin E is required (NRC, 2007). However, if a dietary strategy is to increase the PUFA in ruminant meat further increases in vitamin E supply may be required.

1.5.3.2. The antioxidant defence system

Living organisms produce several types of reactive oxygen species (ROS) as a result of normal metabolic reactions and environmental factors (Birben *et al*., 2012). ROS are highly reactive molecules that have one or more unpaired electrons, thus giving the potential to react with other molecules such as DNA, amino acid and lipids causing oxidative damage (Min and Ahn, 2005). The most physiologically significant ROS are superoxide (O^{2-}), hydrogen peroxide (H2O2) and hydroxyl radical (HO•) (Birben *et al*., 2012). Living organisms have an antioxidant defence system, which is classified based on activity, into enzymatic and non-enzymatic antioxidants that stop or block the harmful effects of ROS (Patekar, 2013). The enzymatic defence system is uniquely produced in living organisms and can be subdivided into primary antioxidants, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) and secondary antioxidants including glutathione reductase (GR) and glucose-6-phosphate dehydrogenase (G6PDH). The action of the enzymatic antioxidants is to break down and remove free radicals through converting dangerous oxidative products into hydrogen peroxide and then to water (Balasaheb and Pal, 2015).

The non-enzymatic defence system is a class of the antioxidants which are not found in the body naturally but are acquired from diet for proper metabolism (Raygani *et al*. 2007). Some of the known non-enzymatic antioxidants are vitamins (vitamin E, C and A), minerals (selenium, copper, iron, zinc, and manganese), carotenoids (β-carotene, lycopene, lutein), polyphenols (phenolic acids, flavonoids, gingerol) (Mcdowell, 2000). The action of the non-enzymatic antioxidants is interrupting free radical chain reactions (Balasaheb and Pal, 2015).

Generally, vitamin E has been shown to be essential for the optimum function of the immune, muscular, nervous, reproductive and circulatory systems (Mcdowell, 2000). Vitamin E is well known as a part of the intracellular defence system against the harmful effect of ROS that is produced from the oxidation of cellular membrane and subcellular organelles membrane (Hidiroglou *et al*., 1992; Gagné *et al*., 2009). The action of an α-tocopherol isoform of vitamin E is to inhibit radical chain propagation within the cell membrane by conversion into α-tocopheryl quinone (oxidises product) (Patekar, 2013).

The antioxidant role of vitamin E becomes very important during the immune response when immune cells produce considerable quantities of hydrogen peroxide and superoxides to destroy foreign organisms (NRC, 2007; Reboul, 2017). The α-tocopherol is also reported to act as an antioxidant in animal tissue post-mortem to delay lipid oxidation and increase the shelf life of meat (Wood and Enser, 1997).

The metabolic antioxidant function of α-tocopherol is closely associated with the enzyme glutathione peroxidases (GPxs) which is selenium (Se) dependent enzyme. Glutathione peroxidases (GPxs) is a general name for an enzyme family with peroxidase activity whose main biological role is to protect the cell from oxidative damage. There are eight isozymes of GPxs which very in cellular location and substrate specificity; GPX1, GPX2, GPX3, GPX4, GPX5, GPX6, GPX7 and GPX8. Glutathione peroxidase (GPX1) is most abundant version and acts in the cytoplasm on the substrate hydrogen peroxide, while GPX4 is found in the cell membrane and mitochondria and acts on hydroperoxides (Chauhan *et al*., 2014), thus protecting unsaturated lipid from oxidation within the cell membrane. Thus, GPxs and α-tocopherol are complementary in their action. Moreover, selenoenzymes such as thioreductase and iodothyroxine deiodinases also act as antioxidant and alter redox status and thyroid hormone metabolism (CSIRO, 2007). Animals that have a deficiency of vitamin E and Se suffer from white muscle disease or nutritional myopathy or muscular dystrophy

(Mcdowell, 2000). This alters their antioxidant defence system by depressing GPxs and increasing lipid degradation within the cell membrane (Chauhan *et al*., 2016).

Excessive Se is toxic, and the recommended dietary level of Se should be 5-10 times less than those found to be toxic (CSIRO, 2007). According to the National Research Council (2007), the tolerable level was increased from 2 mg/kg DM (NRC, 1980) to 5 mg/kg DM (NRC, 2005). This implies that the dietary requirement level for ruminants is between 0.5 - 1 mg/kg DM.

1.5.3.3. Effect of vitamin E on muscle's fatty acids

Feeding animals can improve the nutritional value of meat with vitamin E at a level greater than the requirement (Álvarez *et al*., 2009). One of the nutritional values of meat is the fatty acid profile that can be modified by lipd oxidation. Unsaturated FAs are more susceptable for oxidation than satuarated FAs during meat storage (Liu *et al*., 2013). The protective role of vitamin E against lipid oxidation during storage has been well reported (Wood *et al*., 2008). It has been also reported that lipid oxidation could reduce the content of essential PUFA and long chain PUFA. However, FAs oxidation can be reduced by increasing the vitamin E content of meat. Álvarez *et al*. (2009) reported that lambs supplemented with vitamin E (250, 500 and 1000 mg/kg diet) had unchanged proportion of saturated FA and PUFA in LD muscle during storage under retail conditons after 14, 21 and 28 days. Also, Bellés *et al*. (2018), studied the effect of vitamin supplementation (1000 mg/kg diet) on FA stability in fresh or thawed lamb leg chops (frozen stored for 3, 6 and 9 months) maintained for 9 days under retail conditions. The muscle concentration of α-tocopherol was over 3.5 times higher in supplemented samples compared to the control samples. The supplemented group showed a higher content of PUFA in meat than the control group as a result of a reduction in lipid oxidation. Thus, animal feeding with supranutritional vitamin E can not only delay lipid oxidation but also mantain the nutritional value of meat through the storage period.

1.5.3.4. Effect on shelf life (colour and lipid stability)

Colour is one of the first sensory properties of meat that the consumers judge when purchasing meat (Erasmus and Webb 2014). In red meat, myoglobin is the main component which is responsible for meat colour (Mancini and Hunt, 2005). Myoglobin oxidation during the storage period can result in a brown discolouration on the meat's surface (Kerry *et al*., 2002). Increasing the P:S ratio in ruminant meat can lead to an increased the oxidation of PUFA and consequently produce undesirable flavours, to control these problems, supra-nutritional vitamin E might be beneficial (Channon *et al*., 2003). A reduction in myoglobin and lipid oxidation in meats has been related to the supra-nutritional supply of vitamin E and selenium in ruminant diets (Suman and Joseph, 2013). This reduction is due to vitamin

E acting as an antioxidant and increasing the cell membrane α-tocopherol concentration and increasing glutathione peroxidase activity (McDowell *et al*., 1996). Protecting cell membranes (phospholipids) and cholesterol against oxidation, leads to an increase in the shelf life of meat by preventing the production of undesirable flavour and discolouration (Suman and Joseph, 2013). Vitamin E has been found to improve meat quality in beef (Lavelle *et al*., 1995), lamb meat (Turner *et al*., 2002) and in chicken meat (Galvin *et al*., 1997).

It has been found that finishing lambs on vitamin E at level 450 mg /lamb /day for 56 days can increase the concentration of α-tocopherol levels in muscle and delay the oxidation of myoglobin and lipids to extend the shelf life of fresh lamb cuts by approximately 4 days (Wulf *et al*., 1995). Also, Turner *et al*. (2002) found that when comparing pasture finished to concentrate finished lamb, supplemented with 13.5, 135, 270 mg of vitamin E/kg DM for 71 days, the levels of α-tocopherol in muscle were higher in pasture finished lamb compared to concentrate finished lamb receiving 13.5 and 135 mg. However, lamb finished on concentrates containing 270 mg had a higher tocopherol concentration than pasture-finished lambs. Furthermore, lightness and redness value of SM muscles were higher with 135 and 270 mg of vitamin E than the lambs finished on 13.5 mg.

1.6. Conclusion

In the UK, consumers prefer grass to concentrate finished lamb. However, a proportion is finished off concentrates. Grass finishing is associated with higher welfare, a shorter supply chain and higher eating quality (Fisher *et al*., 2000). Dietary factors such as carbohydrate, PUFA and vitamin E are known to influence carcass chemical composition, shelf life and eating quality of lamb, with grass finished lamb being associated with a higher PUFA and vitamin E content, (Wood, 2005). Within meat, vitamin E acts as an anti-oxidant, reducing PUFA oxidation, extending shelf life, and may contribute to the pastoral flavour of lamb. Therefore, the objective was to investigate a dietary strategies to produce concentrate finished lamb with a similar chemical composition, shelf life and eating quality characteristics to grass finished lamb by using different source of carbohydrate, fat and vitamin E level.

Chapter 2

2.0. General materials and methods

2.1. Proximate analysis

All samples for proximate analysis were analysed in duplicates at Harper Adams University in accordance with the method of Association of Official Analytical Chemists (AOAC, 2016) for Dry matter (930.15), crude protein (968.06), and ash (942.05).

2.1.1. Dry matter (DM)

Dry matter content of all samples (grass, concentrate and meat) was determined according to method 930.15 (AOAC, 2016). A sample was accurately weighed and oven dried (Binder, Tuttlingen, Germany) at 105 °C over-night or freeze dried (Edwards Modulyo freeze dryer, Sussex, UK) until a constant weight. After removal from the oven, samples were cooled down to room temperature in a desiccator and reweighed. Subsequently, dried samples were milled through a 1 mm screen using a cyclon mill (Cyclotec, FOSS, Warrington, UK) and used for the subsequent lab analysis. Dry matter was measured and calculated:

$$\text{DM g/kg} = \frac{Dried\ sample\ weigh\ (g)}{fresh\ sample\ weigh\ (g)} \times 1000 \qquad \text{Equation 2.1.1}$$

2.1.2. Crude protein (CP)

Dried feeds and freeze-dried meat samples were analysed for CP according to method 968.06 (AOAC 2016) operating the Dumas method using an auto analyser LECO FP528 (Corp., St. Joseph, MI, USA) with the use of a standard EDTA (Sweeney, 1989). Approximately 0.15 g of dried sample was weighed in an aluminium foil tray which was placed into the auto analyser. Mixture of gas (O_2 for rapid combustion and Helium as a carrier) were used when samples heated to 950 °C. Warmer copper fillings were used to reduce N2 from N oxidise. CP was measured and calculated:

$$\text{CP g/kg DM} = Nitrogen\ content\ \times 6.25 \qquad \text{Equation 2.1.2}$$

2.1.3. Ash and organic matter (OM)

Dried feed and freeze-dried meat samples were analysed for ash and OM according to method 942.05 (AOAC, 2016) by weighting approximately 2 g of sample into a pre-weighed porcelain crucible. Samples were then ashed in a muffle furnace (Gallenkamp Muffle Furnace, Size 3, GAFSE 620, Gallenkamp, Loughborough, UK) at 550 °C for 4 h. After removal from the muffle furnace samples were cooled down to room temperature in a desiccator and reweighed. Ash content and organic matter were calculated:

Ash g/kg DM = $\frac{Ash\ weigh\ (g)}{Initial\ sample\ weigh\ (g)} \times 1000$ Equation 2.1.3.a

OM g/kg DM = 1000 - ash weigh (g/kg DM) Equation 2.1.3.b

2.1.4. Gross energy (GE)

Gross energy of the dried feed samples was determined using an adiabatic bomb calorimeter (Parr 6200 Instrument Company, Moline, IL, 61265, USA) with Benzoic acid as a standard. Approximately 1 g of dried samples were accurately weighed and placed into a crucible after being pelleted using a 2811 Parr Pellet press (Parr instrument Co., Moline. USA). Fuse wire (10 cm) was inserted through the holes of bomb, ensuring that there was no contact between wire and sample. Apparatus was assembled, filled with O_2 (pressure 450 psi for 1 minute) placed in a bucket containing exactly 2 litres of water after being filled with O_2 and the wires connected. Energy content of the samples was measured by burning samples under constant volume of water and enclosed condition. Produced energy was measured as MJ/Kg DM.

2.1.5. Neutral detergent fibre (NDF)

Neutral detergent fibre content of dried feeds was determined according to Van Soest *et al*. (1991) using Fibertec apparatus (Tecator Fibertec 1020 Hot extractor, FOSS, UK Ltd, Warrington, UK). Approximately, 0.5 g of dried ground sample was accurately weighed into a glass crucible (porosity 1, Soham Scientific, Ely, UK). Crucibles were fitted into the Fibertec apparatus making sure the valves were closed. 25 ml of previously prepared cold NDF reagent (150 g sodium dodecyl sulphate (SDS), 93 g of di-sodium ethylene diamine tetra acetic acid dehydrate (EDTA), 34 g sodium tetraborate ($Na_2B_4O_7$ $10H_2O$), 50 ml tri-ethylene glycol, and 22.8 g anhydrous disodium hydrogen phosphate (Na_2HPO_4) to make 5 L solution with distilled water and pH was adjusted between 6.9 and 7.1) were added to the samples followed by 0.5 ml of Octan-1 reagent grade (Sigma, Aldrich, Dorset, UK) to inhibit foaming. The samples were then boiled for 30 min. Another 25 ml of cold NDF reagent was then added, together with 2 ml of alpha amylase solution (2.8 g of stable alpha-amylase from *Bacillus subtilis* (Sigma, Gillingham, UK) dissolved in mixed of 10 ml of 2-ethlylene glycol and 90 ml of distilled water) and the sample boiled for a further 30 minutes. The sample was then filtered and washed 3x with 25 ml of hot distilled water (~80 °C). A further 2 ml of alpha amylase and 25 ml of hot distilled water (~80 °C) were added to the samples and allowed to stand for 15 minutes. Samples were then filtered and washed again with 3x with 25ml of hot distilled water~80 °C). The crucibles were then removed from the apparatus and dried overnight at 105 °C. After that, the crucibles were placed in a desiccator and weighed. They were then placed in a muffle furnace for 4 h at 550 °C. Crucibles were placed in a desiccator to cool at room temperature, and re-weighted. NDF was calculated as:

NDF g = $(crucible + dry\ fibre\ weigh\ ) - (\ crucible + ash\ weigh)$

NDF g/kg DM = $\frac{NDF\ weigh\ \ (g)}{Sample\ weigh\ (g)} \times 1000$ Equation 2.1.6

2.2. Fatty acid analysis in feeds, muscle and adipose tissue

The analysis of FA in the feed, muscle and adipose tissue was determined after FA methyl ester (FAME) synthesis, according to a modification of the method outlined by O'Fallon *et al*. (2007) using gas chromatography (Agilent 6890 1st experiment and Agilent 7820A 2nd experiment).

Approximately 0.5 g of oven dried feed or freeze dried meat and 0.1g of freeze dried adipose tissue were weighted into a saponification tube, followed by adding 1 ml of internal standard (4 mg of C13:0/ml methanol) and 0.7 ml 10N KOH. To facilitate saponification 5.3 ml of methanol was added to each tube. The tubes were then incubated in a water bath (55 °C, 1.5 h) and shaken for 5 seconds every 20 min. The tubes were then cooled down to ambient temperature to obtain FAME, 0.58 ml 24 N H_2SO_4 was added and the tubes incubated in a water bath (55 °C, 1.5 h, shaking 5 sec/20 min). The samples were cooled down in a cold water bath, and 3 ml of hexane were added and vortexed for 5 min. the tubes were then centrifuged (500 x g, 10 min). The top layer of hexane that contained FAME was then transferred using glass pasture pipette into a GC vials.

Samples were analysed by gas chromatography using a flame ionization detector (Agilent Inc. Wilmington, DE) and a capillary column (CP-SIL 88, 100m x 0.25mm x 0.2µm) (Agilent J and W, GC columns, UK). The GC conditions were as follow: Carrier gas hydrogen ; flow rate 2.1 ml/min; column pressure 29.59 psi; split ratio 100:1; maximum oven temperature 225 °C; starting temperature 70 °C held for 2 min; increased 8 °C/min to 110 °C ; then increased 5 °C/min to 170°C ; finally increased 4 °C/min to 225 °C. The FAME standard Supelco® 37 component FAME mix (Sigma-Aldrich, Dorset, UK) was used for identification of sample peaks by comparison of retention times.

Total FAs content (g/kg DM) were quantified as follows:

Total FA content (g/kg DM) = $\frac{\left(\frac{100 \times weigh\ (g) of\ IS}{\%\ area\ of\ IS}\right) - weight\ (g) of\ IS}{weigh\ (g) of\ sample}\ x\ 1000$

Equation 2.1.8 a

The percentage of each FA (% from total FAs) were corrected by removing the area of internal standard as follows:

Corrected area of each FA % = $\frac{\%\, area\ of\ specified\ area\ fatty\ acid}{100-\%\, area\ of\ Internal\ standard} x\ 100$

Equation 2.1.8 b

The individual FA (g/kg DM) content in the feed sample was quantified as follows:

Individual FA content (g/kg DM) = $\frac{specified\ fatty\ acid\ \%}{100}\ x\ total\ fatty\ acid\ content$ (g/kg DM)

Equation 2.1.8 c

To obtain the individual FA (g/kg muscle) the following equation was used:

Individual FA (g/kg muscle) = $\frac{specified\ fatty\ acid\ \%}{100}\ x\ \frac{total\ fatty\ acid\ (g/kg\ DM)}{DM\ \%\ /100}$

Equation 2.1.8 d

2.3. Vitamin E in feedstuffs

Vitamin E (α- tocopherol) in Feedstuffs was measured according to a modification of the method described by Hidiroglou *et al.,* (1988) using high performance liquid chromatography (HPLC) (Gilson, France for experiment 1 and Agilent 1100, Germany for experiment 2) . Duplicate freeze dry feed samples (0.3 to 1 gm) were weighted into a 50 ml volumetric flask and mixed with 0.05 g α-amylase and 8 ml deionised water (DW) and placed in a water bath (37°C, 16 h). The following day, 0.1 g ascorbic acid, 10 ml ethanol, 2 ml KOH 50% and an appropriate quantity of internal standard rac-5, 7-dimethyltocol (Universal Biological Ltd, Stroud, UK) (taking in the account to the final dilution factors) were added and boiled for 30 minutes, prior to cooling in ice. After that 10 –ml of hexane, 10 ml of deionised water (DW) and 2 ml of ethanol were added to the samples in a volumetric flask. The samples were then vigorously shaken for 30 seconds the top layer transferred into a separating funnel that contained 10 ml of DW. A further 10 ml of hexane were added to the sample, shaken, and separated until enough of the hexane had been collected in a separating funnel. The separating funnel was shaken gently in order to wash the sample, which was then allowed to separate, and the bottom layer run into a waste beaker. The washing process was repeated three times. The extract was then transferred into a 30 ml soveril tube, leaving the aqueous phase behind, evaporated to dryness in a water bath at 60°C under oxygen free nitrogen, made up in 1 ml hexane and transferred into 2ml amber HPLC vial using a 13mm PTFE filter pore size 0.2 µm.

Samples (40 µl) were injected into a 5 µm silica column (250 x 4.6mm, Phenomenex Hyperclone) with a mixed mobile phase 4% 1, 4-Dioxine 97% n-hexane at 40 °C at a flow rate of 1.6 ml/min. The α-tocopherol was detected by fluorescence detection and quantified by a comparison of sample peak area to that of the internal standard using the following equations.

$$\alpha\text{- tocopherol µg/sample wt.} = \frac{\left(\frac{area\ of\ \alpha{-}tocopherol\ in\ sample}{area\ of\ pure\ \alpha{-}tocopherol\ in\ std}\right) * conc.\ of\ \ pure\ \alpha{-}tocopherol\ in\ std}{\frac{area\ of\ DMT\ in\ samples}{area\ of\ DMT\ in\ std}}$$

Equation 2.1.7.a

$$\alpha\text{- tocopherol µg/g} = \frac{\alpha{-}\ tocopherol\ \mathrm{µ}g}{sample\ weight\ (g)}$$

Equation 2.1.7.b

2.4. Vitamin E in muscle

Vitamin E in meat samples was determined according to the modified method described by (Liu *et al.,* 1996). Approximately 1 g of minced and homogenised meat was weighed into a 30 ml soveril tube with a PTFE lined screwcap. To this, 2 ml of ethanolic butylated hydroxytoluene (BHT) (0.1 % w/v) and 2.8 ml of ascorbic acid solution (8.8% w/v) were added and vortexed for 30 seconds. 2.5 ml of alcoholic potassium hydroxide (KOH) (14.52% w/v) was then added, together with 15 µl of Dimethyl tocol (250ug/ml) (DMT) as an internal standard. The mixture was then shaken vigorously for 30 sec and the tubes incubated in a water bath at 80 °C for 20-30 minutes until meat samples had fully dissolved. The samples were then cooled down using a bowl of cold water. Four ml of n- hexane was then added to each tube and they were shaken vigorously for 30 sec, prior to centrifugation for 4 minutes at 2000 x g. The supernatant layer (hexane layer) was then carefully transferred into a 15 ml test tube using a Pasteur pipette and evaporated to dryness under oxygen free nitrogen at 60 °C in a water bath. Exactly one ml of pure hexane was added to the dried sample, mixed well and transferred into 2ml amber HPLC vial using a 13mm PTFE filter pore size 0.2 µm.

Samples (40 µl) were injected into a 5 µm silica column (250 x 4.6mm, Phenomenex Hyperclone) with a mixed mobile phase 4% 1, 4-Dioxine 97% n-hexane at 40 °C at a flow rate of 1.6 ml/min. α-tocopherol was detected by fluorescence detection and quantified by a comparison of sample peak area to that of the internal standard using equations (2.17.a) and (2.17.b).

2.5. Preparation of α- tocopherol standard

Approximately 40 mg of α- tocopherol (≥ 96% Sigma® Chemical Co. St. Louis, MO. USA) was weighed into an amber conical flask and diluted to 100 ml of hexane (original dilution). One more dilution 1:100 (1 ml of original dilution into 99 ml hexane) was prepared. The concentration of α- tocopherol was determined by absorbance of the second dilution using molar extinction coefficient (EmM) of 3.26 at 292 nm by spectrophotometry.

$$C = \Delta bs/EmM \qquad \text{Equation 2.5}$$

Where:

E = molar extinction coefficient
A = Absorbance at 292 nm
C= Molar Concentration
I = path length of cuvette (cm3) = 1

The molar extinction coefficient of α- tocopherol was then converted into a mass using a molecular weight of α- tocopherol (430.71 g/mol). After the exact concentration was determined, 0.7 ml of the original solution was diluted in 99.3 ml of hexane with the aim of getting the concentration to between 3.5 – 4.0 µg/ml. The standard was then stored in amber glass vials and kept at -20 °C until the day of α- tocopherol analysis.

2.6. Blood sampling and analysis

Blood samples were taken from lambs via jugular venepuncture at 11.00 am using a 20-gauge 1.5″ needle (Becton Dickinson Vacutainer Systems, Plymouth, UK) into 2 different vacutainer tubes (Becton Dickinson Vacutainer Systems, Plymouth, UK).

Tubes containing lithium heparin (6 ml green cap) containing 102 IU/tube were used for determination of blood biochemical. Tubes containing fluoride/potassium oxalate (4ml grey cap) containing 10 mg/tube were used for glucose determination.

Tubes containing blood samples were immediately centrifuged for plasma at 1000 x g for 15 minutes at temperature 4 °C using a (Sigma 3-16KL, Germany) centrifuge. The supernatant was transferred into 1.5 ml Eppendorf tubes via disposable pipettes and stored at -20 °C for further analysis.

Blood plasma were used for determination of total protein (Randox Laboratories kit, TP245) albumin (AB362), Non-Esterified Fatty Acids (NEFA) (FA115), β-hydroxybutyrate (BHB) (RB1008), urea (UR221) and glucose (GL1611) using the blood analyser machine Cobas Mira Plus auto- analyser (ABX Diagnostics, Bedfordshire, UK)

2.7. Live weight determination

Lamb live weights (LW) were weighted once a week at 14.00h using a weight scale (Shearwell Data Ltd., Somerest, UK) with an electronic display head (Salter Bracknell LS300 electronic weight scale, Staffordshire, UK). For accuracy and precision, the scale was calibrated with a standard weight (20kg) (F.J. Thornton and Co. Ltd., Wolverhampton, UK) prior to each weighting.

2.8. Back fat and eye muscle depth

Back fat and *Longissimus lumborum* (LL) muscle thickness were scanned and measured by ultrasound device (DP-6900Vet, Mindray Ltd.) according to the procedure of Davis (2010). The third lumbar vertebrate was specified and at 90 degrees to the back bone wool parted. Liquid paraffin oil was applied on the third lumbar vertebrate to give a contact. The transducer was placed on the prepared site and adjusted until a clear image of eye muscle and fat layers appeared. The picture was then frozen and measurement of muscle and fat depth taken. A single measurement of muscle depth was taken from a frozen image at the deepest point and three measures of fat depth were taken at 1 cm interval (Figure 2.1).

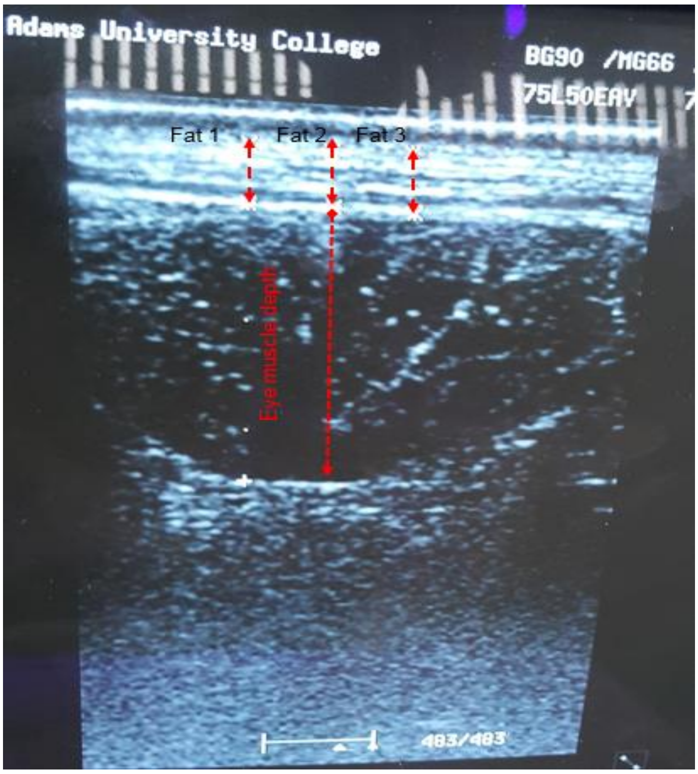


Figure 2. 1. Eye muscle area and fat layer of lamb.

2.9. Rumen fluid sampling and pH

Post slaughter (approximately 15 minutes), the rumen was removed and 50 ml of rumen fluid taken and filtered through two layers of muslin into a 50 ml pot. Rumen pH was measured by pH meter (HACH, H160, Loveland, U.S.A.). The pH probe was calibrated before each batch of slaughtered lambs using a 2 pH buffer solution (pH 7.0 and pH 4.0) (VWR, International Ltd., Poole, UK). Rumen fluid samples pH were acidified by adding a few drops of concentrated hydrochloric acid (HCL) and frozen at – 20 °C for volatile FA analysis.

2.10. Volatile fatty acids (VFA)

Volatile fatty acids in rumen fluid were determined using gas chromatography (GC) according to Cottyn and Boucpue, (1968). Frozen samples (50 ml pot) were defrosted at room temperature, and 5 ml transferred into a 15 ml tube and centrifuged at 1000 x g for 10 minutes at temperature 4 °C using a (Sigma 3-16KL, Germany) centrifuge. One ml of the rumen fluid supernatant was then pipetted into a 15 ml tube. To this, 200 µl of metaphosphoric acid (25%) and formic acid (3: 1, v/v) and 200 µl of internal standard (IS) (2-methylvalerate, 2g/l) were added and after 30 minutes, samples were centrifuged at 3622 x g for 20 minutes at 4 °C. The clear supernatant was pipetted into a GC Vials and run on GC.

The analysis was conducted on a 6890 GC Agilent technologies. Using a column (DB-FFAP, 30m x 0.250mm x 0.2µm) (Agilent J and W, GC columns, UK) and a flame ionization detector (Agilent Inc. Wilmington, DE). The GC conditions were as follow: carrier gas nitrogen ; flow rate 2.7 ml/min; column pressure 11.72 psi; split ratio 30:1; maximum oven temperature 235 °C; temperature programmed on 60 - 200 °C (20 °C/min, 10 min), Injector temperature 250 °C; detector temperature 300 °C. To separate particle of dirt from the sample, a glass wool liner was used in the injector.

A standard solution containing all VFA (acetate, propionate, iso-butyrate, butyrate, iso-valerate, valerate and caproate) and internal standard (2-methylvalerate) was run before and between samples to ensure constant reading. Internal response factor was used to quantify VFA using the following equations:

$$\text{Internal response factor (IRF)} = \frac{IS\ area\ \times\ specific\ VFA\ amount}{IS\ amount\ \times\ specific\ VFA\ area} \qquad \text{Equation 2.6.a}$$

$$\text{Amount of specific VFA} = \frac{IS\ amount\ \times\ Specific\ VFA\ area\ \times\ IRF\ of\ specific\ VFA}{IS\ area}$$

Equation 2.6.b

$$\text{Individual VFA (mmol/l)} = \frac{concentration\ of\ specifid\ VFA\ (mg)}{molecular\ weight\ of\ specified\ VFA} \times 1000 \qquad \text{Equation 2.6.c}$$

$$\text{Individual VFA \%} = \frac{Individual\ VFA\ (mmol/l)}{total\ VFA\ (mmol/l)} \times 100 \qquad \text{Equation 2.6.d}$$

2.11. Slaughter

Lambs were sent to abattoir (Euro Quality Lambs Ltd., Craven Arms, UK) and slaughtered after electrical stunning, without electrical stimulation and dressed up conventionally (Danso *et al.,* 2017). The lambs had free access to water and food prior to being sent to the slaughter house (1 h). Lambs were labelled and slaughtered in order. Carcass were chilled under commercial conditions at 2 °C for 24 h. At the slaughterhouse, the following variables were recoded; hot carcass weight (0.5 h post slaughter), cold carcass weight (24 h post slaughter). Carcass pH was also recorded using a pH probe (HACH, H160, Loveland, U.S.A.) by inserting a probe into *longissimus thorasic* (LT) muscle (12th and 13th ribs) at 45 min and 24 h post slaughter.

2.12. Carcass fatness, conformation and dimensional characteristics

Carcasses were classified for conformation and fatness scores by a trained assessor using the EUROP classification system (Commission Regulation EEC 461/93). There are five shape or conformation classes: E, U, R, O and P according to the carcass profile shape. There are also five main classes for external carcass fat (1 to 5). Carcass 3 and 4 are subdivided into low and high as shown in (Figure 2.2). For analysing carcass classification, numerical values were used as shown in (Table 2.1) (Danso *et al.,* 2017).

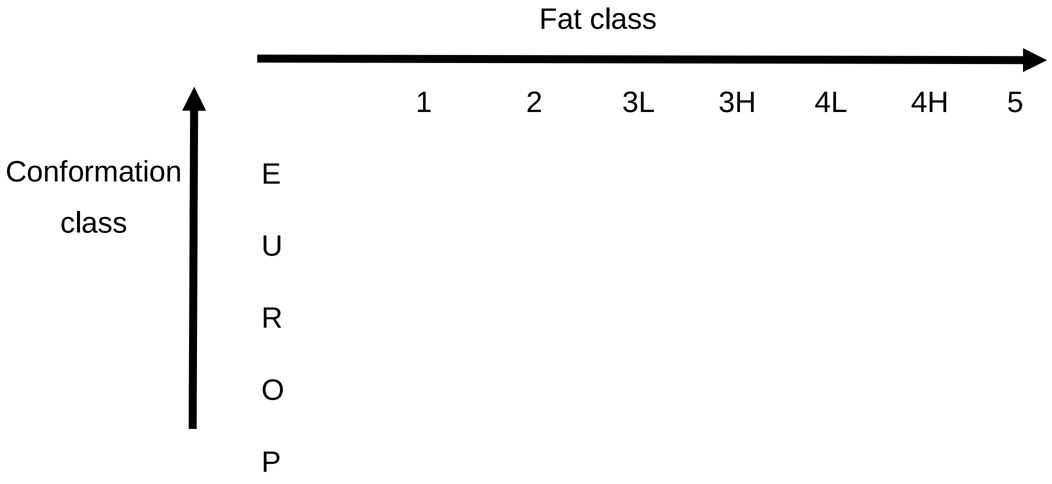


Figure 2. 2. Carcass conformation and fatness scores of lamb.

Table 2. 1. Carcass conformation, fatness scores and their numerical values.

Conformation							
Conformation class	E	U	R	O	P		
Numerical values	5	4	3	2	1		
Fatness							
Fat class	1	2	3L	3H	4L	4H	5
Numerical values	1	2	3	4	5	6	7

Dimensional measurements of carcasses (carcass length, barrel width, chest circumference, chest depth, buttock circumference, and gigot depth) were taken according to Brown and Williams (1979) as shown in (Figure 2.3)

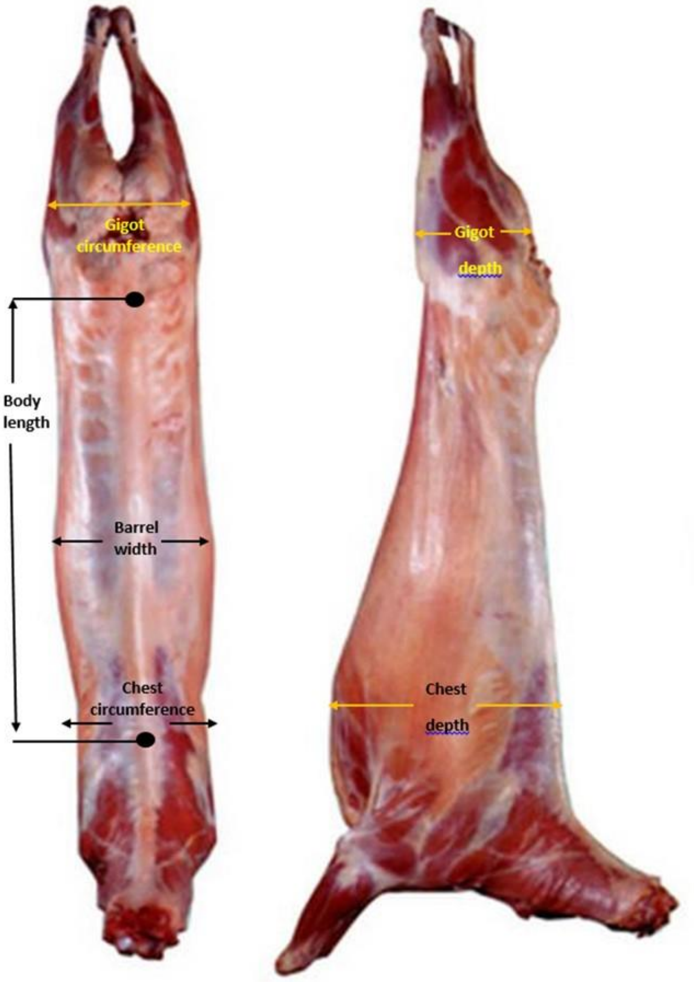


Figure 2. 3. Carcass dimensional measurements of lambs adopted from Brown and Williams (1979).

2.13. Meat quality

2.13.1. Colour evaluation

Colour of *semimembranosus* muscle (SM) was measured using a Minolta Chroma meter (Konica Minolta, CR-400, Japan) with a data processor DP-400 (Konica Minolta sensing, Inc., Japan) and 8mm head diameter. Colour measurements included: L* (lightness), a* (redness) and b* (yellowness). Muscle samples were vacuum packed (vacuum machine; TEPRO, MCV-011, UK), (bag's code; 721530/50, TRE SPADE, Italia) and conditioned at 2-4°C for six days, before transferred into plastic tray (19.7 x 15.5 x 5.5 cm) with modified atmosphere of (75.2% O_2 and 17.5% CO_2) using packaging machine (T100, MULTIVAC, Germany), sealing layer (CRYOVAC, UK, LTD) and subjected to simulate retail display (2-4°C, 700 lux; 16h on and 8h off) for seven days (1st experiment) and 14 days (2nd experiment) to measure colour.

Colour measurements were taken every day at the same time (11.00 am) at two points on the surface area of specified muscle using D65 illuminator at 2^{o} standard observer angle after being calibrated with a white calibration plate.

Colour saturation (the degree of red stability) and Hue angle (the degree of browning) were calculated (Kasapidou *et al*., 2009) using a* and b* values as follows:

$$\text{Saturation (Chroma)} = (a^{*2} + b^{*2})^{1/2} \qquad \text{Equation 2.9.1}$$

$$\text{Hue angle} = \tan^{-1}(b^{*}/a^{*}) \times 180/\pi \qquad \text{Equation 2.9.2}$$

2.13.2. Thiobarbituric Acid Reactive Substances (TBARS) evaluation

Lipid oxidation of meat samples was determined using TBARS assay according to the method of Buege and Aust (1978). Approximately 1 g of minced and homogenised meat was weighed in a 15 ml test tube to which 5 ml of TBARS stock solution was added. One litre of stock solution contained 150 g trichloroacetic acid, 3.75 g thiobarbituric acid and HCL at a final concentration 0.25 N. Samples were then vortexed and incubated in a water bath at 95 °C for 10 minutes until a pink colour appeared. The tubes were then cooled down under running tap water and centrifuged (Sigma 3-16KL, Germany) at 3000 x g for 10 minutes at 4 °C. The supernatant was transferred into a 1 ml cuvette and the absorbance recorded at 532 nm using spectrophotometer (JENWAY 6305, Bibby Scientific Ltd., UK) against a blank (1 ml of deionised water (DW) and 5 ml of TBARS stock solution).

A standard curve was prepared using a 1,1,3,3, tetra-ethoxypropane (TEP). Exactly 31 mg of TEP was dissolved in 1 litre of DW to produce 0.031mg TEP/ml (standard working solution). Serial dilutions were prepared by pipetted 0, 1, 2, 3, 4, 5 and 6 ml of TEP solution into 50 ml test tubes and adding DW to make up 50 ml to obtain 0, 0.00062, 0.00124, 0.00186, 0.00248, 0.0031 and 0.00372 mg of TEP/ml of extraction. One ml was pipetted from each dilution and placed in 15 ml test tube after being vortexed for 30 seconds. samples were analysed using the same procedure as for meat samples except TEP diluted concentrations were used instead of meat samples. A standard curve was constructed by plotting TEP concentrations against TEP absorbance (Figure 2.4). The concentration of TBARS in meat samples as mg of malondialdehyde/kg meat was determined by using the following equations:

$$Y= (153.63x + 0.0041) \qquad \text{Equation 2.8.7.a}$$

$$\text{TBARS mg/kg meat} = \frac{X\ (mg)}{weigh\ of\ meat\ sample\ (g)}\ x\ 1000 \qquad \text{Equation 2.8.7.b}$$

Where y is absorbance of meat samples, x is the unknown concentration of MDA (mg/g) and is obtained from standard curve (Figure 2.4), 1000 is a dilution factor used to obtain TBARS (MDA) mg/kg meat.

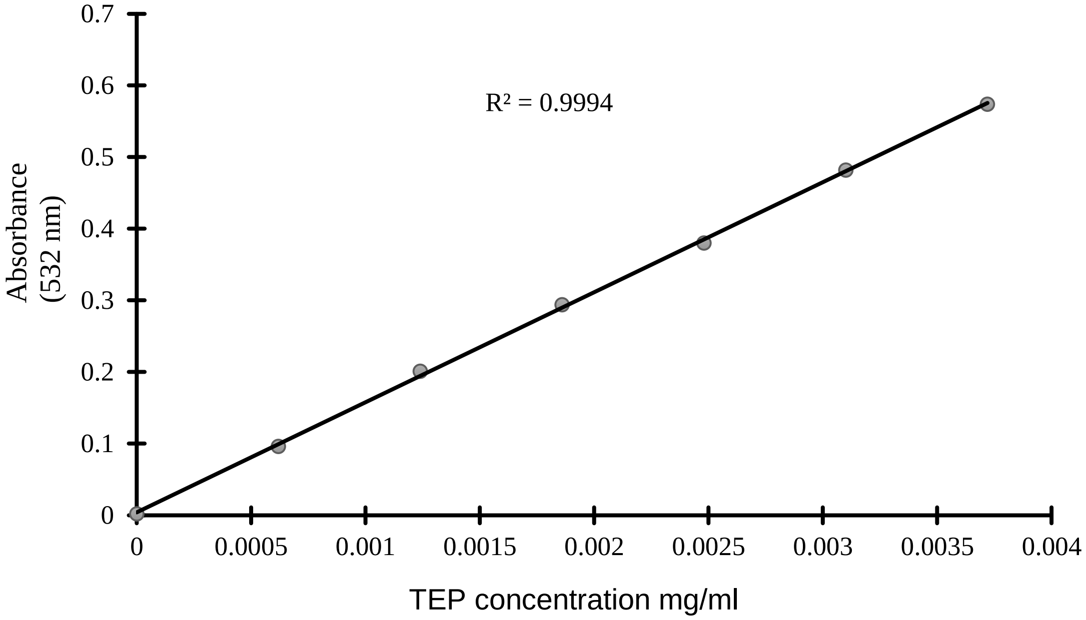


Figure 2. 4. Standard calibration curve of 1,1,3,3,-tetra-ethoxypropane of TBARS determination.

2.13.3. Thawing loss

Thawing loss was determined according to the method of Bonanno *et al*. (2011). Approximately 170 g of meat sample was weighted, vacuum packed and frozen at - 20 °C until the day of analysis. Samples were defrosted at 2- 4 °C for 24 h and reweighed after being dried using a paper towel. Thawing loss was determined and measured as a percentage by using the following equation:

$$\text{Thawing loss \%} = \frac{weigh\ of\ froozen\ sample\ (g) - weigh\ of\ thawed\ sample\ (g)}{weigh\ of\ frozen\ sample\ (g)}\ X\ 100$$

Equation 2.8.10

2.13.4. Cooking loss

Cooking loss was determined according to Sazili *et al*. (2013). Samples were removed from the freezer (-20 °C) and thawed at 2-4 °C for 24 h. Approximately 110 g of the thawed samples were weighed and recorded as initial weight. Samples were then vacuum packed and cooked at 70 °C for 1 h in a water bath (Sous vide supreme, 10ls, China). Cooked meat samples were then cooled down using running tap water for 30 min, dried with paper towel and reweighted as a weight of cooked meat. Subsequently, cooking loss was measured using the following equation:

$$\text{Cooking loss \%} = \frac{Initial\ weigh\ of\ meat\ (g) - weigh\ of\ cooked\ meat(g)}{initial\ weigh\ of\ meat\ (g)}\ X\ 100$$

Equation 2.8.11

2.13.5. Warner-Bratzler shear force

The previous cooked loss samples were taken directly for texture analysis using a texture machine analyser (TA. HD. Plus. Stable Micro Systems, UK) fitted with 30 kg load cell. The crosshead speed was set at test speed 1.5 mm/sec and 12 mm distance. A rectangular slot blade (HDP/WBR) Warner Bratzler blade was set for cutting meat samples. The shape, thickness and fibre orientation of samples were prepared according to Sazili *et al*. (2013). Cooked meat samples were cut into ten subsamples of a rectangular cross section of 20 mm length and 10 mm in cross section, with fibre direction parallel to the long axis. The samples were shear forced at a right angle to the fibre direction and the force required (N) was measured as a peak force.

2.13.6. Sensory analysis

For sensory evaluation, regular lamb consumers were recruited based on the average of lamb consumption at least once per month. Panels consisted of 40 members, male, female and different ages (Maiorano *et al*., 2016) (Table 2.2).

The left *Longissimus thoracis et lumborum* muscles were dissected out, trimmed off from fat, vacuumed packed and aged for 10 days at 2- 4 °C then frozen at -20 °C until the day of analysis. Frozen samples were thawed for 24 h at 2- 4 °C before cooking day. Thawed samples were placed in the centre of the tray, covered by foil and cooked in a kitchen (Regional Food Academy, Harper Adams University). Samples were placed in a preheated convection oven (Rational oven, SCC101) at 200 °C until the internal temperature of the sample reached 71 °C (approximately 20 min) using an internal thermometer probe and sous vide thermometer probe. Meat samples were removed from oven and put in the chiller to make sure that the internal temperature of meat reduced from 71 °C to below 10 °C within 2 h (Alimentarius, 1993), then cooled meat samples transferred in to the fridge (2-4 °C) for next day of sensory evaluation (1st experiment). On the day of the test, 1 h before each session meat samples were taken out from fridge and sliced into four steaks (2.5 cm) then served in a 3-digit code plate according to the serving plan. In the second experiment, meat samples were served directly after being cooked (i.e. cooked and tasted on the same day).

Samples were tested in four sessions with two sessions held in morning and two sessions in afternoon and 10 people per session, resulting in total 40 untrained consumers. The sample-serving plan based on Latin Square design to balance first order and carry-order effects (Macfie *et al.,* 1989). Dummy samples (bought from a commercial shop) were presented first to familiarise consumers with the test scale, followed by the four experimental samples. The samples were served one at a time and four questions asked on each sample (monadic sequential test). The scale used was a 9-point category scale for both hedonic and intensity questions. The anchor words were applied: tenderness (not tender to very tender), juiciness (not juicy to very juicy), liking of flavour (dislike extremely to like extremely) and overall liking (dislike extremely to like extremely). The panels were offered toasted bread and water to rinse their mouth before testing each sample.

Table 2. 2. Panels member characteristics for sensory evaluation of both experiments.

1st Experiment						
Gender		Age				
Male	Female	18-25	26-35	36-45	46	Total
22	18	19	4	8	9	40
2nd Experiment						
Gender		Age				
Male	Female	18-25	26-35	36-45	46	Total
19	21	1	12	14	12	40

Chapter 3

3.0. Effect of concentrate carbohydrate, fat source and vitamin E concentration on the performance, carcass composition and meat quality of lambs.

3.1. Introduction

Lambs fed on concentrate diets generally grow faster and have higher daily gains than those lambs finished on pasture (Priolo *et al*., 2002; Borton *et al*., 2005; Armero *et al*., 2015). However, forage finished ruminants produce meat products with a higher content of *n*-3 PUFA and lower saturated FA compared to those finished on concentrate diets, which improves the P:S and *n*-6:*n*-3 ratios (Wood and Enser, 1997; Nuernberg *et al*., 2005; Kasapidou *et al*., 2012). This reflects differences in dietary fat source, and in particular, that grass is high in α-linolenic acid (C18:3*n*-3) and concentrates are high in linoleic acid (C18:2*n*-6) (Enser *et al*., 1996; Hajji *et al*., 2016). The FA profile in lamb meat can be manipulated to better meet the requirements of the human diet using different FA sources, such as linseed oil, fish oil, marine algae and protected linseed and soybean (Cooper *et al*., 2004; Demirel *et al*., 2003; Wachira *et al*., 2002). However, little attention has been specifically directed at manipulating the FA profile and eating quality of concentrate fed lamb.

Meat from lambs grazing grass compared to that of lambs fed concentrates (which vary in carbohydrates such as fibre, WSC and starch has a different colour (Inserra *et al*., 2014), FA composition (Sinclair, 2007) and flavour (Young *et al*., 2003; Priolo *et al*., 2004). Different sources of carbohydrate produce different concentrations of volatile FAs in the rumen (Ramos *et al*., 2009). Propionate is metabolised differently to acetate and is used preferentially for glucose production, via gluconeogenesis in the liver (Priolo *et al*., 2001). This has implications for glycogen deposition and consequently the ultimate pH and colour of meat (Andersen *et al*., 2005).

A reduction in lipid oxidation in meat has been related to the supra-nutritional supply of vitamin E in ruminant diets (Lynch *et al*., 1999). This reduction is due to vitamin E acting as an antioxidant and increasing the cell membrane α-tocopherol concentration (McDowell *et al*., 1996). Protecting cell membrane (phospholipids) and cholesterol against oxidation increases the shelf life of meat by preventing the production of undesirable flavours and discolouration (Lynch *et al*., 1999). Vitamin E has been found to improve meat quality in beef (Lavelle *et al*., 1995), lamb (Turner *et al*., 2002) and chicken (Galvin *et al*., 1997). In addition, the efficiency of vitamin E absorption varies between diets. Forage fed lambs have a muscle vitamin E concentration of 4 to 7 mg/kg (Whittington *et al*., 2006; Kasapidou *et al*., 2012) whereas, lambs finished on concentrate diets have low tissue vitamin E concentrations of approximately 1 mg/kg (Wachira *et al*., 2002).

The differences in the FA profile of muscle resulting from different diets influence several aspects of meat quality including colour, lipid oxidation and flavour (Wood *et al*., 2004,). An increase in unsaturated FAs can lead to a reduction in shelf life due to an increase in lipid and colour oxidation (Nute *et al*., 2007; Kasapidou *et al*., 2009). Poly unsaturated FAs have a key role in flavour development and are mostly incorporated in the phospholipid fraction (Mottram, 1998; Channon *et al*., 2003) These FAs are oxidised during storage, processing and cooking and the interaction between lipid oxidation products and Maillard reaction compounds can also alter meat flavour (De Brito *et al*., 2017). Therefore, this study aimed to investigate the effect of carbohydrate source, fat source and vitamin E concentration on the performance, carcass composition, shelf life and eating quality of lamb.

3.2. Materials and methods

All animal procedures were conducted according to the UK animals (Scientific Procedures Act) 1986 and were approved by the Harper Adams University Animal Welfare and Ethical Review Board (AWERB). All other aspects of husbandry and management were similar to commercial practice.

3.2.1. Experimental design.

Forty Suffolk cross Texel ewe lambs (mean LW = 29 kg, s.e.d; 0.9) of approximately 11 weeks of age from the Harper Adams University flock were blocked by live weight into four treatments (ten lambs/treatment):

1- Grazed grass (**G**)

2- Barley based concentrate (**B**)

3- Dried grass based concentrate (**DG**)

4- Sugar beet based concentrate (**SB**)

Three concentrate diets were formulated to provide a similar level of crude protein (CP) of 180 g/kg DM, ether extract (EE) of 45 g/kg DM and an effective rumen-degradable protein (ERDP) /fermentable metabolisable energy (FME) ratio >10.0 g/MJ (Table 3.1) (AFRC, 1993). The DG and SB diets provided similar water soluble carbohydrate (WSC), but different proportions of NDF, whereas in diet B the energy was supplied mainly as starch. Diets SB and B were formulated to have a similar metabolisable energy (ME) content, and DG was formulated to have a similar ME content to G. All diets were formulated to have at least 300 g NDF/kg DM to prevent acidosis.

The barley based diet (B) contained Megalac$^{©}$ 18 g/kg (calcium salt of palm fatty acids, HJ lea Oakes Ltd., UK) rich in the saturated FA C16:0. Diets DG and SB contained 15 and 21 g/kg linseed oil (high in C18:3*n*-3, Young Animal Feeds Ltd., UK), respectively, as a precursor of longer chain *n*-3 PUFA EPA and DHA. Due to the inclusion of different fat sources, diets were formulated to provide different levels of vitamin E (α- tocopherol-acetate). Diet B was formulated to contain 60 mg/kg DM and diets DG and SB to contain 250 mg/kg DM vitamin E. The raw materials for the concentrate diets were based on predicted nutrient values (MAFF, 1992), mixed and prepared as course mix at HJ Lea Oakes Ltd., High town Mill Congleton, Cheshire, UK.

Table 3. 1. Raw materials and predicted chemical composition of the experimental diets.

Raw materials (g/kg)	**G**	**B**	**DG**	**SB**
Dried grass	---	---	602	---
Sugar beet pulp	---	---	74	610
Barley	---	590	75	---
Soya hulls	---	---	---	---
Oat feed	---	177	40	86
Soya bean meal	---	116	62	96
Rape seed meal	---	64	34	55
Urea	---	5	5	19
Sucrose	---	---	63	77
Megalac©	---	18	---	---
Linseed oil	---	---	15	27
Mins/vitamins[1]	---	20	20	20
Ammonium chloride	---	5	5	5
Sodium chloride	---	5	5	5
Predicted chemical composition (g/kg DM)				
DM (g/kg)	200	879	906	904
CP	180	180	180	180
NDF[2]	560	301	492	313
ADF[3]	285	139	243	197
Starch	10	363	61	26
WSC	207	37	151	150
Ash	85	67	114	111
Ether extract	20	45	45	45
C18:2*n*-6	2.6	8.4	5.5	8.2
C18:3*n*-3	12	0.7	17.3	19.7
C18:2/C18:3	0.21	12	0.31	0.41
Vitamin E (mg/kg DM)	150	60	250	250
Selenium (mg/kg DM)	0.1	0.5	0.4	0.4
ME[4] (MJ/kg DM)	11.5	12.1	11.4	12.7
FME[5] (MJ/kg DM)	10.8	10.3	9.8	11.1
ERDP[6] (0.05)	124	127	108	112
DUP[7] (0.05)	36	32	43	42
ERDP/FME	11.5	12.3	11	10.1

G: Grass, B: Barley, DG: Dried grass, SB: Sugar beet, [1]Mineral vitamin premix contained: E672a vitamin A 10000IU/kg, E671 vitamin D3 2000 IU/kg, ferrous sulphate monohydrate 556 mg/kg, sodium molybdate 5 mg/kg, sodium selenite 0.7 mg/kg calcium iodate anhydrous 8.25 mg/kg, manganous oxide 121 mg/kg, zinc oxide 167 mg/kg, [2]Neutral detergent fibre, [3]Acid detergent fibre, [4]Metabolisable energy, [5]Fermentable metabolisable energy, [6]Effective rumen-degradable protein, [7]Digestable undegradable Protein.

3.2.2. Experimental routine

Ten lambs were grazed on the Harper Adams University farm on a mixed pasture sward that consisted mainly of perennial ryegrass, with sward height being maintained at between 5-10 cm by adding or removing additional lambs as required. However, the pasture was not determined or specified for growing lambs or rearing sheep. The remaining thirty lambs were housed indoors and randomly allocated to individual pens in a naturally ventilated shed and bedded on sawdust throughout the study period.

Diets were offered twice a day at 08:00 and 16:00h in individual clean plastic buckets and water was available *ad-libitum*. Concentrate diets and fresh grass samples (0.5 kg) were collected weekly at 12:00 pm and stored at -20 °C for further analysis.

Concentrate dry matter intake (DMI) were recorded by offering a fixed amount daily and weighing back refusals twice a week (Saturday and Wednesday). The amount of each diet offered was calculated to supply 1.1x daily consumption during the previous week. Lamb live weight was recorded once a week on Thursday at 14:00h (section 2.7).

Blood samples were taken by jugular venepuncture into green (lithium heparin) and grey tubes (fluoride/potassium oxalate) (section 2.6) from all lambs at three time points throughout the experiment when they achieved a live weight of approximately 29 kg (W1), 34 kg (W2) and 40 kg (W3). In addition, lambs were ultra-sound scanned for back-fat and eye-muscle depth one day before being slaughtered (section 2.8).

3.2.3. Slaughter and measurements

Lambs were slaughtered once they reached half the potential mature weight of approximately 40 kg and each batch was selected from the 10 heaviest lambs (section 2.11). During the slaughter process, the gastrointestinal tract was collected and rumen fluid samples obtained. Rumen pH was measured immediately and the samples acidified by adding a few drops of HCL prior to subsequent VFA analysis. Carcass pH and both hot and cold carcass weight were recorded (sections 2.9 and 2.12). Following slaughter, lamb carcasses were returned to Harper Adams University, where carcass measurements (section 2.12) were taken prior to carcass preparation.

3.2.4. Carcass preparation:

Tail head adipose tissue samples (5 x 5 cm) were collected, vacuum packed and stored at -20 °C for FA analysis. Carcasses were processed according to Cross, (1977) (Figure 3.1). The *Longissimus dorsi* (LD) muscle was dissected from the right side of each carcass, vacuum packed and stored at -20°C for FA and proximate analysis. The *Longissimus* *thoracis* (LT) was dissected from both sides, the LD muscle from the left side was also dissected out, vacuum packed and aged for 10 days at 2-4 °C Samples were then frozen at -20 prior to sensory evaluation (section 2.13.6).

The left leg was cut into three 2 cm thick leg steaks, containing the *semimembranosus* (SM) and all pelvic limb muscles. These steaks were vacuum packed and conditioned at 2-4°C for six days, before being transferred into plastic trays with modified atmosphere package (MAP) (75.2% O_2 and 17.5% CO_2) and subjected to simulated retail display (2-4°C, 700 lux; 16 h on and 8 h off) for seven days to measure colour (section 2.13.1) and lipid oxidation (section 2.13.2). The right legs were vacuum packed and stored at -20°C for subsequent vitamin E determination of the SM (section 2.4).

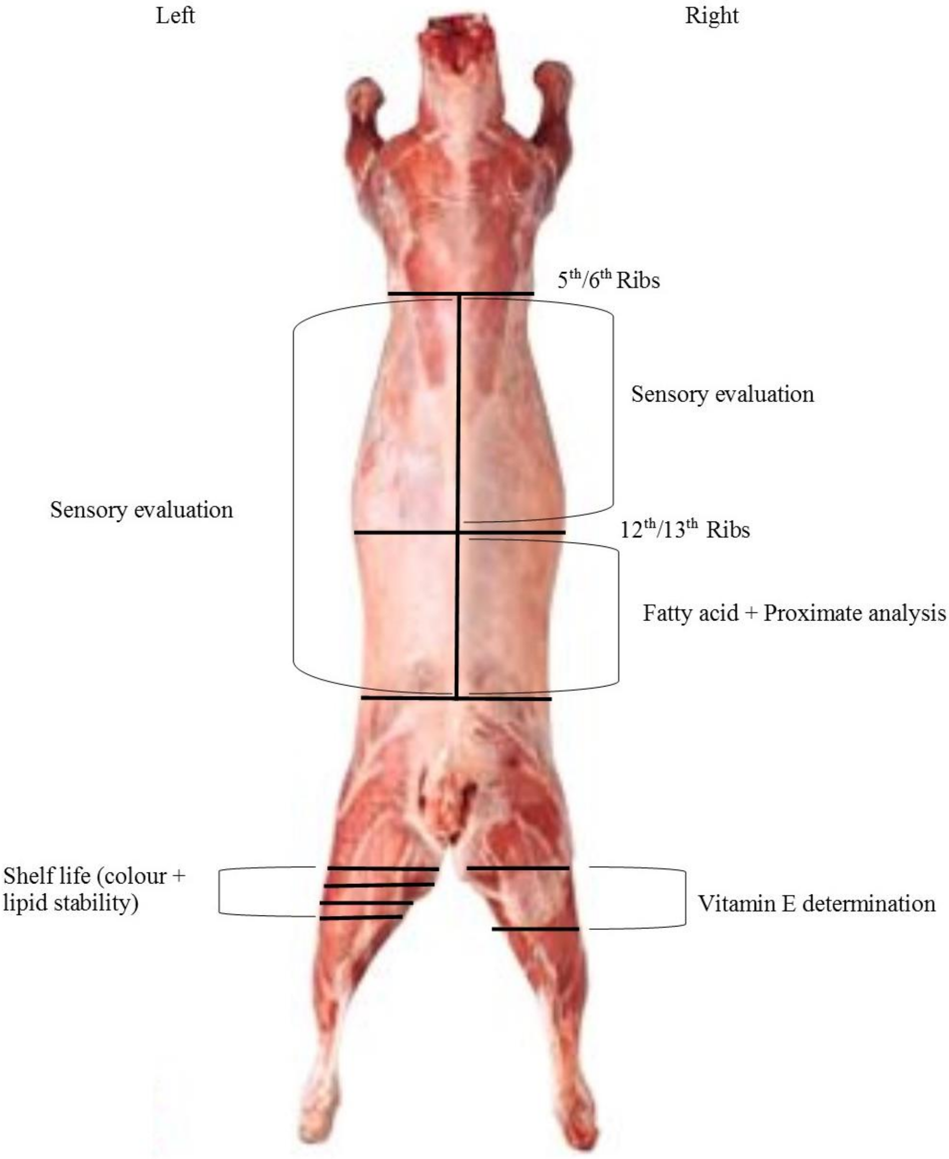


Figure 3. 1. Carcass preparation of lambs in the experiment.

3.2.5. Chemical analysis

Concentrate and grass feed samples were either oven dried or freeze-dried, ground (section 2.1.1) and a similar amount from each week mixed to prepare one sample for each concentrate diet and three samples of grass (each sample represented four consecutive weeks).

Feed sample was analysed for DM, CP, OM, NDF, GE, FAs and vitamin E as described in section 2.1.1, 2.1.2, 2.1.3, 2.1.4, 2.1.5, 2.2 and 2.3, respectively. Feed samples were analysed for selenium, starch and WSC by Trouw Nutrition, GB. Blood samples were analysed for total protein, albumin, glucose, NEFA, BHB and urea using a Cobas Mira Plus auto- analyser (ABX Diagnostics, Bedfordshire, UK) as described in section 2.6. Rumen fluid volatile FAs were determined by the method of Cottyn and Boucpue (1986) as described in section 2.10. The proximate composition (DM, CP and OM) and FA composition of the LD were determined as described in sections 2.1.1, 2.1.2, 2.1.3 and 2.2. The FAs of tail head adipose tissue were determined as described in section 2.2. Vitamin E was determined in the SM according to the method of Liu *et al.* (1996) as described in section 2.4. Lipid oxidation was determined by the method of Buege and Aust (1978) as described in section 2.13.2.

3.2.6. Calculation and statistical analysis

The daily live weight gain (DLWG) for each animal was estimated from the regression of live weight against time. The carcass conformation and fatness scores were converted to numerical values for statistical analysis (Danso *et al*., 2017).

Animal performance, rumen VFA, carcass chemical composition including FA, TBARS and sensory results were analysed by one-way analysis of variance (ANOVA) as a randomised block design using software GenStat 18th (Lawes Agricultural Trust, VSN International Ltd, Oxford, UK). Blood biochemical parameters and meat colour were analysed by repeated measure ANOVA as randomised block design with the main effect of time and treatments. Tukey's multiple range test (α =0.05) was used to determine significant differences between treatments with P<0.10 being identified as a trend.

3.3. Results

3.3.1. Animal health

The lambs fed concentrate diets had no health issues throughout the experiment. In contrast, lambs grazed on grass performed poorly between weeks 4 to 11. In week 8, following veterinary advice, all lambs were dosed for coccidia (10 ml Vecoxan (2.5 mg/ml oral suspension)). Two lambs subsequently died and were excluded from the analysis.

3.3.2. Feed analysis

The chemical compositions of the different diets are presented in Table 3.2. The chemical composition of the three concentrate diets (B, DG, and SB) was similar, with the mean values for DM and CP being 883, and 191 g/kg DM respectively. Similarly, the OM matter and GE values were similar between the four diets. Diet G had the highest content of NDF (537 g/kg DM) and the lowest content of total FA. The starch content in diet B was the highest, while the WSC was the lowest, compared to diets G, DG and SB. The α-tocopherol content of the four diets differed, with the lowest being diet G (34.0 mg/kg DM) and the highest being diet SB (259 mg/kg DM). The inclusion of Megalac in diet B increased C16:0, C18:1*n*-9c, C18:2*n*-6 and total FA compared to G, DG and SB diets. Similarly, adding linseed oil to diets DG and SB increased C18:1*n*-9c, C18:2*n*-6, C18:3*n*-3 and total FA compared to G diet. The chemical composition of the grass varied through the experimental period, especially during G2 (weeks 5 to 8), where DM, CP, WSC, α-tocopherol, C18:0, C18:2*n*-6 and total FA reduced, and NDF increased. In contrast, during G3 (week 9 to 12) CP, α-tocopherol, C18:0, C18:3*n*-3 and total FA increased.

Table 3. 2 Chemical composition of the experimental diets (g/kg DM).

Diets	**G**	**B**	**DG**	**SB**
DM[1] (g/kg)	202	872	896	881
OM[2]	911	929	907	892
CP[3]	144	192	201	183
NDF[4]	537	244	407	314
Starch	<5	411	82	78
WSC[5]	209	76.4	188	296
α-tocopherol (mg/kg DM)	34.0	64.7	224	259
Selenium (mg/kg DM)	0.07	0.27	0.48	0.59
GE[6] (MJ/kg DM)	17.6	18.3	18.1	17.5
Fatty acids (g/kg DM)				
C14:0	0.61	0.32	0.49	-----
C16:0	3.37	14.9	4.87	4.40
C16:1*n*-9	0.33	-----	0.23	-----
C18:0	0.40	1.24	0.82	1.23
C18:1*n*-9c	0.57	12.9	4.58	7.30
C18:2*n*-6c	3.03	14.3	9.59	10.2
C18:3*n*-3	11.3	0.17	15.2	17.7
RFA[7]	1.10	2.81	1.17	0.61
Total FAs	20.7	46.5	36.9	41.4
Grass quality	G1[8]	G2[9]	G3[10]	Mean
DM (g/kg)	209	197	202	202
OM	920	907	906	911
CP	143	140	149	144
NDF	515	553	542	537
Starch	<5	<5	<5	<5
WSC	243	196	189	209
α-tocopherol (mg/kg DM)	33.7	26.2	42.8	34.3
GE (MJ/kg DM)	17.8	17.6	17.5	17.6
Fatty acids (g/kg DM)				
C14:0	0.55	0.59	0.69	0.61
C16:0	3.36	3.19	3.55	3.37
C16:1*n*-9	0.36	0.36	0.28	0.33
C18:0	0.40	0.37	0.43	0.40
C18:1*n*-9c	0.64	0.50	0.56	0.57
C18:2*n*-6c	3.09	2.89	3.10	3.03
C18:3*n*-3	10.8	10.8	12.4	11.3
RFA	0.95	1.08	1.28	1.10
Total FAs	20.2	19.8	22.2	20.7

G: Grass, B: Barley, DG: Dried grass, SB: Sugar beet, [1]Dry matter, [2]Organic matter, [3]Crude protein, [4]Neutral detergent fibre,[5]water soluble carbohydrate, [6]Gross energy, [7]Remainig fatty acid, [8]week 1-4, [9]week 5-9, [10]week 9-12.

3.3.3. Animal performance

The effects of diet on lamb performance are presented in Table 3.3. The initial live weight (LW) of lambs was 29.2 kg and did not differ (P>0.05) between treatment groups. Lambs fed diet G grew slower, and took longer (P<0.001) to finish (Figure 3.2), and were slaughtered at a lower (P<0.001) LW than lambs fed diets B, DG and SB. There was a trend (P=0.096) for the lambs offered diet B to have a lower DM intake compared to DG group (1.48 kg/day vs 1.62 kg/day). However, there was no significant effect of concentrate diet (B, DG and SB) on final live weight, DLWG, FCR and the growth period.

Table 3. 3. Effect of dietary treatments on lamb performance throughout the experiment.

	G	**B**	**DG**	**SB**	**s.e.d.**	**P-value**
Initial LW (kg)	29.2	29.3	29.3	29.1	0.909	0.998
Final LW (kg)	38.1^{b}	42.4^{a}	41.2^{a}	41.9^{a}	0.573	<0.001
DM intake (kg/day)	------	1.48	1.62	1.55	0.059	0.096
DLWG[1] (kg/day)	0.07^{b}	0.38^{a}	0.33^{a}	0.37^{a}	0.028	<0.001
FCR[2] kg DM/kg gain	------	4.22	4.56	4.27	0.337	0.555
Growth period (day)	88.0^{a}	38.3^{b}	41.1^{b}	37.6^{b}	2.052	<0.001

G: Grass, B: Barley, DG: Dried grass, SB: Sugar beet, [1]Daily live weight gain, [2]Feed conversion ratio,
$^{a, b,}$ Means in a row with the same superscript are not different (P>0.05).

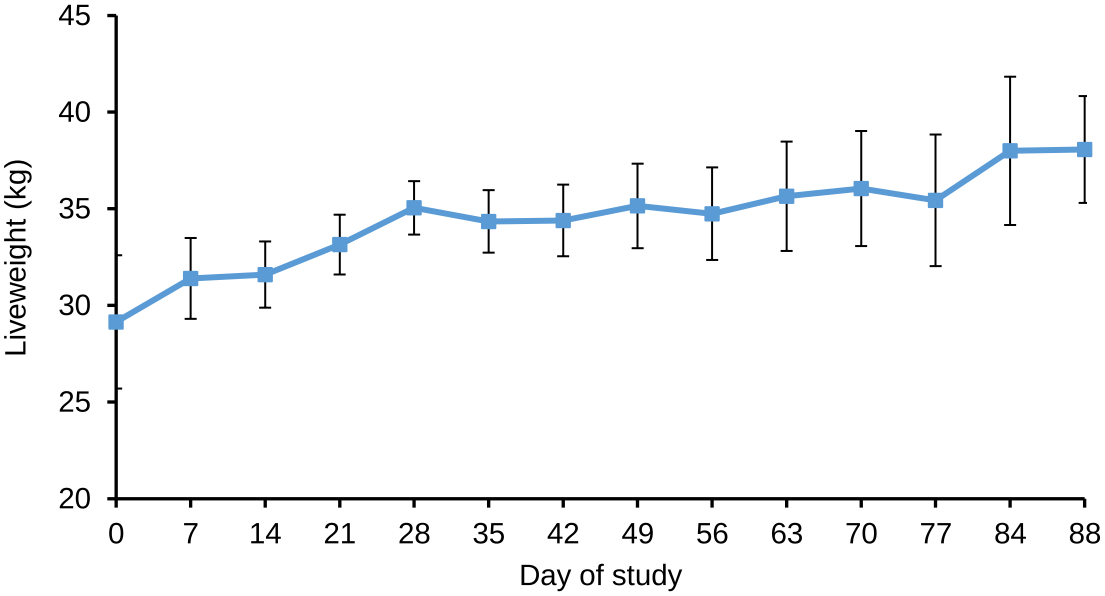


Figure 3. 2. Growth of lambs reared on grass throughout the experiment. Error bar indicates standard deviation (SD).

3.3.4. Carcass characteristics

The effect of the experimental diets on carcass characteristics is shown in Table 3.4. Due to the higher ($P<0.001$) final live weight and DLWG of lambs offered diets B, DG or SB, the hot and cold carcass weights and dressing % were higher ($P<0.001$) than those offered diet G. Based on visual classification, lambs that recieved diet G had a lower ($P<0.01$) conformation score and fat content ($P<0.001$) than those finished on concentrate diets (B, DG and SB). Similarly, the back-fat and eye-muscle depth of lambs scanned using the ultrasound scanner showed that grazed lambs had a lower ($P<0.001$) fat and eye-muscle depth than those finished on diets B, DG or SB. The muscle pH after 45 minutes post slaughter in lambs offered diet G was lower ($P<0.01$) than that of lambs offered diets DG or SB, however, the ultimate muscle pH value of lambs offered diet G was higher ($P<0.05$) than that of lambs offered diet B. The rumen pH was similar ($P>0.05$) across all four treatment although higher ($P<0.05$) in treatment B than treatment SB.

Table 3. 4. Effect of dietary treatments on the carcass characteristics of growing lambs.

	G	**B**	**DG**	**SB**	**s.e.d.**	**P-value**
Hot carcass weight (kg)	16.5^{b}	20.7^{a}	19.5^{a}	20.8^{a}	0.548	<0.001
Cold carcass weight (kg)	16.4^{b}	20.4^{a}	19.3^{a}	20.4^{a}	0.524	<0.001
Dressing %	43.6^{b}	48.0^{a}	46.8^{a}	48.7^{a}	1.051	<0.001
pH, 45 min	6.46^{b}	6.62^{ab}	6.69^{a}	6.66^{a}	0.069	0.012
pH, 24 h	5.77^{a}	5.55^{b}	5.71^{ab}	5.70^{ab}	0.068	0.024
Conformation score	2.42^{b}	3.20^{a}	3.00^{ab}	3.20^{a}	0.239	0.01
Fat score	2.41^{b}	3.30^{a}	3.40^{a}	3.40^{a}	0.310	0.009
Fat thickness (cm)	0.23^{b}	0.32^{a}	0.29^{a}	0.34^{a}	0.017	<0.001
Eye muscle depth (cm)	2.21^{b}	2.94^{a}	2.94^{a}	2.95^{a}	0.102	<0.001
pH, rumen fluid	6.31^{ab}	6.43^{a}	6.18^{ab}	6.00^{b}	0.150	0.041

G: Grass, B: Barley, DG: Dried grass, SB: Sugar beet, $^{a,\ b,}$ Means in a row with the same superscript are not different ($P>0.05$).

3.3.5. Carcass measurements

The effect of the experimental diets on carcass measurements is presented in Table 3.5. Carcass length, chest circumference and chest depth were not different between treatments ($P>0.05$). In contrast, buttock circumference, barrel width and gigot depth were significantly lower ($P<0.05$) for lambs offered diet G compared to those offered diets B, DG and SB. The carcass measurements of lambs offered diets B, DG and SB were not different ($P>0.05$).

Table 3. 5. Effect of dietary treatments on carcass measurements of growing lambs.

	G	**B**	**DG**	**SB**	**s.e.d.**	**P-value**
Carcass length (cm)	56.9	58.3	59.7	59.1	1.512	0.292
Chest circumference (cm)	73.0	74.2	73.5	73.7	0.803	0.502
Buttock circumference (cm)	62.5^{b}	65.0^{a}	64.6^{a}	66.1^{a}	0.703	<0.001
Barrel width (cm)	24.0^{b}	25.9^{a}	25.4^{ab}	26.5^{a}	0.659	0.007
Chest depth (cm)	24.9	24.6	24.2	24.1	0.414	0.227
Gigot depth (cm)	14.4^{b}	15.5^{a}	15.1^{ab}	14.9^{ab}	0.307	0.019

G: Grass, B: Barley, DG: Dried grass, SB: Sugar beet, $^{a,\ b,}$ Means in a row with the same superscript are not different ($P>0.05$).

3.3.6. Blood metabolites

3.3.6.1. Total protein

Repeated measures analysis showed no effect (P>0.05) of time, treatment and time x treatment interaction on blood plasma total protein concentration of lambs fed diets G, B, DG or SB diets, with mean values of 52.0, 54.7, 51.3 and 53.4 mg/ml respectively (Figure 3.3).

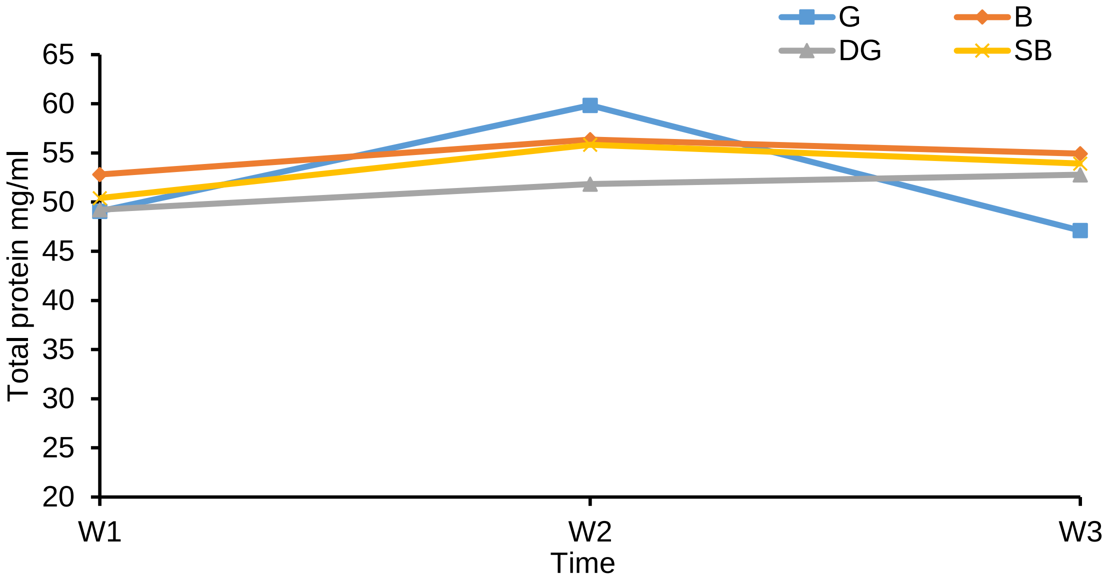


Figure 3. 3. Effect of dietary treatments (G: Grass, B: Barley, DG: Dried grass, and SB: Sugar beet) on blood plasma total protein (mg/ml) concentration of growing lamb throughout the experiment (W1: beginning, W2: middle, W3: end of experiment). (s.e.d values: Treatment=3.797, Time=2.44, Inter=5.504, P-values: Treatment=0.813, Time=0.077, Inter=0.563).

3.3.6.2. Albumin

Repeated measures analysis showed an effect of time (P<0.05) with the mean blood plasma albumin concentration decreasing from 27.3 mg/ml at point W1 to 25.0 mg/ml at point W3 (Figure 3.4). There was no effect (P>0.05) of dietary treatment on blood plasma albumin concentration. There was a time x treatment interaction (P<0.05) in the plasma albumin concentration of lambs offered diet G declining at a faster rate from point W2 to W3 than that of lambs offered diets B, DG or SB.

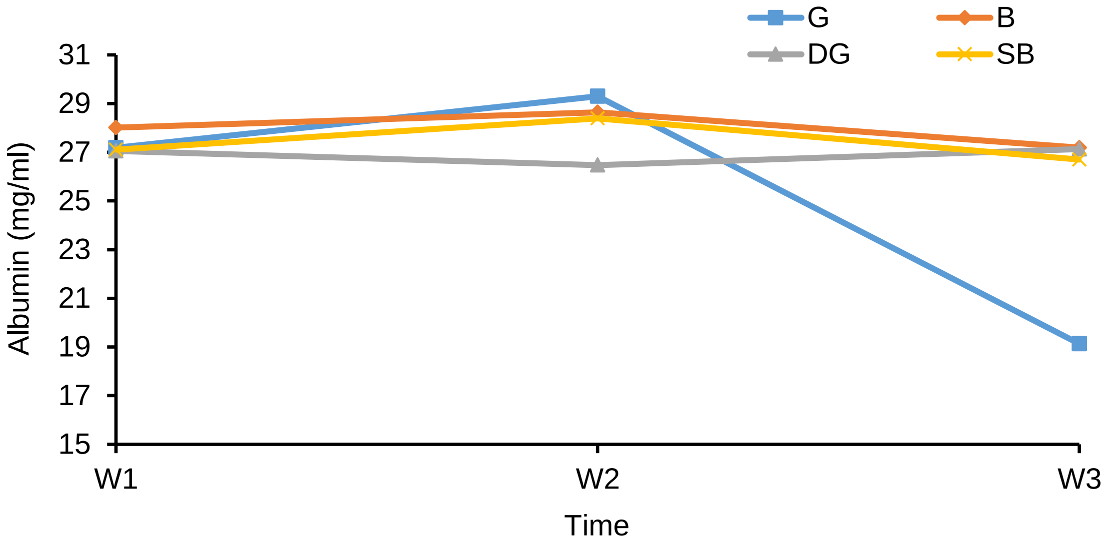


Figure 3. 4. Effect of dietary treatments (G: Grass, B: Barley, DG: Dried grass, and SB: Sugar beet) on blood plasma albumin (mg/ml) concentration throughout the experiment (W1: beginning, W2: middle W3: end of experiment). (s.e.d values: Treatment=1.455, Time=0.941, Inter= 2.117, P-values: Treatment=0.287, Time=0.005, Inter=0.004).

3.3.6.3. Urea

Repeated measure analysis showed an effect (P<0.001) of time on mean urea concentration increasing from 5.45 mmol/l at point W1 to 7.54 mmol/l (Figure 3.5). There was also effect (P<0.05) of dietary treatment on blood plasma urea concentration, with lambs offered G diet having a lower urea concentration than those offered B, DG or SB (4.87 vs 7.31, 7.41 and 7.47 mmol/l, respectively). Similarly, there was a time x treatment interaction (P<0.001) on urea concentration, in contrast to lambs offered diet G, the urea concentration of lambs offered diets B, DG and SB increased throughout the experiment.

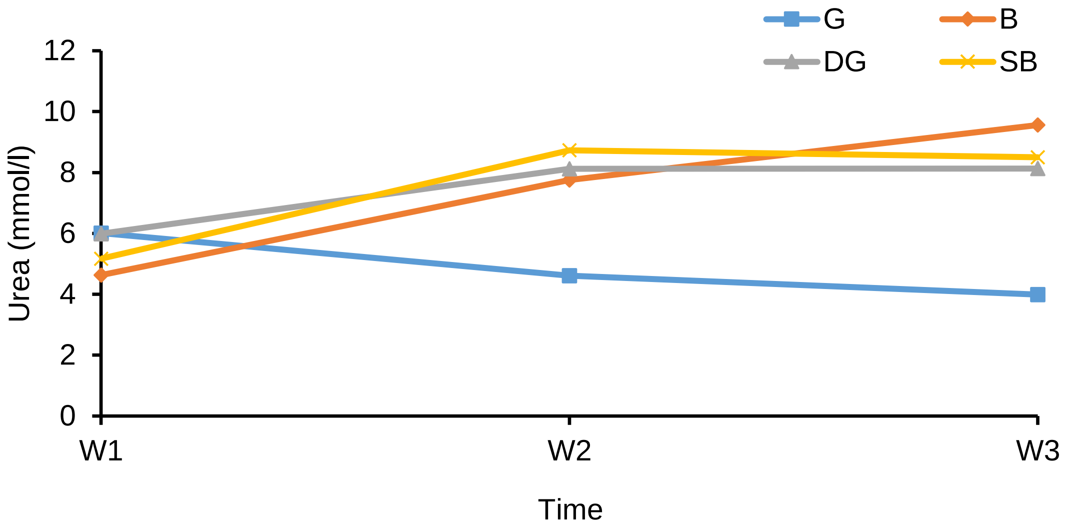


Figure 3. 5. Effect of dietary treatments (G: Grass, B: Barley, DG: Dried grass, and SB: Sugar beet) on blood plasma urea (mmol/l) concentration throughout the experiment (W1: beginning, W2: middle W3: end of experiment). (s.e.d values: Treatment=0.501, Time=0.294, Inter=0.693, P-values: Treatment <0.001, Time <0.001, Inter <0.001).

3.3.6.4. Glucose

Repeated measures analysis showed an effect of time (P<0.001) with mean blood plasma glucose concentration increasing from point W1 to W3 (2.41 vs 2.85 mmol/l) (Figure 3.6). Similarly, there was also an effect of treatment (P<0.05) on blood plasma glucose concentration, with lambs offered diet B having a higher mean blood glucose concentration than those offered diets G, DG and SB (2.77 vs 2.04, 2.03 and 2.04 mmol/l, respectively). Similarly, there was an effect (P<0.05) of time x treatment interaction on glucose concentration with the plasma glucose concentration of lambs offered diet B increasing steadily throughout the experiment, whereas concentration in lambs offered diets DG and SB decreased between points W1 and W2 and then increased to point W3. The plasma glucose concentration of lambs offered diet G decreased throughout the experiment from 2.59 mmol/l at point W1 to 2.2 mmol/l at point W3.

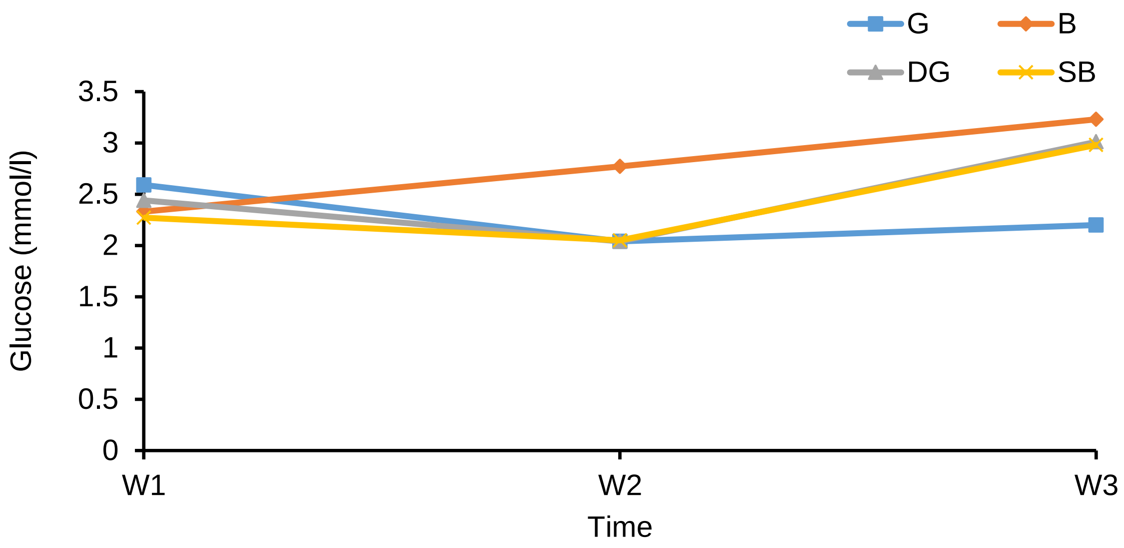


Figure 3. 6. Effect of dietary treatments (G: Grass, B: Barley, DG: Dried grass, and SB: Sugar beet) on blood plasma glucose (mmol/l) concentration throughout the experiment (W1: beginning, W2: middle W3: end of experiment). (s.e.d values: Treatment=0.155, Time=0.137, Inter=0.272, P-values: Treatment=0.024, Time <0.001, Inter=0.012).

3.3.6.5. Non-Esterified Fatty Acids (NEFA)

Repeated measure analysis showed an effect of time (P<0.001) with the mean blood plasma NEFA concentration decreasing from 0.45 mmol/l at point W1 to 0.09 mmol/l at point W3 (Figure 3.7). There was no effect (P>0.05), of dietary treatment on blood plasma NEFA concentration. There was a time x treatment interaction (P<0.05), with lambs offered diet G having a higher value than those offered diets B, DG or SB at point W2. In contrast to lambs offered diet G the plasma NEFA concentration of lambs offered dies B, DG and SB decreased from point W1 to W2 then remained constant until the end of the experiment.

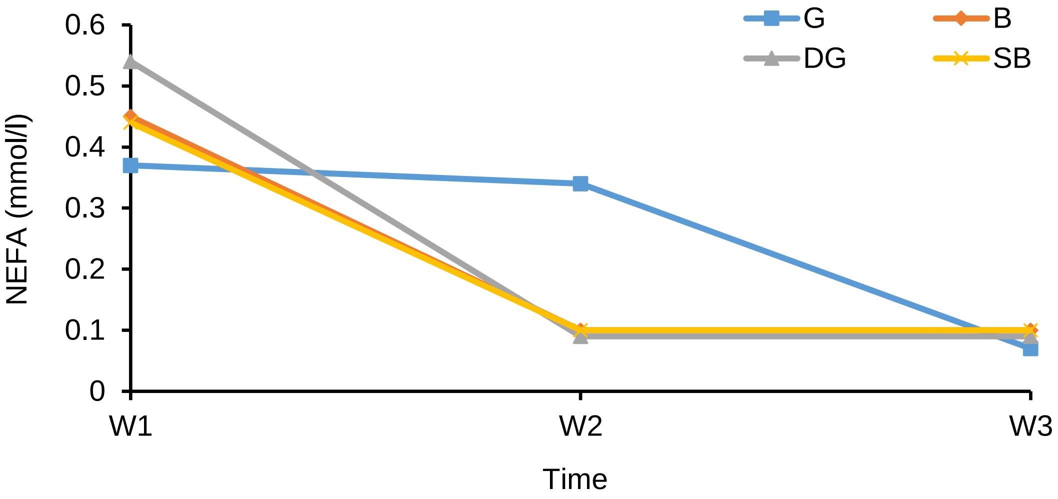


Figure 3. 7. Effect of dietary treatments (G: Grass, B: Barley, DG: Dried grass, and SB: Sugar beet) on blood plasma NEFA (mmol/l) concentration throughout the experiment (W1: beginning, W2: middle W3: end of experiment). (s.e.d values: Treatment=0.042, Time=0.033, Inter=0.068, P-values: Treatment=0.656, Time <0.001, Inter=0.003).

3.3.6.6. β-hydroxybutyrate (BHB)

Repeated measure analysis showed an effect (P<0.001) of time on BHB concentration increasing from 0.29 mmol/l at point W1 to 0.53 mmol/l at point W3 (Figure 3.8). There was also an effect of dietary treatment on blood plasma BHB concentration, with lambs offered diet SB having a higher mean BHB concentration than those offered diets G, B or DG (0.62 vs 0.35, 0.37 and 0.52 mmol/l, respectively). Similarly, there was an effect (P<0.001) time x treatment interaction on BHB concentration on the BHB concentration in all treatments increasing from point W1 to W2, where diet SB had the highest value compared to DG, B or G (0.82 vs 0.64, 0.43 and 0.41 mmol/l, respectively).

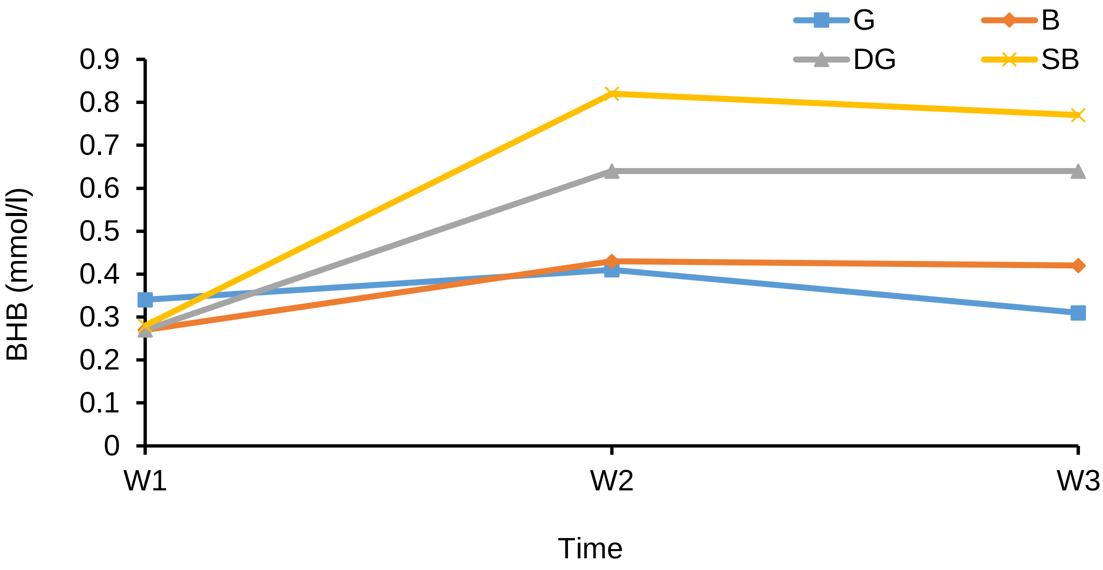


Figure 3. 8. Effect of dietary treatments (G: Grass, B: Barley, DG: Dried grass, and SB: Sugar beet) on blood plasma BHB (mmol/l) concentration throughout the experiment (W1: beginning, W2: middle W3: end of experiment). (s.e.d values: Treatment=0.064, Time=0.042, Inter=0.093, P-values: Treatment <0.001, Time <0.001, Inter <0.001).

3.3.7. Rumen volatile fatty acids (VFA)

The total molar concentration and molar proportion of individual volatile fatty acids are presented in Table 3.6. There was a difference (P<0.001) in rumen fluid of total volatile fatty acid (TVFA) from lambs fed different diets. Lambs fed diet B had a lower (P<0.001) proportion of acetate and higher (P<0.001) proportion of propionate compared to lambs offered diets G, DG and SB. Lambs fed diets DG and SB had a relatively similar proportion of butyrate compared to lambs fed diet G, but not diet B. The proportion of branched chain VFA (isobutyrate, valerate, isovalerate and caproate) increased (P<0.001) in rumen fluid from lambs fed diet B compared to those fed G, DG and SB. The acetic: propionic ratio was different (P<0.001) between treatments, with the lowest (P<0.001) ratio for diet B (1.62), followed by SB (2.64), G (3.20) and DG (3.59).

Table 3. 6. Effect of dietary treatments on total molar concentration and individual volatile fatty acid (molar proportion %) of growing lambs.

	G	**B**	**DG**	**SB**	**s.e.d**	**P-value**
TVFA[1] mmol/l	133^{a}	84.2^{b}	129^{a}	130^{a}	7.600	<0.001
Acetate	63.9^{a}	48.9^{c}	63.2^{a}	59.0^{b}	0.950	<0.001
Propionate	20.4^{bc}	33.9^{a}	18.9^{c}	23.5^{b}	1.372	<0.001
Butyrate	12.1^{bc}	10.3^{c}	14.9^{a}	13.3^{ab}	0.960	<0.001
Isobutyrate	1.23^{b}	1.90^{a}	1.27^{b}	0.89^{b}	0.159	<0.001
Valerate	0.93^{c}	3.00^{a}	1.35^{bc}	1.93^{b}	0.224	<0.001
Isovalerate	1.31^{b}	2.41^{a}	1.49^{b}	1.30^{b}	0.222	<0.001
Caproate	0.45^{b}	0.93^{a}	0.11^{c}	0.57^{b}	0.102	<0.001
BCVFA[2]	3.74^{b}	8.89^{a}	3.78^{b}	4.13^{b}	0.292	<.001
A: P ratio[3]	3.20^{b}	1.62^{d}	3.59^{a}	2.64^{c}	0.144	<0.001

G: Grass, B: Barley, DG: Dried grass, SB: Sugar beet, [1]total volatile fatty acid, [2]Branched chain VFA, [3]Acetic: Propionic ratio, $^{a,\ b,\ c,\ d}$ Means in a row with the same superscript are not different (P>0.05).

3.3.8. Proximate and fatty acid composition of muscle

The results for muscle proximate analysis (g/kg muscle), FA composition of *longissimus dorsi* muscle expressed as (% of total FAs) and total FA as (g/kg muscle) are presented in

Table 3.7. The moisture, CP, ash and total fat of LD muscle were unaffected ($P>0.05$) by the dietary treatments. Muscle from lambs fed diet G had a higher ($P<0.05$) percentage of C14:0 compared to those fed diet SB, but not diets B and DG. In contrast, muscle from lambs fed diet G had a lower ($P<0.001$) percentage of C16:0 compared to those fed diets B and SB, but not to DG. Muscle from lambs fed diet B had the highest ($P<0.001$) percentage of palmitoleic acid (C16:1*n*-9) compared to that of lambs fed diets G, DG and SB. Muscle from lambs fed on concentrate diets (B, DG and SB) had the lowest ($P<0.001$) content of C18:0 compared to muscle from lambs fed G.

The percentage of C18:1*n*-9c and C18:2*n*-6c was highest in lambs fed diet B compared to G, but not to DG and SB. The percentage of C18:1*n*-9t was higher ($P<0.001$) in muscle from lambs fed diets B and SB compared to those fed diets G and DG. The percentage of C18:1*n*-t11 in the muscle of lambs fed diet B was higher ($P<0.001$) compared to those fed diets G, DG or SB. The percentage of α-linolenic acid (C18:3*n*-3) increased ($P<0.001$) four fold in the muscle of lambs fed diet DG (2.19%) and SB (2.14%) (inclusion of linseed oil) followed by G (0.88%) and B (0.50%). In contrast, lambs fed diet SB produced meat with the lowest ($P<0.001$) percentage of C20:4*n*-6, whereas, diets B and DG had a similar ($P>0.001$) percentage of this FA compared to G. The percentage of long chain PUFA EPA in the muscle of lambs fed diets B was lower ($P<0.05$) compared to lambs fed diet DG. There was no effect ($P>0.05$) of dietary treatments on the percentage of DHA. The muscle from lambs fed diets G or DG had a higher ($P<0.05$) percentage content of cis-9, trans-11 (CLA) than those fed diet B. The percentage of total SFA and PUFA was not different ($P>0.05$) in muscle from lambs fed on the different diets. In contrast, the total MUFA percentage of muscle in lambs fed diet B was higher ($P<0.05$) compared lambs fed diets G, DG or SB.

The total FA content and FA composition of the *longissimus thorasic* muscle expressed as mg/100g muscle are presented in Table 3.8. Lambs fed the concentrate diets (B, DG or SB) and G diet had a similar ($P>0.05$) content of C14:0, C18:0, C22:6*n*-3 content. However, the C16:1*n*-9, C18:1*n*-9c, C18:1*n*-9t and C18:2*n*-6c were higher ($P<0.001$) in the muscle of lambs fed concentrate diets, except lambs on DG which had a similar ($P>0.05$) content to lambs offered diet G. There was an effect ($P<0.001$) of dietary treatment on the muscle content of C18:1*n*-t11 with the lambs fed diet B had the highest and G had the lowest content. The muscle from lambs fed diets DG and SB had a higher ($P<0.001$) C18:3*n*-3, C20:5*n*-3 content than that of lambs fed on diets B or G, that had a higher ($P<0.001$) C20:4*n*-6 content. There was a trend ($P=0.059$) in the muscle content of cis-9, trans-11 (CLA) from lambs fed diet G or DG to have a higher content than those fed diet B or SB.

Table 3. 7. Effect of dietary treatment on proximate (g/kg muscle) and fatty acid composition (% of total fatty acids) in longissimus dorsi muscle of lamb.

Proximate composition (g/kg DM)	**G**	**B**	**DG**	**SB**	**s.e.d**	**P- value**
Moisture	754	747	744	746	5.263	0.459
CP[1]	212	215	215	217	4.411	0.663
Ash	14.3	18.1	15.7	16.1	1.654	0.168
Total FA	21.7	24.3	24.5	25.8	1.716	0.119
Fatty acid % total fatty acid						
C14:0	3.21^{a}	2.86^{ab}	3.01^{ab}	2.54^{b}	0.20	0.014
C16:0	20.4^{b}	22.1^{a}	21.5^{ab}	22.4^{a}	0.50	0.001
C16:1*n*-9	1.14^{b}	1.55^{a}	1.13^{b}	1.19^{b}	0.05	<0.001
C18:0	16.7^{a}	13.1^{c}	14.7^{b}	13.5^{c}	0.40	<0.001
C18:1*n*-9c	30.7^{b}	33.8^{a}	31.7^{ab}	31.8^{ab}	0.82	0.004
C18:1*n*-9t	0.52^{b}	1.61^{a}	0.56^{b}	1.47^{a}	0.29	<0.001
C18:1*n*-t11	1.48^{b}	1.81^{a}	1.45^{b}	1.53^{b}	0.063	<0.001
C18: 2*n*-6c	5.49^{b}	6.62^{a}	5.97^{ab}	5.71^{ab}	0.372	0.021
C18:3*n*-3	0.88^{b}	0.50^{c}	2.19^{a}	2.14^{a}	0.121	<0.001
C20:4*n*-6	3.04^{a}	2.74^{ab}	2.20^{ab}	1.92^{c}	0.242	<0.001
C20:5*n*-3 (EPA)[2]	0.51^{ab}	0.42^{b}	0.59^{a}	0.51^{ab}	0.060	0.041
C22:6*n*-3 (DHA)[3]	0.54	0.63	0.55	0.71	0.143	0.595
cis-9, trans-11 (CLA)[4]	0.72^{a}	0.48^{b}	0.71^{a}	0.55^{ab}	0.080	0.006
Σ SFA	40.2	38.6	39.1	38.5	0.841	0.169
Σ MUFA	33.8^{b}	38.7^{a}	34.9^{b}	36.0^{b}	0.889	<0.001
Σ PUFA	11.2	11.4	12.2	11.6	0.682	0.463
RFA[5]	14.8^{a}	11.4^{b}	13.8^{ab}	13.9^{ab}	1.047	0.011
ITFA[6]	85.2^{b}	88.7^{a}	86.2^{ab}	86.1^{ab}	1.047	0.011

G: Grass, B: Barley, DG: Dried grass, SB: Sugar beet, [1]Crude protein, [2]Eicosapentaenoic acid, [3]Docosahexaenoic acid, [4]Conjugated linoleic acid, [5]Remaining fatty acid, [6]Identified total fatty acid, $^{a, b, c}$ Means in a row with the same superscript are not different (P>0.05).

Table 3. 8. Effect of dietary treatment on the fatty acid composition (mg/100g) of longissimus dorsi muscle of lamb.

Fatty acids	**G**	**B**	**DG**	**SB**	**s.e.d**	**P-value**
C14:0	71.0	72.0	75.0	67.0	8.000	0.762
C16:0	449^{b}	552^{ab}	531^{ab}	585^{a}	45.700	0.027
C16:1*n*-9	25.2^{c}	39.4^{a}	28.0^{bc}	33.3^{ab}	3.120	<0.001
C18:0	363.0	318.0	359.0	353.0	27.400	0.348
C18:1*n*-9c	667^{b}	830^{a}	778^{a}	831^{a}	62.910	0.042
C18:1*n*-9t	9.9^{c}	36.7^{a}	15.3^{bc}	34.0^{ab}	7.190	<0.001
C18:1*n*-t11	31.3^{c}	42.1^{a}	35.0^{bc}	38.6^{ab}	2.212	<0.001
C18:2*n*-6c	115.3^{b}	151.5^{a}	141.6^{a}	139.5^{a}	6.511	<0.001
C18:3*n*-3	18.5^{b}	11.8^{b}	52.6^{a}	52.2^{a}	2.741	<0.001
C20:4*n*-6	61.0^{a}	60.5^{a}	51.7^{b}	47.2^{b}	2.539	<0.001
C20:5*n*-3 (EPA)^{1}	10.2^{ab}	9.4^{b}	14.2^{a}	13.5^{a}	1.570	0.006
C22:6*n*-3 (DHA)^{2}	13.2	15.3	14.1	18.5	3.927	0.561
cis-9, trans-11 (CLA)^{3}	16.0	11.7	17.9	13.8	2.331	0.059
RFA^{4}	318	280	338	355	34.00	0.162
ITFA^{5} (mg/100g)	2169	2430	2451	2581	171.6	0.119

G: Grass, B: Barley, DG: Dried grass, SB: Sugar beet, ^{1}Eicosapentaenoic acid, ^{2}Docosahexaenoic acid, ^{3}Conjugated linoleic acid, ^{4}Remaining fatty acid, ^{5}Identified total fatty acid, $^{a,\ b,\ c}$ Means in a row with the same superscript are not different (P>0.05)

3.3.9. Fatty acid composition of adipose tissue

The total FA content (g/kg tissue) and FA composition (% total FA) of the subcutaneous adipose tissue are presented in Table 3.9. There was no effect of (P>0.05) of treatments on the total FA content of adipose tissue. Adipose tissue from the lambs fed diet B had a lower (P<0.001) content of C14:0 and a higher content of C16:0, C16:1*n*-9, C18:1*n*-9c, C18:1*n*-t11, C18: 2*n*-6c and cis-9, trans-11 (CLA) than those fed diet G, DG or SB. The majority of the FA (C16:0, C16:1*n*-9, C18:1*n*-9c, C18: 2*n*-6c and C18:3*n*-3) in the adipose tissue of lambs fed on diets DG and SB was similar ($P>0.05$) to those raised on grass. However, the saturated FAs (C16:0 and C18:0) was lower ($P<0.001$) in lambs fed diets DG or SB. The percentage of total SFA was lowest ($P<0.05$) in lambs fed SB (39.9%), followed by B (42.8%), DG (44.9%) and G (50.0%). Total MUFA percentage was higher ($P<0.001$) in lambs fed diets B or SB; similarly, total PUFA was higher ($P<0.001$) in lambs fed diets DG or SB.

Table 3. 9. Effect of dietary treatment on the fatty acid composition (% of total fatty acids and fat content g/kg) in the subcutaneous adipose tissue of lamb.

Fatty acids	**G**	**B**	**DG**	**SB**	**s.e.d**	**P-value**
C14:0	5.30^{a}	3.23^{bc}	3.88^{b}	3.06^{c}	0.291	<0.001
C16:0	21.4^{b}	22.7^{a}	20.8^{b}	21.1^{b}	0.381	<0.001
C16:1*n*-9	1.03^{b}	1.28^{a}	0.91^{b}	0.94^{b}	0.057	<0.001
C18:0	23.3^{a}	16.9^{c}	20.2^{b}	15.8^{c}	0.900	<0.001
C18:1*n*-9c	26.7^{b}	31.8^{a}	27.3^{b}	27.3^{b}	1.022	<0.001
C18:1*n*-9t	5.35^{b}	6.33^{b}	6.53^{b}	8.74^{a}	0.470	0.001
C18:1*n*-t11	1.19^{b}	1.48^{a}	1.17^{b}	1.24^{b}	0.058	<0.001
C18:2*n*-6c	1.61^{c}	3.14^{a}	2.45^{b}	2.51^{b}	0.146	<0.001
C18:2*n*-6t	0.51^{b}	0.25^{b}	0.83^{b}	3.24^{a}	0.236	<0.001
C18:3*n*-3	1.10^{ab}	0.63^{c}	1.27^{a}	0.89^{bc}	0.097	<0.001
cis-9, trans-11 (CLA)[1]	0.09^{b}	0.13^{a}	0.08^{b}	0.09^{b}	0.005	<0.001
Σ SFA	50.0^{a}	42.8^{b}	44.9^{b}	39.9^{c}	0.848	<0.001
Σ MUFA	34.3^{c}	40.9^{a}	35.9^{c}	38.2^{b}	0.796	<0.001
Σ PUFA	3.32^{c}	4.15^{bc}	4.64^{b}	6.73^{a}	0.341	<0.001
RFA[2]	12.4^{b}	12.1^{b}	14.6^{a}	15.2^{a}	0.377	<0.001
ITFA[3] (g/kg tissue)	676	751	673	728	36.7	0.093

G: Grass, B: Barley, DG: Dried grass, SB: Sugar beet, [1]Conjugated linoleic acid, [2]Remaining fatty acid, [3]Identified total fatty acid, $^{a,\ b,\ c}$ Means in a row with the same superscript are not different ($P>0.05$).

3.3.10. Nutritional indices

The important nutritional indices related to human health are presented in Table 3.10. There were no effects (P>0.05) of the dietary treatments on the SFA, and P:S ratio. The MUFA and PUFA of muscle from lambs fed on the concentrate diets (B, DG and SB) were higher (P<0.05) than that of the muscle from lambs fed diet G. Diet B increased (P<0.05) the *n*-6:*n*-3 and C18:2*n*-6: C18:3*n*-3 ratio compared to diets G, DG and SB. Diets DG and SB reduced (P<0.05) the *n*-6:*n*-3 ratio from 6.68 to 2.53 and 2.30 in diets B, DG and SB, respectively, and of C18:2*n*-6:C18:3*n*-3 from 18.9 to 2.76 and 2.79 in diets B, DG or SB, respectively.

Table 3. 10. Fatty acid classes (mg/100g) and ratios related to human health of longissimus dorsi muscle of lambs offered the experimental diets.

	G	**B**	**DG**	**SB**	**s.e.d**	**P- value**
SFA	882	942	965	1005	77.70	0.459
MUFA	736^{b}	947^{a}	857^{ab}	937^{a}	69.90	0.013
PUFA	234^{c}	261^{bc}	292^{a}	285^{ab}	11.66	<0.001
P:S^{1}	0.28	0.30	0.31	0.31	0.021	0.422
n-6:*n*-3^{2}	4.77^{b}	6.68^{a}	2.53^{c}	2.30^{c}	0.834	<0.001
C18:2*n*-6: C18:3*n*-3	6.13^{b}	18.9^{a}	2.76^{b}	2.79^{b}	2.581	<0.001

G: Grass, B: Barley, DG: Dried grass, SB: Sugar beet, ^{1}P:S is= $\frac{\mathrm{PUFA}}{\mathrm{SFA}}$, ^{2}*n*-6:*n*-3 is = $\frac{\mathrm{18:2n-6+20:4n-6}}{\mathrm{18:3n-3+20:5n-3+22:6n-3}}$

$^{a,\, b,\, c}$ Means in a row with the same superscript are not different (P>0.05).

3.3.11. Vitamin E concentration of muscle

Different levels of dietary vitamin E had an effect ($P<0.05$) on the concentration of vitamin E in SM muscle (Figure 3.9). Lambs fed diets DG or SB had a similar ($P >0.05$) vitamin E content in their SM muscle. Lambs fed diet B had a lower ($P<0.05$) vitamin E content compared to diet G, but not to diets DG and SB, with values of 1.88, 2.61, 2.38 and 2.36 mg/kg muscle respectively.

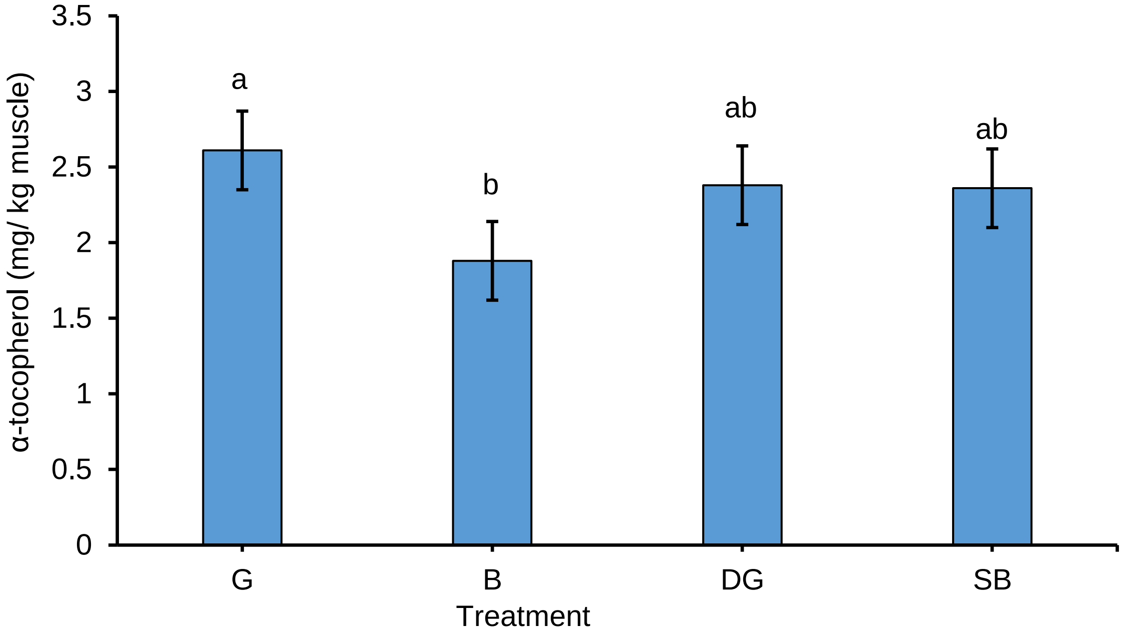


Figure 3. 9. Mean of α-tocopherol concentration in SM from lambs fed (G) grass, (B) barley, (DG), dried grass and (SB) sugar beet (SB). (s.e.d=0.26, P-value=0.046).). $^{a, b}$ Means of dietary treatment with the same superscript are not different ($P>0.05$).

3.3.12. Colour evaluation

3.3.12.1. Lightness

Repeated measure analysis showed an effect (P<0.001) of time on lightness mean values of SM muscle, which increased over the period of the retail display from 43.6 at day one to 44.5 at day 7 (Figure 3.10). There was no effect (P>0.05) of dietary treatments on lightness value, although, there was a trend for the SM muscle of lambs fed diet DG to have a lower value (42.9) (darker meat) compared to that of lambs fed diets G, B or SB with mean values of 44.0, 44.2 and 44.2, respectively. There was no time x treatment interaction (P>0.05) on lightness values.

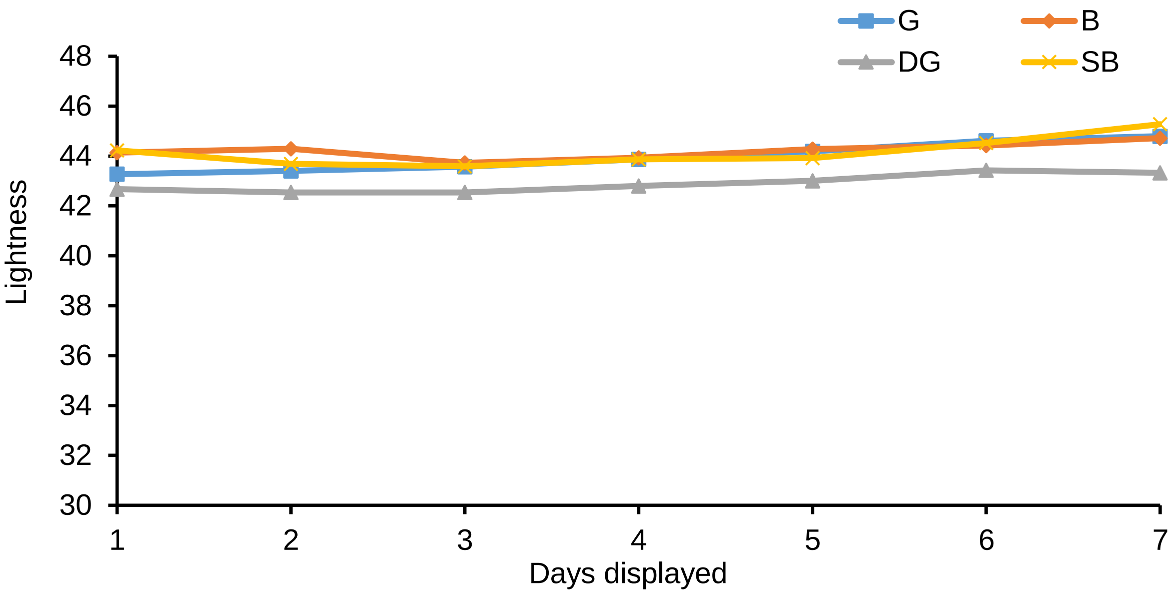


Figure 3. 10. Effect of time displayed on lightness of MAP SM muscle from lambs fed different diets (G: Grass, B: Barley, DG: Dried grass, and SB: Sugar beet). (s.e.d values: Treatment=0.554, Time=0.197, Inter=0.664, P-values: Treatment=0.072, Time <0.001, Inter=0.644).

3.3.12.2. Redness

Repeated measure analysis showed an effect (P<0.001) of time on redness mean values of SM muscle which decreased over the period of the retail display from 19.2 at day one to 15.1 at day seven (Figure 3.11). Similarly, there was an effect (P<0.05) of dietary treatments on redness values with the highest value for lambs fed diet G, followed by lambs fed diets B, DG and SB, with mean values of 17.71, 17.6, 17.2 and 16.9, respectively. There was no time x treatment interaction (P>0.05) on redness value.

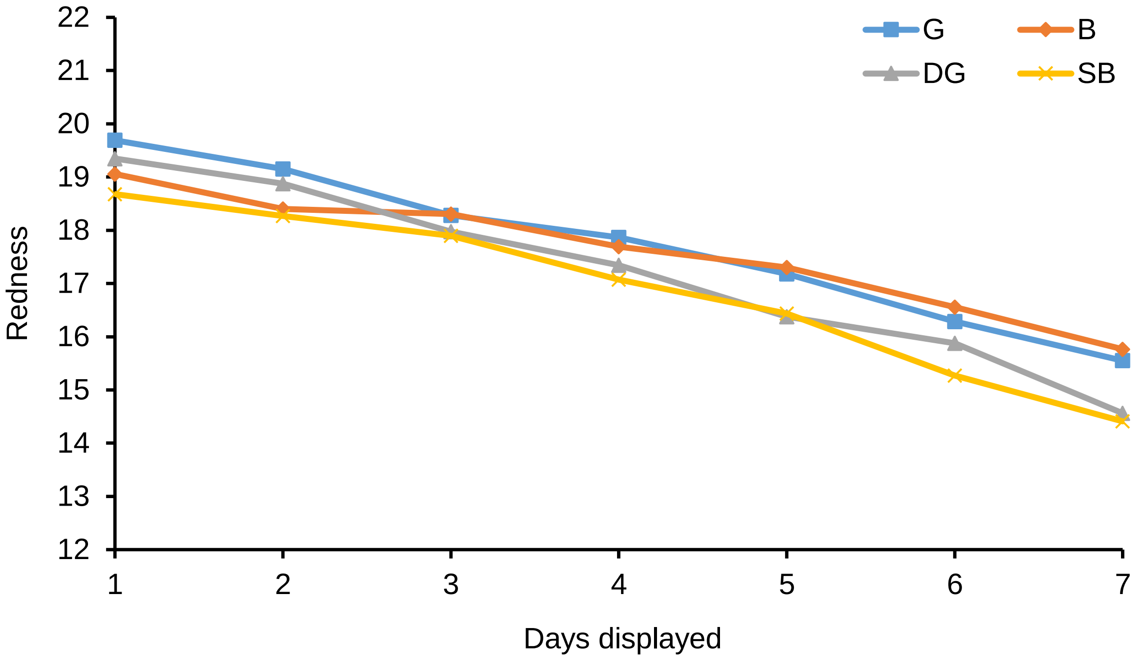


Figure 3. 11. Effect of time displayed on redness of MAP SM muscle from lambs fed different diets (G: Grass, B: Barley, DG: Dried grass, and SB: Sugar beet). (s.e.d values: Treatment=0.297, Time=0.193, Inter=0.465, P-values: Treatment=0.023, Time <0.001, Inter=0.366).

3.3.12.3. Yellowness

Repeated measure analysis showed an effect (P<0.001) of time on yellowness mean values of SM muscle which decreased over the period of the retail display from 10.5 at day one to 9.7 at day seven (Figure 3.12). Similarly, there was an effect (P<0.001) of treatment on yellowness values, where muscle from lambs fed diet G had a higher value compared to that of lambs fed diets B, DG or SB, with mean values of 10.6 vs 9.9, 10.1 and 9.8, respectively. There was no time x treatment interaction (P>0.05) on the yellowness value.

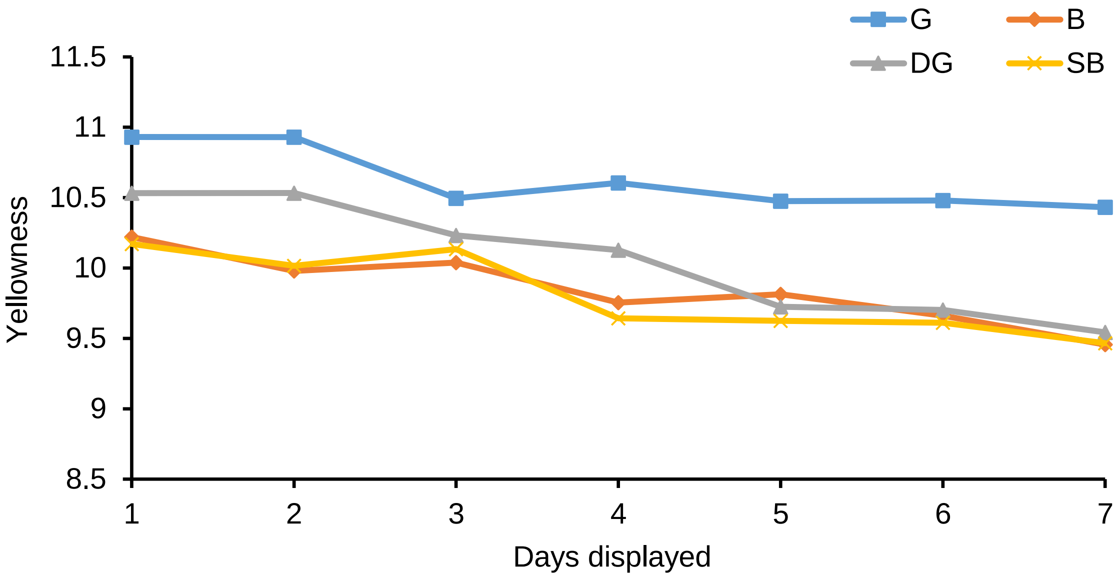


Figure 3. 12. Effect of time displayed on yellowness of MAP SM muscle from lambs fed different diets (G: Grass, B: Barley, DG: Dried grass, and SB: Sugar beet). (s.e.d values: Treatment=0.166, Time=0.091, Inter=0.237, P-values: Treatment <0.001, Time <0.001, Inter=0.311).

3.3.12.4. Saturation

Repeated measure analysis showed an effect (P<0.001) of time on saturation mean values of SM muscle which decreased over the period of the retail display from 21.9 at day one to 18.0 at day seven (Figure 3.13). There was an effect (P<0.05) of dietary treatment on saturation values during retail display with muscle from lambs fed diets G and B had higher saturation values compared to that of those fed diets DG or SB with mean values of 20.7 vs 20.2, 19.9 and 19.6, respectively. There was no time x treatment interaction (P>0.05) on the saturation value.

Shelf life in days for SM muscle from lambs fed different diets was determined using a saturation value of 18.0. Therefore, the SM muscle from lambs fed diets G and B had greater shelf life than the SM muscle from lambs fed diets DG and SB.

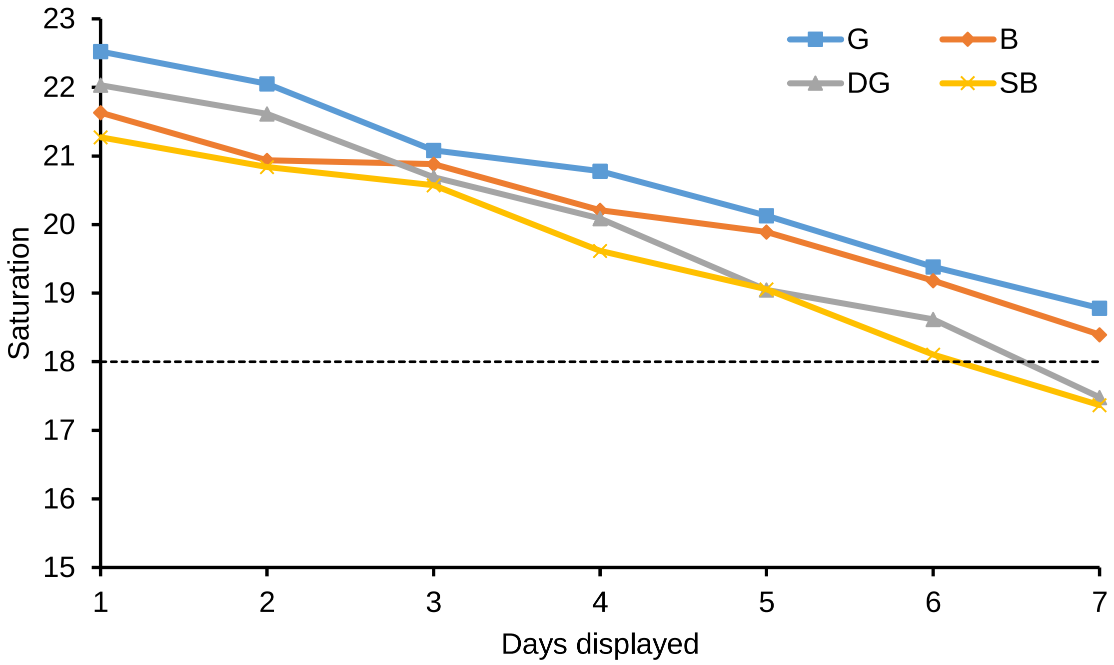


Figure 3. 13. Effect of time displayed on saturation of MAP SM muscle from lambs fed different diets (G: Grass, B: Barley, DG: Dried grass, and SB: Sugar beet). (s.e.d values: Treatment=0.310, Time=0.182, Inter=0.459, P-values: Treatment=0.006, Time <0.001, Inter=0.292).

3.3.12.5. Hue value

Repeated measure analysis showed an effect (P<0.001) of time on hue mean values of SM muscle which increased over the period of the retail display from 28.6 at day one to 33.3 at day seven (Figure 3.14). Similarly, There was an effect (P<0.05) of dietary treatments on hue unit values with muscle from lambs on diet G having a higher hue value compared to that of those fed diet B, with mean values of 31.1 and 29.3, respectively, With lambs fed diets DG or SB having intermediate values of 30.5 and 30.5, respectively. There was no time x treatment interaction (P>0.05) on the hue values.

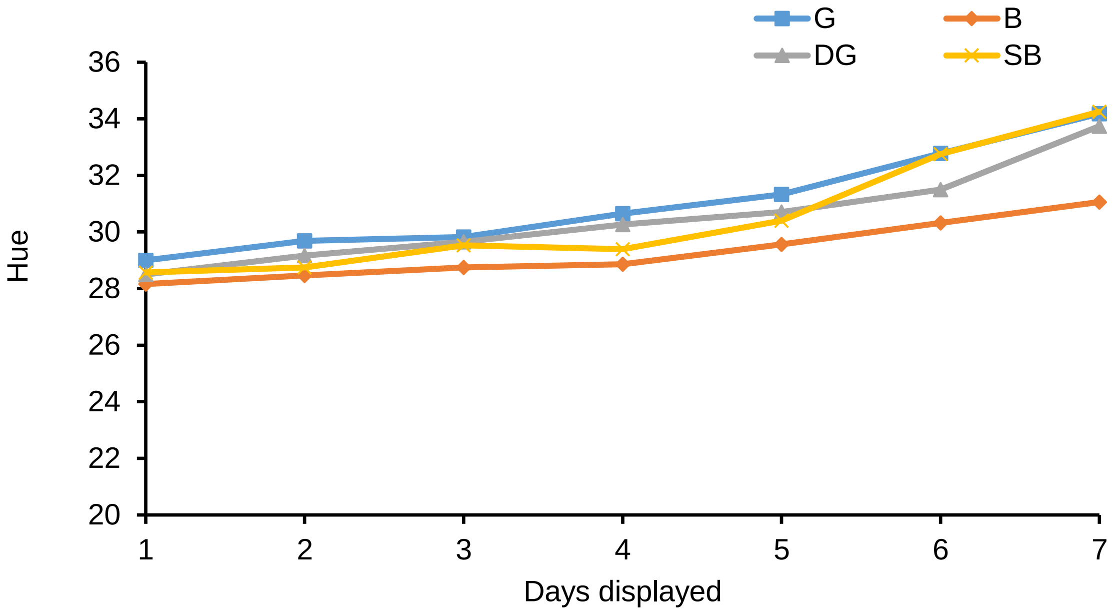


Figure 3. 14. Effect of time displayed on hue value of MAP SM muscle from lambs fed different diets (G: Grass, B: Barley, DG: Dried grass, and SB: Sugar beet). (s.e.d values: Treatment=0.465, Time=0.399, Inter=0.873, P-values: Treatment=0.003, Time <0.001, Inter=0.495).

3.3.13. Lipid oxidation of muscle (TBARS)

Results of SM muscle lipid oxidation are shown in Figure 3.15. At day seven of retail display, there was no effect (P>0.05) of treatment on the lipid oxidation. The SM muscles from lambs grazed diet G had the highest value of TBARS (4.30 mg MDA/kg muscle), whereas, lambs fed diet SB had the lowest value (3.47 mg MDA/kg muscle).

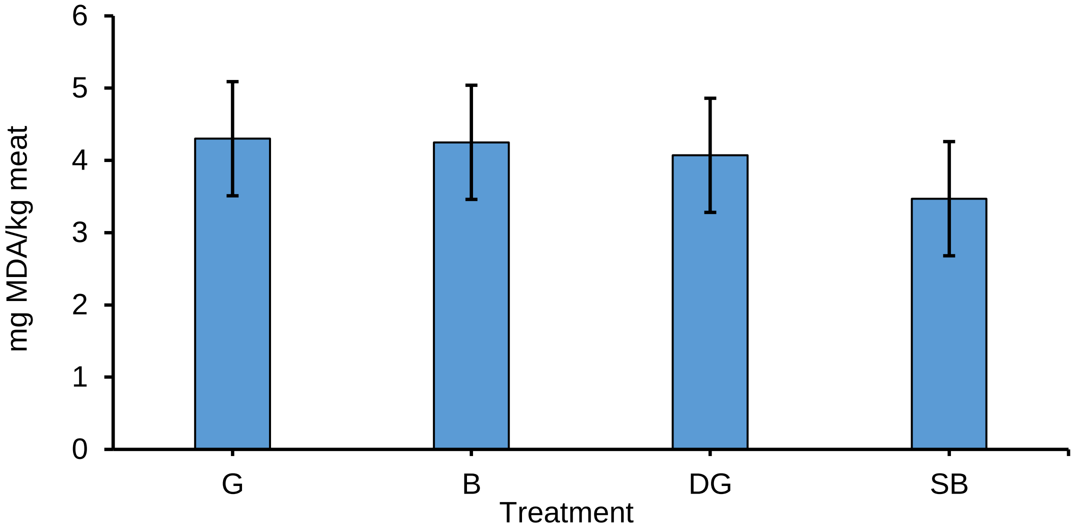


Figure 3. 15. Effect of dietary treatments (G; Grass, B; Barley, DG; Dried grass, SB; Sugar beet) on TBARS (mg malonaldehyde/kg muscle) of semimembranosus muscle at day 7 of simulated retail display in MAP.

3.3.14. Sensory evaluation

The influence of dietary treatments on the eating quality of oven cooked lamb LT muscle is shown in Table 3.11. Tenderness was lower (P<0.05) in muscle from lambs fed diet G compared to that of those fed diet B, but not DG and SB. Juiciness and flavour were not different (P>0.05) between treatments. However, overall acceptability tended (P=0.06) to be higher in muscle from lambs fed diet B.

Table 3. 11. Sensory analysis of lamb meat (evaluated on a scale of 1-9) of different diets.

	G	**B**	**DG**	**SB**	**s.e.d**	**P-value**
Tenderness	5.10^{b}	6.10^{a}	5.88^{ab}	5.73^{ab}	0.351	0.034
Juiciness	5.58	6.18	6.10	5.60	0.312	0.104
Flavour	5.08	5.95	5.65	5.48	0.366	0.119
Overall acceptability	5.13	6.00	5.85	5.54	0.347	0.064

G: grass, B: Barley, DG: Dried grass, SB: Sugar beet, $^{a, b,}$ Means in a row with the same superscript are not different (P>0.05).

3.4. Discussion

3.4.1. Feed analysis

The analysed chemical composition (DM, OM, CP, NDF, starch, WSC, TFA, vitamin E and GE) of the concentrate diets was similar to that predicted from published values (MAFF, 1992). Diet B however, contained a lower NDF, while SB contained higher starch and WSC.

The variability in the dietary FA composition reflected dietary fat sources, diet B was high in C16:0 and C18:2 whereas, diets DG and SB were high in C18:3*n*-3. However, the total FA content of diets DG and SB were lower than the predicted value. Differences between the observed and predicted fat content of dietary treatments could be due to variation in the fat content of different dietary ingredients.

The dietary vitamin E content was similar to that predicted, although, diet DG had a lower content than predicted. The vitamin E content of DG was influenced by the vitamin E content of the grass, which is highly variable ranging from 9 to 400 mg/kg DM (Ballet *et al*., 2000).

Diet G had a lower CP and α-tocopherol content than that predicted (143.8 vs 180 g/kg DM and 34.0 vs 150 mg/kg DM, respectively). Despite this, these concentrations were within the normal range found in ruminant diets (McDonald *et al*., 2011; Ballet *et al*., 2000; Lindqvist *et al*., 2013).

In the current study, the vitamin E content of diet G was in agreement with the finding of Lindqvist *et al.* (2013) who reported an average of 45.5 mg vitamin E/kg DM in red clover + perennial grass in four cuts taken over two years of their experiment. However, it was low compared to the reviewed by Ballet *et al.* (2000), who reported a mean value of grass and legumes of 149 mg/kg DM from 71 samples from different studies. It was also reported that this value decreased to 22 mg/kg DM as the grass increased in maturity (Ballet *et al.,* 2000). A wide variation in the vitamin E concentration in forages is linked to the stage of maturity, where mature grass contains less α-tocopherol than young grass, and the leaves contain 20-30 times as much α-tocopherol as the stems (Mène-Saffrané *et al*., 2017). Therefore, the main factor responsible for the variation in vitamin E content of forages is their stage of maturity and leaf: stem ratio. Although, leaf: stem ratio was not measured in the current study the grass appeared to be less leafy. The concentration of DM in forages increases as the stems formation increases, and there is a negative correlation between DM and vitamin E content of forages (Mène-Saffrané *et al*., 2017).

3.4.2. Animal performance

The initial live weight of lambs was similar between groups, although, the final weight of lambs offered diet G was lower than that of lambs offered diets B, DG or SB. The initial objective was to have a similar slaughter weight between treatments, in order to better study the effect of diets on meat quality. However, poor grass quality and health issues (coccidiosis) in lambs offered diet G affected performance and interpretation of the results. Lambs fed on grass had a low DLWG over 13 weeks and consequently took longer to reach their slaughter weight (88 vs 39 days).

The DLWG was better in lambs fed on the concentrate diets (mean 360 g vs 70 g/day). This was probably due to the consistent supply and quality of nutrient supply to lambs fed the concentrate diets (McClure *et al*., 1994; Armero and Falagan, 2015). However, depending on nutrient availability and pasture type, the growth rate of grazing lambs can be comparable to concentrate fed lambs (Díaz *et al*., 2002; Burnett *et al*., 2012). However, in this study, the grass had a lower concentration of CP and ME compared to published data (MAFF, 1992) (143.0 vs 180 g/kg DM and 10.6 vs 11.5 MJ/kg DM, respectively).

The source of CHO did not significantly affect the performance of lambs fed diets B, DG or SB. However, lambs offered diet B that was rich in starch numerically had a higher performance in terms of DM intake, DLWG and FCR compared DG and SB groups (diets rich in WSC). The dried grass diet had a higher NDF than B diet and an increase in DM intake and FCR of lambs compared to those fed B diet. Galbraith *et al.* (1989) reported that at the same DM intake, lambs fed barley based diets had a higher live weight gain and heavier carcass weights compared with those offered sugar beet pulp. Similarly, Bodas *et al.* (2007) found feed intake, daily gain and FCR were negatively affected when a barley based diet was substituted with sugar beet pulp. This may partially be related to the low feed intake or lower efficiency of energy utilisation due to the nature of the substrates absorbed. The flow rate of microbial protein to the duodenum was reduced in sheep when barley was substituted with citrus pulp (Castrillo *et al*., 2004).

The carcass weight and dressing percentage of lambs reared on diet G were lower compared to those fed the concentrate diets. This is in agreement with the findings of Cifuni *et al.* (2000) who reported that carcass yield and dressing percentage are reduced at a higher age in young lambs (90 vs 45 days, 20.2 and 14.5 kg slaughter weight, 61.1 vs 64.1% dressing, respectively). In contrast, Polidori *et al.* (2017) demonstrated higher carcass weight and dressing percentage in older lambs, slaughtered at 5 vs 2 months at 37.4 vs 23.8 kg slaughter weight, respectively. As slaughter weight increased, there was a concurrent increase in carcass characteristics (hot and cold carcass weight and dressing %). Slaughter weight has an effect on carcass yield (Vergara *et al*., 1999), although, significant differences in carcass weight and dressing out percentage can still be obtained from animals with the same pre-slaughter weight (Santos-Silva *et al*., 2002). This is due to differences caused by the production system, with grass fed animals having a more developed digestive system than those fed on concentrates (Priolo *et al*., 2002). However, Nuernberg *et al.* (2008) found no significant difference in carcass yield from Skudde lambs finished on pasture and concentrate diets.

In the current experiment, differences in conformation and fatness score between lambs fed diet G and those fed the concentrate diets (B, DG and SB) are in line with the result observed by Priolo *et al.* (2002). The aforementioned researchers found that carcasses from lambs raised on pasture had a lower conformation and fatness score. This was confirmed by the results of the back fat and eye muscle scanning, which show that lambs fed diet G had a lower eye muscle depth (2.21 vs 2.94, 2.94 and 2.95 cm) and fat thickness (0.23 vs 0.32, 0.29 and 0.34 cm) compared to those fed dies B, DG and SB, respectively. This could partly be related to increased physical activity, but probably also reflects the fact that they were on a poorer diet, and were lighter at slaughter. The rate of gain is also known to affect carcass composition. The faster an animal grows the higher the proportion of total energy stored as fat (Pethick *et al*.,2004).

There was a decrease in the pH values of the LD muscle between 45 minutes and 24 hours after slaughter in animals fed any of the dietary treatments. This is due to the conversion of glycogen to lactic acid and H^+ (rigor mortis) (Andersen, 2005; Shen and Min Du, 2015). Carbohydrate source had no effect on the ultimate LD muscle pH. It has been reported that high energy diets protect animals from glycogen depletion (Immonen *et al*., 2000). As a result, the ultimate muscle pH of lambs fed diets DG or SB lambs were not significantly different to those fed diet B. The ultimate muscle pH values of lambs fed diet G tended to be higher compared to lambs fed diet B which could be due to the carcass fat as fattier carcasses allow the muscle a slower cooling rate in chill rooms and therefore rigor is attained at higher temperatures. A slower cooling rate corresponds to a faster pH decline and could be responsible for differences in ultimate pH (Priolo *et al*., 2001). Although, there was diffreneces in the carcass fatness of lambs fed diet G compared to those fed B, DG and SB, differences in ultimate pH were only between diets G and B. Therfore, a more likely reason was a carbohydrate sources (grass vs barley). This is in agreement with Adnoy *et al.* (2005) and Priolo *et al.* (2002), who reported that the ultimate pH of the LD muscle tended to be higher for pasture finished lambs. Feeding ruminants with concentrate diets increase volatile fatty acid production in the rumen especially propionate, which is considered to be the only glycogenic volatile fatty acid in ruminants (Priolo *et al*., 2002). Differences in ultimate pH might therefore be related to the muscle glycogen reservoir as well as the stress from gathering outdoor lambs (Terlouw, 2015).

The rumen pH values of lambs in the current study were within the normal range (5.8 to 6.8), which is considered optimal for microbial digestion (Karimizadeh *et al*., 2017). The best rumen pH for starch digesting bacteria is pH 5.5 to 6.0, and fibre digesting bacteria is pH 6.0 to 6.8 (Karimizadeh *et al*., 2017). Also, Olson, (1997) reported that ruminal pH values greater than 5.9 are normal, but considered values of pH 5.6 to 5.8 to be marginal for signs of acidosis.

In the current study, there were differences in rumen pH between lambs fed diets B and SB, with the highest value being for diet B (pH 6.43) and lowest for diet SB (pH 6.0). The ruminal pH value of lambs offered diet B is in contrast to the result of Silveira *et al.* (2007) who reported that rumen pH reduced with increasing starch levels in the diet. Similarly, Suárez *et al.* (2006) reported a reduction in the rumen liquor pH of calves fed a starch diet at 12 wks of age compared to the control. Lechartier and Peyraud, (2010) found that the initial rumen pH before morning meal was higher in cows fed on high starch diets compared to low starch diets, whereas VFA concentration vice versa. In the current study, rumen pH was taken before the morning meal which could have affected the results. Alternatively, higher rumen pH values in lambs offered diet B could be due to dilution effects as a result of a higher water intake, although, water intake was not measured in the current study.

The decline in ruminal pH of lambs fed SB might be due to the rapid fermentation of the high level of WSC (sucrose), resulting in an increase in TVFA and lactic acid concentration (Obara and Dellow, 1994; Rouzbehan *et al*., 1994). Similarly, in an in vitro study, Lee *et al.* (2003) reported a reduction in the pH of a rumen digesta, and lower ammonia-N with increasing WSC inclusion. Diets rich in WSC and low in fibre, reduced rumen pH from pH 6.7 to 5.7, due to an increase in lactic acid and consequently caused acidosis (McDonald *et al*., 2011).

3.4.3. Blood metabolites

Level of feed intake can markedly influence blood metabolic profiles (Connell *et al*., 1997). The mean blood plasma parameters in the current study were within the normal physiological range for sheep (Fraser *et al*., 2004). Total protein and NEFA were similar between treatments, indicating that energy supply was not restricted during the experiment. Lambs exposed to a prolonged fasting period have been reported to have a decrease in total protein and an increase in NEFA level due to increased mobilisation of depot fat (Bowden, 1971). The rate of hepatic albumin synthesis and its plasma concentration is also influenced by nutrient intake. The low concentration of plasma albumin in lambs fed diet G is likely to be a reflection of a lower rate of amino acid uptake, due to lower intake of crude protein from grass compared to the concentrate diets (CP=143.8 g/kg DM vs CP=192 g/kg DM, respectively) (Fraser *et al*., 2004; Fernandes *et al*., 2012). The increase in blood urea that occurred in lambs fed diets B, DG and SB, is probably a response to an excess of ammonia produced from protein degradation in the rumen (Obara and Dellow, 1994). The production of ammonia in the rumen is increased by the intake of highly degradable protein diets, such as grain based diets (Sniffen *et al*., 1992; Karimizadeh *et al*., 2017). Lambs fed the concentrate diets showed a higher concentration of blood glucose compared to those finished on grass. This can be linked to the proportion of rumen propionate, lambs fed concentrate diets had a higher proportion of rumen propionate, which is considered to be the main substrate for gluconeogenesis in the ruminant (Priolo *et al*., 2002). There is a high correlation between the uptake of propionate and blood glucose, which results in an increase in the availability of glucose for peripheral tissues (Fernandes *et al*., 2012). A higher plasma BHB in lambs fed concentrate diets were also found. This is in agreement to the results of Normand *et al.* (2001) and Normand *et al.* (2005) who reported a higher concentration of BHB in lambs finished on concentrate diets, and especially in lambs fed on sugar beet based diets. The higher WSC in the diet SB resulted in a higher production of rumen butyrate and higher plasma BHB concentrations for SB, followed by DG, B and G.

3.4.4. Rumen volatile fatty acid

The concentration of total and individual VFA are highly variable and mainly depend on time after feeding and diet composition (Bergman, 1990; Lechartier and Peyraud, 2010). The TVFA concentrations of rumen fluid were within the normal range observed in adult ruminants of between 60-150 mM (Bergman, 1990). The TVFA concentration found in the rumen of lambs fed diet B was lower than expected and in contrast to the results of Rouzbehan *et al.* (1994) and Bodas *et al.* (2007) who reported that rumen fluid from barley based diets had a higher concentration of TVFA compared to SBP diets. The concentration of VFA in rumen fluid is influenced by the rate of VFA production and the rate of absorption (Dijkstra *et al*., 2012). However, absorption of TVFA can be enhanced by a reduction in ruminal pH, which consequently reduces TVFA (Bergman, 1990; Dijkstra *et al*., 2012). In contrast, in the current study, ruminal pH of lambs fed diet B increased and TVFA decreased, therefore the reduction in TVFA and increased in pH could be due to dilution by increased water consumption, although water consumption was not measured.

The molar proportions of individual VFA are in agreement with the results of Suárez *et al*. (2006); Ramos *et al*. (2009); Jiang *et al*. (2017). Grass, dried grass and sugar beet based diets led to a high proportion of acetate, whereas the barley based diet led to a higher proportion of propionate. Diets based on sugar beet pulp have previously been reported to produce a higher proportion of acetate and butyrate (Van Eenaeme *et al*., 1990; Normand *et al*., 2005).

Ruminal branched chain VFA originate mainly from protein degradation and are considered as an enhancer for fibre degradation microorganisms (Yang, 2002). The higher proportion of branched chain VFA in rumen fluid from the barley-based diet fed lambs could be due to the higher microbial protein synthesis in the rumen. Both branched chain VFA and ammonia N are derived from protein degradation (Eugène *et al*., 2004; Liu *et al*., 2018). The rate of microbial protein synthesis is also mainly dependent on the energy available during fermentation of carbohydrates in the rumen (Dewhurst *et al*., 2000) and the rate of carbohydrate fermentation depends on the carbohydrate source (Rodriguez *et al*., 2007). With microbial growth rate being reduced when cellulose is the only source of energy (Hespell, 1998). In the case of B diet energy was mainly available in the form of starch, which is degraded faster and provides a more energy for the microbial protein synthesis (Cone *et al*., 1989).

3.4.5. Carcass proximate and fatty acids content

The chemical composition (DM, OM and CP) of the LD muscle from lambs fed diets B, DG or SB did not differ to those fed diet G. This indicates that the lower conformation and fatness scores for lambs raised on grass had no effect on the chemical composition of muscle.

The total muscle FA content of concentrate and grass finished lambs were also similar; this is in agreement with the result of Santos-Silva *et al.* (2002) who reported that feeding system had no effect on the total FA content of meat from light lambs finished on grass or concentrate. Similarly, Fisher *et al.* (2000) reported similar results when Suffolk lambs were raised on grass compared to those fed on concentrate and slaughtered at the same live weight.

The percentage of C16:0, C16:1*n*-9, C18:1*n*-9 and C18:2*n*-6 in the muscle of lambs fed concentrate diets, and especially diet B were increased compared to thosed fed G diet. This reflects the dietary concentration of these FAs. Although, PUFAs are extensively hydrogenated in the rumen to produce stearic acid (C18:0), a proportion of PUFA can by-pass the rumen and flow into the duodenum (Wachira *et al*., 2000). Compared with grass, concentrate feeding of lambs also resulted in an increase in saturated C16:0, a decrease in C18:0 and an increase in C18:1*n*-9 (Sinclair, 2007). Santos-silva *et al.* (2002) reported a similar pattern of FA changes in concentrate compared to grass fed lamb. The conversion of C16:0 into C16:1*n*-9 and C18:0 into C18:1*n*-9 is mainly regulated by the enzyme △9 desaturase (Soyeurt *et al*., 2008). As a result, this increases in C18:1*n*-9 and decreases in C18:0 in tissues were reported (Soyeurt *et al*., 2008; Daley *et al*., 2010).

Reported results on the effects of diet on the FA composition of muscle are inconsistent. Slaughter weight has been reported to have a greater effect on the total FA content and FA composition (Santos-Silva *et al*., 2002) and heavier carcasses tend to be fatter than lighter carcasses. Heavier carcasses also tend to contain a higher content of SFA (Sinclair, 2007). Total FA and C16:0, C16:1 and C18:2 were increased when lambs were slaughtered at two different weights of 24 kg vs 30 kg (Santos-Silva *et al*., 2002). In the current study, the mean slaughter weight of lambs fed concentrate diets were higher than the slaughter weight of lambs raised on grass (41.8 kg vs 38.1 kg). This might partly account for lambs raised on grass having less of C16:0, C16:1 and C18:2*n*-6, but the same total FA. The required difference in weight required to make a change in the FA and total FA content at two different slaughter weights however is unclear.

The inclusion of linseed oil in diets, DG and SB increased C18:3*n*-3 in muscle 4 fold compared to diet B, and 2 fold when compared to diet G. This is in accordance with the results of others who have compared the FA composition of meat produced from grass and concentrate diets (Fisher *et al*., 2000; Sinclair 2007). Wachira *et al.* (2002) reported an increase in the percentage of C18:3*n*-3 from 1.4 to 3.1 in muscle when lambs were fed on whole linseed compared the control (Megalac), which reflects an increase in the duodenal flow rate of this FA (Wachira *et al*., 2000).

Muscle EPA increased either when lambs were finished on grass, or when linseed oil was included in the diets. Cooper *et al.* (2004) reported an increase in the percentage of EPA in *longissimus* muscle of lambs fed linseed based diets, however; this proportion significantly increased when lambs were fed on a fish and algae based diet (rich in EPA). There is evidence that the elongation and desaturation of C18:3*n*-3 increases the concentration of EPA compared to diets that have a low concentration of C18:3*n*-3 (Wachira *et al*., 2000 and Cooper *et al*., 2004), although, the efficiency of conversion is low (Chikunya *et al*., 2004). This is due to differences in the pathway of desaturation and elongation of linoleic (*n*-6) and linolenic (*n*-3) acids (Buccioni *et al*., 2012). Fatty acids from the *n*-6 family (linoleic) cannot be converted into members of the *n*-3 family (linolenic) and vice versa (Mattos *et al*., 2000) but long chain *n*-3 and *n*-6 are synthesised by the same enzymes especially, Δ-5 desaturase and △6 desaturase. Diets rich in *n*-3 FAs compete for Δ-5 desaturase activity and undergo a preferentially desaturation and elongation of *n*-3 FAs at the expense of *n*-6 FAs. This was supported by the current findings, as there was a reduction of C20:4*n*-6 in the lambs fed the linseed oil diets (Brenner *et al.*, 1989).

The presence of long chain PUFA in the adipose tissue of lambs was low. This is due to the proportion of phospholipids in adipose tissue being low, and also the incorporation of long chain PUFA into the triacylglycerol fraction of neutral lipid in ruminants is low (Enser *et al*., 1996). Increases in the C14:0, C18:0 and total saturated FA content of adipose tissue of lambs raised on pasture reflected the higher content of these FA in the grass. Rhee *et al*.,(2000) and Rowe *et al.* (1999), argued that fat from grass fed ruminant is more saturated, which is mainly due to higher proportions of C14:0, C18:0 in the grass due to the biohydrogenation of PUFA in the rumen.

Ruminant meat is the main dietary sources of cis-9, trans-11 CLA (Polidori *et al*., 2018) which has many physiological functions and numerous health benefits such as anticarcinogenic, antidiabetic and antiobese (Koba and Yanagita, 2014; Yang *et al*., 2015). Cis-9, trans-11 CLA is produced in rumen by incomplete biohydrogenation of dietary PUFA especially C18:2*n*-6, but is also produced mainly in muscle and adipose tissue by Δ-9 desaturation of trans-11 C18:1 which produced during ruminal biohydrogenation of C18:2*n*-6 and C18:3*n*-3 (Palmquist *et al*., 2005).

In the current study, expected results observed, higher supply of C18:2*n*-6 in diet B decreased percentage of cis-9, trans-11 CLA at the expense of trans-11 C18:1 in muscle and adipose tissues compared to those lambs fed DG, SB or G grass (rich in C18:3*n*-3). Noci *et al*. (2011) found an increase in cis-9, trans-11 CLA in lamb tissues fed linseed oil versus Megalac treatment. In addition, Demirel *et al*. (2004b) reported an increase in CLA by 1.68-fold in IMF when compared to control (Megalac diet). Dietary protection of PUFA by calcium salt (Megalac) would be expected to reduce biohydrogenation intermediates. Thus a decrease in cis-9, trans-11 CLA at the expense of trans-11 C18:1 due to a reduction in the biohydrogenation rate of C18:2*n*-6 and/or decrease in tissue desaturation of trans-11 C18:1. Afore explanation is more likely as diet B had a shorter available time to rumen microorganisms to hydrogenate C18:2*n*-6 and increase muscle C18:2*n*-6 due to a lower content of fibre (NDF) or high level of starch (Oliveira *et al*., 2017) compared to G, DG or SB which had higher NDF contents. It is well reported that diet containing high level of forage to concentrate ratio increase cis-9, trans-11 CLA in meat (French *et al*., 2000; Hajji *et al*., 2016). In addition to the dietary intake of C18:2*n*-6, rumen condition also has an effect on the balance of growth and proliferation of bacterium that are responsible for the synthesis of individual FA of BH intermediates (Palmquist *et al*., 2005). Thus, change in rumen pH has been reported to have an effect on *Butyrivibrio fibrisovens* bacterium to synthesis cis-9,trans-11 CLA (Loor *et al*., 2004). The high concentration of neutral detergent fibre and water soluble carbohydrate that were found in G and DG probably create a good condition for *Butyrivibrio fibrisovens* bacterium to enhance a greater production or decrease in utilization of cis-9, trans-11 CLA by the rumen.

3.4.6. Nutritional indices

The nutritional values of red meat generally depend on three factors; total fat content, PUFA:SFA ratio and *n*-6:*n*-3 ratio (Department of Health, 1994). In the current study, although grass fed lambs had a lower carcass fat content, the fat content of the LD was not affected by treatments. Similarly, Wachira *et al.* (2002) reported that neither breed nor diet affected the total fat content of meat, although Soay lambs had a lower carcass fat score compared to Suffolk and Friesland lambs.

In the current study, the P:S ratio was not affected by treatments and remained lower than the level 0.45 (Department of Health 1994). Linseed oil inclusion increased C18:3*n*-3 FAs, but Megalac inclusion increased C18:2*n*-6, both of which are included in the P:S ratio calculation. The P:S values in the current study are comparable to those reported from the lambs finished on grass (Enser *et al*., 1996), or supplemented with whole linseed or fish oil (Wachira *et al*., 2002). It is difficult to increase the P:S ratio in ruminant meat due to the process of biohydrogenation in the rumen (Palmquist *et al*., 2005). Only in studies that have included a protected source of PUFA in the diets, has the P:S ratio been significantly increased (Cooper *et al*., 2004), with protected linseed and a mix of protected linseed with algae (rich in long-chain PUFA) improving the ratio of P:S to above recommended values (Cooper *et al*., 2004).

The greatest effect of including linseed oil, compared to Megalac, was observed on the *n*-6:*n*-3 and C18:2*n*-6:C18:3*n*-3 ratios. With diets containing linseed oil, these values were considerably lower than the ratio of <4:0 recommended by the Department of Health (1994). The current values are comparable to previous work by Wachira *et al.* (2002), when lambs were fed on whole linseed, and Demirel *et al.* (2004) when lambs were fed protected linseed. These values reflect the greatest concentration of *n*-3 PUFA in the muscle of lambs fed on high dietary sources of *n*-3 PUFA or a reduction in the uptake of *n*-6 into the muscle. It can be argued that a beneficial *n*-6:*n*-3 ratio is more important than the ratio of P:S because the ruminant meats and fish oil are the major sources of *n*-3 PUFA, especially long-chain PUFAs in the human diets (Enser *et al*., 1996).

3.4.7. Muscle vitamin E concentration

The dietary concentration of vitamin E significantly affected muscle vitamin E concentration. High dietary vitamin E (250 mg/kg DM) increased the vitamin E concentration in muscle and produced a similar content to lambs fed diet G (Turner *et al*., 2002). The muscle vitamin E content of lambs finished on diet B was not significantly different to that of lambs finished on diets DG and SB, although it was numerically lower. This was probably due to fat source as dietary PUFA increases, plasma vitamin E concentration tends to decrease (Chikunya *et al*., 2004), although Demirel *et al.* (2004) reported that dietary fat sources did not affect LD vitamin E content of lambs fed either low or high dietary vitamin E supplied with Megalac, linseed or linseed-fish diet. Once α-tocopherol is hydrolysed, it can be exposed to oxidative damage as a result of lipid peroxidation in the intestinal lumen, enterocytes or blood lipoproteins (Gladine *et al*., 2007). Diets DG and SB were expected to have a greater influence on α-tocopherol destruction as they had a high content of C18:3*n*-3 with a higher peroxidizability index, compared to diet B which had a high content of C18:2*n*-6 (Scislowski *et al*., 2005). Another reason is that high PUFA intakes may reduce the efficiency of synthesised chylomicron and VLDL and reduce the rate of micelles diffusion through the mucosal cell membrane due to an increasing micelle size (Bramley *et al*., 2000). The differences between diets can be reduced in ruminants as a result of a higher biohydrogenation of C18:3*n*-3 in the rumen compared to C18:2*n*-6 (Chikunya *et al*., 2004). However, a significant proportion of dietary PUFA is not hydrogenated and can reach the small intestine (Elmore *et al*., 2005). The amount of fat in a food matrix is also found to have a large effect on the bioavailability of vitamin E. Chylomicron and plasma vitamin E concentration were increased when fat level in human diets increased (2.7 g fat to 17.5 g fat per meal) with *RRR*- α- tocopheryl acetate as a source of vitamin E (Jeanes *et al*., 2004). Indeed, the fat content in diet B had a higher content compared to DG and SB (46.5 vs 36.9 and 41.4 g/kg DM, respectively). The efficiency of vitamin E absorption varies between diets (due to the natural form of vitamin E and passage rate) that increased from 1 mg/kg to 4 and 7 mg/kg muscle when the type of the diets changes from concentrate diet to forage and fresh grass, respectively (Wachira *et al*., 2002; Whittington *et al*., 2006; Kasapidou *et al*., 2012).

The concentration of vitamin E in diet G was lower than in diets B, DG and SB with mean values of 34.0 vs 64.7, 224 and 259 mg/kg DM, respectively. However, the vitamin E concentration in the muscle of lambs fed diet G was similar to that of those finished on diets DG and SB, even though the dietary concentration was considerably lower. More than one factor could contribute to increased bioavailability of vitamin E in diet G, including components of digesta, passage rate of intestinal contents and age of the animal. Another factor that may have contributed to the relatively high vitamin E concentration in the muscle of lambs fed diet G is the natural form of vitamin E in the grass, which is more effectively deposited in muscle than the artificial vitamin E (Hidiroglou *et al*., 1988; Kasapidou *et al*., 2012). Jose *et al*. (2016) also reported that lambs fed concentrate diets containing 275 mg/ day of synthetic α- tocopherol acetate had a similar content of muscle vitamin E to those raised on pasture (112 mg/kg DM) for the same fattening period (56 days).

Plasma's α-tocopherol predominates over other forms of vitamin E (β, γ, δ) due to the high affinity of α-tocopherol transfer protein (α-TTP) in the liver (Burton *et al*., 1998), α-TTP can also differentiate between different α-tocopherol stereoisomers (Hosomi *et al*., 1997). Burton *et al*. (1998) reported that the bioavailability of natural *RRR*-α-tocopherol in humans is twice that of synthesised vitamin E (all- rac-tocopherol) with the 2*R* stereoisomers being preferential retained and 2*S* stereoisomers being removed.

The length of the fattening period can also be another factor affecting vitamin E concentration in muscle (Bramley *et al*., 2000). The deposition of vitamin E through the feeding period showed a linear increase and depended on the dosage (Bellés *et al*., 2019). The highest muscle concentration of vitamin E supplementation reached a plateau after 5 weeks while in grass finished lambs just after 4 weeks (Jose *et al*., 2016). In the current study, although, vitamin E content of grass was low (34.0 mg/kg DM), lambs raised on this diet had a longer fattening period (88 vs 39 days) than those offered the concentrate diets.

3.4.8. Shelf life

Generally, there was a trend for lambs fed diet DG to have darker meat. In contrast to the results of Priolo *et al.* (2002) who reported that lambs finished on pasture had darker meat compared to concentrate finished lambs. The cause of this effect is difficult to explain as it may have been affected by more than one factor. The direct effects of diet on meat colour are dependent on muscle myoglobin (Listrat *et al*., 2016), but this was not measured. In addition, the differences in age, muscle energy status, ultimate pH and intramuscular fat (IMF) are considered indirect effects (Priolo *et al*., 2001). Lambs fed diets B, DG or SB were similar regarding feeding conditions, age, ultimate pH and IMF, but still, those receiving DG had darker meat. The highest yellowness value of meat was for lambs fed diet G. This could be related to the intramuscular fat that was recorded. Lambs raised on grass also have yellower fat depots compared to lambs fed concentrates because grass contains more carotenoid pigment (Xanthophyll) (Priolo *et al*., 2002; Webb and Erasmus 2013).

Lambs fed diets G or B produced meat samples that had a longer colour shelf life by at least one day (or longer if colour had been measured for a longer period) compared to lambs fed diets DG or SB. This was expected as the consumption of PUFA fat reduces shelf life while saturated fat increases shelf life (Wood *et al*., 2004). Lambs fed diet G had a higher vitamin E content and lower PUFA content. In addition, recent findings by Vahedi *et al*. (2015) reported that enzyme activities and mRNA gene expressions for glutathione peroxidase and superoxide dismutase in LD muscle from lambs fed pasture were superior compared to those fed concentrate. This indicate that lambs fed pasture have lower oxidative stress due to having a better antioxidant potential in the body. Regardless of the low vitamin E in samples from lambs fed diet B, they produced better colour shelf life compared to lambs fed DG or SB. This could be due to a low PUFA content and higher *n*-6:*n*-3 ratio (Ponnampalam *et al*., 2016).

The TBARS values are used as an indicator of the formation of lipid oxidative substances (rancidity) during retail display (Cheng, 2016). Lipid oxidation values were generally higher when lambs were fed on concentrate diets. This was linked to low vitamin E content, compared to pasture finished lambs (Wood *et al*., 2004; Nute *et al*., 2007; Jose *et al*., 2016). In the current study, TBAR values were within the normal range based on the results of

Berruga *et al*. (2005), 4.2 to 7.5 mg MDA/kg muscle in lamb muscle was required before the acceptability of meat is reduced due to the detection of off-odours by panellists.

In contrast, in beef, 2.3 mg MDA/kg muscle has been reported to be an upper limit of lipid oxidation before off flavour and odours are detectable by sensory panellists (Campo *et al*., 2006). Therefore, in this study, some samples that had TBARS above the threshold (4.2 to 7.5 mg MDA/kg muscle) could be expected to be unsatisfactory. Non-significant differences between dietary treatments on lipid oxidation of meat were also reported. In the current study, vitamin E content was higher in the muscle of lambs fed diets G, DG or SB compared to diet B (2.61, 2.38 and 2.36 vs 1.88 mg/kg muscle, respectively), but was not enough to delay lipid oxidation. Ponnampalam *et al.* (2014) confirmed that muscle vitamin E content should be higher than 3.45 mg/kg muscle to reduce TBARS value to <2.4 mg MDA/kg muscle.

3.4.9. Sensory evaluation

The different degree of tenderness between lambs fed diets G and B could be due to the degree of fatness, as lambs fed diet G had a lower fat thickness (0.23 vs 0.33 cm) and fat score (2.41 vs 3.30). Similarly, Priolo *et al.* (2002) reported a positive correlation between the degree of tenderness and carcass fat from stall-fed lambs compared to pasture fed lambs. Differences in carcass fat could have affected meat tenderness either directly through a fat that was softer than lean or/and indirectly, by slow post-mortem chilling which in turn, improved tenderness by reducing cold muscle fibre shortening (Fiems *et al*., 2000). The effect of dietary treatment or production system was similar on juiciness and flavour, although, numerically samples from lambs fed diet B were ranked higher than those fed diet G. Meat juiciness and flavour are strongly linked to intra-muscular fat content (Young *et al*., 1997; Danso *et al*., 2017). In the current study, there were no differences between treatments in muscle fat content, and hence no treatment effects on muscle juiciness and flavour were reported. In contrast to the findings of Sañudo *et al.* (1998); Priolo *et al.* (2002), reported that lambs finished on concentrate diets have a less intense flavour and lower livery flavour, whereas pasture finished lambs had more off-odour and off-flavour. In contrast, Fisher *et al.* (2000) reported that concentrate fed lambs had higher scores for lamb off flavour and overall liking than grass fed lambs. This variation in overall acceptance of lamb based on the production system is influenced by the panellist’s country and culture (Sañudo *et al*., 1998).

In general, the consumers that evaluated the lamb meat samples in this study reported no significant difference between dietary treatments for overall acceptability, which is a positive result for UK farmer to have more options when selecting different diet sources without affecting sensory attributes. However, consumer numbers and serving temperature could have an effect on sensory attribute scores. The flavour and odour intensity is enhanced by heating (Engelen *et al*., 2003). Fuentes *et al*. (2013) evaluated the effect of three serving temperatures (7 °C, 16 °C and 20 °C) on sensory characteristics of vacuum packed, dry-cured hams. Flavour intensity and texture were increased by increased serving temperature, as at high temperature volatile compound becomes more volatile and the meat matrix becomes more fluid to release more compound from the sample, especially during the first seconds of sample consumption (Engelen *et al*., 2003). Thus, in the current experiment, differences between treatments may not have been apparent at lower temperatures. However, no complaints were recorded about serving meat samples at room temperature.

3.5. Conclusion

Lamb performance on diet G was lower than the expected. Concentrate carbohydrate source, fat source and vitamin E had no effect on animal performance, carcass characteristics or carcass measurements.

Lambs finished on concentrates containing linseed oil and a high vitamin E (DG and SB) had a similar muscle proximate, C18:3*n*-3, EPA, cis-9, trans-11 CLA, *n*-6: *n*-3, C18:2*n*-6: C18:3*n*-3 and vitamin E content to those finished off grazed grass. Lambs fed either grass or concentrate diets had similar lipid stability and sensory evaluation characteristics. Although, lambs finished on diet B had a better colour shelf life and overall acceptability of sensory perception.

Chapter 4

4.0 Effect of concentrate fat source and vitamin E level on performance, carcass composition and meat quality of lambs.

4.1. Introduction

In experiment 1, an attempt was made to replicate the chemical composition of grass by manipulating a number of dietary variables including CHO source, fat source and vitamin E content. Lamb performance and lipid stability did not differ significantly between treatments. However, consumers tended to prefer lamb that had been finished on barley based diet, as the overall acceptability and tenderness of the meat scored higher than that of lambs finished on the other treatments. Also, lambs finished on barley diet had a lower concentration of CLA. This suggests a further study to enhance the eating quality of lamb finished on barley based concentrates was required.

In experiment 1, lambs fed dried grass or sugar beet based diet had a higher percentage of C18:3*n*-3 and cis-9, trans-11 CLA compared to that fed barley based diet. However, the two former diets were supplied with linseed oil compared to the latter that was supplied with Megalac. Demirel *et al*. (2004b) reported an addition of *n*-3 PUFA to the diet based on dried grass could enhance and increase C18:3*n*-3 and cis-9, trans-11 CLA concentration in lamb. Thus, the inclusion of linseed oil to a diet based on barley may increase the CLA concentration in lamb.

The optimum level of dietary vitamin E to optimise meat quality attributes varies. For example, the optimum vitamin E content of muscle to maintain oxidative stability has been reported to be >2.5 mg/kg muscle, which was achieved with a dietary supply of approximately 270 mg/kg diet (Lopez-Bote *et al.,* 2001). In contrast, the optimum level to improve meat surface redness is in the range of 5.3 to 5.6 mg/kg muscle, which corresponds to a dietary inclusion of 550 to 625 mg/kg diet (Lopez-Bote *et al.,* 2001). The NRC (2007) recommended that the optimum level of vitamin E to protect small ruminants from infectious disease and to extend the storage life of lamb meat should be 9 mg/kg LW/day. Therefore, 250 mg/day is approximately equal to 10 mg of vitamin E/kg live weight for a lamb 25 kg weight. Unsaturated fatty acids in mitochondria and microsomal membranes are thought to be the origin of the lipid oxidation (Arnold *et al.,* 1993).

Therefore, the inclusion of linseed oil to increase the CLA and *n*-3 PUFA content of meat from lambs fed barley based diet may also increase the vitamin E requirement, particularly to extend shelf life during retail display.

4.2. Materials and methods

All animal procedures were conducted according to the UK animals (Scientific Procedures Act) 1986 and were approved by the Harper Adams University Animal Welfare and Ethical Review Board. All other aspects of husbandry and management were similar to commercial practice.

4.2.1. Experimental design.

Forty Suffolk cross Texel wether lambs (mean LW= 24.8 kg, s.e.d; 0.14) and 8 weeks of age from the Harper Adams University flock were blocked by LW and allocated to one of four treatments (ten lambs/treatment):

1- Grazed Grass (**FG**)

2- Barley Megalac (**BML**)

3- Barley Linseed oil Low vitamin E (250 mg/kg DM) (**BLL**)

4- Barley Linseed oil High vitamin E (500 mg/kg DM) (**BLH**)

Three iso-energetic and iso-nitrogenous diets, based on barley were formulated to provide similar levels of ME and fat, with an ERDP/FME of >10.0 g/MJ (Table 4.1). Treatment BML contained Megalac© (calcium salt of palm fatty acids, HJ lea Oakes Ltd., UK) as the fat source (rich in saturated FA C16:0) and 250 mg/kg DM vitamin E (α- tocopherol-acetate). Whereas, treatments BLL and BLH contained linseed oil (Young Animal Feeds Ltd., UK) as the fat source (high in C18:3*n*-3), with either 250 or 500 mg/kg DM vitamin E (α- tocopherol-acetate) respectively. The raw ingredients for the concentrate diets were chosen based on their predicted chemical composition (MAFF, 1992). All concentrates were manufactured and pelleted to 4 mm by at HJ Lea Oakes Ltd., High town Mill Congleton Cheshire, UK

Table 4. 1. Raw materials and predicted chemical composition of the experimental diets.

Raw materials (g/kg)	**FG**	**BM**	**BLL**	**BLH**
Barley	----	609	610	610
Oatfeed	----	93	96	96
NIS[1]	----	71	71	71
Soya bean meal	----	77	77	77
Rapeseed meal	----	88	89	89
Urea	----	9	9	9
Megalac©	----	21	----	----
Linseed oil	----	----	18	18
Mins/vitamins[2]	----	20	20	20
Ammonium chloride	----	5	5	5
Sodium Chloride	----	5	5	5
Predicted chemical composition (g/kg DM)				
DM (g/kg)	200	876	876	876
CP	180	185	185	185
NDF[3]	560	314	317	317
ADF[4]	285	140	141	141
Starch	10	333	334	334
WSC	207	37.5	37.5	37.5
Ash	85	80.4	77.3	77.3
Ether extract	20	36.2	36.1	36.1
C18:2*n*-6	2.6	5.3	8.3	8.3
C18:3*n*-3	12	0.7	13.4	13.4
C18:2/C18:3	0.21	7.57	0.62	0.62
Vitamin E level (mg/kg DM)	150	250	250	500
Selenium (mg/kg DM)	0.1	0.5	0.5	0.5
ME[5] (MJ/kg DM)	11.5	12.0	12.0	12.0
FME[6] (MJ/kg DM)	10.8	10.7	10.7	10.7
ERDP[7] (0.05)	124	138	138	138
DUP[8] (0.05)	36	26.1	26.2	26.2
ERDP/FME	11.5	12.9	12.8	12.8

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E,[1]nutritionally improved straw, [2]Mineral vitamin premix contained: E672a vitamin A 10000IU/kg, E671 vitamin D3 2000 IU/kg, vitamin B12 70 mg/kg, vitamin B1 1 mg/kg, ferrous sulphate monohydrate 667 mg/kg, sodium molybdate 4mg/kg, sodium selenite 0.7 mg/kg calcium iodate anhydrous 7.9mg/kg manganous oxide 121 mg/kg, zinc oxide 167 mg/kg, [3]Neutral detergent fibre, [4]Acid detergent fibre, [5]Metabolisable energy, [6]Fermentable metabolisable energy [7]Effective rumen-degradable protein, [8]Digestable undegradable protein,.

4.2.2. Experimental routine

Ten lambs were grazed on the Harper Adams University farm (pasture mainly for cows) on a mixed pasture sward that consisted mainly of perennial ryegrass, with sward height being maintained between 5-10 cm by adding or removing additional lambs as required. The remaining 30 lambs were allocated by LW to one of the three concentrate treatments and housed indoors in individual pens in a naturally ventilated shed and bedded on sawdust throughout the study period.

Diets were offered twice a day at 08:00 and 16:00h using individual clean plastic buckets. Water was available *ad-libitum*. Concentrate diets and fresh grass samples (0.5 kg) were collected weekly at 12:00 pm and stored at - 20 °C prior to subsequent chemical analysis.

Concentrate dry matter intake (DMI) was recorded by offering a fixed amount daily and weighting back refusals twice a week (Monday and Friday). The amount was calculated to supply 1.1x daily consumption during the previous week. Lamb live weight was recorded once a week on a Tuesday at 14:00h (section 2.7).

Blood samples were taken by jugular venepuncture into green (lithium heparin) and grey tubes (fluoride/potassium oxalate) (section 2.6) from all lambs at three time points throughout the experiment at live weight of approximately 25 kg (W1), 32 kg (W2) and 40 kg (W3). In addition, lambs were ultra-sound scanned for back-fat and eye-muscle depth one day before being slaughtered (section 2.8).

4.2.3. Slaughter and measurements

Lambs were slaughtered in four batches over a number of weeks, once they reached half their potential mature weight of approximately (40 kg), with each batch being selected from the 10 heaviest lambs (section 2.11). During the slaughter process, the gastro-intestinal tract was collected and rumen fluid samples obtained. Rumen pH was measured immediately and the samples acidified by adding a few drops of HCL prior to subsequent VFA analysis. Carcass pH and both hot and cold carcass weight were recorded (sections 2.9 and 2.12). Following slaughter, lamb carcasses were returned to Harper Adams University, where carcass measurements (section 2.12) were taken prior to carcasses preparation. The dimensional measurements of the carcass were also recorded (section 2.12).

4.2.4. Carcass preparation

A sample of tail head adipose tissue (5 x 5 cm) was collected, vacuum packed and stored at -20 °C for FA analysis. Carcasses were processed according to Cross, (1977) (Figure

4.1). The *Longissimus dorsi* (LD) muscle was dissected from the right side of each carcass, vacuum packed and stored at -20°C for FA and proximate analysis (section 2.1 and 2.2). The *Longissimus thorasic* (LT) muscles were dissected from both sides and vacuum packed and aged for 10 days at 2-4 °C. Samples were then frozen at -20 °C for sensory evaluation (section 2.13.6). The left LD muscle was vacuum packed and aged for 10 days, then frozen at -20 °C for the determination of thawing loss, cooking loss and shear force (section 2.13.3, 2.13.4 and 2.13.5, respectively).

The left leg was cut into three 2 cm thick leg steaks, and the right leg was cut into one 2 cm steak (total 4 steaks) that contained semimembranosus (SM) and all pelvic limb muscles. These steaks were vacuum packed and conditioned at 2-4°C for six days, before being transferred into plastic trays with modified atmosphere (MAP) (75.2% O2 and 17.5% CO2) and subjected to simulate retail display (2-4°C, 700 lux; 16h on and 8h off) for 14 days to measure colour (section 2.13.1) and lipid oxidation at day 7 and day 14 (section 2.13.2). The remainder of the right leg was also vacuum packed and stored at -20°C for subsequent vitamin E determination of the SM.

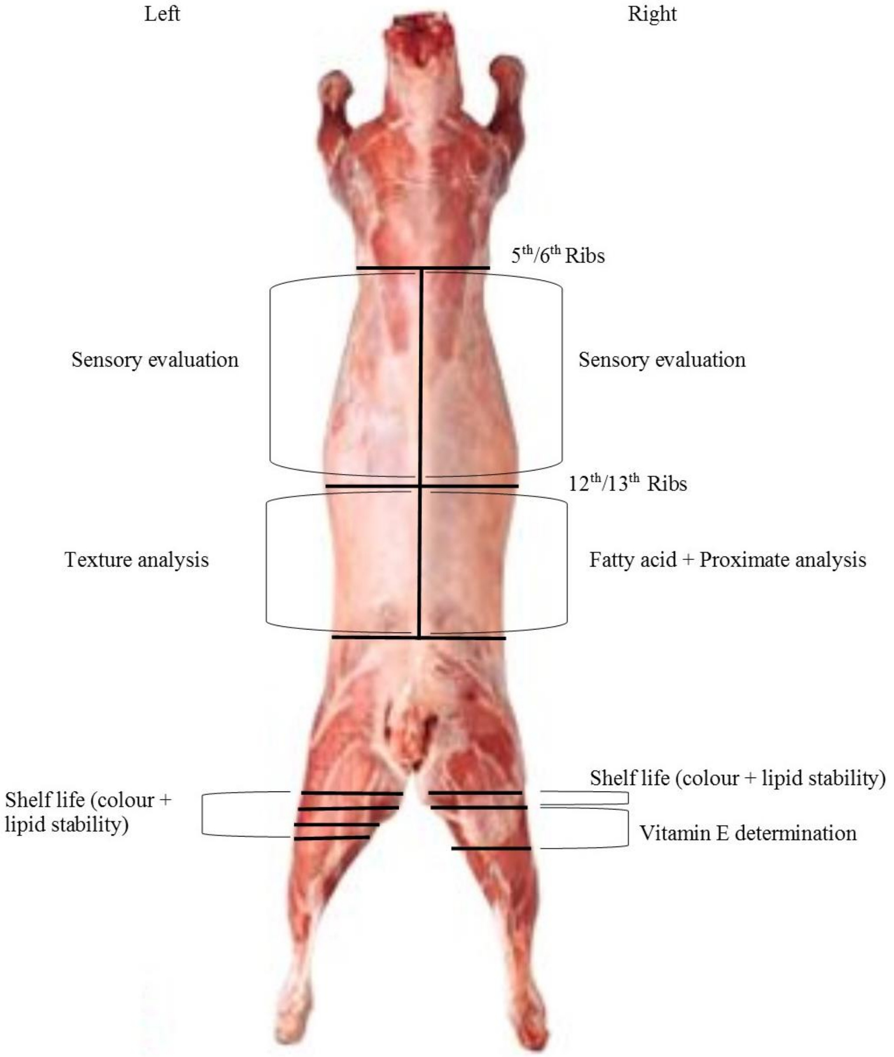


Figure 4. 1. Carcass preparation of lambs in the experiment.

4.2.5. Chemical analysis

Concentrate and grass feed samples were either oven dried or freeze dried, ground (section 2.1.1), and a similar amount from each week mixed to prepare one composite concentrate sample and three composite grass sample (each sample represented four consecutive weeks) for subsequent chemical analysis.

Feed sample was analysed for DM, CP, OM, NDF, GE, FA and vitamin E as described in sections 2.1.1, 2.1.2, 2.1.3, 2.1.4, 2.1.5, 2.2 and 2.3, respectively. Feed samples were also analysed for selenium, starch and WSC by Trouw Nutrition, GB. Blood samples were analysed for total protein, albumin, glucose, NEFA, BHB and urea using a Cobas Mira Plus auto- analyser (ABX Diagnostics, Bedfordshire, UK) as described in section 2.6. Rumen fluid VFA were determined by the method of Cottyn and Boucpue (1986) as described in section 2.10. The proximate composition (DM, CP and OM) and FA composition of the LD were determined as described in sections 2.1.1, 2.1.2, 2.1.3 and 2.2. The FA composition of the tail head adipose tissue was determined as described in section 2.2. Vitamin E was determined in the SM according to the method of Liu *et al.* (1996) as described in section 2.4. Lipid oxidation was determined by the method of Buege and Aust (1978) as described in section 2.13.2.

4.2.6. Calculation and statistical analysis

The daily live weight gain for each animal was estimated from the regression of live weight against time. The carcass conformation and fatness scores were converted to numerical values for statistical analysis (Danso *et al.,* 2017).

Animal performance, rumen VFA, carcass chemical composition including FA, TBARS, thawing loss, cooking loss, shear force and sensory results were analysed by one way analysis of variance (ANOVA) as a randomised block design using software GenStat 18th (Lawes Agricultural Trust, VSN International Ltd, Oxford, UK). Blood biochemical parameters and meat colour were analysed by repeated measure ANOVA as a randomised block design with the main effect of time and treatments. Tukey's multiple range test (α =0.05) was used to determine significant differences between treatments with $P<0.10$ being reported as a trend.

4.3. Results

4.3.1. Animal health

The lambs fed either grass or the concentrate diets had no health issues throughout the experiment.

4.3.2. Feed analysis

The chemical composition of the diets is presented in Table 4.2. The chemical composition of the three concentrate diets (BML, BLL and BLH) was similar with mean values for DM, CP, starch, WSC and Se being 871, 193, 352, 112 and 0.45 mg/kg DM respectively. Similarly, organic matter and gross energy content were similar between dietary treatments. Diet FG had the highest content of NDF and the lowest CP, vitamin E, Se and total FA content compared to the concentrate diets. Inclusion of Megalac (BML) increased C16:0, C18:0, C18:1*n*-9c and C18:2*n*-6, whilst, the inclusion of linseed oil (BLL and BLH) increased C18:0, C18:1*n*-9c, C18:2*n*-6 and C18:3*n*-3.

Table 4. 2. The chemical composition of the experimental diets (g/kg DM).

Diets	**FG**	**BML**	**BLL**	**BLH**
DM[1] (g/kg)	211	874	873	866
OM[2]	909	914	914	915
CP[3]	143	190	198	190
NDF[4]	556	272	284	267
Starch	<5	356	350	349
WSC[5]	250	115	103	118
α-tocopherol (mg/kg DM)	158	287	281	542
Selenium (mg/kg DM)	0.02	0.42	0.53	0.40
GE[6] (MJ/kg DM)	17.8	17.9	17.6	18.0
Fatty acid g/kg DM				
C14:0	0.77	0.84	0.64	0.68
C16:0	3.54	12.7	5.50	5.48
C16:1 *n*-9	0.04	0.04	0.03	0.03
C18:0	0.35	1.11	1.10	1.20
C18:1*n*-9c	0.62	12.3	8.77	9.17
C18:2*n*-6c	3.27	13.46	14.80	14.37
C18:3*n*-3	14.4	1.51	9.77	11.1
RFA[7]	3.99	3.92	3.84	3.90
Total fatty acids	27.0	45.9	44.5	46.0
Grass quality	FG1[8]	FG2[9]	FG3[10]	Mean
DM (g/kg)	232	212	190	212
OM	913	912	900	909
CP	133	123	174	143
NDF	507	567	596	557
Starch	<5	<5	<5	<5
WSC	348	266	136	250
α-tocopherol (mg/kg DM)	178	136	160	158
GE (MJ/kg DM)	17.8	17.8	17.9	17.8
Fatty acids (g/kg DM)				
C14:0	0.74	0.58	1.00	0.77
C16:0	3.41	3.28	3.94	3.54
C16:1*n*-9	0.03	0.04	0.04	0.04
C18:0	0.38	0.30	0.37	0.35
C18:1*n*-9c	0.50	0.70	0.65	0.62
C18:2*n*-6c	3.01	3.33	3.47	3.27
C18:3*n*-3	14.5	11.8	17.0	14.4
RFA	3.94	3.39	4.65	3.99
Total FA content	26.5	23.4	31.1	27.0

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E, [1]Dry matter, [2]Organic matter, [3]Crude protein, [4]Neutral detergent fibre, ,[5]Water soluble carbohydrate [6]Gross energy, [7]Remainig fatty acid, [8]week 1-4, [9]week 5-9, [10]week 9-12.

4.3.3. Animal performance

The effect of dietary treatment on lamb performance is presented in Table 4.3. The initial mean live weight (LW) was 24.8 kg and did not differ (P >0.05) between treatments. Lambs fed diet FG grew slower and took longer (P<0.001) (Figure 4.2) to finish compared to those fed diets BML, BLL or BLH. Lambs receiving FG were also slaughtered at a lower (P<0.001) LW than those fed BML or BLH but not BLL. There was no difference between the three concentrate diets (BML, BLL and BLH) on DM intake, DLWG, FCR and fattening period. However, lambs fed BLL tended (P=0.073) to have a higher FCR compared to those fed the BML or BLH diets.

Table 4. 3. Effect of dietary treatment on lamb performance throughout the experiment.

Animal Performance	**FG**	**BML**	**BLL**	**BLH**	**s.e.d.**	**P-value**
Initial LW (kg)	24.8	24.8	24.8	24.7	0.137	0.977
Final LW (kg)	40.9^{b}	43.3^{a}	41.4^{b}	42.8^{a}	0.907	0.038
DM intake (kg/day)	------	1.48	1.38	1.38	0.051	0.117
DLWG[1] (kg/day)	0.18^{b}	0.41^{a}	0.37^{a}	0.41^{a}	0.020	<0.001
FCR[2] kg DM/kg gain	------	3.73	4.12	3.53	0.245	0.073
Growth period (day)	84.0^{a}	48.0^{b}	49.4^{b}	46.6^{b}	1.924	<0.001

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E, [1]Daily live weight gain, [2]Feed conversion ratio, $^{a,\ b}$ Means in a row with the same superscript are not different (P>0.05).

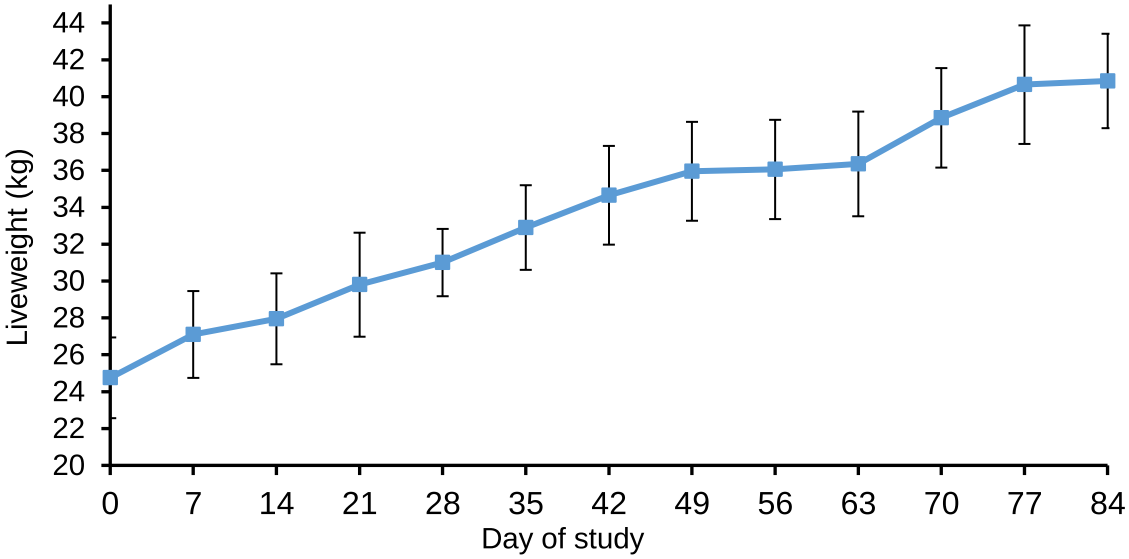


Figure 4. 2. Growth of lambs reared on grass throughout the experiment. Error bar indicates standard deviation (SD).

4.3.4. Carcass characteristics

The effect of dietary treatment on carcass characteristics is presented in Table 4.4. The hot and cold carcass weights and dressing % of lambs fed diets BML, BLL or BLH were higher ($P<0.001$) compared to those fed diet FG. Muscles pH at 45 min and 24hs post slaughter were similar ($P>0.05$) between treatments. Lambs fed diet FG had a lower ($P<0.05$) conformation score compared to those fed BML and a lower ($P<0.05$) fat score compared to those fed BML, BLL or BLH. Similarly, lambs fed diet FG had a lower ($P<0.05$) back-fat depth compared to BML and a lower ($P<0.001$) eye-muscle depth compared to those fed BML, BLL or BLH. Lambs fed diet FG also had a lower ($P<0.05$) ruminal pH compared to those fed BLL.

Table 4. 4. Effect of dietary treatments on carcass characteristics of growing lambs.

Carcass characteristic	**FG**	**BML**	**BLL**	**BLH**	**s.e.d.**	**P-value**
Hot carcass weight (kg)	17.1^{b}	21.3^{a}	20.3^{a}	20.5^{a}	0.478	<0.001
Cold carcass weight (kg)	17.0^{b}	21.1^{a}	20.1^{a}	20.3^{a}	0.465	<0.001
Dressing %	41.6^{b}	48.7^{a}	48.5^{a}	47.5^{a}	0.815	<0.001
pH, 45 min	6.23	6.38	6.48	6.36	0.092	0.088
pH, 24 h	5.60	5.61	5.60	5.61	0.067	0.991
Conformation score	2.5^{b}	3.3^{a}	3.0^{ab}	3.0^{ab}	0.239	0.020
Fat score	2.00^{b}	3.50^{a}	3.00^{a}	2.90^{a}	0.233	<0.001
Fat thickness (cm)	0.27^{b}	0.33^{a}	0.29^{ab}	0.29^{ab}	0.020	0.037
Eye muscle depth (cm)	2.53^{b}	3.12^{a}	3.12^{a}	2.97^{a}	0.104	<0.001
pH, rumen fluid	6.02^{b}	6.40^{ab}	6.60^{a}	6.26^{ab}	0.187	0.031

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E, $^{a,\,b}$ Means in a row with the same superscript are not different ($P>0.05$).

4.3.5. Carcass measurements

The effect of diet on carcass measurements is presented in Table 4.5. There was no difference ($P>0.05$) in carcass measurements between lambs fed diets BML, BLL or BLH. Similarly, there was no difference ($P>0.05$) between lambs fed diets BML, BLL or BLH in chest circumference, barrel width and chest depth. In contrast, the carcass length and gigot depth of lambs fed diet FG was lower ($P<0.05$) compared to those fed diets BML or BLH, and the buttock circumference was lower ($P<0.001$) than those fed diets BML, BLL or BLH.

Table 4. 5. Effect of dietary treatments on carcass measurements of growing lambs.

Carcass measurements	**FG**	**BML**	**BLL**	**BLH**	**s.e.d.**	**P-value**
Carcass length (cm)	56.6^{b}	60.8^{a}	59.0^{ab}	60.0^{a}	1.212	0.012
Chest circumference (cm)	74.1	75.6	74.1	74.6	0.769	0.204
Buttock circumference (cm)	63.2^{b}	67.5^{a}	66.7^{a}	66.2^{a}	0.999	0.001
Barrel width (cm)	24.9	25.5	24.9	25.5	0.395	0.195
Chest depth (cm)	26.4	25.8	24.9	25	0.654	0.114
Gigot depth (cm)	15.9^{b}	17.6^{a}	17.3^{ab}	18.4^{a}	0.587	0.002

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E, $^{a,\,b}$ Means in a row with the same superscript are not different ($P>0.05$).

4.3.6. Blood metabolites

4.3.6.1. Total protein

Repeated measure analysis showed an effect (P<0.001) of time on blood plasma total protein concentration, increasing from 57.5 mg/ml at W1 to 67.4 mg/ml at W3 (Figure 4.3). However, there was no effect (P>0.05) of treatment on total protein concentration of lambs fed diets FG, BML, BLL or BHL, with mean values of 64.7, 62.4, 62.9 and 63.3 mg/ml, respectively. There was a time x treatment interaction (P<0.05) on total protein concentration with the total protein concentration of lambs fed diet FG being lowest at point W1, and highest at point W3, with values of 54.0 mg/ml and 73.5 mg/ml, respectively.

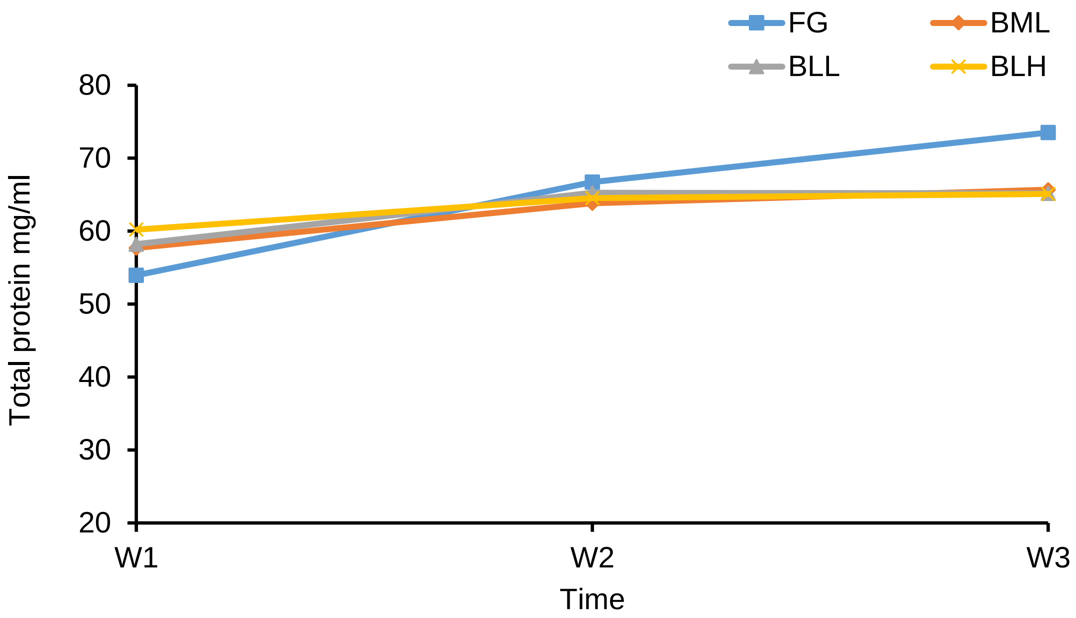


Figure 4. 3. Effect of dietary treatments (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E) on the blood plasma total protein (mg/ml) concentration of growing lamb throughout the experiment (W1: beginning, W2: middle W3: end of experiment). (s.e.d values: Treatment=1.969, Time=1.225, Inter= 2.807, P-values: Treatment=0.673, Time <0.001, Inter=0.003).

4.3.6.2. Albumin

Repeated measure analysis showed an effect (P<0.001) of time on plasma albumin concentration increasing from 31.9 mg/ml at point W1 to 35.0 mg/ml at point W2 (Figure 4.4). There was also an effect (P<0.05) of treatment on albumin concentration, lambs fed FG had a lower concentration compared to those fed diet BML, but not those fed diet BLL or BLH, with mean values of 32.1, 34.4, 33.3 and 34.3 mg/ml, respectively. There was no time x treatment interaction (P>0.05) on plasma albumin concentration.

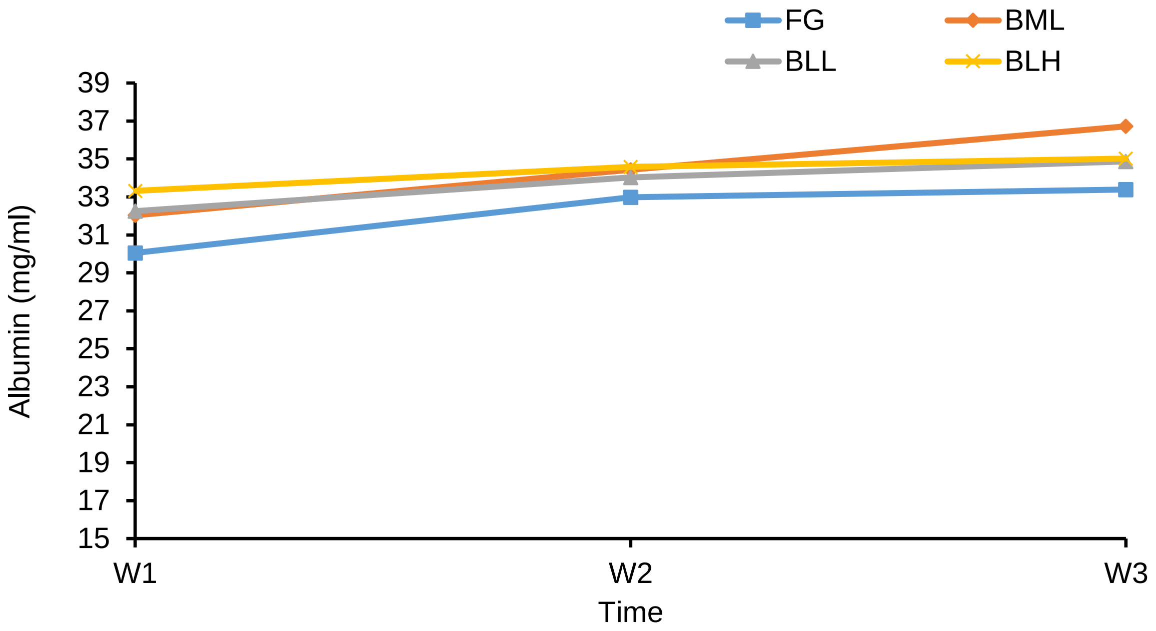


Figure 4. 4. Effect of dietary treatments (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E) on blood plasma total protein (mg/ml) concentration throughout growing lamb period (W1: beginning, W2: middle W3: end of experiment). (s.e.d values: Treatment=0.821, Time=0.450, Inter=1.102, P-values: Treatment=0.038, Time <0.001, Inter=0.319).

4.3.6.3. Urea

Repeated measure analysis showed an effect (P<0.001) of time on plasma urea concentration in lambs increasing from 6.33 mmol/l at point W1 to 12.0 mmol/l at point W3 (Figure 4.5). Similarly, treatment had an effect (P<0.001) on lamb plasma urea concentration with lambs fed FG having a lower mean concentration compared to those fed diets BML, BLL or BLH (6.02 vs 9.50, 11.03 and 10.22 mmol/l, respectively). There was no time x treatment interaction (P>0.05) on lamb plasma urea concentration.

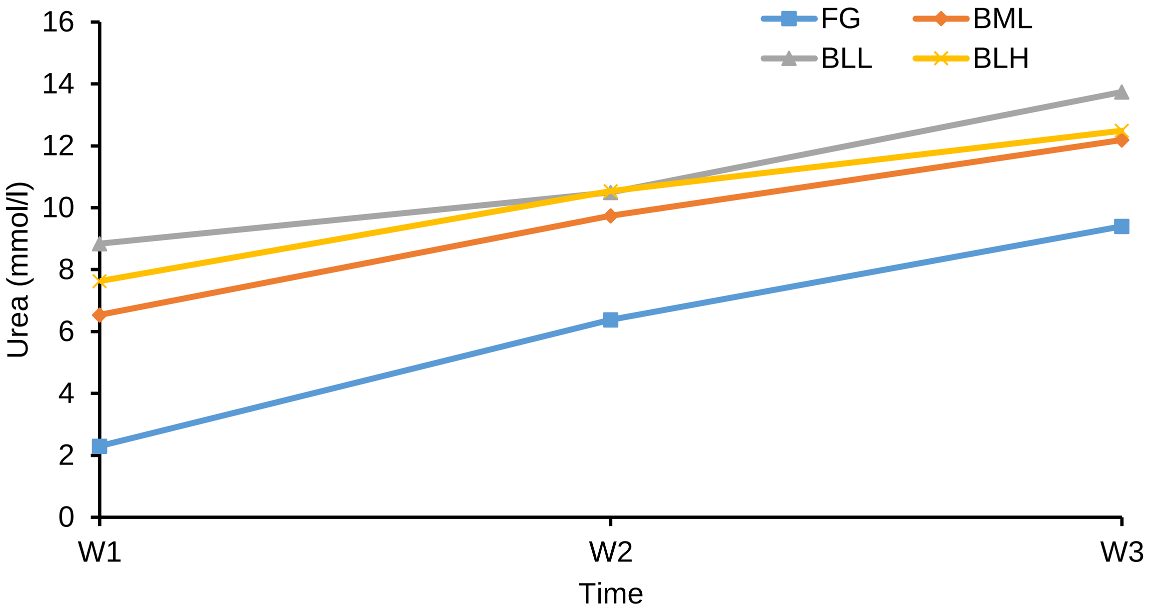


Figure 4. 5. Effect of dietary treatments (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E) on blood plasma total protein (mg/ml) concentration throughout growing lamb period (W1: beginning, W2: middle W3: end of experiment). (s.e.d values: Treatment=0.546, Time=0.353, Inter=0.794, P-values: Treatment <0.001, Time <0.001, Inter=0.155).

4.3.6.4. Glucose

Repeated measure analysis showed an effect ($P<0.05$) of time on lamb plasma glucose concentration decreasing from 4.08 mmol/l at point W1 to 3.48 mmol/l at point W3 (Figure 4.6). There was also an effect ($P<0.05$) of treatment on lamb plasma glucose concentration, lambs fed diet FG having a lower concentration compared to those fed diets BML or BLL, but not those fed diet BLH, with mean values of 4.12, 3.48, 4.02 and 3.97 mmol/l, respectively. There was no time x treatment interaction ($P>0.05$) on lamb plasma glucose concentration.

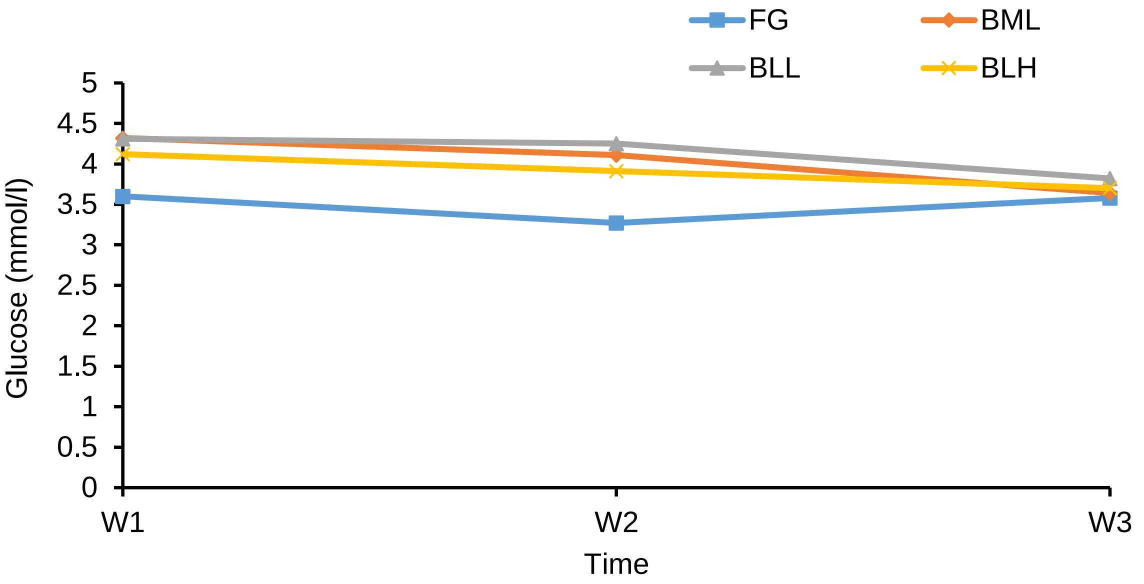


Figure 4. 6. Effect of dietary treatments (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E) on blood plasma total protein (mg/ml) concentration throughout growing lamb period (W1: beginning, W2: middle W3: end of experiment). (s.e.d values: Treatment=0.158, Time=0.108, Inter=0.237, P-values: Treatment=0.002, Time=0.004, Inter=0.192).

4.3.6.5. Non-Esterified Fatty Acids (NEFA)

Repeated measure analysis showed no effect (P>0.05) of time on lamb plasma NEFA concentration (Figure 4.7). In contrast, there was an effect (P<0.05) of treatment on lamb plasma NEFA concentration, with lambs fed diet FG having a higher concentration compared to those fed diet BLL, but not those fed diets BML or BLH with mean values of 0.15, 0.09, 0.12 and 0.11 mmol/l, respectively. There was no time x treatment interaction (P>0.05) on lamb plasma NEFA concentration.

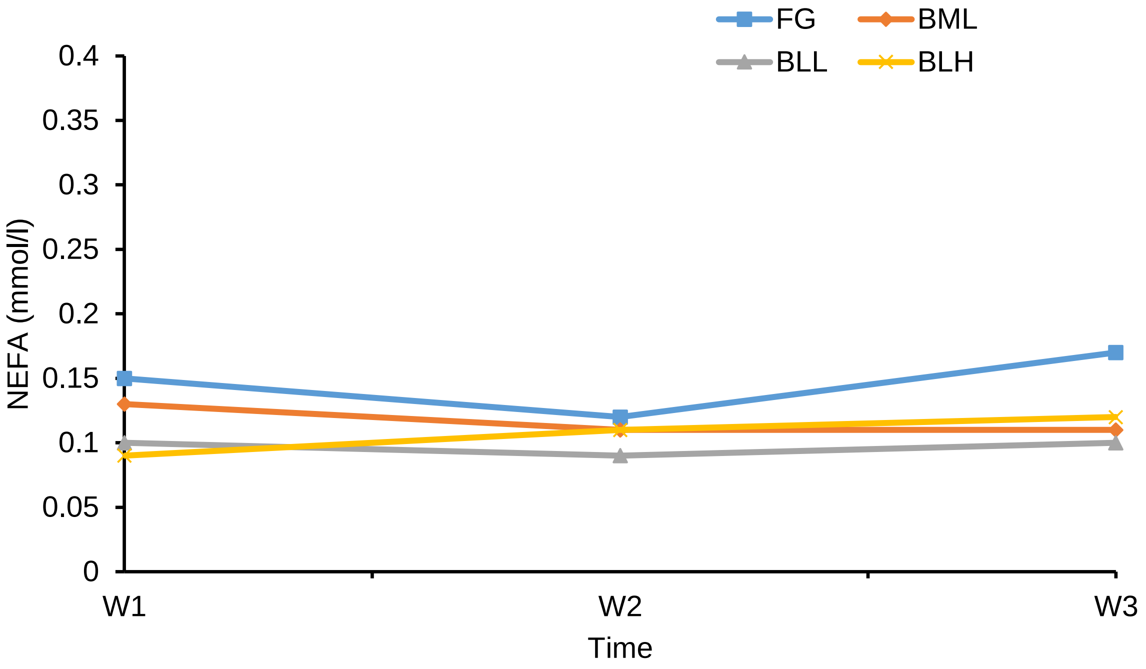


Figure 4. 7. Effect of dietary treatments (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E) on blood plasma total protein (mg/ml) concentration throughout growing lamb period (W1: beginning, W2: middle W3: end of experiment). (s.e.d values: Treatment=0.017, Time=0.009, Inter=0.024, P-values: Treatment=0.04, Time=0.259, Inter=0.411).

4.3.6.6. β-hydroxybutyrate (BHB)

Repeated measure analysis showed an effect (P<0.001) of time on blood plasma BHB concentration increasing from 0.46 mmol/l at point W1 to 0.62 mmol/l at point W3 (Figure 4.8). The plasma BHB concentration of lambs fed diet FG was lower (P<0.05), compared to that of lambs fed diet BML, but not BLL or BLH with mean values of 0.37, 0.60, 0.48 and 0.55 mmol/l, respectively. There was a time x treatment interaction (P<0.001) on lamb plasma BHB concentration, with concentrations in lambs fed diets BML, BLL or BLH increasing from point W2 to reach their highest values at point W3. In contrast, the plasma BHB concentration of lambs fed diet FG decreased from 0.47 mmol/l at W1 to 0.27 mmol/l at point W3.

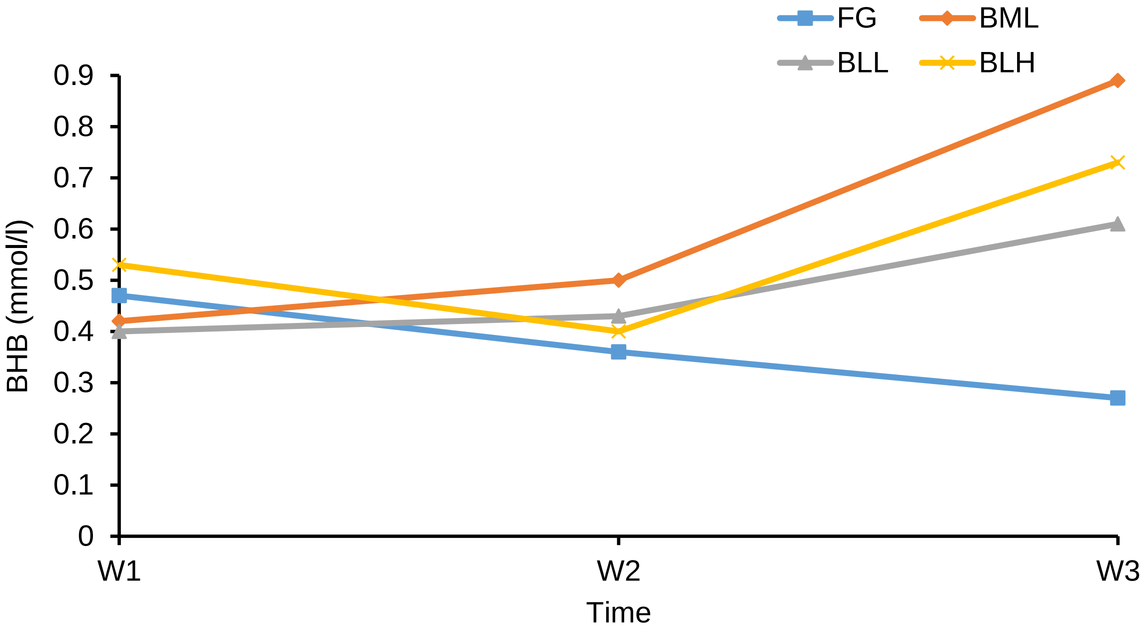


Figure 4. 8. Effect of dietary treatments (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E) on blood plasma total protein (mg/ml) concentration throughout growing lamb period (W1: beginning, W2: middle W3: end of experiment). (s.e.d values: Treatment=0.079, Time=0.048, Inter=0.112, P-values: Treatment=0.032, Time <0.001, Inter <0.001).

4.3.7. Rumen volatile fatty acids (VFA)

The total molar concentration and molar proportions of individual VFA are presented in Table 4.6. Lambs fed diet FG had a higher ($P<0.001$) total VFA, acetate % and A: P ratio, and a lower ($P<0.001$) propionate % and branched chain volatile fatty acids (BCVFA) (isobutyrate, valeric, isovaleric and caproate) compared to those fed diets BML, BLL or BLH. The total VFA, propionate proportion, BCVFA and acetic:propionic ratio in rumen fluid did not change ($P>0.001$) in response to the different concentrate treatments. However, the inclusion of Megalac in diet BML decreased ($P<0.001$) acetate and increased ($P<0.05$) butyrate proportions in rumen fluid.

Table 4. 6. Effect of dietary treatments on total molar concentration and individual volatile fatty acid (molar proportion %) of growing lambs.

	FG	**BML**	**BLL**	**BLH**	**s.e.d.**	**P-value**
TVFA[1] mmol/l	269^{a}	102^{b}	86.5^{b}	96.8^{b}	12.460	<0.001
Acetate	73.8^{a}	58.0^{c}	61.7^{b}	62.4^{b}	1.187	<0.001
Propionate	15.2^{b}	25.9^{a}	25.1^{a}	24.9^{a}	1.337	<0.001
Butyrate	7.97^{b}	10.06^{a}	7.22^{b}	7.52^{b}	0.771	0.002
Isobutyrate	0.84^{c}	1.27^{b}	2.54^{a}	1.43^{b}	0.062	<0.001
Valerate	0.87^{c}	3.23^{a}	3.11^{ab}	2.51^{b}	0.230	<0.001
Isovalerate	1.21^{b}	1.58^{b}	2.12^{a}	1.67^{ab}	0.177	<0.001
Caproate	0.85^{b}	1.66^{a}	1.64^{a}	1.24^{ab}	0.197	0.003
BCVFA	3.07^{b}	6.06^{a}	6.06^{a}	5.26^{a}	0.646	<0.001
A: P ratio[2]	4.82^{a}	2.38^{b}	2.52^{b}	2.72^{b}	0.178	<0.001

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E, [1]total volatile fatty acid, [2]Acetic: Propionic ratio, $^{a,\,b,\,c}$ Means in a row with the same superscript are not different ($P>0.05$).

4.3.8. Proximate and fatty acid composition of muscle

The results for the proximate analysis (g/kg muscle) and fatty acid (% of total fatty acids) composition of the *longissimus dorsi* muscle are shown in Table 4.7. Dietary treatment had no effect (P>0.05) on muscle CP and ash content. However, there was an effect (P<0.05) of treatment on muscle moisture and fat content, with lambs fed diet FG having a higher content compared to those fed diets BML or BLL. Total fat content was different (P<0.001) between treatments with the highest value in BML and BLH, then followed by BLL and FG.

Lambs fed diets BML, BLL or BLH had a higher (P<0.001) proportions of C10:0, C16:0, C17:0, C18:1*n*-9c, C18:1*n*-11t, C18:2*n*-6c and Σ MUAF, and lower (P<0.001) proportion of C15:0, C16:1*n*-9, C18:0, C20:4*n*-6, DHA and EPA, compared to those fed diet FG. Similarly, fat source had an effect on the FA composition of muscle samples. Muscle from lambs fed BLL or BLH diet had a higher (P<0.001) percentage of C18:3*n*-3, DHA, EPA and Σ PUFA and a lower percentage (P<0.001) of C18:1*n*-9c, SFA and Σ MUFA in muscle compared to those fed diet BML. Concentrate diet had no effect (P>0.001) on the percentage of C10:0, C12:0, C14:0, C15:0, C15:1, C16:1*n*-9, C18:0, C18:2*n*-6c, C20:4*n*-6 and cis-9, trans-11 CLA.

The FA composition and total FAs expressed as mg/100 g muscle are presented in Table 4.8. Lambs fed on the concentrate diets produced meat with a higher (P<0.001) concentration of C10:0, C14:0, C16:0, C17:0, C18:1*n*-9c, C18:2*n*-6c and C18:1*n*-11t and a lower (P<0.001) concentration of EPA compared to those fed diet FG. Muscle concentrations of C18:3*n*-3, DHA and EPA were higher (P<0.001) and C18:1*n*-9 and C20:4*n*-6 were lower (P<0.001) in lambs fed diet BLL or BLH compared to BML

Table 4. 7. Effect of dietary treatment on proximate (g/kg muscle) and fatty acid composition (% of total fatty acids) in longissimus dorsi muscle of lamb.

Proximate composition	**FG**	**BML**	**BLL**	**BLH**	**s.e.d**	**P-value**
Moisture	762^{a}	750^{b}	749^{b}	755^{ab}	4.763	0.046
CP[1]	209	212	217	209	4.440	0.279
Ash	16.8	17.9	17.6	19.7	2.201	0.508
Total FA	16.3^{b}	24.2^{a}	20.6^{ab}	23.4^{a}	1.862	<0.001
Fatty acid % total fatty acid						
C10:0	0.11^{b}	0.13^{a}	0.12^{a}	0.13^{a}	0.006	<0.001
C12:0	0.19	0.2	0.17	0.18	0.022	0.728
C14:0	2.02^{b}	2.42^{a}	2.13^{ab}	2.31^{ab}	0.123	0.008
C15:0	0.49^{a}	0.30^{b}	0.30^{b}	0.31^{b}	0.016	<0.001
C15:1	0.09^{b}	0.11^{ab}	0.13^{a}	0.12^{a}	0.008	<0.001
C16:0	17.2^{c}	21.0^{a}	19.6^{b}	20.1^{ab}	0.365	<0.001
C16:1*n*-9	1.21^{a}	0.98^{b}	1.06^{b}	1.08^{ab}	0.052	<0.001
C17:0	1.01^{c}	1.59^{a}	1.42^{b}	1.46^{ab}	0.051	<0.001
C18:0	17.4^{a}	13.0^{b}	13.2^{b}	13.9^{b}	0.400	<0.001
C18:1*n*-9c	28.5^{c}	36.0^{a}	32.5^{b}	32.4^{b}	0.582	<0.001
C18:1*n*-9t	3.84^{ab}	3.12^{b}	4.30^{a}	4.20^{ab}	0.411	0.024
C18:1*n*-11t	1.12^{b}	1.69^{a}	1.80^{a}	1.68^{a}	0.051	<0.001
C18: 2*n*-6c	4.84^{b}	5.89^{a}	6.50^{a}	6.03^{a}	0.318	<0.001
C18:3*n*-3	1.67^{a}	0.52^{c}	1.59^{ab}	1.50^{b}	0.061	<0.001
C20:4*n*-6	2.51^{a}	1.89^{b}	1.74^{b}	1.76^{b}	0.14	<0.001
C20:5*n*-3 (EPA)[2]	0.92^{a}	0.28^{c}	0.62^{b}	0.53^{b}	0.039	<0.001
C22:6*n*-3 (DHA)[3]	1.19^{a}	0.59^{c}	0.92^{b}	0.82^{b}	0.048	<0.001
cis-9,trans-11 CLA[4]	0.61^{a}	0.25^{b}	0.26^{b}	0.24^{b}	0.042	<0.001
trans-10,cis-12 CLA	0.07^{ab}	0.06^{b}	0.08^{a}	0.07^{ab}	0.007	0.02
Σ SFA	38.4^{a}	38.7^{a}	36.9^{b}	38.4^{a}	0.531	0.007
Σ MUFA	34.8^{c}	41.9^{a}	39.8^{b}	39.5^{b}	0.514	<0.001
Σ PUFA	11.8^{a}	9.51^{b}	11.7^{a}	11.0^{a}	0.518	<0.001
RFA[5]	14.7^{a}	9.94^{c}	11.6^{b}	11.16^{b}	0.379	<0.001
ITFA[6]	85.0^{c}	90.1^{a}	88.4^{b}	88.8^{b}	0.379	<0.001

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E, [1]Crude protein, [2]Eicosapentaenoic acid, [3]Docosahexaenoic acid, [4]Conjugated linoleic acid, [5]Remaining fatty acid, [6]Identified total fatty acid, [a, b, c] Means in a row with the same superscript are not different (P>0.05).

Table 4. 8. Effect of dietary treatment on the fatty acid composition (mg/100g) in the longissimus dorsi muscle.

Fatty acids	**FG**	**BML**	**BLL**	**BLH**	**s.e.d.**	**P-value**
C10:0	1.76^{c}	3.18^{a}	2.52^{b}	2.95^{ab}	0.213	<0.001
C12:0	2.99^{b}	4.66^{a}	3.54^{ab}	4.22^{ab}	0.506	0.008
C14:0	32.7^{c}	58.2^{a}	43.8^{b}	53.9^{a}	3.660	<0.001
C15:0	7.92^{a}	7.15^{ab}	6.12^{b}	7.24^{ab}	0.541	0.015
C15:1	1.45^{b}	2.64^{a}	2.60^{a}	2.68^{a}	0.120	<0.001
C16:0	284^{c}	508^{a}	406^{b}	475^{ab}	28.1	<0.001
C16:1*n*-9	19.9^{b}	23.9^{ab}	21.8^{ab}	25.3^{a}	1.980	0.044
C17:0	16.5^{c}	38.3^{a}	29.6^{b}	34.7^{ab}	2.218	<0.001
C18:0	286	316	271	330	24.3	0.069
C18:1*n*-9c	471^{c}	874^{a}	675^{b}	769^{ab}	50.3	<0.001
C18:1*n*-9t	64.5^{b}	75.7^{ab}	86.4^{ab}	95.7^{a}	10.430	0.024
C18:1*n*-11t	12.3^{c}	39.1^{a}	32.0^{b}	34.2^{b}	1.779	<0.001
C18: 2*n*-6c	74.5^{b}	140.1^{a}	131.4^{a}	135.3^{a}	5.948	<0.001
C18:3*n*-3	26.3^{b}	12.5^{c}	32.1^{a}	34.6^{a}	1.417	<0.001
C20:4*n*-6	38.4^{b}	44.5^{a}	35.4^{b}	38.9^{b}	1.596	<0.001
C20:5*n*-3 (EPA)[1]	14.2^{a}	6.7^{c}	12.4^{b}	12.0^{b}	0.561	<0.001
C22:6*n*-3 (DHA)[2]	18.29^{a}	13.79^{b}	18.48^{a}	18.35^{a}	0.572	<0.001
cis-9, trans-11 CLA[3]	7.60^{a}	5.83^{ab}	4.51^{b}	5.77^{ab}	1.005	0.029
trans-10, cis-12 CLA	0.81^{c}	1.80^{a}	1.09^{bc}	1.46^{ab}	0.148	<.001
RFA[4]	246	240	242	258	8.57	0.141
ITFA[5] (mg/100gm)	1627^{b}	2416^{a}	2057^{ab}	2340^{a}	186.2	<0.001

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E, [1]Eicosapentaenoic, [2]Docosahexaenoic acid, [3]Conjugated linoleic acid [4]Remaining fatty acid, [5]Identified total fatty acid $^{a, b, c}$ Means in a row with the same superscript are not different (P>0.05).

4.3.9. Fatty acid composition of adipose tissue

The FA composition of the subcutaneous adipose tissue expressed as % of total FA and FAs content as g/kg tissue are presented in Table 4.9. There was an effect (P<0.001) in the total FA content of adipose tissue, with the lowest value being for lambs fed diet FG, compared to those fed diets BML, BLL or BLH. In general, the adipose tissue of lambs fed the concentrate diets (BML, BLL or BLH) had a higher (P<0.001) percentage of C16:0, C17:0, C18:1*n*-6, C18:1*n*-11t, C18:2*n*-6c and C20:4*n*-6, and a lower (P<0.001) of C18:0 and cis-9, trans-11 CLA compared to those fed FG. Concentrate diet had no effect (P>0.001) on the adipose tissue percentage of C12:0, C14:0, C16:1*n*-9, C17, C18:1*n*-9c, C18:1*n*-9t and C18:2*n*-6c. The percentage of C18:2*n*-6t, EPA and DHA increased and C20:4*n*-6 decreased in adipose tissue from lambs fed BLL or BLH compared to BML. Similarly, the adipose tissue of lambs fed diet BLL had a lower percentage (P<0.001) of C16:0 and C18:0 compared to that of lambs fed diet BML. The total SFA content of adipose tissue from lambs fed any of the three concentrate diets (BML, BLL and BLH) was lower (P<0.001) than that of lambs fed FG. In contrast, the total PUFA increased in adipose tissue in lambs fed diets BLL or BLH compared to those fed BML or FG.

Table 4. 9. Effect of dietary treatments on the fatty acid composition (% of total fatty acids and total fat content g/kg) in the subcutaneous adipose tissue of lambs.

Fatty acids	**FG**	**BML**	**BLL**	**BLH**	**s.e.d.**	**P-value**
C10:0	0.18	0.19	0.18	0.20	0.015	0.701
C12:0	0.39^{a}	0.23^{ab}	0.17^{b}	0.18^{b}	0.067	0.005
C14:0	3.77^{a}	3.00^{ab}	2.70^{b}	3.11^{ab}	0.395	0.05
C15:0	0.93^{a}	0.52^{c}	0.60^{bc}	0.67^{b}	0.041	<0.001
C16:0	18.5^{c}	22.6^{a}	20.4^{b}	21.6^{ab}	0.535	<0.001
C16:1*n*-9	2.06^{b}	2.08^{ab}	2.40^{ab}	2.42^{a}	0.140	0.008
C17:0	0.97^{b}	1.31^{a}	1.34^{a}	1.41^{a}	0.069	<0.001
C18:0	24.1^{a}	16.3^{b}	14.3^{c}	14.96^{bc}	0.700	<0.001
C18:1*n*-9c	24.1^{c}	33.8^{a}	30.5^{ab}	27.6^{bc}	1.420	<0.001
C18:1*n*-9t	9.31^{a}	5.52^{b}	7.0^{ab}	6.21^{ab}	1.220	0.015
C18:1*n*-11t	0.61^{c}	1.13^{a}	1.05^{ab}	0.99^{b}	0.052	<0.001
C18: 2*n*-6c	1.03^{b}	3.02^{a}	3.19^{a}	2.80^{a}	0.156	<0.001
C18: 2*n*-6t	0.78^{b}	0.22^{c}	1.21^{a}	1.43^{a}	0.114	<0.001
C18:3*n*-3	0.94^{b}	0.38^{c}	1.34^{a}	1.40^{a}	0.052	<0.001
C20:4*n*-6	0.09^{c}	0.16^{a}	0.12^{b}	0.12^{b}	0.007	<0.001
C20:5*n*-3 (EPA)[1]	0.06^{b}	0.02^{c}	0.06^{b}	0.07^{a}	0.004	<0.001
C22:6*n*-3 (DHA)[2]	0.18^{a}	0.10^{b}	0.18^{a}	0.18^{a}	0.009	<0.001
cis-9, trans-11 CLA[3]	1.51^{a}	0.46^{b}	0.52^{b}	0.49^{b}	0.071	<0.001
trans-10, cis-12 CLA	0.04^{b}	0.05^{b}	0.08^{a}	0.05^{b}	0.006	<0.001
Σ SFA	48.9^{a}	44.2^{b}	39.6^{c}	42.1^{b}	0.89	<0.001
Σ MUFA	36.1^{c}	42.5^{a}	41.0^{ab}	37.2^{bc}	1.68	0.001
Σ PUFA	4.63^{b}	4.40^{b}	6.62^{a}	6.54^{a}	0.285	<0.001
RFA[4]	10.46^{bc}	8.89^{c}	12.82^{ab}	14.19^{a}	1.18	<0.001
ITFA[5] (g/kg tissue)	613^{b}	718^{a}	705^{a}	700^{a}	25.6	<0.001

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E, [1]Docosahexaenoic acid, [2]Eicosapentaenoic, [3]Conjugated linoleic acid, [4]Remaining fatty acid, [5]Identified total fatty acid, $^{a, b, c}$ Means in a row with the same superscript are not different (P>0.05).

4.3.10. Nutritional indices

The most important nutritional indices related to human health are shown in Table 4.10. Diet BML or BLH increased ($P<0.001$) the muscle SFA content compared to those fed diet FG, but not those fed diet BLL. Similarly, the total muscle MUFA content of lambs fed diets BML, BLL or BLH increased ($P<0.001$) compared to those fed FG. However, lambs fed BLL had a lower ($P<0.001$) muscle MUFA content than those fed diet BML, but not BLH. Diets BML, BLL or BLH, increased ($P<0.001$) muscle PUFA content compared to those fed diet FG. In addition, lambs fed BLH had a higher ($P<0.001$) muscle PUFA content compared to those fed BML. In term of P:S ratio, lambs fed diets BLL or BLH had a similar ratio compared to those fed FG, while, lambs fed diet BML had the lowest ($P<0.001$) ratio compared to those fed FG or BLH. In contrast, the *n*-6:*n*-3 and C18:2*n*-6: C18:3*n*-3 ratios were reduced ($P<0.001$) in lambs fed BLL or BLH compared to those fed diet BML, but were higher ($P<0.001$) than those fed diet FG.

Table 4. 10. Fatty acid classes (mg/100g) and ratios related to human health of longissimus dorsi muscle of lambs fed on grass and concentrate diets.

	FG	**BML**	**BLL**	**BLH**	**s.e.d.**	**P-value**
SFA	631^{c}	935^{a}	762^{bc}	908^{ab}	56.3	<0.001
MUFA	570^{c}	1015^{a}	818^{b}	927^{ab}	56.5	<0.001
PUFA	180^{c}	225^{b}	235^{ab}	246^{a}	8.02	<0.001
P:S[1]	0.31^{a}	0.25^{b}	0.32^{a}	0.29^{ab}	0.016	<0.001
n-6:*n*-3[2]	1.96^{c}	5.73^{a}	2.69^{b}	2.74^{b}	0.219	<0.001
C18:2*n*-6: C18:3*n*-3	2.91^{c}	11.41^{a}	4.12^{b}	4.01^{b}	0.377	<0.001

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E, [1]P:S= $\frac{\mathrm{PUFA}}{\mathrm{SFA}}$, [2]*n-6:n-3*= $\frac{18{:}2n-6+20{:}4n-6}{18{:}3n-3+20{:}5n-3+22{:}6n-3}$, $^{a,\,b,\,c}$ Means in a row with the same superscript are not different ($P>0.05$).

4.3.11. Vitamin E concentration of muscle

The vitamin E content of SM muscle from lambs fed different diets is shown in Figure 4.9. Lambs fed diet BLH had the highest ($P<0.05$) muscle concentration of vitamin E compared to those fed diets BML or BLL, but not diet G (5.36 vs 4.27, 4.12 and 4.48 mg/kg muscle, respectively). However, the vitamin E content of the SM from lambs offered diets BML or BLL had a similar ($P>0.05$) vitamin E content as those fed diet FG (4.27 and 4.12 vs 4.48 mg/kg muscle, respectively).

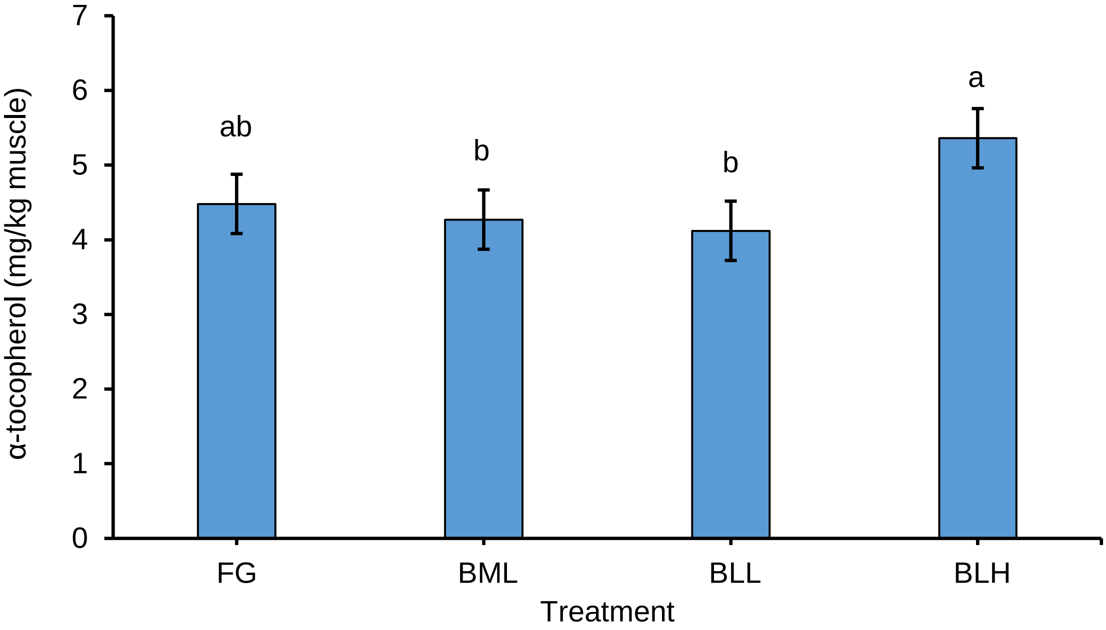


Figure 4. 9. Mean of α-tocopherol concentration in semimembranosus muscle from lambs fed FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E diets. (s.e.d=0.397, P-value=0.013). $^{a,\,b}$ *Means of dietary treatment with the same superscript are not different ($P>0.05$).*

4.3.12. Colour evaluation

4.3.12.1. Lightness

Repeated measure analysis showed an effect (P<0.001) of time on SM muscle lightness with values increasing from 43.8 at 2h to 47.4 at day 14 (Figure 4.10). There was also an effect (P<0.001) of treatment on SM lightness, lambs fed diet FG had darker muscle compared to those fed diets BML, BLL or BLH (43.5 vs 46.7, 46.7 and 45.9 respectively). There was no time x treatment interaction (P>0.05) on lightness values.

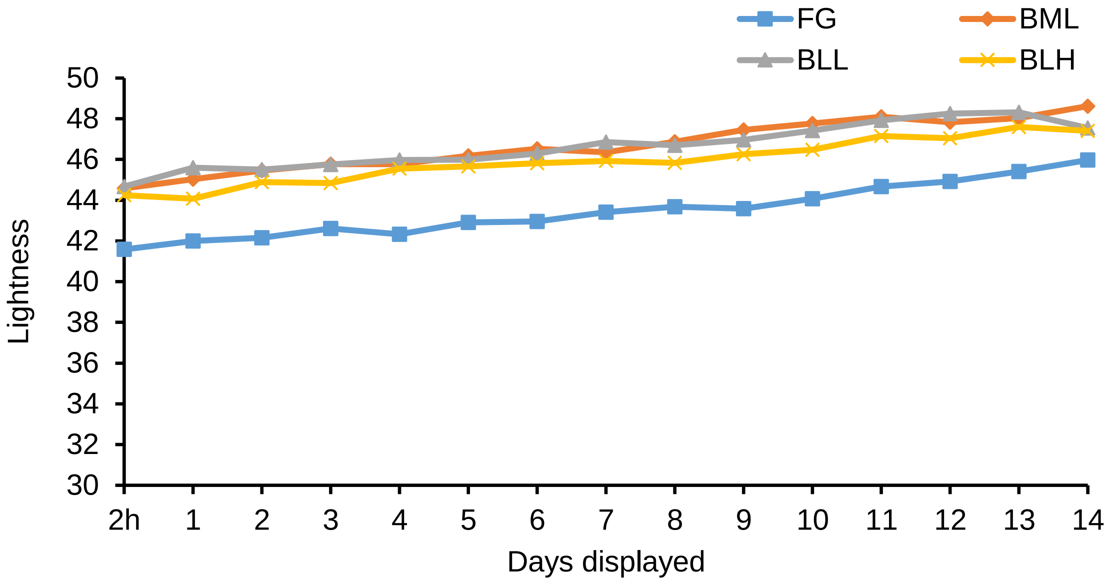


Figure 4. 10. Effect of time displayed on the lightness of MAP SM muscle from lambs fed different diets (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E). (s.e.d values: Treatment=0.619, Time=0.234, Inter=0.767, P-values: Treatment <0.001, Time <0.001, Inter=0.788).

4.3.12.2. Redness

Repeated measure analysis showed an effect (P<0.001) of time on SM muscle redness, which decreased over the period of the retail display from 20.1 at 2h to 12.9 at day 14 (Figure 4.11). There was also an effect (P<0.05) of dietary treatment on SM redness values, lambs fed diet FG had the lowest values compared to those fed diets BML or BLH, but not those fed diet BLL (16.5 vs 17.6, 17.5 and 17.2, respectively). There was a time x treatment interaction (P<0.05) on the SM muscle redness. Muscle redness values of lambs fed diets FG and BLL decreased more rapidly at day 9 and 11, respectively. At the end of retail display, the redness values of lambs fed diets FG or BLL were lower than those of lambs fed diets BML or BLH (10.7, 11.9 vs 14.3, 14.8, respectively).

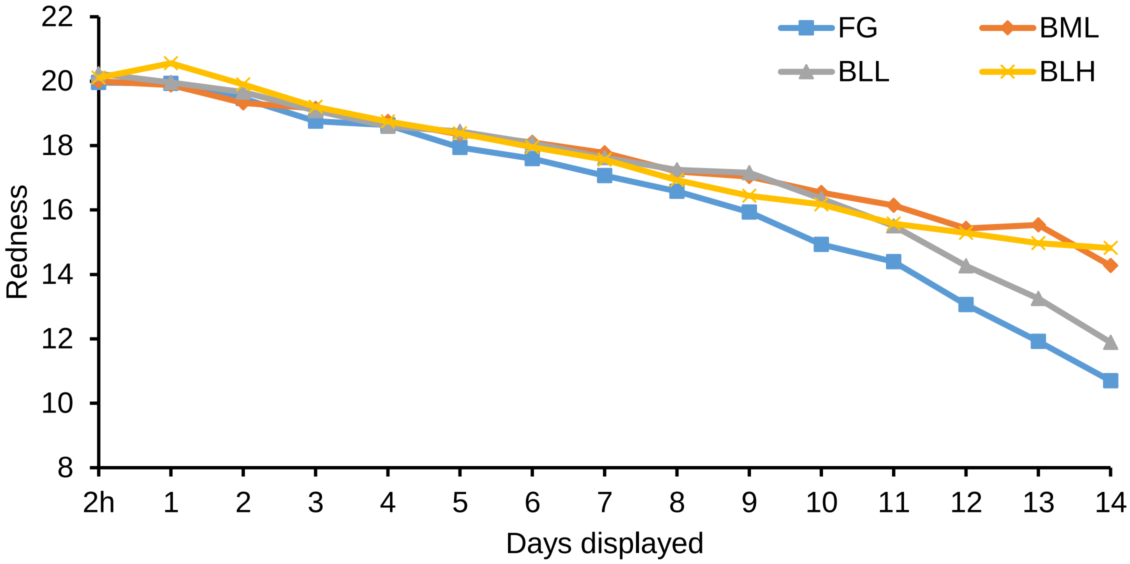


Figure 4. 11. Effect of time displayed on the redness of MAP SM muscle from lambs fed different diets (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E). (s.e.d values: Treatment=0.391, Time=0.251, Inter=0.624, P-values: Treatment=0.023, Time <0.001, Inter=0.005).

4.3.12.3. Yellowness

Repeated measure analysis showed an effect (P<0.001) of time on SM muscle yellowness values, which decreased over the period of the retail display from 10.9 at point 2h to 10.4 at day 14 (Figure 4.12). There was also an effect (P<0.001) of treatment on SM muscle yellowness values, with lambs fed diet FG having lower values compared to those fed diets BML, BLL or BLH (10.2 vs 10.7, 10.9 and 10.7, respectively). There was no time x treatment interaction (P>0.05) on yellowness.

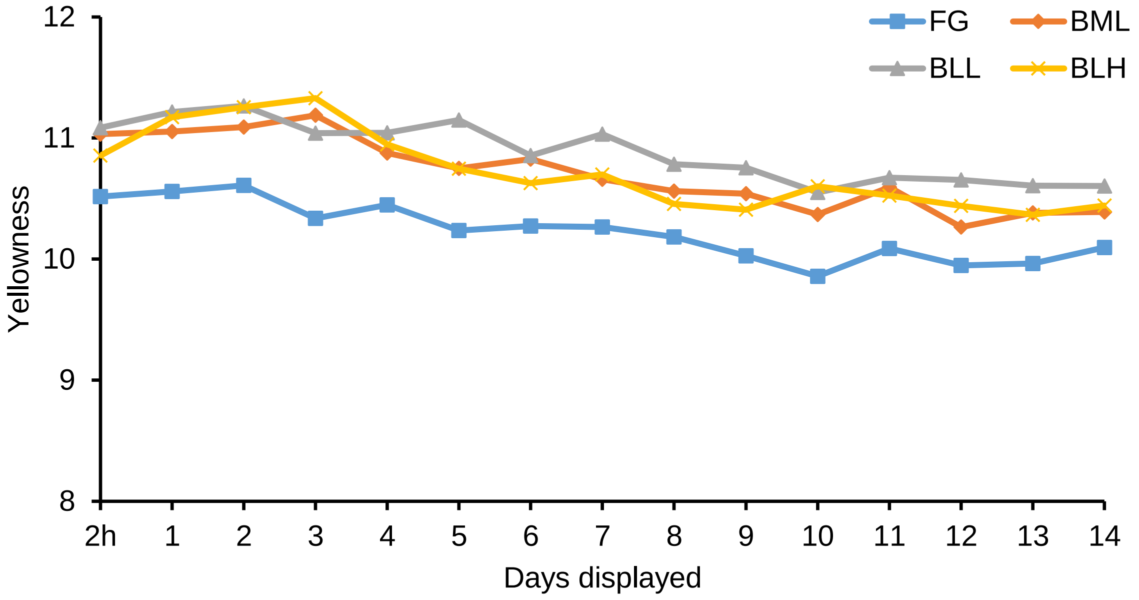


Figure 4. 12. Effect of time displayed on yellowness of MAP SM muscle from lambs fed different diets (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E). (s.e.d values: Treatment=0.163, Time=0.099, Inter=0.251, P-values: Treatment=<0.001, Time <0.001, Inter=0.938).

4.3.12.4. Saturation

Repeated measure analysis showed an effect (P<0.001) of time on SM muscle saturation, which decreased over the period of the retail display from 22.8 at 2h to 16.9 at day 14 (Figure 4.13). There was also an effect (P<0.05) of dietary treatment on SM muscle saturation during retail display, with muscle from lambs fed diet FG having a lower saturation mean compared to those fed diets BML, BLL or BLH (19.5 vs 20.6, 20.4 and 20.6 respectively). There was a time x treatment interaction (P<0.05) on SM muscle saturation value, with lambs fed diet FG declining more rapidly to reach a value of 18 at day 10, followed by lambs fed diet BLL at day 12, and lambs fed diets BML and BLH at day 14.

Shelf life in days for SM muscle from lambs fed different diets was determined using a saturation value of 18.0. Therefore, SM muscle from lambs fed BML or BLH had a greater shelf life by 4 days compared to FG samples and 2 days compared to BLL samples.

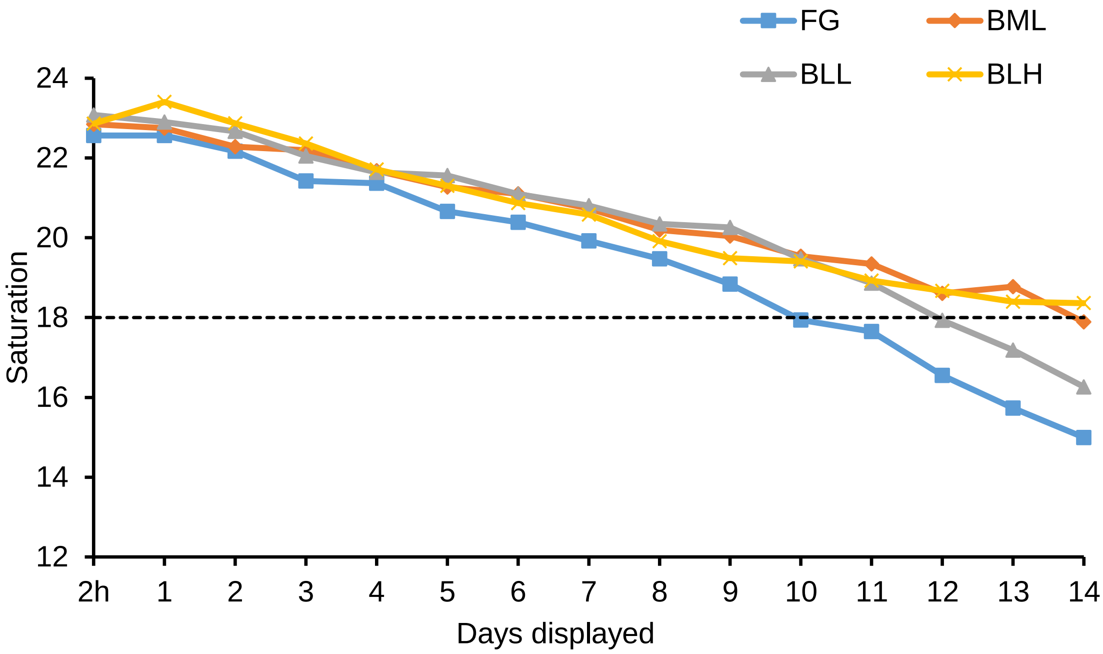


Figure 4. 13. Effect of time displayed on the saturation of MAP SM muscle from lambs fed different diets (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E). (s.e.d values: Treatment=0.334, Time=0.202, Inter=0.514, P-values: Treatment=0.003, Time <0.001, Inter=0.002).

4.3.12.5. Hue value

Repeated measure analysis showed an effect (P<0.001) of time on hue mean values of SM muscle which increased over the period of the retail display from 28.4 at point 2h to reach 40.5 at day 14 (Figure 4.14). There was no effect (P>0.05) of dietary treatments on hue unit with the SM muscle hue values of lambs fed diets FG, BML, BLL and BLH being 32.7, 31.7, 33.1 and 32.0, respectively.

There was, however a time x treatment interaction (P<0.05) on SM muscle hue unit values. There was a difference between lambs fed diets FG and BLL compared to those fed diets BML and BLH, starting at day 11 to increase and reach the highest value at day 14.

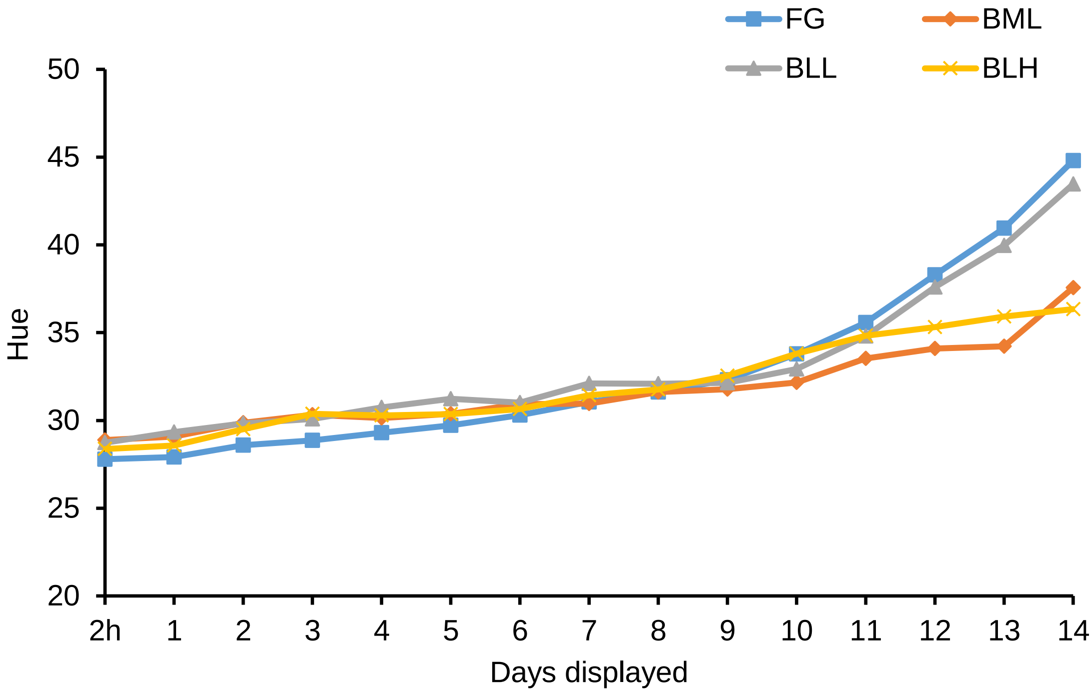


Figure 4. 14. Effect of time displayed on hue value of MAP SM muscle from lambs fed different diets (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E). (s.e.d values: Treatment=0.853, Time=0.641, Inter=1.503, P-values: Treatment=0.359, Time <0.001, Inter=0.03).

4.3.13. Lipid oxidation of muscle (TBARS)

The lipid oxidation results for SM muscles are presented in Figure 4.15. After 7 days of retail display, there was no (P>0.05) differences in the lipid oxidation of the SM muscle from lambs fed diet FG compared with those fed diets BML or BLL (2.59 vs 2.02 and 2.27 mg MDA/kg muscle, respectively). However, the SM muscle samples of lambs fed diet BLH had the highest (P<0.05) lipid stability (lowest lipid oxidation) compared with those fed diet FG (1.48 vs 2.59 mg MDA/kg muscle).

At day 14, lambs offered diets BML or BLH had the lowest (P<0.001) TBARS value compared to those fed diets FG (3.20 and 2.36 vs 5.78 mg MDA/kg muscle, respectively), whereas, lambs fed diet BLL had a similar lipid stability values to those fed diet FG (4.59 vs 5.78 mg MDA/kg muscle).

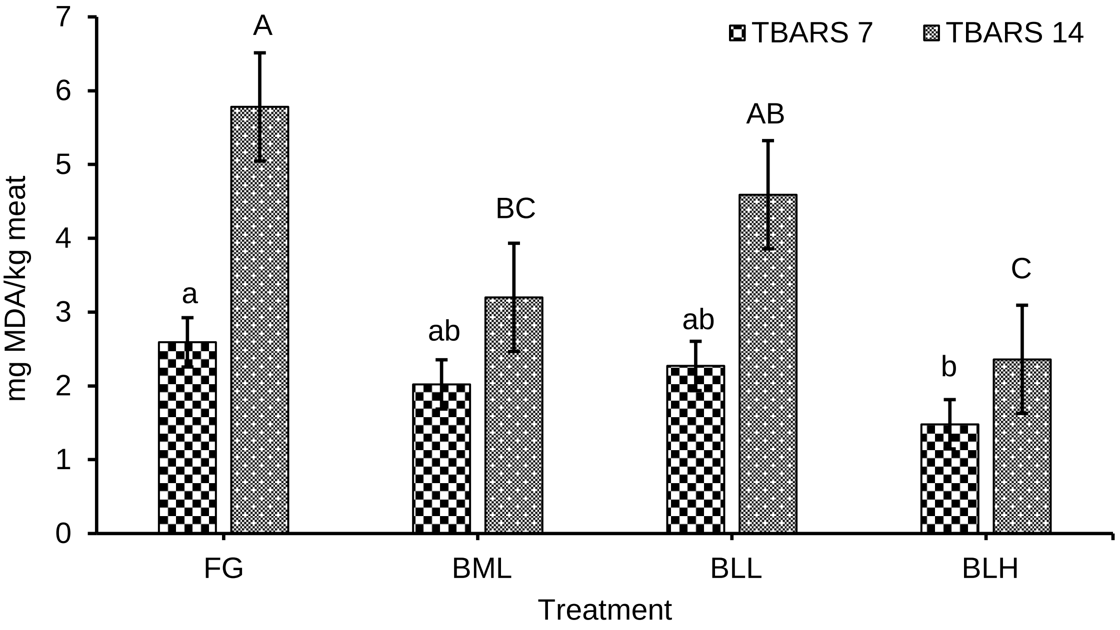


Figure 4. 15. Effect of dietary treatments (FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E) on TBARS (mg malonaldehyde/kg muscle) of SM at day 7 and 14 of simulated retail display in MAP. Similar lowercase letters at day 7 and capital letters at day 14 are not different (P>0.05).

4.3.14. Thawing loss, cooking loss and shear force

The results of thawing %, cooking % and shear (N) force for aged lambs LD muscles fed different diets are presented in Table 4.11. The thawing loss % of aged LD samples from lambs fed the concentrate diets (BML, BLL and BLH) was higher ($P<0.05$) than that of lambs fed diet FG. The required shear force of LD samples from lambs fed diet FG was also lower ($P<0.05$) than that of those fed concentrate diets.

Table 4. 11. Effect of dietary treatments on thawing loss %, cooking loss % and shear force (N) of aged LD muscle.

	FG	**BML**	**BLL**	**BLH**	**s.e.d.**	**P-value**
Thawing loss	7.21^{b}	8.73^{a}	8.16^{a}	8.77^{a}	0.589	0.047
Cooking loss	24.7	25.6	25.6	26.2	0.759	0.321
Shear force N	30.4^{b}	40.3^{a}	40.0^{a}	41.4^{a}	1.24	<0.001

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E, $^{a,\,b}$ Means in a row with the same superscript are not different ($P>0.05$).

4.3.15. Sensory evaluation

The effect of dietary treatment on the eating quality of oven cooked lamb LT muscle is shown in Table 4.12. There was no difference ($P>0.05$) in the sensory evaluation of the LT muscle of lambs fed different diets, although, numerically muscle from lambs fed diet FG had the highest score whereas muscle from lambs fed diet BLL had the lowest score in all the sensory perceptions.

Table 4. 12. Sensory analysis of lamb meat (evaluated on scale 1-9) of different diets.

	FG	**BML**	**BLL**	**BLH**	**s.e.d.**	**P-value**
Juiciness	6.27	6.13	5.66	6.28	0.347	0.247
Tenderness	6.58	6.12	5.90	6.15	0.378	0.337
Flavour	6.33	6.15	6.00	6.23	0.361	0.826
Overall acceptability	6.42	6.19	5.99	6.28	0.33	0.616

FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E, $^{a,\,b}$ Means in a row with the same superscript are not different ($P>0.05$).

4.4. Discussion

4.4.1. Feed analysis

The chemical composition (DM, CP, starch, vitamin E and GE) of the concentrate diets was similar to that predicted from published values (MAFF, 1992), although, the NDF content was slightly lower than predicted (275 vs 316 g/kg DM). In contrast, the WSC and total FA content of the concentrate diets were higher than the predicted values (112.0 vs 37.5 g/kg DM and 45.4 vs 36.1 g/kg DM, respectively). The variability in dietary FA composition reflected the different dietary fat sources. The inclusion of Megalac increased C16:0, C18:1 in diet BML, whereas the inclusion of linseed oil increased C18:3*n*-3 in diets BLL and BLH. Diet FG had a similar DM, NDF and vitamin E content to the predicted values, but a slightly lower CP, higher WSC, and total FA content. The WSC content of FG was higher than the normal range or expected value (MAFF, 1992). However, it is difficult to compare the result with published papers, as the WSC content of the grass is highly variable. Grass variety, environmental condition, seasonal variation, growth rate and maturity are the main factors affecting the WSC content of grass (Miller *et al*., 2001; Watts, 2008). The high content of WSC in the current study could be due to the seasonal variation where the value was at the peak of the beginning of the experiment (FG1, during May) compared to FG2 (June) and FG3 (July) when the values decreased gradually from 348 to 266 and 136 gm /kg DM, respectively. A similar results were reported by Miller *et al*. (2001) found that WSC was over 350 gm /kg DM during May for a high sugar ryegrass compared to the control 240 gm /kg DM (standard variety of perennial ryegrass).

4.4.2. Animal performance and carcass traits

Generally, lambs fed on the concentrate diets (BML, BLL and BLH) had a higher performance and heavier carcasses compared lambs fed diet FG as previously reported (Armero and Falagan, 2015).

Dietary vitamin E concentration had no effect on the animal performance and carcass characteristics of concentrate fed lambs. This result was expected as vitamin E supplementation above recommended levels (ARC, 1980; NRC, 1985; NRC, 2007) has been shown to have no effect on animal performance (Kasapidou *et al.,* 2009; Bellés *et al*., 2019). This is also consistent with the results of Lauzurica *et al.* (2005) who reported no significant differences in the performance of lambs fed diets supplemented with different vitamin E concentrations (0, 250, 500 and 1000 mg/kg). However, in the current study, although there was no significant difference in lamb performance, lambs receiving 500 mg/kg feed with linseed oil, had a numerically higher DLWG (10.8%), and reduced FCR and fattening period (14.3% and 5.66%, respectively) comparing with lambs received 250 mg/kg feed with linseed oil. In contrast, Macit *et al.* (2003) reported an 8.1 % improvement in feed conversion efficiency in lambs received 45 mg/day vitamin E compared to control (0 vitamin E supplementation). This improvement was related to the protection of the ruminal epithelium against the ulcerative action of rumen contents when lambs were fed with vitamin E supplemented diets. However, Belanche *et al.* (2016) reported that vitamin E supplementation as α-tocopheryl acetate vs α-tocopherol increased rumen fermentation, possibly as a result of its antioxidant effect leading to higher protozoal and bacteria levels and increased feed degradability by 8%.

Dietary fat source had also no effect on animal performance and carcass traits of concentrate fed lambs. The DLWG and DM intake of lambs that received Megalac with 250 mg/kg DM vitamin E were numerically higher than that of lambs received linseed oil with 250 mg/kg DM (0.41 kg/day and 1.48 kg/day vs 0.37 kg/day and 1.38 kg/day, respectively). This could be due to variation in the food digestibility between treatments, as the digestibility of DM and EE increased when sheep fed on diet contained calcium salt or Megalac (Bayourthe *et al.,* 1994). Another reason could be due to the toxicity of unsaturated FA, especially α-linoleic acid which has been shown to reduce protein yield by the suppression of microbial protein synthesis at the level of 3% (w/w) (Wang *et al.,* 2018).

4.4.3. Blood metabolites

Feed intake and diet composition can significantly influence blood metabolic profiles (Connell *et al.,* 1997). The mean blood plasma parameters in the current study were within the normal physiological range for sheep (Fraser *et al.,* 2004). Plasma total protein concentrations were similar between treatments, which can be used as an indicator of nutrient restriction in animals (Connell *et al.,* 1997). Protein intake can influence hepatic albumin synthesis, and therefore the low plasma albumin concentration in lambs fed diet FG, may have resulted from a lower absorption of amino acids due to the low concentration of CP in the grass samples compared to the concentrate diets (CP=143 g/kg DM vs CP=193 g/kg DM, respectively) (Yokus and Cakir, 2006). Similarly, the lower concentration of plasma urea found in lambs fed diet FG may have reflected the lower ammonia production due to the less protein degradation in the rumen (Harmeyer and Martens, 1980; Abdoun *et al.,* 2006).

Plasma glucose concentration was higher in lambs fed the concentrate diets compared to those on FG. This is consistent with the results for VFA, as a higher proportion of rumen propionate was recorded in lambs fed the concentrate diets. Propionate is considered to be the main substrate for gluconeogenesis, increasing blood glucose concentration in ruminants (Priolo *et al.,* 2002). A higher concentration of plasma BHB was also found in lambs fed BML which may have resulted from the higher rumen butyrate concentration (Normand *et al.,* 2001). Butyrate produced in the rumen can be absorbed across the rumen wall and converted into BHB (McDonald *et al.,* 2011). Circulating plasma NEFA concentration are derived from adipose tissue release and could be used an indicator for adipose tissue mobilisation (Mears and Mendel, 1974). Generally, the level of plasma NEFA concentration was within the normal range, although in lambs fed FG it was higher compared to those fed diets BLL and, as previously reported, concentrate supplementation leads to a depressive effect on plasma NEFA concentration (Joy *et al.,* 2007*)*.

4.4.4. Rumen volatile fatty acid

In the current study, the TVFA concentration was higher in lambs fed diet FG compared to those fed the concentrate diets (269.4 mmol/l vs 98.5 mmol/l respectively). Although, the TVFA concentration is normally between 60-150 mmol/l, the value can rise to 200 mmol/l when animals graze fresh grass or when fed on starch based diets (Bergman, 1990). This is because the pattern and amount of VFA production depends mainly on diet composition and time after feeding (Jouany *et al.,* 2000; Dijkstra *et al.,* 2012). Therefore, a high concentration of TVFA in lambs fed diet FG is possibly due to the feeding time, as lambs fed fresh grass had free access to grass until an hour before slaughter, while lambs fed the concentrate diets had their last meal in the afternoon of the day before being slaughtered.

The increased ruminal acetate and decreased ruminal propionate proportion in lambs diet FG and vice versa in lambs fed the concentrate diets is consistent with the results of Suárez *et al.* (2006) and Jiang *et al.* (2017). It has generally been reported that diets rich in starch such as cereal grains favour propionate production and less acetate, and the rapid fermentation of starch lowers rumen pH and encourages the growth of microorganisms that produce both propionate and lactate (Hungate *et al.,* 1997).

Concentrate fat source had no effect on TVFA production. Similarly, Chikunya *et al.* (2004) reported that Megalac and formaldehyde treated linseed supplementation had no effect on fermentation rate and TVFA production in the ruminal fluid of lambs. In other studies, unsaturated FA has been found to have a greater inhibitory influence on rumen fermentation than SFA (Palmquist and Jenkins, 1980; Maia *et al.,* 2010). Unsaturated FAs have also been reported to have a toxic effect on cellulolytic microbes (*Butyrivibrio fibrisolvens*) (Maia *et al.,* 2007) through impairing the permeability of microbial cell cytoplasmic membranes (Jenkins *et al.,* 2008). According to Maia *et al.* (2010) PUFA, especially α-linoleic acid rich linseed oil, had the greatest influence on rumen disturbance. This could be the reason for lambs fed linseed oil having a higher ruminal acetate proportion compared to Megalac.

The current study showed an effect of fat source on acetate and butyrate proportion, as Megalac supplementation decreased acetate and increased butyrate proportions compared to linseed oil supplementation. Linseed oil supplementation has been found to increase propionate concentration at the expense of acetate or butyrate especially at higher levels of supply >50 g/kg DM linseed oil (Doreau *et al.,* 2009). However, both Megalac and linseed oil supply had no effect on propionate proportions because starch digestibility is not affected by lipid source (Bock *et al.,* 1990). Jouany *et al.* (2000) reported that saturated and monounsaturated fatty acids could decrease cellulolytic bacteria and change the pattern of VFA production (Machmüller *et al.,* 2000; Nowak and Potkanski, 2011). A well-accepted action of lipids on carbohydrate metabolism is a reduction in organic matter digestibility, especially when the diets are rich in PUFA (Doreau and Chilliard, 1997). This effect is more pronounced for fibre digestibility at the ruminal level (Palmquist and Jenkins, 1980; Oldick and Firkins, 2000), while starch digestibility is less affected (Bock *et al.,* 1990).

4.4.5. Carcass proximate and fatty acids content

The protein and ash content of the LD muscle was similar between dietary treatments. However, the moisture content was higher and the lipid content lower in LD muscle of lambs fed FG compared to lambs offered the concentrate diets. Similar results have been reported by Rowe *et al.* (1999) and Matsushita *et al.* (2010), who stated that muscle moisture was negatively correlated with lipid content in both (pasture and concentrate finishing) system. In addition, a lower fat content has frequently been reported in muscle from lambs offered grass compared to concentrates (Realini *et al*., 2004). This is mainly attributed to the lower energy availability to animals offered grass compared to concentrate diets (De Brito *et al.,* 2017).

Dietary treatment had an effect on the FA composition of the LD muscle. Muscle from lambs fed the concentrate diets containing either Megalac or linseed oil had a higher content of C16:0, C18:2, MUFA and particularly C18:1*n*-9c. This could be due to the concentrate diets having a higher concentration of these FAs, resulting in a proportional increase in muscle FA concentration (Aurousseau *et al.,* 2004; Sinclair, 2007; Boughalmi and Araba, 2016).

Fat source also had an effect on the FA composition of intramuscular fat. Compared to Megalac feeding, linseed oil increased muscle C18:3*n*-3 by 3 fold and even to a higher level than the FG group when based on mg/100g muscle. Similarly, the long chain FAs (DHA, EPA) and Σ PUFA were increased when lambs fed diets containing linseed oil. As expected, lambs were fed diets enriched diets with *n*-3 PUFA, have higher proportions of PUFA in muscle compared to control (Noci *et al.,* 2011). A similar trend in the FA profile has been found in the muscle of lambs fed diets that have included extruded linseed (Realini *et al.,* 2017; Facciolongo *et al.,* 2018) whole linseed (Wachira *et al.,* 2000) or linseed oil (Gallardo *et al.,* 2015). According to the literature, the PUFA content in lamb muscle can be increased by linseed (oil or seed), marine algae and fish oil, since the former is rich in C18:3*n*-3, and the rest are rich in long chain PUFA (DHA and EPA) (Cooper *et al.,* 2004; Fuente-Vázquez *et al.,* 2014). However, fish oil and marine algae are more effective at increasing long chain

PUFA, although lambs fed diets enriched with C18:3*n*-3 have increased long chain PUFA in lamb muscle (Wachira *et al.,* 2000; Cooper *et al.,* 2004; Andrés *et al.,* 2014). As a result of the elongation and desaturation of the *n*-3 family FAs (C18:3*n*-3) by the enzymes elongase, and Δ-5 and Δ-6 desaturases at the expense of the *n*-6 family FAs (Daley *et al.,* 2010), the concentration of C20:4*n*-6 in the muscle of lambs fed diet BM was decreased (Brenner *et al.*, 1989; Raes *et al.,* 2004).

Ruminant meat contains CLA which is produced in the rumen as an intermediate of dietary PUFA biohydrogenation and in tissue by Δ-9 desaturation of C18:1*n*-t11(Palmquist *et al*., 2005). Muscle conjugated linoleic acid (cis-9, trans-11) was lower in lambs fed concentrate diets compared to those off grazed grass, which confirmed the results of other reports in meat (French *et al*., 2000; Realini *et al*., 2004; Hajji *et al*., 2016). Concentrate fat sources had no effect on muscle CLA concentration. However, Wachira *et al*. (2002); Demirel *et al*. (2004b) reported an increase in CLA muscle concentration when lambs were supplied with whole linseed oil in a dried grass diet compared to the control (Megalac). In addition to the dietary fat source, ruminal conditions have also been reported to have a significant effect on the biohydrogenation process (Palmquist *et al*., 2005). Diets rich in starch can cause an inhibition to *Butyrivibrio fibrisolvens* that is responsible for the synthesis of cis-9,trans-11 CLA due to a reduction in ruminal pH (Loor *et al*., 2004; Lourenço *et al*., 2010; Bauman *et al*., 2016). French *et al*. (2000) reported an increase in beef CLA concentration when the grass to concentrate ratio increased at a similar dietary intake of C18:2. This suggests that dietary PUFA with basal diet have a role to increase meat CLA concentration. Thus, a decrease in cis-9, trans-11 CLA could be due to a reduction in the biohydrogenation rate of C18:2*n*-6 and/or decrease in tissue desaturation of trans-11 C18:1.

The FA profile of adipose tissue varied between the dietary treatments, as lambs fed FG had an increase in SFA (mainly C12:0, C14:0 C15:0 and C18:0), C18:3*n*-3, DHA and EPA and a reduction in C18:1*n*-9c and C20:4*n*-6 compared to lambs finished on the concentrate diets. This trend in FA profile was similar to that reported for animals finished on grass (Nuernberg *et al.,* 2005; Nuernberg *et al.,* 2008; Lind *et al.,* 2009). This could be due to the passage rate of food as forage is known to increase ruminal activity and increase biohydrogenation of FA, resulting in an increase in the concentration of SFA (Díaz *et al.,* 2003).

Fat sources also had an effect on FA composition of adipose tissue, especially C18:2*n*-6t, C18:3*n*-3, C20:4*n*-6, DHA and EPA. Diets rich in C18:3*n*-3 (BLL and BLH) increased C18:3*n*-3, DHA and EPA and decreased C20:4*n*-6 concentrations in adipose tissue compared to those fed Megalac, as previously reported by Noci *et al.* (2011). It has been reported that DHA and EPA can be synthesised from C18:3*n*-3 by a series of desaturase and elongase enzymes (Raes *et al.,* 2004). A reduction in C20:4*n*-6 and increase in C18:2*n*-

6t have been frequently observed in the muscle and adipose tissue FA acid profiles, as C18:2*n*-6 is considered a precursor for C20:4*n*-6 synthesis in ruminants (Brenner, 1989).

4.4.6. Nutritional indices

The P:S and *n-6:n-3* ratios are considered to be important indices of the nutritional quality of meat (Scollan *et al*., 2001; Wood *et al*., 2008). Generally, lamb meat has a high SFA concentration and a low P:S ratio (Sinclair, 2007; De Brito *et al.,* 2017). Saturated FA and MUFA increase in meat in accordance with the meat fat content. The higher the fat content, the higher the SFA and MUFA content (neutral lipids). Despite the fat source, Megalac and linseed oil inclusion increased muscle PUFA content compared to lambs fed FG.

The inclusion of either *n*-3 or *n*-6 PUFA in the diet can improve muscle P:S ratio in ruminants (Cooper *et al.,* 2004; Fuente-Vázquez *et al.,* 2014). This was also reported in the current experiment, mainly by the inclusion of linseed oil, although the P:S ratio for all groups was lower than the recommendation (0.45) (Department of Health, 1994). The *n*-6:*n*-3 ratio was also influenced by the diet FA composition (Wachira *et al.,* 2002; Facciolongo *et al.,* 2018). In the current experiment, the lower diet ratio of *n*-6:*n*-3 in the diets (BLL or BLH) decreased the *n*-6:*n*-3 ratio in muscles to the value below the maximum recommended value (4) of Health Department, (1994).

4.4.7. Vitamin E concentration of muscle

The vitamin E concentration in the muscle of lambs fed diet FG was similar to that of those supplemented with either 250 or 500 mg/kg DM dietary vitamin E although, the vitamin E concentration of fresh grass was lower than the concentrate diets. Jose *et al.* (2016) reported that muscle vitamin E concentration was similar between lambs grazed on pasture (112 mg/kg DM of α- tocopherol) and lambs fed a concentrate diet (360 mg/diet of α-tocopherol acetate). Moreover, Kasapidou *et al.* (2012) found a higher muscle concentration of vitamin E in lambs fed grass silage compared to those fed concentrate diets with either 60 mg/kg DM or 500 mg/kg DM of all-rac-α-tocopheryl acetate. Therefore, similar muscle concentrations of vitamin E can be obtained by a lower supply of natural vitamin E compared with synthetic vitamin E. This may be due to differences in the efficiency of vitamin E utilisation from forage diet and synthetic source into animal tissues. The affinity of α-tocopherol transfer protein (α-TTP) in the liver is predominant toward the naturally occurring form of vitamin E (Hosomi *et al.,* 1997). Natural vitamin E appears only as RRR- α-tocopherol, which has the highest bioavailability, whereas, synthetic vitamin E is a mixture of eight stereoisomers of α-tocopherol, among which 2R presents a high bioavailability and 2S low bioavailability (Burton *et al.,* 1998). On the other hand, digestion of vegetal lipids can increase natural vitamin E absorption as it located within forage lipids (Bellés *et al.,* 2019).

In the current study, concentrate vitamin E concentration increased muscle concentration. The deposition of α-tocopherol in muscle depends mainly on the level of supplementation (Wulf *et al.,* 1995; Turner *et al.,* 2002; Kasapidou *et al.,* 2012; Jose *et al.,* 2016). The LD muscle α-tocopherol concentration increased quadratically as dietary vitamin E concentration increased from 13.5 mg/kg DM to 270 mg/kg DM (Turner *et al.,* 2002). In addition, muscle vitamin E concentration was increased 2 and 3 times when lambs were supplemented with either 135 or 360 mg/day vitamin E over a 6 week period compared to those that received 27 mg/day vitamin E (Jose *et al.,* 2016). Thus, a linear increase in the deposition of α-tocopherol was observed through the feeding period, with the slope being dependent on the level of vitamin E supplementation (Álvarez *et al*., 2008).

Similar muscle α-tocopherol concentrations can be obtained with different supplementation levels. Wulf *et al.* (1995) reported 5.9 mg/kg muscle α-tocopherol concentration by supplementation 450 mg/lamb/day over a 56 day period. However, over a similar period, 5.48 mg/kg muscle α-tocopherol was reported when 360 mg/kg diet was supplied (Jose *et al.,* 2016). On the other hands, Turner *et al.* (2002) recorded 4.19 mg/kg muscle α-tocopherol by supplementation of 270 mg/kg DM over 70 days. In contrast, increasing the rate of supplementation to 900 mg/kg diet for 14 days, increased muscle α-tocopherol to 3.91 mg/kg with a reduced period of supplementation (Bellés *et al.,* 2018).

4.4.8. Shelf life

The SM muscle from lambs fed FG had darker meat and a lower value for yellowness (b*) compared to those finished on the concentrate diets. These results were expected as animals finished on forage generally have darker meat compared to those finished on concentrates (Priolo *et al.,* 2001). Nuernberg *et al.* (2005) suggested that animals fed on grass have a higher myoglobin concentration due to the higher physical activity compared to indoor finished animals. A positive correlation was found between oxidative fibres and meat lightness, with darker meat for bulls fed grass compared to those fed concentrates (Vestergaard *et al*., 2000). However, Priolo *et al*. (2001) after reviewing 35 experiments on the effect of production system on meat colour, reported that several factors are responsible for the differences, among them ultimate pH and intramuscular fat played a major role. In the current study, there was no differences in muscle ultimate pH, but there was a significant difference in intramuscular fat content with lambs fed diet FG having the lowest fat content. The lower fat content, the lower lightness value, as fat is lighter in colour than muscle.

Both fat source and dietary vitamin E concentration had an effect on muscle redness, saturation and Hue unit values during retail display, particularly after 9 days of retail display. As expected, Megalac inclusion (BML) and high dietary vitamin E (BLH) with linseed oil resulted in an improvement in redness stability and extended colour shelf life by 4 and 2 days compared to lambs fed diets FG or BLL respectively. After 12 days of retail display, lambs fed diets FG or BLL had the highest Hue unit values (degree of brownness) compared to those fed diets BML or BLH. The SM muscle TBARS values of lambs fed diets BML and BLH after day 14 of retail display were lower compared to those in lambs fed diets FG or BLL. Although, muscle lipid oxidation was significantly increased during the period of retail display (day 7 vs day 14), the SM muscle TBARS values of either day 7 or day 14 of retail display were within the normal range (4.2- 7.5 mg MDA/kg muscle) as previously reported by Berruga *et al*. (2005).

An increase in muscle α-tocopherol concentration with high dietary vitamin E (BLH) supplementation could contribute to extending the colour shelf compared to BLL, which was similar to the previous reports (Wulf *et al*., 1995; Jose *et al*., 2016). Although, the concentration of muscle α-tocopherol was significantly lower in muscle samples from lambs fed diet BML, the muscle long chain PUFA (EPA and DHA) and P:S ratio were also low. Ponnampalam *et al*. (2012) found meat redness and *n*-3, *n*-6 and PUFA are negatively correlated. The higher PUFA in muscle tissue results to a higher level of myoglobin oxidation and deterioration in colour stability that is initiated through lipid oxidation during retail display (Faustman *et al*., 2010).

4.4.9. Thawing loss, cooking loss and shear force

Neither fat source nor vitamin E level had an effect on thawing loss, cooking loss and shear force. The increased thawing loss from the LD muscle of concentrate fed lambs compared to grass, could be due to their growth rate. High growth rate increases myofibrillar density leading to changes in the intra myofabrilla space (Bertram and Andersen, 2007). This is expected to influence the inter and intra myofabrillar myowater properties (Pearce *et al*., 2011). Bertram *et al*. (2002) reported that increased protein deposition increased water muscle content, as high growth rates increased the proportion of glycolytic fibres (Maltin *et al*., 2003) (Dransfield and Sosnicki 1999). Glycolytic fibres have a higher extra myofibrillar fluid space than oxidative fibres (Polak *et al*., 1988). Thus, protein degradation by proteolysis enzyme during ageing (Pringle *et al*., 1993) might have reduced water retention in muscle.

The shear force results were within the range of tenderness value as above 49 N is considered tough (Perry *et al*., 2001; Hopkins *et al*., 2006). Muscle tenderness varies and depends on a number of intrinsic factor such as animal species (Maltin *et al*., 2003), sex (Dransfield, 1994), age (Hopkins *et al*., 2001) and muscle type (Starkey *et al*., 2016), and extrinsic factors such as electrical stimulation (Geesink *et al*., 2011) and ageing period (Starkey *et al*., 2016). The variation in tenderness is mostly due to the change in collagen content and solubility (a myofibrillar and cytoskeletal protein) (Pearce *et al*., 2011). Hopkins *et al*. (2005) reported higher shear force values for topside muscle from animals reared on a low plane of nutrition compared to those reared on a high plane of nutrition (Mandell *et* *al*., 1998) due to the high content of insoluble collagen (Sañudo *et al*., 1998; Díaz *et al*., 2002). Thus, low growth rate animals are older at slaughter weight and produce less tender meat (Young and Braggins, 1993). Based on this, it is surprising that the muscle from lambs fed diet FG had a lower shear force value compared to those finished on the concentrate diets, although, they were older (fattening period 84 vs 47 days, respectively). The reason could be due to ageing as previously found that tenderisation is more intense in the older animals because of the increased action of protease enzymes (calpains) during ageing (Pringle *et al*., 1993).

4.4.10. Sensory evaluation

The results of the sensory evaluation study undertaken as part of the current experiment showed no difference between treatments for sensory attributes, although, the muscle of lambs fed diet FG received numerally higher sensory scores. Diets influence muscle FA content and composition (Nuernberg *et al*., 2008; De Brito *et al*., 2017) and consequently, this could affect meat juiciness and flavour (Young *et al.,* 1997; Nute *et al*., 2007; Wood *et al*., 2008). Previous studies (Sañudo *et al*., 1998; Fisher *et al*., 2000; Nute *et al*., 2007; Font i Furnols *et al*., 2009) found that lambs finished on pasture had a stronger flavour and overall liking compared to those finished on concentrate diets. Similarly, Priolo *et al*. (2002) and Resconi *et al*. (2009) reported that lambs finished on pasture had a higher flavour intensity but a lower overall liking.

In the current study, despite the FA composition and total fat content of LD muscle were varying between treatments, consumers did not detect a stronger flavour intensity from grazed lamb meat, and this could be due to testing samples by a lower consumer panel numbers. In addition, meat samples were offered without subcutaneous fat, as known this is to be the main source of aroma and flavour compounds (Young *et al*., 1997).

4.5. Conclusion

Lambs fed concentrate diet had higher animal performance than those finished on grass. Concentrate fat source and vitamin E levels had no effect on lamb performance, although, lambs finished on BLH tended to have a reduced feed conversion ratio compared to those fed BLL diet.

Compared to lambs fed diet BML (Megalac), the C18:3*n*-3, C20:5*n*-3 (EPA) and C22:6*n*-3 (DHA) content of lambs fed diets BLL or BLH were increased, although, cis-9, trans-11 CLA in lamb muscle remained low. Similarly, the *n*-6: *n*-3 and C18:2*n*-6: C18:3*n*-3 ratios were improved by inclusion of linseed oil.

Supplementation with 500 mg/kg DM vitamin E and linseed oil (BLH) or 250 mg/kg DM vitamin E with Megalac (BML) in the diet significantly enhanced shelf life (colour and lipid stability) of SM muscle compared to those supplied with 250 mg/kg DM vitamin E with linseed oil (BLL) or finished on FG. Neither grass nor concentrate diets had an effect on the sensory attributes of lambs perceived by consumer panellists

Chapter 5

5.0. General discussion

The main objective of these experiments was to manipulate the diet formulation of concentrate fed lambs in order to achieve a similar carcass composition and eating quality to those finished on grass. The study considered the effects of carbohydrate and fat source, and level of vitamin E supplementation on the performance, carcass composition, shelf life and eating quality of lambs. This chapter aims to bring together the main results of both experiments and discuss their implications and application. Two experiments were conducted to investigate the effect of dietary carbohydrate source, fatty acid composition and vitamin E content on the performance, chemical composition, shelf life and sensory characteristics of concentrate fed lamb.

5.1. Animal performance

The study aimed to modify muscle FA composition and vitamin E concentration by manipulating diets using different CHO source. Therefore, it was important to know whether or not these dietary changes have an adverse effect on animal performance including DM intake and DLWG. In the current study, lambs fed concentrate diets had a higher DLWG and heavier carcass weights compared to those fed grass as previously reported (Priolo *et al*., 2002; Armero and Falagan, 2015).

Dietary supplementation of barley based diets with Megalac$^{©}$, or dried grass and sugar beet based diets with linseed oil had no effect on food intake and daily live weight gain. Whereas, supplementation of barley based diets with linseed oil and moderate vitamin E supplementation (250 mg/kg DM) tended to increase FCR compared to those supplied with 500 mg/kg DM vitamin E. Other authors have also reported no effect of dietary unsaturated FA source (linseed oil) on food intake and FCR either with low (100) or high (500) dietary vitamin E mg/kg DM (Demirel *et al*., 2004a), or with similar levels of vitamin E supplementation to those used in the current study (De la Fuente-Vázquez *et al*., 2014). However, previous studies have demonstrated that unsaturated FAs are unfavourable to rumen bacteria and protozoa. In an *in-vitro* study, Wang *et al*. (2018) observed that α-linolenic acid reduced microbial protein yield as a result of reduced the recycle rate of the bacterial crude protein. The deleterious effect of dietary unsaturated FAs on rumen protozoa and bacteria growth could be overcome by dietary supplementation with 500 mg/kg vitamin E due to its anti-oxidant effect (Belanche *et al*., 2016).

5.2. Effect of dietary fat source on their concentration in muscle.

In the current study, Megalac© (saturated fat) and linseed oil (unsaturated fat) were used as sources of dietary fat. Previous studies have shown that fat supplementation can increase the concentration of PUFA in meat, and meet consumer dietary recommendations (Demirel *et al*., 2004; Noci *et al*., 2011; Facciolongo *et al*., 2018). However, in the current study, the transfer of *n*-3 PUFA from the diets to the muscle was low, due to extensive biohydrogenation of C18:3*n*-3 in the rumen (Cooper *et al*., 2004; Demirel *et al*., 2004). Various methods have been used to protect C18:3*n*-3 from rumen microbes by using formaldehyde, whole linseed, and calcium salt (Chikunya *et al*., 2004). Despite extensive biohydrogentation, some PUFAs can pass through the rumen to the duodenum and are incorporated into muscle (Demirel *et al*., 2004; Noci *et al*., 2011; Urrutia *et al*., 2016). In lamb studies, linseed oil supplementation either as whole seed, or protected by formaldehyde increased muscle C18:3*n*-3 and other long chain PUFA such as C20:5*n*-3 and C22:6*n*-3 due to the desaturation and elongation of *n*-3 FAs (Noci *et al*., 2011; de la Fuente-Vázquez *et al*., 2014). In the current study, linseed oil supplementation increased muscle C18:3*n*-3, C20:5*n*-3 and CLA concentration in experiment 1, but only muscle C18:3*n*-3, C20:5*n*-3 and C22:6*n*-3 concentration in experiment 2. When lambs were fed barley based diets, the inclusion of linseed oil did not increase muscle CLA concentration compared to those lambs fed grass or dried grass based diets. The difference between experiments 1 and 2 in muscle CLA concentration could be due to the basal diets as previously discussed (section 3.4.5 and 4.4.5). Two main factors; dietary intake of C18:2*n*-6 and rumen conditions determine the amount of CLA absorbed from the duodenum. Changes in the rumen environment due to change in basal diet (grass vs grain) could affect the growth and activity of the *Butyrivibrio fibrisolvens* bacterium which is responsible for the synthesis of CLA (French *et al*., 2000; Palmquist *et al*., 2005; Bauman *et al*., 2016). In the current study, dietary intake of C18:2*n*-6 was similar between the concentrate treatments. This suggests that the dried grass based diet favoured the growth of *B. fibrisolvens*. The high concentration of neutral detergent fibre found in the grass and dried grass based diets could have created a rumen environment that promotes the production of CLA, or reduced CLA utilisation in the rumen. In general, valuable changes in the PUFA content of lamb muscle can be achieved by dietary linseed oil inclusion.

5.3. Effect of dietary vitamin E level on its concentration in muscle

The current study investigated the effect of dietary vitamin E level on the vitamin E content of muscle. The concentration of vitamin E in muscle increased linearly ($P<0.001$) as dietary vitamin E intake increased on both the grass and concentrate based diets (Figure 5.1). This is in agreement with the published literature (Wulf *et al*., 1995; Turner *et al*., 2002; Álvarez *et al*., 2008). López-Bote *et al*. (2001) reported that dietary inclusion of 270, 520 or 1020 mg/kg vitamin E, resulted in muscle vitamin E concentrations of 3.6, 5.2 and 6.9 mg/kg muscle respectively. In the current study, there was a difference in dietary vitamin E intake (mg/day) or dietary vitamin intake (mg/kg LW) between the experimental groups (Table 5.1). In experiment 1, lambs fed diets based on dried grass or suger beet pulp (250 mg/kg DM vitamin E) had a higher (P<0.001) vitamin E intake compared to lambs fed diet grass (34 mg/kg DM), similarly, in experiment 2, lambs fed diets based on barley (250 or 500 mg/kg DM vitamin E) had a higher (P<0.001) vitamin E intake compared to those fed grass (158 mg/kg DM). However, no differences were observed in muscle vitamin E concentration between lambs fed on the concentrate and grass based diets. Baldi *et al*. (2019) reported that the muscle vitamin E concentration of lambs fed grain based diets was significantly lower compared to that of lambs fed lucerne based diets (2.53 vs 3.43 mg/kg muscle), even though the vitamin E content of the barley based diet was higher than that of the lucerne based diet (42 vs 37 mg/kg, respectively). Differences in the bioavailability of natural and synthetic forms of vitamin E could influence the accumulation of vitamin E in muscles as previously discussed (section 3.4.7 and 4.4.7). In general, muscle vitamin E concentration in the second experiment was higher compared to the first experiment, although similar levels of dietary vitamin E were supplied. The differences in the growth period and total body gain between the two experiments may have affected muscle vitamin E concentration (Arnold *et al*., 1993).

Table 5. 1. Dietary vitamin E intake and muscle vitamin E concentration of lambs in experiments 1 and 2.

1st experiment						
	G	**B**	**DG**	**SB**	**s.e.d**	**P-value**
Dietary vitamin E intake, mg/day	48.2^{d}	96^{c}	364^{b}	401^{a}	11.45	<0.001
Dietary vitamin E intake, mg/kg LW	1.27^{c}	2.27^{b}	8.86^{a}	9.57^{a}	0.295	<0.001
Muscle vitamin E, mg/kg	2.61^{a}	1.88^{b}	2.38^{ab}	2.36^{ab}	0.259	0.046

2nd experiment						
	FG	**BML**	**BLL**	**BLH**	**s.e.d**	**P-value**
Dietary vitamin E intake, mg/day	207^{c}	424^{b}	388^{b}	747^{a}	22.26	<0.001
Dietary vitamin E intake, mg/kg LW	5.07^{c}	9.81^{b}	9.37^{b}	17.4^{a}	0.379	<0.001
Muscle vitamin E, mg/kg	4.48^{ab}	4.27^{b}	4.12^{b}	5.36^{a}	0.397	0.013

G: Grass, B: Barley, DG: Dried grass, SB: Sugar beet, FG: Grass, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E. $^{a, b, c, d}$ Means in a row with the same superscript are not different (P>0.05). Dietary intake of grass finished lambs was predicted based on 4% of lambs weight (AHDB, 2016).

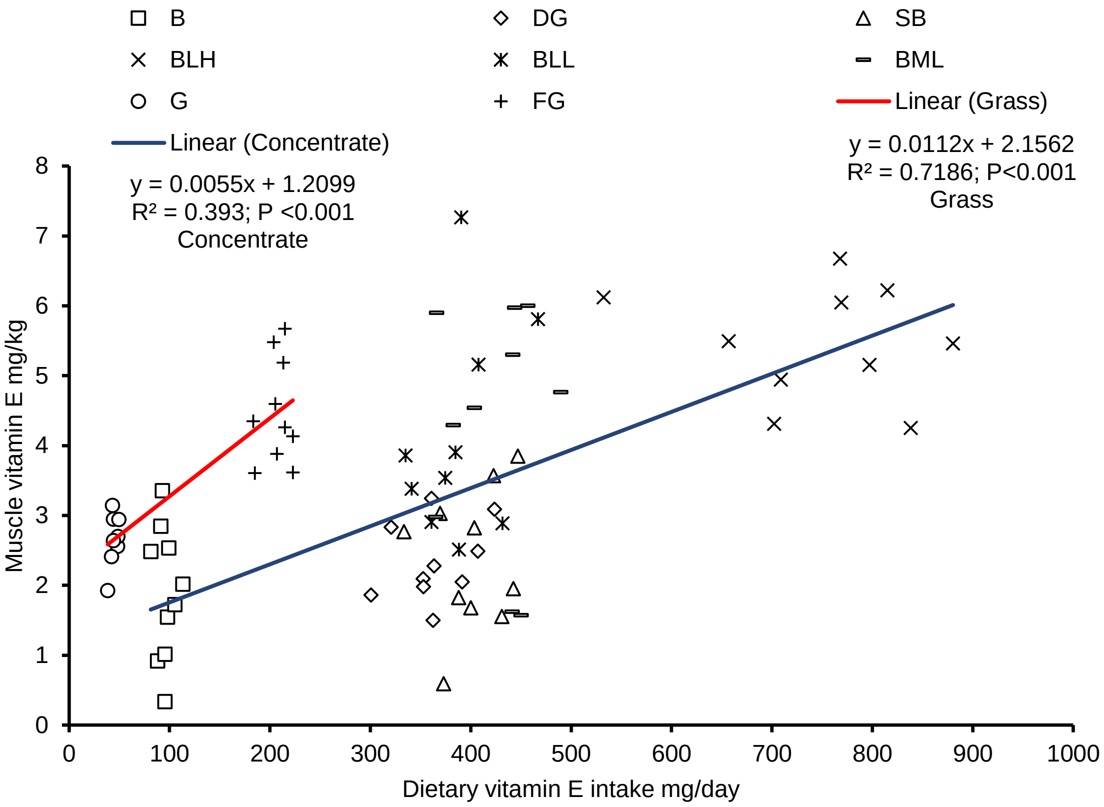


Figure 5. 1. The relationship between dietary vitamin E intake and muscle vitamin E concentration of lambs fed either grass (G: Grass, FG: Grass), or concentrate based diets (B: Barley, DG: Dried grass, SB: Sugar beet, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E).

5.4. Effect of muscle's FA and vitamin E on meat quality

In addition to improving the muscle FA composition of concentrate fed lambs, the objective of this study was also to investigate the effect of increasing muscle PUFA and vitamin E concentration on meat colour, lipid oxidation and sensory evaluation (flavour).

5.4.1. Colour

As expected, the muscle of lambs fed diets that included linseed oil had a shorter colour shelf life due to increased lipid oxidation (Ponnampalam *et al*. 2012). A decline in saturation value was evident during retail display in all dietary treatments based on a threshold level of 18, which is generally accepted as the minimum for consumer acceptability of lamb meat (MacDougall, 1982; Kasapidou *et al*., 2012). The inclusion of linseed oil decreased muscle colour shelf life in both experiments 1 and 2. In contrast, the inclusion of Megalac extended muscle colour shelf life as the saturation value was maintained at >18 for longer. The higher level of PUFA in the muscle of lambs fed linseed oil resulted in higher levels of myoglobin oxidation and deterioration in colour stability that is initiated through lipid oxidation during retail display (Faustman *et al*., 2010).

In the current study, there was a positive relationship ($P<0.05$) between saturation values and muscle vitamin E concentration (Figure 5.2a and 5.2b). Although, the concentration of α-tocopherol was significantly lower in muscle samples from lambs fed diets contained Megalac, the muscle long chain PUFA (EPA and DHA), P:S ratio were also significantly lower. Ponnampalam *et al*. (2012) reported that muscle redness and *n*-3, *n*-6 and PUFA were negatively correlated. Another mechanism could also be possible, as muscle vitamin E could directly reduce pigment oxidation (Cheah *et al*., 1995). Muscle redness is related to the haem iron (haem pigment), and muscle vitamin E concentration. Faustman *et al*. (2010) proposed that muscle PUFA oxidation could be an initiator of myoglobin oxidation and vice versa, which means that muscle vitamin E concentration is a fundamental factor in maintaining the shelf life of meat.

In the current study, muscle FA composition varied with the basal diet and fat source, but colour shelf life was mainly related to muscle vitamin E concentration, with 1.5 and1.9 mg/kg muscle being required to maintain colour shelf life above 18 for more than 7 days in lambs offered grass or concentrate based diets, and 1.0 and 2.6 mg/kg muscle being required to maintain colour shelf life above 18 in lambs offered diets containing either Megalac or linseed oil as the fat source. This was confirmed in experiment 1, where the vitamin E concentration of muscle from lambs fed diets containing linseed oil was lower than the value of 2.6 mg/kg muscle which required to prevent colour deterioration. The value of 2.6 mg/kg muscle vitamin E was achieved when the dietary intake of vitamin E was 260 mg/day (176 mg/kg DM), although, in the 1st experiment, dietary vitamin E intake was higher than 260 mg/day (Table 5.1). Whereas, in the 2nd experiment, the vitamin E concentration of muscle from lambs fed a similar dietary vitamin E intake to those in experiment 1 (406 vs 383 mg/day) was higher. Differences in the growth period and total body gain between the two experiments may have affected muscle vitamin E concentration (Arnold *et al*., 1993). Colour shelf life was extended by 14 days when muscle vitamin E concentration was 5.02 mg/kg (Figure 5.2b), which correspond to a dietary vitamin E intake of 700 mg/day (476 mg/kg DM). Lopez-Bote *et al.* (2001) also reported that the optimum level to improve meat surface (LD) redness in MAP for 9 storage days was in the range of 5.3 to 5.6 mg/kg muscle.

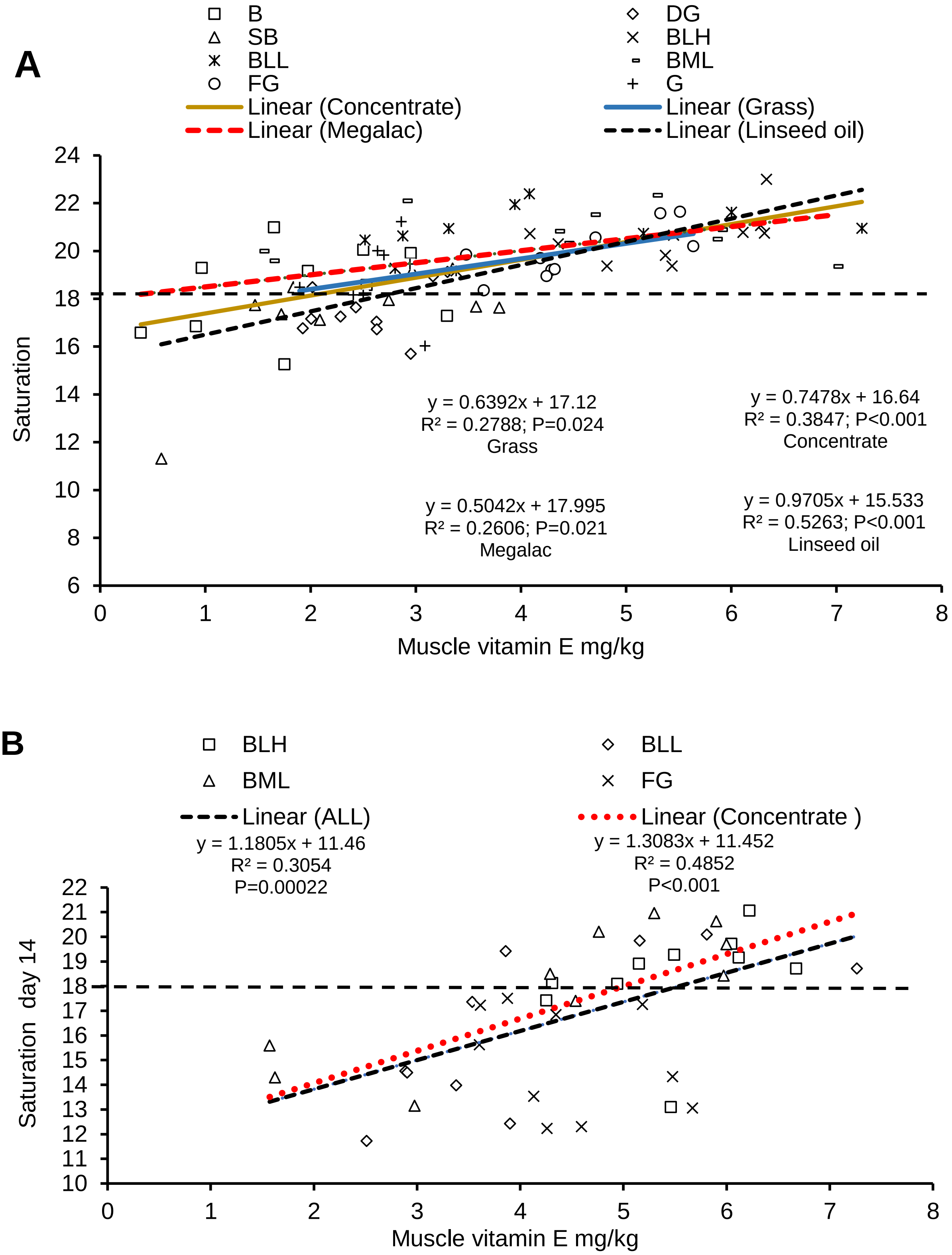


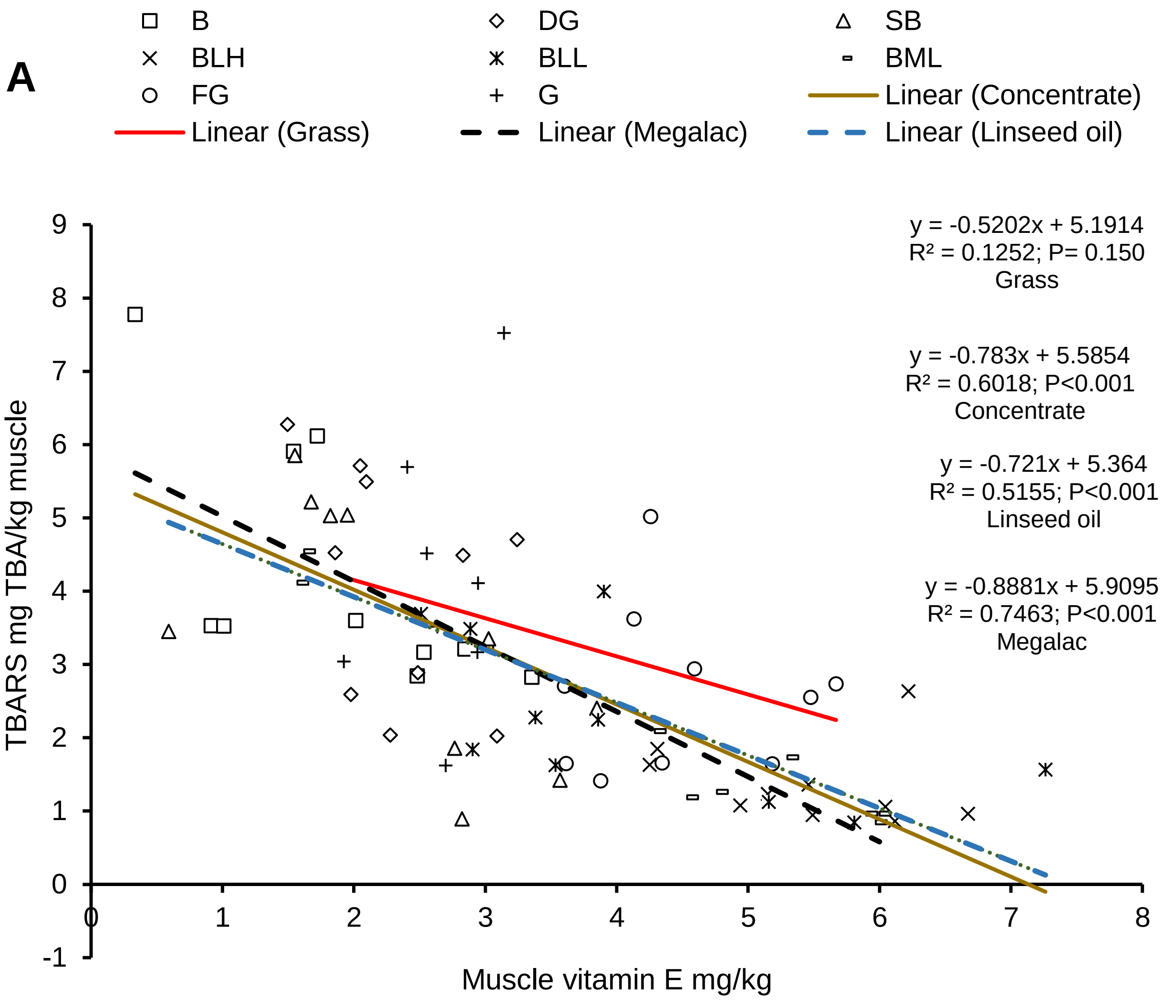
Figure 5. 2. The relationship between muscle vitamin E concentration and saturation of SM muscle from lambs fed either (A) grass (G: Grass, FG: Grass), or concentrate based diets (B: Barley, DG: Dried grass, SB: Sugar beet, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E) at day 7 or (B) at day 14 only for the 2nd experiment.

5.4.2. Lipid oxidation

In the current study, lipid stability was assessed by lipid oxidation (TBARS). In meat product, PUFA with more double bonds are more susceptible to lipid oxidation during storage or conditioning (Morrissey *et al*., 1998; Nieto and Ros, 2012). In both experiments 1 and 2, dietary inclusion of linseed oil increased muscle PUFA content compared to inclusion of Megalac. However, lipid oxidation was higher in experiment 1, compared to experiment 2. Thus as a general hypothesis, the higher the muscle PUFA, the higher the level of lipid oxidation, when muscle vitamin E concentration is not taken into account. The study of Ponnampalam *et al*. (2014) reported that muscle TBARS value was positively correlated to muscle haem iron and PUFA concentration when muscle vitamin E concentration was below 2.95 mg/kg muscle. In the 1st experiment, the highest level of muscle vitamin E was 2.61 mg/kg muscle (diet G) which was below the 2.95 mg/kg muscle reported by Ponnampalam *et al*. (2014). Thus, the high PUFA muscle content and possibly haem iron (not available for the current study) concentration of muscle from lambs on diets based on dried grass or sugar beet pulp may have had a greater effect on lipid oxidation than vitamin E content. Although the lipid stability of the SM muscle was similar between treatments, colour was less stable than muscle derived from lambs on diets based on grass or barley. In contrast, in experiment 2, muscle vitamin E concentration had a greater effect on lipid stability at both day 7 and 14 of retail display.

However, there was a significant negative relationship between muscle vitamin E concentration and TBARS value ($P<0.001$) (Figure 5.3a and 5.3b). This indicates that increasing muscle vitamin E concentration reduces SM muscle TBARS during retail display. López-Bote *et al*. (2001) reported that for LD muscle stored under aerobically refrigerated conditions for 9 days, muscle vitamin E concentrations of 2.0, 3.6, 5.2 or 6.9 mg/kg muscle, produced TBARS value of 3.1, 2.3, 1.3 or 0.5 mg MDA/kg muscle, respectively. Similarly, Kasapidou *et al*. (2012) reported that TBARS values were significantly lower in vitamin E enriched lamb SM muscle (3.73 mg vitamin E/kg muscle) compared to control lambs (0.73 mg vitamin E /kg muscle) after 6 days of MAP storage.

The study of Ponnampalam *et al*. (2014) reported that TBARS value was positively correlated to the muscle haem iron and PUFA concentration when muscle vitamin E concentration was below 2.95 mg/kg muscle. This confirms that more than one factor affects lipid stability, and to reduce the deleterious effect of these factors muscle vitamin E concentration needs to be greater than 2.95 mg/kg muscle. As expected, the SM muscle of lambs conditioned for 14 days had higher TBARS values than the muscle of those conditioned for 7 days. As display time increases, lipid oxidation also increases (Kasapidou *et al*., 2012). Therefore, a higher muscle vitamin E concentration was required to reduce TBARS values during retail display (Figure 5.3b). The vitamin E concentration of muscle required to keep TBARS value below 4.2 mg MDA/kg muscle (Berruga *et al*., 2005) for lambs fed concentrate based diets by day 7 was 1.75 mg/kg muscle, which equates to 100 mg/day of dietary intake (68 mg/kg DM). However, the concentration required to keep TBAR values below 4.2 mg MDA/kg muscle after 14 days of retail display was 4 mg/kg muscle vitamin E for concentrate treatments (Figure 5.3b) which corresponds to a dietary supplementation of 520 mg vitamin E/day (354 mg/kg DM).



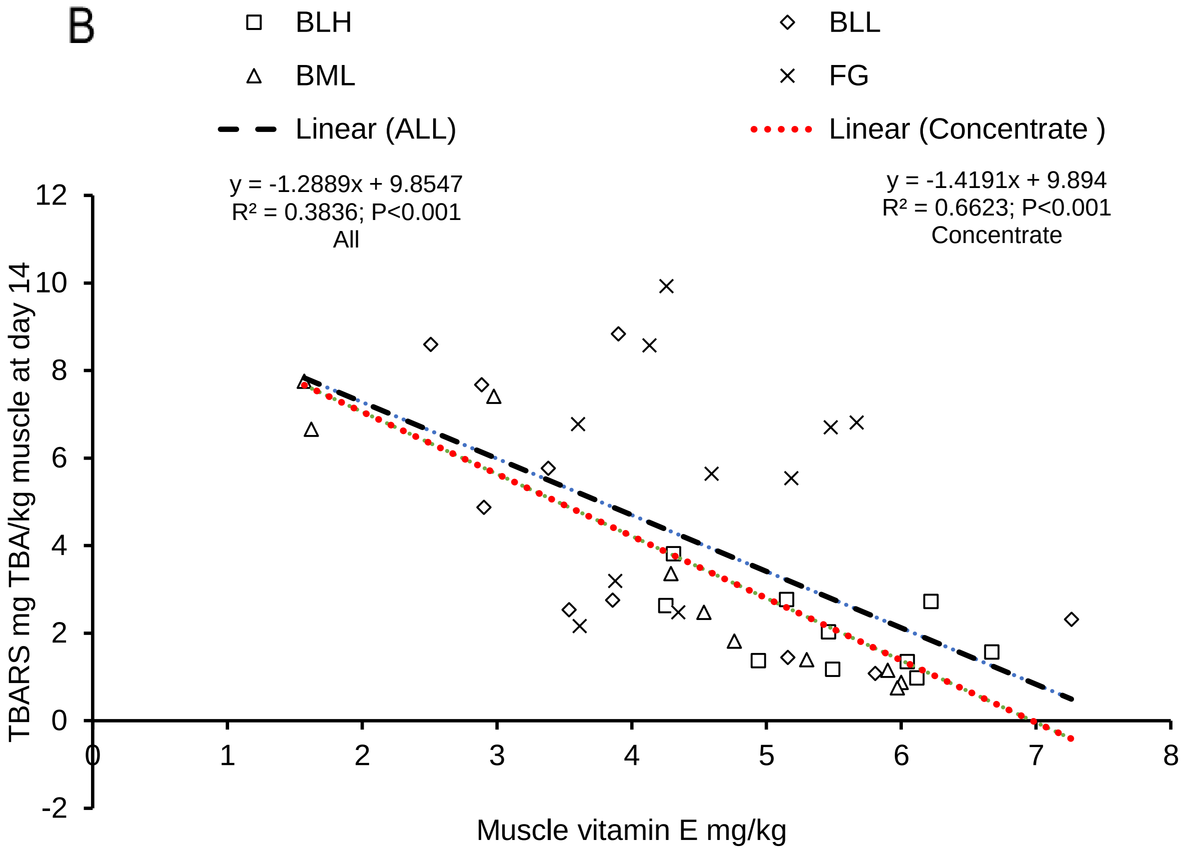


Figure 5. 3. The relationship between muscle vitamin E concentration and lipid oxidation of SM muscle from lambs fed either (A) grass (G: Grass, FG: Grass), or concentrate based diets (B: Barley, DG: Dried grass, SB: Sugar beet, BML: Barley Megalac, BLL: Barley linseed oil low vitamin E, BLH: Barley linseed oil high vitamin E) at day 7 or (B) at day 14 only for the 2nd experiment.

Generally, the effect of high muscle vitamin E concentration was not the same on both parameters of shelf life (colour and lipid oxidation). In the current study, a higher muscle vitamin E concentration was required to improve colour shelf life for a storage period of either 7 or 14 days (2.6 and 5.02 mg/kg muscle vitamin E, respectively). Whereas a lower muscle vitamin E concentration was required to extent lipid stability for a storage period of either 7 or 14 days (1.75 and 4 mg/kg muscle vitamin E, respectively). Similarly, Dufrasne *et al*. (2000) reported that a higher muscle vitamin E concentration was required to improve colour shelf life than lipid stability in the LD muscle of bulls supplemented with 1000 mg vitamin E/day. Therefore, taking into account the length of retail display and the vitamin E required to maintain colour shelf life, the optimum concentration would be 5.02 mg/kg muscle, which correspond to a dietary vitamin E intake of 700 mg/day (476 mg/kg DM).

5.4.3. Sensory evaluation

In general, the study has shown that the chemical composition of meat can be enhanced in term of FAs, nutritional indices and vitamin E content. This can be achieved either by feeding diets containing α-linolenic acid and vitamin E. These desirable changes in the carcass chemical composition did not affect lamb sensory attributes when evaluated by consumer panellists. However, these results contradict other published works (Fisher *et al*. 2000; Priolo *et al*. 2002; Resconi *et al*. 2009) who reported significant differences in the sensory attributes of lambs finished on different production system, particularly lamb flavour when lamb meat was assessed by trained panellists. They also reported that lambs flavour intensity score was higher for the lambs fed grass compared to those fed concentrate, although, overall liking score was contradicted due to the panellist's countries origin. The advantage of using trained panellists that are trained in the description and identification of flavour, is that they produce a more repeatable results, with a more normal distribution than untrained panellists. However, the disadvantage is that these results are not usually representative of the results from consumer panellists (Channon *et al*., 2003; Mancini and Hunt, 2005). The results of the current project have significant implications for diet formulation ratios to improve meat quality of lambs finished on concentrate diets without affecting the sensory attributes of consumer preferences.

5.5. General conclusion

- Lambs fed concentrate diets had higher animal performance and heavier carcasses compared to those fed grass.
- Concentrate carbohydrate source, fat source and vitamin E level had no effect on animal performance, carcass characteristics and carcass measurements.
- Carcass chemical composition and eating quality of grass finished lamb can be replicated by inclusion of linseed oil and vitamin E 250 mg/kg DM.
- The CLA content of muscle can be improved from a human health perspective by inclusion of dried grass and linseed oil in concentrate diets.
- Inclusion of linseed oil with supra nutritional vitamin E 500 mg/kg DM extended lamb shelf life by 4 days compared to grass finished lambs.
- Desirable changes in the chemical composition of lamb muscle did not affect lamb sensory attributes in consumer preference tests.

5.6. Further study

1. Muscle form lambs fed barley based diet with high dietary PUFA and 500 mg/kg DM of vitamin E increased lamb shelf life, so does it follow that a muscle from lambs fed dried grass or sugar beet pulp based diet with high dietary PUFA and 500 mg/kg DM of vitamin will increase lamb shelf life?

2. The vitamin E content of lamb muscle is increased by increasing the fattening period. What will be the optimum dietary vitamin E supplementation, to maximise vitamin E deposition in lamb muscle over shorter fattening period?

3. The CLA content of lamb muscle can be increased if dried grass is used as the basal diet. However, it is not increased when other raw materials such as sugar beet pulp or barley are used. Further work is required to investigate why.

4. The vitamin E content of lamb muscle does not influence the colour and lipid stability in the same way. If vitamin E is not solely responsible for shelf life, what other factors could be influencing shelf life? Therefore, further investigation is required to understand why.

5. Due to a low bioavailability and high cost of synthetic forms of vitamin E, using an alternative natural source of vitamin E (i.e. agro industrial by products) will be beneficial for farmer and consumer.

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