Improving resource use efficiency and nutritional quality of grazing-based dairy production

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ABSTRACT

The dairy industry has received recent negative media coverage due to perceived poor sustainability, low animal welfare and high saturated fat content. However, evidence suggests that efficient, forage-based dairy production supports the environment and provides a nutritious product. While industrial dairy production has focused on yield as a measure of productivity, there is little research into optimising efficiency AND nutritional composition through farm management (such as breed, forage allowance, grazing strategy, etc.). Classic measures of production efficiency (kg feed/litre of milk) are not the sole priority in low-input systems, which also aim for improved health, fertility, forage conversion and milk quality. Holstein-Friesians were traditionally bred for high milk yields, which often correlate negatively with functional traits, such as fertility and health. For low-input and/or pasture-based systems, alternative breed choices are preferable, and UK dairy farmers have used several crossbreeding practices.

Milk fat composition is examined alongside farm management strategies (conventional, organic, low-input and pasture-based) to identify if there is a management practice, breed and/or individual cows that are most suitable to the farming system. Consistently, farms and cows that had higher proportions of forage in their diets produced milk with a higher concentration of nutritionally beneficial fatty acids such as, omega-3 and CLA9. Despite evidence that efficient cattle have less of an impact on the environment, the metric used for efficiency in pasture-based systems is just inputs vs outputs. This thesis explores the definition of efficiency and tries to find alternative metrics that include cow health, milk quality and productivity and finds breeds and cows that perform well under these conditions.

Researching the distinguishing factors of nutritional milk quality is key to sustainable production and addresses increasing media and scientific scrutiny regarding human health effects and ecological impacts of dairy products. Evidence of dairy farming systems that support the environment and provide nutritious food are essential to supporting UK dairy farmers.

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LIST OF ABBREVIATIONS

AHDB- Agriculture, Horticulture Development Board

ANOVA- Analysis of Variance

CIDR- Controlled Internal Drug Release (fertility treatment)

D- value- Measure of digestibility

DIM- Days In Milk

DM- Dry Matter

DMI-Dry Matter Intake

ECMY- Energy Corrected Milk Yield

ECMYP- Energy Corrected Milk Yield with Premium

EU- European Union

FU- Functional Unit

GB- Great Britain

GC- Gas Chromatography

GFE- Gross Feed Efficiency

GHG- Green House Gas (Emissions)

Grassmilk^{TM}- Organic Valley Certified 100% Pasture Fed Milk Brand

GxE- Gene - Environment Interaction

F1- First generation cross breed

FA- Fatty Acids

ID- Identification

LCFA- Long Chain Fatty Acids

LI- Low Input

LW- Live Weight

ME- Metabolisable Energy

NA- Not Applicable

NMR- National Milk Recording

PB- Pasture-Based

PCA- Principal Components analysis

PE- Production Efficiency

PFLA- Pasture For Life Association

RDA- Redundancy Analysis

RFI- Residual Feed Intake

RSP- Residual Solids Production

RW- RumiWatch

SCC- Somatic Cell Count

TB- Bovine Tuberculosis

TMR- Total Mixed Ration

UK- United Kingdom

VFAs- Volatile Fatty Acids

Common Cow Breeds:

AYR-Ayrshire

BF-British and unknown Friesian

BS-Brown Swiss

DS- Dairy Shorthorn

HF -Holstein/Friesian

JE-Jersey

MO-Montbelliarde,

MRI-Meuse Rhine Issel

NZF-New Zealand Friesian

RP-Red Poll

SH-Shorthorn

SIM- Simmental

SR-Scandinavian Red

XX-crossbred with unknown breed composition.

Common Milk Fatty Acids

ALA- α-Linolenic Acid (c9 12, 15 C18:3)

C12:0-Lauric Acid

C14:0-Myristic Acid

C16:0-Palmitic Acid,

CLA9-Conjugated Linoleic Acid (C18:2, c9t11 isomer)

DHA-Docosahexaenoic Acid (c4,7,10,13,16,19 C22:6)

DPA- Docosapentaenoic Acid (c7,10,13,16,19 C22:5)

EPA- Eicosapentaenoic Acid (c5,8,11,14,17 C20:5)

LA- Linoleic Acid (c9,12 C18:2)

MUFA- Monounsaturated Acid

OA- Oleic Acid (c9 C18:1)

VA- Vaccenic Acid (t11 C18:1)

n-3- Omega-3

n-6- Omega-6

PUFA- Polyunsaturated Acid

SFA- Saturated Fatty Acid

DECLARATION

I declare that this thesis was written by myself and that the work within is my own unless explicitly stated otherwise. The work in this thesis has not been submitted for other degrees or qualifications.

Publications arising from this work

There are two publications arising from this research.

Chapter 4:

Davis, H., Stergiadis, S., Chatzidimitriou, E., Sanderson, R., Leifert, C., and Butler, G. (2020) 'Meeting breeding potential in organic and low-input dairy farming' *Frontiers in Veterinary Science - Animal Behavior and Welfare,* 7: 544149.

Chapter 5:

Davis, H., Chatzidimitriou, E., Leifert, C. and Butler, G. (2020) 'Evidence That Forage-Fed Cows Can Enhance Milk Quality', *Sustainability,* 12(9), p. 3688.

CHAPTER 1. INTRODUCTION

1.1 SUSTAINABLE INTENSIFICATION

Originally, sustainable development was framed as "Development that meets the needs of the present without compromising the ability of future generations to meet their own needs." (Brundtland *et al.*, 1987). This was later considered under three sub-groups: social, economic and environmental, which, when working in harmony, would create 'sustainable development'. However, assessing sustainability to create a decision-making framework that is effective across the world is nearly impossible. The three 'pillars' fit nicely into policy, already existing expertise and datasets, yet they often missed the mark in 'developing' nations and environmental considerations were often side-lined when convenient (Gibson, 2006).

In modern agriculture, sustainable development seems like an oxymoron because of a generally agreed upon principal that more food must be grown to feed an increasing population. This gave rise to Sustainable Intensification (SI): 'a process or system where agricultural yields are increased without adverse environmental impact and without the conversion of additional non-agricultural land' (Pretty and Bharucha, 2014). Quantitative assessments found that the continuation of land clearing (extensification) in poorer nations and further intensification in richer nations would have much higher global green-house gas (GHG) emissions and impacts on the environment than moderately intensifying production on previously cleared land by sharing technologies and using more efficient management practices (Tilman *et al.*, 2011). According to Smith (2013) and Pretty and Bharucha (2014), agriculture has to intensify and global food systems must change to meet population demands. Many studies have agreed that behavioural change by policy makers (Foresight, 2011), consumers (Godfray *et al.*, 2010) and farmers (Smith *et al.*, 2007) are essential to achieve SI and food security. This involves changing diets, the distribution of food/feed and fossil fuel use (Smith, 2013). However, this near impossible task of total food system transformation, is beyond the scope of this PhD and this thesis will focus on how sustainable development has been adopted into dairy systems.

Intensification in the European dairy industry has meant a huge increase in yield per cow by feeding higher energy and protein feed (e.g. wheat, soybean meal and crimped maize (AHDB Dairy, 2012)) instead of pasture. High-energy feeding, as well as keeping cattle inside (where animals burn less energy from restricted activity and farmers have more control over their diet) and selective breeding (high-yielding Holstein/Friesian (HF)) resulted in a 94% increase in yield per cow from 1975 to 2018 (4099 to 7959 L/cow/year), but only a 12% increase in total

domestic milk production over the same time period, because the number of farms and cows decreased (Ubero, 2020). However, intensively reared cattle consume food suitable for human consumption, have poor health traits, fertility and milk fat composition (Butler, 2014), suggesting that cows able to maintain health and productivity with lower-inputs (e.g. diet, health and fertility treatments) are required to improve sustainability.

A strong criticism of SI is the use of the word intensification, which seems to contradict the reality of lower outputs. Under SI dairy systems, yields decrease and more land is necessary to grow forages to feed cattle, but less land to grow concentrates (often off-farm), compared to mainstream or conventional systems. However, ruminant livestock have traditionally produced food from marginal land unsuitable for arable crops or rotational, short-term grassland established to improve soil fertility or reduce weed pressure in arable rotations (Eisler *et al.*, 2014). Efficient, grazing-based dairy production is not only an effective way of utilising permanent and rotational grassland under UK conditions, but also fits clearly within the sustainable development goals (e.g. Goal 2: improved nutrition and promotion of sustainable agriculture and Goal 12: ensure sustainable consumption and production patterns) (United Nations, 2015). There are clear nutritional, environmental, social, economic and ecological benefits to pasture-based dairy compared to intensive systems. Grazing-based systems produce milk of substantially better nutritional quality (e.g. higher in omega-3 fatty acid) (Stergiadis *et al.*, 2012; Stergiadis *et al.*, 2015b), deliver better economic returns to farmers (AHDB Dairy, 2015; PFLA, 2016) and have similar/lower environmental impacts, especially with legumes in forage swards (as opposed to carbon heavy nitrogen fertiliser) (Peyraud *et al.*, 2009). Therefore, the environmental gain, lower inputs and more nutritious milk might be considered to outweigh potentially lower yield.

There are research-driven arguments against dairy farming *per se* that depict ruminants, especially bovine livestock, as the largest emitters of GHGs, particularly under extensive grazing systems with moderate milk yields/growth rates (Hristov *et al.*, 2013a). Additionally, sceptics of organic and low-input systems claim the lower yield is less sustainable as more land is required to produce less milk, leading to further biodiversity loss and deforestation (Seufert *et al.*, 2012). However, this approach of measuring emissions per unit product (rather than unit of land) does not consider ruminants grazing where other crops cannot grow or net carbon emissions (e.g. carbon sequestration offsets) and, as discussed by Salou *et al.* (2017), different metrics need to be considered. These systems of analysis are not prepared to encompass the full picture of low-input, sustainability-driven farms, which is necessary to consider the future potential of sustainable dairy production.

1.2 FARMING SYSTEMS

During the early to mid-20th century, the use of synthetic fertilisers and crop protection came into practice, which dramatically increased output and decreased labour in large-scale agriculture. The widespread adoption of *chemicals* in agriculture led to a divergence in management practices on farms: those that adopted this technology and those that did not. The use of mineral nitrogen and pesticides to grow crops and historically prophylactic antibiotic use in livestock to maximise yield is typically called conventional farming. In the early 1900s, the term 'organic farming' was introduced then more widely adopted and standardised after the Second World War, as awareness about soil health, animal welfare and environmental impact was developing. Organic farming in Europe is governed by standards set by the EU (European Comission, 2008) and country-specific certifying bodies, e.g. The Soil Association (Soil Association, 2018), that aim to support the natural environment and wildlife species, use no artificial fertilisers or herbicides and have high standards of animal welfare.

Table 1.1 The different farming systems considered in this thesis

Farming System	Conventional	Organic	LI	PFLA	PB
Certifying Body	NA	European Commission, Soil Association (UK)[a]	NA	PFLA[b] (UK)	NA
Forage from diet (as dry matter intake)	Typically [c], ~50%	>60%	Typically [c], >50-75%	100%	>85%
Access to outdoors	0-200 days	Average 215 days	Typically >150 days	>215 days	>215 days

[a] Standards are governed by the European Commission, but individual countries have their own certifying bodies (European Comission, 2008; Soil Association, 2018), the Soil Association is just one of the UKs certifying bodies.
[b] The PFLA have their own set of standards (PFLA, 2018), are independent from organic certification and receive 100% of their diet from forage (no concentrates are permitted).
[c] There is not a standard but a typical proportion of forage in these systems. The conventional system may have 0% grazing, whereas all other systems rely on spring/summer grazing. The low-input system is based on the explanations used in a meta-analysis by Lorenz *et al.* (2019) and research by Butler *et al.* (2009).

While the organic label is designated by complying with specific standards, many farmers follow similar principals but are not certified organic. These farms are generally referred to as 'low-input' (LI) and typically follow most organic principles, but might add nitrogen fertiliser to grazing land and/or use non-organically sourced feed for livestock (Butler *et al.*, 2009; Bijttebier *et al.*, 2017; Lorenz *et al.*, 2019). While low-input conventional farmers use both organic and non-organic practices, some follow additional standards. For example, the Pasture for Life Association (PFLA, 2018) in the UK, is a certification for feeding 100% forage-based rations, e.g. diverse grazing swards, silage and hay (no concentrates are permitted), which does

not require organic certification. Some farmers choose to go beyond organic standards (with or without certification) and feed their ruminant livestock a pasture-based (PB) diet, which in this thesis will be defined as a dairy diet with at least 85% forage. Whilst other farming systems exist, this thesis will use the following definitions to describe five farming systems: conventional, organic, low-input, pasture-based and PFLA (Table 1.1). Generally, there is a lot of overlap between organic, LI, PFLA and PB farming principles and in some instances names may be used interchangeably, but the descriptions above and in Table 1.1 outline the main differences in management.

1.3 UK DAIRY INDUSTRY

1.3.1 CURRENT TRENDS

Globally, consumption of milk and meat is expected to double by 2050 (FAO, 2008), which is expected to put pressure on global agricultural systems and drive intensification. The UK is the one of the largest producers of milk in the world (11th), accounting for 16.9% of global output and producing 15 million litres in 2018 (Ubero, 2020), additionally, importing 1,363,000 tonnes and exporting 1,458,000 tonnes of dairy products during 2019 (AHDB, 2020b). As a result of fluctuating milk prices (AHDB, 2020g), farmers have had to intensify in a bid for financial sustainability, demonstrated by the UK dairy industry seeing a 0.4% increase in liquid milk yield from 2019-2020 and a 2% decrease in cow numbers (2.57 million cows) (AHDB, 2020b). But this intensification has not necessarily coincided with improved efficiency or sustainable development.

While milk prices fluctuate in the conventional dairy industry, demand for organic and pasture-based milk has increased. UK organic milk was valued at £351 million in 2018 with over 25% of UK households purchasing organic milk, representing 5.1% of retail milk sales and 3.9% of total dairy produced in UK additionally (OMSCo, 2019). There has been a shift in consumer awareness of sustainability and environmental impacts of ruminant production (Lang and Barling, 2013) and this is reflected in the overall global growth in the organic sector (compound annual growth rate of 8%) (OMSCo, 2019). A major scrutiny of the dairy system is the production of crops to feed livestock. Globally, one third of total arable area is used to produce livestock feed (Steinfeld *et al.*, 2006), and in 2019 Great Britain (GB) produced over 4 million tonnes of feed for the cattle industry (AHDB, 2020d), providing evidence that GB land under arable management is still used for cattle production. The demand for supplementary feed is highest in systems that are unable to fully utilise forage, such as year-round calving and intensive indoor systems. Currently, around 81% of farmers use year-round calving, whilst 4%

use spring, 8% autumn and 7% have spring and autumn block calving (AHDB, 2020e). Year-round calving has the clear advantage of a constant milk supply, but additional labour and feed costs, whilst block calving condenses labour demand. By reducing the cost of production through utilising forage growth and consumption, the AHDB (Agriculture and Horticulture Development Board, i.e. the UK levy board funded by farmers and growers) suggests that combined spring and autumn blocks could be more profitable and still provide a year-round milk supply. Additionally, on farms with pasture access, spring calving allows farmers to utilise quality forage as feed at a time when the cows' nutritional need is highest, significantly reducing costs and improving farm efficiency (Shalloo *et al.*, 2004). These current trends in UK dairy point towards continuing intensification, but with some support from the AHDB and organic certifying bodies, there seems to be movement towards greater forage utilisation and lowering inputs.

1.3.2 CHALLENGES AND OPPORTUNITIES

Regardless of the type of system, dairy production faces particular challenges in this moment, with opportunities for pasture-based dairy as UK producers face reduced demand for milk and must strive to adapt to climate change, shifting dietary preferences, Brexit and disruption to the supply chain in a global pandemic (Covid-19) (AHDB, 2020b).

A major advantage of ruminants over pigs and poultry is that they can graze (consume cellulose) on land which cannot grow crops for direct human consumption, to produce milk and meat. However, utilisation of cellulose requires a complicated digestive tract relying on extensive microbial fermentation, which results in methane emissions from ruminants. Greenhouse gases (GHG) from livestock and associated supply chains are thought to represent 14.5% of all 'human-induced' emissions, with 4.6Gt CO_2 equivalents from dairy and beef cattle (Metz *et al.*, 2007; Gerber *et al.*, 2013b). The three main GHGs emitted from the agricultural sector are (mostly enteric) methane (CH_4), nitrous oxide (N_2O) and carbon dioxide (CO_2). There is a lot of pressure for the dairy industry to reduce emissions (Gerber *et al.*, 2013a; Gerber *et al.*, 2013b), but no consensus on how to manage this under current production systems. GHG emissions and their impact will continue to loom large for dairy production, with pressure on farmers and consumers to move away from intensive livestock products and towards more plant-based diets.

Recently there has been a sharp rise in veganism, vegetarianism, environmentalism and consumer awareness around intensive agriculture and animal welfare (Trent Grassian, 2020). Additionally, the International Panel on Climate Change has suggested that moving to plant-

based diets will help reduce the risks of climate change (IPCC, 2019). Despite these recommendations, dairy produce is an incredibly nutrient dense food group providing protein (including essential amino acids), essential fats, calcium and many other nutrients (Drewnowski, 2018; Feeney and McKinley, 2020). Thus, veganism could result in nutrient deficiencies, such as vitamins B_{12} and D, iron, calcium and zinc (McGirr *et al.*, 2017). Plant-based 'milks' have become popular, but despite being designed to look like cow's milk, their nutrient profiles are not similar (Sethi *et al.*, 2016). Moving towards a plant-based diet by increasing fresh fruit and vegetables is reasonable, and not necessarily a threat to British dairy, but in the UK, where ruminants can be sustainably farmed, excluding ruminant produce from the national diet entirely (other than with allergies), is unnecessary. While the overall market trend toward plant-based alternatives is perceived as a threat to the dairy industry, the reality is cow milk will remain a dietary staple (FAO, 2008) and the increased awareness of sustainability and animal welfare will provide additional opportunities for low-input, organic and pasture-based dairy.

Currently, the UK dairy industry is preparing for an unknown Brexit agreement, with different potential outcomes: a hard deal, no-deal or something else altogether, which will impact imports, exports, various tariffs, time delays and permissions (AHDB, 2019). The main fear is the increase in imports from countries with lower standards than UK (such as USA), lowering the cost of imported products, whilst the price of home production continues to increase (AHDB, 2019; Allen, 2019). Despite this uncertainty, the rhetoric around Brexit also includes an emphasis on 'buying British', with potential opportunities for UK dairy domestically. Thus far, the dairy industry is awaiting the outcomes of Brexit negotiations, but there is an opportunity to meet the demands of a British public motivated not only by sustainability and nutritional concerns, but also a desire to support UK agriculture.

This year in particular has been tumultuous for the dairy industry, as COVID-19 has seen disruption of supply chains and dramatically reduced the demand for dairy produce, causing milk price cuts for more than 5200 of Great Britain's 9200 dairy farmers (some over 4p/L) and 2200 farmers having to reduce milk output (AHDB, 2020f). The combination of Brexit and COVID-19 has led to uncertain times for the dairy industry and over the next couple of years farmers will need to develop sustainable and resilient approaches to production to withstand further uncertainties from Brexit, COVID-19, climate change and any other global factors.

1.4 THESIS AIM

This thesis aims to investigate a combination of milk quality, human nutrition, farm management systems and individual cow performances, to identify key factors that influence production efficiency in organic, low-input and pasture-based dairying.

1.5 SCOPE OF EACH CHAPTER

1.5.1 CHAPTER 2: LITERATURE REVIEW

The Literature Review aims to explore and identify background information on each of the key areas of interest covered in this thesis, including:

i. Production efficiency and dry matter intake,
ii. Farm management, such as breeding and grazing strategies,
iii. The environmental impact of dairy production
iv. Milk quality, fat composition and impact on human health

1.5.2 CHAPTER 3: METHODS

The Methods Chapter outlines the main laboratory and statistical analytical approaches used throughout this thesis by:

i. Explaining how milk lipid was extracted and quantified using gas chromatography.
ii. Describing the statistical methods used to explore, define and drive production efficiency.
iii. Outlining the main statistics used to analyse collected data, including explanations of interpretation.

1.5.3 CHAPTER 4: EVIDENCE THAT FORAGE-FED COWS CAN ENHANCE MILK QUALITY

Chapter 4 aims to explore differences in milk fat composition between own-brand conventional and organic milk from supermarkets and PFLA (100% forage-fed) milk in the UK by:

i. Sampling and analysing conventional and organic supermarket and PFLA milk.
ii. Identifying differences in fatty acid profile between different management systems and season.
iii. Discussing the potential implications of switching to PFLA milk.

1.5.4 CHAPTER 5: MEETING BREEDING POTENTIAL IN ORGANIC AND LOW-INPUT DAIRY FARMING

Chapter 5 aims to use an existing dataset to identify breed choice within low-input and organic dairy systems that maintain health and yield whilst producing milk with a beneficial FA profile by:

i. Defining variables most relevant to low input and organic farming and observed differences in management systems
ii. Identifying breeds and crosses that are similar across the farms
iii. Developing a score for low-input-production (LI-P) to identify breeds and crosses that best suit low-input and organic management in terms of production, health and milk composition with respect to consumer health.

1.5.5 CHAPTER 6: POTENTIAL TO SELECT FOR PRODUCTION EFFICIENCY IN PASTURE-BASED DAIRY SYSTEMS

Chapter 6 aims to determine variability in performance between farms as well as between cows under common management by:

i. Following cows from three pasture-based farms through one lactation, estimating dry matter intake by measuring grazing and ruminating behaviour.
ii. Assessing if there is scope for cow selection, within PB herds, based on efficiency and milk quality.
iii. Determining if any variables could be used to identify efficiency beyond input vs output.

1.5.6 CHAPTER 7: GENERAL DISCUSSION

Chapter 7 draws common threads from the results of the previous Chapters and discusses the potential direction of future research.

CHAPTER 2. LITERATURE REVIEW

2.1 INTRODUCTION

Ruminant agriculture is currently experiencing intense scrutiny as environmentalists and others question the sector's land use and greenhouse gas emissions and dairy's nutritional credentials have been in the spotlight for a number of years. Much of this reaction is directed at industrial dairy production, with more recent indications that pasture-based systems have lower net emissions and greater nutritional benefits. With increasing attention on forage-based dairy, both globally and in the UK, understanding these management systems and their metrics of evaluation are essential to support sustainable agriculture.

This Chapter aims to explore and identify background information on each of the key areas covered in this dissertation. This includes: definitions of production efficiency and dry matter intake and the specific considerations for calculating/measuring these metrics in pasture-based systems; farm management, such as breeding and grazing strategies; the environmental impacts of dairy production; milk quality, fat composition and impact on human health.

2.2 PRODUCTION EFFICIENCY

To farm sustainably economic efficiency is essential, but for environmental sustainability physiological efficiency is important. Traditionally economic production efficiency (PE) is a measure of input cost against profit or output; typically feed efficiency (kg dry matter intake (DMI)/litre of milk) is used in dairy systems to measure physical production efficiency (e.g. Milkbench+ system (DairyCo, 2012)). An efficient dairy farm helps minimise costs and reduce environmental impact by breeding cows that eat less but produce the same yields or eat the same and produce more milk.

Whilst the importance of profit is not overlooked in organic and LI dairying, management priorities are different. By focusing on selecting to improve functional health traits and fertility, farmers are able to lower overall costs (e.g. vet, feed, labour) and by using grazing strategies that enhance soil health, sward growth and quality (e.g. mob grazing) cows consume richer pasture, potentially resulting in more nutritious, value-added products (Scollan *et al.*, 2017). Performance in conventionally managed HF cattle is predominantly defined by the highest-yielding, but these farms and cows typically have high energy requirements, poor fertility and health traits (Miglior *et al.*, 2005). Whereas performance in LI and/or PB farming is a combination of a farm's/cow's yield, health and fertility, and because these cows are not solely

pushed for output their milk yields are inevitably lower than the intensive dairy systems (de Haas *et al.*, 2013). However, cows selected for 'performance' on predominantly forage diets have better health, fertility and efficiency of forage conversion into milk, while producing milk with a beneficial fatty acid profile (Stergiadis *et al.*, 2012; Benbrook *et al.*, 2018). To account for these less tangible improvements in sustainability (animal health and product quality) farm management systems need different measurements of PE.

Current PE assessments were designed by advisory networks for mainstream conventional production and are ideal to compare and improve PE on those relatively intensive farms (AHDB, 2018a). However, intensifying milk production (increased yield per hectare) often results in increased environmental impacts per ha (Crosson *et al.*, 2011). Additionally, the functional units (mass-based (per kg milk) vs area-based (per hectare)) often used to explain differences between management systems environmental impact, PE, or sustainability influences results and conclusions (Salou *et al.*, 2017), further discussed in Section 2.6.3.

However, as identified, LI systems have different priorities and need alternative methods to fairly compare farms and/or cows to identify superior examples and thus, improve efficiency in these systems. So, to compare these management systems under the same metric creates the impression that lower intensity farming is less productive and efficient and therefore less sustainable. More appropriate methods are needed to judge performance and priorities in lower-input systems.

One way of measuring biological efficiency is 'Gross Feed Efficiency' (GFE), which is the ratio of feed input (either as kg of dry matter (DM) or units of energy) to milk output (yield, milk solids or energy corrected milk) (Connor, 2015). Another measure of efficiency is 'Residual Feed Intake' (RFI), which calculates the difference between actual intake and predicted intake based on performance using a regression model (Koch *et al.*, 1963) that accounts for the energy necessary for recorded production, maintenance and metabolic liveweight. However, RFI was designed to assess efficiency in beef cattle and is more complicated than GFE to calculate and interpret for dairy cows. There are considerable changes in liveweight and body composition throughout the lactation cycle so these growth-based models are ineffective, especially in LI systems where yield and liveweight changes are not monitored regularly. Another alternative method for analysing production efficiency is 'Residual Solids Production' (RSP) defined by Coleman *et al.* (2010), who also compared GFE and RFI on different dairy management systems. Similar to RFI, RSP uses a regression model but regresses milk solids on DMI, metabolic bodyweight and body condition score. The best approach to modelling efficiency in dairy production is unclear, and the priorities and

measurements in PB systems are different from intensive output driven farming. Throughout this thesis, PE is explored and Chapter 5 and Chapter 6 attempt to find alternative approaches that suit LI and PB management.

2.3 DRY MATTER INTAKE

As discussed, most measures and/or estimates of efficiency rely on a measure or estimate of intake. In an indoor system feeding a total mixed ration (TMR), the amount of feed supplied and rejected is easily measured (usually accurately weighted into the mixer wagon), especially in controlled, experimental sheds (Bani *et al.*, 2014). Additionally, estimating DMI for dairy cows is important to calculate rations and supplements suitable for the size of cattle, stage of lactation and milk yield. By targeting cattle nutrition accurately, the system becomes more efficient with less wastage, especially when supplementary forage or expensive concentrates are given. In LI and PB systems without supplementation, estimating DMI has been less important, other than to allocate grazing areas. However, by ignoring DMI in LI and PB systems where reliance is on intake from pasture, the opportunities to improve system efficiency are limited. Unfortunately, due to the outdoor nature of PB farming, measuring and estimating DMI in PB systems are notoriously difficult, as further discussed in the paragraphs below.

Many methods of measuring DMI have been developed, and the most simple is weighing all feed intake and refusals (often in tie-stalls) (Kmicikewycz and Heinrichs, 2014). Whilst this method is simple and useful for modelling and validating other methods, it is not representative of cows at pasture which are able to pick and graze rather than be given a set diet. Another common method is using inert n-alkanes (made from saturated hydrocarbons in plant wax, given as an oral bolus, with a faecal marker) to estimate feed intake and faecal outputs (Dove and Mayes, 2006). This method was intended for use in grazing dairy cows, they are dosed with a known quantity of even-chained alkane, faecal samples give concentrations of this and naturally occurring odd-chain alkane. Dry matter intake is then calculated from the daily dose rate in the dietary and faecal concentrations (Dove and Mayes, 1996). However, this method is labour intensive (especially with total faecal collection), time sensitive and nearly impossible to validate in a grazing-based system. There are many studies that have combined and validated the weighing and n-alkane methods (Bani *et al.*, 2014; Wright *et al.*, 2019; Wright *et al.*, 2020), but in a grazing system neither of these approaches are feasible, especially on commercial farms.

Various studies have generated models of varying accuracy to estimate DMI, often in indoor and/or HF systems (Huhtanen *et al.*, 2011). Many of these models have been compared and

ranked on their accuracy (Krizsan *et al.*, 2014; Jensen *et al.*, 2015), but in PB systems, where cattle are outdoors grazing, both measuring and estimating DMI is more complex. In PB management, DM cover of the vegetation can be assessed pre- and post-grazing to estimate herd or group consumption. This can be refined to use an area of grazing with a known DM cover before and after grazing and allocate DMI for individual cows based on relative yield and live-weight (LW) but, the n-alkane method is the most commonly used method for forage diets (Macoon *et al.*, 2003). Unfortunately, many of the DMI measuring methods are difficult or impossible to implement in PB systems (Decruyenaere *et al.*, 2012). PB grazing is dependent on the DM available, the quality and digestibility of the sward (which can vary across each paddock) and will effect rumination behaviour (Prendiville *et al.*, 2010) and grazing behaviour traits of the cows, which vary both within and between breeds (McCarthy *et al.*, 2007). To further complicate estimating DMI in PB systems, when cows are in a set location for many days grass will continue to grow, in these situations exclusion cages are used to assess DM growth during grazing (where cuts are taken daily to mimic grazing) (Sim and Moot, 2019).

If we are to predict DMI and therefore production efficiency in grazing cattle, eating and ruminating behaviour needs to be understood. Once eating and ruminating behaviour were recognised as drivers of DMI, studies used human observers to count the number of chews, boluses, ruminating bouts, etc., in a bid to model DMI (among other variables) more accurately (Tager and Krause, 2011). This was incredibly labour intensive, inherently prone to error and variation in interpretation, therefore halters or collars were developed to take real time recording of grazing and ruminating behaviour. The 'Hi-Tag' (SCR Engineers Ltd., Netanya, Israel) was one of the first collars developed to collect ruminating behaviour but was only able to collect data over two eleven-hour periods (Schirmann *et al.*, 2009).

More recently the 'RumiWatch' (RW) halter was developed (Itin+Hoch, 2015) to estimate eating, ruminating and drinking behaviour. This has been validated and a small systematic error with eating and drinking time and random errors with ruminating and eating time have been identified (Ruuska *et al.*, 2016). The RW system was further validated in a grazing system (Rombach *et al.*, 2018; Werner *et al.*, 2018a), where errors in recording grazing and eating chews, rumination boluses and time spent eating and ruminating existed, but noted the limitations of their study (the data converter is meant for hourly intervals and they were only able to record for ten minutes). RW was validated in a tie-stall study, where measurements were deemed to be accurate for 'scientific measurements', but did not highlight number of chews (Zehner *et al.*, 2017). Additionally, the software that converts the raw data into logical, manageable spreadsheets has been validated (Werner *et al.*, 2018a). Rumiwatch halters have

successfully been used in research to explore differences in the grazing and ruminating behaviour of cows on different grazing rotations (Kidane *et al.*, 2018), with different pasture allowance (Werner *et al.*, 2019) and the effect of concentrate feeding level on grazing behaviour (Romanzin *et al.*, 2018). Whilst the RW does not directly measure DMI, analysis by Guilherme Amorim Franchi (2017) identified and compared models that predict DMI. The RW halter is further discussed and used to explore grazing and ruminating behaviour and model DMI in Chapter 6.

2.4 SELECTIVE BREEDING

The selective breeding approach to conventional and intensive dairy production is not suitable for LI or PB farming, because the priorities in each system are different (Simianer and Bieber, 2012). Where in LI systems with a tight six-week spring calving block, fertility is essential, in a more intensive year-round calving system fertility is not prioritised. This is one example why the LI and PB sector have had to develop an approach to selective breeding, which for some farmers has been an inter-generational learning experience, but the farmers who are now looking for more sustainable approaches to dairying find it difficult to replicate. Hence, the importance of research into breeding strategies for these systems.

The success and sustainability of a system is affected by the breeds or genetic potential of cows selected. The strengths of a number of different breeds are often combined in LI farming, resulting in composite crossbreeds that suit specific management style and aims on individual farms. To some extent, the UK dairy industry is starting to diversify breeding strategies away from conventional HF, to cater for different management styles. The AHDB breeding objectives have both spring and autumn calving indices, in an attempt to highlight the importance of forage utilisation by the former (AHDB Dairy, 2018b). Despite the range of dairy breeds available in the UK, the index falls short for many PB farmers because they want and need to be able to select cattle that are more targeted for their specific needs. For the research to confirm or deny farmers' hypotheses (that less intensive systems have reduced emissions, enhanced soil health and biodiversity, purpose selected cattle, etc.), breeding objectives and dairy scientists must expand beyond traditional, intensive practices dominated by milk yield and basic composition, this is further discussed in Chapter 5.

2.4.1 TRAITS

For the last 50 years, HFs have been at the forefront of high yielding dairy production globally. Holsteins have been primarily selected for their production traits (milk yield), while more

traditional Friesians can be selected for functional traits (health and fertility). However, HF cows are not well-suited to LI and organic systems as they require relatively high levels of both concentrate feeds and veterinary inputs to achieve maximum yield potential (Butler, 2014). Instead, more traditional breeds from around the world, able to maintain health and productivity with low-inputs, are preferred, such as New Zealand Friesians (health and fertility (NZAEL, 2019)), Scandinavian Reds (udder health (Clasen *et al.*, 2019)) and Jerseys (milk solids (Auldist *et al.*, 2004)). Production traits (higher yield) are often negatively correlated with functional traits, portrayed by the decline in fertility and health reported in HF (Simianer and Bieber, 2012). In order to exploit the contrasting potential of both alternative and high-yielding breeds, LI and organic dairy systems have increased interest in cross-breeding dairy cattle, combining genetics from both robust and productive breeds (Sørensen *et al.*, 2008). Additionally, functional traits are heavily influenced by the local environment (G x E interactions) and also have low heritability (Simianer and Bieber, 2012), making it difficult to select genetic lines to improve health and fertility. For this reason, LI and organic systems benefit from crossing productive breeds with those known to have stronger functional traits.

2.4.1.1 FAT COMPOSITION

Milk fat content and its composition are essential traits for the dairy industry as they have an effect on processing abilities and consumer health perceptions (Cruz *et al.*, 2019). However, breeding for milk fat composition is complicated by the fact that it is primarily driven by dairy diet/management (Stergiadis *et al.*, 2012; Średnicka-Tober *et al.*, 2016), stage of lactation (Nantapo *et al.*, 2014) and season (Butler *et al.*, 2008), rather than genetics. However, there is some evidence that it is possible to alter saturated fatty acid content by selecting bulls with high or low saturated fat breeding values (Poulsen *et al.*, 2020). There are polymorphisms on genes (predominantly diacylglycerol-O-acyltransferase 1 (DGAT1) and stearoyl-CoA desaturase 1 (SCD1)) that have strong associations with milk composition and fat quality (Bouwman *et al.*, 2012; Cruz *et al.*, 2019) and multiple trait analysis described the heritability (h^2) of short, medium, long-chain, saturated and unsaturated fatty acids (h^2=0.24, 0.32, 0.23, 0.33, 0.21, respectively) through lactation (Narayana *et al.*, 2017). These studies have identified the potential to manipulate the FA profile through genetics, but, as with so many other traits, are limited to and focused on HF cattle, which may or may not be similar across other breeds, so further research is needed.

2.4.1.2 PRODUCTION EFFICIENCY

As discussed (above, Section 2.2), GFE is a common method of estimating the efficiency of an individual cow and/or a farm/system. Additionally, the GFE trait is moderately heritable (h^2 = 0.32) (Spurlock *et al.*, 2012), suggesting that there can be genetic progress with GFE. However, due to the associated increased energy demand, increased production is negatively correlated with days open (poor fertility), body condition and energy balance (Spurlock *et al.*, 2012), therefore cows that appear to be the most efficient could be losing body condition. Additionally, there are studies that identified correlations and heritability using RFI (Vallimont *et al.*, 2011; Connor *et al.*, 2012; Vallimont *et al.*, 2013), but all conducted in indoor, intensive systems or with non-lactating heifers, therefore more research using RFI is needed before it is a comprehensive, reliable measure for genetic improvement in dairy efficiency.

The criticisms of selecting on GFE and RFI are similar and while RSP is a better model with a different trait, more research is needed to identify genetic correlations with RSP (Coleman *et al.*, 2010). Typically, many efficiency traits combine multiple performance traits (e.g. yield and live weight) and their contribution to efficiency will vary according to stage in lactation (Hurley *et al.*, 2017). This adds to the complication of breeding for efficiency traits in a dairy system. An additional concern is the long-term (and permanent) nature of selecting for specific traits, it could take many generations before mistakes are noticed or implications fully understood. Hence the benefits of breeding indices that cover many traits where progress may be slower than selecting solely for yield, but slowness in the right direction is better than the alternative, especially health and production traits improve at different rates (Simianer and Bieber, 2012). Ultimately, there is ongoing debate on how to select for efficiency in dairy cows and the impact of unintended consequences (body condition score, etc.) (Pryce *et al.*, 2014; Connor, 2015; Hurley *et al.*, 2017).

2.5 GRAZING STRATEGIES

Low-input and PB dairying relies on pasture to provide most of the cows' nutrition, therefore it is essential that farmers maximise herbage growth, optimise sward/soil health and in turn, grazing utilisation and conversion into healthy milk and soil carbon. Grazing management influences all these factors and there are many strategies that farmers can adopt to suit various farming styles. Scientific research into the different grazing strategies in the UK is extremely limited and has largely centred on DM production from temporary, permanent and rough-grazing grassland (Qi *et al.*, 2018). Farmers have traditionally implemented a set or continuous grazing strategy (Table 2.1), with very little movement from pasture to pasture during the

grazing season; this low labour method poorly utilises grazed grassland. There is evidence that regularly moving cows to fresh pasture improves utilisation and extends the grazing season (Lawrence *et al.*, 2019). When paddocks are given time to rest during the grazing season,

Table 2.1 Common grazing strategies (modified from (AHDB, 2018b; Billman *et al.*, 2020) and personal communications)

Grazing Strategy	Set/ Continuous	Strip (no back fence)	Paddock/ Cell	Mob/ Tall-grass
Frequency of new pasture	Once or twice over the entire season	Every day, but same area expanded	Every 24-72 hours	Every 12-24 hours
Rest period [a]	NA	0-60 days	20-30 days	30-60 days
Utilisation	50-60%	65-70%	85%	50-90%
Predominant sward species	Perennial rye grass	Perennial rye grass	Perennial rye grass and clover mix	Mix of grasses, herbs, legumes
Digestibility (D-value)	68-72	68-72	68-72	60-68[b]
Stocking density [c]	Low	Low	Medium	High
Pre- grazing cover	Not measured	~2500-2900kg DM/ha	2500-2900kg DM/ha	>3000kg DM/ha
Post- grazing cover (residual)	Not measured	~1250kg DM/ ha, often less chance for regrowth	1250-1500kg DM/ha	>1500-2000kg DM/ha

[a] Rest period will vary throughout the season
[b] Overall D- value is low but cows can select more digestible parts of the plants and sward
[c] There are different ways of assessing stocking density (cows/Ha while grazing or cows/Ha/year), also varies by season, cover and growth

vegetation and pasture production is improved, thus providing more, good quality forage than set stocking and improving utilisation (Lawrence *et al.*, 2019). So, to maximise herbage growth and utilisation, the AHDB encourages a paddock or rotational grazing approach and advises grazing to 'three leaves' of perennial rye grass growth, as there are only ever three live leaves (AHDB, 2018b). This management is optimum for herbage harvest and supplies grass with high digestibility (Table 2.1).

Additionally, many farmers operating paddock grazing visualise herbage available as a 'wedge', where they record DM cover, using a plate meter (described in Chapter 6, Section 6.2.1.5), before and after grazing each paddock (known as the residual) (Table 2.1). They also record DM cover periodically throughout the rest period (to assess cover and growth rates) and identify the size of paddock and number of cows grazing. This is more labour intensive than set-stocking, but gives farmers a better understanding of DM availability across the farm, preventing overgrazing and extending the grazing season.

Many PB farmers go further and adopt a mob/ tall-grass-grazing strategy (Table 2.1). This normally involves diversifying swards from solely perennial rye grass using meadow grasses,

legumes and herbal leys and extending the grazing rotation. By diversifying the sward (many with different root lengths) and giving pastures longer to rest between grazing soil structure and quality is improved resulting in diverse swards that can be productive, drought resistant, weed suppressive, nutritious, reduce nitrogen leaching and increase yields (Sanderson *et al.*, 2005; Woodward *et al.*, 2013; Cranston *et al.*, 2015; Romera *et al.*, 2017; McCarthy *et al.*, 2020). Additionally, mob-grazing is thought to improve soil carbon by using a "graze a third, trample a third and leave a third" ethos (Zaralis and Padel, 2019). However, nearly all studies on herbal leys and sward diversity are conducted in New Zealand, Australia or the USA. Grazing strategy is another example of industry driven recommendations differing from the goals of PB farmers. Where the focus for most farmers is maximising herbage yield and utilisation, PB farmers are diversifying swards and looking to protect the environment for longer term sustainability. UK grazing science needs robust trials to identify swards and grazing strategies that target both ruminant nutrition and environmental impacts.

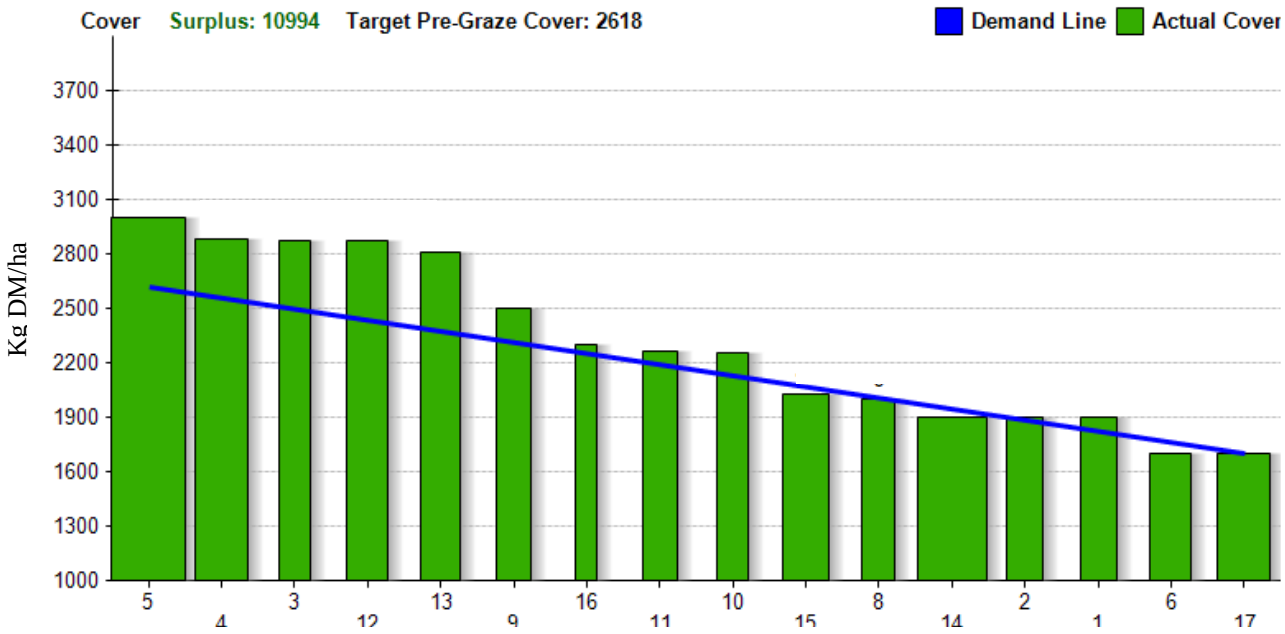


Figure 2.1 Grazing wedge displaying DM cover in each paddock (green bars, width indicates size of paddock), the blue line indicates whether growth rate is expected to meet demand. Data lifted from Newcastle University, Nafferton Farm, July 2020, wedge supplied by www.agrinet.ie

2.6 ENVIRONMENTAL IMPACT

2.6.1 EMISSIONS

The demand for milk and meat has been predicted to double by 2050 (FAO, 2008) and an increasing number of ruminants is inevitable (Turk, 2016). Therefore, reducing ruminant numbers to mitigate emissions is not possible and understanding factors that influence ruminant

emissions to develop mitigation strategies is essential. However, there are different approaches to reducing (or mitigating) emissions: a. reducing individual or group emissions (such as feeding to reduce emissions, discussed below), which typically favours the more intensive systems or b. reducing net emissions for the whole system (such as accounting for carbon sequestration and carbon fluxes), which typically favours extensive systems.

Anaerobic manure storage and processing contribute to CH_4 and N_2O emissions but most emissions from ruminants are in the form of enteric CH_4, produced in the rumen during fermentation of the feed consumed, especially forages. Bacterial, protozoal and fungal species begin digesting the feed, resulting in volatile fatty acids (predominately acetate, butyrate and propionate, all absorbed) and the gases CO_2 and H_2 (Hungate, 1967; Buddle *et al.*, 2011). Methanogens (microbes in the rumen which generate methane) get their energy from H_2 and create methane (CH_4), which the host animal then eructates and releases into the atmosphere (Buddle *et al.*, 2011). Residual feed and rumen microbes are subsequently digested on their journey through the abomasum, small and large intestines (Figure 2.2). Reducing the supply of H_2 to methanogens (e.g. through diet manipulation) can briefly suppress methane production. However, the effectiveness and efficiency of the rumen relies on the process that converts complex carbohydrates to fermentable sugars (McAllister and Newbold, 2008).

There are a few management practices that have been investigated to reduce emissions. Methane production in the rumen is closely linked to dry matter intake and efficiency (the more efficient the system the lower the emissions) when measured as emissions per DMI or emissions per output (milk yield or live weight gain) (Hegarty *et al.*, 2007; de Haas *et al.*, 2011; Hayes *et al.*, 2013). Additionally, there is evidence that future breeding directly for reduced emissions, rather than selecting for efficiency, may be possible (Hayes *et al.*, 2013; Hristov *et al.*, 2013b; López-Paredes *et al.*, 2020) and currently ruminants lose an average of 7% of food energy as methane (McDonald *et al.*, 2011). Another method to reduce GHG emissions is to manipulate the diet. 'Inhibitors', such as chloroform, cyclodextrin and bromochloromethane, have been shown to inhibit CH_4 production *in vivo* (Lila *et al.*, 2004; Knight *et al.*, 2011; Mitsumori *et al.*, 2012). However, these chemicals are not widely accepted and there is no long-term evidence for their success. Another option is 'plant bioactive compounds' such as tannins (often derived from forage such as sorghum and chicory). Tannins may reduce enteric emissions but decrease feed digestibility, effecting efficiency and potentially performance (Jayanegara *et al.*, 2012). 'Dietary lipids' have been another route of reducing emissions through diet. Increasing dietary fat (tallow, oil seeds, etc.) in the ruminant diet reduces CH_4 production (as they cannot be utilised by microbes) by decreasing DM available for fermentation but maintaining production

(Rabiee *et al.*, 2012) and increased efficiency reduces enteric methane (emission intensity) (Jiao *et al.*, 2014). There are other types of feed additive available and well-reviewed by Hristov *et al.* (2013b), but the main conclusion is that the rumen microbial populations adapt to diet-change and over time, emission levels often return to near pre-treatment and/or studies have not been long enough to determine the long-term effects of feed additives on enteric methane emissions.

There is evidence that including grain (starch/concentrates) in the ruminant diet reduces methane emissions relative to forage diets through rumen production of propionate, rather than acetate and butyrate (further discussed below, Section 2.7.3) (Bannink *et al.*, 2008; Beauchemin *et al.*, 2011; Moate *et al.*, 2020). However, there is also evidence that increasing concentrates in the ruminant diet increases methane emissions (Harper *et al.*, 1999; Lovett *et al.*, 2005; Muñoz *et al.*, 2015). This conflict exists because most studies do not take the whole management system into consideration. Some studies only directly measure emissions from cows in isolation, which develop management methods to mitigate emissions within systems. Whilst others take wider land use into consideration, which gives a more robust representation of emission fluxes at a farm scale (for example, including carbon sequestration potential). Even the way that emissions are measured or estimated (in situ vs. in vivo methods, reviewed by (Hill *et al.*, 2016)) have differences, strengths and weaknesses. Additionally, the number of management systems, types of feed and the many ways to measure emissions (absolute value, intensity, emission per unit of product/area) further complicate interpretation of results. A meta-analysis is needed to evaluate the different systems, feeding styles and emission levels to better understand how feeding intensity affects enteric methane production (and whole system emissions), but is outside the scope of this thesis.

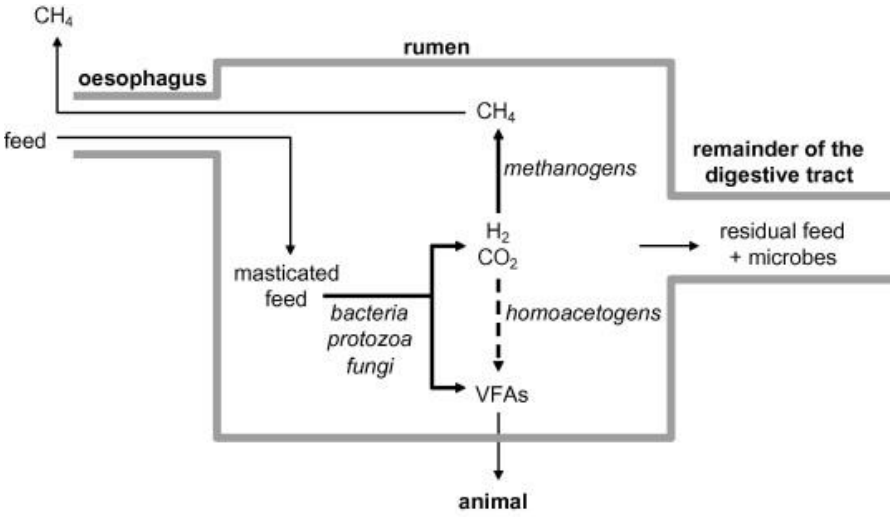


Figure 2.2. Simplified rumen processes with microbial fermentation (thick arrows) of feed to volatile FAs, hydrogen and carbon dioxide. Diagram extracted from Buddle *et al.* (2011).

2.6.2 SOIL HEALTH

There is more to the environmental impact of ruminant systems than enteric methane emissions. A major component of the sustainability of agricultural systems is soil quality and how it changes with management practices (Zani *et al.*, 2020). There are many studies that found organic management, whether due to mixed farming systems, longer crop rotations (with grass/clover), less cultivation, limited fertiliser and pesticide use or other methods, has a positive effect on various soil quality markers, such as pH, total carbon, aggregate stability and others (Gattinger *et al.*, 2012; Lori *et al.*, 2017; Cooper *et al.*, 2018; Zani *et al.*, 2020). Specifically, including grazing (or grass/clover) in an arable rotation can improve soil carbon accumulation (which indirectly stimulates root growth), nutrient cycling and utilisation (Assmann *et al.*, 2017; Lori *et al.*, 2017; Zani *et al.*, 2020). Additionally, there is evidence that when land is converted from arable to grazed grassland it can shift from a carbon emitting system to a carbon sink (Beauchemin *et al.*, 2011). Utilising ruminants' ability to convert this forage into nutritious product by optimising farming practices to improve soil health is essential for future sustainability.

2.6.3 LIFE CYCLE ASSESSMENT

A common and popular way of investigating and comparing the environmental aspects of sustainability in farming systems is through Life Cycle Assessment (LCA) (Baldini *et al.*, 2018). However, LCA rarely takes the full system into consideration and has been shown to favour more intensive farming practices. For example, LCA models do not necessarily depict nitrogen left in the system (which impacts eutrophication, biodiversity etc), and nitrogen emission models are based on mineral nitrogen fertiliser, not organic fertilisers, which have a different mode of action (such as farm yard manure), this results in biased LCA models that do not accurately depict the nitrogen available for emissions (Meier *et al.*, 2015). Additional differences are in part due to the metrics used to assess sustainability, for example, mass-based (emissions per kg milk) and area-based (emissions per hectare) LCA give different results (Salou *et al.*, 2017). Current LCAs use area-based units which do not capture the environmental impact caused by agricultural intensification (Salou *et al.*, 2017).

In LCA analyses of food products, the predominant functional units (FUs) used in the calculation are mass (e.g. per 100g product) or energy (e.g. per 100 calories) (Drewnowski et al., 2009). Unfortunately, these metrics are only useful when comparing similar food groups, because mass FU disadvantages foods with high water content and energy FU disadvantages energy dense foods (for example, one kg of milk has more water than one kg of cheese but

contains less energy) (Hallström et al., 2018). While LCAs designed for diet quality acknowledge that a nutritional quality FU is required, they do not take nutrient density in combination with environmental impact into consideration (Saarinen et al., 2017). A few studies have tried to develop nutritional quality indices and assess environmental impact, but this is incredibly complex because nutritional value combines many components (fatty acids, protein, vitamins, minerals, etc.) (Hallström et al., 2018; McAuliffe et al., 2020; Sonesson et al., 2019). Current LCA models are not yet able to determine the impact of nutritional value and environmental effects on animal production systems, a capability that is still under development.

This manipulation of FU also results in discrepancies between comparisons of agricultural system efficiency. For example, cattle produce 5.45 kg CO_2 equivalent per kg liveweight (LW) (green-house-gas emissions including enteric and manure CH_4 and manure N_2O), whilst pigs produce 3.97 kg CO_2 equivalent per kg LW (Zervas and Tsiplakou, 2012). This would indicate that pig production is more efficient than cattle production, with a lower environmental impact. However, this does not consider how or where the feed was produced, or the amount of feed suitable for human consumption diverted to livestock. For ruminants, 2.8kg of human edible feed is needed to produce 1kg meat, whereas this value is 3.2kg for pigs (Mottet *et al.*, 2017). Additionally, these results also obscure production, nutrition and emission factors. These studies highlight how FU can be used to rank systems to make food look more or less efficient/ sustainable based on selection preferences, again suggesting the necessity for a more holistic approach to LCA modelling.

LCAs will continue to be developed and methods improved, but at least for the moment, the published literature comparing intensive to less intensive agriculture is questionable, unless all factors (and functional units) are considered.

2.7 MILK QUALITY

Milk quality is defined differently depending on stakeholder perspective. Farmers consider characteristics that will affect their final margin: good milk quality is more than 4% butterfat, 3.3% protein, somatic cell count (SCC) under 200,000 cells/ml milk and total bacterial count below 100,000/ml milk, because, depending on contracts, farmers receive a premium for meeting these targets (DairyCo, 2013; MilkPrices, 2019). Whilst these characteristics are important, milk quality can also be viewed from a nutritional perspective and evaluated by fat composition, including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), poly-unsaturated fatty acids (PUFA) and macronutrient (e.g. calcium and phosphorous) and

micronutrient (e.g. iodine, vitamin A and D) content (Dror and Allen, 2014). Milk quality is influenced by feeding and also, within and between, breed genetics. Milk quality also varies by season (Butler *et al.*, 2011b; Kliem *et al.*, 2013) and management (including breed, feed and stage of lactation) (Jensen, 2002; Stergiadis *et al.*, 2015b; Średnicka-Tober *et al.*, 2016; Benbrook *et al.*, 2018). The following sections will explore milk quality, the potential health implications and the impact of management on the FA profile.

2.7.1 DAIRY PRODUCTS AND HUMAN HEALTH

According to the Eat Well guide, the average British consumer should have three portions of low-fat and low-sugar dairy per day, representing around 8% of the daily diet (Public Health, 2016). These guidelines specify 'low' to reduce saturated fat and sugar intake (e.g. from added sugar yoghurts), suggesting that the high saturated fat associated with dairy is still a concern in dietetics despite numerous studies suggesting the benefits of moderate whole milk consumption (Pereira, 2014). The evidence points toward milk and dairy products having a positive and sometimes protective effect on human health, as discussed in the following sections, and there is no evidence to suggest increased incidence of disease directly from dairy consumption in UK (Kliem and Givens, 2011; Thorning *et al.*, 2016).

2.7.2 FATTY ACIDS

Dietary fat is mostly comprised of FAs (99%) and all fat from food consumed will have varying amounts and types of FA. Fatty acids are carboxylic acids classified by the length of their carbon chains, whether they have double bonds and the configuration of the hydrogen atom (Figure 2.3) (Jensen, 2002). There are over 400 different FAs in milk, of which approximately 74 have been classified and named and far fewer have been studied for their effect on human/calf health and sensory characteristics (Jensen, 2002). Based on their chemical structure, FAs were split into PUFA (~2.3% in milk fat), MUFA (~27.7%) and SFA (~70%) and many of the FAs within each class have a range of actions and effects (Grummer, 1991; Lindmark Månsson, 2008). Early dietary guidance grouped and researched FAs of the same class (e.g. SFAs), whereas more recent research have studied the impact of individual FAs (e.g. linoleic acid) for their effect on human health. But, explaining the health impacts of each FA to consumers would be a confusing approach to nutrition, instead well-rounded dietary advice from FAs is needed (Calder, 2015a). Discovering the FAs that are beneficial to human health and manipulating human (and dairy) diet to create a desirable profile is possible, but the human health implications of converting from conventional to grass-fed dairy (despite being known to impact fat composition) have not been well investigated.

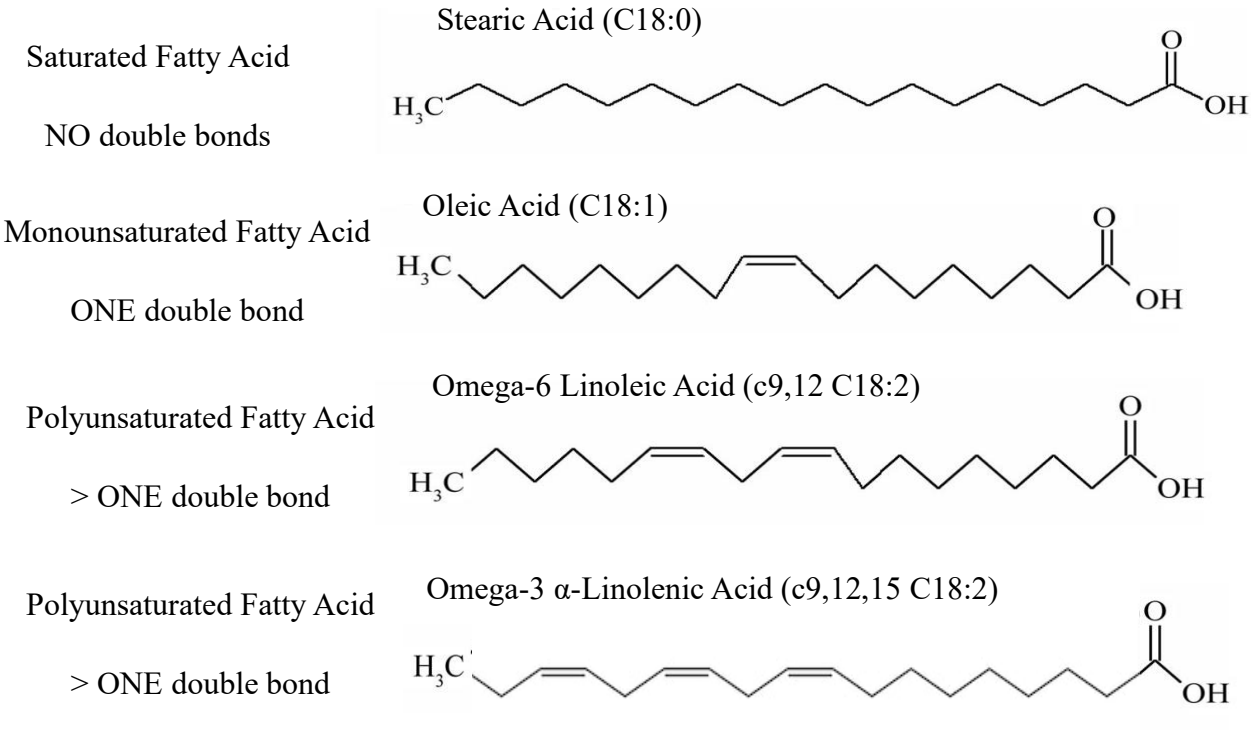


Figure 2.3 Chemical structure of example FAs: SFA (Stearic Acid), MUFA (Oleic Acid) PUFAs (Linoleic and α-Linolenic Acid). Structures adapted from Goli *et al.* (2012).

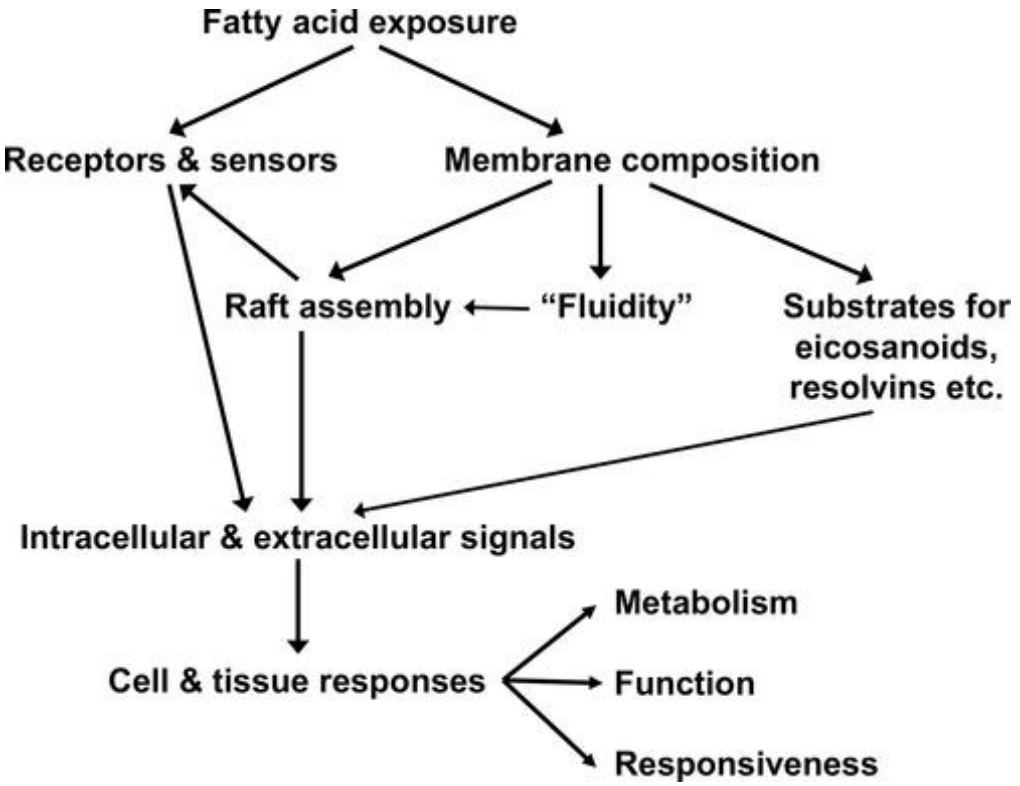


Figure 2.4. Mechanisms involved in the human exposure to FAs (Calder, 2015a)

The complexity of milk fat composition is still not fully understood. However, many FAs have been associated with positive and/or negative health outcomes for consumers and in some cases, the relative concentrations or ratios of one to another may be of more importance than absolute values. The human body is able to synthesise most FAs with the exception of the two *essential* FAs linoleic acid (LA) and α-linolenic acid (ALA) (both depicted in Figure 2.3), which must be obtained through the diet (Calder, 2015a). The metabolism of many FAs is well understood (Figure 2.4), for example arachidonic acid (C20:4 n-6) is the main precursor for producing eicosanoids, which have many regulatory roles, whilst some FAs are important for controlling gene expression and others help regulate metabolic processes (Calder, 2015a). Through the many effects of FAs, there are links to health and risk of disease indicating the importance and moderation of fat consumption.

2.7.2.1 SATURATED FATTY ACIDS

Saturated FAs have no double bonds in the carbon chain (hence, saturated) and historically, SFAs have been demonised in the human diet (De Souza *et al.*, 2015). The main SFAs in dairy products are: lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acids. SFAs are most common in fats that are solid at room temperature (e.g. butter or tallow). Early research suggested that diets high in saturated fat are associated with obesity and type-2 diabetes, which are both risk factors for heart disease, however, in most cases, results were either inconclusive or found some protective effect of SFAs (Crichton *et al.*, 2011; Louie *et al.*, 2013; Soedamah-Muthu *et al.*, 2013). Some SFAs (lauric, myristic and palmitic) have been shown to have cholesterol-increasing properties and high cholesterol levels are an indicator of risk of coronary heart disease (CHD) (Mensink *et al.*, 2003). Generally, low density lipoprotein cholesterol (LDL-C) is associated with a higher risk of heart/artery disease than high density lipoprotein cholesterol (HDL-C), which is protective (Stein and Stein, 1999; Garg *et al.*, 2003). The links and mechanisms between SFA, cholesterol and CHD are complicated and oftentimes results are conflicting, as individual SFA have been linked to positive, neutral and negative effects on heart disease (Mensink *et al.*, 2003; Lordan *et al.*, 2018). Forouhi *et al.* (2014) found even chain SFAs (C14:0, C16:0 and C18:0) were positively associated with incidence of type two diabetes, while odd chain SFAs (C15:0 and C17:0) (which are of rumen origin and found at minor concentrations) were inversely associated. Khaw *et al.* (2012) also reported even chain SFAs were positively associated with CHD risk. Overwhelmingly, meta-analyses and cohort studies are finding that the saturated fat (originally thought to increase risk factors associated with CHD) is potentially protective (Elwood *et al.*, 2010; Siri-Tarino *et al.*, 2010; Aune *et al.*, 2013; Gao *et al.*, 2013). It is clear not all SFAs affect human health uniformly, and these studies

highlight that further subgrouping and identifying individual FAs for their specific function may help identify risk factors for human health.

Despite potential protective effects, there is no hiding from the fact that dairy is a major contributor to SFA intake and the average UK consumer eats more SFA than the recommended daily allowance (<11% of total energy intake) (Roberts *et al.*, 2018). Nutritional advice is to reduce SFA intake, but fails to consider the 'whole-food' effect and does not discriminate between type of SFA (potential for protective vs harmful effects) (Thorning *et al.*, 2017). Until these dietary recommendations are updated, health guidance must focus on reducing SFAs, whilst retaining the positive components, in dairy products. Evidence has shown that SFAs in milk can be reduced by increasing the proportion of FAs with over 18 carbons (long-chain) in the mammary gland to reduce synthesis of short and medium chained SFAs, most of which are saturated (Givens and Shingfield, 2006). This can be achieved by supplementing with seed/plant oils (Glasser *et al.*, 2008) and/or by increasing forage, especially grazing in the dairy diet (Kalač and Samková, 2010). This seems like a straightforward method to reduce SFAs, but farmers do not currently receive a premium for milk nutritional quality and so are unwilling to incur costs to supplement with expensive oils or risk lower yields by increasing forage proportion. Until this is addressed it is unlikely that SFA content of the majority of industrially produced dairy will be reduced.

2.7.2.2 MONOUNSATURATED FATTY ACIDS

Monounsaturated FAs have one double bond somewhere along the carbon chain with the 'remaining' hydrogen in either the cis or trans configuration, for example, oleic (OA) (c9 C18:1) and vaccenic (VA) (t11 C18:1) acids. Oleic acid is the most abundant MUFA and is commonly found in animal fats, olive oil, nuts and avocados, while VA is found in ruminant fats (Field *et al.*, 2009; Tarantino and Finelli, 2016). The most common MUFAs in milk are palmitoleic (c9 C16:1), oleic and vaccenic acid (Butler *et al.*, 2011b). Meta-analyses have shown that replacing SFAs with cis-MUFAs can reduce LDL and increase HDL (small effect size) (Mensink and Katan, 1992; Mensink *et al.*, 2003), although this could be due to the reduction of SFAs and/or carbohydrates rather than an increase in oleic acid. This has been shown by another meta-analysis, which did not distinguish between cis- and trans-MUFAs and found no difference in health outcomes between the FA consumed (Chowdhury *et al.*, 2014) and a more recent study reported a reduced risk of cardiovascular disease when SFAs are replaced with MUFAs, although this mechanism is not yet fully understood (Vafeiadou *et al.*, 2015). If studies reduce specific FAs and replace them with other FAs, is it the reduction in

SFA content or the replacement that is responsible? Since the proportion of all FAs and FA groups are interlinked (all expressed as a proportion of their total), considering them in isolation does not make sense. At the moment, it is accepted that increasing MUFAs (e.g. oleic acid), especially when replacing SFAs, in dairy products could be beneficial to human health and increasing forage in the dairy diet will increase milk MUFAs, typically increasing both oleic and vaccenic acid (Butler *et al.*, 2008; Stergiadis *et al.*, 2015b).

2.7.2.2.1 TRANS MONOUNSATURATED FATTY ACIDS

There are two main MUFAs that are also sources of trans fatty acids (TFAs) in the human diet: ruminant products and partially hydrogenated vegetable oils (which have been banned in many countries). The main trans FA in dairy is vaccenic acid (t11 C18:1), whereas the predominant trans FA in industrially hardened, hydrogenated oils is elaidic acid (t9 C18:1), an important distinction because their metabolism is different (Jahreis and Dawczynski, 2020). There are many common TFA isomers in both ruminant and industrially hydrogenated products (Nestel, 2014), but in very low concentrations (Jahreis and Dawczynski, 2020). There have been many health concerns surrounding TFAs including associations with CHD, obesity and insulin resistance (Mozaffarian *et al.*, 2009). Vaccenic acid is metabolised to CLA9, which is beneficial for human health, in the mammary gland and adipose tissue of both animals and humans (discussed below, Section 2.7.3) whereas elaidic acid has been closely linked to CHD, steatohepatitis and obesity (Qiu *et al.*, 2018). The naturally occurring TFAs found in dairy may not be harmful (Nestel, 2014) but due to the challenges of isolating TFAs and examining their direct effect on human health, there is no considered conclusive safe amount. Currently, the evidence does not point towards ruminant-derived TFAs negatively impacting human health, especially when the full FA profile of dairy is considered, though research is ongoing.

2.7.2.3 POLYUNSATURATED FATTY ACIDS

Polyunsaturated FAs research has become very popular in dairy and human nutrition, as in the Western world, typical consumption of PUFAs is below recommended levels (Benbrook *et al.*, 2018). PUFAs are categorised as having more than one double bond and most are classified into two main groups: omega-3 (n-3) FAs have a double bond between the third and fourth carbon from the end methyl group, and omega-6 (n-6) FAs have a double bond between the sixth and seventh carbon from the end methyl group (Russo, 2009). As mentioned, many FAs can be metabolised and synthesised by the human body but, there are two main essential PUFAs that must come from the diet: n-6 linoleic acid (LA) and n-3 α-linolenic acid (ALA) (Russo, 2009). Interventional and observational studies demonstrate that replacing SFAs in the diet with

PUFAs significantly reduces CVD risk (Mozaffarian *et al.*, 2010; De Souza *et al.*, 2015; Vafeiadou *et al.*, 2015). Current research suggests that increasing PUFAs in the diet (especially in place of SFAs) is beneficial for human health.

2.7.2.3.1 OMEGA 3

The main omega 3 fatty acid is the essential ALA, which metabolises to the long chain FAs (LCFAs) Eicosapentaenoic Acid (EPA, C20:5), Docosapentaenoic Acid (DPA, C22:5) and Docosahexaenoic Acid (DHA, C22:6) (n-3 FAs with more than 20 carbons). DHA is an important part of all cell and organelle membranes and is found in the brain and retina (Swanson *et al.*, 2012). LCFAs are found in fish and fish oil products and in much smaller quantities in meat, eggs and dairy, but research suggests that conversion of ALA to EPA, DPA and DHA is very limited (1-8% ALA converted to EPA depending on method of analysis and LA concentration in diet) (Goyens *et al.*, 2005; Goyens *et al.*, 2006; Brenna *et al.*, 2009), signifying the importance of getting these nutrients from the diet (Kris-Etherton *et al.*, 2009). These FAs are vital for foetal development (Dunstan *et al.*, 2007), healthy aging and neuro- development and degeneration (Janssen and Kiliaan, 2014), controlling inflammation, FA metabolism and may have a protective role against CVD (Calder, 2015a) and prevent some cancers (Gleissman *et al.*, 2010). In recent history there has been a decline in Western n-3 consumption (generally, eating less fish and pasture-raised ruminant produce) (Simopoulos, 2013), so even as specific omega 3 FAs have a range of functions, the research highlights the benefits of consuming a diet rich in n-3 FAs overall.

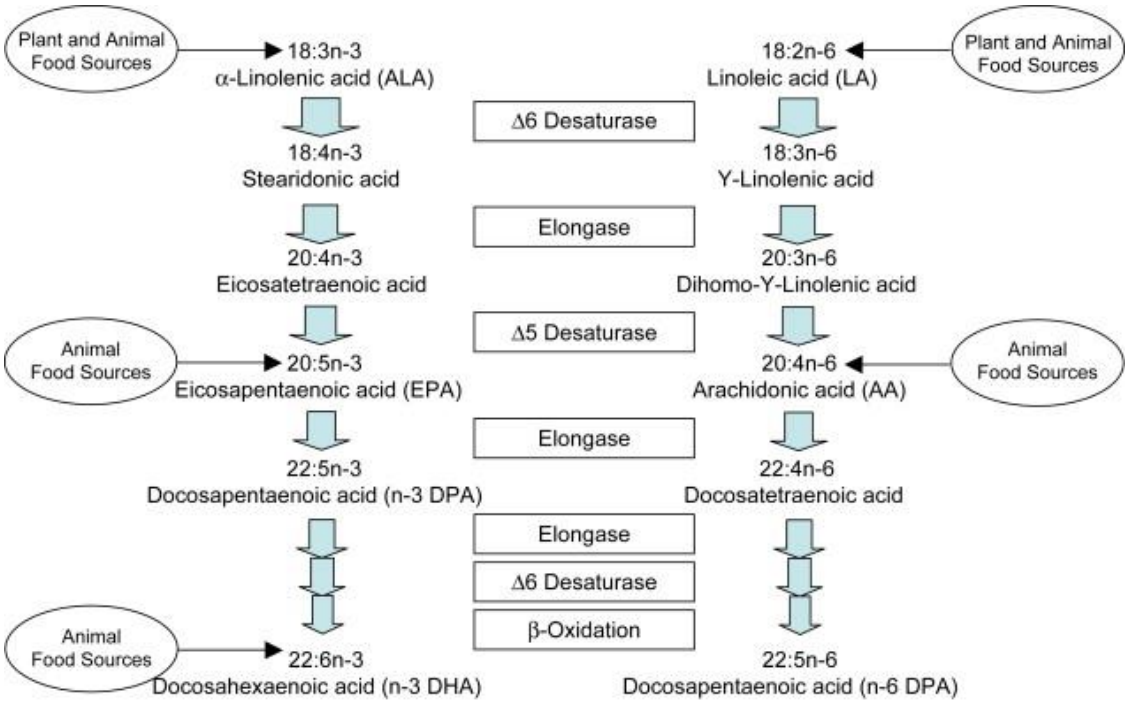


Figure 2.5. The metabolic pathways of alpha-linolenic acid to EPA, DPA and DHA and linoleic acid to AA. Figure from Milligan and Bazinet (2008).

Whilst dairy produce is not a major source of essential FAs in the diet, a swap from conventional to organic milk could raise n-3 consumption from 11% to 16% of daily FA intake in half a litre of full fat milk, demonstrating that changing the management of the dairy system can increase n-3 FAs in the diet without increasing calorie intake, since more LCFAs can be found in organic than conventional milk (Średnicka-Tober *et al.*, 2016; Benbrook *et al.*, 2018). Evidence suggests that the higher the proportion of forage in the dairy diet, the higher the concentration of n-3 in milk, but the specific effects that this may have on human diet and health is unclear (Benbrook *et al.*, 2018).

2.7.2.3.2 OMEGA 6

The most prevalent n-6 in animal and human diets is the essential linoleic acid (LA), found in plants and seeds and is metabolised to the long chain FA arachidonic acid (AA) (also found in ruminant products) (Calder, 2015a). LA is important for skin barrier function (Rabionet *et al.*, 2014) whilst AA has an important role in brain development and function (Camandola *et al.*, 1996) and synthesis of eicosanoids (Calder, 2015b). These FAs are generally pro-inflammatory (Harris *et al.*, 2009), which helps to defend against pathogens, but if there is a loss in the regulation of inflammation, disease can occur (Calder, 2015b). In modern Western diets, consumption of n-6 has risen sharply over the previous 150 years with the increased use of vegetable oils and cereal grains (along with a decrease in fresh vegetables, protein and complex carbohydrates) (Simopoulos, 2008). This over consumption of n-6, and under consumption of n-3, has potentially led to inflammatory processes which have been linked to an increase in diabetes, obesity and atherosclerosis (Donahue *et al.*, 2011; Simopoulos, 2016). However, replacing SFAs with LA has been shown to lower blood cholesterol and LDL (Mensink *et al.*, 2003), suggesting that LA could lower CVD when replacing SFA and Chowdhury *et al.* (2014) found no association that n-6 intake effected CHD. Despite this, some eicosanoids promote tumour growth, which is speculated to be in response to increased AA levels, but conversion of LA to AA is extremely low (around 0.5%) (Czernichow *et al.*, 2010). Ultimately, LA is an essential FA and current research suggests that any increase in consumption of PUFAs is advantageous and reducing overall n-6 consumption is not advised.

2.7.2.3.3 OMEGA-6/OMEGA-3 RATIO

Linoleic acid and alpha-linolenic acid share a complement and competition metabolic pathway (Baker *et al.*, 2016). During metabolism of LA to AA and ALA to EPA, DPA and DHA, both LA and ALA go through elongation (addition of carbon atoms) and desaturation (removing hydrogen, hence introducing double bonds in the carbon chain) and these reactions are mediated by elongase and desaturase enzymes (Zárate *et al.*, 2017) (Figure 2.5). The metabolites are then

activated and further metabolised to the inflammatory n-6 derived eicosanoids and the anti-inflammatory n-3 derived eicosanoids (Kang and Weylandt, 2008). During metabolism, there is competition for the enzymes, which makes the ratio of n-6:n-3 (or LA:ALA) important, however, LA is more efficient metabolising to AA than ALA to EPA, DPA and DHA (Kang and Weylandt, 2008), again making the ratio an important aspect of animal and human nutrition and health.

Modern Western diets have an n-6/n-3 ratio of about 7-20:1, far from the 1:1 which is thought to have been the norm during human evolution (Simopoulos, 2001; Calder, 2015a). Historically, n-3 came from meat (reared on pasture), fish, leafy green vegetables, nuts and berries, but these have decreased whilst n-6 consumption has increased with the inclusion of vegetable oils (including sunflower, soya bean, corn/maize oil), cereal grains and animal products produced from grain-based, rather than forage, diets over the last 50 years (Simopoulos, 2008). There is increasing evidence that this excess LA consumption and increase in dietary n-6/n-3 ratio has contributed to a sharp rise in obesity, atherosclerosis and diabetes (Harris *et al.*, 2009; Donahue *et al.*, 2011; Simopoulos, 2016) and an imbalance in this ratio towards n-6 is highly proinflammatory and prothrombotic (Simopoulos, 2016). Therefore, working towards a n-6/n-3 ratio closer to 1-4:1, is considered beneficial for human health (Simopoulos, 2008). However, much of this evidence seems to be based on pathways and not robust dietary intervention trials or long-term health studies, therefore, at least for now, the evidence does not suggest that n-6 consumption should decrease. But increasing consumers' dietary n-3 would increase total PUFA intake (preferably by replacing SFAs) and decrease the n-6/n-3 ratio.

There has been a lot of research into manipulating the FA profile of dairy produce through changes in cows' diet and management, which has a significant effect on PUFA concentration and the n-6: n-3 ratio. Typically, increasing the proportion of forage decreases the n-6/n-3 ratio in milk, a pattern that has also been associated with differences in management, e.g. conventional, organic and 100% pasture fed milk (Butler *et al.*, 2011a; Stergiadis *et al.*, 2012; Benbrook *et al.*, 2018; Davis *et al.*, 2020), as the organic and PFLA standards require more forage in the dairy diet than conventional management (PFLA, 2018; Soil Association, 2018). Additionally, summer milk has a lower n-6/n-3 ratio than winter milk, thought to be connected to the amount of fresh forage or grazing available in the summer compared to winter (Butler *et al.*, 2008; Kliem *et al.*, 2013). Furthermore, there is evidence that there can be as much variation within breeds as between breeds on the same diet (Soyeurt *et al.*, 2006; Stergiadis *et al.*, 2013), but more thorough analysis is needed to determine within system genetic effects on the n-6/n-

3 ratio. The evidence strongly suggests that the more fresh forage in the dairy diet the lower the n-6/n-3 ratio in the cows diet and that this will reduce the ratio in dairy products (Średnicka-Tober *et al.*, 2016; Butler and Stergiadis, 2020).

2.7.2.3.4 CLA9

A group of isomers of linoleic acid are conjugated (CLAs) (having double bonds on adjacent carbon atoms) the most abundant being rumenic acid (c9, t11 CLA (CLA9)), which is mainly found in ruminant milk and meat, contributing around 90% of human dietary intake (Tricon *et al.*, 2005). Technically, many are trans-fats, but as they also have a cis- double bond are mostly left out of the trans-fat category (Benbrook *et al.*, 2018). CLA9 has attracted attention due to identified anticancer properties and health benefits associated with the immune system and cardiovascular health (Wahle *et al.*, 2004; Benjamin and Spener, 2009) and potential to reduce adiposity (Kennedy *et al.*, 2010). Much of the research examines pathways and mechanisms or discusses studies based on animals (predominantly modified mice). However, Dilzer and Park (2012) reviewed studies involving humans and Yang *et al.* (2015) reviewed health and mechanistic studies, both concluding that there is evidence of health benefits from CLA, but the observed effects of CLA are larger in mice than humans and more research is needed to understand the dose effect in humans.

There are many studies that show CLA9 concentrations in milk increase as forage content increases in the dairy diet (similarly to omega-3) with management (Średnicka-Tober *et al.*, 2016; Benbrook *et al.*, 2018; Davis *et al.*, 2020) and season (Butler *et al.*, 2008; Kliem *et al.*, 2013). However, there do not appear to be any studies demonstrating direct health benefits from increasing ruminant milk or meat consumption in the human diet, therefore the health impacts of increasing consumption of ruminant produce with high CLA9 is unknown.

2.7.2.4 FATTY ACID SUMMARY

Generally, the higher proportion of forage (and less concentrate) in the dairy diet, the greater the concentration of PUFAs (especially omega-3), CLA9 and MUFAs (typically oleic and vaccenic acid) and the lower the concentration of SFAs. Any reduction in SFAs and increase in PUFAs is thought to be beneficial for human health. Despite evidence that switching to forage-based and/or organic milk would have an effect on FA consumption and thus human health, these conclusions are derived from modelling work, which have clear health implications but not yet evidenced impact.

2.7.3 LINKING DAIRY DIETS TO MILK FAT COMPOSITION

The dairy diet is predominantly (up to 70% of DM) carbohydrates (cellulose and starch), which is broken down in the rumen to simple sugars and converted to pyruvate. Pyruvate is then converted to the volatile fatty acids (VFAs) acetate, propionate and butyrate (and carbon dioxide and methane) in the rumen (McDonald *et al.*, 2011). The proportion of the VFAs is influenced by the diet and time elapsed since the previous meal. For example, fibrous forages create more acetate, whereas more digestible forages increase the concentration of propionate. However, when concentrates make up >60% of the diet, the amount of propionate increases at the expense of acetate (McDonald *et al.*, 2011). These energy providing fermentation end-products are essential for a healthy rumen; acetate and butyrate are then used for *de novo* fat synthesis and propionate is used for glucose (precursor for lactose and amino acids) in the mammary gland during lactation (Erickson and Kalscheur, 2020).

Additionally, fat is an important part of the dairy diet (up to 8% of DM), and the FA profile of forages (such as grasses and clovers) are predominantly ALA (~62% of the total), LA (~20%) and palmitic acid (C16:0) (~17%), which combined equate to ~93% of the profile, but the relative proportion of FAs offered by each forage is variable (Walker *et al.*, 2004; Clapham *et al.*, 2005). In contrast, cereal lipids (in concentrate feeds) have a higher proportion of LA (~58%) compared to ALA (~4%) and palmitic acid (~20%) (Ryan *et al.*, 2007; Wojtkowiak *et al.*, 2018). Therefore, fresh forages have a strong supply of n-3 and CLA precursors whilst diets containing grain have a supply of LA and SFA precursors (Buccioni *et al.*, 2012). Both carbohydrates and lipid sources, influenced by feeds in the diet, will have an impact on the FA profile of milk, directly via the FA supplied and indirectly from rumen fermentation and VFAs produced. This indicates that targeting dairy nutrition affects the milk FA profile, potentially impacting human health.

There are two main sources of FAs in milk (through many complex pathways) (Chilliard *et al.*, 2000; Chilliard *et al.*, 2007):

i. Uptake of FAs from blood circulation (around 40% of the total) mostly originating from the diet but modified in the rumen (and the mammary gland)
ii. From *de novo* synthesis within the mammary gland (around 60% of the total, almost all C4:0-C14:0 and ~50% of C16:0) synthesised from acetate and butyrate produced in the rumen.

The rumens' bacteria and protozoa hydrolyses esterified fat to unsaturated free fatty acids, phospholipids and glycolipids (and other organic compounds) (Buccioni *et al.*, 2012). Then, most unsaturated FAs are biohydrogenated, resulting in mostly saturated free FAs leaving the rumen, predominantly palmitic (C16:)0) and stearic acid (C18:0) (Bauman *et al.*, 2011) (Figure

2.6). Despite this, there are intermediate pathways that give rise to many, more minor, FAs (Destaillats *et al.*, 2005) and these intermediates (which can escape the rumen and are found in milk) are heavily influenced by diet (Chilliard *et al.*, 2007).

Another well studied FA pathway is that of CLA9. The trans-FA vaccenic acid is the main precursor for CLA9 and is synthesised in two main ways. Some CLAs isomers, including CLA9, are produced in the rumen, along with VA which go through circulation to the mammary gland where the Δ^9-desaturase enzyme desaturates VA (removes two hydrogen atoms), synthesising more CLA9 (Jahreis and Dawczynski, 2020), which is by far the most significant source in milk (Butler *et al.*, 2009). These pathways and processes of converting feed to milk are complicated and have both been well reviewed but are not completely understood or predictable.

Ruminants readily synthesise short and medium chain FA, but not many long-chain FAs that are desirable in milk; it is therefore essential for long chain FA to be consumed in the ruminant diet for them to be secreted into milk (Lock and Garnsworthy, 2002; Elgersma *et al.*, 2006). However, forage FA profile varies by species, method of conserving (hay vs silage), nitrogen fertilisation, quality/maturity, light intensity and across the grazing season (Dewhurst and King, 1998; Boufaïed *et al.*, 2003; Elgersma *et al.*, 2003a; Elgersma *et al.*, 2003c; Glasser *et al.*, 2013). But the three stages that have the biggest impact on FA profile of the forage are maturation, after grazing or cutting and through storage (Elgersma *et al.*, 2006). As forage ages or after it is cut the proportion of lipid decreases, as does the proportion of ALA. Wilting before ensiling also reduces total FA concentration (~30%) and reduces ALA (~40%), but as long as compaction and anaerobic conditions are effective and/or silage additives are used (e.g. formic acid) losses are reduced, preserving the CLA precursors (Dewhurst and King, 1998; Doreau and Poncet, 2000). Consequently, hay and haylage making incur greater losses in FA concentration (~50%), mostly at the expense of ALA (Doreau and Poncet, 2000; Elgersma *et al.*, 2003b). Ruminants grazing fresh forage provides the most beneficial FA profile for rumen health and most nutritious milk FA profile.

Generally, the proportion of ALA in grasses are higher than in clover and forbs (herbs) (Elgersma *et al.*, 2013). Of the most common grass species, timothy has the highest palmitic acid (18.8g/100g) and LA (20.3g/100g) concentration compared to rye-grass and fescue (palmitic acid: 16.7g/100g, LA: 12.3, 13.4g/100g respectively), while rye-grass has the highest concentration of ALA (61.0g/100g) compared to timothy (49.9g/100g) and fescue (55.9) (Glasser *et al.*, 2013). Additionally, there is evidence that increasing the proportion of ALA in ryegrass increases the concentration of CLA9 in milk (Elgersma *et al.*, 2003a). Between

common legumes, alfafa/lucerne has the highest concentration of palmitic acid (23.2g/100g) and LA (19.9g/100g) compared to red and white clover (C16:0: 18.0g/100g, 15.3g/100g and LA: 19.8g/100g, 16.5g/100g), whilst white clover has the highest proportion of ALA (58.0g/100g) compared to alfafa/lucerne and red clover (ALA: 41.7g/100g, 49.0g/100g) respectively (Glasser *et al.*, 2013). Additionally, the maturity of the plant impacts the FA profile, and the timing of maturation varies with species and weather; typically the greener and leafier the plant, the higher the concentration of ALA and as it grows it loses leaves and becomes stemmy (cell walls increase relative to cell membranes), increasing fibre and decreasing fat concentration, eventually going to seed, increasing the concentration of LA and palmitic acid (Dewhurst *et al.*, 2001; Buccioni *et al.*, 2012; Khan *et al.*, 2012; Glasser *et al.*, 2013). This provides clear evidence that sward type and species impact sward FA profile which, as discussed above, has implications on the milk FA profile. These differences and changes support a grazing management approach that encompasses a wide mix of sward varieties. By making sure plants are at different stages of maturation throughout the season will provide cows with a continuous supply of fresh leafy green plant matter.

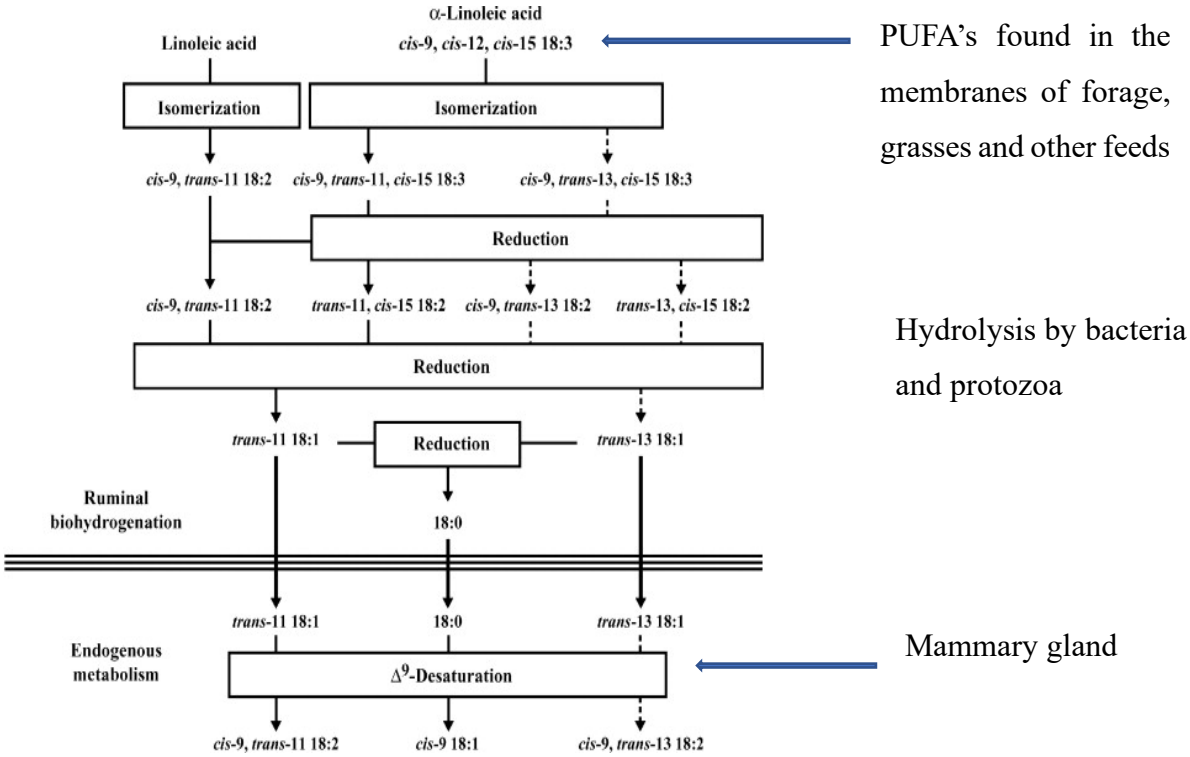


Figure 2.6 Biohydrogenation of dietary linoleic and linolenic acid, adapted from Destaillats *et al.* (2005).

With the rise in consumers wanting to understand the authenticity of their food and forage-fed becoming a marker of health and high welfare (Arnott *et al.*, 2017), there is some evidence that it may be possible to distinguish between LI and conventional milk through FA analysis (in combination with other analytics) (Engel *et al.*, 2007; Prache *et al.*, 2020). A meta-analysis is

needed that identifies the effects of different grasses, legumes and forbs on milk FA profile to provide further validation and better understand these processes. Alongside the meta-analysis an education policy that informs consumers about product quality is required.

2.7.4 THE DAIRY MATRIX

As evidenced above, research into fatty acid nutrition and human health has typically and simplistically focussed on individual components and associated health outcomes (e.g. SFAs impact on cholesterol). However, dairy products are very complex and variable foods, with many constituents that affect human health, including fat, protein, minerals, sodium and components of the milk fat globule membrane, this combination of nutrients and components is known as the dairy matrix (Thorning *et al.*, 2017). Therefore, the whole food or even diet needs to be taken into consideration when discussing health outcomes from a specific product; humans eat a combination of foods, not nutrients (fat, protein, minerals, etc) in isolation. Because whole foods and dietary patterns are known to impact health outcomes, assessing the whole food in relation to health outcomes may be an appropriate method (Mozaffarian and Ludwig, 2010). There is an abundance of research displaying the differences in the dairy matrix between yoghurt, cheese, milk, etc. (Thorning *et al.*, 2017; Feeney and McKinley, 2020), yet dietary recommendations treat dairy as a singular food group (Willett *et al.*, 2019). Treating each food independently would result in a more specific analysis and discussion of health outcomes. A good example of this is cheese, a very rich source of SFAs (approximately 17-25g/100g cheese) and a big contributor to total SFA intake (Feeney *et al.*, 2016). However, meta-analyses have associated cheese consumption with a reduced risk of CVD, hypertension and stroke (De Goede *et al.*, 2015; Drouin-Chartier *et al.*, 2016). This evidence suggests that despite the SFA content, other nutrients in cheese counteract the known negative effects of SFAs and supports recommendations to research whole food effects.

2.8 CONCLUSION

It is clear from recent studies that pasture-based, low-input and organic dairy systems produce milk with a beneficial fatty acid profile (increased omega-3 and CLA9) compared to intensive, concentrate-fed cows, as well as potentially reducing environmental impacts through improved pasture management (e.g. improved soil quality and biodiversity). Pasture-based farmers are already developing their own breeding programmes, reducing inputs (from antibiotics to mineral nitrogen fertiliser) and developing grazing strategies that utilise forage growth which are supportive of the local environment. Knowing both the nutritional and environmental benefits of grazing-based dairy systems, it is imperative that research includes breeding

programmes and production efficiency to suit the needs of these systems, including health, fertility and milk quality parameters.

CHAPTER 3. METHODS

3.1 INTRODUCTION

As discussed in Chapter 2, Section 2.2, LI systems do not have a pre-determined model of production efficiency. Low-input dairy cows demonstrate their suitability to a forage-based system with a relatively high yield compared to the amount of forage/feed they consume, low incidence of illness, high fertility and milk composition beneficial for human health (n-3, CLA and low n-6:n-3 ratio), as defined by the Organic Valley (Benbrook *et al.*, 2018), each of these components will be explored in Chapters 5 and 5. Data collected through this thesis allows scope to explore the relationship between these key variables and the potential drivers of PE. The complexity of the datasets provided the scope to explore multiple statistical approaches and simple modelling procedures.

3.2 ETHICS

Ethical approval was required for work undertaken in Chapters 4, 5 and 6. All procedures were acceptable to internal ethical review, in accordance with EU Directive 2010/63/EU for animal experiments and approved by the Animal Welfare and Ethical Review Body at Newcastle University.

3.3 MILK PREPARATION AND ANALYSIS

3.3.1 MILK COLLECTION AND STORAGE

After collection, all milk included in this thesis was transported for no longer than 3 days, at 4^{o}C, to Newcastle University and frozen at -20^{o}C. Samples sent to National Milk Records (NMR, 2019) for basic composition analysis (discussed below in Section 3.3.4) were preserved in bronopol in pre-prepared sample bottles before transportation to the NMR lab. After NMR analysis the samples were posted to Newcastle University (ambient temperature) and frozen at -20^{o}C. The following sample preparation and analysis took place at Newcastle University unless otherwise noted.

3.3.2 LIPID EXTRACTION

3.3.2.1 MILK SAMPLE PREPARATION

Prior to extraction, milk was defrosted over-night at 4^{o}C, aliquoted into two 20g portions and put in the freeze dryer for a weeklong programme. Lyophilised milk samples were put in the desiccator then weighed, before lipid extraction.

3.3.2.2 EXTRACTION

Dried milk samples were methylated and esterified in preparation for Gas Chromatography (GC). Lipid extraction for each individual milk sample occurred in the same order using the following methods adapted from Chilliard *et al.* (2009):

- A 130mg aliquot was placed in clean, dry test tubes.
- 2ml Hexane added, then tubes vortexed.
- 2ml 0.5M Sodium Methylate added, then tubes vortexed.
- Tubes placed on the hot block at 50^{o}C for 15 minutes then cooled to room temperature.
- 75µl 12N Hydrochloric Acid added, then tubes vortexed and left to stand for 15 minutes.
- 3ml Hexane added, then tubes vortexed.
- 3ml deionised water added, then tubes vortexed.
- Tubes placed in the centrifuge at 1,160g for 5 minutes at 5^{o}C.
- 400µl of the upper layer collected and decanted into 400µl GC vials.

3.3.3 GAS CHROMATOGRAPHY

The prepared fatty acid methyl esters (FAME) were separated and quantified using GC (Shimadzu, GC-2014, Kyoto, Japan). The GC had a flame ionisation detector and a 100m x 0.25mm ID with 0.2 µm film thickness Varian CP-SIL 88 fused silica capillary column. The original method described by Chilliard *et al.* (2009) and further described by Stergiadis *et al.* (2014) was used and adapted to ensure clear peak separation (Table 3.1). Individual FAs were identified against peaks generated by a 74 methyl FA standard, where the area under each peak was integrated using Shimadzu GC Solution software. Quantification of individual FAs was based on the peak area of each FA, reported as percentage of total peak areas for quantified FAs. A fatty acid methyl ester standard and published chromatograms (Loor *et al.*, 2004; Shingfield *et al.*, 2006) were used to identify the FAs and correction factors for short chain FAs were applied using the method described by Stergiadis *et al*. (2014). The identification of peaks for this thesis are available in Appendix A Figure A.1. The chemicals used for extraction of

FAs, correction factors for short-chain FAs (C4:C10:0), analytical standards and identification of peaks followed the methodology of and are described by Stergiadis *et al.* (2014).

Table 3.1 The programme used for GC separation and quantification of milk fatty acids

Rate (°C/min)	Temperature (°C)	Hold time (min)
-	50	2
2.00	188	20
2.00	240	44

Total runtime: 150 minutes

The set up for the GC was as follows:

- Column: CP-SIL 88, 100m x 0.25mm ID x 0.20μm FT
- Injector temperature: 250°C
- Detector temperature: 275°C
- Injection Mode: Split
- Flow Control Mode: Linear Velocity
- Pressure: 109.9 kPa
- Total Flow: 21.9 ml/min
- Column Flow: 0.43ml/min
- Linear Velocity: 9.5 cm/sec
- Split Ratio: 50.0

3.3.4 NATIONAL MILK RECORDING

Milk samples collected for Chapter 5 and Chapter 6 were preserved with Bronopol and kept at ambient temperature during transportation to a commercial NMR lab (National Milk Recording, 2018). Basic milk composition was analysed using Milkoscan FT 6000 (Foss Electric, Hillerød, Denmark) (milk fat, protein, urea and lactose content) and Somatic Cell Count (SCC) was recorded using a Fossomatic instrument (Foss Electric). The samples were then transported at ambient temperature to Newcastle University where they were frozen at -20^{o}C. Bronopol preserves milk for more than five days and is effective unless 'temperatures are consistently high' (Ruttan, 1993); ambient temperature varied by season but milk was frozen within four days of collection. There is some evidence that Bronopol may have a small impact on minor long-chain FAs (Butler *et al.*, 2011a) and protein concentration (Barbano *et al.*, 2010), but all milk samples included in this thesis followed the same protocol making the various 'treatments' comparable.

3.4 THE DATA

There are three main datasets explored and analysed though the upcoming chapters. All datasets contain full fatty acid (FA) profiles (74 response variables, expressed as a proportion of total FAs), with independent factors including farm management (conventional, organic, low-input, pasture-fed, farm ID) and source (supermarket and/or farm and cow ID). Two datasets

(discussed in Chapter 5 and Chapter 6) also have individual cow and farm background data including milk yield, animal health, NMR milk quality analysis (protein, lactose, urea, SCC), fertility, breeding and various farming methods (type of rotation, grazing management, AI/bull etc). Additionally, Chapter 6 reports data collected on the eating, grazing and ruminating behaviour of individual cows, followed through lactation.

3.5 DATA HANDLING

All raw data from chromatograms (the 74 FAs) was uploaded and manipulated (calculations and ratios including SFAs, MUFAs, PUFAs, omega-3, omega-6, omega-6: omega-3 ratio and very long chain FAs) in Excel and organised using 'R' (R Core Team, 2017). All FAs, classifications and calculations are included in Appendix A, all other raw data was manipulated, organised, visualised and analysed in 'R'. 'R' is a powerful open source statistical programming software that has many functions, from data handling and visualisation to statistical analysis and computer programming. For additional functionality and to simplify coding, packages are developed and shared by other programmers or users. These packages are downloaded and can run alongside others within 'R'. Due to the open source nature of 'R', they are continually updated, debugged and developed.

3.6 STATISTICAL ANALYSIS

With all statistical approaches there are many models and methods that can be used to explore and explain the data. There is not a single 'best' method of data analysis that can be used in all circumstances, instead the approach used in this thesis has been to explore several avenues for data modelling and identify the most appropriate. The ideal model is one that is relatively simple, and thus is easier to interpret biologically, whilst still explaining as much of the variation in the raw data as possible. This is sometimes described as "Occram's razor" or "law of parsimony", in that the simplest explanation is more likely to be of practical value (Crawley, 2012). The data analysis approach to this PhD has also included a large amount of data visualisation to guide model choice and enhance interpretation of model outputs.

All statistical analysis was conducted in 'R' (R Core Team, 2017). In most cases, data was first visualised using 'ggplot2' (Wickham, 2009) and in many cases had to be organised, subset, grouped and filtered using 'tidyverse' functions (Wickham, 2017). The main statistical concerns were:

i. Missing data
ii. Large number of response variables

iii. Numerous continuous variables that could be argued as both response and explanatory. For example, milk yield is both a response to intake (the more a cow eats the more milk it will give) but can explain intake (a high yielding cow will need high intake to maintain condition and produce milk) and possibly milk composition.

3.6.1 MISSING DATA (EXPLANATIONS)

In Chapter 5 and Chapter 6 there are considerable missing data, mostly because working with commercial farms, cattle and technology are all challenging. There are missing observations for entire farms (where a farm missed a data recording session) and specific cows on particular dates (if a cow is on medication it may not have been milked with the rest of the herd) and sections where lab results were not returned (milk sent to NMR occasionally was not forwarded to the university and/or results were inaccurate) or technology failed (there were numerous times when RumiWatch halters stopped working mid-recording). Steps were taken to mitigate the impact and prevent missing data occurring and re-occurring (e.g. rewiring halters and numerous phone and email exchanges), but unfortunately missing results were inevitable.

In most statistical analysis, missing data results in rows of data deletion, which leads to information loss. There are many 'R' packages that work with missing values ('MICE', 'Amelia', 'Hmisc') that use a machine learning approach of multiple imputations to obtain the most likely missing values. Typically, these methods produce many potential datasets that can be combined (averaged), but an assumption of these packages is randomness of the missing values. Unfortunately, imputing missing data was not an option because the missing results in this thesis are not random throughout the data (Harrell, 2015), but often systematic (e.g. specific to a farm or a cow). In practice this meant that sections of data were sometimes missing and if multiple imputations are applied to a whole or partial row of missing data the algorithm and thus the response is unreliable.

Given that imputation was not an option, the overall approach was to leave missing values in the datasets as NAs and adjust based on specific analysis. Data was subset (using 'tidyverse' functions) before each individual analysis to retain as much appropriate data as possible. Some of the calculations required other known data. For example, to calculate energy-corrected milk yield, fat and protein content are prerequisites. In a few instances where fat and/or protein content were missing but other data for that cow were available, fat and/or protein content were calculated by averaging other results from that cow. This is controversial because using means to replace missing values introduces bias by reducing data noise and mean values shrink standard errors, among other concerns (Little *et al.*, 2013). However, list-wise deletion (removing an entire record if data is missing) also creates bias and removes important data. For

this reason, using means where missing values are not random and included only for further calculation (on very small subsections of data) is justified. Where entire FA profiles or halter recordings were missing, no imputation methods were used. The implication of this missing data will be discussed in the relevant sections throughout the thesis.

3.6.2 DATA SIMPLIFICATION

Despite the missing data, there were still numerous variables recorded, many of which were potentially collinear with each other, increasing the challenges for data analysis and interpretation. These include the FA data (74 variables, that always add up to 100%, plus calculations) from all cows over numerous dates and farms, background farm information and, in Chapter 6, the automated data collection by RumiWatch halters (all eating, grazing and ruminating behaviour, recorded hourly, but totalled daily). Therefore, data reduction techniques were required due to the large amount of data collected for Chapter 5 and Chapter 6. Initially, there was no clear definition of PE, which made identifying explanatory variables difficult, especially as yield acts as both a response and explanatory variable. Many packages were used to determine if step-wise reduction techniques were applicable and to evaluate the strengths and weaknesses of each approach. Many of these were subsequently discarded, but the exercises proved a valuable learning experience, generating objective scrutiny to decide which methods or packages were appropriate. The figures shown in the following sections are used as descriptive statistics and will be used to illustrate the methods described; the data will not be discussed in this section.

Aim: Reduce the data by identifying variables that can help define production efficiency and are important to production efficiency.

3.6.2.1 VEGAN

The ‘vegan’ (Oksanen *et al.*, 2017) package was developed for community ecology and provides ordination and analysis functions. Ordination has two main methods: unconstrained (e.g. principal components analysis- PCA) and constrained (e.g. redundancy analysis- RDA) (Anderson and Willis, 2003). Unconstrained ordination is useful for visualising patterns among groups and was used extensively in all experimental chapters to better understand the data. However, constrained ordination uses prior hypotheses to produce plots, relate response variables (e.g. FA proportions) to predictor variables (e.g. Farm ID) and is used in permutation tests (ANOVA) (Anderson and Willis, 2003). Using unconstrained ordination, permutation

tests were used to identify which explanatory variables influence different response variables (e.g. the FA profile). This is further discussed below in Section 3.6.4.

Using constrained ordination such as redundancy analysis (RDA), an ANOVA can then identify significance within the selected model. 'Vegan' has a command, 'ordistep', which uses a step-wise approach to refine the model that best fits the data. However, Harrell (2001) outlines the limitations with this approach and ultimately concludes that this step-wise method results in a high likelihood of Type 1 errors (false positives), violates assumptions and produces p-values that are biased to 0. Additionally, this approach deviates from the main aim of identifying variables that effect efficiency and was ultimately abandoned. There is no scope here to define production efficiency and determine the important drivers.

3.6.2.2 RPART

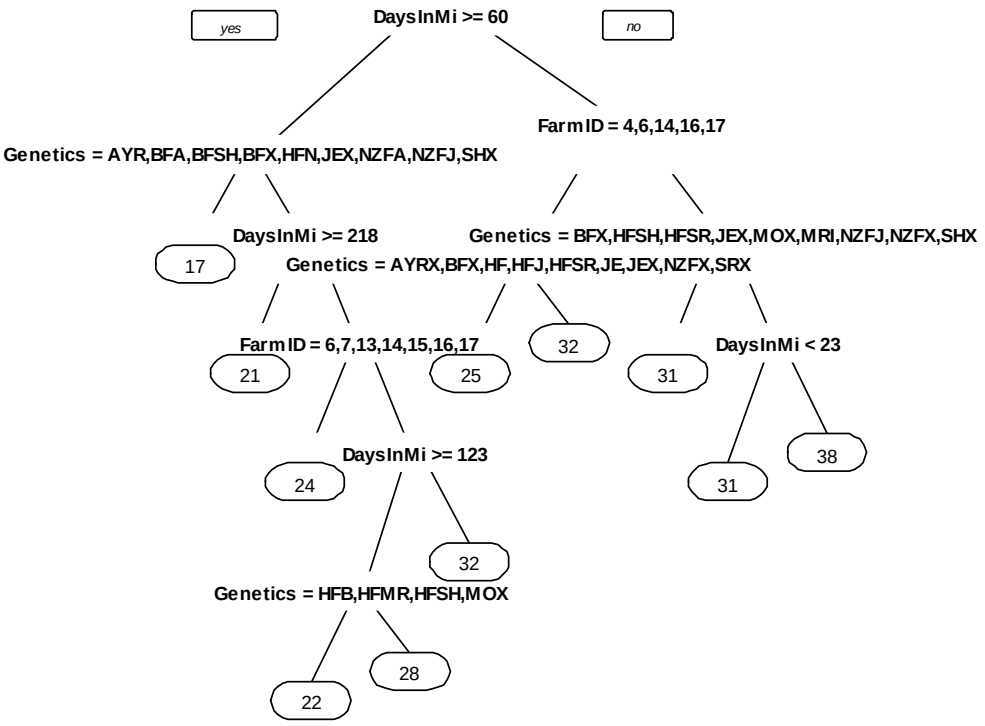


Figure 3.1 Decision tree generated from 'rpart' using data collected for Chapter 5. The numbers represent the predicted outcome (Yield in L/day) within each classifier.

The 'rpart' (Therneau *et al.,* 2017) package uses decision trees to split covariates and fit a model for each split, which is a powerful tool for prediction and interpretation. However, this approach was abandoned because the trees are susceptible to over fitting (Hothorn *et al.,* 2008) and although it allows numerous outcomes there is only one predictor (response variable). For example, yield could be predicted given Farm, Intake, Genetics and Days in Milk, but this

would create an unreliable tree because not all potential yield predictors are accounted for or included in this list (Figure 3.1). Additionally, the model doesn't have the scope to identify the variables that could drive or predict PE, which is determined by a combination of many variables.

3.6.2.3 RANDOM FOREST

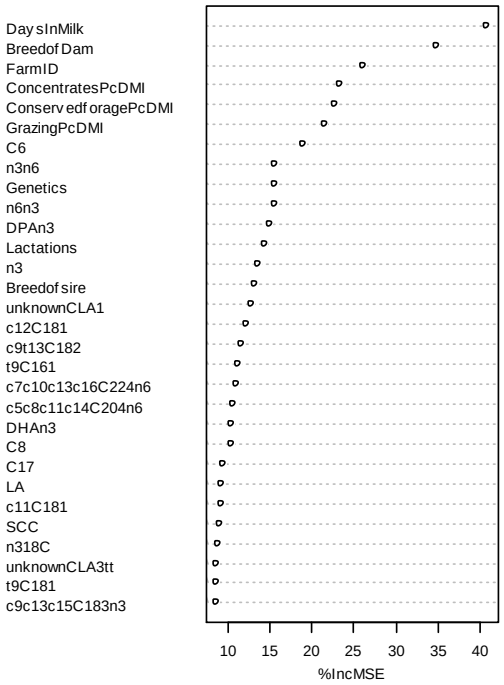


Figure 3.2 Plot generated from 'randomForest' with the most important variables (to Yield) at the top of the plot using data collected in Chapter 5. Variables will be explained in further chapters, this figure serves as an example only.

The 'randomForest' (Liaw and Wiener, 2002) package uses a classification algorithm to generate decision trees (a large number of uncorrelated models) and combines them to make a random forest, which dictates the class (or group) to which an observation might belong. Each tree created is uncorrelated due to bagging (taking a random sample replacement so that models are trained on different sets of data) and features randomness (Datla, 2015). This protects each tree from the others' errors and develops a very powerful prediction tool. The model learns from training data (the majority of the data, 75-80%) Kursa (2014), assesses all relationships within that subset to create a model, then the model is tested or validated on the remaining data to evaluate the accuracy of model predictions. This relies on high quality data and the ability to generate uncorrelated models. There is evidence that random forests have been effective in life sciences (Touw *et al.*, 2012; Kursa, 2014), particularly the -omics (genomics, proteomics etc) and ecology, but there are very few published examples in dairy science (Parker Gaddis *et al.*,

2016). Whilst this was an interesting way to explore the data, it did not ultimately help identify PE or scale down the quantity of data (Figure 3.2).

3.6.2.4 BORUTA

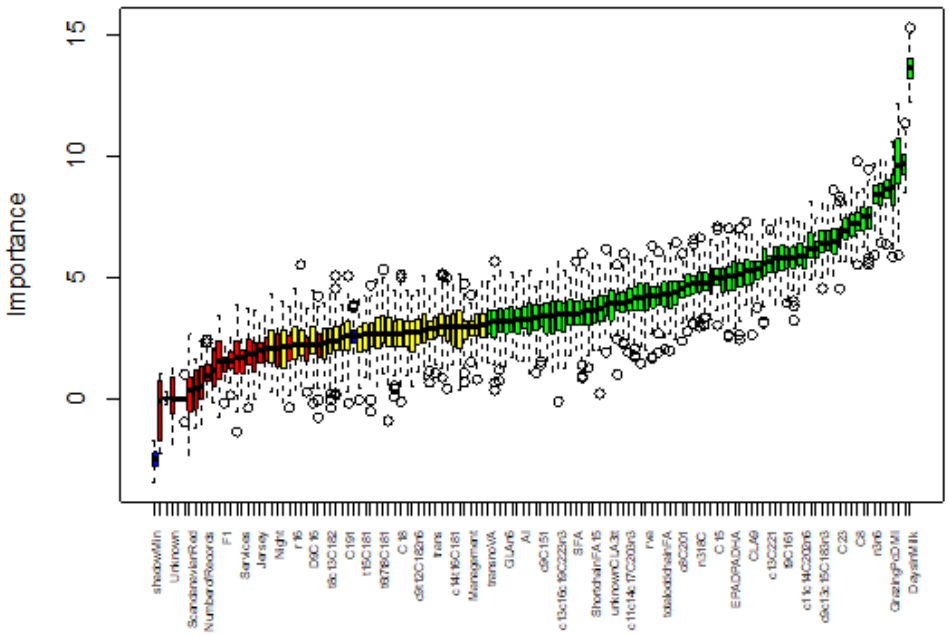


Figure 3.3 Boruta plot, using machine learning and the data from Chapter 5. The red indicates that variables are not important to Yield, the blue are dummy variables, the yellow are tentative and green are important to model Yield.

The 'Boruta' (Kursa, 2010) package uses a machine learning approach (built around the Random Forest algorithm) to reduce the dimensionality of large datasets with redundant data. This type of approach often uses Principal Components Analysis for feature selection, but PCA assumes multivariate normal distribution (which requires transformation when data is skewed) and treats variables independently, therefore it doesn't take into consideration the information between variables (how each variable is related to each other). Boruta on the other hand, does not require these assumptions. However, it does require only one outcome variable (e.g. Yield). Boruta determines how important variables/features are to one another as well as to the outcome variable. The variables are classified as important, tentative or not important. Additionally, Boruta inserts dummy variables to test their importance to the outcome variable (Figure 3.3). The package then compares the median importance score of the tentative variables to the dummy variables to make the decision whether to confirm or reject features (Figure 3.4). Using data collected for Chapter 5, the important features were taken forward for further investigation (below- Bayesian Network). Despite the number of variables in Chapter 5, Boruta was unable to identify any that could be excluded as being unimportant to yield, thus was not a useful method for this data exploration.

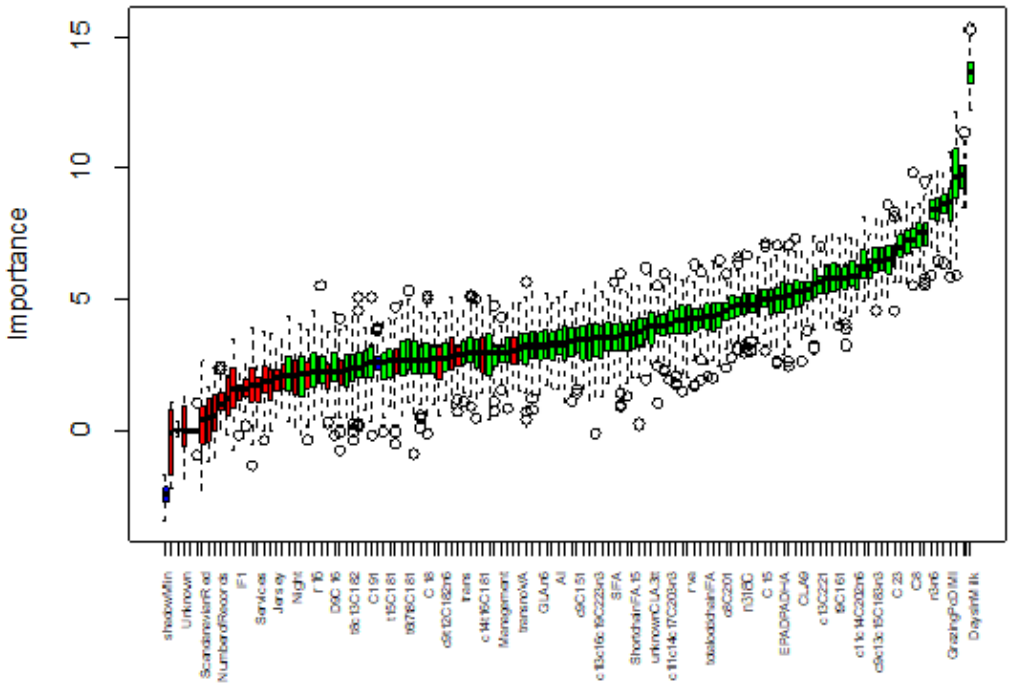


Figure 3.4 Boruta plot using machine learning and the data from Chapter 5. Red indicates rejected features and green confirmed features.

3.6.2.5 BAYESIAN NETWORK

Bayesian networks express variable relationships through graphical separation models, encoding the between variable conditional probability. The 'bnlearn' (Scutari, 2010) package created a network for all of the data available for Chapter 5 using the greedy, 'hill -climbing' algorithm (explained by Tsamardinos *et al.* (2006)). The structure can then be viewed and edited based on the analyser's prior knowledge of the network and, once the structure makes sense, inference by means of probability can be extracted. In this example, the software 'GeNIe' (BayesFusion, 2017) was used to visualise the model (Figure 3.5). All variables were converted to categorical by splitting numerical variables into three groups with equal numbers of observations. Once the model has been trained it can be used to find probability under certain conditions to discover relationships between variables. For example, the network in Figure 3.6 (from Chapter 5) has selected all New Zealand Friesian cross Jersey cows (far right), all of these cows were in the lowest concentration group of the saturated FA C16:0, 60% had the lowest concentration of omega-6 (n6), 38% in the middle concentration group and so on. Whilst this was an interesting way to explore the data and discover relationships between variables, extracting clear and robust results (while focussing on the research question) was confusing and the model generated was unstable.

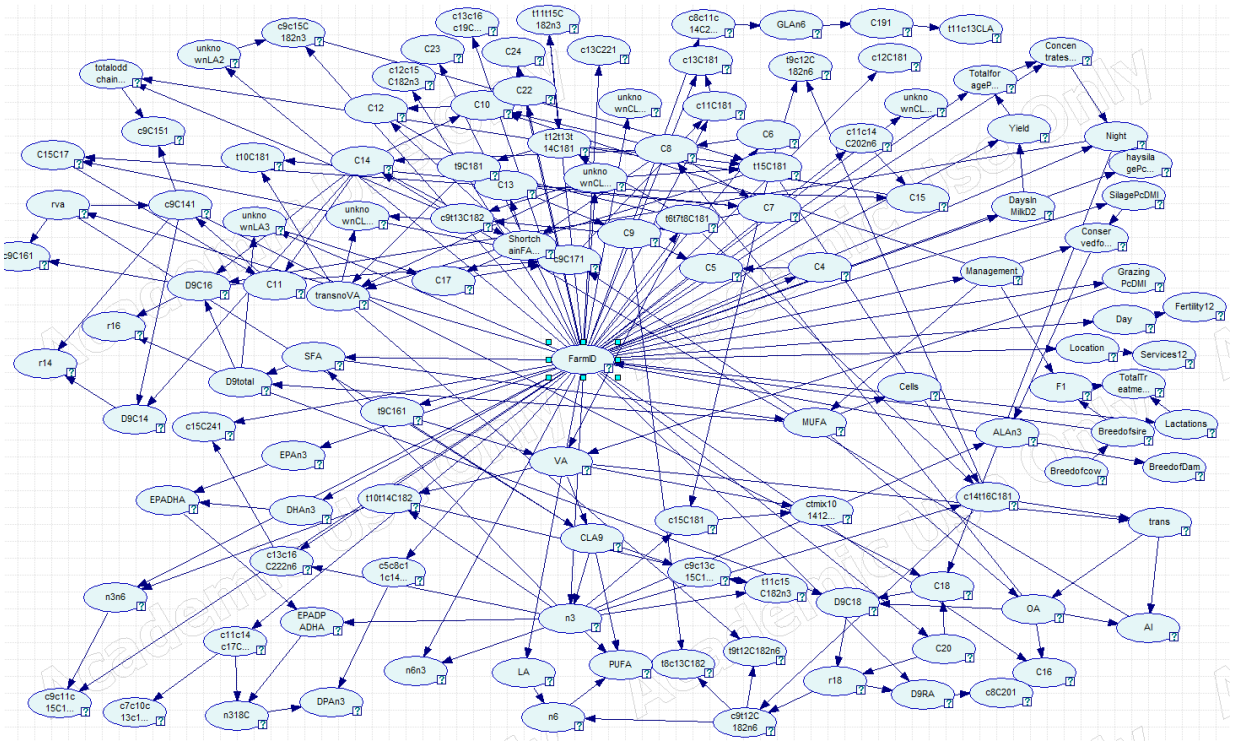


Figure 3.5 Bayesian Network using data collected for Chapter 5, relationships between all variables deemed important by Boruta, visualised using GeNIe.

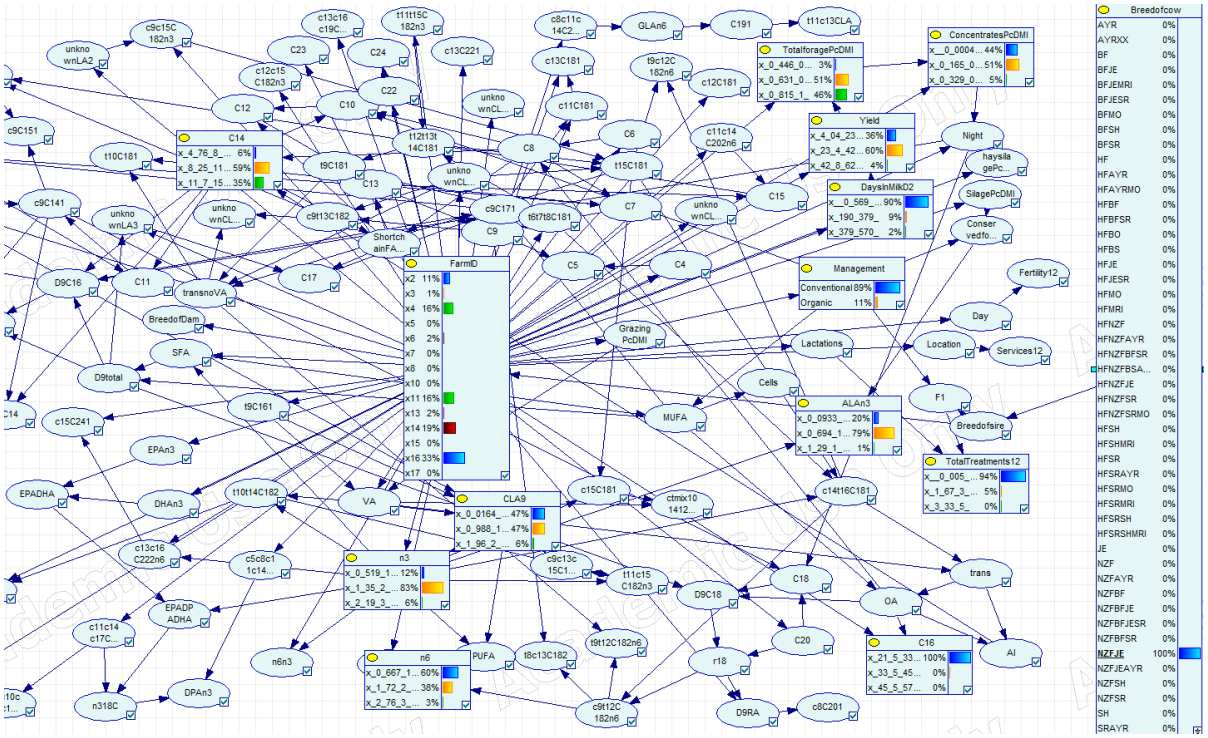


Figure 3.6 Bayesian Network using data collected for Chapter 5, relationships between all variables deemed important by Boruta, visualised using GeNIe, evidence set to only New Zealand Friesian x Jersey cows.

3.6.2.6 CONCLUSION

Despite the number of packages and functions available for data reduction, no single method could be used in subsequent Chapters. All packages helped explore and understand the datasets, but did not reduce variables for further analysis.

3.6.3 REFINING THE DATA

Due to these flaws in the data reduction stage, it became clear that production efficiency needed to be explored more closely and ultimately defined by combining relevant traits into a single response variable. This was approached slightly differently in Chapter 5 compared to Chapter 6 due to differences in the type of data collected, and will be discussed further in those sections.

3.6.4 MULTIVARIATE ANALYSIS

Ordination techniques are a good way to reduce many response variables into a single point and discover patterns (similarities and differences between data subsets), making it good to visualise the data and thus, interpret the results (as discussed previously, Section 3.6.2.1). Packages like 'vegan' (Oksanen *et al.*, 2017) make visualising and interpreting ordination results relatively straightforward.

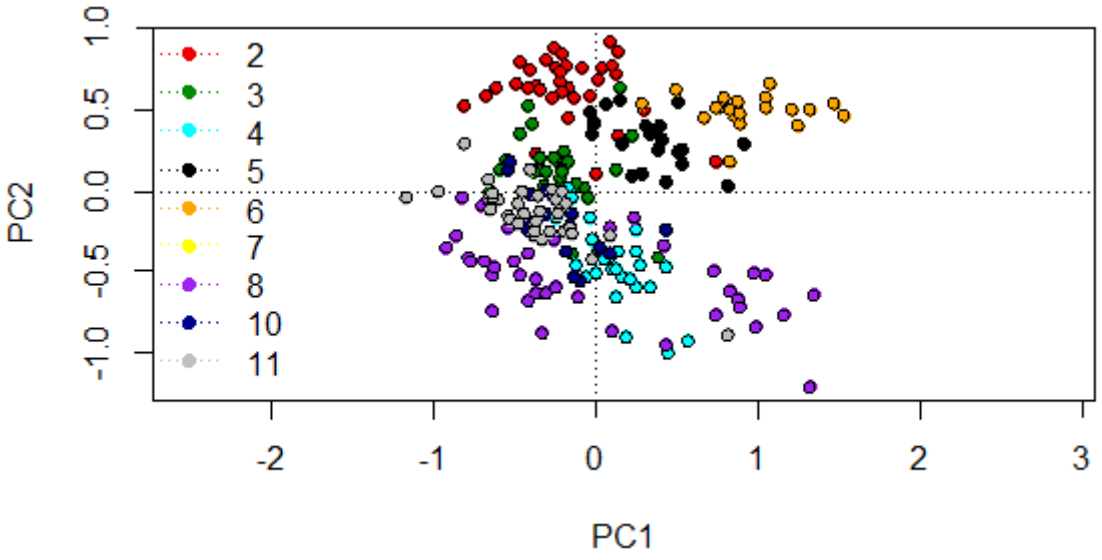


Figure 3.7 PCA displaying data from Chapter 5, each point represents the FA profile of the cow, highlighted by Farm ID. PC1 accounts for 16.9% and PC2-11% of the variation.

Principal Components Analysis was used to look at patterns in the overall FA profile of milk (74 proportional response variables) and production efficiency (in Chapter 5). Prior to multivariate analysis, all data were standardised using Hellinger transformation (divide by the sum (of the variable for example, yield), then square root) (Rao, 1995) or normalised (to mean

of zero and standard deviation of one) (Milligan and Cooper, 1988) to give each element the same weight (such as, elements of production efficiency). PCA then transformed the correlated variables into fewer uncorrelated variables or principal components. This method is deemed appropriate for community composition data (Legendre and Gallagher, 2001), which is not the same as FA data. However, this technique meets the assumptions for PCA and there is not a more specific technique that is best for all data (Lepš and Šmilauer, 2003). The response variables (as single points representing the profile of all FA concentrations) can be coloured by levels within factors (potential explanatory variables). This gives a clear indication of separation or overlap by levels, indicating if factors do, or are unlikely to, affect the response variables. In many circumstances, constraining the data by explanatory variables allows a bi-plot to be generated in which the size of arrows indicate the strength of relationships and their direction indicates how the factors could be related/unrelated. An ANOVA can then provide statistical evidence of the relationship between response and explanatory variables. However, in the case of data in Chapter 5, many of the factors were related (colinear), which would confound the results of a bi-plot and ANOVA. Therefore, the PCA was left unconstrained and colour was used to discern the factors.

3.6.5 MIXED EFFECTS MODELS

Mixed effects models to identify differences within data sets differ from linear models and analysis of variance (ANOVA) due to categorising two types of explanatory variables: fixed effects and random effects. Fixed effects are assumed to influence the mean values of the response variable (y) whilst random effects influence the variance within the y values (Barr *et al.*, 2013).

As an example, in Chapter 5, fixed factors (effects) are Farm ID and the sampling round and the individual cow number (and Farm ID combined with Round) is treated as a random factor. Typical models rely on the assumption that errors are independent (not correlated), but random effects represent grouping and can be correlated. Mixed effect models are useful when there is pseudo-replication, such as temporal (repeated measures) or spatial (nested designs/split plot) replication (Crawley, 2012). If the model has a linear structure, a linear mixed effects model (lme) can be used, otherwise a non-linear mixed effects model (nlme) is preferred (Boisgontier and Cheval, 2016).

A standard linear model can be described as:

$$Y = X + \epsilon$$

Where:
Y = the response variable
X = matrix explanatory variable(s)
ε = error variation
In a mixed effects model the error variation is expanded so that

$$\varepsilon_{ij} = \phi_j + \omega_j + \psi_{ij}$$

Where:
φj = cow number (random) variation
ωj = cow –specific variation in the effect of Farm ID and sampling round
ψij = individual cow variation (unexplained error variation)
For most of the models generated in this PhD, the package 'nlme' (Pinheiro *et al.,* 2017) is used to generate linear mixed effects models on un-transformed data. Using a mixed effects model prevented statistical bias due to pseudo-replication but meant that the same cow could be followed through numerous stages of lactation or farms could have milk sampled throughout the year. Other types of models and methods were explored (general mixed effects, Monte Carlo approximations, etc.), but the data met the assumptions of nlme most of the time and, where possible, a robust post-hoc analysis was conducted.

When there are multiple levels within an explanatory variable (such as the many farms within Farm ID or breeds within the Breed factor), a post-hoc test is used to explore the pairwise comparison of means and identify which values differ from each other. The function 'glht' in the 'multcomp' (Hothorn *et al.,* 2008) package is used, as it does not assume normal approximation but uses the t-distribution. Additionally, specifying Tukey Honest Significant Difference (Tukey HSD) accounts for random effects and controls family-wise error. When the post hoc analysis assumptions were not met (discussed further in Chapter 5), controlling the family-wise error rate was not possible (Smith *et al.,* 2002). Throughout this thesis managing Type 1 errors was not possible, thus clear separation of multiple levels within factors was not always obtainable and is discussed at each instance.

CHAPTER 4. EVIDENCE THAT FORAGE-FED COWS CAN ENHANCE MILK QUALITY

4.1 INTRODUCTION

Fats from dairy products have undergone major scrutiny by both the media and scientific community. Though ruminant agriculture has been criticised for its land use and emissions, a recent study indicates that pasture-based (PB) production has lower emissions compared to intensive systems (Lorenz *et al.*, 2019). Additionally, despite claims that dairy products are unnecessary sources of saturated fat and calories, dairy fats are a complex of important fatty acids (FA) (Mills *et al.*, 2011), many beneficial to human health. The more forage that cows consume, the higher the concentration of beneficial fatty acids (Stergiadis *et al.*, 2015b; Benbrook *et al.*, 2018), linking environmental benefits with nutritional gains.

Dairy herd management has the biggest impact on milk fat composition; cows fed a forage-based diet produce milk with more nutritionally beneficial FA compared to grain fed herds (Ellis *et al.*, 2006; Butler *et al.*, 2008; Stergiadis *et al.*, 2012; Stergiadis *et al.*, 2015b; Średnicka-Tober *et al.*, 2016; Prache *et al.*, 2020). The Pasture For Life Association (PFLA, 2018) is a UK certifying body that ensures 100% grass and forage-fed (maize and maize silage is not permitted) and high welfare beef, lamb and dairy products. PFLA farmers choose robust breeds that stay healthy and reach maturity under pasture-based forage only diets and utilise diverse vegetation swards consisting of herbs, red clover, grasses and wild-flowers. This is designed to reduce costs, increase profitability and regenerate and improve soil fertility, farm land and the environment (PFLA, 2018). A recent nationwide American study (Benbrook *et al.*, 2018) compared conventional, organic and Grassmilk^{TM} (a trademark label from Organic Valley, similar to PFLA certification) milk quality and found significantly different FA profile in the three milk types. Grassmilk^{TM} was higher in n-3, CLA9 and EPA and had a lower n-6/n-3 ratio, all attributes shown to lower cardiovascular and metabolic diseases (Swanson *et al.*, 2012; Markey *et al.*, 2014). These recent findings suggest a marked difference in the nutritional quality of pasture-only milk with the potential to consume more FA beneficial for human health, which has yet to be investigated in the UK.

This chapter aims to explore differences in milk fat composition between own-brand conventional and organic milk from supermarkets and PFLA milk in the UK. The objectives are to sample and analyse conventional and organic supermarket and PFLA milk, identify differences in fatty acid profile between different management systems and season and discuss the potential implications of switching to PFLA milk.

4.2 METHODS

4.2.1 EXPERIMENTAL DESIGN

Milk was collected from eight PFLA dairy farms (across southern UK) and five supermarkets (located centrally to the PFLA farms, i.e. within 10 miles (16 km) west of Bristol) in April (4^{th}–6^{th}), July (4^{th}–6^{th}) and October (10^{th}–12^{th}) 2017 (Figure 4.1). There is no winter sampling because PFLA farmers predominantly spring calve, therefore cows do not produce milk from November to January/February. A representative 500 ml milk sample was taken from the bulk tank on each farm (after stirring to disperse the cream) on the morning of collection. This coincided with the purchase of half-litre (500 ml), own-brand organic and conventional whole, pasteurised and homogenised milk from five supermarkets: Asda, Marks and Spencer, Sainsbury's, Tesco and Waitrose. None of the dairy farms supplied milk to the supermarkets.

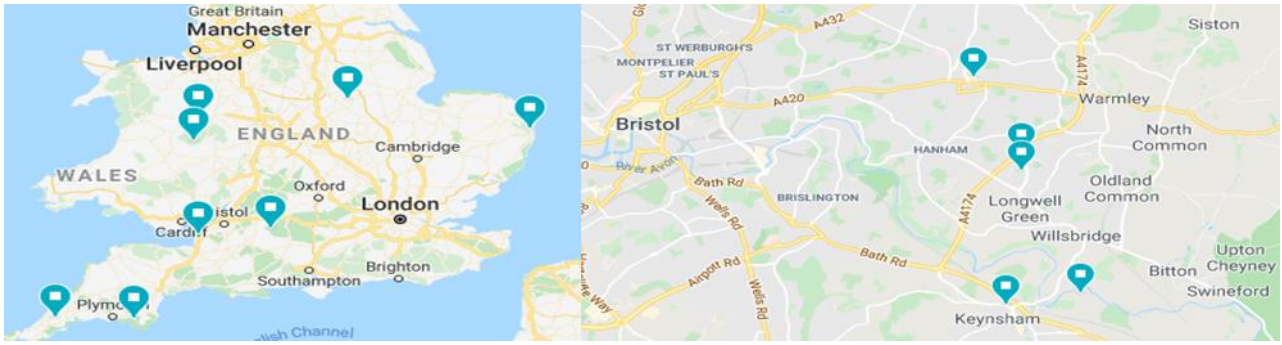


Figure 4.1 Map of the PFLA farms sampled on the left and the supermarkets sampled on the right [(Google., n.d.)].

4.2.2 MILK ANALYSIS

All samples were kept below 4 °C during transportation (maximum 3 days) and then frozen at −20 °C until analysis. Milk from supermarkets was purchased on the same day as each farm sampling date. All were in-date and their shelf life went beyond the date they were frozen. The samples were thawed overnight at 4 °C, freeze dried and then 130 µg of lyophilized milk was methylated and esterified to prepare for gas chromatography (GC), using the method described by Chilliard *et al.* (2009) and Stergiadis *et al.* (2015a) and in Chapter 3, Section 3.3.2.

Fatty acid methyl esters (FAMEs) were separated and quantified using GC (Shimadzu, GC-2014, Kyoto, Japan). The GC had a flame ionisation detector and a 100 m × 0.25 mm ID, 0.2 µm film thickness, Varian CP-SIL 88 fused silica capillary column. To optimize peak separation, modifications to the chromatographic conditions from the original method by Chilliard *et al.* (2009) were updated as described by Stergiadis *et al.* (2014) and Chapter 3, Section 3.3.3.

Although purchased milk was pasteurised and homogenised and farm milk was in a raw state at sampling, milk FA profiles are not changed by these processes (Butler *et al.*, 2011b), making this an appropriate comparison. Fatty acid results are expressed as a percentage of the whole FA profile, thus there is no need to adjust for differences in fat content between the whole supermarket milk (3.5% fat) and the raw farm milk (~3.5-4.5% fat).

4.2.3 DATA ANALYSIS

Microsoft Excel was used for data handling and statistical analysis was completed using 'R' (R Core Team, 2017).

The FAs thought to influence human health described in Chapter 2 (C12:0, C14:0, C16:0, Oleic Acid, Vaccenic Acid, Conjugated linoleic acid, omega-3, omega-6, omega-6/ omega-3 ratio, Eicosapentaenoic Acid + Docosapentaenoic Acid + Docosahexaenoic Acid) were standardised (normalisation to mean of zero and standard deviation of one) (Milligan and Cooper, 1988) for principal components analysis. The 'R' package 'vegan' (Oksanen *et al.*, 2017) was used to visualise the effects of management on the influential FAs. Two plots were produced of the same analysis: one that displays the FAs and the second displays the influential FAs from each source (supermarket and farm) and management (conventional, organic and PFLA) on each date, which are highlighted by their management. The closer two points are to each other in PCA ordination space, the more similar their characteristics (in terms of milk FA profile), as discussed in Chapter 3. Superimposing one plot over the other should help to identify which FAs are associated with which management.

Tests included linear mixed effects model (R package 'nlme'(Pinheiro, 2017)) to allow for nested random effects and unbalanced design, analysis of variance and Tukey's Honest Significant Difference. The factors were Month (April, July, October (n=18 each month)) and Management (Conventional (n=15), Organic (n=15), PFLA (n=24)) with Source (supermarket name and farm name) treated as a random factor. The concentrations of FA identified as having either a positive or negative impact on human health are explored in the results.

4.3 RESULTS

Milk fat composition varied by management system but rarely by month and interactions between management and month were not significant. The concentrations of the main nutritionally relevant FAs are presented in Table 4.1. Unless stated otherwise, all differences mentioned here and in the discussion were significant ($p<0.05$).

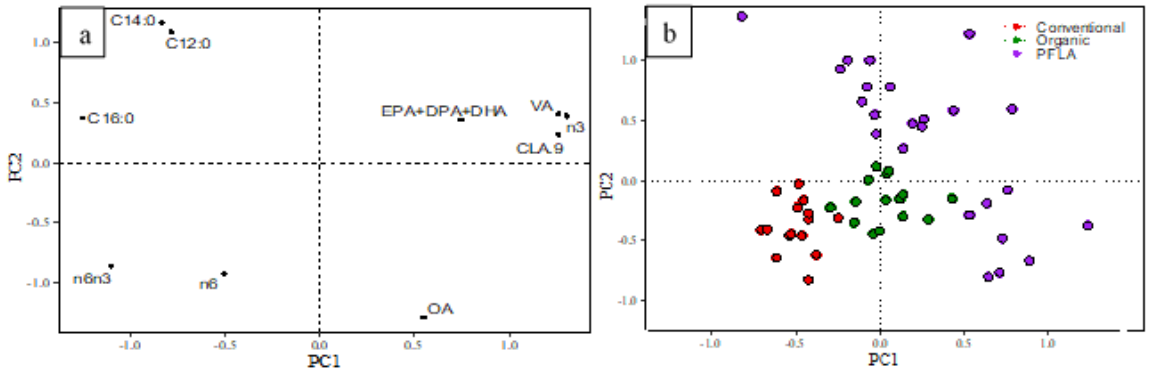


Figure 4.2 a. PCA displaying fatty acids thought to influence health. b. PCA of milk samples designated by Management system (each dot represents a milk sample.

C12:0= Lauric Acid, C14:0= Myristic Acid, C16:0= Palmitic Acid, OA= Oleic Acid (c9 C18:1), VA= Vaccenic Acid (t11 C18:1), CLA.9=Conjugated linoleic acid (C18:2, c9t11 isomer), n3=omega-3, n6=omega-6, n6n3= omega-6/ omega-3 ratio, EPA+DPA+DHA= EPA=Eicosapentaenoic Acid + DPA=Docosapentaenoic Acid + DHA=Docosahexaenoic Acid

4.3.1 PCA

In the PCA plots, PC1 explains 43% and PC2 explains 23% of the data (Figure 4.2). There is clear clustering by management with conventionally sourced supermarket milk (red) in the negative PC1 and PC2 axis and organically sourced supermarket milk (green) mostly clustered across 0 on the PC1 axis and slightly negative on the PC2 axis. The PFLA milk samples (purple) are spread out across the positive PC2 axis and in the positive PC1 and negative PC2 axis (Figure 4.2 b). Superimposing Figure 4.2 a over Figure 4.2 b suggests that the conventional milk is closely associated with n-6 and the n-6/n-3 ratio. Whilst the PFLA samples are spread out they are more associated with n-3, VA, CLA9 and the long chain FAs than conventional and organic samples. There is one PFLA sample that is close to the SFAs (C12:0, C14:0, C16:0) and a small cluster closer to OA.

4.3.2 EFFECT OF MANAGEMENT ON COMPOSITION

Table 4.1 Effect of Management (Conventional, Organic and PFLA) and Month (April, July and October) on the concentrations of nutritionally relevant FA in milk. Mean FA proportion, standard deviation (SD) and ANOVA p-values for each FA, expressed as a percentage of the entire FA profile.

	Management			Month			p-Value		
	Conv.‡	Org.	PFLA	April	July	October	Man	Mon	Man x Mon†
Fatty Acids	n=15	n=15	n=24	n=18	n=18	n=18			
OA	19.0 ± 0.68	19.3 ± 0.75	18.4 ± 2.93	18.5 ± 2.58	19.3 ± 1.90	18.7 ± 1.52	ns	ns	ns
VA	1.1 ^{c} ± 0.23	1.9 ^{b} ± 0.28	2.5 ^{a} ± 1.09	2.0 ± 1.37	1.8 ± 0.60	1.9 ± 0.81	***	ns	ns
ALA‡	0.47^{c}± 0.020	0.79^{b}± 0.032	1.1^{a}± 0.06	0.79± 0.066	0.82± 0.078	0.86± 0.083	***	ns	ns
CLA9	0.62^{c}± 0.018	0.92^{b}± 0.036	1.2^{a}± 0.09	0.95± 0.118	0.90± 0.056	0.98± 0.082	***	ns	ns
n-3	1.2^{c}± 0.05	1.9 ^{b}± 0.10	2.3^{a} ± 0.10	1.8± 0.13	1.8± 0.14	2.00± 0.14	***	ns	ns
EPA+DHA +DPA	0.25^{b}± 0.022	0.39 ^{a}± 0.038	0.38 ^{a}± 0.016	0.36± 0.032	0.34± 0.028	0.35± 0.025	**	ns	ns
C12:0	3.5± 0.06	3.3± 0.04	3.4± 0.12	3.6^{a}± 0.11	3.3^{b}± 0.08	3.3^{b}± 0.08	ns	**	ns
C14:0	11.0± 0.10	11.0± 0.08	11.1± 0.24	11.2± 0.22	11.0± 0.21	11.0± 0.14	ns	ns	ns
C16:0	31.1^{a}± 0.30	28.6 ^{b}± 0.32	28.0^{b}± 0.80	29.0± 0.94	28.6± 0.64	29.5± 0.54	*	ns	ns
n-6	2.7^{a}± 0.06	2.5^{a} ± 0.06	1.6^{b}± 0.09	2.1± 0.15	2.1± 0.13	2.2± 0.14	***	ns	ns
n-6/n-3	2.2^{a}± 0.10	1.3^{b}± 0.06	0.73^{c}± 0.021	1.4± 0.17	1.3± 0.16	1.2± 0.14	***	t	ns
SFA	67.7 ± 0.87	66.3 ± 1.49	66.9 ± 4.54	67.7 ± 4.12	66.6 ± 2.95	66.6 ± 2.16	ns	ns	ns
MUFA	27.0 ± 0.80	27.6 ± 0.97	27.1 ± 3.64	26.6 ± 3.35	27.6 ± 2.30	27.3 ± 1.52	ns	ns	ns
PUFA	5.3 ± 0.41	6.2 ± 0.80	6.0 ± 1.52	5.7 ± 1.13	5.8 ± 1.09	6.1 ± 1.26	ns	ns	ns

†$^{a-c}$ Mean values for management or date in a row with different letters are significantly different, according to Tukey's honestly significant difference test (P-values < 0.05). ***: P < 0.001, **: P < 0.01; *: P < 0.05, t: P < 0.1, and ns: P > 0.1. ‡ Conv.= conventional, Org.= organic, PFLA= Pasture for Life Association, Man= management, Mon= month, and SD= standard deviation. OA= c9 C18:1, VA= t11 C18:1, ALA=alpha-linolenic acid, CLA9=conjugated linoleic acid (C18:2, c9t11 isomer), n-3=omega-3, EPA=eicosapentaenoic acid, DPA=docosapentaenoic acid and DHA=docosahexaenoic acid, C12:0=lauric acid, C14:0=myristic acid, C16:0=palmitic acid, n-6=omega-6, SFA= Saturated Fatty Acids, MUFA= Monounsaturated acids and PUFA= Polyunsaturated Acids.

Generally, nutritionally beneficial FAs had a gradient in concentrations, being lowest in conventional supermarket milk and highest in the PFLA milk (Table 4.1), with organic supermarket milk intermediate. There were higher concentrations of VA (+127%), ALA (+134%), CLA9 (+94%), n-3 (+92%) and EPA+DPA+DHA (+52%) in PFLA milk compared with conventional milk. There were also higher concentrations of C16:0 (+11%) and n-6 (+69%) and a higher ratio of n-6/n-3 (201%) in conventional milk compared to PFLA milk.

When compared to organic milk, PFLA milk had higher concentrations of VA (+32%), ALA (+39%), CLA9 (+30%) and n-3 (+21%), lower concentrations of n-6 (−36%) and a lower ratio of n-6/n-3 (−44%). Additionally, organic milk had higher concentrations of VA (+73%), ALA (+41%), CLA9 (+33%), n-3 (+37%) and EPA+DPA+DHA (+36%) but lower concentrations of C16:0 (−8%) and a lower ratio of n-6/n-3 (−41%) compared to conventional milk.

4.3.3 EFFECT OF SEASON ON COMPOSITION

The only FA that showed seasonal variation was C12:0, which was ~8% higher in April compared with milk collected in July and October. There was no seasonal variation in total SFA, MUFA, PUFA, omega-3 or omega-6.

4.3.4 EFFECT OF MANAGEMENT AND SEASON

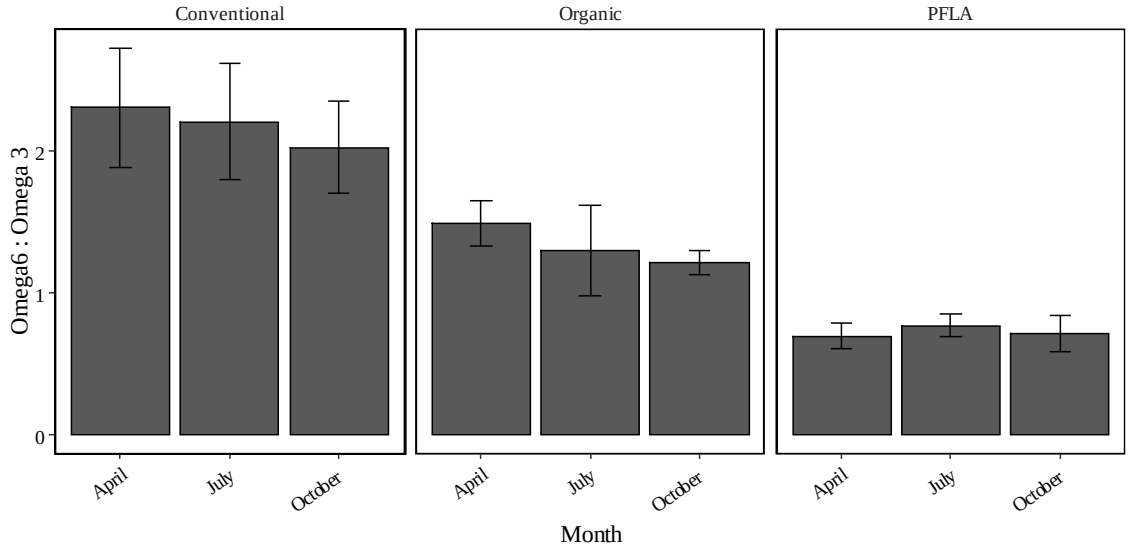


Figure 4.3 Ratio of n-6/n-3 by month and management from conventional and organic supermarket and PFLA milk (mean values with error bars as SD).

There was no significant interaction between management and sampling month for the fatty acid profiles. However, Figure 4.3 demonstrates that for conventional and organic milk, the n-6/n-3 ratio was significantly higher in April compared with other months. PFLA milk had a more consistent ratio across the three sampling dates and had greater consistency between the individual farms compared with variation between samples bought from the various supermarkets (as indicated by the standard deviation). This is further examined in Figure 4.4, where the standard deviation is much smaller on some of the PFLA farms compared to the organic and conventionally sourced milk samples.

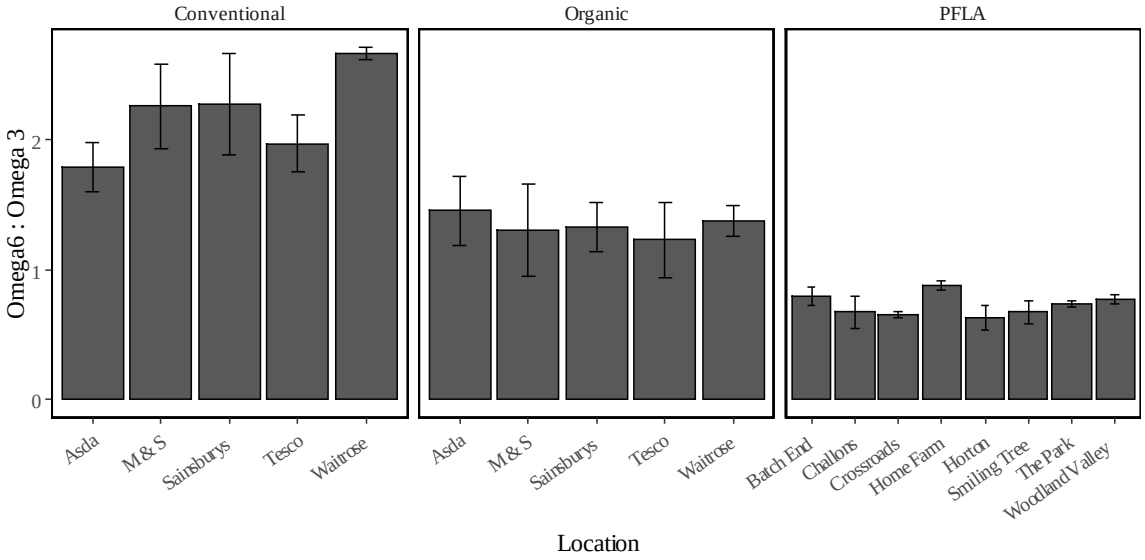


Figure 4.4 Ratio of n-6/n-3 by source of the milk from conventional and organic supermarkets and PFLA farms (mean values with error bars as SD) averaged over the three sampling dates.

4.4 DISCUSSION

4.4.1 MILK FAT COMPOSITION

This study explored differences in milk fat composition between conventional and organic UK supermarket own-brand and PFLA products and found significant differences between the three types of milk. Overall, these findings support previous studies (Table 4.2 showing benefits in organic compared with conventional milk, but also, for the first time, demonstrate the scope for further nutritional improvement beyond that of typical organic milk in the UK, through forage only feeding.

4.4.1.1 OMEGA-6 AND OMEGA-3

The main n-6 (LA) and n-3 (ALA) PUFA must both come from our diet as they cannot be synthesised by humans however, the balance of these two essential FA is important. Both are metabolised into long chain FA; LA to arachidonic acid and ALA to EPA, DPA and DHA. However, ALA and LA compete for the same enzymes for elongation/desaturation resulting in lower EPA, DPA and DHA generation from ALA at high dietary LA intakes (Simopoulos, 2016). In the past 150 years, the n-6/n-3 ratio in our diet has increased dramatically- from around 1/1 to 10-25/1 (Simopoulos, 2008). Historically n-3 came from meat (reared on pasture), fish, leafy green vegetables, nuts and berries however, this has decreased whilst n-6 consumption has increased with the inclusion of vegetable oils (including sunflower, soya bean, corn/maize oil), cereal grains and animal products produced from grain-based, rather than

forage diets over the last 50 years (Simopoulos, 2008). There is increasing evidence that this excess n-6 consumption and increase in dietary n-6/n-3 has contributed to a sharp rise in obesity, atherosclerosis and diabetes (Harris *et al.*, 2009; Donahue *et al.*, 2011; Simopoulos, 2016). Therefore, working towards a n-6/n-3 ratio, closer to 1-4/1, is thought to be important for human health (Simopoulos, 2008). Although the n-6/n-3 ratio of conventional milk (2.2) was within the recommended range, it was three times lower in PFLA grass-fed milk (0.73), reflecting more n-3 than n-6, with the potential to counterbalance high n-6 intakes in many western diets.

Results from other studies (Table 4.2) show lower n-6/n-3 ratio in milk from organic compared with conventional production and for low-input/forage-based systems, an even lower ratio. Details of the diet and management system used on farms producing the conventional and organic supermarket milk assessed in this study were not available. However, results from previous farm surveys including feeding details (Table 4.2) also showed clear positive associations between n-6/n-3 and forage consumption in dairy diets. For example, PFLA-type farms producing Grassmilk^{TM} in the US, Benbrook *et al.* (2018) showed that the highest proportion of forage in the diet had the lowest n-6/n-3 ratio (0.95), low-input, organic farms using moderate levels of concentrate had an intermediate ratio (2.3), while milk from conventional feedlot dairy systems had the highest n-6/n-3 ratio (5.8). It is well known that the inclusion of grain in livestock diets increases n-6 and suppresses n-3 in both dairy and meat produce (Daley *et al.*, 2010; Średnicka-Tober *et al.*, 2016; Benbrook *et al.*, 2018). Additionally, modelling by Benbrook *et al.* (2018) suggests a dietary switch from moderate consumption of conventional dairy products to high consumption of Grassmilk^{TM} products reduces the LA/ALA ratio (LA and ALA are the major PUFA and this ratio tends to mirror that of n-6/n-3) in the total diet by 47%, hypothesised to have a direct benefit to human health. The milk collected from PFLA farms here (n-6/n-3: 0.73) show similar results to those reported for Grassmilk^{TM} in the US (n-6/n-3: 0.954) by Benbrook *et al.* (2018) and other studies (Table 4.2 suggesting these systems maximising grazing and other forages in dairy diets significantly alters the FA composition of milk, providing potential health benefits to consumers.

Table 4.2 Omega-6/Omega-3 ratio from studies examining milk fat composition across management systems both at the farm and retail level

Source	Location	Management	Omega-6/Omega-3	Reference
Retail studies	NE England	Conventional	3.8	Butler *et al.* (2011b)
		Organic	2.6	
	USA	Conventional	5.8	Benbrook *et al.* (2018)
		Organic	2.3	
		Grassmilk™	0.95	
	England	Conventional	2.2	Current study
		Organic	1.3	
	South England	Conventional	2.6	Stergiadis *et al.* (2019)
		Organic	1.7	
	NW England & Wales	Conventional	2.5	Ellis *et al.* (2006)
		Organic	1.5	
	UK	Conventional	2.7	Butler *et al.* (2008)
		Organic	1.3	
		Conventional Low-Input[a]	1.1	
Farm surveys	Denmark	Conventional	4.7	Slots *et al.* (2009)
		Organic	1.9	
	UK	Low-Input	1.0	
	Wales	Conventional	3.1	Stergiadis *et al.* (2015b)
		Organic	1.7	
		Conventional Low-Input	1.4	
	USA	100% forage-fed	0.95	Benbrook *et al.* (2018)
	England	PFLA	0.73	Current study

[a]Conventional low-input farms are not certified organic but generally follow some organic principles, often are spring calving and feed very little or no concentrate during lactation (the outdoor season), but, unlike organic farms, may use nitrogen fertiliser.

4.4.1.2 CONJUGATED LINOLEIC ACID

The main CLA in cow's milk is the isomer CLA9, which comes almost exclusively from ruminant animal fats including dairy products (Murphy *et al.*, 2008). This study found higher concentrations of CLA9 in milk from cows on pasture, and this is supported by previous research (Kelly *et al.*, 1998). Milk from conserved forage diets has less CLA than milk from cows grazing fresh forage although many studies have supplemented silage based dairy diets with oilseeds, fish and/or various vegetable oils to improve the milk FA profile by elevating CLA concentrations (Glasser *et al.*, 2008; Murphy *et al.*, 2008; Chilliard *et al.*, 2009). In this study, the 100% grass-fed milk had double the concentration of CLA9 (1.2%) compared to the conventional supermarket milk (0.62 %), suggesting it may not be necessary to feed expensive oils or seeds, when high quality, fresh forage could be used.

4.4.2 EFFECT OF MANAGEMENT AND SEASON

Seasonal variation in milk composition is expected due to the difference in feeding throughout the year in most systems (often cows are outdoors grazing during the summer and indoors on silage or hay diets during winter) (Butler *et al.*, 2008). Conventional and organic milk follow

the expected trend of a high ratio of n-6/n-3 in April (when high-yielding cows might still be fed conserved forages with grains or cereal by-products) and a decrease in the ratio across July and October, when diets are likely to have increasing proportions of fresh forage from grazing (Figure 4.4). In both the conventional and organic milk, n-3 concentrations gradually increased across the three sampling dates whilst n-6 either remained the same (conventional) or decreased (organic), which is a similar pattern reported by Butler *et al.* (2011b) and Kliem *et al.* (2013) (only conventional) in seasonal retail milk comparison studies. However, the PFLA milk did not follow this pattern; both n-3 (higher in PFLA than the other milks) and n-6 (lower than the other milks) increase incrementally from July to October resulting in the n-6/n-3 ratio remaining relatively constant. Additionally, PFLA n-6/n-3 had a smaller standard deviation (SD) (±0.1) (Figure 4.3) than in conventional (±0.4) and organic (±0.2) milk, suggesting greater variability in milk composition across the five supermarkets than the eight individual PFLA farms. PFLA farms were spread across the southern UK with a wide range of herd sizes, breeds, sward types and management, but the main constant between farmers was their feeding policy. PFLA does not feed any grains, only forage, suggesting the continuity in forage-based feeding creates a more consistent milk fat composition, especially n-6/n-3.

There were observed differences between the brands, especially the conventionally sourced milk (Figure 4.4). All conventional supermarket brands were Red Tractor assured, which does not require an outdoor summer grazing period (Red Tractor, 2017). However, M&S (M&S, 2016) and Waitrose (Waitrose, 2018) brand milk go beyond Red Tractor standards and ensure at least 100 and 120 days grazing per year respectively. Despite this, both these brands of milk had an n-6/n-3 ratio above 2, while Asda and Tesco brands had an n-6/n-3 ratio below 2. Most UK dairy farmers (~95%) have a mixed indoor and grazing system or have year round grazing access (DEFRA, 2019), providing access to fresh forage during the year. Therefore, regardless of assurance guidelines, the difference in n-6/n-3 ratio between milk brands suggests that the dairy diet had a high proportion of forage. Without more detailed management information (including farms the supermarket milk was sourced from), especially fresh forage to concentrate ratio, the differences in milk quality between supermarket brands are difficult to explain, as evidenced by Waitrose brand milk producing the highest n-6/n-3 ratio despite guaranteeing 120 days of grazing per year. Farmers may over-compensate for a longer grazing requirement by additional concentrate feeding, but without knowing the total amount of concentrate feed, final conclusions are presumptuous.

Organic standards require at least 60% of the dairy diet to come from forage (Soil Association, 2018) and the organic supermarket milk had a more consistent and lower n-6/n-3 ratio than the

conventional milk, suggesting that despite grazing access, the proportion of the diet that comes from concentrate remains higher under conventional management. Likewise, the consistency and n-6/n-3 ratio below 1 of PFLA milk is indicative of 100% of the diet coming from forage (Benbrook *et al.*, 2018). Although the supermarket milk samples may have come from a smaller geographic area, they had a wide variation, partially attributed to the unknown of how many farms contributed to each sample. Despite this inherent variability, likelihood is high (especially in conventional systems) that cows come from a similar genetic lineage, suggesting that management, including feeding, is diverse in farms supplying the different supermarkets.

4.4.3 FORAGE IN THE DIET

Despite evidence that increased forage in the dairy diet reduces SFAs and increases MUFA and PUFA (Chilliard *et al.*, 2007; Kliem *et al.*, 2013; Elgersma, 2015), this study found no difference in total SFA, MUFA or PUFA across the management systems. However, within each group there were important differences that have been linked to forage consumption (Figure 4.2, Table 4.1). MUFA vaccenic acid is the most abundant trans FA in milk and is the precursor to CLA9 in both the mammary gland and during metabolism (Qiu *et al.*, 2018) and so increasing VA and thus CLA9 is thought to be beneficial for human health (Yang *et al.*, 2015). This study found more than double the concentration of VA in PFLA (2.5%) compared to conventional milk (1.1%), supporting findings by Butler *et al.* (2011b) who linked increased VA to increasing forage in the diet. Whilst some SFAs have been shown to be protective and others pose a health risk, it is generally accepted that Western consumption levels of saturated fat are too high (Thorning *et al.*, 2017). There was a slightly higher concentration of the most abundant SFA, palmitic acid (C16:0), in conventional milk (31.1%) compared to organic and PFLA milk (28.6% and 28.0%), which along with other even chained SFAs has been linked to increased risk of CHD (Mensink *et al.*, 2003; Forouhi *et al.*, 2014). Despite the lack of difference between SFA, MUFA and PUFA in this study, it does support evidence that increasing forage consumption (and decreasing concentrate supplementation) creates a milk FA profile that is more nutritionally beneficial for human health.

A common theme of this study is that concentrations of nutritionally beneficial FA were significantly higher with pasture-based feeding. Fatty acid manipulation/improvement has been extensively researched with oils (Glasser *et al.*, 2008) and/or forage (Kalač and Samková, 2010) however, it is evident from this paper and work by Benbrook *et al.* (2018) and others (Table 4.2) that this can also be achieved by increasing forage within dairy diets. This is a cheaper and more effective way of modifying FA whilst supporting the natural function of cow digestion

and increasing beneficial FA in the human diet. Increasing forage (and decreasing concentrate feed) could lower yields but the cost saved on inputs could justify the compromise, pending a full economic analysis, which is outside the scope of this thesis. The PFLA farm-business case for feeding ruminants solely on pasture and conserved forages (PFLA, 2016) finds that, with the right management, raising ruminants on forage can be profitable and support the environment. Additionally, as mentioned, in the USA, Grassmilk^{TM} farmers receive a 15% premium (over and above that for organic milk) if concentrations of beneficial FA reach a threshold (Benbrook *et al.*, 2018). Whilst this premium is not available in the UK, consumers are increasingly interested in the nutritional composition of foods and encouraging high welfare and sustainable farming standards.

4.5 CONCLUSION

In conclusion, this study found that milk from 100% forage-based dairy systems has a nutritionally more desirable FA profile compared to conventional and organic supermarket brand milk. Of particular interest is the ratio of n-6/n-3, which decreases dramatically from conventional to organic to PFLA milk. Additionally, the ratio of n-6/n-3 remained constantly low over the seasons in PFLA milk yet peaked (end of winter) and troughed (end of summer) in conventional and organic milk. Whilst these findings are consistent with previous studies, the novel finding in this study shows further improvement in the FA profile beyond organic milk. This provides scope for further and larger-scale research into producing 'more nutritious' milk and how dairy consumption can alter the ratio of n-6/n-3 in the total diet.

CHAPTER 5. MEETING BREEDING POTENTIAL IN ORGANIC AND LOW-INPUT DAIRY FARMING

5.1 INTRODUCTION

Organic farming in the UK is defined by EU regulations (European Comission, 2008) and certifying bodies such as The Soil Association (Soil Association, 2018) and Organic Farmers and Growers (Organic Farmers and Growers, 2013). However, many farms operate LI systems, which are not certified organic nor formally defined or regulated. Low- input farming refers to the practice of using less inputs than conventional agriculture but not necessarily adhering to organic or other quality assurance standards. Motivations towards low-input farming include economic, environmental, and social parameters (Bijttebier *et al.*, 2017). The main criticism of organic and LI farming is that, compared with intensive systems, lower yields require more land to produce the same amount of food, leading to poorer biodiversity if semi-natural vegetation is converted to agriculture (Seufert *et al.*, 2012). However, rejecting organic production methods by emphasising yield productivity ignores opportunities for practices that enhance sustainability, therefore alternative metrics are required to assess LI systems.

Over the past 60 years, dairy farming has typically focused on making better use of inputs, maximising profit, relying heavily on high yields and improved feed efficiency (kg dry matter intake/litre of milk) (e.g. Milkbench+ system (DairyCo, 2012)). However, in organic and LI dairying, priorities are different; whilst profit is still essential, the production system involves fewer inputs. Feed efficiency is equally important, but the pathway to achieve this is mainly on reducing external inputs rather than maximising outputs; a practice which may also benefit herd health, as health traits are negatively correlated with production traits (Wagenaar *et al.*, 2011). Reducing the intensity of production lowers veterinary bills and costs associated with inseminations whilst using optimal grazing strategies (such as mob-grazing) to enhance soil and sward health, meaning cows consume a richer pasture and produce more nutritious milk (Stergiadis *et al.*, 2015b; Scollan *et al.*, 2017). A robust method to determine sustainability, accounting for animal health/welfare, nutritional quality and environmental/social impacts, is needed.

Breaking from the conventional Holstein/Friesian (HF) breeding approach discussed in Chapter 2, Section 2.4, organic and LI systems often rely on crossbreeding strategies to optimise their herd health and yield potential. A strong reason for crossbreeding is the resulting heterosis or hybrid vigour in the first generation (F1). Crossbred offspring (including HF crosses) outperformance relative to the parental average is one way to improve functional traits (Kargo

et al., 2014) without a negative impact on milk production. However, to extend the benefit beyond the first generation, a carefully designed system is required for rotational crossbreeding: crossing two F1 individuals only expresses half the hybrid vigour, whereas introducing a third breed preserves up to 86% of the heterosis (Sørensen *et al.*, 2008). Cross-breeding high-production HF with traditional breeds better suited to LI management (with high forage diets) shows potential. For example, recent studies comparing breeds and cross-breeding regimes in Switzerland and the UK showed more traditional breeds or cross-breeding with traditional breeds can significantly improve the economic performance and milk quality in LI grazing based dairy systems (Butler, 2014; Stergiadis *et al.*, 2015a). The indicators from these studies are positive, but further research is needed to identify the key mechanisms required to produce predictable, repeatable, efficient and effective crossbreeds.

There is very little recognised research into breeding for cross-bred cattle in smaller LI and organic dairy systems, yet these farms have progressed with cross breeding for many generations within their herds, each employing a different strategy to search for breed combinations that perform within their system (Nauta *et al.*, 2009). Therefore, there is not a clear breed (or crossbreed) that typically out-performs others in LI systems, in the way that HF dominates conventional production. In addition, most scientific research has focused on HF because they account for 95% of the EU dairy cow population (Oltenacu and Broom, 2010).

UK organic milk was valued at £351 million in 2018 with over 25% of UK households purchasing organic milk, representing 5.1% of retail milk sales (OMSCo, 2019), which highlights the increased need to develop appropriate crossbreeding schemes for such production chains. Studies from a range of countries argue that, due to genetic x environment (GxE) interactions, optimal genetic progress requires either independent breeding programmes or an index (to rank sires against requirement) specific for each farming system (Nauta *et al.*, 2006; Rozzi *et al.*, 2007; Garnsworthy *et al.*, 2010; Yin *et al.*, 2012; Martin-Collado *et al.*, 2015; Rodriguez-Bermudez *et al.*, 2018). This approach would directly benefit LI and organic systems.

The complexity of breeding support for LI dairying is not well established in the UK. As discussed in Chapter 2, in LI and organic dairy systems the diet is predominantly forage, therefore it is beneficial to have cows that efficiently convert forage, especially grazing, to milk (Buckley *et al.*, 2005). However, current UK breeding objectives available do not include forage conversion as a desirable trait when calculating economic values of genetic gain. Instead, the AHDB breeding index for year-round calving focuses on milk production (34.4%), health (21.8%), fertility (15.3%) and temperament, among other traits (AHDB Dairy, 2018b). The

AHDB also has a Spring Calving Index, aimed at herds making use of grazed grass by assigning 71.6% of the weighting to fitness traits, but the dominant individual driver is still milk production (27.4%) and the link between efficiency (with an emphasis on forage conversion) in LI systems has yet to be fully explored. Typically, LI and organic management supports animal health and mastitis is the main concern (Richert *et al.*, 2013), but the risk of illness (for example, acidosis (Plaizier *et al.*, 2008)) is much reduced compared to conventional systems. Although UK resources for dairy breeding selection exist, other options (with less focus on intensive production) seem more appropriate for organic and LI production.

Milk quality has gained a lot of media attention recently, continuing the debate around the role of milk in human diets and the environment (Thorning *et al.*, 2016). Milk fatty acid (FA) profile is strongly influenced by management and there is a clear difference in the FA profiles of organic and conventional milk (Średnicka-Tober *et al.*, 2016; Benbrook *et al.*, 2018; Stergiadis *et al.*, 2019), between the different stages of lactation (Nantapo *et al.*, 2014) and seasonally (Butler *et al.*, 2008; Kliem *et al.*, 2013). Additionally, FAs can vary as much within- as between-breeds (Soyeurt *et al.*, 2006; Stergiadis *et al.*, 2013), making it harder to isolate breeds that could give an 'optimal' FA profile within a specified management system.

Some FAs have been studied closely for their effects on human health, as described in Chapter 2, Section 2.7. The main FAs considered to have a positive effect on human health are: Alpha-Linolenic Acid (ALA), Eicosapentaenoic Acid (EPA), Docosapentaenoic Acid (DPA), Docosahexaenoic Acid (DHA), Oleic Acid (OA) and cis-9 trans-11 Conjugated Linoleic Acid (CLA9). In contrast, FAs highlighted as undesirable in human nutrition, are: lauric (C12:0), myristic (C14:0) and in particular palmitic (C16:0) acids. Additionally, essential FA Linoleic Acid (LA) when consumed in excess, as prevalent in Western diets, is undesirable. Especially when considering the dietary ratio of n-6/n-3 which, when too high (exact optimal ratio is unknown) may cause inflammations and increase CHD risk (Simopoulos, 2008; Simopoulos, 2016).

Whilst there is currently no premium in UK linked to milk fat composition, in the USA, CROPP's organic "Grassmilk^{TM}" receives a 15% premium above standard organic milk prices for meeting minimum requirements for the n-6/n-3 ratio, total n-3 and CLA (Benbrook *et al.*, 2018). This demonstrates the potential for other sectors and countries to create premium dairy products with increased concentration of beneficial FAs.

Historic approaches to breeding in dairying have not taken a whole system view, generally resulting in poor health traits and concentrate-dependent cows (Oltenacu and Broom, 2010). If

robust methods to identify cattle best suited to a particular system are to be developed, there is the potential to improve animal health and welfare, production, nutritional quality, milk FA profile and efficiency. This Chapter aims to identify breeds within low-input and organic dairy systems that can maintain health and yield whilst producing milk with a beneficial FA profile. The objectives are to: a) define the variables most relevant to LI and organic farming and observe differences in management system (individual farms), b) identify breeds that are similar across the farms and quantify differences, c) develop a score for low-input-production (LI-P) to identify breeds that best suit LI and organic production in terms of production, health and milk composition with respect to consumer health.

5.2 MATERIALS AND METHODS

5.2.1 DATA COLLECTION

Table 5.1. Background information from each farm sampled.

Farm ID	Management	No. of cows included	Calving	Breeds & crosses represented [a]
1	Organic	40	Spring	AYR, JE, HF, NZF, SR, SH
2	Organic	42	Year-round	HF, JE, SR
3	Low-Input	55	Spring	BS, JE, HF, SR
4	Low-Input	52	Spring	NZF, JE, HF
5	Organic	49	Year-round	HF, SR, SH, MRI
6	Low-Input	28	Spring	HF, JE, SR
7	Organic	61	Autumn (late)	AYR, HF, SH, SR
8	Low-Input	113	Year-round	BF, HF, SR, SH, MRI
9	Low-Input	60	Autumn (early)	BF, JE, HF, NZF, SH
10	Organic	55	Autumn (early)	BF, BS, HF, MO, SR
11	Low-Input	66	Spring	JE, NZF, BF, HF
12	Low-Input	27	Spring	BF, SR, JE
13	Low-Input	84	Year-round	AYR, BF, HF, SR, MO, NZF
14	Low-Input	76	Spring	BF, JE, NZF, SR, HF, MRI
15	Organic	93	Autumn	AYR, HF, MO, SR, JE
16	Low-Input	97	Spring	AYR, JE, HF, NZF
17	Organic	72	Autumn	AYR, HF, SH, XX

[a] HF =Holstein/Friesian, NZF=New Zealand Friesian, BF=British and unknown Friesian, JE=Jersey, SR=Scandinavian Red, SH=Shorthorn, AYY=Ayrshire, MO=Montbelliarde, BS=Brown Swiss, MRI=Meuse Rhine Issel, and XX=crossbred with unknown breed composition.

Data for this study was collected from 17 dairy farms (7 organic and 10 low-input-conventional) throughout England and Wales between November 2011 and October 2012. All herds were a mix of both purebred and different crossbred cows (Table 5.1). Herd sizes ranged from 150-550 cows and a total of 1,070 cows were recorded, to encompass a broad range of breeds and crosses from each farm. A one-off questionnaire was completed to gain information on pre-

survey health and parity as well as a breeding pedigree for all individual cows (according to the farmers' records). Milk from each cow was sampled over four dates: Autumn 2011 (D1), Spring (D2), Summer (D3) and Autumn 2012 (D4). A corresponding questionnaire for each farm and cow was used to record husbandry practices on all sampling dates, including milk yield, disease incidence, health treatments, cow diet, calving intervals, milking and grazing management. Organic farming standards require concentrate feed to be sourced organically and have strict land management application practices (Soil Association, 2018), whereas LI follow similar practices but are not certified organic. Organic and LI farms fed similar levels of concentrate per cow and organic farms typically fed more conserved forage (Appendix C, Table C.1.). Access to grazing varied across the year and by individual management (Appendix C, Table C.2). All milk samples were analysed for basic composition, somatic cell count and fatty acid profile, as described in Chapter 3, Section 3.3.

5.2.2 MILK ANALYSIS

Table 5.2. The number of cows with complete records on each sampling date (D1, D2, D3, D4) and the number of genotype combinations on each farm (the number of cows within each genotype combination) with the total number of possible genotype combinations.

	Number of Cows with Full Records				Number of Genotype Combinations [a]			
Farm ID	D1	D2	D3	D4	D1	D2	D3	D4
1	0	0	0	0	N/A	N/A	N/A	N/A
2	34	33	34	28	4 (2- 22)	4 (2-18)	4 (2-20)	4 (1-16)
3	30	42	21	35	4 (1-15)	4 (1-20)	3 (5-9)	4 (1-18)
4	41	34	35	24	8 (1-14)	7 (1-13)	8 (1-14)	8 (1-9)
5	30	32	29	19	4 (5-8)	4 (6-10)	4 (5-8)	4 (3-8)
6	19	22	27	26	3 (3-9)	3 (4-10)	3 (5-12)	3 (5-12)
7	22	56	47	37	3 (1-12)	4 (2-20)	4 (2-16)	4 (1-13)
8	50	87	75	51	6 (1-15)	9 (1-24)	9 (1-21)	9 (1-11)
9	0	0	52	40	N/A	N/A	7 (1-12)	7 (1-10)
10	32	53	46	42	5 (1-15)	6 (1-20)	5 (4-17)	6 (1-13)
11	41	44	55	54	7 (1-19)	9 (1-15)	9 (1-20)	8 (1-16)
12	0	0	0	0	N/A	N/A	N/A	N/A
13	0	60	68	52	N/A	8 (1-16)	8 (1-17)	8 (1-14)
14	0	70	74	61	N/A	14 (1-13)	14 (1-13)	14 (1-11)
15	0	86	72	68	N/A	7 (3-32)	7 (2-28)	7 (2-26)
16	0	85	83	35	N/A	6 (1-18)	6 (1-19)	5 (5-10)
17	0	53	52	40	N/A	4 (6-18)	4 (8-16)	4 (5-14)
Total	299	757	770	612	24 (1-53)	33 (1-119)	32 (1-103)	33 (1-100)

[a] For example, 4 (2-22): 4 different genotype combinations and the number of cows from each combination range from 2-22.

A representative raw milk sample was collected from each cow during milking in the parlour on each sampling date. As described in Chapter 3, Section 3.3, milk samples, were preserved with Bronopol and kept at ambient temperature during transportation to a commercial NMR lab (National Milk Recording, 2018), where basic milk composition was analysed (milk fat, protein, urea, lactose content, Somatic Cell Count (SCC)). The lipid was extracted using the method described in Chapter 3, Section 3.3.2 and by Chilliard *et al.* (2009). Gas chromatography (Shimadzu, GC-2014, Kyoto, Japan) was used to analyse content of the FAs. The gas chromatography method has been previously described by Stergiadis *et al.* (2014) and in Chapter 3, Section 3.3.3.

5.2.3 DATA HANDLING

5.2.3.1 BREED COMBINATIONS

All animals were categorised by the farmers' breeding records. Cows were given a code based on their sire, dam and predominant breed, for example, a pure-bred Jersey=JE; sire Jersey and dam Ayrshire =JEAYR; sire Jersey x Shorthorn and dam Jersey x Ayrshire =JEX (Table 5.1). The X indicates a majority genetic contribution and/or a back cross. Including the sire and dam breeds for all cows across the study resulted in around 40 different breed combinations of varying population sizes, depending on the sampling date. This ranged from a single representative on one farm (British Friesian x Montbelliarde) to 119 Holstein-Friesian individuals across all farms for D2. To rationalise the number of crossbreed combinations in this study, there is no differentiation between the contribution of genetics by parents' sex. For example, both a cross from a Jersey sire and HF dam and from a HF sire and Jersey dam are labelled HFJE.

5.2.3.2 DATA ANALYSIS

Microsoft Excel was used for data handling whilst all statistical analysis was completed using 'R' (R Core Team, 2017). The background information on the farms and monitored cows is displayed in Table 5.1. Other statistical approaches used to explore and identify best approaches for this data are reviewed in (Chapter 3).

5.2.3.2.1 LI-P AND PRINCIPAL COMPONENTS ANALYSIS

The initial data collection involved 1070 cows, but for some farms and/or cows on some sampling dates there are missing and incomplete records. For the observational statistics, the cows selected had records on any given date for: production, health, and FA composition results (explained below). This resulted in 299, 757, 772 and 613 cows on D1, D2, D3 and D4

respectively. Table 5.2 demonstrates the number of records that met the selection criteria and the wide number of genotypic combinations and cows within each combination.

Focusing on the available data, using a combination of farm records and results from milk analysis and the priorities of typical LI practices, the variables selected to define LI-P were split into three main criteria:

1. Production:
    i. Milk yield (L/day).
    ii. Total fat and protein solids (kg/day)
2. Health:
    i. Udder health; Somatic cell count ($x10^3$ cells/mL milk).
    ii. Treatments, including antibiotic (e.g. for mastitis or metritis) or other (e.g. for lameness, milk fever or pain/inflammation).
3. Fatty acid profile:
    i. Percentage of total profile with desirable FAs (n-3, OA, CLA9, EPA+ DPA+ DHA).
    ii. Percentage of total profile with FAs often consumed in excess and undesirable (C12:0, C14:0, C16:0, n-6, and n-6/n-3 ratio).

The elements of LI-P had different units (FAs were proportional; yield in litres/cow/day; SCC $x10^3$ cells/mL milk, etc.), thus the data were standardised (normalisation to mean of zero and standard deviation of one) (Milligan and Cooper, 1988) to give each element of LI-P the same weight. Principal Components Analysis (PCA) in the package 'vegan' (Oksanen *et al.*, 2017) was used to aid visualisation of the effects of Farm ID (2-17) on LI-P. Two sets of graphs were produced from the PCA. First, graphs in which points represent samples/records from cows: under each management system (Figure 5.1), at each stage of lactation (Figure 5.2) and at each farm (Figure 5.3) (one graph for each of the 4 dates, each figure displays the four plots for each date). As described in (Chapter 3, Section 3.6.4) in these graphs, the closer two points are to each other in PCA ordination space, the more similar their characteristics (in terms of production, health, and milk FA profile); points in these graphs were colour-coded by management/stage of lactation/farm identity to aid interpretation. Second, PCA graphs (Figure 5.4) of PE characteristics, in which points close together are indicative of co-occurrence on similar farms or farming systems. This second set of PCA graphs were also broken down by date. In other words, the characteristics that are grouped together in Figure 5.1, Figure 5.2 and Figure 5.3 can be associated with cows and/or farms that occupy similar ordination space in Figure 5.4.

5.2.3.2.2 DESCRIPTIVE STATISTICAL ANALYSIS

For the descriptive statistical analysis, additional inclusion criteria were considered: on any sampling date, records existing for at least six cows of the same breed (combination) from at least three different farms. These criteria resulted in the most breed combinations and ensured comparison between breeds rather than individual farm management style. After these additional inclusion criteria were applied there were eight breeds for comparison: Ayrshire cross (AYRX, n=100), Holstein/Friesian (HF, n=325), HF x Jersey (HFJE, n=184), HF x Scandinavian Red (HFSR, n=274), Jersey cross (JEX, n=121), New Zealand Friesian cross (NZFX, n=90), Dairy Shorthorn (SH, n=80) and Scandinavian Red cross (SRX, n=140).

The 'R' package 'nlme' (Pinheiro *et al.*, 2017) was used to model 'Breed' against the variables described for the LI-P, with Season and Farm ID as random factors. The linear mixed effects model accounts for variation explained by the fixed effects (Breed) and random effects (Season and Farm ID). Since farms were observed across the four sampling dates, these related measures would violate the independence assumptions made by a linear model hence, the use of 'Farm ID' and 'Season' as random factors. Days in milk did not differ between the breeds (F-statistic=1.50, p=0.165) allowing breed to be compared without differentiating or adjusting for stage of lactation. On each date all cows from the same farm were fed the same ration, not as individuals (Appendix C, Table C.1.). The feed data did not meet the assumptions of the model, therefore mean and standard deviations are given, but a p-value is not provided. Observationally, there was not a big difference in the amount of concentrate fed between the breeds, but there was a notable difference in the amount of conserved forage fed between breeds (Table 5.3). Concentrate feeding is thought to have the biggest impact on fatty acid profile (Butler *et al.*, 2011b), therefore no corrections were made to the data prior to analysis.

Traditionally, post-hoc Tukey Honest Significant Difference (Tukey HSD) tests are used for multiple comparisons of levels within a factor. However, due to the complexity of this data set with multiple levels of comparison (8), some with few replicates, controlling the family-wise error rate even by this approach would risk numerous Type 1 errors (false positives) and would be mis-leading (Fletcher *et al.*, 1989; Smith *et al.*, 2002; Cramer *et al.*, 2016).

5.2.3.2.3 LOW-INPUT PRODUCTION SCORE

To create a universal score for each record common units are required. Using the variables selected, for LI-P, scores were created for each cow record to assess the best performing breed. Milk yield, total fat and protein solids, SCC and proportions of desirable and un-desirable FAs were (higher rankings indicate more beneficial qualities) scored as described below.

i. Production records (milk yield in L/day and total fat and protein solids in kg) were allocated into five groups of equal observations, rated 1-5 with 5 the highest and 1 the lowest. Scores were combined to make a total production score out of 10.

ii. Somatic cell count ($x10^3$ cells/mL milk) was allocated into five groups of equal observations rated 1-5 with 5 the lowest and 1 the highest. For veterinary treatments, cows were given a 1 if they received no treatments and 0 if they had been given antibiotics or an alternative (e.g. for mastitis or metritis or other e.g. for lameness, milk fever or pain/inflammation) at least once since the previous collection date, which was added to the SCC category resulting in a total health score out of 6.

iii. For desirable FAs (OA, CLA9, n-3 and EPA+ DPA+ DHA) concentrations were ranked and allocated to 5 equal groups with a score of 5 given to the highest and 1 to the lowest group whilst undesirable FA (often consumed in excess) (C12:0, C14:0, C16:0, n-6, and n-6/n-3 ratio) scores were reversed, 5 to the lowest group. Fatty acid categories were combined to create a total FA score out of 45.

These individual assessments were then used to calculate a single score (out of one) for each cow record using two alternative approaches. The score weightings are based on organic and LI values, the AHDB Spring and Autumn calving indices (AHDB Dairy, 2018) and the premium offered for FA quality by Organic Valley's Grassmilk^{TM} (Benbrook *et al.*, 2018):

- Weighted health score: the scores were weighted at: 30% production, 50% health and 20% FA.
- Weighted production score: 60% production, 30% health and 10% FA

For example: Weighted health score=

30% * (production score/10) + 50% * (health score/ 6) + 20% * (FA score/45)

5.3 RESULTS

5.3.1 LI-P AND PCA

Despite the clutter in Figure 5.1, D2, D3 and D4 all have a relatively clear separation by management practice (either LI or organic). This is mirrored in Figure 5.2, where D3 and D4 have the clearest spread with late in lactation records occurring in the negative PC1 axis and spreading vertically while mid and late lactation records spread vertically on the positive PC1 axis. This near mirroring of results identifies collinearity. Most of the organic herds were autumn calving and many LI were spring calving (Table 5.1). Due to this collinearity, evident in the PCA plots (Figure 5.1 and Figure 5.2), it is statistically difficult to identify which factor (management or stage in lactation) has the greatest effect on LI-P.

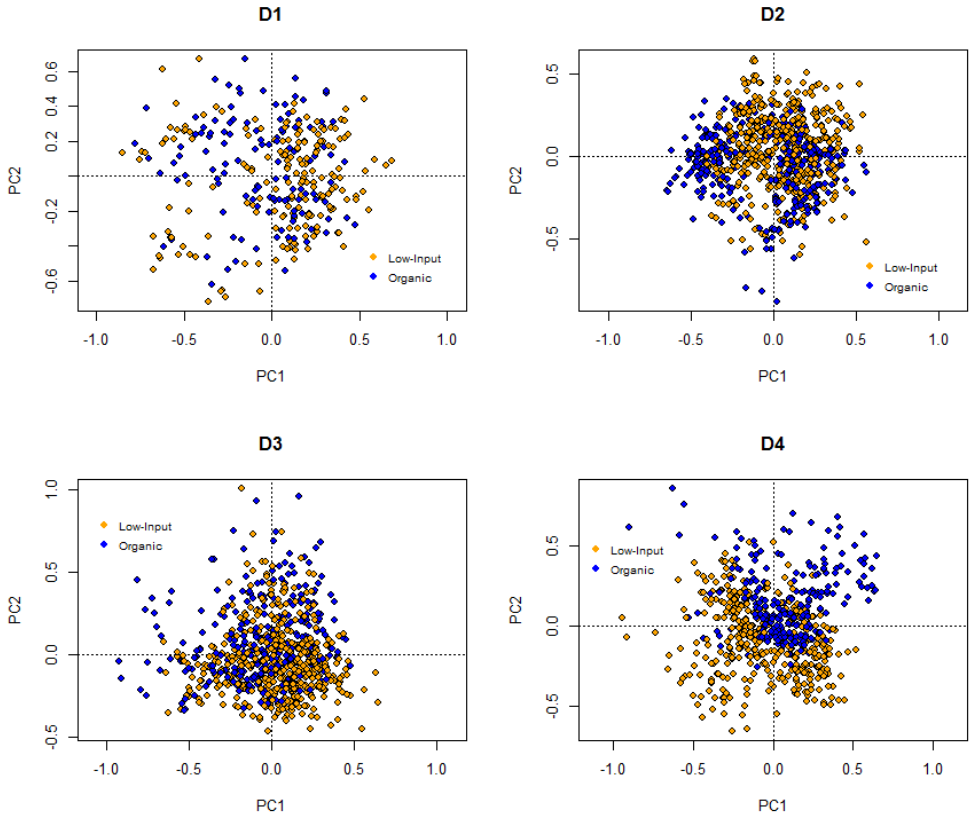


Figure 5.1 Principal components analysis based on production efficiency, highlighted by management system. D1= Autumn 2011, D2=Spring, D3=Summer, D4= Autumn 2012

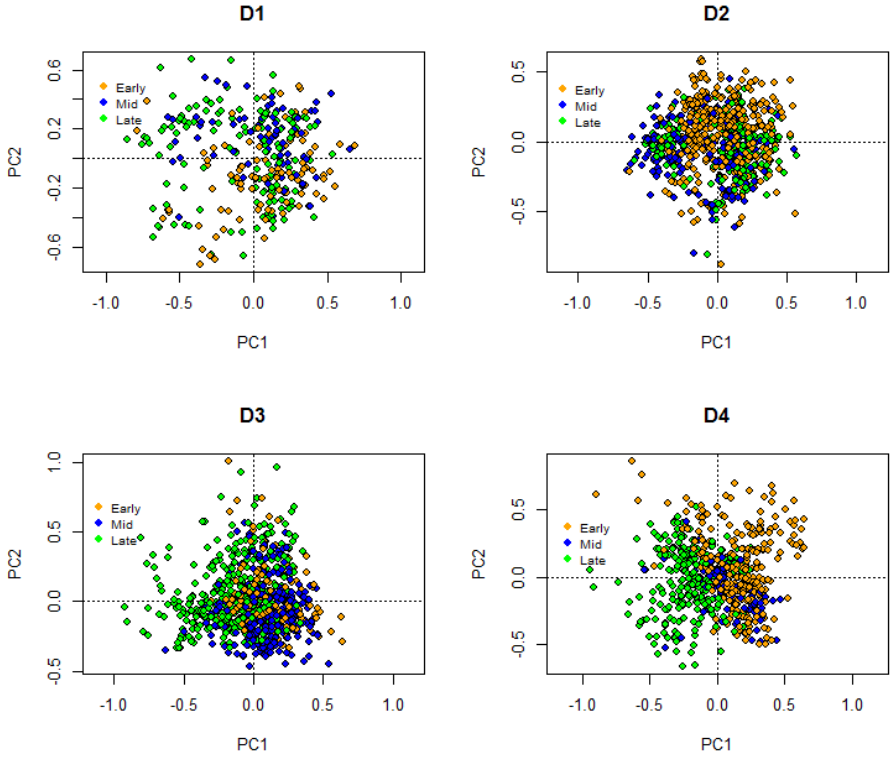


Figure 5.2 Principal components analysis based on production efficiency, highlighted by stage of lactation. D1= Autumn 2011, D2=Spring, D3=Summer, D4= Autumn 2012

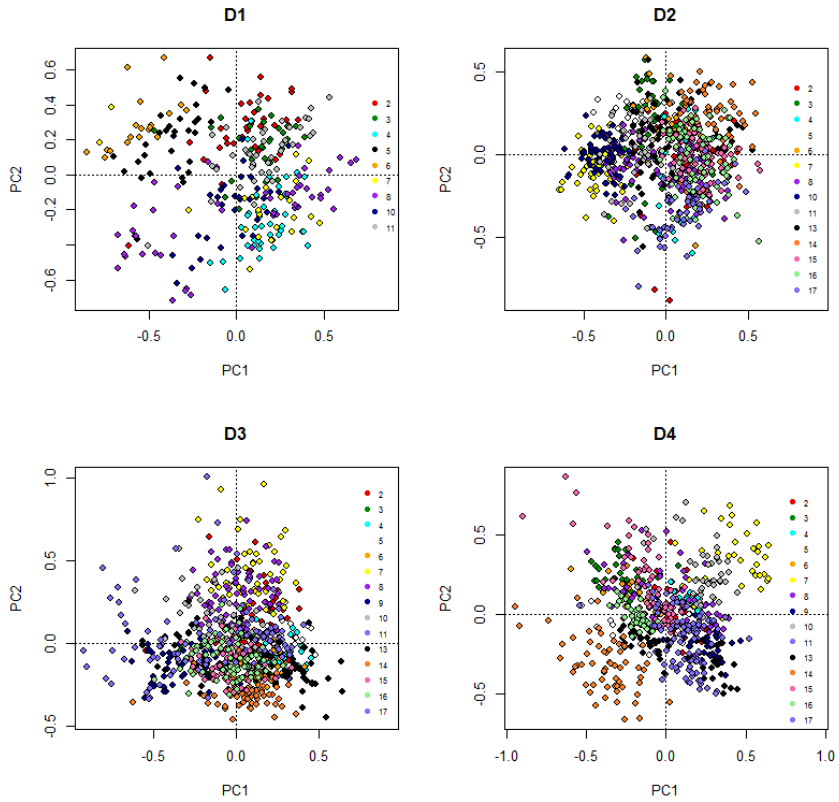


Figure 5.3 Principal components analysis based on production efficiency, highlighted by Farm ID. D1= Autumn 2011, D2=Spring, D3=Summer, D4= Autumn 2012

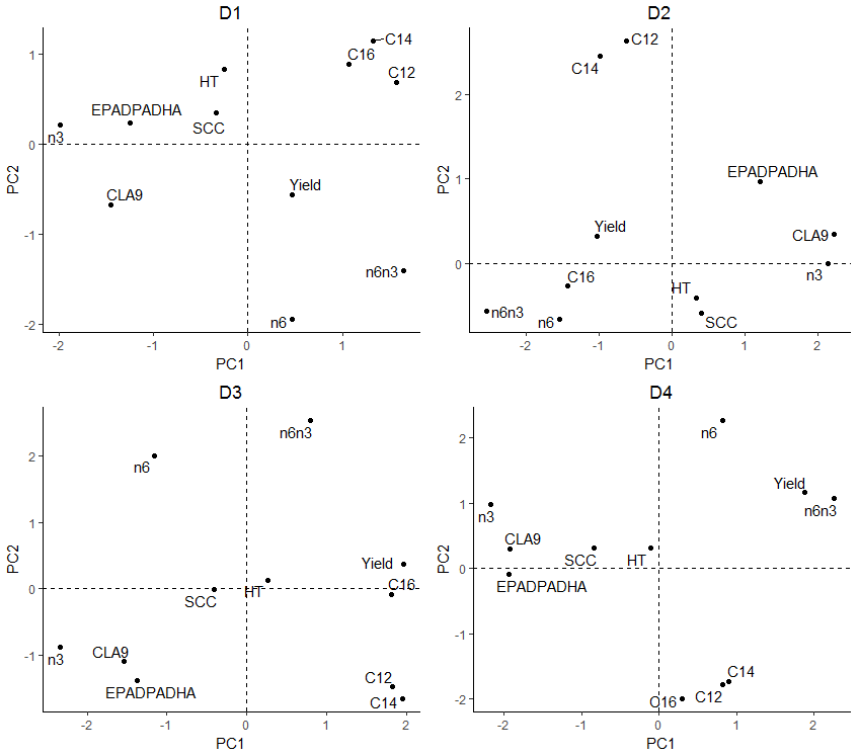


Figure 5.4. Principal components analysis displaying characteristics of production efficiency. D1= Autumn 2011, D2=Spring, D3=Summer, D4= Autumn 2012. C12=C12:0, Lauric Acid, C14=C14:0, Myristic Acid, C16=C16:0, Palmitic Acid, CLA9=Conjugated linoleic acid (C18:2, c9t11 isomer), n3=omega-3, n6=omega-6, n6n3= omega-6/ omega-3 ratio, EPADPADHA= EPA=Eicosapentaenoic Acid, DPA=Docosapentaenoic Acid, DHA=Docosahexaenoic Acid, SCC= Somatic Cell Count, HT= Health Treatments

The individual farm had major influences on LI-P, especially on D4 (Autumn 2012) (Figure 5.3) where cows from the same farm are clearly clustered together. Farm 7 cows are tightly clustered in the negative PC1 axis and positive PC2 axis whilst Farm 14 cows are clustered in the negative PC1 and PC2 axis. Interpretation of Figure 5.3 is aided by cross-referencing with Figure 5.4, for example, on date D1, many cows from Farm 8 are associated with high levels of CLA9 and OA in milk. In contrast, Farms 2, 3 and 11 have higher saturated FA; C12:0, C14:0 and C16:0 concentrations. However, on D2 cows from Farms 6, 15 and 16 are associated with the beneficial FA EPA+DPA+DHA and CLA9 and Farm 17 with n-6 and a high ratio of n-6/n-3. Across all four sampling dates, Farm 7 (yellow) stands out for producing milk with elevated n-6 content and n-6/n-3 although no farm is consistently associated with beneficial FA in milk.

Figure 5.4 displays the characteristics of LI-P, the positive FA n-3 and CLA.9 are on the same PCA axis whilst the detrimental saturated FA C12:0 and C14:0 are together, but on the opposite axis on all four sampling dates. This suggests that cows with high C12:0 will also have high C14:0 and cows with high n-3 will have high CLA.9. The individual elements of LI-P can be superimposed over the individual cow observations to give a view of which observations were associated with LI-P characteristics. Figure 5.3 compared with Figure 5.4 suggests that on D1, many cows from Farm 8 are associated with CLA9. Most cows from Farms 5 and 6 were associated with n-3 and health treatments. In contrast, Farms 2, 3 and 11 are associated with the saturated FAs C12:0, C14:0 and C16:0 and Farms 4 and 7 with n-6 and a high n-6/n-3. However, on D2 Farms 6, 15 and 16 are associated with the beneficial FA EPA+DPA+DHA and CLA9 and Farm 17 with n-6 and a high n-6/n-3. Across all four sampling dates, Farm 7 (yellow) stands out for being associated with n-6 and n-6/n-3 but no farm is consistently associated with the beneficial FA.

5.3.2 EFFECT OF BREED ON LI-P

The mean values for the components of LI-P for the eight most common breeds and crosses are shown in Table 5.3. Averaging data (over four dates) from multiple farms with similar breed combinations indicated that the individual parameters used to define LI-P did significantly differ between breeds although, again, there was no difference in stage of lactation between the breeds in this data set. The highest yielding breed was the HF (21.2L) and the HF crosses (HFJE; 21.8L and HFSR; 21.9L) and HFJE had the highest fat and protein solids (1.8kg).

Table 5.3 Effect of breed on components of low-input-production: production (milk yield and total fat and protein solids), health (health treatments and SCC), nutritionally relevant FA in milk (expressed as a percentage of the entire FA profile). Results presented as mean ± standard deviation (SD) and ANOVA p-values.

	AYRX[a]	HF	HFJE	HFSR	JEX	NZFX	SH	SRX	Sig[b]
n	100	325	184	274	121	90	80	140	
Days in milk	154 ± 94.3	182 ± 96.9	157 ± 102.2	161 ± 94.3	134 ± 85.2	138 ± 96.9	153 ± 106.9	151 ± 93.3	ns
Concentrate Feed (kg/day)[c]	3.0 ± 1.54	3.3 ± 2.15	3.6 ± 1.68	4.5 ± 2.45	3.1 ± 2.49	3.5 ± 3.00	3.3 ± 1.49	3.1 ± 2.55	NA
Conserved Forage(kg/day) [c]	3.7 ± 4.91	6.1 ± 5.25	6.5 ± 5.65	5.6 ± 5.48	3.1 ± 3.80	1.7 ± 2.94	8.2 ± 4.78	3.2 ± 4.09	NA
Production									
Yield (L/day)	20.2 ± 7.33	21.2 ± 8.67	21.8 ± 8.89	21.9 ± 9.50	17.9 ± 7.47	20.1 ± 7.08	17.8 ± 8.81	19.7 ± 7.68	***
Solids (fat and protein) (Kg/day)	1.7 ± 0.80	1.6 ± 0.62	1.8 ± 0.70	1.7 ± 0.63	1.7 ± 0.65	1.7 ± 0.56	1.5 ± 0.76	1.6 ± 0.52	***
Health									
SCC (x 10^3cells/mL milk)	243 ± 454.2	234 ± 584.5	248 ± 782.1	293 ± 885.4	247 ± 664.1	232 ± 944.9	261 ± 688.2	170 ± 400.8	ns
Treatments [d]	0.41 ± 0.818	0.34 ± 0.713	0.35 ± 0.670	0.24 ± 0.549	0.12 ± 0.369	0.08 ± 0.343	0.09 ± 0.284	0.18 ± 0.527	**
Median SCC (x 10^3cells/mL milk)	78.5	78.0	70.0	73.0	84.0	56.5	89.0	73.0	
FA Profile [e]									
C12:0	3.2 ± 0.80	3.3 ± 0.81	4.0 ± 0.92	3.6 ± 1.00	3.9 ± 0.83	3.4 ± 0.82	3.7 ± 0.84	3.8 ± 0.77	**
C14:0	10.9 ± 1.82	11.4 ± 1.53	12.2 ± 1.69	11.8 ± 1.56	11.6 ± 1.45	10.7 ± 1.74	11.6 ± 1.49	11.9 ± 1.40	**
C16:0	29.6 ± 4.79	32.5 ± 4.92	32.9 ± 6.23	31.4 ± 3.99	31.4 ± 6.32	29.6 ± 5.10	29.9 ± 3.93	31.3 ± 5.68	***
n-6	1.6 ± 0.30	1.7 ± 0.53	1.6 ± 0.42	1.6 ± 0.46	1.4 ± 0.43	1.6 ± 0.44	2.1 ± 0.45	1.4 ± 0.44	**
n-6/n-3	1.0 ± 0.31	1.4 ± 0.66	1.4 ± 0.44	1.3 ± 0.49	1.1 ± 0.53	1.3 ± 0.77	1.9 ± 0.89	1.1 ± 0.51	*
OA	20.3 ± 3.87	18.8 ± 3.87	16.6 ± 4.02	19.5 ± 4.01	17.8 ± 4.45	20.2 ± 4.22	20.2 ± 3.46	18.6 ± 4.20	**
CLA9	0.99 ± 0.418	0.88 ± 0.507	0.67 ± 0.451	0.79 ± 0.416	0.93 ± 0.491	1.03 ± 0.454	0.74 ± 0.602	0.91 ± 0.417	ns
EPA+DPA+ DHA	0.23 ± 0.074	0.20 ± 0.073	0.19 ± 0.056	0.19 ± 0.046	0.23 ± 0.070	0.22 ± 0.085	0.20 ± 0.083	0.21 ± 0.056	**
n-3	1.7 ± 0.46	1.4 ± 0.51	1.3 ± 0.45	1.3 ± 0.30	1.4 ± 0.41	1.5 ± 0.50	1.4 ± 0.65	1.4 ± 0.30	***

[a] AYRX= Ayrshire cross, HF=Holstein/Friesian, HFJE= Holstein/Friesian x Jersey, JEX= Jersey cross, NZFX= New Zealand Friesian cross, SH= Shorthorn, SRX= Scandinavian Red cross.
[b] P-values<0.05. ***: P< 0.001, **: P< 0.01; *: P< 0.05, t: P<0.1, ns: P>0.1.
[c] The amount of concentrate feed and conserved forage offered to each cow
[d] The number of treatments each cow recieved between sampling
[e] C12:0=Lauric Acid, C14:0=Myristic Acid, C16:0=Palmitic Acid, n-6=omega-6, n-6/n-3= omega-6/ omega-3 ratio, OA=Oleic Acid, CLA9=Conjugated linoleic acid (C18:2, c9t11 isomer), EPA+DPA+DHA= Eicosapentaenoic Acid+ Docosapentaenoic Acid+ Docosahexaenoic Acid, n-3=omega-3

However, HF and the crosses had the lowest concentrations of long chain n-3 fatty acids, EPA+DPA+DHA (HF:0.20%, HFJE:0.19%, HFSR:0.19%) and HFJE and HFSR had the lowest total n-3 (both 1.3%). Additionally, HFJE had the highest concentrations of C12:0 (4%), C14:0 (11.2%) and C16:0 (32.9%). AYRX had the lowest concentration of C12:0 (3.2%), C16:0 (29.6%), n-6/n-3 (1.0) and also had the highest concentration of OA (20.3%), CLA9 (0.99%-not significant), EPA+DPA+DHA (0.23%) and n-3 (1.7%). SH had the lowest average daily yield (17.8L) and solids (1.5kg), a high average cell count (261 x10^3cells/mL milk), the highest concentration of n-6 (2.1%) and n-6/n-3 (1.9) and had a low concentration of EPA+DPA+DHA (0.20%), n-3 (1.40%) and CLA9 (0.74%).

There was no difference in SCC between breeds, but 12% of SCC recordings from individual cows were above the EU standard, ranging from 400,000-9,000,000 cells/mL milk. This resulted in SCC having a very wide standard deviation, therefore the median values were included in Table 5.3 (as well as mean values) for a more representative SCC status. The median cell counts for each breed are below 90,000 cells/mL milk. Most health treatments were given to the AYRX (0.41) whilst the NZFX (0.08) and SH (0.09) received the least.

5.3.3 LI-P SCORE

The two LI-P scores for each breed combination are presented in Table 5.4. The NZFX was the highest scoring breed, ranking first under both the weighted health and production scenarios, whilst SH was the lowest scoring breed, ranking last in both scenarios. The largest change in LI-P score with the different weightings was HFJE, which scored fourth in the health score, but second with more emphasis on production.

Table 5.4 Effect of breed on health score and production score ± standard deviation.

	NZFX [a]	AYRX	HFJE	SRX	HFSR	JEX	HF	SH	Sig[b]
n	90	100	184	140	274	121	325	80	
Health [c] Score	0.60[d] ± 0.136	0.60 ± 0.167	0.58 ± 0.164	0.58 ± 0.143	0.57 ± 0.163	0.57 ± 0.167	0.57 ± 0.165	0.50 ± 0.133	*
Rank	1	2	4	3	5	7	6	8	
Production Score	0.61 ± 0.169	0.60 ± 0.202	0.61 ± 0.194	0.59 ± 0.170	0.59 ± 0.198	0.59 ± 0.199	0.57 ± 0.198	0.50 ± 0.197	**
Rank	1	3	2	4	5	6	7	8	

[a] AYRX= Ayrshire cross, HF=Holstein/Friesian, HFJE= Holstein/Friesian x Jersey, JEX= Jersey cross, NZFX= New Zealand Friesian cross, SH= Shorthorn, SRX= Scandinavian Red cross.
[b] P-values<0.05. ***: P< 0.001, **: P< 0.01; *: P< 0.05, t: P<0.1, ns: P>0.1.
[c] Maximum possible score = 1
[d] Where mean values are equal the lower standard deviation dictates the rank.

5.4 DISCUSSION

The data collected for this chapter provides valuable information from commercial farms of direct practical application for farmers, in an area lacking in the scientific literature. As a study monitoring on-farm activities, many variables are not controlled, but the statistical model mitigates some of these effects. The data collected is of sufficient quality and range to provide invaluable insights into LI production systems in the UK. This includes the effects of breed combinations on LI-P and determining how and why breeds are suited to different farms. Whilst this chapter does not draw definitive conclusions, it explores the current status of dairy breeding strategies and highlights how farmer's decision-making should direct future LI (cross) breeding research.

5.4.1 LOW-INPUT PRODUCTION

The influence of farm management (e.g., breed, diet, calving date and nutrition) on milk composition, yield and animal health has been well documented (Kalač and Samková, 2010; Schwendel *et al.*, 2015; Średnicka-Tober *et al.*, 2016; Benbrook *et al.*, 2018). These effects are seen in the PCA analyses (Figure 5.3), where each farm system clusters (apart from D1, with fewer records). Most organic cows were autumn calving and many LI were spring calving (Table 5.1). Due to this collinearity, it would be statistically difficult to identify if management (organic vs LI) or stage in lactation had an effect on LI-P. Additionally, the collinearity violates the assumptions of most statistical models on the independent influence of factors; it would therefore be incorrect to separate these in an attempt to identify whether management or lactation stage has the strongest influence on LI-P. While the inclination is to identify drivers that can explain the dataset, the reality (shown in Figure 5.3) is that LI-P is very closely clustered by individual farms. The specific aims and preferences of individual farmers result in decisions about suitable breeds for that particular system, and as these management decisions are unique to each farm, the effect of breed on LI-P is multifaceted.

5.4.2 FEEDING

Whilst the scoring system aimed to identify breeds well suited to low-input farming, there were differences in supplementary feeding between breeds which could influence findings. The amount of concentrate feed offered was fairly consistent across breeds (from 3.0-4.5 kg per head per day), although conserved forage offered was more variable, ranging from 1.7-8.6kg per head per day. Increasing fresh forage in the diet influences milk fat composition, raising CLA9 and omega-3 (Benbrook *et al.*, 2018; Davis *et al.*, 2020) and if we assume fresh forage consumption is indirectly proportional to the amounts of other feeds offered (Butler *et al.*,

2008), we could expect the ranking of the breeds to follow a similar pattern, driven by the positive influence of milk fat composition to these composite scores. This logic holds for the best and worst ranked breeds under both scores, as NZFX, ranked first on both scores, had the lowest supplementary feeding, the highest concentration of CLA9 (1.03%) and second highest concentration of n-3 (1.5%) and SH, ranked eighth on both scores, had the highest level of supplementation offered. However, the ranking of all other breeds does not follow combined supplementary feeding rates. The AYRX outranked both SRX and JEX in health and production scores whilst HFJE outranked JEX in both scores and SRX in production score, yet both AYRX and HFJE received more supplementary feed than JEX and SRX. Despite receiving higher levels of supplementary feed than JEX and SRX, milk from AYRX cows had the highest concentration of n-3 (1.7%) and second highest CLA9 (0.99%) among all the breeds. At the other end of the health and production ranking, JEX cows were judged 7th and 6th respectively, yet were offered the 2nd lowest level of supplementary feeding, hence expected to have a relatively high grazing intake. Despite the evidence that feed management has the greatest impact on FA profile (Kalač and Samková, 2010; Benbrook *et al.*, 2018) this study sampled milk from a wide variety of farms and breeds so that the effect of diet was possibly minimised, potentially displaying differences between the breed.

5.4.3 ANIMAL HEALTH

Somatic cell count is an indication of udder health, cow welfare and milk quality. Generally, if SCC is below 100,000 cells/mL milk the cow is considered healthy, while above 200,000 cells/mL milk the cow is likely to have at least one mastitic quarter and, although some cows naturally have higher SCC, above 400,000 cells/mL milk is deemed unfit for human consumption by the EU (AHDB Dairy, 2018a). During the study, only 19% of high SCC (>400,000 cells/mL milk) cows received a health treatment (veterinary or other). Under EU organic guidelines, cows are expected to resist infection through effective management (Council Regulation, 1999), suggesting that the farmers in this study were more likely to allow cows to build immunity to fight infection rather than treat with antibiotics. Interestingly, HF and HF crosses were responsible for 41% of the high cell counts, whereas only 4% of cows that had SCC over 400,000 cells/mL milk were the *best* performing breed (NZFX), supporting evidence that NZ genetics have effective health traits (Harris and Kolver, 2001). Additionally, Scandinavian Reds have a reputation for good udder health (Clasen *et al.*, 2019) and the SRX had the lowest average SCC (170,000 cells/mL milk), but the HFSR had the highest average SCC (294,000cells/mL milk). Interestingly, the median of both SR crosses was the same (73,000 cells/mL milk). This suggests that farms with a high mastitis challenge might cross HF

with SR due to their reputation and breeding history, instead of changing management to reduce infection risk. Additionally, this portion of animals with high cell counts highlights the need and potential benefit of breeding for improved health traits, especially in organic production systems when prophylactic treatment is not an option. A recent UK governmental report found antibiotic use in livestock decreased 40% from 2013-2017 (Government, 2019), but there is still pressure on dairy industries to reduce antibiotic use due to antimicrobial resistance, which already impacts human and animal health (Hoelzer *et al.*, 2017).

5.4.4 BREEDING OBJECTIVES

The effect of forage diets on milk FA profile has been well researched (Kalač and Samková, 2010; Butler *et al.*, 2011a; Benbrook *et al.*, 2018), but forage conversion by diverse breeds in LI systems has not. Most of the research into forage conversion has predominantly focused on Holstein/Friesians (Tozer *et al.*, 2004; McCarthy *et al.*, 2013). Other studies have suggested that the JE x HF cross is better suited to a pasture based system (Prendiville *et al.*, 2011; Beecher *et al.*, 2014; Coffey *et al.*, 2017), but only compared to purebred HF. As a generalisation, Holstein/Friesians were bred for their production traits rather than milk composition or health traits (Miglior *et al.*, 2005). This was reflected in this study, as cows with HF genetics had the highest yield (21.2-21.9L/day) and HFJE had the most protein and fat solids yield (1.8kg/day), but SCC was highest for HFSR (294,000cells/mL milk) and HFJE (3rd highest: 248,000 cells/mL milk). Whilst HFs are important in the UK and their crosses have worked well in some grazing based systems, further research into forage conversion in more diverse breeds is needed to improve LI and organic dairy systems. Whilst cattle diets might be the dominant factor controlling milk FA profiles there is also evidence that heritability affects milk fat composition both within and between breeds (Soyeurt *et al.*, 2006; Garnsworthy *et al.*, 2010). This suggests a combination of feeding forage and selective breeding may optimise FA composition for consumer health. Despite breeding bodies and milk purchasers currently prioritising milk fat and protein content, there is currently no premium to reward fat composition in the UK. Organic Valley's "Grassmilk.^{TM}" (USA) receives a 15% premium above organic prices for n-3, CLA9 content and n-6/n-3 ratio (Organic Valley, 2019). This demonstrates a market for optimising milk fat composition and thus potentially creates a marketing opportunity for UK milk.

An alternative benchmark for LI dairy is the New Zealand National Breeding Objectives, in which grazing is emphasised and priority placed on forage conversion, yield of milk components (protein and fat %), health and fertility (NZAEL, 2019). Based on the importance of forage in NZ dairying, it is unsurprising that the NZ Friesian cross outperformed all other

genotypes in this study, ranking first in both performance scores (Table 5.4). Although breed is an important component of management, diet is the strongest factor that influences FA composition in milk (Butler *et al.*, 2011a), whilst high intakes of forage in the diet increases milk n-3 concentrations and reduces n-6/n-3 ratio (Średnicka-Tober *et al.*, 2016; Benbrook *et al.*, 2018). The contribution of milk FA profile to the LI-P score identifies a breed's ability to graze and use grass efficiently, therefore the concentrations of n-3, CLA9 or n-6/n-3 ratio in milk could be used to predict how well forage is converted to milk.

5.4.5 EFFECT OF BREED ON LOW-INPUT-PRODUCTION

The results of this study confirm that although management on individual farms affects LI-P, breed also plays an important role. Despite ranking last under both scenarios (Table 5.4), Shorthorns are well known for their positive temperament, high fertility and efficiency in converting forage to milk (The Shorthorn Society, 2019), which are all metrics important for LI dairying although not formally analysed in this study. In terms of desirable milk-fat composition, AYRX had the most desirable FA profile. However, AYRX yielded less milk (20.2L/day) than the more productive HF crosses (21.2-21.9L/day) and came fourth for SCC (243,000 cells/mL milk). Despite this, the AYRX ranked second in health and third in the production score. Ayrshire's are commonly used in organic systems because of their ease of management, efficiency in forage to milk conversion and overall health and longevity (Ayreshire Cattle Society, 2018). The Jersey crosses did not rank well (rank= 7^{th} weighted health and 6^{th} in production score) but, the Jersey has many desirable traits for organic and LI systems (Jersey Cattle Society, 2019). The Ayrshire, Shorthorn and Jersey breeds are selected by farmers for merits that were beyond the scope of this study to measure and the low UK population of these breeds offers less scope for selection than the more popular HF.

The breeds in this study are generally popular and well-suited to organic and LI farming. Despite this, many of the desirable traits for organic and LI dairying were not measured in this study (forage conversion, fertility, temperament, ease of calving, etc.). It is easy to pick and choose the characteristics that could make a breed look 'better' or 'worse', it can be subjective but, farmers make their decisions based on their priorities and what works best for their specific system and despite the low score for LI-P, many of these breeds are all essential for LI and organic dairying. The ideal scenario would be for farmers to access an interactive flow chart to guide them through breed selection based on inputs, constraints and priorities within their system, resulting in an indexing system unique to each farm.

5.4.6 HETEROSIS

Another important factor to consider in a crossbreeding programme is heterosis and the effects of back-crossing, as demonstrated from the breeding approach used on these organic and LI farms. All farms had at least three core breeds (Table 5.1), most of which are crossed and back-crossed. In this study, of the 1070 cows selected from 17 farms, 40% were F1 (first generation crosses), 40% were F2 or subsequent generations and only 20% of cows were pure-bred. This confirms that in these LI and organic systems, cross breeding is essential to develop robust, productive cows. As discussed, much of the published research is centred on HF crosses, which, as demonstrated by this study, are not representative of LI and organic management practices on the UK farms studied. Additionally, maximizing the benefits of hybrid vigour can be complicated and unpredictable, but challenging organic conditions often make heterosis worthwhile (de Haas *et al.*, 2013). Partially due to the emphasis on specific breeds, such as Holstein/Friesian, there is little readily available, independent advice for farmers with alternative breeds, regarding heterosis. Further studies are needed using a diverse range of breeds to fully understand this effect and the benefits it offers (Rodríguez-Bermúdez *et al.*, 2019), but as demonstrated by the predominance of crossbreeding in this study, the industry is ahead of the science; farmers are investigating the effects for themselves.

5.4.7 GXE

The Genotype by Environment interaction (GxE) is key to distinguishing between intensive and LI or organic breeding programmes. Nauta *et al.* (2006) first explored the GxE differences between organic and conventional dairying and reported heritabilities of SCC and production traits that warrant a re-ranking of dairy bulls for organic systems. This was because the size of the GxE interaction was different between organic and conventional management, suggesting that the breeding values for these traits should be adjusted, which would result in a re-ranking of bulls (Nauta *et al.*, 2006). The abundance of cross-breeding in this study indicates that farmers are learning about how (cross) breeds interact with their environments, potentially observing heterosis and GxE independently, suggesting that for LI and organic breeding objectives to be successful, the science will have to align with farming practices. Rodriguez-Bermudez *et al.* (2018) concludes that by breeding for intensive systems, organic cows will not meet their potential due to the impact of GxE interactions on performance. To improve efficiency in LI/organic dairying, genotypes must be well adapted to their systems, which has less emphasis on production but greater focus on fertility and resilience (Rodríguez-Bermúdez *et al.*, 2019). Keeping the GxE interaction in mind when developing and evaluating breeding

programmes is essential to allow livestock to meet their potential, regardless of the system they are kept in.

5.5 CONCLUSION

To conclude, this Chapter highlights weaknesses in current UK breeding programmes for LI and organic dairying due to limited past research on forage conversion to healthy milk and a bias towards Holstein/Friesians. The lack of robust scientific evidence necessary to advance breeding systems has resulted in the science-base often being behind best farming practices. Evidence from this study indicates that New Zealand Friesian and Ayrshire genetics could suit some LI/organic farms. Thorough further research is needed to explore the GxE and forage intake and conversion to meet the true potential of cows under these management systems.

CHAPTER 6. POTENTIAL TO SELECT FOR EFFICIENCY IN PASTURE-BASED DAIRY SYSTEMS

6.1 INTRODUCTION

Typically, dairy systems focus on increasing production and lowering costs; however, driving for economic efficiency has neglected sustainability and led to very high-output but poor health and fertility in cattle (Oltenacu and Broom, 2010). The PB[1] approach to sustainable dairy production emphasises soil and sward health, aiming to optimise pasture growth and utilisation and match availability to cow demand, thus avoiding concentrate feeding. This management system aims to rear cattle that are well adapted to their low-input systems, selecting breeds and family lines that stay healthy and efficiently convert forage to milk without reliance on antibiotic, fertility treatments or other inputs. Inevitably, these farms are lower yielding than intensive Holstein Friesian (HF) farms, but the input costs are greatly reduced, resulting in a profitable system (PFLA, 2016).

Pasture-based dairy farmers are not focussed on the quantity of production and tend to keep breeds that are more suited to their system than the typical HF found on most UK dairy farms; instead, PB systems are more akin to New Zealand or Irish dairying (McCall and Clark, 1999; Butler *et al.*, 2008; Bijttebier *et al.*, 2017). Farmers often have generations of acquired knowledge and through years of trial and error their farming methods are effective and system specific, resulting in breed and replacement decisions based on farmer experience and personal preference (Nauta *et al.*, 2009). Many PB farmers spring calve to optimise pasture utilisation, so a tight calving block is essential to ensure calving during the same period the following year. However, in year-round calving systems there is less emphasis on exploiting pasture, so fertility is less essential and longer calving intervals are generally accepted. The average calving interval for UK year-round dairy production in 2017/18 was 396 days, compared to 377 days in spring calving herds (AHDB, 2018a). This highlights that in a PB system it is crucial that cows can effectively convert pasture into milk whilst maintaining body condition, health and fertility.

There are many methods used to consider production efficiency and feed conversion, as explained in Chapter 2 and Chapter 5. However, these methods are often developed in intensive, indoor dairy farms and/or using heifers. A major problem in estimating dairy efficiency, feed conversion and dry-matter-intake (DMI) is the fluctuations of their energy balance throughout lactation, specifically energy mobilised from body fat reserves soon after calving (Connor,

[1] For the purpose of this study PB will be defined as cattle that receive over 85% of their diet from forage, compared to organic, which is at least 60% forage (Soil Association, 2018).

2015), which is why growth rate in heifers (with zero lactations) are used to develop methods. For example, dairy efficiency may look good during early lactation for cows that lose more weight (cows are eating less per unit of output, but losing body condition), but this is likely to have a negative impact on fertility (Westwood *et al.*, 2002). This variation complicates genetic progress in efficiency related traits and could negatively impact traits such as energy balance (Pryce *et al.*, 2014). In PB systems the priorities and grazing strategies are different from more intensive systems, suggesting that an alternative metric for production efficiency is required compared to traditional methods (such as the ratio of feed intake (kg) to milk production (kg)) used in the UK. Despite this, improved efficiency is still vital for improved environmental and economic sustainability (Mulliniks *et al.*, 2015).

Pasture-based dairying emphasises grazing strategy to optimise sward/soil health and in turn grazing utilisation and production efficiency (PE, i.e. how many kg of grass dry matter (DM) need to be grown for every litre of milk produced). The farmers go beyond the cow's nutritional requirement and consider herbage utilisation, building soil carbon, supporting biodiversity, and sowing diverse swards that are resistant to adverse weather (Zaralis and Padel, 2019). Whilst there is little concrete scientific literature supporting these claims in the UK, early results are supportive (Zaralis and Padel, 2019). As discussed in Chapter 2, Section 2.5 the strategic grazing management system often selected by PB farmers uses 'tall grass' or 'mob' grazing, which includes a longer rotation (30-60 days) than typical paddock/cell grazing (21-28 days), a higher un-grazed dry matter (DM) cover with the aim of grazing a third, trampling a third and leaving a third ungrazed, and a higher stocking density (Tracy and Bauer, 2019). By improving grazing systems there are opportunities to optimise management and identify the cows that are best suited to a PB system.

As discussed in Chapter 2, Section 2.7, milk nutritional quality is an important indicator of forage content in the cow's diet, with potential implications for human health. This chapter will explore differences in milk quality between individual cows on the same farm as well as between farms and determine if there are interactions between fatty acid content and production efficiency.

Aim: To determine variability in performance between farms as well as between cows under common management and assess if there is scope for selection of cows, within PB herds based on efficiency and milk quality, highlighting any variables that farmers could use to identify efficiency.

6.2 MATERIALS AND METHODS

6.2.1 FARM RECRUITMENT AND COW SELECTION

This study required three commercial farms willing to participate in research from calving to drying off (approximately February to November) during 2018. To qualify for this study, farms had to be spring calving, pasture-based (at least 85% forage in the cows' diet) and have at least 30 second and/or third lactation cows (to limit variability caused by parity across the farms). Initially, farmers that had previously taken part in university studies were contacted via email and Newcastle University's Agriculture Twitter account was used to find additional farmers. Seven farmers responded to the call, of which five met the specification. After explaining fully what the study would comprise, three farmers remained. All farms were organic with cows receiving over 85% of their diet from forage and based in the Southern Midlands, UK (farm background information can be found in Table 6.1).

Table 6.1 Background information of the three farms (A, B and C) involved in this study, further information on individual cows (including fertility) is presented in Appendix D, Table D.3.

Question	A	B	C
Calving 2018	25th March+ 6weeks	20th February+ 10weeks	14th February+ 8weeks
Farm Size (Ha)	300	230	140
No. of milking cows	400	300	218
No. 2nd calvers	25%	20%	4%
No. 3rd calvers	15%	17%	32%
Grazing 2018	25thMarch-December	20th February- 20th December	February- November
Records yield	No	No	No
Weighing facilities	No	No	No
Certification	Organic, PFLA	Organic	Organic
Pasture	Diverse grass, clover and herbal ley	Diverse grass, clover and herbal ley, 0%perennial- rye	Grass, clover and herbal ley
Percentage of diet pasture/ forage	100%	85%	90% (additional hay summer 2018- drought)
Grazing Rotation	Fresh pasture every 12 hours, 30-55 day rotation	Fresh pasture 12-24 hours, 30-40 day rotation	Fresh pasture every 12-24 hours, 25- 30 day rotation
Average pre-grazing pasture cover) kg DM/ha)	2800	2800	2300
Average post-grazing pasture cover (residual) kg DM/ha	1600	1600	1600
Insemination policy	Bull	AI (sweeper bull)	AI
Average Cow Liveweight (kg)	534±66.9	539±64.9	634±59.1
Antibiotic Policy	None needed	Only where essential	In line with organic standards

Further selection occurred within each farm, as 23 second or third lactation cows were selected at random by each farmer. Farmers were asked to select a range of cattle within the age range to reflect diversity of breed, size and productivity. However, all farmers confessed that the cows selected were the first 23 that met the criteria to come through the milking parlour. The cows wore RumiWatch halters (explained below in Section 6.2.2.2) for two weeks at three key periods in 2018, designated by number of days in milk (DIM): early (E; <100 DIM), mid (M; >101 and <200 DIM) and late (L; >201 DIM) lactation (Appendix D, Table D.1.). The same cows on each farm were followed throughout this study. The assessment timetable was considerate to the needs of the farmer (calving, holidays, staff, etc.), but still required time and labour from each participant. During all rounds of data collection the farmers were required to record yield twice (24-hour yield), and take a representative milk sample for National Milk Recording (NMR, 2019). Additionally, farmers had to bring cows in and separate them to put-on and remove the halters, followed by health checks to ensure the halters did not rub or cause sores.

6.2.2 DATA COLLECTION

6.2.2.1 FARM AND COW DETAILS

Initially records were collected for background management information on the size of the farm and herd, feeding and calving plan and the facilities available on site (yield recording, weighing, pedometers). Further records were collected for the individual cows on yield, additional feeding, supplementation and medications at the time of recording. Additionally, data were collected on breeding, date of birth, calving and servicing dates. Throughout this chapter, the three farms are designated as Farm A, Farm B and Farm C (Table 6.1).

All farmers had a very tight spring calving block of six to ten weeks. As such, fertility in all cases was very good (Appendix D, Table D.3), all cows on Farms A and B and over half from Farm C became pregnant following first service) and (although highly relevant to this system) was not used as a variable in assessing the suitability of cows to a system. Additionally, on two of the Farms (A and B) there were no incidences of illness (including mastitis) or dry cow therapy administered. Farm C, however, had three incidences of mastitis, one metritis case and three fertility treatments. Due to the limited number of disease and fertility treatments between the farms, the only health measurement metric used for statistical analysis was somatic cell count (SCC). Additionally, three cows from Farm C tested positive for TB between mid and late lactation and were removed from the recorded herd, although their results from early and

mid-lactation were retained. Individual cow background information including health and fertility treatments is in Appendix D, Table D.3.

6.2.2.2 RUMIWATCH HALTERS

The 'RumiWatch' (RW) halter (Itin+Hoch, 2015) is a pressure-based recording system that can estimate eating, grazing, ruminating and drinking behaviour, based on head position and jaw movements. RumiWatch (RW) is currently the only validated hardware able to record real-time eating and ruminating behaviour in-field over numerous days and weeks (Rombach *et al.*, 2018; Werner *et al.*, 2018a). RumiWatch halters sourced from (Itin+Hoch, 2018) were used to record grazing and ruminating behaviour, which drive DMI.

Figure 6.1 Dairy cow wearing a correctly fitted RumiWatch halter (Itin+Hoch, 2015). The halter slipped over the cow's nose, a strap passed over the top of the head and fastened on the left cheek, was loose enough so that it did not affect jaw or mouth movement.

There are two different software packages, one used to manage the halters (RW Manager 2, Version 2.2.0.0) and one to analyse the data (RW Converter, Version 0.7.4.13 (FW00.62)). The halter has a data logger, a 3-axis accelerometer and an oil-filled noseband pressure sensor. Mouth movements during eating and ruminating cause the pressure in the noseband to change which is recorded at a resolution of 10Hz. Each halter has a 4GB SD-card with the data logger in a box on the right side of the halter and two 3.6V batteries in the left side box. The halter has fully adjustable straps and were fitted and synched to each cow (Figure 6.1) following instructions in the RW handbook (Itin+Hoch, 2015). At the end of the two-week period, halters were removed, cleaned and disinfected. Between each farm, the batteries were checked and

replaced if under 20% and data from the SD cards were downloaded. The SD cards were wiped and reset.

Based on rhythm, frequency and acceleration patterns, the RW Converter detects the jaw movements then defines the type of activity (e.g. grazing or ruminating) and the time spent on each. The RW Converter calculates the activity in one-minute summaries, which can then be converted to hourly or 24-hour values for each behaviour: eating (head up), grazing (head down), drinking and ruminating. Additionally, they record temperature, other jaw movements and any activity changes. Further information outlining the technical set-up, design and how the RW Manager and Converter function can be found in papers by Zehner *et al.* (2012) and (2017), Werner *et al.* (2018a) and (2018b).

Despite consistent application of halters, checking batteries, downloading data and re-setting the SD card, many halters failed. Nosebands and data loggers were replaced and errors were troubleshooted with the manufacturers regularly. Additionally, there was a heatwave (with daytime temperatures regularly above 25°C) during the summer of 2018, which may have affected the hardware in some of the halters, as many stopped recording a few days into mid-lactation at Farm C and never recovered. The manufacturer sent new hardware to replace some of the broken halters, but the missing data is reflected in the number of cows in the RW data, in the top row of results in Table 6.2.

6.2.2.3 MEASUREMENTS

None of the farms had facilities for weighing, so heart girth and body length were measured to estimate live-weight (LW) at the beginning of each recording period (Appendix D, Table D.1.) during halter set up. The most accurate estimate of LW when weighing facilities are not available is Schaeffer's formula (Wangchuk *et al.*, 2018), which was used to estimate all individual cow liveweights:

Live-weight (lbs (1lb= 0.454kg)) = Heart Girth^2 (inches) x Body Length (inches) /300.

Despite weight fluctuations through lactation, the three weight estimates were averaged to create one weight recording per animal, accounting for inaccuracies during measurements.

Mouth circumference was measured using a soft tape measure after halter set up and used in the model for estimating DMI (explained below. Section 6.2.3.1). The cows were prone to moving during this measurement, therefore the mouth circumference was also averaged across the three stages of lactation.

6.2.2.4 MILK YIELD AND COMPOSITION

None of the farms had the infrastructure to record daily milk yield, therefore yield data were collected twice during each two-week block. The farmers installed Waikato meters (Waikato, 2019) in the milk line during each recording. Milk was sampled (50ml) and sent to National Milk Records (NMR, 2019) for somatic cell count (SCC), protein, lactose and fat analysis each time the farmer recorded yield. NMR provided 50ml sampling tubes with the preservative bronopol, to which farmers or an NMR technician added a representative, raw milk sample. Once NMR completed analysis, milk samples were forwarded to Newcastle University where they were immediately frozen at -20^{o}C (within one week of sampling) until analysis. Unfortunately, some milk samples and results were lost or destroyed before reaching Newcastle, but there is at least one complete milk sample from each round and farm, apart from Farm A at early lactation. The samples were thawed, freeze-dried then prepared for Gas Chromatography (GC) to assess FA, using the method described by Chilliard *et al.* (2009) and in Chapter 3, Section 3.3. During round 3, an additional 50ml milk sample (without bronopol) was collected for iodine analysis; the iodine analysis was beyond the scope of this PhD but will be included in a forthcoming paper.

6.2.2.5 SWARD

All farmers managed their pasture through paddock grazing and Farms A and B used a tall-grass (mob) grazing approach, moving their herds onto fresh pasture every 12-24 hours (Table 6.1). On each visit to the farm, a representative sward sample was collected from a recently grazed pasture and from a pasture to be grazed in the next 24 hours to be analysed for content of vitamins, minerals and trace elements (these data will be used in a future paper). Additionally, during early- and mid- lactation visits, a plate meter was used to assess dry matter cover before and after grazing (Table 6.1). All farmers highlighted that standard plate meters are not accurate in their systems and they judge dry matter cover by eye. Standard plate meters are calibrated to estimate yields of perennial rye-grass, which is stalkier in a tall-grass setting, whereas herbal leys as grown on these farms remain more leafy. This suggests that, at any given estimate of herbage cover, the DM available to the cattle (the cows tend not to chew on the stalks of perennial rye-grass) is higher in a diverse, herbal ley and the recordings under-estimate the actual field values. Despite this, the plate meter was used to estimate an approximate value. The plate-meter number was recorded before and after fifty ‘plonks’ randomly distributed across the pasture, avoiding the areas around gateways and water troughs. The calculation used to estimate dry matter is based on guidance from the UK Dairy board (AHDB Dairy, 2019):

Kg DM per hectare = average difference between plate meter readings x 125 +640

6.2.3 DATA HANDLING AND ANALYSIS

Data was collected in Excel and organised, subset, visualised and analysed in 'R' (R Core Team, 2020), as discussed in Chapter 3, Section 3.5.

6.2.3.1 DRY MATTER INTAKE

Calculating ruminant dry matter intake in *ad lib* forage-based systems is notoriously difficult as there is no quantifiable amount of feed given to individual cows; it is their preference how much they eat. Many studies have researched models to predict DMI and, most recently, Guilherme Amorim Franchi (2017) compared 16 models and generated an equation to estimate DMI, involving numerous factors, which will be used to estimate DMI in this chapter:

DMI [kg/d] = 6.65 + (0.001 × Age [d]) – (0.00001 × Lactation length^2 [d]) + (3.17 × Lactation length$^{0.178}$ [weeks]) – (0.598 × Mouth Circumference [cm]) + (12.04 × Milk Protein Yield [kg/d]) + (0.000018 × Liveweight^2 [kg]) + (0.012 × Eating Time [min/d]) + (0.178 × Ruminating Chews [n/min])

This model was generated using data from indoor systems, but in this Chapter will be used to give an estimated grazing dry matter intake (EDMI). Dado and Allen (1994) and National Research Council (2001) suggests that normal intake by dairy cows can range from 15-27kg DM/day under intensive management. In this chapter, there were a few instances where DMI was assessed below 15kg DM/day, but some of the cows from this RumiWatch study were much smaller, estimated at 400-700kg, than the HF cited (weight unknown, but HF average 650kg (Oklahoma State University, 2007)).

6.2.3.2 MILK YIELD

To compare milk from different farms and cows producing milk with different solid contents, energy corrected milk yield (ECMY) was calculated using an equation adapted from Tyrrell and Reid (1965) and cited in Sigl *et al.* (2013). Milk is corrected to 3.5% fat and 3.2% protein:

ECMY (kg/day) = (milk yield (kg/day) × 0.327) + (milk fat (kg/day) x 12.86) + (milk protein (kg/day) x 7.65)

Additionally, individual cow records were also given a 15% premium based on Organic Valley Grassmilk^{TM} standards, to acknowledge the benefits from cows producing milk of high nutritional quality. Economic analysis was beyond the scope of this PhD, therefore 15% was

added to the yield of each milk sample, creating an energy corrected milk yield premium (ECMYP), when milk met the following minimum standards defined for Grassmilk™ (Benbrook *et al.*, 2018):

i. 39mg total omega-3/100g milk
ii. 26.6mg total conjugated linoleic acid/100g milk
iii. Omega 6: omega 3 ratio less than 1.2

Farmers were only able to record milk yield twice during each assessment stage, which were averaged to give a single entry for yield and composition per round. Despite halters recording daily, an average value is used for ECMY, ECMYP and EDMI, per cow per round.

6.2.3.3 PRODUCTION EFFICIENCY

Despite the importance of health and fertility in PB systems, they could not be built into an efficiency model here, since at least two of the farms were exemplars of this system. Farms A and B had no health or fertility treatments during this trial (Appendix D, Table D.3), so the sole health indicator was SCC. Whilst this parameter offers insight into udder health, if cows are able to fight off the infection without the assistance of an antibiotic, as suggested by EU organic standards (Council Regulation, 1999), SCC could be a marker of good health, rather than ill health. Therefore, creating efficiency models that include health and fertility based on data from farms that are already achieving near perfect health and fertility outcomes is impossible. For this reason, the simple conversion approach of forage input to energy corrected milk output was the only viable option for an efficiency metric (conventional approach to PE) with data collected from these farms.

The dairy diets were predominantly forage, at least 85% (Table 6.1) with up to 15% of the diet coming from organic concentrate. Despite this, this chapter will refer to feed and forage conversion efficiency as production efficiency (PE) unless otherwise specified. Production efficiency in these systems was defined as EDMI divided by ECMY, where the lower the value the more efficient the cows (i.e. the amount of feed it takes to produce 1 litre of milk).

The aim of this study was to compare cows under common management, therefore cows were compared within their own system as well as across systems. So, to identify similarities between the most/least efficient cows, cows from each farm were allocated into five groups by their PE and assigned ‘One’ for the most efficient 20%, through to ‘Five’ for the least efficient 20%.

Milk fat concentration was highest in the most efficient group. ECMY favours milk fat, so another efficiency was also calculated as EDMI divided by liquid milk yield, for comparison

and discussion. These results are in Appendix D, Table D.2 to not confuse the PE grouped results mentioned above.

6.2.3.4 EATING BEHAVIOUR

Data from the RW Halters were converted into daily averages and paired with the milk analysis, yield and composition data. Eating rate is defined as EDMI divided by time spent eating; similarly, rumination rate is EDMI divided by time spent ruminating.

6.2.3.5 MILK FAT QUALITY

The calculation to transform percentage of milk FAs to g/kg of milk includes milk fat percentage. This was not available for all milk samples, therefore, to retain as much data as possible, FAs are presented as a proportion of the FA profile (%). Simple addition was used to calculate total mono-unsaturated FAs, saturated FAs, polyunsaturated FAs, omega-6, omega-3 and very long chain n-3 FAs. Omega-6 to omega-3 ratio was calculated as the latter divided by the former (these calculations are explained in Appendix A).

6.2.4 STATISTICAL ANALYSIS

Three models were used to identify differences and similarities between:

i. Farms (A, B, C) and stage of lactation (E, M, L), null hypothesis (H_0)= there are no differences between farms and stages of lactation
ii. The combination of farm and stage of lactation (A-E, A-M, A-L, B-E, etc.), H_0= there are no differences between the combination of farms and stages of lactation.
iii. Cows with the best to worst PE from each farm (One, Two, Three, Four, Five), H_0= there are no differences in PE.

The variables selected for comparison included milk FA composition and ruminating and eating behaviours. It is also important to note that Farm A only milked once per day, which may prevent fair comparisons between systems.

All three models were generated using the 'R' package 'nlme' (Pinheiro *et al.,* 2017). The linear mixed effects model accounts for variation explained by the fixed effects and random effects (typically repeated measures). As discussed in Chapter 3, Section 3.6.5, traditional post-hoc Tukey Honest Significant Difference (Tukey HSD) tests are used for multiple comparisons of levels within a factor, but controlling the familywise error rate using this approach risks numerous Type 1 errors (false positives) and would be misleading (Fletcher *et al.*, 1989; Smith *et al.*, 2002; Cramer *et al.*, 2016). P-values give an indication of whether the null hypothesis

can be accepted or rejected, but interpretation of multiple comparisons is left to the author and readers discretion.

6.3 RESULTS

6.3.1 DRY MATTER INTAKE

There were significant differences in EDMI between Farms (p=0.010). Farm C cows had a higher DMI (22.3kg DM/day) than A (17.0kg DM/ day) and B (17.4kg DM/ day). EDMI was fairly consistent from early (20.0kg DM/ day) to mid (20.4 kg DM/day) but fell by late lactation (15.7kg DM/day) (p=0.017) (Table 6.2).

On all three farms EDMI decreased across lactation (p=0.000); Farm A decreased by 30%, Farm B by 13% and Farm C by 23%. Whilst Farm A and B reduced DMI throughout lactation, Farm C increased to mid- lactation and then decreased by late lactation (Table 6.3).

By definition of efficiency, EDMI was lowest in Group One (17.8kg/cow/day), highest in Group Five (19.6kg/cow/day), although this was only a trend (p=0.097) (Table 6.4), when EDMI was based on EDMI/ECMY.

6.3.2 YIELD

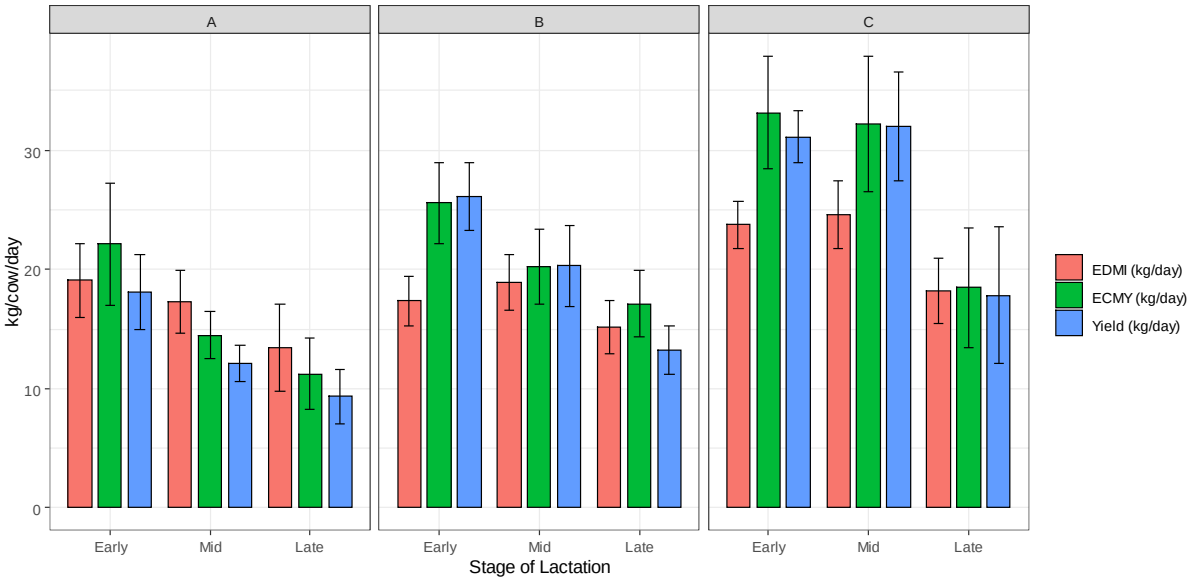


Figure 6.2 Relationship between estimated dry matter intake (EDMI), energy corrected milk yield (ECMY) and liquid milk yield on each Farm (A, B, C) at each stage of lactation (Early, Mid and Late)

Energy corrected milk yield followed the same pattern as EDMI, highest on Farm C (28.5 kg/day) followed by Farm B (21.4 L/day) and Farm A (17.0 L/day) (p=0.026) and between the rounds from early (26.8 L/day) to mid (23.0 L/day) and falling by late lactation (15.7 L/day)

(p=0.030) (Table 6.2). Liquid milk yield followed the same pattern, highest on Farm C (27.3 L/day), followed by Farm B (20 L/day) and Farm A (14 L/day) (p=0.012). Milk yield decreased through lactation (E=24.9 L/day, M=22.1 L/day, L=13.7 L/day) (p=0.020).

On all three farms ECMY decreased across lactation (p=0.000); Farm A decreased by 50%, Farm B by 33% and Farm C by 44% (Table 6.3). Liquid milk yield decreased on all farms through lactation (p=0.000); Farms A and B decreased by 49% and Farm C by 47%. When the premium was applied (ECMYP), there were marginal notional *improvements* in the yield from cows on Farm A and no improvement on B or C, since more cows from Farm A met the premium requirements than on farms B or C.

When PE was based on EDMI/ECMY, both ECMY and liquid milk yield was highest in Group One cows (26 L/day, 21.6 L/day) and lowest in Group Five cows (18.5 L/day, 19.0 L/day) (p=0.000), (Table 6.4). Similarly, when efficiency was based on EDMI/Yield, ECMY and liquid milk yield was highest in Group One cows (24.5 L/day, 23.6 L/day) and lowest in Group Five cows (21.1 L/day, 18.3 L/day) respectively (p=0.000) (Appendix D, Table D.2.).

6.3.3 PRODUCTION EFFICIENCY

Farms B and C had similar PE (EDMI/ECMY) (0.84) but Farm A was less efficient (1.07) (p=0.040). Efficiency deteriorated (where a numeric increase is a reduction in efficiency) throughout lactation (E:0.77, M:0.95, L:1.06) (p=0.040) (Table 6.2). The relationship between EDMI, ECMY and liquid milk yield is visualised in Figure 6.2.

On all three farms efficiency worsened from early to late lactation (p=0.000); Farm A decreased by 40%, Farm B by 33% and Farm C by 42% (Table 6.3).

Group One was the most efficient (0.72) and group Five was the least efficient (1.14) (p=0.000) (Table 6.4). The variation in PE between cows that were present for two and three stages of lactation is visualised in (Figure 6.3).

Table 6.2 Mean eating, ruminating, health and fatty acid analysis ± SD by Farms (F) (A, B and C) and Rounds (R)(Early, Mid and Late). Fatty Acid results are expressed as a percentage of the total fatty acid profile.

	A[a]	B	C	Early	Mid	Late	F	R	F	R
n	45	51	49	57	47	41	p-val	p-val	Sig	Sig
Days in Milk	123± 65.1	104± 63.3	130± 66.6	51± 16.1	125± 17.0	207± 15.5	NA	NA	NA	NA
SCC[b] ($x10^3$ cells/ml milk)	104± 153.6	76± 102.2	167± 419.5	103± 210.2	62± 57.5	194± 424.3	0.312	0.179	ns	ns
EDMI (Kg/day)	17.0± 3.92	17.4± 2.68	22.3± 3.68	20.0± 3.59	20.4± 3.98	15.7± 3.51	0.010	0.017	**	*
ECMY(L/day)	17.0± 6.36	21.4± 4.71	28.5± 8.60	26.8± 6.44	23.0± 8.53	15.7± 5.00	0.026	0.030	*	*
EDMI/ECMY	1.07± 0.297	0.84± 0.166	0.84± 0.218	0.77± 0.159	0.95± 0.202	1.06± 0.311	0.044	0.040	*	*
Yield (L/day)	14.0 ± 4.70	20.7 ± 5.86	27.3 ± 7.72	24.9 ± 6.09	22.1 ± 8.58	13.7 ± 5.29	0.012	0.020	*	*
EDMI/Yield	1.28 ± 0.314	0.90 ± 0.236	0.87 ± 0.227	0.84± 0.197	1.01 ± 0.288	1.23 ± 0.344	0.011	0.018	*	*
Eating Time (min/day)	548± 110.4	466± 106.2	534± 59.2	522± 79.4	560± 72.7	452± 122.0	0.297	0.206	ns	ns
Ruminating Time (min/day)	417± 73.8	431± 65.7	439± 81.9	430± 52.1	453± 64.8	403± 98.6	0.865	0.678	ns	ns
Rumination Boluses (n/day)	503± 101.5	481± 70.4	488± 94.1	507± 81.5	510± 64.1	444± 106.8	0.990	0.556	ns	ns
Chews per Bolus (n/bolus)	49.5± 6.02	55.6± 6.20	54.9± 6.90	51.7± 5.95	56.1± 6.88	53.0± 7.35	0.090	0.268	t	ns
Eating Rate	0.03± 0.005	0.04± 0.006	0.04± 0.007	0.04± 0.007	0.04± 0.009	0.04± 0.007	0.184	0.840	ns	ns
Rumination Rate	0.04± 0.011	0.04± 0.009	0.05± 0.015	0.05± 0.013	0.05± 0.009	0.04± 0.016	0.074	0.399	t	ns
Fat (%)	4.7±0.93	3.8±1.22	3.7±1.37	4.0±1.19	3.8±1.28	4.5±1.27	0.272	0.489	ns	ns
Protein (%)	3.6±0.19	3.3±0.44	3.3±0.23	3.4±0.34	3.3±0.30	3.6±0.33	0.417	0.475	ns	ns
Lactose (%)	4.5±0.15	4.5±0.21	4.5±0.17	4.6±0.18	4.5±0.16	4.5±0.17	0.758	0.326	ns	ns
n	25	50	49	37	47	40				
ECMYP (kg/day)	13.2± 3.28	21.8± 4.71	28.9± 8.60	29.4± 5.75	23.5± 8.62	16.2± 5.25	0.032	0.124	*	ns
EDMI/ECMYP	1.19± 0.298	0.82± 0.163	0.82± 0.208	0.70± 0.117	0.93± 0.197	1.04± 0.306	0.038	0.096	*	ns

Table 6.2 continued. Mean eating, ruminating, health and fatty acid analysis ± SD by Farms (F) (A, B and C) and Rounds (R)(Early, Mid and Late). Fatty Acid results are expressed as a percentage of the total fatty acid profile.

Milk FA Concentrations (%)	A[a]	B	C	Early	Mid	Late	F	R	F	R
n	45	51	49	57	47	41	p-val	p-val	Sig	Sig
C14:0	11.6± 0.69	12.1± 1.37	11.9± 1.14	11.6± 1.44	11.9± 0.97	12.1± 1.07	0.448	0.275	ns	ns
C16:0	31.4± 4.06	28.6± 3.56	28.5± 3.57	26.3± 2.41	29.6± 3.23	31.0± 4.21	0.475	0.377	ns	ns
VA	1.8± 0.42	2.8± 0.95	2.2± 0.85	2.8± 1.03	2.2± 0.83	2.2± 0.70	0.476	0.767	ns	ns
OA	18.1± 2.32	17.9± 2.31	19.5± 3.02	19.3± 3.11	18.4± 1.88	18.1± 2.97	0.293	0.593	ns	ns
LA	0.9± 0.14	1.5± 0.33	1.0± 0.26	1.6± 0.33	1.2± 0.35	1.0± 0.22	0.016	0.039	*	*
ALA	1.1± 0.18	0.7± 0.17	0.8± 0.13	0.8± 0.12	0.8± 0.17	0.8± 0.28	0.166	0.844	ns	ns
CLA.9	0.8± 0.18	1.1± 0.27	1.0± 0.47	1.0± 0.35	0.9± 0.26	1.1± 0.41	0.573	0.545	ns	ns
SFA	69.4± 2.84	68.9± 3.37	67.1± 4.40	66.3± 4.00	69.1± 2.56	69.0± 4.18	0.425	0.389	ns	ns
PUFA	4.9± 0.61	5.2± 0.80	5.1± 1.09	5.8± 0.80	4.9± 0.79	4.8± 0.76	0.907	0.341	ns	ns
MUFA	25.7± 2.44	25.9± 2.82	27.8± 3.68	27.9± 3.53	26.0± 2.17	26.2± 3.64	0.309	0.522	ns	ns
n-3	2.0± 0.28	1.5± 0.31	1.6± 0.27	1.7± 0.24	1.7± 0.27	1.7± 0.47	0.247	0.685	ns	ns
n-6	1.5± 0.19	2.1± 0.36	1.7± 0.52	2.3± 0.36	1.7± 0.39	1.5± 0.26	0.099	0.070	t	t
n6/n3	0.7± 0.09	1.4± 0.21	1.0± 0.22	1.4± 0.22	1.0± 0.21	1.0± 0.35	0.028	0.252	*	ns
EPADPADHA	0.35± 0.036	0.26± 0.053	0.28± 0.047	0.26± 0.037	0.31± 0.046	0.28± 0.076	0.078	0.442	t	ns

[a] Mean values for Farm (F) (A, B and C) or Round (R) (Early, Mid and Late) where there is a difference between means (P-values<0.05). ***: P< 0.001, **: P< 0.01; *: P< 0.05, t: P<0.1, ns: P>0.1. NA= Not applicable.

[b] SCC=Somatic Cell Count, EDMI= Estimated Dry Matter Intake, ECMY= Energy Corrected Milk Yield, ECMYP= Energy Corrected Milk Yield with Premium, EDMI/ECMYP= 'Production Efficiency' ration of Estimated Dry Matter Intake to Energy Corrected Milk Yield with Premium, C12:0=Lauric Acid, C14:0=Myristic Acid, C16:0=Palmitic Acid, VA= Vaccenic Acid, OA= Oleic Acid, LA= Linoleic Acid, ALA=alpha-linolenic acid, CLA9=Conjugated linoleic acid (C18:2, c9t11 isomer), SFA= Saturated Fatty Acid, PUFA=Poly-Unsaturated Fatty Acids, MUFA= Mono-Unsaturated Acids, n-3=omega-3, n-6=omega-6, n-6/n-3=, EPADPADHA=Eicosapentaenoic Acid +Docosapentaenoic Acid + Docosahexaenoic Acid, F= Farm, R= Round, SD= Standard Deviation.

Table 6.3 Mean eating, ruminating, health and fatty acid analysis ± SD by Farm (A, B, C) and Round combined (Early (E), Mid (M) and Late (L)). Fatty Acid results are expressed as a percentage of the total fatty acid profile

Farm	A[a]			B			C			P-val[b]	Sig
Stage of Lactation[c]	E	M	L	E	M	L	E	M	L		
n=	20	12	13	19	19	13	18	16	15		
Days in Milk	58± 7.4	139± 6.6	209± 5.6	36± 11.1	111± 11.3	194±11.9	59± 17.0	130± 16.4	215± 17.7	NA	NA
SCC (x 10^3 cells/ml milk)	115±209.3	88± 50.6	102±120.0	63± 71.8	48± 64.1	134±156.7	132± 297.1	58± 49.8	326± 671.6	0.313	ns
Eating and ruminating behaviour											
EDMI (kg/day)	19.1± 3.13	17.2± 2.58	13.5± 3.67	17.3± 1.97	19.0± 2.37	15.0± 2.33	23.7± 1.95	24.5± 2.82	18.2± 2.65	0.000	***
ECMY (L/day)	22.3± 5.37	14.5± 2.10	11.2± 3.11	25.6± 3.50	20.3± 3.25	17.1± 2.90	33.1± 4.84	32.6± 6.34	18.5± 5.22	0.000	***
EDMI/ECMY	0.89± 0.166	1.19± 0.133	1.25± 0.394	0.69± 0.099	0.95± 0.099	0.90± 0.164	0.73± 0.128	0.77± 0.141	1.04± 0.244	0.000	***
Yield (L/day)	18.2 ± 3.26	12.0 ± 1.55	9.3 ± 2.34	26.1 ± 2.93	20.3 ± 3.51	13.2 ± 2.12	31.1 ± 2.28	31.8 ± 4.80	17.8 ± 5.94	0.000	***
EDMI/Yield	1.1 ± 0.13	1.4 ± 0.18	1.5 ± 0.40	0.7 ± 0.08	0.9 ± 0.11	1.2 ± 0.21	0.8 ± 0.08	0.8 ± 0.10	1.1 ± 0.27	0.000	***
Eating Time (min/day)	583± 41.9	589± 64.5	455± 158.3	437± 47.9	574± 68.7	351± 48.9	544± 58.8	523± 71.5	535± 45.5	0.000	***
Rumination Time (min/day)	444± 36.0	432± 58.2	361± 99.9	405± 47.5	416± 36.8	492± 83.8	441± 63.6	512± 54.9	361± 47.2	0.000	***
Rumination Boluses (n/day)	562± 48.7	499± 78.8	416± 119.6	439± 49.6	485± 45.7	536± 87.5	518± 87.5	547± 56.7	389± 44.1	0.000	***
Chews per Bolus	48.6± 5.16	52.2± 7.13	48.5± 5.88	54.4± 5.08	55.3± 6.34	57.9± 7.29	52.3± 6.33	59.9± 5.60	52.6± 6.17	0.000	***
Eating Rate	0.03± 0.005	0.03± 0.004	0.03± 0.007	0.04± 0.005	0.03± 0.005	0.04± 0.005	0.04± 0.004	0.05± 0.004	0.03± 0.005	0.000	***
Rumination Rate	0.04± 0.007	0.04± 0.007	0.04± 0.019	0.04± 0.006	0.05± 0.006	0.03± 0.007	0.06± 0.019	0.05± 0.012	0.05± 0.011	0.000	***
Fat (%)	4.8 ± 1.09	4.7 ± 0.88	4.6 ± 0.74	3.3 ± 0.78	3.4 ± 0.64	5.3 ± 1.27	3.8 ± 1.18	3.6 ± 1.74	3.7 ± 1.21	0.000	***
Protein (%)	3.6 ± 0.20	3.6 ± 0.19	3.6 ± 0.17	3.0 ± 0.24	3.2 ± 0.29	3.9 ± 0.33	3.4 ± 0.21	3.3 ± 0.25	3.4 ± 0.24	0.000	***
Lactose (%)	4.6 ± 0.19	4.5 ± 0.07	4.5 ± 0.07	4.5 ± 0.19	4.6 ± 0.19	4.3 ± 0.20	4.6 ± 0.17	4.4 ± 0.14	4.5 ± 0.13	0.000	***
Premium Calculations											
n=	0	12	13	19	19	12	18	16	15		
ECMYP		15.0± 2.31	11.6± 3.27	25.7± 3.61	20.8± 3.39	17.3± 2.91	33.4± 4.93	33.1± 6.66	19.2± 5.53	0.000	***
EDMI/ECMYP		1.2± 0.14	1.2± 0.40	0.7± 0.10	0.9± 0.10	0.9± 0.17	0.7± 0.13	0.8± 0.15	1.0± 0.24	0.000	***

Table 6.3 continued. Mean eating, ruminating, health and fatty acid analysis ± SD by Farm (A, B, C) and Round combined (Early (E), Mid (M) and Late (L)). Fatty Acid results are expressed as a percentage of the total fatty acid profile

Farm		A[a]			B			C		P-val[b]	Sig
Stage of Lactation [c]	E	M	L	E	M	L	E	M	L		
Milk FA Concentration (%)											
C12:0		3.2± 0.279	3.8± 0.428	4.3± 1.091	4.6± 0.708	4.7± 0.793	4.5± 0.828	3.4± 0.400	3.7± 0.639	0.000	***
C14:0		11.4± 0.560	11.8± 0.759	11.3± 1.505	12.3± 1.119	12.8± 1.023	11.8± 1.358	12.1± 0.895	11.8± 1.138	0.000	***
C16:0		31.7± 2.50	31.2± 5.24	26.3± 1.46	26.7± 1.77	33.0± 2.24	26.4± 3.12	30.4± 3.00	28.6± 3.47	0.000	***
VA		1.7± 0.301	1.9± 0.496	3.4± 0.759	3.0± 0.790	1.8± 0.503	2.3± 0.985	1.7± 0.415	2.8± 0.677	0.000	***
OA		18.5± 1.36	17.8± 2.99	19.5± 2.59	17.5± 1.59	16.7± 1.75	19.2± 3.62	19.2± 2.26	20.0± 3.11	0.000	***
LA		0.98± 0.107	0.86± 0.144	1.81± 0.227	1.58± 0.247	1.19± 0.156	1.32± 0.215	0.92± 0.136	0.89± 0.175	0.000	***
ALA		0.96± 0.080	1.14± 0.205	0.75± 0.091	0.87± 0.174	0.58± 0.072	0.83± 0.128	0.66± 0.078	0.75± 0.104	0.000	***
CLA.9		0.72± 0.109	0.92± 0.189	1.10± 0.272	1.12± 0.222	0.97± 0.295	0.93± 0.409	0.79± 0.213	1.48± 0.455	0.000	***
SFA		69.5± 1.71	69.3± 3.68	66.5± 3.17	68.7± 2.24	71.5± 2.69	66.0± 4.74	69.2± 3.43	66.0± 4.28	0.000	***
PUFA		4.7± 0.405	5.1± 0.714	5.7± 0.527	5.6± 0.520	4.3± 0.519	6.0± 0.988	4.3± 0.727	5.0± 0.789	0.000	***
MUFA		25.8± 1.52	25.6± 3.14	27.8± 2.94	25.7± 1.91	24.2± 2.31	28.0± 4.11	26.5± 2.84	29.0± 3.71	0.000	***
n3		1.9± 0.161	2.2± 0.305	1.6± 0.173	1.8± 0.239	1.2± 0.142	1.8± 0.264	1.5± 0.224	1.6± 0.204	0.000	***
n6		1.5± 0.139	1.4± 0.226	2.3± 0.266	2.1± 0.282	1.7± 0.214	2.3± 0.435	1.4± 0.221	1.4± 0.216	0.000	***
n6/n3		0.79± 0.056	0.66± 0.047	1.46± 0.223	1.22± 0.164	1.44± 0.159	1.27± 0.183	0.95± 0.103	0.87± 0.100	0.000	***
EPADPADHA		0.35± 0.025	0.36± 0.044	0.25± 0.029	0.30± 0.044	0.21± 0.038	0.26± 0.043	0.29± 0.048	0.27± 0.046	0.000	***

[a] Farm= A, B or C. Round: E=Early, M=Mid, L=Late

[b] Mean values for Farm or Round where there is a difference between means (P-values<0.05). ***: P< 0.001, **: P< 0.01; *: P< 0.05, t: P<0.1, ns: P>0.1.

[c] SCC=Somatic Cell Count, EDMI= Estimated Dry Matter Intake, ECMY= Energy Corrected Milk Yield, ECMYP= Energy Corrected Milk Yield with Premium, EDMI/ECMYP= 'Production Efficiency' ration of Estimated Dry Matter Intake to Energy Corrected Milk Yield with Premium, C12:0=Lauric Acid, C14:0=Myristic Acid, C16:0=Palmitic Acid, VA= Vaccenic Acid, OA= Oleic Acid, LA= Linoleic Acid, ALA=alpha-linolenic acid, CLA9=Conjugated linoleic acid (C18:2, c9t11 isomer), SFA= Saturated Fatty Acid, PUFA=Poly-Unsaturated Fatty Acids, MUFA= Mono-Unsaturated Acids, n-3=omega-3, n-6=omega-6, n-6/n-3=, EPADPADHA=Eicosapentaenoic Acid +Docosapentaenoic Acid + Docosahexaenoic Acid.

Table 6.4 Cows allocated by production efficiency (based on EDMI/ECMY); One is the most efficient and Five the least, combining data from all 3 farms over the 3 dates

	One	Two	Three	Four	Five	p-val [a]	sig
n=	32	27	29	27	31		
Days in Milk	120 ± 66.8	114 ± 64.5	121 ± 68.3	116 ± 65.8	123 ± 65.0	0.443	ns
SCC[b] ($x10^3$ cells/ml milk)	162 ± 371.7	142 ± 358.8	94 ± 165.7	73 ± 112.7	101 ± 211.2	0.135	ns
Eating and Ruminating Behaviour							
EDMI (kg/day)	17.8 ± 4.78	19.6 ± 3.68	19.0 ± 4.30	18.8 ± 3.93	19.6 ± 4.06	0.097	*
ECMY (L/day)	26.0 ± 9.60	25.1 ± 7.30	22.1 ± 8.16	20.2 ± 6.31	18.5 ± 6.61	0.000	***
EDMI/ECMY	0.72 ± 0.156	0.82 ± 0.172	0.92 ± 0.194	0.97 ± 0.178	1.14 ± 0.306	0.000	***
Yield (L/day)	21.6 ± 8.31	23.2 ± 7.77	20.2 ± 8.44	20.1 ± 7.79	19.0 ± 8.48	0.000	***
EDMI/Yield	0.88 ± 0.216	0.91 ± 0.248	1.05 ± 0.306	1.03 ± 0.301	1.18 ± 0.405	0.000	***
Eating Time (min/day)	487 ± 112.0	514 ± 90.9	519 ± 109.8	518 ± 96.6	539 ± 88.5	0.081	t
Rumination Time (min/day)	416 ± 79.9	445 ± 61.3	431 ± 89.9	432 ± 63.1	430 ± 73.6	0.331	ns
Rumination Boluses (n/day)	470 ± 87.5	504 ± 72.7	502 ± 115.9	498 ± 80.6	486 ± 85.5	0.201	ns
Chews per bolus	51.4 ± 6.70	55.0 ± 6.27	53.0 ± 8.13	53.1 ± 6.50	55.0 ± 6.25	0.294	ns
Eating Rate	0.04 ± 0.008	0.04 ± 0.007	0.04 ± 0.008	0.04 ± 0.008	0.04 ± 0.007	0.595	ns
Rumination rate	0.04 ± 0.009	0.05 ± 0.009	0.05 ± 0.014	0.05 ± 0.012	0.05 ± 0.019	0.492	ns
Fat (%)	4.7 ± 1.29	4.0 ± 0.83	4.1 ± 1.34	3.5 ± 1.18	3.3 ± 1.10	0.000	***
Protein (%)	3.4 ± 0.42	3.3 ± 0.29	3.4 ± 0.36	3.4 ± 0.32	3.4 ± 0.29	0.187	ns
Lactose (%)	4.5 ± 0.16	4.5 ± 0.13	4.5 ± 0.15	4.5 ± 0.26	4.5 ± 0.16	0.916	ns
n=	28	23	24	23	27		
ECMYP (kg)	26.1 ± 10.21	25.5 ± 7.74	23.0 ± 8.77	20.8 ± 6.60	18.8 ± 6.99	0.000	***
EDMI/ECMYP	0.71 ± 0.156	0.80 ± 0.173	0.90 ± 0.200	0.96 ± 0.175	1.13 ± 0.318	0.000	***

Table 6.4 continued. Cows allocated by production efficiency (based on EDMI/ECMY); One is the most efficient and Five the least, combining data from all 3 farms over the 3 dates

	One	Two	Three	Four	Five	p-val [a]	sig
n=	32	27	29	27	31		
Milk FA Concentration (%)							
C12:0	4.1 ± 0.83	4.0 ± 0.78	3.9 ± 0.59	4.0 ± 0.90	4.0 ± 0.92	0.875	ns
C14:0	12.0 ± 1.26	11.8 ± 0.93	11.8 ± 1.02	11.9 ± 1.33	11.7 ± 1.25	0.903	ns
C16:0	29.9 ± 4.00	28.4 ± 3.70	28.7 ± 3.55	27.9 ± 3.61	28.8 ± 4.77	0.384	ns
VA	2.3 ± 0.83	2.5 ± 0.98	2.4 ± 0.99	2.4 ± 1.08	2.5 ± 0.90	0.953	ns
OA	17.8 ± 2.68	19.2 ± 2.28	18.8 ± 2.15	19.5 ± 3.47	18.7 ± 3.22	0.210	ns
LA	1.2 ± 0.38	1.3 ± 0.34	1.2 ± 0.36	1.3 ± 0.46	1.3 ± 0.43	0.474	ns
ALA	0.77 ± 0.233	0.83 ± 0.179	0.87 ± 0.218	0.81 ± 0.196	0.82 ± 0.201	0.562	ns
CLA.9	0.90 ± 0.256	1.11 ± 0.371	1.00 ± 0.462	1.04 ± 0.483	1.07 ± 0.279	0.312	ns
SFA	69.7 ± 3.69	67.0 ± 3.53	68.0 ± 3.09	67.0 ± 4.86	67.5 ± 4.29	0.062	t
PUFA	4.8 ± 0.91	5.5 ± 0.81	5.2 ± 0.90	5.3 ± 1.09	5.4 ± 0.89	0.011	*
MUFA	25.6 ± 3.04	27.5 ± 2.93	26.8 ± 2.67	27.7 ± 4.19	27.0 ± 3.67	0.149	ns
n3	1.6 ± 0.37	1.7 ± 0.33	1.8 ± 0.37	1.7 ± 0.34	1.8 ± 0.36	0.279	ns
n6	1.7 ± 0.46	1.9 ± 0.38	1.8 ± 0.43	1.9 ± 0.56	1.9 ± 0.58	0.050	*
n6/n3	1.1 ± 0.31	1.1 ± 0.28	1.0 ± 0.30	1.1 ± 0.33	1.1 ± 0.38	0.642	ns
EPADPADHA	0.26 ± 0.058	0.28 ± 0.048	0.30 ± 0.065	0.27 ± 0.060	0.30 ± 0.058	0.058	t

[a] Mean values for PE where there is a difference between means (P-values<0.05). ***: P< 0.001, **: P< 0.01; *: P< 0.05, t: P<0.1, ns: P>0.1.

[b] SCC=Somatic Cell Count, EDMI= Estimated Dry Matter Intake, ECMY= Energy Corrected Milk Yield, ECMYP= Energy Corrected Milk Yield with Premium, EDMI/ECMYP= 'Production Efficiency' ratio of Estimated Dry Matter Intake to Energy Corrected Milk Yield with Premium, C12:0=Lauric Acid, C14:0=Myristic Acid, C16:0=Palmitic Acid, VA= Vaccenic Acid, OA= Oleic Acid, LA= Linoleic Acid, ALA=alpha-linolenic acid, CLA9=Conjugated linoleic acid (C18:2, c9t11 isomer), SFA= Saturated Fatty Acid, PUFA=Poly-Unsaturated Fatty Acids, MUFA= Mono-Unsaturated Acids, n-3=omega-3, n-6=omega-6, n-6/n-3=, EPADPADHA=Eicosapentaenoic Acid +Docosapentaenoic Acid + Docosahexaenoic Acid.

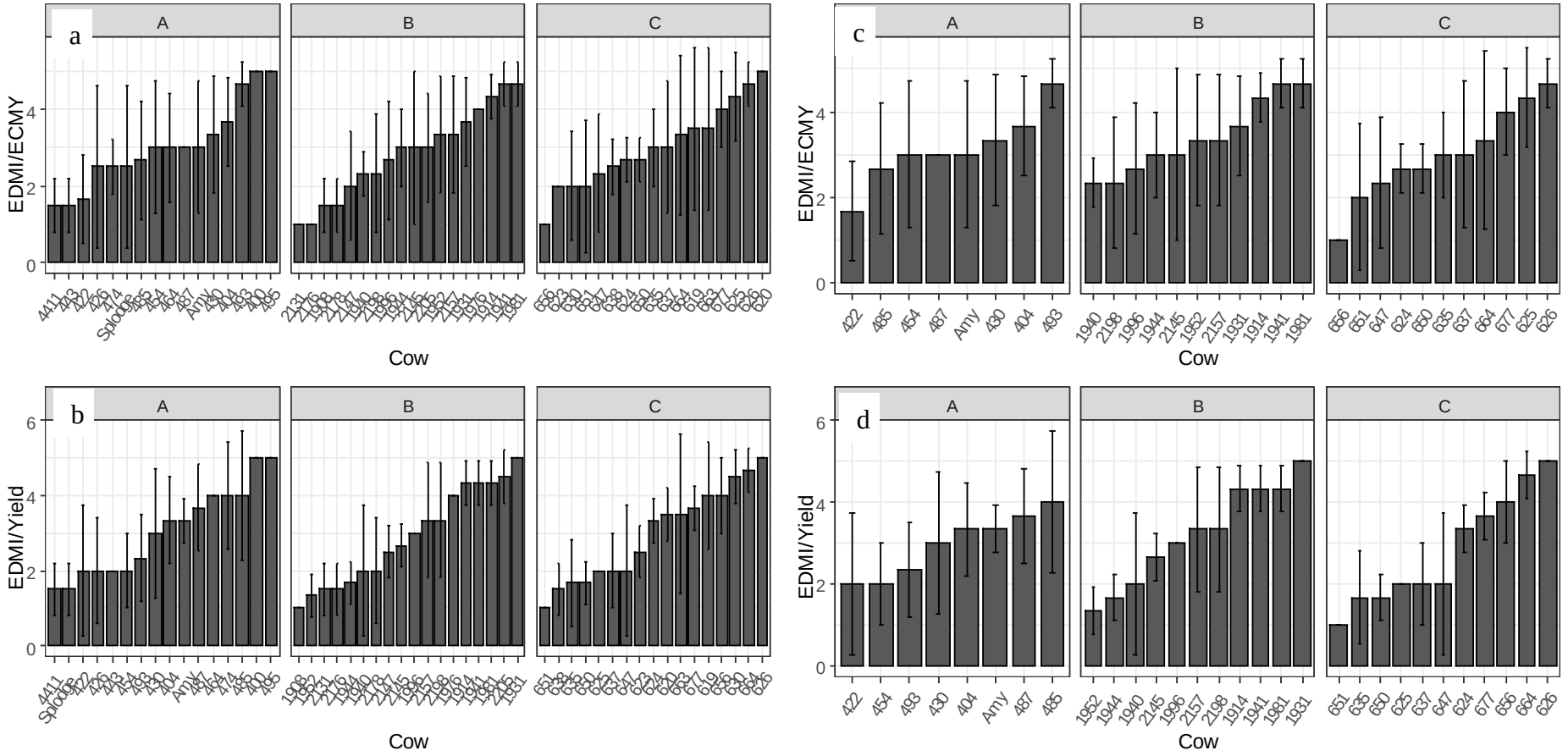


Figure 6.3. a The average efficiency of cows that were present for two stages of lactation, on each Farm (A, B and C) where PE is EDMI/ECMY and b. where PE is EDMI/Yield. c. The average efficiency of cows that were present for all three stages of lactation where PE is EDMI/ECMY and d. where PE is EDMI/Yield.

6.3.4 EATING BEHAVIOUR

There were no significant differences between the Farms or Rounds for time spent eating or ruminating (p>0.1), although observationally both eating and ruminating time decreased from early to late lactation (p=0.206). There was no difference in the number of rumination boluses between the Farms or Rounds, but cows on Farm A tended to chew less per bolus (49.5 chews/bolus) compared to those on Farm B (55.6 chews/ bolus) and C (54.9 chews/bolus) (p=0.090) (Table 6.2).

The time spent eating on all farms decreased from early to late lactation. Both Farms A and B had a small increase from cows in early (A: 583, B: 437 min/day) and mid-lactation (A:589, B:574 min/day), then dramatically decreased between mid- to late-lactation (A:455, B:351 min/day) (p=0.000) (both fell by over 130 minutes/cow/day or 30-60%). Whereas cows from Farm C decreased eating time from early-mid lactation then increased in eating time from mid-late lactation (E:544, M:523, L:535 minutes/cow/day). Conversely, Farm A had a steady decline throughout lactation (E:544, M:523, L:535 min/day) and did not have a sudden drop during late lactation. During late-lactation, cows from Farm C were spending a lot more time eating than Farm A and B (p=0.000) (Table 6.3).

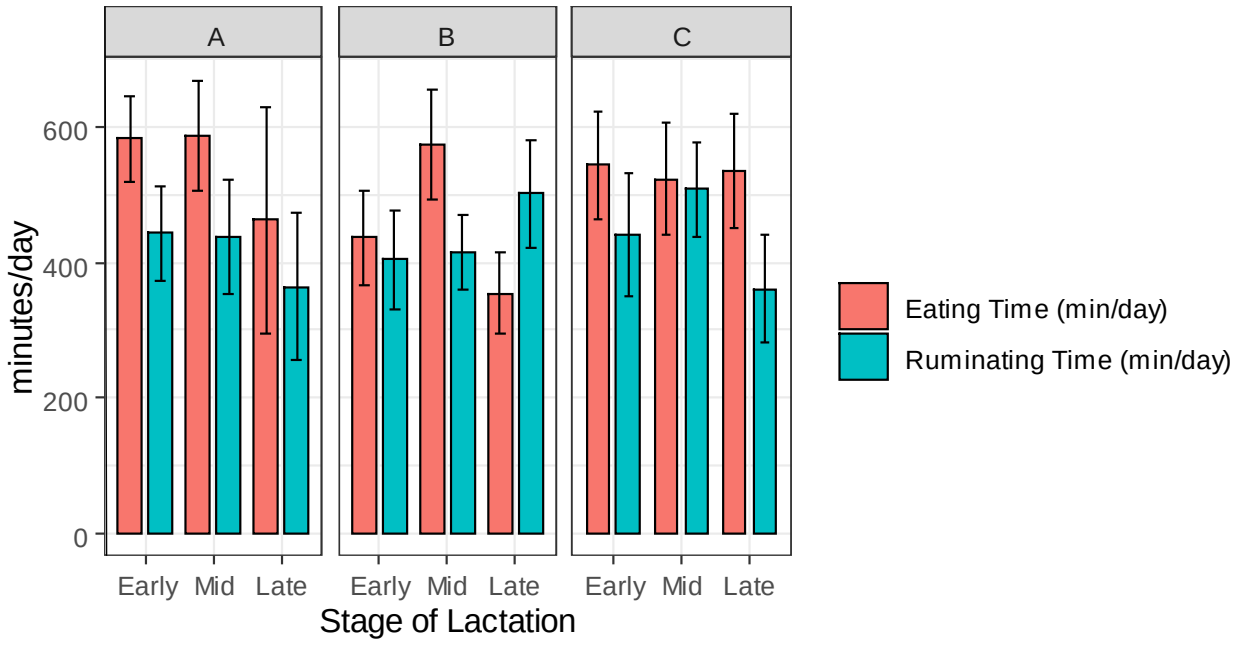


Figure 6.4 Mean time (± SD) spent eating and ruminating on each Farm (A, B, C) at each stage of lactation (Early, Mid and Late)

The pattern throughout lactation for daily ruminating time differed between farms; cows on Farm A decreased by 19% (E:444, M:432, L:361 min/day), on Farm B increased by 21% (E:405, M:416, L:492 min/day) and on Farm C increased by 16% then decreased by 30%

(E:441, M:512, L:361 min/day) throughout lactation (p=0.000). The number of boluses produced followed the same pattern as rumination time on all farms (p=0.000) (Table 6.3).

In terms of PE, Group One cows tended to spend least time eating (487min/day) whilst Group Five spent the most time eating (539min/day) (p=0.081). Additionally, although not significantly different, Group Five cows spent more time ruminating each day (3.4%) (p=0.331), produced more rumination boluses (3.4%) (p=0.201) and chewed more per bolus (7.8%) (p=0.294) than Group One (Table 6.4).

6.3.5 MILK QUALITY

There was no difference in the milk protein, fat or lactose content between the Farms and Rounds (p>0.2). Linoleic acid (LA) concentration in the milk was highest on Farm B (1.5%) and lowest on Farm A (0.9%) (p=0.016). LA was highest during early lactation (1.6%) and lowest in late lactation (1.0%) (p=0.039). Observationally (but not significant), cows on Farm A had the highest concentration of α-Linolenic Acid (ALA) (1.1%) compared to those on B (0.7%) and C (0.8%) (p=0.166). The ratio of n-6/n-3 was lowest in milk on farm A (0.7) and highest on farm B (1.4) (p=0.028) (Table 6.2).

Milk fat concentration was highest on Farm A during early (4.8%) and mid- lactation (4.7%) and on Farm B during late lactation (5.3%). Fat percentage was consistent on Farms A (~4.7%) and C (~3.7%) but on Farm B fat was lowest during early-(3.3%) and mid-(3.4%) lactation then dramatically increased during late lactation (p=0.000). Similarly, milk protein content was highest on Farm A at early (3.6%) and mid- lactation (3.6%) and on Farm B at late-lactation (3.9%) and followed the same pattern as milk fat (p=0.000) (Table 6.3).

Milk FA data were absent for Farm A during early lactation. Linoleic Acid decreased throughout lactation on all farms, while α-Linolenic acid increased on Farm A (M:0.96%, L:1.14%), increased then decreased on Farm B (E:0.75%, M:0.85%, L:0.58%) and decreased then increased on Farm C (E:0.83%, M:0.66%, L:0.75%) (p=0.00) (Table 6.3).

The most notable significant difference (p=0.00) was that the most efficient cows in group One had the highest average milk fat concentration (4.7%) and the lowest concentration was in group Five (3.3%). Additionally, the most efficient group had the highest concentration (marginally, p>0.3) of all relevant individual and total SFAs (C12:0: 4.1%, C14:0: 12.0%, C16:0: 29.9% and overall SFA: 69.7%). The most efficient group had the lowest concentration of Oleic Acid (17.8%) (p=0.210) and the lowest overall MUFA (25.6%) (p=0.149); they also had the lowest

concentration of n-3 (1.6%, p=0.279), n-6 (1.7%, p=0.050) PUFA (4.8%, p=0.011) and the very long chain fatty acids EPA+DPA+DHA (0.26%, p=0.058) (Table 6.4).

When efficiency is measured as EDMI/Yield (Appendix D, Table D.2) the least efficient group (Five), had the highest concentration of fat (4.5%, p=0.035) and protein (3.5%, p=0.000). Group Five also had the highest concentration of C16:0 (30.5%, p=0.083) and the lowest concentration of oleic acid (17.5%, p=0.079).

The number of cows that met the milk quality criteria to receive the Organic Valley Grassmilk^{TM} premium were highest on Farm A (85% in mid and late lactation) whereas, Farm B peaked mid lactation (E-10%, M-52%, L-0%) and Farm C peaked at the end of lactation (E-17%, 31%, 87%) (Table 6.5).

Table 6.5 Number of cows meeting milk quality criteria under Organic Valley Grassmilk^{TM} Standards to receive a premium by Farm (A, B, C) and Round combined (Early (E), Mid (M) and Late (L).

Farm	A		B			C		
Lactation stage	M	L	E	M	L	E	M	L
n	13	13	19	19	12	18	16	15
No	2	2	17	9	12	15	11	2
	11	11	2	10	0	3	5	13

6.4 DISCUSSION

This study collected valuable information on milk quality and animal eating and ruminating behaviour, and thus, production efficiency from three pasture-based farms. There is missing and incomplete data that has resulted in a less statistically robust study than hoped. The resulting incomplete dataset was beyond the control of this study, due to labs losing milk samples, halter hardware failing, cows unwilling to be measured and farmers unable to milk record or catch the correct cows. Despite this, the data collected offers valuable insights into dairy cows/farming under PB management.

6.4.1 DRY MATTER INTAKE

Estimated dry matter intake was calculated using data collected by RumiWatch halters, and modelled using an equation generated by Guilherme Amorim Franchi (2017). Whilst the RW halters have been validated to estimate DMI (Ruuska *et al.*, 2016; Zehner *et al.*, 2017; Werner *et al.*, 2018a; Werner *et al.*, 2018b) and measurements were carefully taken (consistent in data recording approach; keeping cows calm and ensuring no difference in recording practice between cows and farm), the specific model used has not been widely validated, leaving

potential for human and technical errors. Despite this, EDMI, time spent eating and ruminating were comparable to another grazing-based study (Rombach *et al.*, 2019).

Dry matter intake can be influenced by numerous factors including; diet quality, digestibility, supplementation and availability, sward density and composition, size and breed of the cows, stage of lactation, milking frequency, weather, stress and illness (Kertz *et al.*, 1991; Holmes *et al.*, 1992; Delaby *et al.*, 2001; Aikman *et al.*, 2008; Oudshoorn *et al.*, 2013; Yadav *et al.*, 2013; Rombach *et al.*, 2019; Munksgaard *et al.*, 2020). Some of the differences in DMI in this study can be attributed to the milking frequency, size and breed of the cows. Farm A milks once daily, possibly resulting in the cows having more time to graze, but because there is less pressure for milk production on these cows, they have a lower DMI (Holmes *et al.*, 1992). However, Farms A and B had a very similar EDMI (A:17.0kg/day, B:17.4kg/day), suggesting that milking frequency did not affect recorded EDMI. Notably, early in lactation average days in milk for Farm B was only 36 days, compared to Farm A's 58 days and these extra twelve days at this stage of lactation could have been when Farm B's EDMI was still increasing post calving, whilst cows on Farm A were nearer peak intake (around 28-70 DIM (Kertz *et al.*, 1991)). This indicates that there could be more of a difference in DMI, especially early in lactation, between Farm A and B than captured in this study. Typically, the larger the cow the bigger the rumen and the more feed it requires for maintenance. Farm C had predominantly HF and New Zealand Friesian (NZF) with an average LW of 635kg/cow, whereas Farm A had mostly Jersey crosses and Farm C had a mixture of British Friesian crosses, Dairy Shorthorn crosses and others. These much smaller breeds result in an average LW of 534kg/cow on Farm A and 539kg/cow on Farm B. Given the more intensive, larger breeds on Farm C the increased EDMI is easily explained.

The energy balance of a dairy cow changes through lactation and gestation, which makes predicting DMI from energy output in milk complicated, especially in a PB system where the metabolisable energy (ME), fibre and crude protein content of the sward will also vary across the season (Holden *et al.*, 1994; Marshall *et al.*, 1998). It is well recognised that DMI changes throughout lactation; after the four to ten-week increase in DMI from calving, studies have found DMI increases, decreases and/or plateaus before tapering off at the very end of lactation, which can vary by system intensity and quality of pasture and/or total mixed ration (TMR) (Kertz *et al.*, 1991; Holden *et al.*, 1994; Friggens *et al.*, 1998; Faverdin *et al.*, 2011; Munksgaard *et al.*, 2020). In this study, cows had access to a diverse (especially Farms A and B) fresh pasture every twelve hours, but during mid lactation on Farm C limited pasture allowance was supplemented with hay (further discussed in the below Section 6.4.4), which could have resulted in longer eating time and thus, a higher EDMI (as eating time is a function within the

EDMI model). From the 3 isolated measurements in this RumiWatch study, EDMI dropped on all farms from early to late lactation (Farm A: 9%, B: 13%, C: 23%), but cows on Farms B and C had a small increase from early to mid- lactation (this is visualised alongside yield in Figure 6.2). These differences could indicate that cows on Farms B and C had not reached peak DMI during the early lactation sampling and/or Farm C's increase could be due to the supplementary hay, or perhaps the pattern of change through lactation (especially in PB systems) is not yet understood. More research is needed to understand these fluctuations and determine if they are important for production and efficiency.

6.4.2 MILK YIELD

In PB systems, milk yield is driven by DMI but limited by the quality of the diet. The predominant limiting factor affecting yield in PM systems is metabolisable energy (ME) (Kolver, 2003). Modelling work suggests that for 500-600kg cows grazing good quality pasture (ME density of pasture: 11.5MJ/kg DM), consuming 17-19kg DM/day should support cows producing 25L milk/day, assuming no loss in body weight (Kolver, 2003). Liquid yield (27.3L/day) and EDMI (22.3kg DM/day) achieved were slightly above this example with the bigger cows on Farm C, but in this study, abundant, good quality pasture (ME and other constituents, e.g. fibre, protein, etc. (not measured)) was available 24/7. Despite the lack of measurements confirming the quality of the pasture, on all farms cows were allocated fresh pasture every 12 hours and pastures were never overgrazed (residual cover >1600kg DM/ ha) indicating that DM supply from pasture was probably not a limiting factor for milk yield. But, if yield were a priority on these PB farms, supplementing pasture with concentrate could increase yield.

As expected, ECMY fell consistently from early to late lactation (Farm A: 50%, B: 33%, C: 44%). Farm B's smaller decrease in EDMI is reflected in the smaller decrease in ECMY, suggesting that cows on Farm B had greater within-herd persistency (rate of yield decline during lactation). Alternatively, the smaller drop in yield and intake could indicate that cows on Farm B had not reached peak yield during early lactation sampling (36 days) and typically, peak yield is 4-8 weeks after calving (Webster, 2020). If Farm B's yield increased after the early lactation sampling period, the reduction from early to late lactation would be greater. When liquid milk yield is observed next to ECMY (Figure 6.2), Farm A's liquid milk yield from predominantly Jersey cross cows was consistently increased by correcting for fat and protein (ECMY), because average fat content was above 3.5% and 3.2% protein, which was consistent across lactation. This is consistent with other studies that have found once a day milking results in a higher

concentration of fat and protein in milk, but fat and protein yield is less than in twice a day milking (Davis *et al.*, 1999; Clark *et al.*, 2006).

Feeding strategy impacts peak lactation and the shape of the lactation curve. There is evidence that in more intensive feeding systems, even with grazing, the lactation curve has a more pronounced peak and tail (Horan *et al.*, 2005). Whereas, in a PB system, cows are typically given a flat rate of feeding (Hills *et al.*, 2015) so their lactation curve tends to be flatter with a greater persistency. However, as discussed before, if Farm A and C were not at peak lactation during the early lactation round, the differences between stages of lactation may appear less pronounced than reality. The large decrease in ECMY on Farm A supports findings from other research that once a day milking results in lower persistence (Hickson *et al.*, 2006; Dobson *et al.*, 2007). Overall, EDMI and ECMY follow the expected pattern throughout lactation on all three farms, yet there are clear differences between the farms.

6.4.3 EFFICIENCY

Feed conversion in the dairy industry has dramatically improved, mainly due to milk yield per cow doubling over the last 40 years (Oltenacu and Broom, 2010). Feeding less per animal whilst maintaining production traits improves the economic and environmental sustainability of dairy farming by producing less potentially polluting nutrient loss and greenhouse gas emissions (Hurley *et al.*, 2017). However, feed conversion efficiency is often left behind in breeding objectives and forage conversion efficiency is completely neglected. This could, in part, be due to the myriad of traits already involved in breeding objectives, the complicated genetic variability of those associated with feed efficiency and the fact they also vary throughout lactation (Hurley *et al.*, 2017).

Production/feed efficiency is both complicated to define and influenced by many different factors. In this study, PE was defined as EDMI/ECMY, additionally EDMI/liquid milk yield and EDMI/ECMYP (the nutritionally enhanced milk) were calculated for comparison. Other studies have calculated PE similarly (Table 6.6), whilst others have used net energy intake in place of EDMI or DMI/100kg LW (Prendiville *et al.*, 2011; Hurley *et al.*, 2017; Al-Marashdeh *et al.*, 2020) (and discussed in Chapter 2, Section 2.2). The various methods of determining efficiency all have related genetic traits, whose heritability varies across lactation (Hurley *et al.*, 2017). However, the interactions of efficiency with reproduction and health traits are still unquantified, further complicating these relationships and definitions. The composition of the swards was not measured in this study, so the effect of energy consumed on efficiency is unknown. However, pasture quality (i.e. ME, protein and trace element supply and digestibility)

along with how energy is used (mobilising or adding to reserves, pregnancy, activity, etc.) will influence PE (Kolver, 2003; Doyle *et al.*, 2006; Al-Marashdeh *et al.*, 2020). If cows are not getting the correct nutrient and trace element supply, this could affect how and if they are able synthesise milk. Additionally, efficient cows will synthesise milk rather than gaining bodily reserves. The greater the cows ability to synthesise milk the more feed they will voluntarily consume, therefore increased nutrient consumption results from increasing production (Baumgard *et al.*, 2017). Whilst nutrient accessibility could have influenced milk synthesis and thus PE on these farms, the farmers here are satisfied with their management strategies and were confident that their swards were meeting the nutritional demands of the cows they selected to suit their herds and management.

Production efficiency on Farm A was highest (EDMI/ ECMY=1.07) whilst Farms B and C had the same efficiency (0.84). Farm B had a lower ECMY than Farm C, but the cows appeared to be just as efficient at converting pasture to milk, because they had the same level of efficiency. Despite the similar PE of Farms B and C there are clear differences between their systems including the health, fertility, sward mixture and the breed choice. These differences in management and cow breed give insight into cow performance within the two systems, suggesting that cows from Farm B were well suited to their system.

Feed conversion was examined more closely by assigning PE to individual cows (Table 6.4); the most efficient cows from each farm were given a score of 'One'. Despite individual management style throughout lactation on the 3 farms having the biggest impact on most of the variables measured and estimated (Table 6.2), there is evidence that the most efficient cows were consistent throughout lactation, whether using ECMY or actual recorded liquid yield (Figure 6.3), suggesting the potential to select for efficiency in PB systems. There was one cow (ID:656) from Farm C who had the best efficiency score during all three stages of lactation (Group One), no health or fertility treatments and held to her first insemination. However, the trade-off associated with selecting for traits that are a ratio of two component traits (EDMI:ECMY, for example) can have unexpected consequences (Gunsett, 1984). There is a strong negative correlation between perceived efficiency and body condition score and live weight (Vallimont *et al.*, 2011) and selecting for yield negatively impacts many health, fitness and fertility traits (Collard *et al.*, 2000). However, the studies that have identified trait correlations with feed efficiency have predominantly been in intensive, Holstein Friesian based systems, and breed can effect heritability and variability within and between traits (Muuttoranta *et al.*, 2019). Despite the negative correlations highlighting the difficulties of selecting for feed efficiency, paying attention to changes throughout lactation shows potential for selecting these

traits without negative consequences (Connor, 2015). This suggests that, with caution, there is scope to select for forage efficiency in PB systems.

Production efficiency in this study ranged from 0.69 to 1.25, which is slightly poorer than other studies (Table 6.6). However, this study included a once a day milking farm (A), which are typically less feed efficient than twice a day milking systems (Hickson *et al.*, 2006) and no other study cited had more than 70% forage in the dairy diets. This suggests that despite many of these studies (Table 6.6) claiming to be forage/grazing based, they are managed in a way that still prioritises yield, which is not the primary goal for the participant farmers in the current study, making direct PE comparisons unfair.

6.4.4 EATING AND RUMINATING BEHAVIOUR

Access to grazing, sward composition and digestibility (Mangwe *et al.*, 2018; Mangwe *et al.*, 2020a), weather and stress (e.g. illness) (Yadav *et al.*, 2013), oestrus (Reith and Hoy, 2012), parity, size, breed, DMI, stage of lactation and yield (Aikman *et al.*, 2008; Watt *et al.*, 2015) can all impact eating and ruminating time and behaviour. Lactating dairy cows are reported to spend approximately 270 minutes/day eating (range: 144–510 minutes/day) and 420 minutes/day ruminating (range: 150–630 minutes/day) (Beauchemin, 2018). Cows in this study spent more time eating than expected (351-589 minutes per day) but were in the expected range for ruminating (361-512 minutes per day) (Table 6.3). However, eating and ruminating time in this study are similar to that of other studies using RW halters (Leiber *et al.*, 2016; Werner *et al.*, 2019). Across all stages of lactation there was no difference in the behaviours between the farms, but when broken down by stage of lactation and farm, the differences in eating and ruminating behaviour become much clearer. All farms had a different pattern of eating and ruminating behaviour throughout lactation (Figure 6.4), yet all cows were in their second or third lactation and had 24/7 access to pasture (other than milking time).

There is some evidence that sward composition can influence grazing behaviour, with cows grazing a sward containing plantain and chicory spending more time grazing than cows grazing rye-grass and white clover swards (Mangwe *et al.*, 2018). Farms A and B had a very diverse sward with many herbs and minor grass varieties (including chicory, plantain, lucerne and many more), both over sown and through natural sward persistence, whereas Farm C had a more mainstream composition of perennial rye grass and clover mix, suggesting that some differences in eating and ruminating behaviour could be attributed to sward diversity. Despite similarities in sward and grazing strategy, Farm A and B had the opposite patterns in eating and ruminating behaviour (Farm A cows spent less time eating and ruminating as lactation

progressed, whereas Farm B cows increased time spent ruminating, but increased then decreased their time spent eating early- to mid- to late lactation (Figure 6.4)). This confirms there is more to eating and ruminating behaviour than differences in sward composition.

Cows from Farm C were, on average 100kg heavier than the cows on Farms A and B (Table 6.1) and predominantly had HF and NZ genetics, whereas Farm A was mostly Jersey crosses and Farm B had a wide range of smaller breeds (Appendix D, Table D.3). There is some evidence that Holsteins spend more time ruminating than Jerseys (Aikman *et al.*, 2008; Prendiville *et al.*, 2010), which is not supported by this study. Additionally, cows milked once daily are expected to spend more time eating, as there is less disruption to their natural grazing behaviour, but have a similar ruminating time to cows milked twice daily (O'Driscoll *et al.*, 2010). Again, the lactation average showed no difference in eating and ruminating time between the farms; it is examining the within lactation behaviours that expose the differences, and most of the studies cited only take one stage of lactation into consideration. There is very little evidence in the literature that explain the differences (shown in Figure 6.4) within farms across the different stages of lactation.

A major factor that can affect grazing and rumination are extremes in the weather (Yadav *et al.*, 2013). During July 2018 (mid-lactation) at Farm C, the weather was very warm for the UK and in a prolonged incredibly dry spell. The Midlands average temperature was 19°C with only 11.5mm rainfall in June and 30.8mm rainfall in July, considerably less rainfall than previous years (Met Office, 2020). It was so dry that the farmer reported having to supplement cows with hay due to a lack of grass growth. Grass growth rates in 2018 were around a third of 2019 rates during July (21kg DM/ha, 2018 compared to 64kg DM/ha, 2019 (AHDB, 2020c). Additionally, 2018 weather data from Shrewsbury (9 miles from Farm C) indicated that five days out of the two-weeks that halters were worn saw temperatures above average (25°C) and all other days were above 21°C (TimeandDate, 2018). Cows are prone to heat stress when the ambient air temperature is above 25-26°C (Kadzere *et al.*, 2002) and no other halter rounds experienced this heat, suggesting that cows during mid-lactation on Farm C could have been subject to heat stress at the same time as limited grass availability. This could be why eating and ruminating behaviour was so different on Farm C compared to A and B. However, it does not explain why the cows spent so much time ruminating during mid-lactation, as heat stress would usually result in less time spent ruminating and depress DMI (Yadav *et al.*, 2013). Grass has a higher moisture content and generally shorter particle size than hay (which is stemmier), therefore hay requires more chewing than fresh, leafy grass/ clover, so the supplementary hay probably increased both eating and ruminating time (Beauchemin *et al.*, 2008). There is a possibility that

the halters mistook lying behaviour in the absence of grazing due to a lack of grass, for ruminating, but the number of ruminating boluses produced by Farm C during mid-lactation is consistent with the rumination time. Perhaps the sward was of poorer quality towards the end of lactation and cows needed to spend more time eating to get the desired nutrition, or, as most were HF, they may have lost condition through lactation and continued eating to regain condition.

There are many reasons that explain why eating and ruminating behaviours in PB cattle vary from farm to farm, but what is evident from this research and discussed by Ben Meir *et al.* (2019) is that there is a lack of evidence identifying 'normal' behaviour and explanations for differences. Given the emphasis on feed intake and conversion in the intensive dairy industry, there is an expectation that the behaviours throughout lactation are well understood, yet this is not the case for alternative, less studied systems. Additional research and resources should be allocated to investigate grazing and ruminating behaviours in PB dairy systems to fully understand the effect on herbage intake and efficiency.

6.4.5 FORAGE

The strategic grazing management system (as discussed in Chapter 2, Section 2.5) often selected by PB farmers uses 'tall grass' or 'mob' grazing, which includes a longer rotation than typical paddock/cell grazing (50-60 rather than 20-40 days), with a higher stocking density and leaving more un-grazed vegetation (a higher residual). This is a popular method on some farms in the USA and is thought to improve soil carbon, water- and nutrient-holding capacity, vegetation composition, nutritive value and biomass compared to continuous grazing under set-stocking (Müller *et al.*, 2007; Teague *et al.*, 2011; Tracy and Bauer, 2019; Billman *et al.*, 2020). The grazing strategy implemented on all farms in this study was similar. Farm C used a paddock/cell grazing system but Farms A and B use a 'mob' or 'tall grass' grazing system, although Farm B describes their system as strip grazing with a back fence. Farm C had a lower average DM cover (approximately 2300kg DM/ha) when cows went into paddocks, compared to Farms A and B (approximately 2800kg DM/ha), but all Farms left a residual of around 1600kg DM/ha (Table 6.1). These DM covers are above the recommended pre (2000-2500kg DM/ha) and post grazing (1250-1500kg DM/ha) amounts for intensive grazing management (AHDB, 2018b), confirming these farmers commitment to taller grass grazing. Despite growing interest in mob grazing amongst PB farmers, other than a single case-study (Leach *et al.*, 2014) there has been very little farm-scale scientific research into its benefits on UK land (Leach *et*

al., 2014). These gaps in knowledge expose opportunities for research into best practice in vegetation management, which could go hand in hand with identifying the most suitable cows.

The proportion of cows that met the criteria for milk quality premium (39mg total omega-3/100g milk, 26.6mg total conjugated linoleic acid/100g milk, omega 6: omega 3 ratio less than 1.2 (Benbrook *et al.*, 2018)) varied throughout lactation on Farms B and C but was more consistent on Farm A, although results from early lactation were missing due to loss of milk samples. The higher proportion of cows meeting the requirements during mid-lactation may reflect their diverse pastures that were better able to withstand drought conditions of summer 2018, whereas Farm C (with predominantly ryegrass/clover swards) resorted to supplementing with hay as vegetation growth and cover was poor. However, 87% of monitored cows on Farm C meet the requirements during late lactation, and no cows on Farm B. This again could be a result of the sward diversity, yet the sward on Farm B was similar to Farm A, where 85% of cows qualified for the premium. It is surprising that no cows met the milk quality premium requirements during late lactation on Farm B (whilst over 50% of cows met premium requirements during mid lactation), but average fat (5.3%) and protein (3.9%) concentration were highest (across all farms and stages of lactation) on Farm B during late lactation. This combination could indicate that later in the growing season (September) there had been a long regrowth period, which could reduce omega-3 and CLA9 in milk (Elgersma *et al.*, 2006), whilst creating a more fibrous sward which could increase fat and protein concentration (Oba and Allen, 1999). Whilst this speculation may not reflect the true reasons for these differences, it highlights the seasonal variability in forage and hence milk quality.

Access to forage and different varieties of vegetation has an effect on FA profile (Elgersma *et al.*, 2006) and many studies have looked at the FA profile in milk from various swards (Cabiddu *et al.*, 2009; Elgersma *et al.*, 2013). Additionally, there is some evidence that swards including chicory and/or plantain increases omega-3 in milk and swards with rye-grass and white clover increase CLA (Mangwe *et al.*, 2020b). There is also evidence that swards including clover produce milk with a higher concentration of omega-3, whilst swards grown with nitrogen fertiliser increase CLA9 in milk (Butler *et al.*, 2008). However, there is little research into the impact that different forage species (other than clover) have on the milk FA profile.

6.4.6 MILK QUALITY AND PRODUCTION EFFICIENCY

A variable that stood out as differing between PE groups (based on ECMY) was milk fat content. Despite lactose and protein being consistent across efficiency groups, fat concentration was highest in the most efficient group (4.7%) and gradually reduced to the least efficient group

(3.3%). Generally, the drive for efficiency within the dairy industry has led to a decline in milk fat concentration, typically due to high quantities of concentrate in the dairy diet (>50%) (Palmquist *et al.*, 1993). The calculation for ECMY gives a higher weighting to fat content, which probably explains why the apparent most efficient cows have the highest milk fat percentage. However, when efficiency is defined as EDMI/liquid milk yield (Appendix D, Table D.2) the most efficient groups (One: 3.9% and Two:3.7%) have the lowest milk fat concentration, as this milk will need less energy to produce each litre. Additionally, under both definitions of efficiency there is evidence of individual cows that are consistently the most or least efficient within a system (Figure 6.3). This re-emphasises the importance of defining efficiency so that farmers can appropriately select cows that best fit their management style. As previously mentioned, there are potential trade-offs and unintended consequences of selecting for efficiency (Collard *et al.*, 2000; Vallimont *et al.*, 2011), but these studies have predominantly examined animal health and fertility but not milk quality. This could offer new insights into the relationship between efficiency and milk quality.

6.4.6.1 LINOLEIC ACID CONCENTRATION IN MILK

The biggest difference in FA profile between farms was the predominant essential n-6 FA, LA ($p<0.01$). Farm B had the highest concentration (1.5%) of LA compared to Farm A (0.9%) and C (1.0%). Farm A cows were 100% forage fed whereas B and C had up to 15% of their diet from organic concentrate (Table 6.1). Therefore, LA did not differ based on access to forage (as A and C have very similar LA concentration), which suggests that in addition to access to forage, sward quality and/or diversity, may be an additional factor that influences LA concentration in milk; this hypothesis will be followed up in a future paper where sward samples will have been analysed.

The seasonal variation in LA followed the same pattern as other studies (Butler *et al.*, 2011b; Kliem *et al.*, 2013), which was highest at the start (1.6%) and decreasing throughout the grazing season (1.2% mid- and 1.0% late lactation). However, this seasonal variation may be misleading because milk analysis from Farm A is missing from early season and since this had a lower LA concentration than the other farms (on the other dates) it would probably reduce the average LA concentration from early lactation. Whilst the seasonal variation may exist it is probably not as wide as suggested here.

6.4.6.2 OMEGA 6: OMEGA 3 RATIO

The difference in LA concentration resulted in a significant difference in the ratio of n-6/n-3 between farms. Farm A had the lowest ratio, most beneficial for human health (0.7) compared to Farm B (1.4) and Farm C (1.0), although all are very low. These much lower results compared to the conventional milk sampled in Chapter 4 (2.2) are reflective of the high forage content in these cows' diets and the benefits of a low n-6/n-3 ratio have been highlighted extensively in previous chapters. The very low ratio across lactation from Farm A is possibly a reflection of 100% of the cow's diet coming from diverse forage compared to only 85-90% from Farms B and C. This continues to strengthen the argument that the n-6 and n-3 content of cow's milk is a reflection of the forage content of their diet (Butler *et al.*, 2008; Stergiadis *et al.*, 2015b). Given the similarity between farms in terms of access to grazing and forage, the similarity of FA profile between farms is unsurprising. Perhaps future analysis of trace elements and antioxidants will provide clearer insights into the relationship between milk quality and type of forage cows are consuming and the underlying soil health (Grace and Knowles, 2012; Gulati *et al.*, 2018).

Despite the difference in grazing strategy and sward diversity, the FA profiles between farms are relatively similar. This suggests that sward diversity may influence FA profile but the predominant driver is proportion of forage and other feeds in the diet, this is further discussed in Chapter 7, Section 7.3.

Table 6.6 Comparison of efficiency (EDMI/ECMY) from other studies with multiparous cows. Data were adapted from the references given.

Stage of Lactation	Diet from forage	EDMI/ECMY	Reference
Early	60%	0.74	Ramin *et al.* (2017)
Early	62%	0.49	Aikman *et al.* (2008)
Early	70%	0.48	Kaufmann *et al.* (2011)
Early	90%	0.77	This study
Mid	67%	0.72	Brito and Silva (2020)
Mid	68%	0.60	Talmón *et al.* (2020)
Mid	90%	0.95	This study
Late	65%	0.68	Barros *et al.* (2017)
Late	65%	0.72	Talmón *et al.* (2020)
Late	90%	1.06	This study

6.4.7 COW HEALTH

Cows from Farm C had considerably more health treatments (fertility and antibiotic, etc.) than Farms B and A (no treatments throughout the trial). However, for context, in Chapter 5 on average all cows received at least one treatment (antibiotic, fertility or other) through one lactation, highlighting that even Farm C has low treatment levels (under 50% of cows received

a treatment) compared to other organic and low-input farms. Farm C had the highest milk yield (28.5L ECMY /day) and many studies indicate that high yielding cows are more prone to mastitis (O'Reilly *et al.*, 2006; Nyman *et al.*, 2007; Clasen *et al.*, 2019). In this case, the highest yielding individual cow on Farm C in early lactation (Cow ID 688, 38L/day) had mastitis and became the lowest yielding cow in mid- (23L/day) and late-(6L/day) lactation, despite apparently recovering fully from the infection. Additionally, Cow ID 647 was treated for mastitis twice between mid and late lactation sampling; she had the third highest yield early in lactation (33L/day), but her yield did not decrease as far as Cow ID 688 by the end of lactation (13L/day). This could be a result of their lactation curves following a different pattern or the type of mastitis bacteria they were exposed to (Hertl *et al.*, 2014). These are only two examples of relatively high yielding cows with mastitis, but it is worth noting that they were two of the three highest yielding cows in early lactation.

Somatic cell count is often used as an indicator of udder health, where SCC is greater than 200,000 cells/ml milk it is likely that there is at least one mastitic quarter (AHDB Dairy, 2018a). The EU Organic guidelines indicate that cows should be able to resist infection through effective management (Council Regulation, 1999), so whilst SCC is a sign of infection, in organic, low-input and PB systems it can also be viewed as a healthy response to a challenge (Lund, 2006). Only 10% of all records in this RW study had cell counts above 200,000 cells/ml milk, split evenly across the farms, many of which were the same cows across lactation, suggesting a few cows with persistent high cell count rather than isolated bouts of mastitis. The lack of treatments on Farms A and B indicates their management strategy effectively supports the cows' immune system and that cows are able to effectively respond to a mastitis challenge. provides some evidence ($p>0.1$- ns) that the most efficient cows (Group One) across all farms had the highest SCC (162,000cells/ml milk) whilst cows in groups with poorer efficiency had lower SCC (Groups Three-Five: 73-101,000cells/ml milk). These mean values are all relatively low and below the range which suggests clinical infection might be present, but generally, the lower the SCC the lower the chance that cows are experiencing an immune response. Evidence from this study supports previous findings that low intensity dairy farming supports fertility and immune function compared to more intensive dairying (Washburn *et al.*, 2002; Arnott *et al.*, 2017).

6.4.8 IDENTIFYING BEST AND WORST PERFORMING COWS

This research found there are cows from each farm that are consistently efficient or inefficient, through lactation and also found cows whose PE changes through lactation (Figure 6.3). This

suggests that there is scope for PB farmers to select efficient cows in their systems in early lactation, and decide which cows to inseminate for replacements, if efficiency is their desired outcome. The most efficient cows produced the most ECMY per kg of DM consumed, spent less time eating ($p<0.05$), ruminating (ns), regurgitated fewer boluses (ns) and chewed less per bolus ($p<0.05$) than those in the other efficiency groups. This supports an earlier study that suggested efficient cattle are also efficient with their eating and rumination behaviours (Dado and Allen, 1994). Most research looks at interventions to improve efficiency or milk yield (e.g. different forages and particle sizes) (Adin *et al.*, 2009; Suarez-Mena *et al.*, 2013). Thorough grazing and ruminating behavioural monitoring are needed without intervention to understand efficiency prior to or alongside intervention studies. Studies that identify the heritability of various traits related to production efficiency such as DMI, LW and ECMY (heritability: 0.27-0.63, 0.63-0.72, 0.12-0.62, respectively) (Spurlock *et al.*, 2012) are useful, but without understanding if and how behaviours effect these traits, there is a large knowledge gap. Additionally, without careful consideration, selecting for these traits in isolation could result in unintended consequences, potentially with an impact on health, fertility (Spurlock *et al.*, 2012; Van de Haar *et al.*, 2016) and milk quality, as discussed in Chapter 2, Section 2.4.

6.5 CONCLUSION

This chapter aimed to determine if there is scope for individual cow selection, within pasture-based herds, based on efficiency and milk quality. As discussed, efficiency in PB systems is poorly defined and understood. In these PB systems with limited recording facilities, other than milk fat (due to contribution to ECMY), there were no standout features that would indicate to the farmer which cows would be the most/least efficient. Agriculture research is framed as beneficial to farmers in assisting with decision-making and to identify practices that will best suit their needs. In the case of PB dairy systems, farmers have already adapted to choose breeds and cows that best suit their system. This provides an opportunity for research to continue the development of sustainability in dairy production, giving farmers and industry more confidence in pasture-based and LI management.

CHAPTER 7. GENERAL DISCUSSION

7.1 INTRODUCTION

This thesis aimed to investigate a combination of milk quality, human nutrition, farm management systems and individual cow performances, to identify key factors that influence production efficiency in organic, low-input and pasture-based dairying. This thesis includes three separate studies covering the relationship between dairy management and milk nutritional quality: Chapter 4 found 100% forage-fed cows produce milk with more omega-3, CLA9 and a lower omega-6: omega-3 ratio than organic and conventional milk. Chapter 5 redefined production efficiency based on low-input priorities (yield, cow health and milk quality) and found that, based on these criteria, the New Zealand Friesian cross out-performed other breeds. Chapter 6 found cow grazing and ruminating behaviour greatly varied between the three study farms and cows and the most consistently efficient cows produced milk with the highest concentration of milk fat.

While the results of these chapters are valuable to interpret and discuss individually, it is also useful to consider them together. These studies demonstrate that organic, LI and PB dairy production produces milk of a substantially better nutritional quality than conventional management. The nutritional quality of the milk is dependent on the management practice, particularly the amount and quality of forage in the diet, which is dependent on the grazing strategy and forage utilisation.

Additional research beyond the scope of this thesis also needs to be considered, either with respect to other aspects of dairy sustainability or further evaluation of similar topics covered here. For example, establishing that PB dairy production supports sustainability with respect to animal and human health but has not addressed the concern of methane emissions from dairy production, yet more efficient cows produce less emissions. Pulling together results from this thesis allows these areas to be explored through this discussion.

7.2 NUTRITIONAL CONTENT

This thesis, along with many other studies, highlights the benefits of increasing the forage: concentrate ratio in dairy cow diets, to increase the proportion of FAs beneficial for human health in milk. Additionally, for the first time in the UK, this thesis has evidenced further improvements by feeding 100% and/or increasing forage in the dairy diet. While chapter results cannot be compared statistically (inconsistent sampling across years, stages of lactation, etc.),

observational comparisons reveal overall trends and limitations across the full thesis (Table 7.1).

As a result of the scrutiny that dairy foods receive for potential high SFAs and the associated health implications, many FAs (as discussed in Chapter 2, Section 2.7) have been investigated for their impact on human health. Evident from results in this thesis (Table 7.1), when averaged across many factors (e.g. stage of lactation, season, breed, etc.) a lot of the diversity is hidden. The most striking results across all three chapters are the relatively high concentration of omega-3 in milk from 100% pasture-fed cows, resulting in considerably lower omega-6:omega-3 ratio than other diets. Levels of vaccenic acid, ALA and CLA9 are noticeably lower in the supermarket conventional milk from Chapter 5 than all milk sampled across other chapters. These are all FAs that have been highlighted as beneficial for human health, supporting evidence that increasing forage in the dairy diet and increasing non-conventional milk in the human diet will increase concentrations of these '*healthy'* FAs (Stergiadis *et al.*, 2015b; Benbrook *et al.*, 2018; Qiu *et al.*, 2018; Jahreis and Dawczynski, 2020). However, the main nutritional concern for dairy produce is the high SFA content (De Souza *et al.*, 2015) and this thesis does necessarily support the evidence that increasing forage in the dairy diet reduces total SFA (Table 7.1). However, as discussed in Chapter 1 and Chapter 2, the evidence does not support removing dairy from the human diet and suggests that the total dairy matrix is supportive of human health.

Table 7.1 Summary of fatty acid (expressed as percentage of the FA profile) results from Chapter 4 (Conventional, Organic and PFLA), Chapter 5 (Low-Input and Organic) and Chapter 6 (100%, 90% and 85% Forage-Fed).

Chapter	Chapter 4			Chapter 5		Chapter 6		
Man [a]	Conv	Org	PFLA	LI	Org	100% Forage	90% Forage	85% Forage
n	15	15	24	1493	948	44	66	68
C12:0	3.5±0.23	3.3±0.15	3.4±0.57	3.8±1.00	3.5±0.80	3.5±0.46	3.9±0.79	4.5±0.88
C14:0	11.0±0.38	11.0±0.33	11.1±1.18	11.7±1.58	11.7±1.66	11.6±0.69	11.9±1.14	12.1±1.37
C16:0	31.1±1.17	28.6±1.23	28.0±3.92	31.3±5.71	32.3±4.31	31.4±4.06	28.5±3.57	28.6±3.56
OA	19.0±0.68	19.3±0.75	18.4±2.93	18.8±4.44	18.5±3.61	18.1±2.32	19.5±3.02	17.9±2.31
VA	1.1±0.23	1.9±0.28	2.5±1.09	2.1±1.17	1.5±0.85	1.8±0.42	2.2±0.85	2.8±0.95
LA	1.8±0.17	1.6±0.23	0.9±0.32	0.9±0.38	1.3±0.39	0.9±0.14	1.0±0.26	1.5±0.33
ALA	0.5±0.08	0.8±0.12	1.1±0.27	0.6±0.20	0.8±0.27	1.1±0.18	0.7±0.13	0.7±0.17
CLA9	0.6±0.07	0.9±0.14	1.2±0.43	0.9±0.50	0.7±0.39	0.8±0.18	1.0±0.47	1.1±0.27
SFA	67.7± 0.87	66.3± 1.49	66.9± 4.54	68.8±5.90	69.8± 5.03	69.4± 2.84	67.1±4.40	68.9±3.37
MUFA	27.0± 0.80	27.6± 0.97	27.1± 3.64	26.9± 5.15	25.6± 4.36	25.7±2.44	27.8±3.68	25.9±2.82
PUFA	5.3±0.41	6.2±0.80	6.0±1.52	4.3±1.06	4.6±1.01	4.9±0.61	5.1±1.09	5.2±0.80
EPA+DPA+ DHA	0.3±0.09	0.4±0.15	0.4±0.08	0.2±0.06	0.2±0.07	0.4±0.04	0.3±0.05	0.3±0.05
Omega-3	1.2±0.20	1.9±0.37	2.3±0.48	1.3±0.41	1.5±0.51	2.0±0.28	1.6±0.27	1.5±0.31
Omega-6	2.7±0.22	2.4±0.23	1.6±0.45	1.4±0.44	1.8±0.42	1.5±0.19	1.7±0.52	2.1±0.36
n-3:n-6 ratio	2.2±0.38	1.3±0.23	0.7±0.10	1.2±0.56	1.4±0.61	0.7±0.09	1.0±0.22	1.4±0.21

[a] Man= Management, LI= Low-Input, Org.= Organic, Conv.= Conventional, PFLA= Pasture for Life Association (100% forage-fed) C12:0=Lauric Acid, C14:0=Myristic Acid, C16:0=Palmitic Acid, OA= Oleic Acid, VA= Vaccenic Acid, LA= Linoleic Acid, ALA=alpha-linolenic acid, CLA9=Conjugated linoleic acid (C18:2, c9t11 isomer), n-3=omega-3, n-6=omega-6, n-6/n-3=, EPADPADHA=Eicosapentaenoic Acid + Docosapentaenoic Acid + Docosahexaenoic Acid, SFA= Saturated Fatty Acids, MUFA= Mono-Unsaturated Acids, PUFA=Poly-Unsaturated Fatty Acids

7.2.1 HUMAN HEALTH IMPLICATIONS

As noted, the 100% forage-fed results have the highest concentrations of omega-3 and the lowest omega-6: omega-3 ratio, which are highly relevant for human health. However, with these dairy products at a low proportion of the typical UK human diet, would switching to 100% forage-fed milk significantly impact human health? The only way to know is to: a) evaluate/model the impact of increasing intake of 100% forage-fed dairy products on a typical diet or b) conduct long-term human intervention dietary trials. In the absence of the latter, the former has been attempted recently by Butler and Stergiadis (2020), who suggest that by switching from conventional to organic milk, consumption of n-3 would increase by 6-11% across all age groups for both sexes in the UK (based on recommended daily intake (RDI) (Bates *et al.*, 2014)). Additional modelling work in two American studies by Benbrook *et al.* (2013) and (2018) suggest that women (19-30 years old) on a moderate fat diet (33% of energy) would significantly increase their total ALA consumption from dairy fat by switching from conventional to organic milk (organic milk had 59% more ALA than conventional) and increase again with a change from organic to Grassmilk^{TM} (52% more than organic and 141% higher than conventional). This has a dramatic impact on the LA/ALA ratio of the full diet; by increasing servings of 100% forage-fed dairy produce to 4.5 per day (from 3 servings of conventional milk), the ratio was reduced from 11.33 to 5.95 (Benbrook *et al.*, 2018). The modelling work from the UK and USA both suggest a dramatic increase in n-3 and decrease in n-6/n-3 by switching from conventional to organic (Benbrook *et al.*, 2013; Butler and Stergiadis, 2020) and from conventional or organic to 100% forage-fed milk (Benbrook *et al.*, 2018). This dietary change of switching from conventional to 100% forage-fed milk and increasing dairy fat (butter) in the diet (in place of vegetable oil) would not have the same impact in UK because UK conventional milk has less LA than milk in the USA (further discussed below) (Table 7.2) resulting in a lower LA/ALA ratio (UK:1.60-4.03, USA:6.27). Despite this, UK 100% forage-fed milk has an even lower ratio than conventional and organic milk, so we can hypothesise that increasing dairy fat from 100% forage-fed cows (at the expense of other dietary fat with a higher LA/ALA, i.e. conventional dairy or vegetable oils) would decrease the overall dietary LA/ALA. This modelling work suggests clear impact on a typical diet composition, but it does not describe any impact on human health. As these studies are the first of their kind, more work is needed on forage-fed milk before conclusions and dietary interventions can be studied.

Nutrition research, from dietary interventions to meta-analysis (but no population studies), seem to conclude that there are benefits of decreasing the LA/ALA ratio during adulthood to

lower the risk of CVD (Studer *et al.*, 2005; De Caterina, 2011), type 2 diabetes (Djoussé *et al.*, 2011; Gao *et al.*, 2013) and obesity (Donahue *et al.*, 2011; Simopoulos, 2016). However, current nutrition guidance is to decrease dietary SFA and replace with unsaturated FA, preferably PUFA, with increases in dietary n-3, but not n-6 (Public Health England, 2019). This is a roundabout way of reducing the LA/ALA ratio but these specific guidelines are difficult to interpret and potentially confusing for consumers. Despite this, forage-fed milk fits the requirements of this nutritional guidance, suggesting that consuming forage-fed milk is advantageous for human health. However, as discussed in Chapter 4, there is no premium in the UK for milk that meets these nutritional guidelines, so why would farmers risk a yield dip with no financial support? Additionally, PFLA milk (the only UK certification for 100% forage-fed dairy) is not yet available in UK supermarkets, as most farmers sell directly to consumers, from whom they receive a premium. Identifying a nutritional standard like those in the USA, France, Germany and the Netherlands (Borreani *et al.*, 2013) provides evidence of success and a clear opportunity for UK retail to support sustainable dairy production and human health. If government priorities are to improve sustainability and health benefits of food production, a major shift is needed to make sustainable and healthy dairy accessible (price and availability) to the average consumer.

Table 7.2 Comparison of milk α-linolenic acid (ALA), linoleic acid (LA) and the ratio of LA to ALA from results in this thesis and papers by Benbrook *et al.* (2013) and (2018) in conventional, organic and 100% forage fed milk. Results display percentage of the total FA profile.

	Conventional		Organic		100% Forage fed	
FA	Thesis[a]	Benbrook *et al.* (2013)	Thesis [b]	Benbrook *et al.* (2013)	Thesis [c]	Benbrook *et al.* (2018)
ALA (%)[d]	0.47-0.65	0.51	0.73-0.84	0.82	1.05-1.06	1.23
LA (%)	0.94-1.81	2.76	1.05-1.57	2.05	0.92-0.93	1.25
LA/ALA	1.60-4.03	6.27	1.79-2.05	2.57	0.87-1.03	1.04

[a] The range covers conventional milk collected in Chapter 4 and Chapter 5. Low-input is included in conventional, despite increased forage from the diet compared to typical conventional diets as both would be sold in the supermarket as conventional milk.
[b] The range covers organic milk collected in Chapter 4, Chapter 5 and Chapter 6
[c] The range covers 100% Forage- fed milk collected in Chapter 5 and Chapter 6
[d] ALA=Alpha linolenic acid, LA= Linoleic acid, LA/ALA= Linoleic acid/ alpha linolenic acid

7.2.2 THE DAIRY MATRIX

There is much more to dairy nutrition than just the FA profile, but examining the wider dairy matrix was beyond the scope of this thesis. Despite this, evidence suggests that organic milk has higher concentrations of α-tocopherol, β-carotene, lutein, and zeaxanthin (Stergiadis *et al.*, 2012; Schwendel *et al.*, 2015; Średnicka-Tober *et al.*, 2016), potentially due to higher intakes

of fresh forage, containing more carotenoids than concentrate feeds, and using breeds (e.g. Jersey) that transfer carotenoids more efficiently into milk (Noziere *et al.,* 2006). Examining dairy and other food groups as a sum of their nutrients would be a more robust approach to nutrition and researching interactions with other foods during digestion and metabolism would provide more robust nutrition guidance (Feeney and McKinley, 2020). However, this computational task is not yet possible, so for now, dietary guidelines must be based on nutrient contents, reaffirming the need to understand what influences milk's FA profile.

7.3 GRAZING STRATEGIES

Determining that milk is increasingly supportive of human health with more forage in the dairy diet confirms that grazing strategy and forage utilisation are essential considerations for farm management. As discussed in Chapter 2, Section 2.7.3, the balance of milk FAs is the result of a combination of biohydrogenation of ingested FA in the rumen, synthesised *de novo* in the mammary gland (from rumen VFAs) or modified in the mammary gland by desaturase enzyme activity (Destaillats *et al.*, 2005; Chilliard *et al.*, 2007; Bauman *et al.,* 2011; Mangwe *et al.*, 2020a). Milk FA profile is predominantly dictated by diet, and forage lipids supply mostly ALA (~62% of total FA), whilst cereal lipids mostly supply LA (~58%) (from concentrate feeds) (Walker *et al.*, 2004; Ryan *et al.*, 2007; Wojtkowiak *et al.*, 2018). This thesis supports evidence that increasing forage content in the cow diet directly increases milk ALA, with the 100% forage-fed milk supplying the highest concentration of ALA compared to all other milk analysed (Table 7.1).

All chapters discuss enhancing milk FA profile to support human health through increasing pasture feeding, but to support these swards an optimum grazing strategy or harvesting option must be established. An optimum grazing strategy needs to support cow health and nutrition, prevent biodiversity degradation and overgrazing and utilise available land. Historically, the UK grazing strategy has been set or continuous stocking (where cows are in one or a few fields the entire grazing season), using predominantly heavily selected perennial ryegrass and nitrogen fertiliser. But evidence suggests that this method only utilises 50-60% of the herbage (compared to 85%, paddock grazing) (AHDB, 2018b). As discussed in Chapter 5, one of the main differences between LI and certified organic management is the use of mineral nitrogen fertiliser in LI systems whilst organic standards encourage sowing legumes such as red and white clover (Soil Association, 2018). This difference in plant fertility management could explain the differences in ALA (LI:0.65%, organic:0.84%) and LA (LI: 0.94%, organic:1.33%) concentrations in milk (Table 7.1). Clover has a higher concentration of ALA and LA than

perennial rye grass (clover ALA: ~59.6g/100g, LA:~20.2g/100g, rye grass ALA:~51.6g/100g, ~LA:13.2g/100g) and is more rapidly digestible at comparable crop maturity (McDonald *et al.*, 2011). This suggests that there is more ALA and LA in the diet from clover under organic management compared with fertilised grass swards. Additionally, clover is more digestible than grass and moves through the rumen faster, resulting in more ALA (and potentially LA) leaving the rumen before biohydrogenation, arriving at the mammary gland intact (Stergiadis *et al.*, 2018). FA profiles of different forage species under the same management have different FA profiles (Dewhurst *et al.*, 2001), but due to the complex nature of ruminant lipid metabolism, more research is needed to understand the impact of forage FA profiles on milk FA profiles.

The milk FA profile is quick to adapt to the diet (Vlaeminck *et al.*, 2010) and there is some evidence that grazing management strategy impacts FA profile. In a continuous grazing setting, digestibility and palatability is high at the beginning of the season but drops by the end of the season, especially around dung deposits, resulting in cows consuming poorer quality forage by Autumn, whilst rotational grazing allows recovery, providing consistent good quality pasture throughout the grazing season (Coppa *et al.*, 2009; Coppa *et al.*, 2011). Forage available in spring (in a diverse pasture) allows cows to be selective under continuous management, whilst the tighter stocking density in rotational grazing discourages selection. This constant supply of fresh leafy forage in rotational grazing results in a constant supply of ALA from forage (Elgersma *et al.*, 2006; Coppa *et al.*, 2011), whereas under continuous management supply of ALA decreases as vegetation matures. However, this is not necessarily reflected in the milk FA profile, and depends on the proportion of forage in the diet (Coppa *et al.*, 2011; Farruggia *et al.*, 2014), yet this might explain the lack of seasonal variation in the PFLA milk and Chapter 6 milk. A slightly unexpected insight to the different grazing systems explored by Farruggia *et al.* (2014) and Coppa *et al.* (2015) is increased milk yield/ha and persistence under rotational management, potentially due to the decreasing herbage quality through the grazing season and in continuous management as the season progresses cows have to work harder/walk further to find the more palatable/digestible grasses. This could also be because diverse swards containing various legumes (such as red and white clover and birdsfoot trefoil) improve pasture productivity and enhance seasonal distribution of DM production (Sleugh *et al.*, 2000). This was not explored in this thesis, but further supports the move towards diverse swards and paddock grazing.

7.3.1 MOB GRAZING

Many of the farmers involved in trials for this thesis (many of the PFLA farmers in Chapter 4 and two participant-farmers in Chapter 6) have gone far beyond rotational grazing to use paddock (as recommended by (AHDB, 2018b)) and mob/tall-grass grazing. As discussed in Chapter 2, Section 2.5, despite the (weakly) evidenced environmental benefits of these grazing strategies and nearly all farmers recruited through this thesis using some form of mob grazing, there is very little scientific research into its effects on cow nutrition, health, welfare, milk or sward FA profiles, forage productivity or utilisation and/or environmental impacts in the UK. But, mob grazing aims to increase soil organic matter, forage utilisation and nutrient cycling through tightly stocking paddocks for short periods of time (12-24 hours), grazing a third, trampling a third and leaving a third of the vegetation (Zaralis and Padel, 2019). Despite taller plants becoming more mature, less palatable and digestible, because of the amount of DM available (cover >3000kg DM/ha), cows can self-select the most palatable or digestible parts of the plants, often then trampling the stems into the ground. In addition to grazing strategy, farmers utilising mob-grazing typically sow a more diverse sward which, as discussed in Chapter 2, Section 2.5, improves soil structure and quality resulting in drought resistant, weed suppressive pastures and reduced nitrogen leaching (Sanderson *et al.*, 2005; Woodward *et al.*, 2013; Cranston *et al.*, 2015; Romera *et al.*, 2017; McCarthy *et al.*, 2020). Since data was collected for this thesis, a farmer-led mob grazing group has been set up in Scotland (Soil Association, 2019), demonstrating further that farmers are interested and willing to try new farming methods to improve the sustainability of their farms, but without a strong UK evidence-base the benefits are hearsay.

7.3.2 ANIMAL HEALTH

In addition to improving FA profile and sustainability, there is evidence that grazing strategy impacts animal welfare. Grazing stock continuously on a couple of pastures throughout the season increases parasitic burden, which can lead to anthelmintic resistance (Waller, 2006). Effective grazing strategies can prevent worm burdens by limiting grazing in one paddock for a short enough time to prevent re-infestation (<1 week), and each paddock should be left un-grazed for long enough that the infective parasites die off (>30 days) (timings dependent on local ecologies) before being eaten by a potential host animal (Waller, 2006). In addition to rotation, including forage species with anthelmintic properties (condensed tannins) such as sainfoin, birdsfoot trefoil and chicory are advantageous (Hoste *et al.*, 2006), especially in organic systems where anthelmintic use is restricted and management to support the immune system is encouraged (Soil Association, 2018), ensuring a diverse leafy sward is essential. It is

therefore important to promote animal health by managing pastures effectively and efficiently (Leiber *et al.*, 2014). Farmers A and B in Chapter 6 reported they could not remember the last time they used anthelmintics, but regularly test for worm counts, further suggesting the effectiveness of diverse swards and long grazing rotations. Typically, the more diverse the sward, the more cows can self-select the most palatable and digestible (leafy) (or possibly medicinal) parts of the plants, avoiding additional parasitic burden and supporting cow welfare (Villalba *et al.*, 2010). Despite evidence from only two farms, this provides additional support for moving away from set and continuous setting towards rotational and paddock grazing.

Evidence in this section identifies benefits for animal health, welfare and productivity by moving away from set and continuous grazing towards rotational or paddock grazing with more diverse swards. This provides an opportunity for UK research institutes to establish grazing trials and scientifically assess the environmental, health and welfare implications of different grazing strategies, as well as reinforce their nutritional impact for cows and consumers.

7.4 GENOTYPES

In all farming systems, selecting genotypes that suit management practice is important for efficiency and sustainability. As discussed in Chapters 2, 4, 5 and 6, management systems vary widely and farmers have different priorities, so selecting high-input, high-output HFs is unsuitable for forage-based dairy production (Simianer and Bieber, 2012). The diversity of breeding strategies implemented in LI and organic farming were highlighted in Chapter 4, with over 40 different breed combinations from a sample of cows on 17 farms, and again in Chapter 6 with 32 breed combinations from just 3 PB farms, many first generation crosses (F1), but also third and fourth generation crosses. This variety of genotypes indicates that: a) there is not a singular breed or combination that best suits all LI dairy farming, b) farmers use trial and error to find out what works best for their system, c) farmers research breeds known for health, fertility and add these genetics to their systems when appropriate and d) academic and industry (AHDB) research into breeding for LI dairy production is not robust enough to support these systems in practice. As discussed in Chapter 5, ideally farmers need access to a flow chart to guide them through breed selection based on inputs, constraints and priorities within their system, resulting in an indexing system unique to each farm.

In the UK, there are different breeding indices offering guidance on how likely an individual animal will pass its genetic traits onto its offspring: a Profitable Lifetime Index (£PLI), a Spring Calving Index (£SCI) and an Autumn Calving Index (£ACI) (AHDB, 2020a). The indices selected are used to help farmers make decisions about the genetics and improvements they can

make to their herds. However, since before the 1980s focus was on improving production in HFs, and, more recently, on improving health and fertility traits. As a result, the reliability of genomic evaluations for HF are around 65-75%, but only around 55% for other breeds (AHDB, 2020a). Therefore, genomic 'progress' lags in the dairy industry for breeds that are more suited to LI systems, so farmers have had to make progress through trial and error. For example, if a farmer with a spring- calving herd needed to improve fertility (h^2=0.03) and locomotion (for walking to and from paddocks, h^2=0.10), they might bring in New Zealand Friesian genetics, then want a higher percentage of fat (h^2=0.68) and protein (h^2=0.68), they could add some Jersey genetics (h^2 from (AHDB, 2020a)). Additionally, forage conversion is incredibly important to PB farmers, but as discussed in Chapter 2, Section 2.4.1.2, efficiency is typically a combination of traits, the interactions of which are still poorly understood. This example highlights that progress in PB systems are slow, with important, functional traits having a low heritability. Thus, LI farmers are not able to improve traits as quickly as in intensive systems as discussed in Chapter 5), where bulls (within breeds) are ranked based on traits and suitability for £PLI, £SCI or £ACI. Globally, organic and LI farming is expanding, therefore research into breeding programmes needs to reflect the efforts that have gone into HF production, especially as PB farming and utilising forage (through spring and extended season grazing) increase in the conversation about sustainability and welfare.

7.5 PRODUCTION EFFICIENCY

Production efficiency, especially forage conversion, is essential for sustainability under LI and PB management, yet modelling efficiency in these systems is notoriously difficult. The major hurdle to advancing PE in PB systems is identifying farmers' aims and priorities to provide bulls/semen (across a range of breeds) that will progress their herd to meet these goals. This was attempted in this thesis, using physical (or possibly phenotypic) measurements rather than genetic analysis (using SNPs (single nucleotide polymorphisms), a measure of genetic variability, often using data from thousands of animals (AHDB, 2020a) to assess breed combinations across different farms (Chapter 5) and individual cows within farms (Chapter 6). The results indicated that, based on described criteria, there were breed combinations and individual cows that consistently outperformed others. But there was no clear distinguishing factor that would identify a 'superior cow' within a single farm system. The process of defining PE in LI and PB systems was explored in Chapter 3, identifying pitfalls in statistical approaches highlighted the complexity of modelling with numerous explanatory and response variables. This thesis did not find an effective way to measure PE in LI and PB systems, but different methods explored identified that farmers may be able to improve production efficiency, by

selecting the best performing cows and breeds (based on metrics that include health, fertility and milk quality). This is something that these particular farmers have already been doing for some time, but for farmers moving towards and interested in LI and PB farming, this thesis provides useful pointers in support of these farming practices.

7.6 ENTERIC METHANE EMISSIONS AND CLIMATE CHANGE

Consumption of ruminant milk and meat produce is continuously scrutinised for impacts on greenhouse gas (GHG) emissions and the wider environment, from producing soy through deforestation to feed ruminants, to land use where human food could be grown, to enteric methane emissions. This thesis has so far demonstrated that dairy cows can be productive under organic, 100% forage-fed and PB management, i.e. in systems where no soy is fed. However, enteric methane production has not been discussed. Despite its contribution to GHGs, the demand for ruminant produce is still expected to increase (Gerber *et al.*, 2013b), and it is therefore essential that mitigating methane emissions are prioritised.

As discussed in Chapter 2, Section 2.6.3, LCA analysis often biases methane emissions, due to modelling (based on HF and conventional practice (mineral nitrogen fertiliser)) and the functional units used, but recently studies have attempted to model and predict emissions from DMI (Charmley *et al.*, 2016), milk quality parameters (Negussie *et al.*, 2017) and FA profiles (Dijkstra *et al.*, 2011). Milk fat and protein have been shown to be poor predictors of emissions (Negussie *et al.*, 2017; van Gastelen *et al.*, 2018), but DMI shows promise (Hristov *et al.*, 2018). Two robust meta-analyses have been conducted by van Lingen *et al.* (2014) and Bougouin *et al.* (2019) to create models to predict methane emissions from FA content using studies with a wide range of feeding and management practices. Many of the FAs used in these models were quantified in this thesis, except branch- chain FA (such as, *iso* C16:0 and *anteiso* C17:0). These are mostly synthesised by bacteria in the rumen and thought to be related to rumen fermentation and CH_4 yield (Vlaeminck *et al.*, 2006). However, research by van Engelen *et al.* (2015) generated methane yield models based solely on FAs quantified in this thesis. Two of these models (Appendix E, Table E.1.) were used to predict CH_4 yield using the FA data collected in Chapters 4, 5 and 6 (Table 7.3).

Dry matter intake is known to effect methane production; generally the more feed consumed, the more DM fermented in the rumen producing VFA and gasses (Bannink *et al.*, 2010), so methane yield (g/kg DMI) is used. The results are in a similar range (18.35-22.99g/kg DMI) to those calculated using the equations of Dijkstra *et al.* (2011) and van Engelen *et al.* (2015) and were relatively similar across the different management systems and chapters. The evidence

suggests that the higher the forage:concentrate ratio, the higher the methane production, possibly due to the promotion of acetate and butyrate production in the rumen, and thus CH_4 (Vlaeminck *et al.*, 2006; Bannink *et al.*, 2008; Bougouin *et al.*, 2018; Moate *et al.*, 2020). Based on this, conventional milk from Chapter 4 would be expected to have the lowest emissions, yet the results in Table 7.3 predict the lowest CH_4 yield is from the 85% forage-fed farm from Chapter 6 (Farm B). But Chapter 6 does see a steady decline in CH_4 yield as forage in the diet decreases. Of course, this is just a snapshot of potential CH_4 yield, and research needs to further validate the models created in these various analyses.

It is important to also consider changes over time, as the rumen microbial population will adapt to changes in diet and forage quality (Moate *et al.*, 2020). Additionally, the relationship between forage:concentrate ratio and CH_4 changes. For example, when the dairy diet has less than 30% concentrate, the relationship is linear (Charmley *et al.*, 2016), yet above this the relationship seems to curve linearly (CH_4 decreasing at high DMI) (Knapp *et al.*, 2014), therefore PB diets, which have different FA profiles, will also have different predicted emissions (Dijkstra *et al.*, 2016). Whilst modelling methane emissions from cows will help identify potential needs for mitigation, there are many factors to consider. Despite the meta-analysis including a wide range of dietary studies, acknowledging that these relationships are important, suggests that a meta-analysis that models CH4 emissions covering PB systems is required.

Table 7.3 Modelled methane emissions (g/kg DMI ± SD) using FA data from Chapter 4, Chapter 5 and Chapter 6 and Model 1 and 2 (Appendix E, Table E.1.) from van Engelen *et al.* (2015)

	Management	Model 1. CH_4 (g/kg DMI)	Model 2. CH_4 (g/kg DMI)
Chapter 4	Conventional	20.0±1.73	22.3±0.66
	Organic	20.1±2.16	21.1±0.68
	PFLA	18.5±3.39	20.5±2.07
Chapter 5	Low-Input	19.2±3.08	21.8±2.32
	Organic	21.1±2.56	23.0±2.13
Chapter 6	100% forage	21.1±0.97	22.9±0.68
	90% forage	19.4±2.84	21.5±1.62
	85% forage	18.4±2.72	20.2±2.17

7.7 DRY MATTER INTAKE

Feed quality and quantity will also impact emissions, so estimating DMI is essential. In PB systems (such as in Australia and New Zealand) CH_4 emissions were not predicted well without accurate DMI estimates (Hristov *et al.*, 2018). But, as discussed in Chapter 2, Section 2.3 and Chapter 6, Section 6.4.1, predicting DMI in PB systems is difficult. The model currently widely

used to predict DMI to estimate methane emissions (in LCAs) (Hristov *et al.*, 2018) is from the American National Research Council (NRC, 2001):

DMI (kg)= (0.372 x 4% fat corrected milk +0.0968 xliveweight$^{0.75}$)x(1-e$^{(0.192(\text{week of lactation}+3.67))}$)

The data collected in Chapter 6 found DMI predicted using the RumiWatch halters to be relatively similar (within the standard deviation) to the NRC formula (Table 7.4). However, the formula by Guilherme Amorim Franchi (2017) using the RW estimations gave higher DMI than estimated using the NRC formula during early- and mid- lactation, whilst the opposite was true for late- lactation. Whilst neither formula has been effectively validated in grazing-based systems (as this is impossible), these differences would impact CH_4 modelling results. This reemphasises the importance of accurately estimating DMI for both efficiency and methane emissions.

Table 7.4 Estimated dry matter intake (kg/day ± SD) using data from Chapter 6, modelled from and Guilherme Amorim Franchi (2017) and NRC (2001)

Farm	Stage of Lactation	RW EDMI (kg/day)	NRC EDMI (kg/day)
	Early	19.2±3.09	16.4±2.38
Farm A	Mid	17.3±2.61	15.6±1.47
	Late	13.5±3.64	14.5±1.81
	Early	17.3±2.04	15.8±1.38
Farm B	Mid	18.9±2.35	17.1±1.38
	Late	15.2±2.27	16.8±1.49
	Early	23.7±2.01	20.8±1.95
Farm C	Mid	24.6±2.86	22.6±2.20
	Late	18.2±2.74	18.5±2.13

7.8 IMPACT

The data collected for this dissertation supports previous research that increasing forage content in the dairy diet increases the CLA9 and omega-3 concentration in milk, reducing the omega-6:omega-3 ratio and goes beyond published results to find 100% forage-fed dairy further improves these concentrations, potentially supporting human health. This thesis has also found that New Zealand Friesian and Ayrshire genetics perform well in LI and organic systems and identified the potential for farmers to select for efficiency within PB systems. There is no question that PB dairy farming, (especially on marginal land) using a paddock or mob grazing strategy with a diverse sward, promotes sustainability, cow welfare and human health. Mob grazing using a diverse sward improves soil structure and quality, preventing nitrogen leaching, improving biodiversity and weed suppression and decreasing reliance on energy-intensive fertilisers for grain and grass feed production. By selecting breeds well suited to low-input

systems, reliance on antibiotics and anthelmintics is reduced and fertility is improved. If the UK is to improve agricultural sustainability and promote human health through nutrition, research funding and governmental policy support for LI and PB farming practices must be improved. This could be in the form of the AHDB supporting and investing in alternative breeding programmes, developing policy that supports farmers to provide at least 70% of the dairy diet from forage and/or stabilising milk prices to allow farmers to develop more sustainable management practices.

7.9 CONCLUSIONS

This thesis aimed to identify key factors influencing production efficiency in organic, low-input and pasture-based dairying through investigation of milk quality, human nutrition, farm management systems and individual cow performances. The multiple studies included throughout these chapters clearly demonstrate that access to pasture significantly improves milk FA profile, which could have a positive effect on human health. There is clear evidence that breed selection can be tailored to benefit individual low-input and organic dairy systems, with New Zealand Friesian and Ayrshire demonstrating potential, following methods that farmers are already implementing. This thesis also concludes that with supporting research, pasture-based dairy farmers can select for production efficiency and that efficiency itself should consider qualities beyond yield (nutritional quality, animal health, etc.) when applied to low-input systems. While the benefits of grazing are clear, there is a current lack of dairy research focused on pasture-based systems, with farmers leading the way in innovation. The conclusions of this thesis support what these farmers management practices and shows that there are opportunities for dairy research to further study production efficiency in these systems.

APPENDIX A CHAPTER 2

Fatty Acid Calculations

Saturated FA (SFA): C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0.

Monounsaturated FA (MUFA): c9 C14:1, c9 C15:1, c9 C16:1, t9 C16:1, c9 C17:1, t6,7,8 C18:1, t9 C18:1, t10 C18:1, t11 C18:1, t12,13,14 C18:1, c9 C18:1, t15 C18:1, c11 C18:1, c12 C18:1, c13 C18:1, c14 t16 C18:1, c15 C18:1, C19:1 c8 C20:1, c13 C22:1, c15 C24:1.

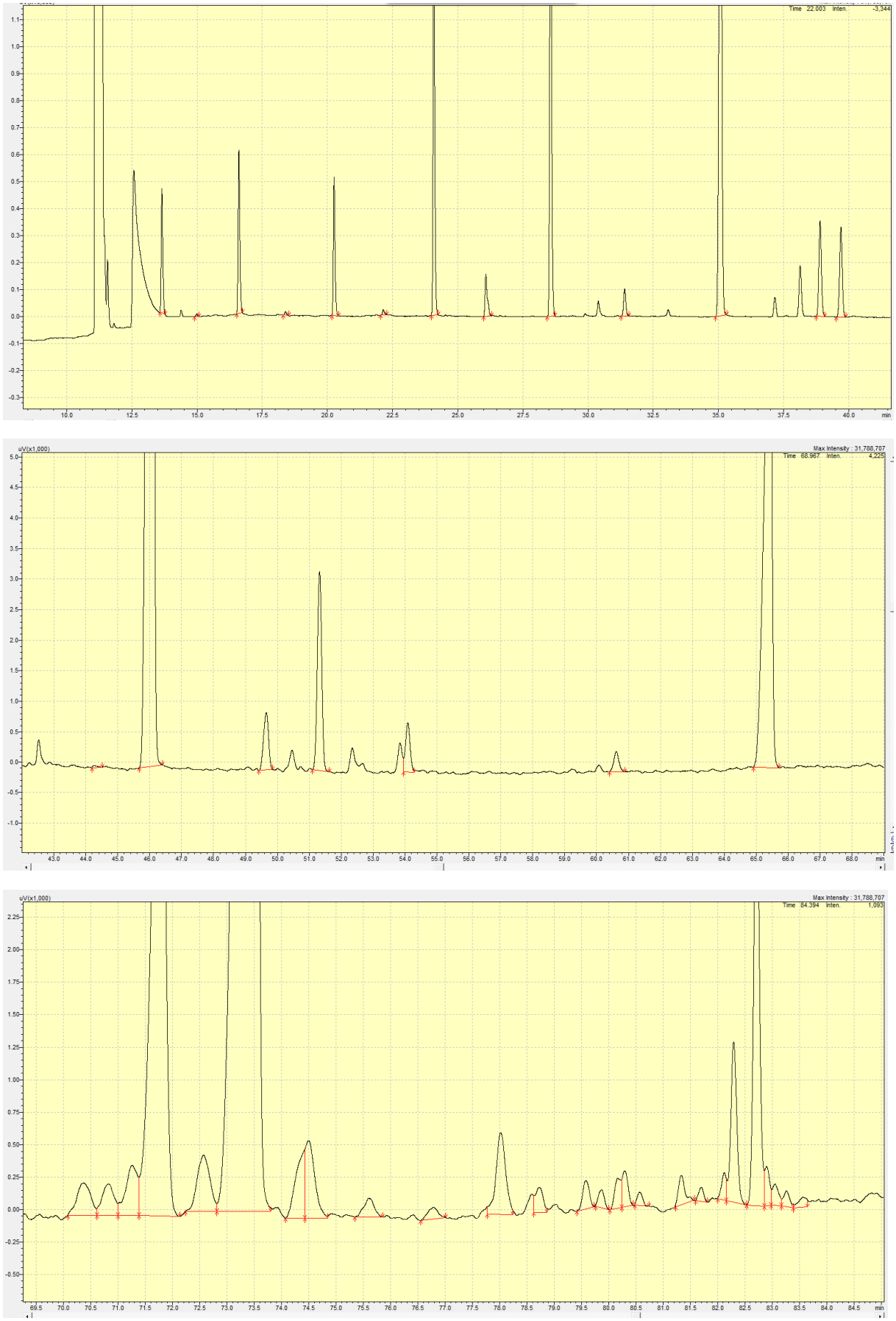
Polyunsaturated FA (PUFA): t11,15 C18:2 (n3), t10,14 C18:2, c9t13, C18:2, t9,12 C18:2 (n6), t8c13 C18:2, c9t12 C18:2 (n6), t9c12 C18:2 (n6), ct mix 10,14,12,16 C18:2, t11c15 C18:2 (n3), c9,12 C18:2 (n6) (LA), Unknown LA2, Unknown LA3, c9,15 C18:2 (n3), c12,15 C18:2 (n3), c6,9,12 C18:3 (n6), c9 12, 15 C18:3 (n3) (ALA), c9t11 C18:2 (CLA9), t11c13 CLA, unknown CLA1, Unknown CLA2tt, Unknown CLA3tt, Unknown CLA6tt, c9,13,15 C18:3 (n3), c11,14 C20:2 (n6), c9,11,15 C18:3 (n3), c8,11,14 C20:3 (n6), c11,14,17 C20:3 (n3), c5,8,11,14 C20:4 (n6), c13,16 C22:2 (n6), c5,8,11,14,17 C20:5 (n3) (EPA), c13,16,19 C22:3 (n3), c7,10,13,16 C22:4 (n6), c7,10,13,16,19 C22:5 (n3) (DPA), c4,7,10,13,16,19 C22:6 (n3) (DHA)

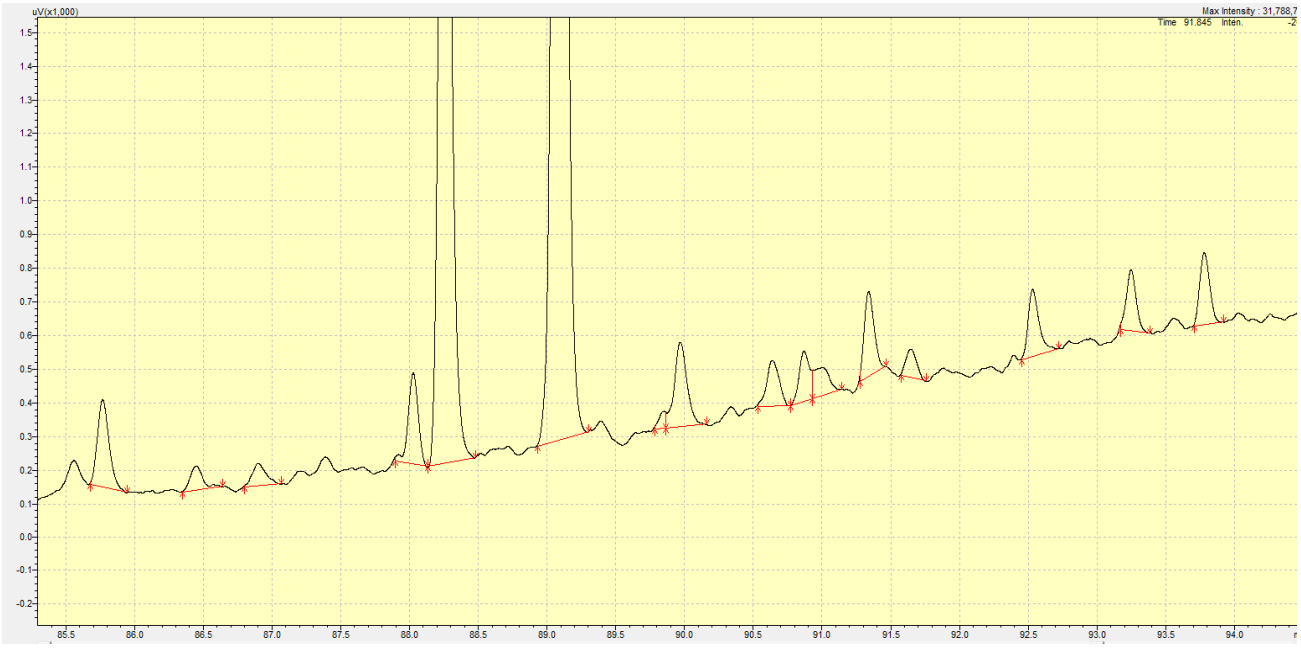
Omega-3 FA (n-3): t11,15 C18:2, t11c15 C18:2, c9,15 C18:2, c12,15 C18:2, c9 12, 15 C18:3 (ALA), c9,13,15 C18:3, c9,11,15 C18:3, c11,14,17 C20:3, c5,8,11,14,17 C20:5 (EPA), c13,16,19 C22:3, c7,10,13,16,19 C22:5 (DPA), c4,7,10,13,16,19 C22:5 (DHA)

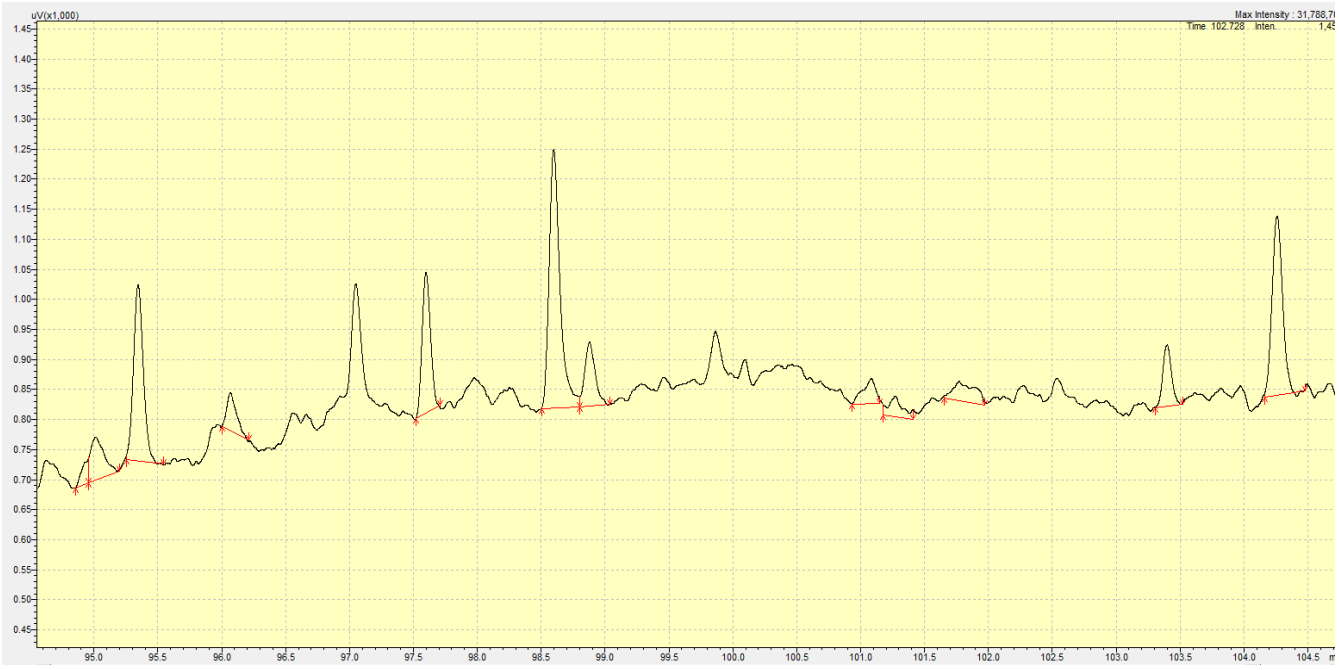
Omega-6 FA (n-6): t9,12 C18:2, c9 t12 C18:2, t9 c12 C18:2, c9,12 C18:2 (LA), c6,9,12 C18:3, c11,14 C20:2, c8,11,14 C20:3, c5,8,11,14 C20:4, c13,16 C22:2, c7,10,13,16 C22:4

Very long chain omega-3 FAs: c5,8,11,14,17 C20:5 (EPA), c7,10,13,16,19 C22:5 (DPA), c4,7,10,13,16,19 C22:5 (DHA)

Figure A.1. Example chromatogram with 74 fatty acids, each fatty acid identified has a red line under the peak associated with the specific FA.




uV(x1,000)
Max Intensity : 31,788,7
Time 91.845 Inten.


uV(x1,000)
Max Intensity : 31,788,7
Time 102.728 Inten.

APPENDIX B CHAPTER 4

Table B.1. Effect of Management (Conventional, Organic and PFLA) and Month (April, July and October) on the concentrations of nutritionally relevant FA in milk (from the study in Chapter 4). Mean FA proportion ± standard deviation (SD) and ANOVA p-values for each FA, expressed as a percentage of the entire FA profile.

	Management			Month			ANOVA p-value[a]		
	Conv.	Org.	PFLA [b]	April	July	October	Man	Mon	Man x Mon
Fatty Acid	n=15	n=15	n=15	n=18	n=18	n=18			
C4:0	3.0 ± 0.159	3.1 ± 0.127	3.0 ± 0.221	3.1 ± 0.171	3.1 ± 0.151	3.0 ± 0.235	ns	ns	ns
C5:0	0.13 ± 0.084	0.13 ± 0.124	0.09 ± 0.060	0.11 ± 0.072	0.10 ± 0.055	0.13 ± 0.126	ns	ns	ns
C6:0	2.5 ± 0.096	2.5 ± 0.092	2.5 ± 0.218	2.6 [a] ± 0.154	2.5 [b] ± 0.151	2.5 [b] ± 0.113	ns	***	t
C7:0	0.08 ± 0.066	0.08 ± 0.049	0.06 ± 0.046	0.06 ± 0.051	0.07 ± 0.050	0.08 ± 0.059	ns	ns	ns
C8:0	1.3 ± 0.085	1.3 ± 0.093	1.3 ± 0.144	1.4 [a] ± 0.109	1.3 [b] ± 0.115	1.3 [b] ± 0.078	ns	***	ns
C9:0	0.08 [a] ± 0.041	0.08 [a]± 0.044	0.06 [b] ± 0.024	0.06 [b]± 0.030	0.07 [b]± 0.024	0.09 [a] ± 0.045	*	**	**
C10:0	2.8 ± 0.191	2.7 ± 0.097	2.9 ± 0.495	3.0 [a] ± 0.422	2.7 [b] ± 0.299	2.7 [b] ± 0.241	ns	**	t
C11:0	0.30 ± 0.029	0.31 ± 0.040	0.31 ± 0.054	0.29 ± 0.031	0.31 ± 0.058	0.31 ± 0.040	ns	ns	ns
C12:0	3.5 ± 0.230	3.3 ± 0.146	3.4 ± 0.568	3.6 [a] ± 0.485	3.3 [b] ± 0.344	3.3 [b] ± 0.329	ns	**	ns
C13:0	0.22 ± 0.038	0.23 ± 0.052	0.21 ± 0.050	0.23 ± 0.039	0.20 ± 0.046	0.22 ± 0.054	ns	ns	ns
C14:0	11.0 ± 0.383	11.0 ± 0.325	11.1 ± 1.179	11.2 ± 0.947	11.0 ± 0.887	11.0 ± 0.610	ns	ns	ns
c9 C14:1	1.0 [a] ± 0.069	0.9 [b] ± 0.085	0.9 [b] ± 0.193	0.9 [c] ± 0.148	0.9 [b] ± 0.135	1.0 [a] ± 0.131	**	**	ns
C15:0	1.1 [b] ± 0.062	1.2 [b] ± 0.117	1.4 [a] ± 0.208	1.3 ± 0.242	1.3 ± 0.176	1.3 ± 0.200	***	ns	ns
c9 C15:1	0.05 ± 0.034	0.07 ± 0.050	0.06 ± 0.036	0.06 ± 0.038	0.05 ± 0.029	0.07 ± 0.050	ns	ns	t
C16:0	31.1 [a] ± 1.17	28.6 [b] ± 1.23	28.0 [b] ± 3.92	29.0 ± 3.97	28.6 ± 2.70	29.5 ± 2.29	*	ns	ns
t9 C16:1	0.41 [b] ± 0.091	0.52 [a]± 0.091	0.59 [a] ± 0.096	0.51 ± 0.118	0.52 ± 0.101	0.52 ± 0.139	**	ns	ns
C16:1	1.9 ± 0.151	1.8 ± 0.146	1.7 ± 0.315	1.7 ± 0.330	1.8 ± 0.163	1.8 ± 0.225	ns	ns	ns
C17:0	0.55 [b] ± 0.080	0.61 [b]± 0.089	0.68 [a] ± 0.129	0.61 ± 0.122	0.67 ± 0.113	0.59 ± 0.111	**	t	ns
c9 C17:1	0.23 ± 0.045	0.27 ± 0.058	0.29 ± 0.095	0.25 ± 0.057	0.26 ± 0.070	0.30 ± 0.096	t	t	ns
C18:0	9.5 [b] ± 0.619	10.5 [a]± 0.609	11.2 [a] ± 1.892	10.6 ± 1.708	11.0 ± 1.445	10.1 ± 1.272	**	ns	ns
t6,7,8 C18:1	0.27 ± 0.084	0.30 ± 0.075	0.23 ± 0.123	0.25 ± 0.123	0.25 ± 0.105	0.28 ± 0.082	ns	ns	ns
t9 C18:1	0.20 ± 0.089	0.20 ± 0.086	0.18 ± 0.106	0.17 ± 0.093	0.17 ± 0.097	0.23 ± 0.085	ns	ns	ns
t10 C18:1	0.37 [a] ± 0.099	0.26 [b] ±0.131	0.19 [b] ± 0.115	0.22 ± 0.125	0.29 ± 0.160	0.26 ± 0.119	**	ns	ns
t11 C18:1	1.1 [c] ± 0.234	1.9 [b] ± 0.284	2.5 [a] ± 1.091	2.0 ± 1.369	1.8 ± 0.601	1.9 ± 0.809	***	ns	ns
t12,13,14 C18:1	0.42 ± 0.065	0.36 ± 0.101	0.32 ± 0.110	0.38 ± 0.097	0.36 ± 0.126	0.35 ± 0.090	t	ns	ns

Table B.1 continued. Effect of Management (Conventional, Organic and PFLA) and Month (April, July and October) on the concentrations of nutritionally relevant FA in milk (from the study in Chapter 4). Mean FA proportion ± standard deviation (SD) and ANOVA p-values for each FA, expressed as a percentage of the entire FA profile.

	Management			Month			ANOVA p-value[a]		
	Conv.	Org.	PFLA [b]	April	July	October	Man	Mon	Man x Mon
Fatty Acid	n=15	n=15	n=15	n=18	n=18	n=18			
c9 C18:1	19.0 ± 0.676	19.3 ± 0.752	18.4 ± 2.931	18.5 ± 2.582	19.3 ± 1.898	18.7 ± 1.521	ns	ns	ns
t15 C18:1	0.27 ± 0.094	0.25 ± 0.081	0.25 ± 0.112	0.26 ± 0.110	0.24 ± 0.069	0.27 ± 0.113	ns	ns	*
c11 C18:1	0.53 [a] ± 0.177	0.36 [b]± 0.155	0.41 [b] ± 0.137	0.38 ± 0.151	0.46 ± 0.176	0.44 ± 0.167	*	ns	ns
c12 C18:1	0.26 [a] ± 0.096	0.17 [b]± 0.116	0.11 [b] ± 0.064	0.16 ± 0.102	0.18 ± 0.115	0.16 ± 0.111	**	ns	ns
c13 C18:1	0.10 ± 0.064	0.10 ± 0.092	0.10 ± 0.055	0.09 ± 0.070	0.10 ± 0.065	0.11 ± 0.072	ns	ns	ns
c14, t16 C18:1	0.33 ± 0.084	0.35 ± 0.104	0.36 ± 0.120	0.34 ± 0.119	0.35 ± 0.111	0.36 ± 0.089	ns	ns	ns
c15 C18:1	0.10 ± 0.044	0.17 ± 0.120	0.13 ± 0.071	0.16 ± 0.111	0.13 ± 0.076	0.12 ± 0.059	ns	ns	ns
t11,15 C18:2 (n3)	0.05 ± 0.041	0.05 ± 0.033	0.05 ± 0.026	0.04 ± 0.026	0.05 ± 0.032	0.05 ± 0.038	ns	ns	ns
t10,14 C18:2	0.06 ± 0.046	0.06 ± 0.046	0.07 ± 0.049	0.05 ± 0.019	0.08 ± 0.048	0.08 ± 0.058	ns	t	ns
c9 t13, C18:2	0.08 ± 0.036	0.09 ± 0.040	0.11 ± 0.070	0.10 ± 0.068	0.09 ± 0.054	0.09 ± 0.043	ns	ns	ns
t9,12 C18:2 (n6)	0.11 ± 0.059	0.10 ± 0.039	0.08 ± 0.047	0.09 ± 0.049	0.09 ± 0.046	0.10 ± 0.056	ns	ns	ns
t8 c13 C18:2	0.14 ± 0.067	0.09 ± 0.057	0.11 ± 0.097	0.09 ± 0.058	0.12 ± 0.070	0.14 ± 0.102	ns	ns	ns
c9 t12 C18:2 (n6)	0.10 ± 0.061	0.11 ± 0.044	0.10 ± 0.066	0.09 ± 0.038	0.11 ± 0.060	0.11 ± 0.072	ns	ns	ns
t9 c12 C18:2 (n6)	0.05 ± 0.033	0.07 ± 0.059	0.06 ± 0.044	0.05 ± 0.041	0.07 ± 0.048	0.06 ± 0.048	ns	ns	*
ctmix10,14,12,16 C18:2	0.09 ± 0.056	0.06 ± 0.030	0.06 ± 0.039	0.07 ± 0.042	0.07 ± 0.047	0.07 ± 0.047	t	ns	ns
t11 c15 C18:2 (n3)	0.16 [b] ± 0.097	0.25 [b]± 0.112	0.40 [a] ± 0.133	0.27 ± 0.164	0.28 ± 0.156	0.32 ± 0.152	**	ns	ns
c9,12 C18:2 (n6) (LA)	1.8 [a] ± 0.168	1.6 [a] ± 0.232	0.9 [b] ± 0.321	1.4 [a] ± 0.548	1.4 [a] ± 0.430	1.3 [b] ± 0.441	***	*	***
C19:1	0.09 ± 0.033	0.10 ± 0.054	0.10 ± 0.074	0.08 ± 0.029	0.11 ± 0.054	0.11 ± 0.080	ns	ns	t
Unknown LA2	0.09 ± 0.046	0.09 ± 0.052	0.11 ± 0.066	0.08 [b]± 0.046	0.09 [b]± 0.047	0.13 [a] ± 0.066	ns	*	ns
Unknown LA3	0.05 ± 0.025	0.08 ± 0.068	0.07 ± 0.068	0.06 ± 0.030	0.08 ± 0.062	0.08 ± 0.077	ns	ns	t
c9,15 C18:2 (n3)	0.03 ± 0.029	0.04 ± 0.024	0.04 ± 0.040	0.03 ± 0.041	0.04 ± 0.030	0.04 ± 0.029	ns	ns	ns
c12,15 C18:2 (n3)	0.05 ± 0.029	0.06 ± 0.045	0.05 ± 0.041	0.05 ± 0.041	0.06 ± 0.043	0.06 ± 0.034	ns	ns	ns
C20:0	0.15 [b] ± 0.034	0.18 [b]± 0.054	0.22 [a] ± 0.058	0.18 ± 0.046	0.20 ± 0.064	0.18 ± 0.062	**	ns	ns

Table B.1 continued. Effect of Management (Conventional, Organic and PFLA) and Month (April, July and October) on the concentrations of nutritionally relevant FA in milk (from the study in Chapter 4). Mean FA proportion ± standard deviation (SD) and ANOVA p-values for each FA, expressed as a percentage of the entire FA profile.

	Management			Month			ANOVA p-value[a]		
	Conv.	Org.	PFLA [b]	April	July	October	Man	Mon	Man x Mon
Fatty Acid	n=15	n=15	n=15	n=18	n=18	n=18			
c6,9,12 C18:3 (n6)	0.04 ± 0.029	0.07 ± 0.046	0.05 ± 0.032	0.04 [b]± 0.027	0.05[ab]± 0.031	0.07 [a] ± 0.043	ns	*	**
c8 C20:1	0.08 ± 0.033	0.11 ± 0.034	0.11 ± 0.038	0.09 ± 0.037	0.10 ± 0.039	0.11 ± 0.034	t	ns	ns
C18:3 (n3) (ALA)	0.47 [c] ± 0.079	0.79 [b]± 0.125	1.06 [a] ± 0.274	0.78 ± 0.279	0.82 ± 0.331	0.86 ± 0.353	***	ns	ns
c9 t11 C18:2 (CLA9)	0.62 [c] ± 0.071	0.92 [b]± 0.138	1.16 [a] ± 0.431	0.95 ± 0.502	0.90 ± 0.238	0.98 ± 0.348	***	ns	ns
t11 c13 CLA	0.05 ± 0.031	0.10 ± 0.053	0.09 ± 0.064	0.08 ± 0.062	0.07 ± 0.048	0.09 ± 0.059	t	ns	ns
unknown CLA1	0.05 ± 0.034	0.08 ± 0.055	0.08 ± 0.054	0.06 ± 0.035	0.07 ± 0.052	0.08 ± 0.062	ns	ns	ns
Unknown CLA2tt	0.07 ± 0.022	0.08 ± 0.052	0.07 ± 0.049	0.07 ± 0.038	0.08 ± 0.041	0.07 ± 0.053	ns	ns	ns
Unknown CLA3tt	0.06 ± 0.043	0.11 ± 0.069	0.11 ± 0.081	0.08 ± 0.069	0.10 ± 0.072	0.10 ± 0.076	ns	ns	ns
Unknown CLA6tt	0.06 ± 0.055	0.07 ± 0.065	0.07 ± 0.065	0.07 ± 0.058	0.06 ± 0.053	0.08 ± 0.072	ns	ns	ns
c9,13,15 C18:3 (n3)	0.05 ± 0.032	0.06 ± 0.027	0.06 ± 0.028	0.05 ± 0.036	0.07 ± 0.020	0.06 ± 0.026	ns	ns	*
c11,14 C20:2 (n6)	0.05 ± 0.028	0.09 ± 0.058	0.06 ± 0.032	0.06 ± 0.024	0.07 ± 0.054	0.07 ± 0.042	ns	ns	ns
c9,11,15 C18:3 (n3)	0.08 [b] ± 0.040	0.13 [a]± 0.068	0.10 [ab] ± 0.043	0.10 ± 0.047	0.11 ± 0.067	0.10 ± 0.046	*	ns	ns
C22:0	0.11 ± 0.057	0.13 ± 0.019	0.12 ± 0.040	0.12 ± 0.041	0.12 ± 0.031	0.12 ± 0.053	ns	ns	ns
c8,11,14 C20:3 (n6)	0.14 [a] ± 0.052	0.10[ab]± 0.030	0.08 [b] ± 0.036	0.10 ± 0.048	0.10 ± 0.040	0.11 ± 0.051	*	ns	ns
c13 C22:1	0.10 ± 0.059	0.08 ± 0.052	0.07 ± 0.047	0.06 ± 0.039	0.09 ± 0.057	0.10 ± 0.054	ns	t	ns
c11,14,17 C20:3 (n3)	0.07 ± 0.061	0.08 ± 0.058	0.07 ± 0.052	0.07 ± 0.046	0.05 ± 0.059	0.09 ± 0.057	ns	ns	ns
c5,8,11,14 C20:4 (n6)	0.21 ± 0.122	0.19 ± 0.090	0.14 ± 0.074	0.17 ± 0.089	0.16 ± 0.096	0.19 ± 0.110	ns	ns	ns
C23:0	0.06 [b] ± 0.033	0.10 [a]± 0.033	0.08 [b] ± 0.023	0.07 ± 0.038	0.08 ± 0.033	0.08 ± 0.025	*	ns	ns
c13,16 C22:2 (n6)	0.08 ± 0.046	0.10 ± 0.041	0.10 ± 0.030	0.10 ± 0.039	0.08 ± 0.035	0.10 ± 0.039	ns	ns	ns
c5,8,11,14,17 C20:5 (n3) (EPA)	0.09 [b] ± 0.044	0.17 [a]± 0.089	0.16 [a] ± 0.052	0.14 ± 0.082	0.14 ± 0.070	0.15 ± 0.061	**	ns	ns
C24:0	0.09 ± 0.054	0.15 ± 0.117	0.14 ± 0.094	0.12 ± 0.089	0.14 ± 0.107	0.14 ± 0.088	ns	ns	ns
c15 C24:1	0.06 ± 0.062	0.07 ± 0.042	0.05 ± 0.019	0.06 ± 0.023	0.06 ± 0.057	0.05 ± 0.039	ns	ns	ns
c13,16,19 C22:3 (n3)	0.04 ± 0.029	0.04 ± 0.025	0.04 ± 0.022	0.04 ± 0.028	0.03 ± 0.019	0.04 ± 0.026	ns	ns	*
c7,10,13,16 C22:4 (n6)	0.05 ± 0.024	0.06 ± 0.031	0.05 ± 0.036	0.05 [b]± 0.023	0.04 [b]± 0.018	0.07 [a] ± 0.041	ns	**	t
c7,10,13,16,19 C22:5 (n3) (DPA)	0.12 [b] ± 0.030	0.16 [a]± 0.067	0.18 [a] ± 0.046	0.17 ± 0.057	0.15 ± 0.054	0.15 ± 0.056	**	ns	ns

Table B.1 continued. Effect of Management (Conventional, Organic and PFLA) and Month (April, July and October) on the concentrations of nutritionally relevant FA in milk (from the study in Chapter 4). Mean FA proportion ± standard deviation (SD) and ANOVA p-values for each FA, expressed as a percentage of the entire FA profile.

	Management			Month			ANOVA p-value[a]		
	Conv.	Org.	PFLA [b]	April	July	October	Man	Mon	Man x Mon
Fatty Acid	n=15	n=15	n=15	n=18	n=18	n=18			
c4,7,10,13,16,19 C22:5 (n3) (DHA)	0.05 ± 0.062	0.06 ± 0.037	0.04 ± 0.023	0.05 ± 0.034	0.05 ± 0.058	0.05 ± 0.025	ns	ns	ns
SFA [c]	67.7 ± 0.868	66.3 ± 1.487	66.9 ± 4.536	67.7 ± 4.123	66.6 ± 2.948	66.6 ± 2.159	ns	ns	ns
MUFA [d]	27.0 ± 0.800	27.6 ± 0.972	27.1 ± 3.636	26.6 ± 3.353	27.6 ± 2.295	27.3 ± 1.522	ns	ns	ns
PUFA [e]	5.3 ± 0.412	6.2 ± 0.797	6.0 ± 1.522	5.7 ± 1.130	5.8 ± 1.091	6.1 ± 1.261	ns	ns	ns
n-3 [f]	1.2 [c] ± 0.197	1.9 [b] ± 0.374	2.3 [a] ± 0.480	1.8 ± 0.539	1.8 ± 0.590	2.0 ± 0.596	***	ns	ns
n-6 [g]	2.7 [a] ± 0.217	2.4 [a] ± 0.234	1.6 [b] ± 0.455	2.1 ± 0.633	2.1 ± 0.552	2.2 ± 0.573	***	ns	ns
n3n6	0.47 [c] ± 0.083	0.77 [b]± 0.136	1.40 [a] ± 0.202	0.96 ± 0.488	0.94 ± 0.394	1.01 ± 0.448	***	ns	t
n-6/n-3	2.2 [a] ± 0.377	1.3 [b] ± 0.230	0.7 [c] ± 0.102	1.4 ± 0.727	1.3 ± 0.665	1.2 ± 0.587	***	t	ns
EPA+DPA +DHA	0.25 [b] ± 0.086	0.39 [a]± 0.147	0.38 [a] ± 0.080	0.36 ± 0.136	0.33 ± 0.119	0.35 ± 0.105	**	ns	ns

[a] Mean values in a row, within management or month, with different letters are significantly different, according to Tukey's honestly significant difference test (P-values<0.05). [2] ***: P< 0.001, **: P< 0.01; *: P< 0.05, t: P<0.1, ns: P>0.1.

[b] Conv.= Conventional, Org.= Organic, PFLA= Pasture for Life Association, Man= Management, Mon= Month, SD= Standard Deviation.

[c] Saturated FA (SFA): C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0.

[d] Monounsaturated FA (MUFA): c9 C14:1, c9 C15:1, c9 C16:1, t9 C16:1, c9 C17:1, t6,7,8 C18:1, t9 C18:1, t10 C18:1, t11 C18:1, t12,13,14 C18:1, c9 C18:1, t15 C18:1, c11 C18:1, c12 C18:1, c13 C18:1, c14 t16 C18:1, c15 C18:1, C19:1 c8 C20:1, c13 C22:1, c15 C24:1.

[e] Polyunsaturated FA (PUFA): t11,15 C18:2 (n3), t10,14 C18:2, c9t13, C18:2, t9,12 C18:2 (n6), t8c13 C18:2, c9t12 C18:2 (n6), t9c12 C18:2 (n6), ct mix 10,14,12,16 C18:2, t11c15 C18:2 (n3), c9,12 C18:2 (n6) (LA), Unknown LA2, Unknown LA3, c9,15 C18:2 (n3), c12,15 C18:2 (n3), c6,9,12 C18:3 (n6), C18:3 (n3) (ALA), c9t11 C18:2 (CLA9), t11c13 CLA, unknown CLA1, Unknown CLA2tt, Unknown CLA3tt, Unknown CLA6tt, c9,13,15 C18:3 (n3), c11,14 C20:2 (n6), c9,11,15 C18:3 (n3), c8,11,14 C20:3 (n6), c11,14,17 C20:3 (n3), c5,8,11,14 C20:4 (n6), c13,16 C22:2 (n6), c5,8,11,14,17 C20:5 (n3) (EPA), c13,16,19 C22:3 (n3), c7,10,13,16 C22:4 (n6), c7,10,13,16,19 C22:5 (n3) (DPA), c4,7,10,13,16,19 C22:5 (n3) (DHA)

[f] Omega-3 FA (n-3): t11,15 C18:2, t11c15 C18:2, c9,15 C18:2, c12,15 C18:2, c9 12, 15 C18:3 (ALA), c9,13,15 C18:3, c9,11,15 C18:3, c11,14,17 C20:3, c5,8,11,14,17 C20:5 (EPA), c13,16,19 C22:3, c7,10,13,16,19 C22:5 (DPA), c4,7,10,13,16,19 C22:5 (DHA)

[g] Omega-6 FA (n-6): t9,12 C18:2, c9 t12 C18:2, t9 c12 C18:2, c9,12 C18:2 (LA), c6,9,12 C18:3, c11,14 C20:2, c8,11,14 C20:3, c5,8,11,14 C20:4, c13,16 C22:2, c7,10,13,16 C22:4

APPENDIX C CHAPTER 5

Table C.1. The average concentrate feed and conserved forage (kg DM/cow/day) fed during each season.

Farm	Management	Concentrate (kg DM)				Conserved forage (kg DM)			
		D1	D2	D3	D4	D1	D2	D3	D4
1	Organic	1.8	1.8	0.9	1.8	0.8	0.0	0.0	0.0
2	Organic	4.4	1.7	2.1	5.0	11.9	0.0	0.1	15.5
3	Low-Input	5.0	3.6	1.8	3.0	6.0	0.0	0.0	5.0
4	Low-Input	10.1	1.8	1.3	3.6	7.1	0.0	0.0	6.0
5	Organic	7.9	2.8	1.6	2.0	9.4	5.2	1.2	6.3
6	Low-Input	4.4	1.8	1.8	3.5	0.0	0.0	0.0	3.5
7	Organic	4.3	4.3	2.9	2.8	10.5	9.3	7.0	12.9
8	Low-Input	8.6	5.0	3.5	4.3	11.8	4.6	1.3	11.4
9	Low-Input	5.3	4.0	0.6	6.0	12.0	0.0	0.0	14.0
10	Organic	8.5	7.4	0.4	8.1	11.8	15.1	0.0	14.9
11	Low-Input	5.4	5.5	2.6	1.8	10.4	2.0	4.9	10.6
12	Low-Input	6.2	5.3	5.3	3.1	12.0	2.4	2.5	8.0
13	Low-Input	3.8	3.8	3.3	4.6	6.0	3.0	0.0	10.7
14	Low-Input	2.0	0.0	0.0	3.0	1.0	0.5	0.0	3.0
15	Organic	5.2	1.5	1.0	4.8	16.4	7.0	0.0	11.9
16	Low-Input	1.8	3.5	2.6	2.6	2.5	0.0	0.0	5.0
17	Organic	6.8	2.6	1.8	6.7	13.0	7.0	0.0	13.0
	Mean Low-Input	5.2	3.4	2.3	3.6	6.9	1.2	0.9	7.7
	Mean Organic	5.5	3.1	1.5	4.5	10.5	6.2	1.2	10.6

D1= Autumn 2011, D2=Spring, D3=Summer, D4= Autumn 2012

Table C.2. Access to pasture during the day and/or night on each farm and sampling date.

		D1		D2		D3		D4	
Farm	Management	Day	Night	Day	Night	Day	Night	Day	Night
1	Organic	Y	Y	Y	Y	Y	Y	Y	Y
2	Organic	Y	N	Y	N	Y	Y	N	N
3	Conventional	Y	Y	Y	Y	Y	Y	Y	Y
4	Conventional	Y	Y	Y	Y	Y	Y	Y	N
5	Organic	Y	Y	Y	Y	Y	Y	Y	Y
6	Conventional	Y	N	Y	Y	Y	Y	Y	N
7	Organic	N	N	N	N	Y	Y	N	N
8	Conventional	Y	N	Y	Y	Y	Y	Y	N
9	Conventional	Y	N	Y	Y	Y	Y	N	N
10	Organic	Y	N	Y	Y	Y	Y	N	N
11	Conventional	Y	Y	Y	Y	Y	Y	Y	N
12	Conventional	Y	Y	Y	Y	Y	Y	Y	Y
13	Conventional	N	N	Y	N	Y	Y	N	N
14	Conventional	Y	Y	Y	Y	Y	Y	Y	Y
15	Organic	N	N	Y	N	Y	Y	N	N
16	Conventional	Y	Y	Y	Y	Y	Y	Y	Y
17	Organic	N	N	Y	Y	Y	Y	N	N

D1= Autumn 2011, D2=Spring, D3=Summer, D4= Autumn 2012, Y= Yes, N=No

APPENDIX D CHAPTER 6

Table D.1. The 2018 timetable for each farmer and round

Name	Calving	Round 1 Start	Round 1 End	DIM*	Round 2 Start	Round 2 End	DIM	Round 3 Start	Round 3 End	DIM
A	25/3/18	25/5/18	8/6/18	61	8/8/18	22/8/18	136	22/10/18	5/11/18	211
B	20/2/18	9/4/18	23/4/18	48	22/6/18	6/7/18	122	12/9/18	26/9/18	204
C	14/2/18	25/4/18	10/5/18	70	9/7/18	23/7/18	145	2/10/18	16/10/18	230

*DIM calculated from the start date of each round and the start of calving for each farmer- this enabled data collection early, mid and late lactation for each cow.

Table D.2. Cows allocated by production efficiency (based on EDMI/Yield), One is the most efficient and Five the least, combining data from all 3 farms over the 3 dates.

	One	Two	Three	Four	Five	p-val	Sig [a]
n=	32	27	29	27	31		
SCC[b] (x103 cells/ml milk)	106 ± 150.2	139 ± 340.6	126 ± 390.5	109 ± 183.9	99 ± 214.2	0.979	ns
Days in Milk	115 ± 64.4	117 ± 70.6	123 ± 62.8	123 ± 68.1	117 ± 64.9	0.037	*
EDMI (kg/day)	18.0 ± 4.84	18.6 ± 4.25	19.0 ± 3.91	19.1 ± 3.54	20.0 ± 4.23	0.059	t
ECMY (kg/day)	24.5 ± 8.22	22.2 ± 6.90	22.6 ± 9.21	21.4 ± 7.56	21.1 ± 8.77	0.026	*
EDMI.ECMY	0.77 ± 0.172	0.88 ± 0.191	0.92 ± 0.249	0.96 ± 0.212	1.05 ± 0.338	0.000	***
Yield (Kg/day)	23.6 ± 9.04	21.9 ± 8.28	20.4 ± 7.76	19.5 ± 7.27	18.3 ± 7.83	0.000	***
EDMI.Yield	486 ± 120.1	501 ± 104.0	516 ± 80.8	524 ± 88.6	551 ±95.1	0.007	**
Eating Time (min/day)	422 ± 79.7	420 ± 85.6	449 ± 67.9	429 ± 56.9	433 ± 79.4	0.531	ns
Rumination Time (min/day)	479 ± 90.9	467 ± 99.0	522 ± 79.8	490 ± 67.8	499 ± 101.2	0.092	t
Rumination Boluses (n/day)	52.0 ± 6.62	54.4 ± 5.67	52.0 ± 7.50	54.3 ± 5.73	54.8 ± 8.23	0.150	ns
Chews per bolus	0.04 ± 0.009	0.04 ± 0.006	0.04 ± 0.007	0.04 ± 0.008	0.04 ± 0.008	0.792	ns
Eating Rate	0.04 ± 0.010	0.05 ± 0.014	0.04 ± 0.008	0.05 ± 0.009	0.05 ± 0.020	0.197	ns
Rumination rate	0.82 ± 0.213	0.93 ± 0.243	1.01 ± 0.265	1.07 ± 0.295	1.23 ± 0.396	0.000	***
Fat %	3.9 ± 1.16	3.7 ± 1.23	4.0 ± 1.14	4.2 ± 1.28	4.5 ± 1.39	0.035	*
Protein %	3.3 ± 0.36	3.3 ± 0.34	3.5 ± 0.33	3.4 ± 0.28	3.5 ± 0.32	0.000	***
Lactose %	4.5 ± 0.18	4.5 ± 0.13	4.5 ± 0.12	4.5 ± 0.23	4.5 ± 0.21	0.707	ns

Table D.2 continued. Cows allocated by production efficiency (based on EDMI/Yield), One is the most efficient and Five the least, combining data from all 3 farms over the 3 dates.

	One	Two	Three	Four	Five	p-val	Sig [a]
Fatty Acids n=	28	23	24	23	27		
C12:0	3.8 ± 0.47	3.9 ± 0.91	4.1 ± 0.86	4.0 ± 0.65	4.2 ± 1.01	0.282	ns
C14:0	11.7 ± 0.97	11.7 ± 1.43	11.9 ± 1.06	12.0 ± 1.08	12.0 ± 1.25	0.752	ns
C16:0	28.3 ± 4.20	28.4 ± 3.01	28.2 ± 3.39	28.5 ± 3.76	30.5 ± 4.93	0.083	t
VA	2.4 ± 0.89	2.4 ± 0.85	2.2 ± 0.64	2.8 ± 1.20	2.2 ± 0.99	0.137	ns
OA	19.1 ± 3.13	19.6 ± 2.79	18.9 ± 2.73	18.7 ± 2.58	17.5 ± 2.67	0.079	t
LA	1.3 ± 0.41	1.3 ± 0.37	1.2 ± 0.37	1.2 ± 0.43	1.2 ± 0.40	0.600	ns
ALA	0.86 ± 0.214	0.83 ± 0.223	0.82 ± 0.206	0.83 ± 0.217	0.75 ± 0.171	0.439	ns
CLA.9	1.0 ± 0.35	1.1 ± 0.35	0.9 ± 0.21	1.2 ± 0.53	0.9 ± 0.36	0.175	ns
SFA	67.3 ± 4.58	66.9 ± 3.75	68.0 ± 3.42	67.7 ± 3.91	69.7 ± 3.87	0.056	t
PUFA	5.4 ± 0.99	5.3 ± 0.90	5.1 ± 0.76	5.3 ± 1.06	4.9 ± 0.96	0.144	ns
MUFA	27.3 ± 3.81	27.8 ± 3.15	26.9 ± 3.18	27.0 ± 3.11	25.4 ± 3.27	0.083	t
n3	1.8 ± 0.36	1.7 ± 0.40	1.7 ± 0.34	1.7 ± 0.36	1.6 ± 0.31	0.238	ns
n6	1.9 ± 0.53	1.9 ± 0.47	1.8 ± 0.43	1.8 ± 0.43	1.8 ± 0.57	0.380	ns
n6/n3	1.1 ± 0.33	1.1 ± 0.32	1.1 ± 0.30	1.1 ± 0.30	1.1 ± 0.37	0.715	ns
EPADPADHA	0.28 ± 0.048	0.29 ± 0.068	0.28 ± 0.064	0.28 ± 0.069	0.28 ± 0.048	0.875	ns

[a] Mean values for PE where there is a difference between means (P-values<0.05). ***: P< 0.001, **: P< 0.01; *: P< 0.05, t: P<0.1, ns: P>0.1.

[b] SCC=Somatic Cell Count, EDMI= Estimated Dry Matter Intake, ECMY= Energy Corrected Milk Yield, ECMYP= Energy Corrected Milk Yield with Premium, EDMI/ECMYP= 'Production Efficiency' ratio of Estimated Dry Matter Intake to Energy Corrected Milk Yield with Premium, C12:0=Lauric Acid, C14:0=Myristic Acid, C16:0=Palmitic Acid, VA= Vaccenic Acid, OA= Oleic Acid, LA= Linoleic Acid, ALA=alpha-linolenic acid, CLA9=Conjugated linoleic acid (C18:2, c9t11 isomer), SFA= Saturated Fatty Acid, PUFA=Poly-Unsaturated Fatty Acids, MUFA= Mono-Unsaturated Acids, n-3=omega-3, n-6=omega-6, n-6/n-3=, EPADPADHA=Eicosapentaenoic Acid +Docosapentaenoic Acid + Docosahexaenoic Acid.

Table D.3. Background information on all cows included in the halter study on participant farms.

ID	Dam	Sire	D.O.B	Parity	Calved 2018	No. of Services*	Treatments
Farm A							
400	JEX	NR	07/03/2014	3	24/03/2018	1	None
404	JEX	MSD	09/03/2014	3	24/03/2018	1	None
422	JEX	JEX	09/04/2014	3	25/03/2018	1	None
426	JEX	JEX	15/03/2014	3	08/04/2018	1	None
430	JEX	SDX	16/03/2014	3	04/04/2018	1	None
443	AYR	JEX	20/03/2014	2	31/03/2018	1	None
451	JEX	RP	07/04/2014	3	03/04/2018	1	None
454	JEX	JEX	22/03/2014	2	06/04/2018	1	None
457	JEX	JEX	23/02/2014	3	09/04/2018	1	None
464	JEX	JEX	23/04/2014	2	25/03/2018	1	None
470	JEX	RP	26/03/2014	3	05/04/2018	1	None
474	JEX	JEX	26/03/2014	2	16/03/2018	1	None
475	JEX	JEX	26/04/2014	2	29/03/2018	1	None
485	JEX	NR	31/03/2014	3	24/03/2018	1	None
487	JEX	NRX	01/04/2014	3	01/04/2018	1	None
493	JEX	JEX	05/04/2014	3	30/03/2018	1	None
495	JEX	JEX	22/03/2014	2	01/04/2018	1	None
4405	JEX	RP	17/04/2014	3	11/04/2018	1	None
4406	JEX	JEX	18/04/2014	3	22/03/2018	1	None
4411	JEX	AYR	22/04/2014	3	12/04/2018	1	None
Amy	JEX	MSD	27/04/2013	1	31/03/2018	1	None
Splodge	JEX	MSD	22/03/2014	1	02/04/2018	1	None
Toast	JEX	MSD	24/03/2014	1	28/03/2018	1	None
Farm B							
1908	BF	BF	31/01/2014	3	09/03/2018	1	None
1914	DS	DS	03/02/2014	3	03/03/2018	1	None
1917	BF	BF	04/02/2014	3	18/03/2018	1	None
1931	DS	BF	07/02/2014	3	28/03/2018	1	None
1940	BF	HF	09/02/2014	3	15/03/2018	1	None
1941	DS	DSX	09/02/2014	3	16/03/2018	1	None
1944	DSX	SIM	01/02/2014	3	15/03/2018	1	None
1952	BF	DS	13/02/2014	3	19/03/2018	1	None
1969	DS	DS	19/02/2014	3	12/03/2018	1	None
1976	BF	DS	23/02/2014	3	26/02/2018	1	None
1981	BF	BF	24/02/2014	3	22/02/2018	1	None
1996	BF	DSX	28/02/2014	3	19/02/2018	1	None
2082	DSX	JE	28/01/2015	2	21/03/2018	1	None
2131	BF	JE	13/02/2015	2	28/02/2018	1	None
2145	BF	NXHF	15/02/2015	2	25/03/2018	1	None
2147	NZHF	NZHF	15/02/2015	2	20/03/2018	1	None
2157	HF	NRX	17/02/2015	2	28/02/2018	1	None
2176	DSX	NZHF	24/02/2015	2	12/03/2018	1	None
2178	DS	NRX	24/02/2015	2	23/03/2018	1	None

Table D.3 continued. Background information on all cows included in the halter study on participant farms.

ID	Dam	Sire	D.O.B	Parity	Calved 2018	No. of Services*	Treatments
2183	HF	MBX	26/02/2015	2	23/03/2018	1	None
2198	BF	MBX	02/03/2015	2	27/02/2018	1	None
2205	BF	JE	08/03/2015	2	01/03/2018	1	None
2216	BF	BS	14/03/2015	2	17/03/2018	1	None
Farm C							
602	HF	HFNZ	10/02/2014	3	24/02/2018	2	None
610	HFCZ	HFNZ	06/02/2014	3	05/03/2018	1	None
615	HF	HFNZ	08/02/2014	3	02/04/2018	1	None
619	HFNZ	NR	02/02/2014	3	16/02/2018	2	Eye infection
620	HFNZ	HFNZ	05/02/2014	3	17/02/2018	3	CIDR
623	HF	HF	05/02/2014	3	07/03/2018	1	Metacam (Splits), TB-CULL
624	HFNZ	HFNZ	06/03/2014	3	09/02/2018	1	None
625	HF	HFNZ	10/03/2014	3	14/02/2018	3	None
626	HF	HFNZ	21/02/2014	3	15/03/2018	2	CIDR
630	HF	ACS	25/03/2014	3	26/03/2018	2	CIDR, TB-CULL
635	HFNZ	HFNZ	15/03/2014	3	24/03/2018	3	None
637	HFMB	HFNZ	22/03/2014	3	30/03/2018	1	Metritis, Lame
638	HF	HFNZ	07/03/2014	3	05/04/2018	2	None
644	HFCZ	HF	27/02/2014	3	23/03/2018	1	None
647	HF	HFNZ	10/03/2014	3	05/03/2018	2	Lame, Mastitis x2
650	HFNZ	HFNZ	13/02/2014	3	26/03/2018	1	None
651	HFCZ	HFNZ	01/03/2014	3	28/02/2018	2	None
655	HF	ACS	18/03/2014	3	19/02/2018	1	None
656	HF	ACS	19/02/2014	3	20/02/2018	1	None
663	HFNZ	HFNZ	12/02/2014	3	23/02/2018	1	None
664	HF	HFNZ	26/02/2014	3	11/03/2018	1	Eye infection
677	HF	NR	06/03/2014	3	23/02/2018	1	None
688	NR	NR	03/02/2014	3	26/02/2018	1	Mastitis, TB re-test

*Number of services 2017, held to calving 2018.

APPENDIX E CHAPTER 7

Table E.1. Models used to predict methane yield based on milk FAs. Models extracted from van Engelen *et al.* (2015).

CH4	Equation	Model Performance
Model 1 (g/kg of DMI)	21.0 (±1.09) + 10.5 × iso C16:0 (±2.32) − 2.7 × cis-11 C18:1 (±0.54) − 0.8 × trans-10 C18:1 (±0.13) − 1.1 × cis-9,cis-12 C18:2 (±0.28)	R^2 = 0.82
Model 2 (g/kg of DMI)	28.60 − 1.13 × C4:0 + 0.36 × C18:0 – 2.57 × C18:1 trans-10+11 – 9.29 × C18:1 cis-11.	R^2=0.70

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