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An Investigation of Red Deer Milk in New Zealand and the Implications for Future Production

A Dissertation
submitted in partial fulfilment
of the requirements for the Degree of
Bachelor of Agricultural Science with Honours

at
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by
Abbey Marie Dowd

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The development of a deer milking industry in New Zealand to produce niche and gourmet products for growing markets has been explored. Studies are lacking on the characteristics of deer milk, including whether there are seasonal changes in its content and how it compares to other animal milks. This study aimed to characterise the fatty acid (FA) profile of red deer milk from hinds grazing lucerne at Aratiatia, Taupō, New Zealand. Milk samples were collected from 43 red deer hinds in March, with a second sample collected from 22 of the original hinds collected in April. These milk samples were analysed using FAME evaluation to determine the FA profiles of individual deer. The samples were compared between mid- and late-lactation, as well as a comparison being made to the FA profiles of dairy cows in New Zealand. Large variations in FA proportions were observed between deer and the proportion of dietarily desirable FAs such as conjugated linoleic acid (CLA) within the sampled population. This suggests that future selection for deer with desirable fatty acid profiles may be possible, but the practicality of doing this within an evolving industry may be challenging. All of the deer milk FA proportions measured differed significantly between mid- and late-lactation (all $P < 0.05$). The changes observed tended to be opposite to that typically seen for cows' milk in New Zealand, and it is suggested that the changes may be due to increased fat content and changes in energy balance towards the end of lactation observed in cervid species. The skewed distribution of essential fatty acids suggested a 'baseline physiological limit'. This may be due to the lesser extent of domestication of the species and the requirements of the neonate. When the deer FA profiles were compared to cows' milk, many differences were observed. Deer milk had higher proportions of short-chain fatty acids SCFAs, lower proportions of long-chain fatty acids (LCFAs) and higher proportions of polyunsaturated fatty acids (PUFAs) relative to cows' milk ($P < 0.05$). Deer milk also had higher proportions of the omega-3 FA group and reduced proportions of conjugated linoleic acid (CLA) compared to cows milk ($P < 0.05$). Using some of these findings to our advantage, deer milk could be marketed as a 'healthy product' containing higher levels of omega-3 relative to other animal milks. The differences observed between individual deer over the two stages of lactation may allow for further manipulation of the milk to obtain more desirable FA profiles, but at this stage of development of the industry, more focus may be placed on increasing production and developing

markets. Further research is required to confirm some of these findings and could be directed towards further characterisation of deer milk FA profiles, including how feed and the stage of lactation affects the contents of deer milk.

Keywords: fatty acids, milk, seasonal variation, animal variation, *Cervus elaphus*, *Medicago sativa* dairy industry, New Zealand

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Chapter 1

Introduction

Deer were first introduced to New Zealand as a game animal in the 1880's. They flourished in the New Zealand environment and numbers quickly multiplied in the wild. Early deer industry pioneers recognised the opportunity of the species, capturing and bringing them to land to be farmed. These deer were farmed for their venison, velvet and co-products with strong markets being established around the globe. Currently there are around 840,000 deer being farmed across New Zealand.

The New Zealand dairy industry is well established. Although predominately cattle; the sheep and goat industries are also involved in both domestic and export markets. New Zealand is well known for its dairy production and demand for the countries produce is high. Other novel dairy industries including a buffalo milk industry have also been established in New Zealand.

The establishment of a viable deer milking industry in New Zealand has been explored. There are currently three farms milking deer throughout the country. Products have been developed that are sold in domestic markets, as well as the formation of export markets. The demand for dairy products is expected to continue rising, as well as the demand for products produced in pasture-based systems as they are deemed to be 'clean and green'. The global reputation of deer products from New Zealand is high, and this along with the high regard that our dairy cattle systems are held in, may allow for the formation of a successful new industry.

The FA profile of dairy products have become increasingly important. Consumers are becoming more aware of the effects of different FAs on their health. This includes beneficial FAs and desirable FA proportions in food. The FA profile also impacts the properties and processing of end-products. Therefore, when establishing a new industry, it is important to gain insight into FA profiles and how these may affect industry development. This potentially includes the manipulation and selection of animals to achieve desirable outcomes.

Non-cattle milks are viewed positively as a potential source of human nutrition. Due to the perception of them having health benefits, they are of interest to consumers and to industry. However, investigations into the characteristics of alternative milks are rare and only small amounts of research has been undertaken to understand both the nutritional and physiochemical properties of these milks. Studies into the characteristics and composition of deer milk are lacking. This includes the effects of diet, stage of lactation and individual animal factors.

Accordingly, this study aimed to characterise the FA profiles of red deer grazing lucerne (*Medicago sativa*) in Taupō, New Zealand. It reveals individual animal variation in milk FA profiles and seasonal changes in FA profiles over mid- to late-lactation. The data from this investigation was then compared

to data sourced from 407 Holstein-Friesian x Jersey (HFxJ)-cross dairy cows of 3 to 10 years of age grazing a perennial ryegrass and white clover pasture (Li *et al.*, 2020). This allowed for a baseline comparison between deer and cow's milk.

Chapter 2

Review of literature

A review of the New Zealand deer industry, its potential to produce milk and characterisation of the fatty acids (FA) in deer milk

Seven species of deer were ultimately introduced to New Zealand as game animals for hunting, with introduction starting in the 1880's. The first deer brought to the country originated from England and Scotland (Wiklund *et al.*, 2014), and because there were no natural predators and the climate was typically temperate, established populations soon flourished. The populations soon reached levels at which they were deemed pests as they were causing damage to crops and native forests. The introduced species were then targeted by cullers on the ground and in and helicopters, with some of the meat (venison) being collected and consumed or sold. In the 1960s an export industry was established as a value proposition emerged around the harvested wild meat.

It was at this stage that some of the early industry pioneers recognised the potential for farming deer. Techniques were developed and adapted to suit helicopter use in New Zealand's mountainous terrain, and this enabled the live capture of deer. These were transported to pastoral farmland, often in the foothills, to be farmed. By the 1970s deer were regularly being captured and the establishment of commercial deer farms began in earnest. These first farmed deer for their venison and velvet production, with the main venison market being Europe during the winter months, where people prefer to eat the meat of game animals. The velvet was mainly exported to Asian traditional medicine markets that perceive velvet to be a health product with many benefits. Majority of the deer farmed are *Cervus elaphus* (red deer), with smaller numbers of *Cervus canadensis* (Wapiti) and *Dama* (fallow deer).

Deer farming continued to spread throughout New Zealand. The pioneering deer farmers began to use knowledge and genetics derived from Europe and North America to develop their stock, which led to gains in production, and profitability as the markets grew (Wiklund *et al.*, 2014). Currently there are around 840,000 deer farmed over 1,500 farms in New Zealand, making it one the largest farmed deer populations in the world (Shadbolt *et al.*, 2008).

The market for deer products

Some of the venison produced in New Zealand is sold into the domestic market, but majority is exported overseas with Germany, the United States of America, China, and Belgium being the major offshore markets. In the Northern Hemisphere autumn, the game market will pay a premium for chilled meat (Archer & Amer, 2009), but this traditional market is highly seasonal. Market variation

creates industry vulnerability; hence emphasis has been placed on the development and diversification of markets to achieve price consistency and premiums.

Deer velvet is also an export product with approximately 200,000 velveting stags on New Zealand farms (<https://www.deernz.org/home/our-great-products/deer-velvet/>, Deer Industry New Zealand, accessed 2022). This makes New Zealand the world's largest exporter of both farmed venison and velvet. High regard is held for New Zealand deer products, with the emphasis in marketing those products being placed on the natural, extensive, pastoral grazed nature of deer farming.

In 2021, New Zealand venison exports were worth \$155 million NZD, with 14,542 tonnes of product exported. Prior, to COVID-19 venison exports earned \$198 and \$188 million for 2018 and 2019 respectively. Velvet reached an export value of \$79 million NZD in 2021. Co-products such as pizzles, tails and sinews, as well as hides and leather, also have value propositions and are also exported, earning NZ\$24 million in 2021 (<https://www.deernz.org/home/deer-industry-new-zealand/statistics/>, Deer Industry New Zealand, accessed 2022).

Average farmed venison prices peaked in 2018 with the schedule for hot carcass weight (\$/kg) declining from \$10.64 per kilogram to \$7.60 per kilogram in 2020 and \$5.72 per kilogram in 2021 (<https://www.deernz.org/home/deer-industry-new-zealand/statistics/>, Deer Industry New Zealand, accessed 2022). Price trends for velvet also peaked in 2018, going from an average of \$132.50 per kilogram in 2018 to \$102.5 per kilogram in 2021 (<https://www.deernz.org/home/deer-industry-new-zealand/statistics/>, Deer Industry New Zealand, accessed 2022).

Venison production

Globally, New Zealand is the largest source of farm-raised venison (Verkhoturov *et al.*, 2022). The meat is higher in iron and protein content than other farmed red meats (Serrano *et al.*, 2019), with low intramuscular fat levels (Volpelli *et al.*, 2003) and a high polyunsaturated fatty acid (PUFA) content (Bureš *et al.*, 2015). It is sold as a premium meat, creating a niche market for fine dining and as a healthy lifestyle option. The New Zealand deer industry has marketed its products in ways that fulfil this niche.

Velvet production

Male deer develop antlers within their first year of life. These are generally removed prior to being transported (for slaughter) due to safety regulations (Ward *et al.*, 2014). In New Zealand trained personnel can remove the antler when it is in the immature stage, that is as velvet. Velvet is used as an important Asian traditional medicine and health product, with claims that it can treat various diseases and provide health benefits (Je *et al.*, 2010). This demand creates a value proposition for the harvested antler and has resulted in the development of specific velvet-farming systems. The top market for

velvet export is China, followed by Korea. In 2018, velvet export values reached NZ\$42 and NZ\$20 million NZD for the countries respectively.

Deer have been bred and selected for meat and velvet producing qualities for many years and remarkable productivity gains have been made in increasing velvet weight due to the high heritability of the trait (0.45-0.85 heritability estimates; Ward *et al.*, 2014; Van den Berg & Garrick, 1997). Breeding and genetic evaluation programmes are well established, and artificial insemination and embryogenic transfer programmes are commonly used on deer farms throughout New Zealand. There is also a genetic database known as DeerSelect, which contains trait data and pedigree records for a range of established breeding values (<https://www.deernz.org/deer-hub/breeding/deer-select/>, Deer Industry New Zealand, accessed 2022).

The reproductive performance of New Zealand deer

The deer farmed in New Zealand are seasonal breeders. They have a programmed seasonal physiology, paired with a short mating season, which results in them giving birth at a time of the year that allows for optimal survival and offspring growth (Asher, 2011). The seasonal reproductive cycles are affected by photoperiod changes that are recognised endogenously, and mating activity is usually initiated by shortened day lengths (in autumn). In New Zealand, deer enter a period of hyper-sexual activity over a 4–5-week period (the rut period), with this occurring through March to April (Lincoln, 1992), and this typically results in parturition occurring in November/early summer.

Most inefficiencies in the reproductive performance of deer occur between birth and weaning. Pregnancy rates are generally relatively high, with Patel *et al.* (2018) describing how 85.8% of 22,130 yearlings and 93.3% of 36,223 mixed age hinds were pregnant by late-autumn. Pre-parturition foetal losses are also relatively low with Campbell *et al.* (2000) reporting an average 1% abortion rate in fifteen mixed-age deer herds, and Patel *et al.* (2018) demonstrating rates of 2.8% and 1.2% for yearling and mixed age hinds respectively. Although, calving rates are high in deer, weaning rates (post-natal calf mortality) can often reveal signs of lost reproductive potential (Asher & Wilson, 2011; Audige *et al.*, 1999). Weaning rates have been demonstrated to vary between 78-88% (Subharat *et al.*, 2011), with Deer Industry NZ (2017) setting a 93% target for weaning percentage.

While venison and velvet are currently key exports for the deer industry, pressure is mounting to investigate other market opportunities for deer-derived products. Among these opportunities, the production of deer milk is emerging as an area of interest. Like all mammals, deer produce milk to feed their young, but little is known about that milk. To better understand this opportunity, one must first understand a little more about milk production systems in other livestock in New Zealand, and the opportunities and benefits that might be derived from commercial deer milk production.

The consumption of dairy products from different livestock species and the dairy industry in New Zealand

Milk, especially human milk, is an excellent nutritional source for humans, and particularly in the early years of life. It contains water, FAs, proteins, carbohydrates, vitamins, and minerals (Wang *et al.*, 2017). Around 20-28% of human protein requirements for those living in western countries are met by milk and dairy products (Vissers *et al.*, 2011). Consumption of animal milk has occurred throughout recorded human history. There is documented evidence of consumption occurring around 1000-2000_{BCE} (Prasad, 2017; Wolf *et al.*, 2008) and the earliest reliable records of dairy production, indicate details of herd size and cheese and butter production around 4000_{BCE} (Toussaint-Samat, 2009). The earliest evidence of the domestication of sheep dates around 9000_{BCE} in Iraq and Romania, with cattle thought to be domesticated between 7000 and 8000_{BCE} (Wolf *et al.*, 2008). Milk was likely harvested and consumed from these animals.

The New Zealand dairy cattle industry

Following the development of refrigeration in the 1880s, which created the ability to export products, the New Zealand dairy cattle industry developed rapidly. The first dairy factories opened in the mid-1880s, with both butter and cheese being produced. In 1920, there were approximately six hundred dairy factories in New Zealand, of which 85% were cooperatively owned (<https://teara.govt.nz/en/dairying-and-dairy-products/page-3>, Te Ara, accessed 2022). Subsequently, increased efficiencies resulted in larger factories being built and the merge of the cooperatives. Today, cattle are the dominant dairy industry in New Zealand, contributing to around 3% of gross domestic product (GDP; albeit this only reflects the pre-farm gate industry), and accounting for 20% of total exports (TDB Advisory, 2020).

Dairy cattle numbers in New Zealand remained stable from the 1970s to 2000s (around 3-4 million cows), but from 2000 numbers increased reaching around five million in 2014-2016, before declining to around 4.9 million in 2021 (Dairy NZ & LIC, 2021). In the 2020-21 season Dairy NZ & LIC, (2021) reported that dairy companies processed 21.7 billion litres of milk, a 2.6% increase from last season, despite a 0.36% decrease in the cow population. The average milk production per cow was 397 kilograms of milk solids (222 kg milkfat, 175 kg protein; Dairy NZ & LIC, 2021).

Due to New Zealand's temperate climate, pasture remains the predominate feed for the dairy cattle industry. This creates a competitive advantage due to the typically lowered cost of livestock feeding. It also contributes to the 'brand,' creating a value proposition for New Zealand dairy products. However, growing environmental regulations and changing consumer demands, has resulted in increasing pressure being exerted on New Zealand dairy cattle farmers. The environmental impacts of dairy cattle are well documented (Foote *et al.*, 2015; Clark *et al.*, 2007), and the high nitrogen loading of urine patches and methane emissions from dairy cattle are of particular concern. Accordingly, alternative

dairy animal industries may provide opportunities for the New Zealand dairy industry to extend its 'clean and green' producer image.

The New Zealand dairy sheep industry

Sheep milking industries are well developed in countries around the world, such as in China, Turkey, and France. In 1979, approximately 1,087 million ewes were being milked globally producing approximately 7,250 billion litres of milk (Mills, 1982). New Zealand's history as pioneers in developing a major sheep industry and as an international leader in bovine dairy production, highlighted the potential to develop a competitive sheep dairy industry, but the development of a commercial industry in New Zealand has only occurred recently.

In the 1990s East Friesian milking sheep were imported to New Zealand, initiating the commercial 'set-up' phase of a dairy sheep industry. Following this, the industry has fluctuated due to challenges with its scale, the instability of supply, a lack of operator experience and limited domestic consumption (Griffiths, 2014). In 2014, there were five dairy sheep operations in New Zealand (Peterson & Prichard, 2015), and increasing interest in sheep dairy has been generated due to changing consumer demands and other economic and environmental factors (Lees & Lees, 2016).

Sheep milk products occupy around a 1.4% share of the global dairy market, but it has been estimated that the demand for sheep dairy products is growing by around 10-20% each year (Downie & Melrose, 2014). Small producers may supply cheese and milk to niche markets, with larger scale operators recognising opportunities in the milk powder market. Dairy plants have been established in New Zealand that process milk powder, which is then exported to Indonesian, Chinese, Taiwanese, and Korean markets. The growing demands of health-conscious consumers has accelerated demand for sheep dairy products. Sheep milk has been identified to have properties more favourable for cheese, yoghurt, and butter manufacture, as well as higher calcium and Vitamin D levels relative to cows' milk (Downie & Melrose, 2014).

The New Zealand dairy goat industry

The global dairy goat industry is expanding. From 2007 to 2017 the world dairy goat population increased by around 22% (Miller & Lu, 2019). In New Zealand dairy goat numbers have been estimated at around 66,100 goats over ninety-two farms (Scholtens *et al.*, 2017). Demand for dairy goat products in both traditional and new markets is rising. Like sheep milk, the nutritional benefits relative to cows' milk have been identified (Haenlein, 2004).

Unlike other ruminant production systems in New Zealand, dairy goats are predominately managed in housed systems (Gautam, 2012). They are typically fed fresh forage, which is either brought onto farm or 'cut and carried' to the barns (Scholtens *et al.*, 2017). Although production in New Zealand is

insignificant relative to world production (51,127 tonnes in 2016; Smith *et al.*, 2017) verses (18,340,016 tonnes worldwide in 2014; FAO, 2014), New Zealand developed the first goat milk infant formula in 1988 and continues to be a world leader in goat milk powders (Stafford & Prosser, 2017). Exported products mainly comprise nutritional formula, milk powder and goat milk tablets. The New Zealand Dairy Goat Cooperative manages around 80% of the nation's goat population and it is considered by some to be the leading international manufacturer of goat milk powders. The industry targets access to niche markets producing high value products.

The New Zealand dairy buffalo industry

The New Zealand dairy buffalo industry comprises two small-scale farms. The first farm was established in 2007 and operates with 200 buffalo producing a range of cheeses and yoghurts (<https://www.clevedonbuffalo.co.nz/>, Clevedon Buffalo, n.d.). Another farm also exists, targeting local domestic niche markets. Although, an overseas export market has not been developed, buffalo milk has also been found to contain more protein and calcium than cows' milk, and less cholesterol. It has also been found to be a popular alternative for those who suffer from other dairy intolerances (<https://wairiribuffalo.nz/stockists-farmers-markets/>, Wairiri Buffalo, 2022). Farm owners have reported high demands for their buffalo milk products, both from restaurants and local customers (The Country, 2017).

The potential for novelty milks and milk products

Dairy markets and consumption

World dairy production accounted for around 887 million metric tonnes of milk in 2021 with approximately 81% cow milk, 15% buffalo milk and 4% for goat, sheep, and camel milk (OECD & FAO, 2022). As incomes and populations increase globally, more dairy products are expected to be consumed (Figure 2.1). The FAO estimates the *per-capita* milk consumption in the developing world will increase by 1.3% a year between 1999 and 2030; an increase of 50% over 30 years (Rischkowsky & Pilling, 2007). The increase in import demand from Asian countries is driven by economic and population growth, with a shift towards livestock products. Global milk production is estimated to grow at 1.8% per annum over the next decade to 2031 (OECD & FAO, 2022).

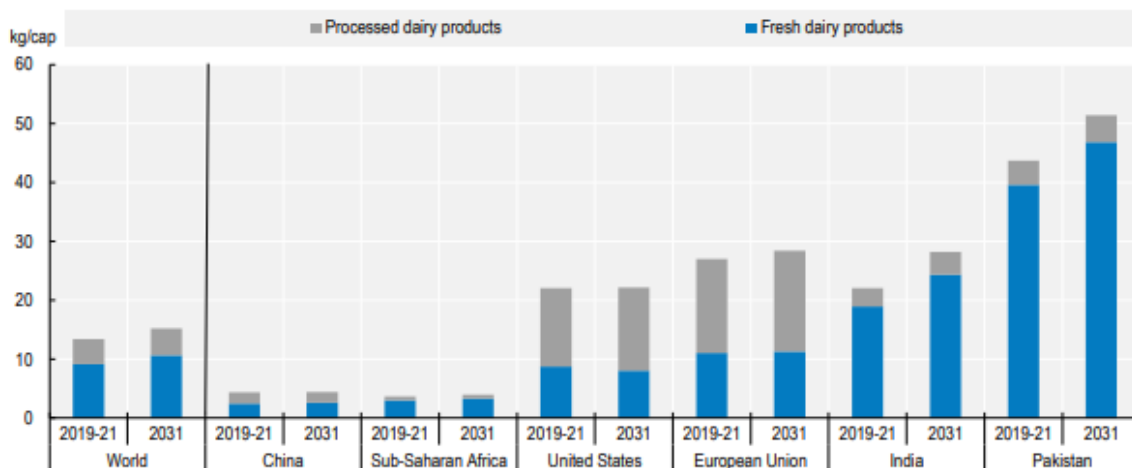


Figure 2.1. Global per-capita consumption of processed and fresh dairy products in milk solids
Source: OECD & FAO, 2022

New Zealand has been able to supply this growing market demand. Although it only produces 2.5% of world milk, it is the most export-orientated country (OECD & FAO, 2022). Dairy export revenue and the export market growth rate has correspondingly risen in recent decades (Figure 2.2). New Zealand is the primary source of butter and whole milk powder on the international market, with the market shares estimated to be around 39% and 58% respectively (OECD & FAO, 2022).

From 2015 to 2022 the price of butter has been higher than skim milk powder. This has been attributed to the stronger demand in international markets for milk fat relative to other milk solids. The demand for products high in dairy fat, such as full-fat milk and cream, could be attributed to recent studies highlighting the health benefits of dairy fat consumption, and contrary to what was believed in the 1990-2000s (OECD & FAO, 2022).

Image removed for Copyright compliance

Figure 2.2. Average annual growth rate in dairy exports from New Zealand, 2006-2016
Source: Ballingal & Pambudi, 2017

New Zealand product quality and reputation

As a consequence of New Zealand's temperate climate, young and fertile soils, and water availability, it has a competitive advantage in dairy farming. Allowing animals to access pasture year-round not only ensures the supply of cheap forage, but it also lays a good foundation for the marketing of the dairy products produced. High regard is held for the quality of New Zealand dairy products derived from pasture-fed systems. Findings, such as reports that milk from cows eating pasture-based diets relative to concentrates have higher levels of beneficial FAs such as conjugated linoleic acid (CLA) as well as being richer in omega-3 fats and beta-carotenes (Dhiman *et al.*, 1999), further reinforce the brand.

New Zealand deer products are also viewed positively in overseas markets (Shadbolt *et al.*, 2008). In Asian markets, deer products are commonly found in products promoting wellness, strength, and immunity (Fraser, 2009). The properties and perceptions of New Zealand deer products have resulted in markets being established for pet food, athletes, and the aging population, but new demand is growing from customer, such as young professional working adults.

Overseas novelty dairy industries

Although cow's milk has replaced other forms of milk in many areas of the world, novelty milks remain a staple diet for communities around the world. Industrial scale dairy operations have been developed to deliver camel and goat milk from remote villages to retailers and neighbouring countries such as Mauritania and Africa (Mathias & Mundy, 2010). Reports on the health benefits has driven the potential for camel milk to be a growing market for Western countries. However, this has been unsuccessful due to the remoteness, cost of transportation and perishability of the milk. Research is continuing with the hopes of developing a niche market in Europe.

The demand for high quality nutritious dairy products is expected to continue increasing. New Zealand's reputation and competitive advantage give a prime opportunity to develop niche markets that focus on high-value products. Therefore, the success of venison/velvet production and bovine/ovine dairy provides an opportunity to adapt these capabilities to develop an innovative deer dairy market.

The current deer milk industry

Deer milk is considered to be a highly nutritious product that has elevated levels of essential vitamins and minerals. It has higher levels of protein and calcium compared to cow's milk and is rich in vitamin A (<https://deermilkingnz.com/our-research>, Deer Milking NZ, n.d.). The use of reindeer milk, the products formed from it and milking habits are of great importance to the reindeer peoples of the northern Eurasia and taiga regions (Holand *et al.*, 2017; Mirov, 1945). Reindeer have been milked for

over two-thousand years in the Taiga region of eastern Siberia, further spreading to nearby ethnic groups in Russia, Mongolia, and China (Fondahl, 1989).

As New Zealand has one of the largest populations of farmed deer in the world, this makes it economically viable to investigate and potentially exploit new products (Wang *et al.*, 2017). It has been recognised that there is the potential for a market of deer milk, but the commercialisation of deer dairy herds is a new concept. There are currently only three known commercial deer milking operations in New Zealand.

Of these, Pāmu, a New Zealand State-Owned Enterprise (SOE) is the first enterprise to commercially raise deer for their milk (Nosowitz, 2018). There is currently one farm in Southland run by the McIntyre's (<https://pamunewzealand.com/deer-milk>, Pāmu, n.d) and one based in Taūpo. Deer Milking New Zealand Limited, in Lincoln Hills, Canterbury, is the third farm owned by Mark Faulks and Graham Carr (Williams, 2019).

The deer from these three farms are typically milked once a day and the milking season runs from January to May (Williams, 2019). Each hind may produce around a couple hundred litres of milk per season (Nosowitz, 2018), and this low level of production and short season means that prices are expected to be high for deer milk and products.

New Zealand deer milk is typically dried to powder and formulated into products. The milk powder produced is said to be nutritionally superior and that it is an exclusive and rare ingredient (<https://pamunewzealand.com/deer-milk>, Pāmu, n.d.). Currently the milk from the hinds at Lincoln hills is used for a range of products. Milk from Lincoln Hills is sent to cheese-makers and made into deer cheese such as gouda and Harvati (Williams, 2019). The potential for formation of cosmetic products using deer milk has also been realised. People involved in handling the milk discovered it had skin smoothing properties, and this has led to the development of beauty products, such as face cream.

Pāmu currently supply milk to restaurants for desserts as well as to cosmetics manufacturers. They have signed a partnership with Yuhan to supply Pāmu deer milk for their range of cosmetic products (called Deerest) in the Korean beauty market (Skerrett, 2019). While the product has been developed into many markets, it remains a niche high-value ingredient, but there is potential for the product to be developed further (Durrell, 2021).

Many parts of Asia have strong affinities with deer. In China there are historical references to deer milk, with the milk often being saved for the royals and acknowledged for its healing properties (Koe, 2021; Durrell, 2021). There are also ongoing studies onto the nutritional benefits of deer milk that can

also contribute to growing demands and may lead to the commercialisation of a product that could lead to a new flourishing industry in New Zealand (Koe, 2021).

Deer milk composition

The nutrient compositions of milk among deer breeds varies. Red deer milk has been found to range from 6.8-10.6 grams of protein per 100 grams of whole milk, with fallow and reindeer ranging from 6.5-8.0 g/100 g and 7.6-11.1 g/100 g respectively (Table 2.1). This suggests reindeer have a slightly higher concentration of protein in their milk.

Milk fat content among species also differs. Studies examining the fat content of deer milk are more challenging to interpret. The fat content of milk varies markedly depending on the stage of lactation, the animal’s diet, and its physiological status (Wang *et al.*, 2017). Literature has also tended to focus on the fat content of deer milk from wild deer, but one report suggests that deer milk has a mean fat content of around 11 grams of total fat per 100 grams of milk, which is high relative to other mammals (Malacarne *et al.*, 2015). Red deer were found to have higher fat concentrations (7.0-19.7 grams of fat per 100 grams of whole milk) relative to both fallow and reindeer (Table 2.1). Within subspecies variation was also high in the red deer fat levels. Lactose levels have been found to be similar between the different subspecies, ranging from around 2.5-6.2 grams pf lactose per 100 grams of whole milk (Table 2.1).

Table 2.1. Nutrient composition (g/100 g) in milk from different deer subspecies

Subspecies	Protein g/100 g	Fat g/100 g	Lactose g/100 g	Reference
Red deer	6.8 - 10.6	7.0 – 19.7	2.6 – 6.2	Malacarne <i>et al.</i> , 2015, Gallego <i>et al.</i> , 2006, Landete-Castillejos <i>et al.</i> , 2000, Ha <i>et al.</i> , 2014, Gomez <i>et al.</i> , 2002
Fallow deer	6.5 – 8.0	8.4 – 15.0	3.6 – 6.1	Malacarne <i>et al.</i> , 2015, Bovolenta <i>et al.</i> , 2013
Reindeer	7.6 – 11.1	11 – 16.9	2.5 – 3.7	Gjøstein <i>et al.</i> , 2004

Milk protein is a source of amino acids for neonates. Deer milk has the highest total protein and casein content among human, cow, sheep, goat, buffalo, camel, llama, yak, horse, and donkey milk (Wang *et al.*, 2017). It appears to have a protein content that is more than twice that of bovine milk (Opatha Vithana *et al.*, 2012). These higher protein contents could indicate to increased yields of protein-based dairy products and will lead to different textural and sensorial properties compared to products from more common milks.

The combination of high protein and high energy content of the milk suggests that deer milk has been optimised to meet the growth and energy demands of fawns, thus favouring survival. What-is-more, it

has been suggested that during late-lactation this concentration increases to compensate for declining rates of milk intake by the young (Wang *et al.*, 2017).

Literature of the FA profiles of deer milk are limited. The FA composition of milk among the deer subspecies has been revealed to be quite similar (Krzywiński *et al.*, 1980; Glass *et al.*, 1967). The most abundant FAs include palmitic (C16:0), oleic (C18:1), stearic (C18:0), and myristic (C14:0) acid. However, these studies all had low sample numbers (n = 1-3 deer) and the studies are quite old.

Factors affecting deer milk production and composition

Deer milk production reduces quickly post-partum. In one study, milk production of red deer dropped from 2104 mL at week 2, to 264 mL at week 34, giving an average daily milk yield of 910 mL (Landete-Castillejos *et al.*, 2000). The lactation spanned 105 days, which is similar to the length observed in natural conditions (Gallego *et al.*, 2006). In this study production peaked at 2500 mL at week 2, decreasing to 1000 mL at week 18. Factors affecting this production have been identified as variation in body weight and condition, and various indirect factors including social rank and age (Landete-Castillejos *et al.*, 2000).

The composition of deer milk is affected by the stage of lactation. Both protein and fat content in red deer milk has been reported to increase with the stage of lactation, with maximum levels recorded at 16 weeks (Landete-Castillejos *et al.*, 2000; Malacarne *et al.*, 2015). These changes are likely to affect the physiochemical properties of the milk, and thus this should be noted as it will impact the further processing of the milk into end-products.

Composition of milk from various species

The composition of milk naturally varies between species. This is dependent upon the breed, region in which the animal was farmed and its feeding. For example, milk from donkeys and horses have found to be high in lactose. Horse milk is also found to contain elevated levels of whey proteins, PUFAs and vitamin C (Park & Haenlein, 2008). Donkey milk has also found to be useful in skin and cosmetic products (Cosentino *et al.*, 2013). Buffalo and sheep milk tend to have the highest fat and energy contents, while goat milk tends to have a lower lactose content, but be rich in protein (Prasad, 2017). The proportion of fat in animal milk differs, with cows having around 3.3% - 4.4% fat by volume, goats 3.25% - 4.2%, and ewe milk containing around 7.1% (Markiewicz-Kęszycka *et al.*, 2013). Whereas marine mammals tend to produce milk with extremely high fat contents (Ofstedal, 1993; Park & Haenlin, 2008). A review by Roy *et al.* (2020) examined the milk composition of a range of species, finding red deer milk to have the highest total solids and fat concentrations amongst all the reviewed species (cattle, buffalo, goat, sheep, camel, horse, donkey, and human).

The variation present amongst species can be attributed to different environments, evolutionary pathways and any selection pressures that may have been placed on the animal. The adaptive

significance of these differences is not always clear, but often can be attributed to neonate requirements, or constraints on secretory processes (Oftedal, 1993). The needs of the neonates are influenced by factors of their developmental biology, such as birth size, litter size, growth rate, metabolic rate, and composition of gain (Ramsay & Dunbrack, 1986). The protein content of milk is related to the growth rate of the offspring, with high growth rates correlated to increased protein levels (Fox *et al.*, 2015). The fat content of milk ultimately reflects the energy requirement of the species, e.g., animals indigenous to cold environments secrete high concentrations of lipids (Fox *et al.*, 2015).

Furthermore, the variation of to what extent different species rely on tissue nutrients for milk production is large. Some species, such as whales and bears, will accumulate body fat prior to parturition then will undergo a period of fasting through lactation relying on body reserves for milk fat synthesis (Oftedal, 1993). Whereas other species will rely primarily on increasing their metabolisable energy intake throughout lactation. Therefore, the composition of milk from various species reflects substrate availability and favours the evolution of milk of a certain composition.

The history of selection of dairy animals has not always been well documented. Therefore, the composition of highly domesticated species relative to its 'natural' state could be quite different. As selection on lactating animals has been continuously placed on milk volume and production, the question has been raised regarding the potential eventual dilution of milk and corresponding lipid levels. The extent of domestication of lactating animals tends to result in decreased milk lipid contents. Animals that have been domesticated to a lesser extent, tend to have higher lipid levels (Park, 2011; Arman, 1979). This is likely due to the selective pressure on volume placed on dairy animals and the natural survival mechanism for milk to contain fat for offspring survival and growth. Meaning semi-domestic and wild ruminants tend to give a richer milk (Park, 2011).

The fatty acid (FA) composition of milk

Ruminant milk fat is a mix of tri- and diglycerides (TAGs and DAGs), complex lipids and other liposoluble substances (Arnould & Soyeurt, 2009). It can contain as many as 400-500 different FAs (Toral *et al.*, 2018; Barlowska & Litwinczuk, 2009). The major component is TAG, comprising glycerol, esterified with three FA chains. In the unesterified form, these FAs are carboxylic acids, with aliphatic chains that vary in length and the number of double-bonded carbons (known as the saturation level) present in the chain. The chain can be classified as saturated FA (SFA; with no intra-chain double bonds), monounsaturated FA (MUFA; with a single intra-chain double bond) and PUFA which contain multiple double bonds (Arnould & Soyeurt, 2009).

In their natural state, the milk lipids are present in the form of water-soluble globules in the aqueous emulsion that is milk (MacGibbon & Taylor, 2006). These fat globules are formed in the epithelial

cells in the alveoli of the mammary gland and sequestered into the aqueous phase of the milk (Lindmark Mansson, 2008).

Milk constituent fat has major influences on its processing performance, as well as being a carrier of both taste and aroma (Markiewicz-Kęszycka *et al.*, 2013). Milk FA profiles can therefore affect the type and quality of the end-products made from milk. As an example, FA profiles have been demonstrated to have major effects on butter properties (MacGibbon, 1996), with increased amounts of C18:1 and C18:2 FA, and reduced amounts of C8:0 and C14:0 FA resulting in a more spreadable butter at room temperature (MacGibbon & McLennan, 1987).

There are many factors that may influence the concentrations of the different FAs in an animal's milk. These include diet (Dewhurst *et al.*, 2006), genetic variation between animals (Soyeurt *et al.*, 2006; Stoop *et al.*, 2008), the stage of lactation (MacGibbon & Taylor, 2006) and the breed of animal of any given species (Beaulieu & Palmquist, 1995).

In ruminant animals, a broad range of FAs can be found in milk. The most common are the SFAs that typically contain 4 to 18-carbon atoms. These account for around 60-70% of ruminant milk FAs (Grummer, 1991). In most mammals the main SFA found in milk fat is C16:0. Around 20-35% of the milk fat in sheep, cow and goat milk are MUFAs, with oleic acid ($C_{18}H_{33}O_2$) typically being the most common (Mayer & Fiechter, 2012). Trans fatty acids (TFAs) can also be present in milk, with conjugated linoleic acid (CLA; a family of at least 28 isomers of linoleic acid, $COOH(CH_2)_7CH=CHCH_2CH=CH(CH_2)_4CH_3$) and vaccenic acid (C18:1 trans-11, $C_{18}H_{32}O_2$) being the most predominant. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are found in low amounts in cow, sheep, and goat milk with around 3-4% of total milk fat being PUFA (Devle *et al.*, 2009), and the predominant omega-3 FA being α -linolenic acid.

Origin of the milk FAs

The biosynthesis of milk fat is a complicated process and includes the coordination of a range of metabolic pathways and cellular processes. Bovine milk FAs arise from two main sources: *de-novo* synthesis in the mammary glands and FA derived from blood plasma lipids that are obtained from the diet. These two mechanisms form FA which differ in structure.

De-novo synthesis produces both short and medium-chain FAs, ranging from C4:0-C14:0, as well as some C16:0. In contrast, C18 FAs and some C16:0 originate from plasma lipids. Approximately 45% of total milk FAs are formed through *de-novo* synthesis, with the remainder originating from dietary origin lipids (MacGibbon & Taylor, 2006).

Conclusion

From the above reviewed literature, it can then be concluded that there remains a large potential for New Zealand deer milk in the current market. Therefore, the composition and components of deer milk, including the factors affecting these changes should be further investigated. The ability to identify and determine differences in deer milk, both intra- and inter-species creates exploration of potential marketing qualities and the potential to exploit the variation and factors affecting these. This investigation then aims to characterise the FA profile of deer milk from deer grazing lucerne in New Zealand. This data will then be compared to a data set of milk from New Zealand dairy cows to identify any potential differences in FA profiles.

Chapter 3

Materials and methods

Animal and milk sample collection

A total of 43 red deer hinds, of 2-7 years of age, and originating from two separate farms were investigated. All the hinds calved over the months November-December and were then grazed on lucerne at the Aratiatia Deer Milk farm (Wairakei, Taupō, New Zealand) over the course of the experiment (Figure 3.3). The deer were also offered *ad-libitum* access to ‘Deer Elite nuts’ during their time in the milking parlour (around 10 minutes). The nuts contained ingredients selected from wheat, maize grain, barley, canola meal, legumes (peas or beans), grain by-products, grass fibre, molasses, limestone flour, dicalcium phosphate, magnesium oxide, salt, NZ vegetable oil, trace mineral and vitamin premix delivering 45mg/kg added copper, 2mg/kg added iodine and 1mg/kg added selenium (<https://nrm.co.nz/spec-sheets/deer-elite-nuts/>, NRM, n.d.).

The first milking of the season commenced on the 10th of January and the hinds were milked once a day throughout the lactation period (from calving to late-April). A milk sample was collected from each hind in a single milking in mid-March (days in milk (DIM) \approx 70 days; Figure 3.1). Prior to the hinds drying off, a second milk sample was taken from 22 of the previously sampled hinds in mid-April (DIM \approx 94 days). These milk samples were frozen at -20°C and transported to Lincoln University where they were freeze-dried and ground to a fine powder in preparation for component analysis.

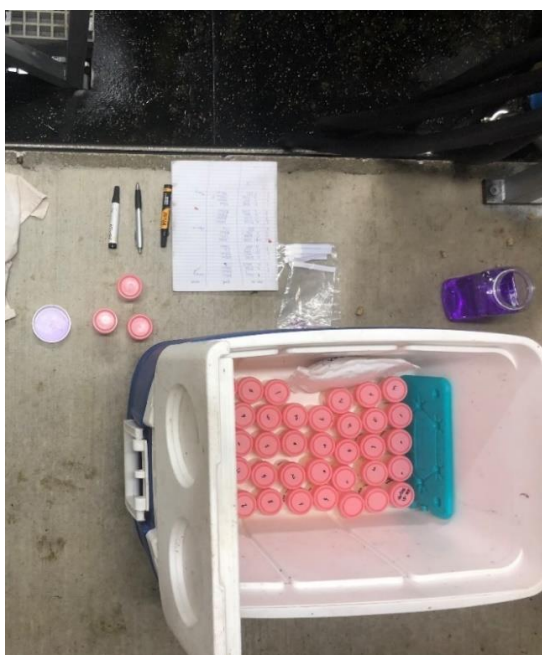


Figure 3.1. Photo showing the deer milk samples following collection at Pāmu Aratiatia, Taupō

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Figure 3.2. A) one of the deer from Pāmu Aratiatia being milked inside the specifically designed milking parlour, B) a part of the milking equipment that measures each deer's milk output. This was one of the top hinds, producing just over 2 L per day



Figure 3.3. The deer milking herd following milking on their Lucerne grazing platform

Analysis of the fatty acid (FA) components of the milk samples using gas chromatography

Prior to being analysed by gas chromatography (GC) as FA methyl esters (FAME), the milk FAs were methylated and extracted in n-heptane. To do this, sub-samples (0.1-0.15 g) of the powdered milk samples were weighed into 10-mL Kimax tubes and 100 μ L of internal standard (C21:0 ester, 5 mg/mL in n-heptane) and 900 μ L of n-heptane (100% AR grade) was added to each tube, followed by 3.0 mL of 0.5 M NaOH in dry methanol. Each tube was vortexed, then incubated in a block heater (Ratek Instruments, Australia) at 50°C for 15 minutes.

Following incubation, the tubes were cooled to room temperature and vortexed. Another 2.0 mL of n-heptane and 1.0 mL of distilled water was added to each tube before they were vortexed again. The tubes were then centrifuged for 5 minutes at 1500 g (Megafuge 1.0R, Heraeus, Germany). The top layer of n-heptane was transferred with a Pasteur pipette into a GC vial and stored at -20°C prior to GC analysis.

For analysis, samples were removed from the freezer and thawed. A 1.0- μ L aliquot of the n-heptane sample was injected into a GC column (0.25 mm x 100 m, 0.2 μ m film thickness capillary column, CP7420, Varian) with a 1:60 split ratio, using an AOC-20i auto-sampler fitted to a Shimadzu GC-2010 gas chromatograph.

The separation was completed using helium as a carrier gas and was run for 92 minutes. The temperature of both the injector and the detector was set at 250°C. The thermal profile of the column consisted of an initial oven temperature of 70°C, increasing to 170°C (ramp rate of 5°C/minute) where it was held for 25 minutes. It was then increased to 240°C (ramp rate of 2°C/minute) and held for 8.5 minutes. Finally, as a final 'bake-off' it was increased to 250°C (ramp rate of 25°C/minute) and held for 5 minutes.

The individual FAMES were then identified using peak retention time compared to commercially obtained external standards (ME61, ME93, BR3, BR2, ME100 from Laroden AB, Sweden; GLC411 and GLC463 from Nu-Chek Prep, Inc., USA). Quantification of the individual FAMES was undertaken using peak area assessment and comparison with internal and external standards. The threshold for peak area on the chromatogram was a 500-unit count, with peaks under this being ignored. The calculated minimum component detection of an individual FAME was therefore 0.01 g per 100 g of total FA.

Following individual FA measurement, the FAs detected were arranged into various groups as follows: short chain FAs (SCFA) = C4:0 + C6:0 + C8:0; medium chain FAs (MCFA) = C10:0 + C12:0 + C14:0; long chain FAs (LCFA) = C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C24:0; omega-3 FAs = C18:3 cis-9,12,15 + C20:5 cis-5,8,11,14,17 + C22:5 cis-7,10,13,16,19; omega-6 FAs = C18:2 cis-9,12 + C18:3 cis-6,9,12 + C20:3 cis-8,11,14 + C20:4 cis-5,8,11,14; monounsaturated FAs (MUFA) =

C10:1 + C14:1 cis-9 + C16:1 cis-9 + C18:1 trans-11 + C18:1 cis-9 + C18:1 cis-(11 to 13) + C20:1 cis-5 + C20:1 cis-9 + C20:1 cis-11; polyunsaturated FAs (PUFA) = C18:2 trans-9,12 + C18:2 cis-9,trans-13 + C18:2 cis-9,trans-12 + C18:2 trans-9,cis-12 + C18:2 cis-9,12 + C18:3 cis-6,9,12 + C18:3 cis-9,12,15 + CLA + C20:3 cis-8,11,14 + C20:4 cis-5,8,11,14 + C20:5 cis-5,8,11,14,17 + C22:5 cis-7,10,13,16,19; total branched FA= C13:0 iso + C13:0 anteiso + C15:0 iso + C15:0 anteiso + C17:0 iso; total UFA = MUFA + PUFA; and total SFA = C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C19:0 + C20:0 + C22:0 + C24:0.

Statistical Analysis

Genstat19 was used to analyse the collected data. First, the distribution of individual FA levels in the hinds were tested for normality of distribution using an Anderson-Darling test. The skewness of these were then calculated. Next, the FA levels for the hinds were compared individually between mid- and late-lactation using paired sample t-tests. Lastly, population-level sample analyses between mid- and late-lactation were completed, with the means of both the individual FAs and the grouped FAs being compared using linear mixed models. Acceptance of significance was at $P < 0.05$.

The deer milk results were then compared with dairy cow data collected by Li *et al.* (2020). These data were taken from milk testing on 407 Holstein-Friesian x Jersey (HFxJ)-cross dairy cows of 3 to 10 years of age. All these cows were grazed on a perennial ryegrass and white clover pasture on the Lincoln University Dairy Farm (LUDF; Canterbury, NZ). The cows calved over August-September and were milked twice daily, with milk samples taken in mid-January. The samples were then stored and analysed in accordance with the same methods as described above. These data were then compared to the deer milk results using two-sample t-tests.

Chapter 4

Results

The distribution of CLA c9, t11

The CLA c9, t11 values for both mid- and late-lactation do not follow a normal distribution (Figure 4.1; Figure 4.2). An Anderson-Darling normality test revealed the data was not normally distributed for both mid- ($P < 0.005$) and late-lactation ($P < 0.002$). The mid-lactation measurements for CLA c9, t11 were highly skewed (1.58), meaning the data distribution is asymmetrical, and longer on the right side of its peak than on the left (with a mean of 0.57 ± 0.018 g CLA c9, t11/100 g total FA). The late-lactation CLA c9, t11 measurements were also highly skewed (1.15), with a mean of 0.71 ± 0.042 g CLA c9, t11/100 g total FA.

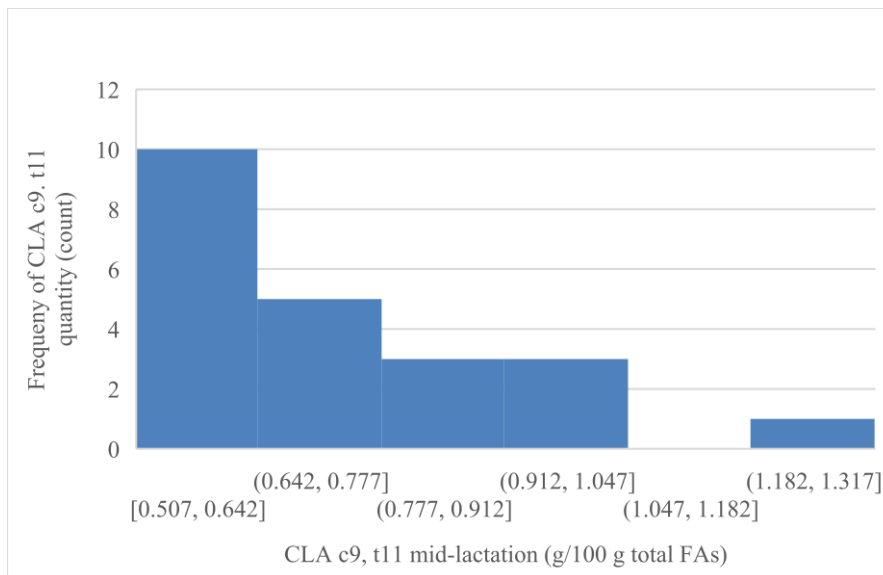


Figure 4.1. A histogram demonstrating the frequency distribution of CLA C9, t11 proportions in deer milk collected in mid-lactation.

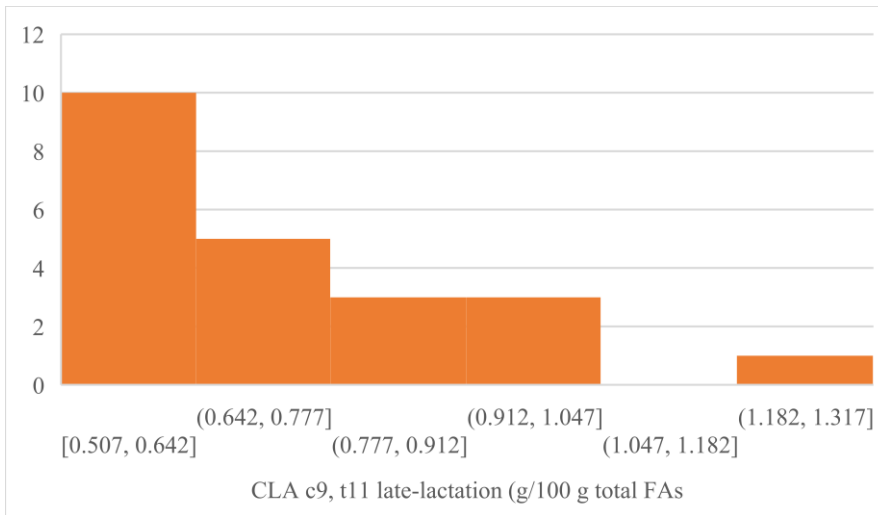


Figure 4.2. A histogram demonstrating the frequency distribution of CLA C9, t11 proportions in deer milk collected in late-lactation.

The spread of the data for the CLA c9, t11 proportions in late-lactation milk appears to be greater (Figure 4.3), and there are also outliers present for both mid- and late-lactation milk samples. These were not removed as they were considered to be valid results. On closer investigation it was revealed that the outlier present in the late-lactation data set, had a much lower CLA c9, t11 proportion in mid-lactation (0.49 g/100 g FA) relative to late-lactation (1.32 g/100 g FA).

Of the outliers present in mid-lactation, that were measured again in late-lactation, both deer continued to demonstrate high levels of CLA c9, t11 relative to the rest of the sample group, 0.87 g/100 g FA and 0.82 g/100 g FA in mid-lactation relative to 0.96 g/100 g FA and 0.84 g/100 g FA respectively.

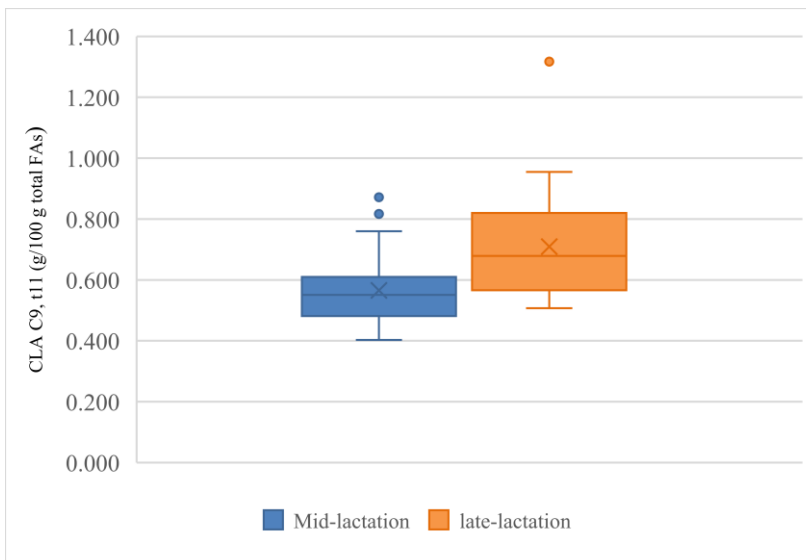


Figure 4.3. A box and whisker graph demonstrating the frequency distribution of CLA C9, t11 proportions in deer milk in mid-lactation and late-lactation.

The distribution of essential and non-essential FAs

An Anderson-Darling normality test revealed the data for mid-lactation proportions of C18:2 c9,12 (an essential FA; EFA) was normally distributed ($P = 0.176$). The mid-lactation measurements were moderately skewed (-0.62) meaning the data distribution is moderately asymmetrical, being longer on the left side of its peak than on the right, with a mean of 2.17 ± 0.046 g C18:2 c9,12/100 g total FA (Figure 4.4).

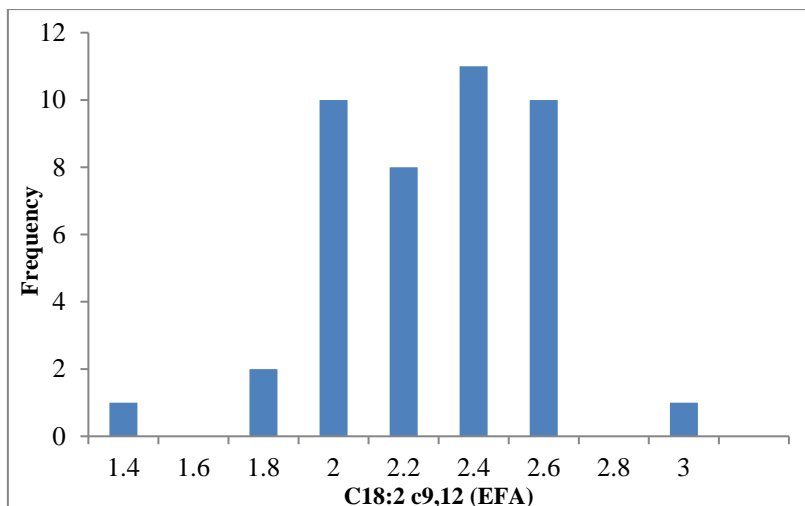


Figure 4.4. Histogram showing the frequency distribution of C18:2 c9,12 proportions in mid-lactation deer milk.

An Anderson-Darling normality test revealed the data for mid-lactation quantities of C18:3 c9,12, 15 (EFA) was trending towards not being normally distributed ($P = 0.085$). The mid-lactation measurements were moderately skewed (0.68) meaning the data distribution is moderately asymmetrical, being longer on the right side of its peak than on the left, with a mean of 2.64 ± 0.080 g C18:3 c9,12, 15/100 g total FA (Figure 4.5).

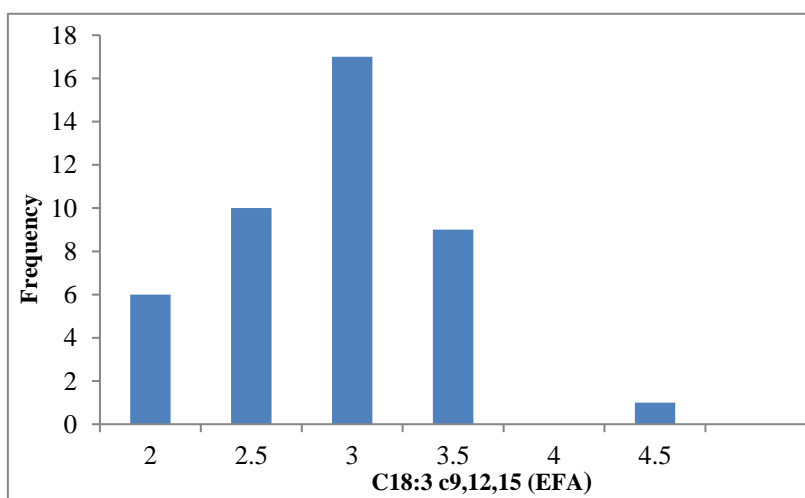


Figure 4.5. Histogram showing the frequency distribution of C18:3 c9,12,15 proportions in mid-lactation deer milk.

An Anderson-Darling normality test revealed the data for mid-lactation proportions of C18:2 c9 t13, (EFA), was not normally distributed ($P < 0.005$). The mid-lactation measurements were highly skewed (1.66) meaning the data distribution is asymmetrical, being longer on the right side of its peak than on the left, with a mean of 0.45 ± 0.027 g C18:2 c9 t13/100 g total FA (Figure 4.6).

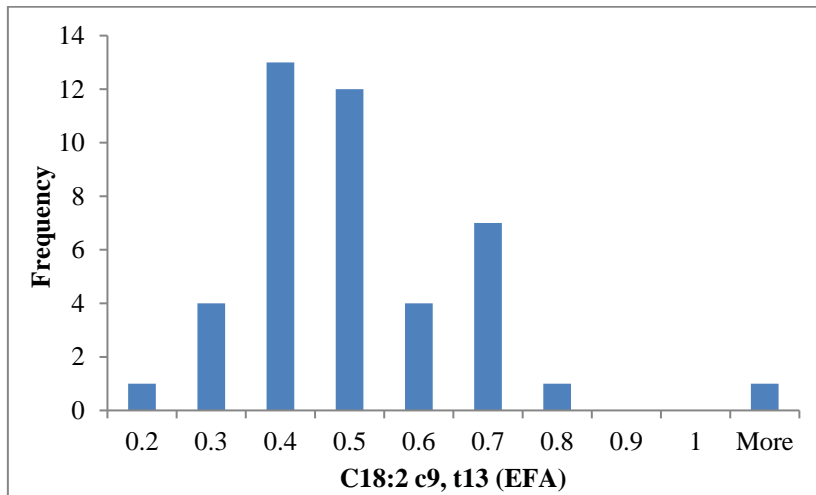


Figure 4.6. Histogram showing the frequency distribution of C18:2 c9, t13 proportion in mid-lactation deer milk

An Anderson-Darling normality test revealed the data for mid-lactation quantities of C14:0, a non-essential FA was normally distributed ($P = -0.18$). The mid-lactation measurements were not symmetrical, with a skewness of -0.18, and a mean of 11.61 ± 0.204 (FA g/100 g total FAs).

An Anderson-Darling normality test revealed the data for mid-lactation quantities of C16:0, a non-essential FA was normally distributed ($P = 0.35$). The mid-lactation measurements were symmetrical, with a skewness of 0.35, and a mean of 29.73 ± 0.369 (FA g/100 g total FAs).

Quantitative data analysis

Individual FAs

A summary of the average proportions of individual milk FAs (g/100 g total FAs) is presented in Table 4.1. Only FAs that proportionately accounted for more than 0.5% of total FA are included in the table.

The C16:0 FA (palmitic acid) was the most abundant FA in both the mid- and late-lactation deer milk samples, with an average proportion of 29.73 ± 0.369 g/100 g and 27.09 ± 0.405 g/100 g for mid- and late-lactation deer milk respectively (Table 4.1). Other FAs that contributed majorly to the composition of the deer milk FA included: C14:0 (myristic acid; 11.61 ± 0.204 g/100 g and 10.24 ± 0.358 g/100 g), C18:0 (stearic acid; 13.69 ± 0.286 g/100 g and 16.29 ± 0.436 g/100 g) and C18:1 c9 (oleic acid; 16.85 ± 0.218 g/100 g and 19.56 ± 0.267 g/100 g) for mid- and late-lactation respectively (Table 4.1).

Table 4.1. Average proportion of individual milk fatty acid methyl esters (FAME; means \pm SE) in mid- and late-lactation in mixed-age New Zealand (NZ) red deer hinds grazing lucerne.

Fatty Acid	Mid-lactation	Late-lactation
	(g/100 g total FA \pm SE)	(g/100 g total FA \pm SE)
C4:0	2.59 ± 0.032	2.39 ± 0.059
C6:0	1.49 ± 0.032	1.34 ± 0.043
C8:0	0.98 ± 0.032	0.88 ± 0.044
C10:0	1.81 ± 0.070	1.66 ± 0.100
C12:0	2.41 ± 0.075	2.23 ± 0.113
C14:0	11.61 ± 0.204	10.24 ± 0.358
C15:0	1.01 ± 0.015	1.09 ± 0.022
C16:0	29.73 ± 0.369	27.09 ± 0.405
C16:1 c9	0.58 ± 0.020	0.64 ± 0.026
C17:0 iso	0.60 ± 0.032	0.66 ± 0.016
C17:0	0.76 ± 0.010	0.81 ± 0.015
C18:0	13.69 ± 0.286	16.29 ± 0.436
C18: t11	1.24 ± 0.050	1.55 ± 0.110
C18:1 c6	0.68 ± 0.025	0.47 ± 0.027
C18:1 c9	16.85 ± 0.218	19.56 ± 0.267
C18:1 t15/c10	0.73 ± 0.025	0.48 ± 0.021
C18:1 c11	0.57 ± 0.016	0.69 ± 0.027
C18:1 c14/t16	0.90 ± 0.025	0.62 ± 0.025
C18:2 c9,12	2.17 ± 0.046	2.51 ± 0.088
C18:3 c9,12,15	2.64 ± 0.080	2.18 ± 0.087
CLA c9 t11	0.57 ± 0.018	0.71 ± 0.042

Grouped FAs

The average proportions for each FA group at mid- and late-lactation are presented in Table 4.2. The most abundant FA group were the LCFAs at both mid- (45.33 ± 0.293 g/100 g) and late-lactation (45.44 ± 0.428 g/100 g). The MUFAs were the second most abundant group in both mid- (20.15 ± 0.218 g/100 g) and late-lactation (23.31 ± 0.310 g/100 g) (Table 4.2).

Table 4.2. Average proportion of grouped milk FAME (means \pm SE) in mid- and late-lactation.

Fatty Acid Group	Mid-lactation	Late-lactation
	(g/100 g total FA \pm SE)	(g/100 g total FA \pm SE)
SCFA	5.06 ± 0.064	4.60 ± 0.086
MCFA	15.83 ± 0.304	14.14 ± 0.502
LCFA	45.33 ± 0.293	45.44 ± 0.428
Omega-3	3.00 ± 0.083	2.48 ± 0.088
Omega-6	2.30 ± 0.047	2.64 ± 0.089
MUFA	20.15 ± 0.218	23.31 ± 0.310
PUFA	7.05 ± 0.174	6.56 ± 0.220
total branched FA	1.48 ± 0.024	1.60 ± 0.035
total UFA	27.19 ± 0.293	29.87 ± 0.444
total SFA	66.57 ± 0.390	64.59 ± 0.571

Variation in FA levels between mid- and late-lactation

A mixed linear model was used to compare mid-lactation and late-lactation proportions for the individual FAs. Deer tag number was selected as a random effect to account for clustering in the data. This was done because a repeated measure ANOVA was not a viable option as the data was not balanced due to there being more measurements taken at mid-lactation compared to late-lactation (i.e., some of the deer that had measurements taken in March were dried off or removed from the herd for various reasons by the time of the April measurements).

When comparing the composition of the FA profile between mid- and late-lactation, differences were observed. Table 4.3 reveals that all the FA proportions were significantly different in late-lactation compared to mid-lactation. Major difference between the two stages of lactation were observed for C18:0 (13.69 g/100 g vs 15.96 g/100 g) and C18:1 c9 (16.82 g/100 g vs 19.58 g/100 g), for mid- and late-lactation respectively (Table 4.3).

Table 4.3. Results of a linear mixed-model analysis comparing the predicted mean FA proportions and standard error of difference (SED) between mid- and late-lactation red deer.

Fatty Acid	Predicted means (g/100 g total FA)		SED	P-value
	Mid-lactation	Late-lactation		
C4:0	2.60	2.43	0.034	< 0.001
C6:0	1.49	1.32	0.024	< 0.001
C8:0	0.98	0.87	0.020	< 0.001
C10:0	1.81	1.60	0.044	< 0.001
C12:0	2.40	2.13	0.050	< 0.001
C14:0	11.63	10.26	0.164	< 0.001
C15:0	1.01	1.20	0.003	< 0.001
C16:0	29.73	27.11	0.584	< 0.001
C16:1 c9	0.58	0.65	0.030	0.035
C17:0 iso	0.60	0.66	0.010	< 0.001
C17:0	0.76	0.81	0.013	< 0.001
C18:0	13.69	15.96	0.218	< 0.001
C18: t11	1.24	1.54	0.089	0.002
C18:1 c6	0.68	0.45	0.021	< 0.001
C18:1 c9	16.82	19.58	0.241	< 0.001
C18:1 t15/c10	0.73	0.45	0.023	< 0.001
C18:1 c11	0.59	0.69	0.029	< 0.001
C18:1 c14/t16	0.90	0.60	0.022	< 0.001
C18:2 c9,12	2.16	2.48	0.043	< 0.001
C18:3 c9,12,15	2.64	2.18	0.078	< 0.001
CLA c9 t11	0.57	0.71	0.034	< 0.001

A mixed linear model was also used to compare the grouped FAs proportions between mid- and late-lactation. Deer tag number was again selected as a random effect to account for clustering. All the FA groups except for the LCFAs were significantly different between mid- and late-lactation. Large differences were observed for the MCFAs (15.84 g/ 100g vs 14.00 g/ 100g), the MUFAs (20.11 g/ 100g vs 23.32 g/ 100g) and the PUFAs (7.03 g/ 100g vs 6.52 g/ 100g; Table 4.4)

Table 4.4. Results of a linear mixed-model analysis comparing the predicted mean FA levels and standard error of difference (SED) between mid- and late-lactation red deer.

Fatty acid group	Predicted means (g/ 100g total FA)		SED	P-value
	Mid-lactation	Late-lactation		
SCFA	5.07	4.62	0.059	< 0.001
MCFA	15.84	14.00	0.243	< 0.001
LCFA	45.39	45.70	0.245	0.219
Omega-3 FAs	3.00	2.48	0.081	< 0.001
Omega-6 FAs	2.28	2.61	0.043	< 0.001
MUFA	20.11	23.32	0.269	< 0.001
PUFA	7.03	6.52	0.119	< 0.001
total branched FAs	1.47	1.60	0.026	< 0.001
total UFA	27.12	29.86	0.260	< 0.001
total SFA	66.65	64.73	0.309	< 0.001

A sub-set of the data was then used to run a paired sample t-test between mid- and late-lactation from deer that had samples taken in both March and April (n = 22). Averages were calculated so that only the deer milk in this sub-set data was included. Table 4.5 demonstrates that all FAs detected differed significantly between mid- and late-lactation. Large differences were revealed for C14:0 (11.60 ± 0.324 g/100 g vs 10.24 ± 0.358 g/100 g), C16:0 (29.08 ± 0.521 g/100 g vs 27.09 ± 0.405 g/100 g), C18:0 (14.11 ± 0.422 g/100 g vs 16.29 ± 0.436 g/100 g) and C18:1 c9 (16.82 ± 0.279 g/100 g vs 19.56 ± 0.267 g/100 g) for mid- and late-lactation respectively (Table 4.5).

Table 4.5. Results of a paired sample t-test comparing individual deer changes in FA proportions between mid- and late-lactation (means ± SE).

Fatty Acid	Mid-lactation (g/100 g total FA ± SE)	Late-lactation (g/100 g total FA ± SE)	P-value
C4:0	2.55 ± 0.045	2.39 ± 0.059	< 0.001
C6:0	1.51 ± 0.051	1.34 ± 0.043	< 0.001
C8:0	1.00 ± 0.050	0.88 ± 0.044	< 0.001
C10:0	1.87 ± 0.111	1.66 ± 0.100	< 0.001
C12:0	2.51 ± 0.121	2.23 ± 0.113	< 0.001
C14:0	11.60 ± 0.324	10.24 ± 0.358	< 0.001
C15:0	1.00 ± 0.021	1.09 ± 0.022	< 0.001
C16:0	29.08 ± 0.521	27.09 ± 0.405	0.006
C16:1 c9	0.56 ± 0.027	0.64 ± 0.026	0.017
C17:0 iso	0.60 ± 0.013	0.66 ± 0.016	< 0.001
C17:0	0.76 ± 0.015	0.81 ± 0.015	0.001
C18:0	14.11 ± 0.422	16.29 ± 0.436	< 0.001
C18: t11	1.27 ± 0.066	1.55 ± 0.110	0.012
C18:1 c6	0.70 ± 0.036	0.47 ± 0.027	< 0.001
C18:1 c9	16.82 ± 0.279	19.56 ± 0.267	< 0.001
C18:1 t15/c10	0.76 ± 0.036	0.48 ± 0.021	< 0.001
C18:1 c11	0.60 ± 0.019	0.69 ± 0.027	< 0.001
C18:1 c14/t16	0.92 ± 0.034	0.62 ± 0.025	< 0.001
C18:2 c9,12	2.20 ± 0.062	2.51 ± 0.088	< 0.001
C18:3 c9,12,15	2.63 ± 0.125	2.18 ± 0.087	< 0.001
CLA c9 t11	0.56 ± 0.025	0.71 ± 0.042	< 0.001

Comparison with Cow milk FA profiles

The deer milk FA data for mid-lactation were then compared with data for dairy cows described in Li *et al.* (2020), using two-sample t-tests. Data from the mid-lactation sampling was used because of the larger number of samples in that group, relative to the late-lactation sampling.

All the detected individual FAs were significantly different to cow's milk. Large differences were observed particularly for C16:0 (29.73 ± 0.369 g/100 g vs 37.62 ± 0.160 g/100 g), C18:0 (13.69 ± 0.286 g/100 g vs 8.65 ± 0.062 g/100 g), C18:1 c9 (16.58 ± 0.218 g/100 g vs 13.05 ± 0.077 g/100 g) and CLA c9, t11 (0.57 ± 0.018 g/100 g vs 0.97 ± 0.016 g/100 g) for mid-lactation deer and cow's milk respectively (Table 4.6; Figure 4.7).

Table 4.6. Results of a two-sample t-test comparing the individual mean FA proportions (means \pm SE) between mid-lactation deer milk from mixed-age New Zealand red deer grazing lucerne and cow's milk from New Zealand mixed age Holstein-Friesian x Jersey-cross cows

Fatty Acid	Deer mid-lactation (g/100 g total FA \pm SE)	Cow* (g/100 g total FA \pm SE)	P-value
C4:0	2.59 \pm 0.032	1.27 \pm 0.006	< 0.001
C10:0	1.81 \pm 0.070	3.23 \pm 0.018	< 0.001
C12:0	2.41 \pm 0.075	3.91 \pm 0.024	< 0.001
C14:0	11.61 \pm 0.204	12.45 \pm 0.042	< 0.001
C15:0	1.01 \pm 0.015	1.49 \pm 0.008	< 0.001
C16:0	29.73 \pm 0.369	37.62 \pm 0.160	< 0.001
C16:1 c9	0.58 \pm 0.020	1.27 \pm 0.012	< 0.001
C18:0	13.69 \pm 0.286	8.65 \pm 0.062	< 0.001
C18:1 c9	16.85 \pm 0.218	13.05 \pm 0.077	< 0.001
C18:2 c9,12	2.17 \pm 0.046	0.70 \pm 0.001	< 0.001
C18:3 c9,12,15	2.64 \pm 0.080	0.82 \pm 0.005	< 0.001
CLA c9 t11	0.57 \pm 0.018	0.97 \pm 0.016	< 0.001

*Raw data for cow milk sourced from Li *et al.* (2020).

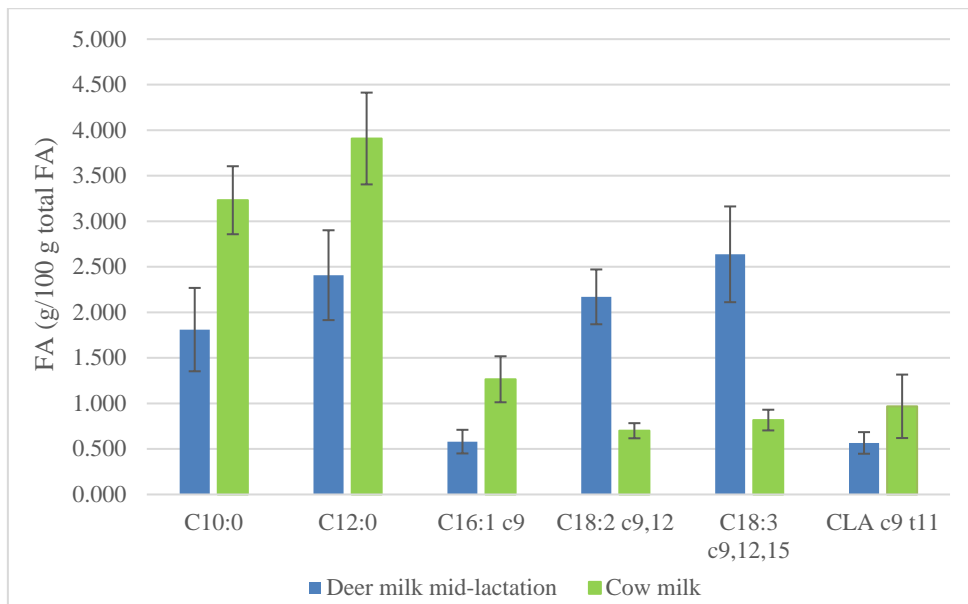


Figure 4.7. A bar graph with standard deviation error bars, representing the differences between deer milk in mid-lactation and cow's milk for selected FAs with between 0.5 and 4 g/100g total FA proportion.

The FA groups were also then compared using a two-sample t-test. All deer milk mid-lactation FA group proportions were significantly different to FA groups derived from cow's milk (Table 4.7). The largest differences were seen in the MCFAs (15.83 ± 0.304 g/100 g total FA vs 19.59 ± 0.076 g/100 g total FA), the omega-3 (3.00 ± 0.083 g/100 g total FA vs 1.04 ± 0.006 g/100 g total FA) and omega-6 groups (2.30 ± 0.047 g/100 g total FA vs 0.85 ± 0.004 g/100 g total FA), and the PUFAs (7.05 ± 0.0174 g/100 g total FA vs 4.10 ± 0.025 g/100 g total FA) for deer milk mid-lactation and cow's milk respectively (Table 4.7; Figure 4.8).

Table 4.7. Results of a two-sample t-test comparing the groups mean FA proportions (means \pm SE) between deer milk in mid-lactation and cows' milk.

Fatty acid group	Deer Mid-lactation	Cow	P-value
	(g/100 g total FA \pm SE)	(g/100 g total FA \pm SE)	
SCFA	5.06 ± 0.064	4.02 ± 0.013	< 0.001
MCFA	15.83 ± 0.304	19.59 ± 0.076	< 0.001
LCFA	45.33 ± 0.293	49.04 ± 0.139	< 0.001
Omega-3	3.00 ± 0.083	1.04 ± 0.006	< 0.001
Omega-6	2.30 ± 0.047	0.85 ± 0.004	< 0.001
MUFA	20.15 ± 0.218	19.79 ± 0.099	0.092
PUFA	7.05 ± 0.174	4.10 ± 0.025	< 0.001
total branched FA	1.48 ± 0.024	1.61 ± 0.007	< 0.001
total UFA	27.19 ± 0.293	23.89 ± 0.118	< 0.001
total SFA	66.57 ± 0.390	68.91 ± 0.131	< 0.001

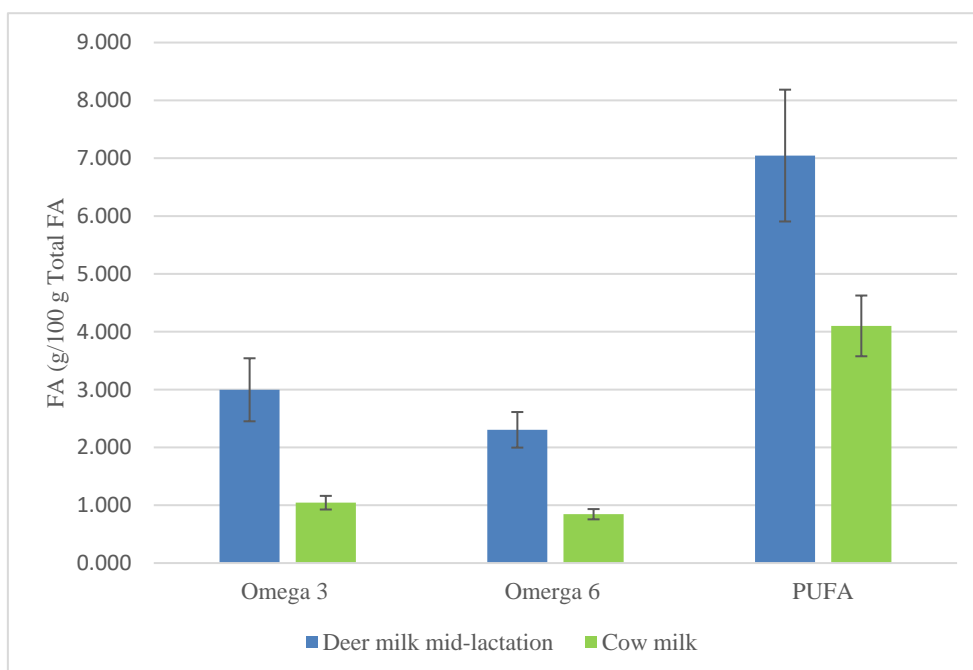


Figure 4.8. A bar graph with standard deviation error bars, representing the differences in omega-3, omega-6 and PUFA groups between deer milk in mid-lactation and cows' milk

The individual omega-3 and omega-6 FA groups were then broken down into individual FA components to examine the underlying causes of difference between the deer and cows' milk. All the individual FA proportions in the omega-3 group were significantly different to the proportions in the cows' milk (Table 4.8). Deer milk had significantly more C18:3 c9,12,15 (2.63 ± 0.080 g/100 g total FA) relative to cow's milk (0.82 ± 0.005 g/100 g total FA), as well as more C22:5 c7,10,13,16,19 (0.29 ± 0.008 g/100 g total FA vs 0.13 ± 0.001 g/100 g total FA; Table 4.8).

All of the individual FAs comprising the omega-6 FA group were also significantly different (Table 4.8). Deer milk from mid-lactation had a significantly greater proportion of C18:2 c9,12 (2.17 ± 0.046 g/100 g total FA) relative to cow's milk (0.70 ± 0.004 g/100 g total FA). Other differences in individual FAs included in the omega-3 and omega-6 groups were also observed, but due to their small relative proportion, their differences are not as notable.

Table 4.8. Component analysis of the differences between omega-3 and omega-6 FA group proportions (means \pm SE) between deer milk in mid-lactation from mixed-age New Zealand (NZ) red deer and mixed age Holstein-Friesian x Jersey-cross cows.

Omega-3 Fatty Acids	Deer Mid-lactation (g/100 g total FA \pm SE)	Cow (g/100 g total FA \pm SE)	P-value
C18:3 c9,12,15	2.63 \pm 0.080	0.82 \pm 0.005	< 0.001
C20:5 c5,8,11,14,17	0.07 \pm 0.002	0.09 \pm 0.001	< 0.001
C22:5 c7,10,13,16,19	0.29 \pm 0.008	0.13 \pm 0.001	< 0.001
Omega-6 Fatty Acids			
C18:2 c9,12	2.17 \pm 0.046	0.70 \pm 0.004	< 0.001
C18:3 c6,9,12	0.02 \pm 0.001	0.08 \pm 0.001	< 0.001
C20:3 c8,11,14	0.01 \pm 0.002	0.03 \pm 0.000	< 0.001
C20:4 c5,8,11,14	0.10 \pm 0.002	0.04 \pm 0.000	< 0.001

The main contributing factor to the observed differences in the omega-3 FA proportions between deer in mid-lactation and cows' milk is the large difference in C18:2 c9,12 levels between the two species (Figure 4.9). The other FAs in the omega-3 group occur in lower proportions and constitute proportionately less of the omega-3 FA levels.

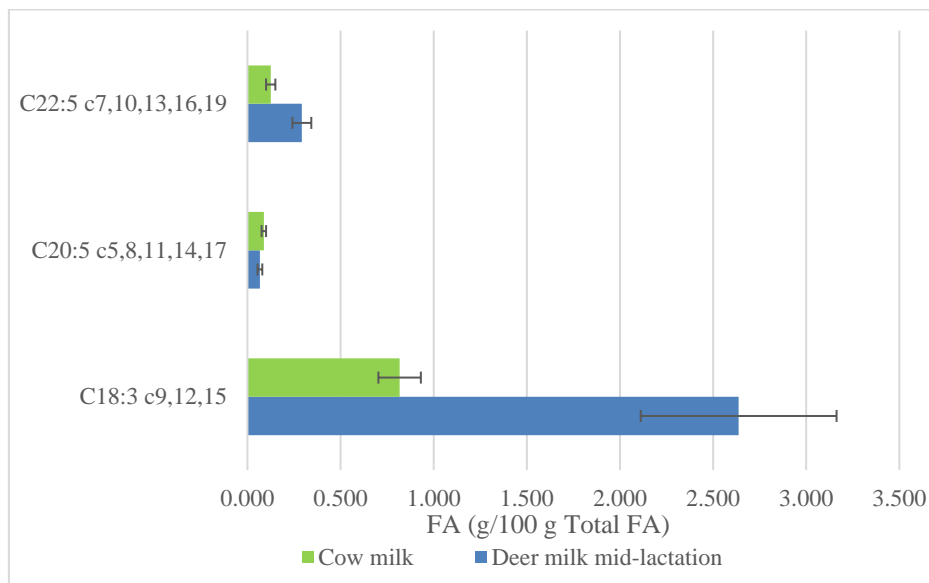


Figure 4.9. A bar graph with standard deviation error bars demonstrating a further FA component analysis of the differences of the omega-3 FA group between deer milk in mid-lactation and cows' milk

The main contributing factor to the differences in omega-6 proportions between the two species milks is also largely due to one FA. The proportion of C18:2 c9,12 is significantly different between the cows' and deer milk in mid-lactation (Figure 4.10). Once again, while there are differences between the species for the other omega-6 FAs, these FAs occur at proportionately lower levels and the difference is mainly due to differences in C18:2 c9,12 proportions.

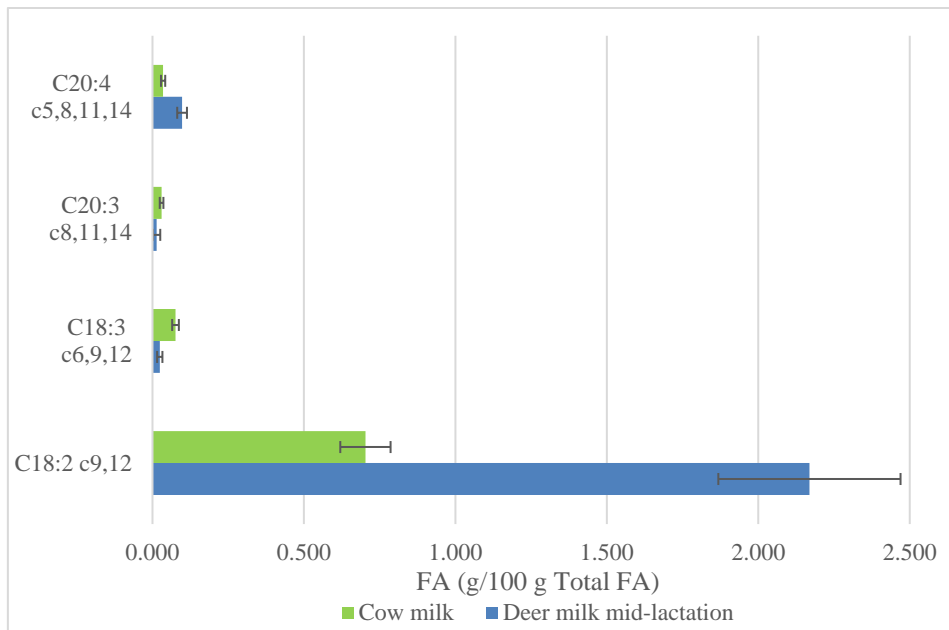


Figure 4.10. A bar graph with standard deviation error bars demonstrating a FA component analysis of the differences in the omega-6 FA groups between deer milk in mid-lactation and cows' milk

Chapter 5

Discussion and conclusion

The aim of this investigation was to characterise the FA profile of milk from red deer in New Zealand. This is the first time the characterisation of the milk FA profile on a large number of deer has been achieved. This enabled an insight into the FA profile of a particular deer population in a specific farming system, but it also enabled understanding of how FA profiles changed over the course of lactation. The deer results were compared with results from Holstein-Friesian dairy cows, giving us greater insight into inter-species variation in milk FA profile.

The fatty acid profile of deer milk

As a consequence of the lack of research into the FA profiles of milk from deer, it is difficult to compare the results from the red deer studied here, with other deer findings. For example, previous research has mainly involved wild deer and with small samples studied (Krzywiński *et al.*, 1980; Glass *et al.*, 1967). Improvements in gas chromatography techniques since these earlier studies, have also advanced our understanding of milk FAs, whilst also possibly providing a reason for the discrepancies in data between what has been reported in the past, and what was observed in this study.

Future research may reveal differences in deer milk FA profiles that can be specifically attributed to factors such as diet. This may enable the design of feeding systems to produce deer milk with unique FA profiles and attributes better suited to specific markets. In that respect, it is known that the FA composition of mammalian milk can be manipulated by changing the nutrition of an animal (Givens & Shingfield, 2006), and within the sampled population, variation was observed within the FA profiles, even though all the deer were kept together in one herd and managed similarly throughout the lactation period. This stated, at milking the deer were offered *ad-libitum* access to a diet supplement (deer elite nuts). Some deer would eat a lot of these nuts, while some would not, suggesting there is preference in feed choice. This study could not accommodate or correct for these preferences. This stated, the variation in feed type was probably only a minor effect, as the deer were only offered the nuts for a short period of time and just once a day. Lucerne was therefore likely to be the primary dietary influence on milk FA levels in the deer studied.

Individual variation in deer milk FA profile

Knowledge of milk composition and individual variation is important when evaluating a species' potential as a dairy animal (Holand *et al.*, 2017). Variation within specific traits or qualities, if heritable, provides a basis for selection. It enables animals to be selectively bred for improvement in that trait, and thereby opportunity to make improvement within a population (Givens & Shingfield, 2006). In this context, variations within individual FA levels within the deer population, were likely also because of individual animal effects, including genetic effects. Although variation within specific FAs in the deer milk may allow for the selection of the animals that have desirable FA quantities, the practicality of selecting for specific FAs in a new and emerging industry is probably less viable. Study of the variation that does occur, does however give us insight into current variation and the potential to develop new products such as cosmetics, or cheese that may have different and desirable FA profiles.

Increased interest in the chemical composition of animal milk fats has come about because of reports describing both beneficial and negative effects of various fats on human health. This has led to increased interest in altering FA milk profiles to produce milk with unique attributes (Samkova *et al.*, 2012), including interest in increasing the content of nutritionally important FAs such as CLA and MUFAs, while reducing the content of other undesirable FAs (Samkova *et al.*, 2012). In this respect, variation present in dairy cows' milk FA profile, when fed the same diet, has allowed Bobe *et al.* (2003) to select for animals with differing FA compositions. This has led to innovations such as allowing the production of butter with higher unsaturated fat levels, which can create a more desirable textural parameters for butter, such as being more spreadable, softer, and less adhesive.

Conjugated linoleic acid (CLA) levels in deer milk

High levels of variation in deer CLA levels observed between individual animals in the present investigation. The CLA c9, t11 levels in mid-lactation had an average of 3.0 mg/g of FA, with data ranging from 1.9 to 5.2 mg/g of FA. Similar results were observed in late-lactation with an average of 3.5 mg/g of FA, and with the individual levels ranging from 2.4 to 6.0 mg/g of FA. This suggests the level of variation within the present study may be due to genetic variation among individuals

Among the various FAs identified in deer milk, CLAs are considered beneficial to human health, making them desirable in milk products (Dhiman *et al.*, 2005). The cis-9, trans-11 isomer is the primary form of CLA found in dairy products and it is a product of endogenous synthesis via the Δ^9 -desaturase enzyme, with the substrate being C18:1 t11 (Kelsey *et al.*, 2003). The content found in milk varies dependant on breed, age, diet, management factors and individual variation.

Various studies have reported high levels of variation in CLA levels from cow to cow, in animals that have been fed the same diet, and at similar stages of lactation (Jiang *et al.*, 1996; Kelly *et al.*, 1998a). For example, Kelsey *et al.* (2003) reported an average of 4.3 mg/g of FA with a range of around threefold (2.3 to 7.2 mg/g of FA) among individuals managed under the same conditions. In this study

it was suggested that the variation among individuals was likely due to rumen output of C18:2 t11 and to the amount of $\Delta 9$ -desaturase activity.

Variation in CLA levels with cows managed in grazing systems is elevated relative to animals fed in confinement (Castillo *et al.*, 2006; Kelly *et al.*, 1998b). For example, cows primarily grazing lucerne in Argentina were found to have CLA concentrations ranging from 6.7 to 18.7 mg/g of FA (Castillo *et al.*, 2006). Variation in CLA levels for New Zealand dairy cows grazing pasture have also been demonstrated, and large variations were found amongst and between herds in milk obtained from forty-four dairy cattle herds in a single-sampling period (MacGibbon *et al.*, 2001).

Medium-chain saturated fatty acid levels in deer milk

High levels of variation were observed for C8:0, C10:0 and C12:0 levels, during mid- and late-lactation for the deer milk in this investigation. Stoop *et al.* (2009b) also found high levels of variation in the amount of these FAs in cows' milk, and which had elevated phenotypic standard deviations relative to the other FAs in milk.

Historical negative perceptions regarding excessive intakes of saturated fats have highlighted the potential to reduce the amounts of these substances in milk. Dairy fat has often been associated with an increased likelihood of cardiovascular disease, metabolic syndrome, and obesity (Lock & Bauman, 2004). However, more recent scientific evidence has suggested otherwise (Gomez-Cortes *et al.*, 2018), and the consumption of MCSFA has had demonstrably beneficial effects on weight control and lipid metabolism (Mumme & Stonehouse, 2015), and increases in the lean body mass of obese adults have been observed following an enriched dairy MCSFA diet (Bohl *et al.*, 2017). The differences in saturated and unsaturated acid contents in dairy products also affects properties such as the softness, flavour, and colour of dairy products (Knutsen *et al.*, 2018), while in comparison, higher ratios of saturated FAs result in hardness and the poor spreadability of butter (Bobe *et al.*, 2003), as mentioned above.

A significant quantitative trait locus for C6:0 and C8:0 FA levels has been reported on bovine chromosome 6 (BTA6), in near proximity to the PPARGC1A gene. This gene is involved in the regulation of milk-fat synthesis in the mammary gland (Khatib *et al.*, 2007), and potentially explains some of the variation in C8:0 quantities. Knutsen *et al.* (2018) also identified several genetic variants associated with the synthesis of short- and medium-chain FAs, and these genetic variations may explain some of the differences observed in the present study.

Conflicting evidence and consumer perceptions suggests that further research is required to better understand the role of milk SFAs on human health and before selection pressure or breeding is used to either increase or reduce milk SFA contents. The level of dairy product required to achieve the reported positive health-benefits are also unknown, hence the question can be raised as to whether these differences are sufficiently large to be of practical importance. This stated the value proposition

placed on altering the saturated FA profile of milk may be more suited to altering the technical properties of deer milk products, than those of cattle.

Seasonal Variation in FA profile

This investigation was the first of its kind to characterise the FA profile of deer milk from a large number of animals. What-is-more is that it allowed for an interesting comparison over the later stages of lactation. There were major differences between the FA profile observed in mid- and late-lactation deer milk, and all individual FAs that accounted for more than 0.5% of the total FA profile were found to differ significantly between the two samplings. The deer were fed and managed the same way between the two samplings, meaning these differences are likely due to physiological changes within the animal and its FA synthesis pathways. The FA found in milk originates from four main pathways: from the diet, from *de-novo* synthesis in the mammary glands, from formation in the rumen by biohydrogenation, and by release from body fat stores (MacGibbon & Taylor, 2006). Alterations in the composition of FA profile throughout lactation suggests changes occur in the activity of one or more of these pathways and because of shifts in animal energy status (Stoop *et al.*, 2009a).

In the pre-ruminant deer calf, milk nutrients are used for growth and maintenance. As milk production declines and the rumen develops, rumen metabolites are then used to meet broader nutritional requirements. Milk nutrients are used at maximum efficiency for growth, whilst rumen metabolites meet maintenance requirements (White, 1992), thus it has been suggested that in temperate environments the level and quality of milk intake in late lactation may be important for maintaining the growth rate of calves as feed quality declines. Supporting this concept, Clutton-Brock *et al.* (1982) claimed red deer milk fat concentrations increase with the age of the calf, with similar changes in milk richness occurring in other cervids (White, 1992).

The impact of negative energy balance

Milk fat composition is affected by the stage of lactation. During early lactation, the energy requirements for milk production of cattle exceeds their energy intake. Cattle therefore enter a period of negative energy balance, resulting in the mobilisation of long-chained FAs from adipose tissue and subsequent incorporation into milk fat. This inhibits the *de-novo* synthesis of short-chain FA (Givens & Shingfield, 2006). During this period, mammary function has metabolic priority, and available nutrients are directed to milk synthesis for the survival of the offspring (Useni *et al.*, 2018).

The alterations in milk FA composition coinciding with the progression through lactation are thought to be a consequence of the effect of mobilised adipose tissue on mammary FA supply and the inhibitory nature of increased mammary uptake of LCFA on *de-novo* FA synthesis (Hanus *et al.*, 2018). Therefore, SFAs are generally at their lowest proportion in milk immediately following birth,

but increase as the energy balance improves. It has been revealed that cattle produce milk with lower levels of C6:0 to C12:0 in early lactation relative to mid- and late-lactation (Palmquist *et al.*, 1993), but in this study of deer, the opposite appears to have occurred. The C4:0 to C14:0 SFA levels decreased from mid- to late-lactation, with this possibly suggesting that deer do not enter a period of negative energy balance following the onset of lactation because of their reduced milk output relative to dairy cows.

Research on another cervid species, reindeer, has revealed that although milk production declines rapidly after peaking, the milk nutrient content increases during this period, compensating for the decline in volume (Chan-McLeod *et al.*, 1994). This highlights that during this stage of lactation, the nutrient transfer of resources from the calve to offspring is energetically demanding. It has been suggested that genetic selection and targeted supplementary feeding could extend the lactation curve, but this has not been put into practice (Holand *et al.*, 2017). The effects on the further reproductive performance of these animals are also unknown (Holand *et al.*, 2006).

The impact of the rut period, and feed intake patterns

Oleic acid (18:1) is the primary FA in adipocytes. It is released through lipolysis during periods of negative energy balance. It has been suggested that elevated levels of C18:1 c9 in cows' milk fat is a marker for negative energy balance in both early lactation and other stages when fasting and ketosis occur (Van Haelst *et al.*, 2008). Additionally, dairy cows deliberately put into a negative energy balance through feed restriction, demonstrated FA profiles similar to those observed in early lactation energy deficit (Gross *et al.*, 2011).

The proportion of C18:1 c9 increased significantly between mid- and late-lactation in the present study of deer. From the above, it could then be suggested that the deer could have been under some form of restricted feed intake. At the time of late-lactation sampling, the deer were just beginning to enter the rut period and stags were amongst the herd. It may therefore be possible that the deer were eating less as they were entering a period of hyper-activity needed to mate.

It has been noted that temperate cervids, such as red deer, have distinct seasonal patterns of voluntary feed intake, which is closely related to photoperiod changes and endogenous cycles signalled by melatonin (Barry *et al.*, 1991). Adam *et al.* (1996) found that deer exposed to natural photoperiod had decreased voluntary feed intakes (VFI) during autumn and winter relative to deer kept in conditions of constant intermediate daylength. Supporting this contention, Semiadi *et al.* (1995) found the VFI of red deer hinds peaked in January (summer), and then declined, reaching its lowest point in June (winter). This coincided with a 32% decline in VFI during the breeding season, and from March, a decline in liveweight was observed to result from the reduced VFI. Clutton-Brock *et al.* (1982) reported that lactation in wild red deer was associated with a rapid decline in the mother's body

reserves, meaning the differences between mid- and late-lactation in deer milk could be due to increased mobilisation of body reserves for milk fat synthesis.

The impact of the environment and evolutionary pathways

Reindeer have been farmed for many years, with their milk often used as a food source for the people of northern Eurasia and Taiga regions (Holand *et al.*, 2017). In reindeer, the short lactation period and consequent rapid decline in milk production is an adaptation to short arctic summers and challenging environments. The mother then typically controls weaning, to allow adequate time to improve body condition to ovulate during the mating period (Holand *et al.*, 2012). Although, red deer do not typically encounter the same harsh conditions, some underlying mechanisms in the evolution of the species could explain the pattern of lactation.

It has been reported that in red deer the cost of lactation affects next year's reproduction (Chan-McLeod *et al.*, 1994). This suggests that the cost of milk production will be traded against the hinds' requirements to replenish body reserves and secure future (re)production. Therefore, when considering the future of deer milk production systems, it will be important to acknowledge the complex interactions between the physiology, reproductive performance, and survival of the deer. The effects of artificially extended lactation on subsequent reproductive performance will most certainly need to be monitored.

Seasonal variation of conjugated linoleic acid proportions

The CLA content of deer milk was significantly higher in late-lactation compared to mid-lactation. Studies of variation in CLA content of cow's milk have revealed variation throughout the lactation period (Lock & Garnsworthy, 2003; Stoop *et al.*, 2009b). Levels tend to be higher in the spring and summer months, where dairy cows were receiving higher levels of fresh pasture in housed dairy systems. Contrastingly, Kelsey *et al.* (2003) reported little to no effects of the stage of lactation on CLA levels in dairy cows, with days in milk accounting for less than 2% of the variation found within CLA concentrations.

In New Zealand dairy cow outdoor grazing systems, the CLA concentration tends to be higher in the spring and autumn, and lower in the summer (MacGibbon *et al.*, 2001; Auld *et al.*, 1998). Similar results in cattle to the present investigation of deer were reported by Auld *et al.* (1998). The stage of lactation resulted in a small change in CLA content of dairy cows grazing pasture increasing from 0.74 to 0.94 g/100 g total FA from mid- to late-lactation respectively. It was suggested the increased fat content overall, which is usually observed in late lactation (Auld *et al.*, 1995) may have been responsible for this change. The Auld *et al.* (1995) study was designed to have calving occurring every 3 months to allow the observation of FA profiles at different stages of lactation at different times

of the year. It was found the overall effects of the time of the year were greater than the effects of stage of lactation, with this suggesting that seasonal variation in milk fat composition is more important to milk FA properties, than the stage of lactation (Auld *et al.*, 1998).

Pasture grazing vs housed production systems

Higher levels of CLA are generally observed in animals grazing pasture compared to animals fed total mixed rations (Kelly *et al.*, 1998b; Atti *et al.*, 2006). Dhiman *et al.* (1999) reported that dairy cows grazing pasture and receiving no supplemental feed, had 500% more CLA in milk compared to cows fed 50:50 forage to grain ratio diets. Biohydrogenation in the rumen and hence FA synthesis is affected by the amount and type of FA substrate, the forage to grain ratio and the nitrogen content of the feed, and this affects the supply of intermediate and end-products of biohydrogenation, thus influencing the milk CLA content (Kelly *et al.*, 1998a). The effects of diet on CLA levels may, therefore, explain the variation in CLA content in this investigation

Other dietary implications

Although, the contents of the diet were kept constant throughout the lactation period, it may be possible that the chemical composition of the lucerne differed between times of mid- and late-lactation, but this was not monitored. The effects on the FA profile of milk from animals grazing lucerne is also poorly understood, although Castillo *et al.* (2006) reported a high proportion of grazed lucerne in the diet was associated with increased CLA levels. A tendency towards higher CLA values in autumn (May) relative to summer (January) was also observed. These changes may represent changes due to stage of lactation or the dietary influences of the changing chemical composition of the feed.

Ribeiro (2005) suggested that the higher sucrose concentration in fresh lucerne relative to lucerne hay may result in higher vaccenic acid flow to the duodenum, and with this increasing milk CLA. A study investigating the sugar content of lucerne in the United States of America, revealed that in the autumn months sugar content increased from September to October, corresponding to declining soil temperatures (Ireland, 1939). The sugar content of various lucerne cultivars roots were also found to increase in autumn (Cunningham & Volenec, 1998). It could therefore be suggested that the sugar content of lucerne in the present deer study has increased during the autumn months (March to April).

In summary, the above information suggests that the increase in CLA may be due to increased fat contents seen in deer milk in late lactation (Park, 2011), or because of changes in the composition of the diet, or both. The effects of stage of lactation and dietary changes could not be separated in this investigation, hence further investigation is required to identify the relationship between lucerne sugar contents and increasing CLA.

The importance of essential fatty acids (EFAs)

Milk contains many components which are important for new-borns, including vitamins and essential and non-essential FAs (Blum *et al.*, 1997). Linoleic (C18:2 c9,12) and α -linolenic acid (C18:3 c9,12,15) are classified as essential fatty acids (EFAs). These are required for mammalian growth and function, and due to their inability to synthesise them, mammals must acquire them from dietary sources (Uken *et al.*, 2021). These molecules are important as structural components of membranes, acting as ligands regulating transcription factors, and helping to modulate cell metabolism. The lactating mother supplies these EFAs through gestation and via the intake of colostrum and milk by the neonate (Garcia *et al.*, 2014).

The importance of human infants gaining EFAs through breastmilk has been explored (Innis, 2000). These EFAs are used to synthesise long chain polyenoic fatty acids containing 20 or more carbons, with three or more double bonds, named LCPs. Deposition of LCP in the central nervous system is rapid during brain growth and is dependent on the quantity and balance of fatty acids received by the neonate through the placenta and in the diet following birth (Innis, 1991). The failure to acquire these essential nutrients and accomplish specific stages of brain growth can result in irreversible damage. The minimum daily intake of C18:3 c9,12, 15 in infants has been described as above 0.71% Kcal (Innis, 1991). It is recognised that the diet has a large influence on the EFA concentration in human breast milk, but the omega-3 and omega-6 content in breast milk of well-nourished mothers always either meets or exceeds the FA requirement of the infant (Innis, 1991). This may explain the levels of these EFAs in milk.

Implications of EFA supply on the FA profile

When deer are first born, they are deemed pre-ruminant and must rely solely on milk to acquire necessary nutrients. Therefore, the principles applied to human infants could also be extrapolated to neonatal pre-ruminant deer. The tendency of essential FAs in this investigation to have a skewed distribution may allow speculation as to the 'baseline physiological requirement' for the neonate. The FAs α -linolenic acid and C18:2 c9, t13 were moderately skewed to the right, and the essentiality of these FAs may mean there is a baseline or minimum level that all milk contains to ensure adequate nutrition for the neonate. This may explain the moderate right skew, whereas the non-essential FAs that were observed (C14:0 and C16:0) were not skewed, and with this suggesting that these FAs primarily serve as an energy source for the suckling young (Drackley, 2008).

As intakes of C18 EFA increase in humans, tissue concentrations of C20:4 and 22:6 increase rapidly and then plateau (Innis, 1999). This may help to explain the skew of the data for these FAs in the deer results as once the required EFA concentration is met, levels above this may not provide additional benefits to the neonate. Whilst high levels of some EFAs are found in individual deer, the energy

demands of synthesising these complex FAs may mean it is only efficient for the mother to synthesise the required minimum concentration, and little more.

Linoleic acid (C18:2 c9,12), another EFA was moderately skewed to the left. This may suggest the possibility of a maximum threshold, meaning milk containing more than this limit may have negative consequences for the neonate. The left skew of C18:2 c9,12 may be explained by the potentially adverse consequences of having higher omega-6 intake relative to omega-3. The importance of the omega-3 to omega-6 ratio was demonstrated by Arbuckle *et al.* (1994), with high dietary C18:2n-6 to C18:3n-3 ratios resulting in 22:6n-3 deficiencies in piglets. This may suggest that there is an upper limit to what is allowed to maintain an optimum omega-3 to omega-6 ratio. It could also be that the skewness of EFAs (in either direction) do not indicate evidence of baseline physiological limits and are due to other unknown factors. Further investigation into baseline limits for these EFAs may determine more significant relationships.

CLA which is primarily formed in the rumen from EFA and in the mammary tissue are also important for energy metabolism in calves. In the present study CLA content in both mid- and late-lactation was not normally distributed and skewed to the right. Therefore, it may also be hypothesised that a baseline limit for CLA in terms of neonate nutrition is present, explaining the distribution of the data. The baseline limit in this investigation appears to be around 0.5 g/100 g total FAs.

These baselines may be more pronounced in deer milk relative to other domesticated dairy species as they have not been selected for milk production as intensively. This may mean their milk reflects that of the more 'natural state' that would be found in the wild, which primary purpose is for neonate nutrition.

Comparison to cow milk

Wild and semi-domestic ruminants tend to have a richer milk, particularly in late-lactation relative to domestic species (Park, 2011). This suggests that selection for increased yields in domesticated dairy animals has resulted in a consequent reduction in the concentration of key milk components (Arman, 1979). The secretion of concentrated milk is an adaptive mechanism, and a mix of high protein, high fat and low lactose is deemed optimal to meet the energy requirements of the deer calf in harsh environments (White & Luick, 1984). Differences are therefore expected to be observed between the milk of deer and dairy cows.

Can we validly compare deer and cow milk and what is to be learnt from this?

The differences observed between cows' and deer milk in this study, must however be treated with some caution as there are factors such as differences in the diets of the cows and deer that could not be

accounted for in this study. Whilst the data is presented there are weaknesses with the comparison involving factors including different species, diets and animal production systems. This investigation does however allow for a baseline comparison between the FA profiles of cow and deer milk. Further investigation, such as research into FA profile of milk from deer grazing ryegrass/white clover pastures may allow for a more direct comparison that can help to differentiate dietary effects.

Short- and long-chain fatty acids

Deer milk had higher SCFA and lower LCFA compared to cow's milk in the current investigation. Genetic selection for increased milk fat percentage has been found to increase the proportions of SCFA in cows' milk and decrease the proportions of LCFA (Palmquist *et al.*, 1993). Cattle have probably been selected for increased milk yield (volume) for hundreds of years, and this has possibly resulted in a reduction in milk fat content. In contrast, farmed deer have not had selective pressure placed on them for milk production. However, it may also be likely that having higher milk fat content increases neonate survival in deer (Clutton-Brock *et al.*, 1982), and that this explains the increased milk fat content relative to cow's milk.

Another potential explanation could be the inclusion of lucerne in the diet of the deer. Castillo *et al.* (2006) reported decreasing concentration of LCFA and SFAs in milk from dairy cattle grazing diets with higher proportions of lucerne pasture.

Omega-3 and omega-6 fatty acids (FAs)

In the current investigation, deer milk was found to have significantly higher levels of omega-3 and omega-6 FAs relative to milk from dairy cows grazing ryegrass and white clover pastures. The main difference was attributed to higher levels of C18:3 c9,12,15 and C18:2 c9,12. Dietary effects on FA profiles are likely as a result of differences in the rumen environment. Different microbial populations occur with different diets, and this can change biohydrogenation intermediates and the proportion of FAs that leave the rumen and end up in the milk (Ström, 2012). The effects of lucerne as the primary diet for grazing animals on milk FA profiles are not well understood.

Ström (2012) reported higher levels of linoleic acid (C18:2) and linolenic acid (C18:3) in dairy cows fed diverse pastures relative to simple pastures (ryegrass/ white clover). Of the diverse pastures investigated, cows fed on the treatment containing lucerne silage had the highest levels of linoleic and linolenic acid. All diverse pastures had a base of chicory, plantain, and prairie grass, with either red clover, big trefoil or lucerne additions. Therefore, as the only difference between the diverse treatments is the latter addition, it could be argued that the addition of lucerne is causing the effect. It has been suggested that the likely cause of differentiation in FA profiles from cows grazing diverse pastures is likely due to the microbial population being affected by different secondary metabolites in diverse plant species, resulting in reduced lipolysis and hydrogenation of linoleic and linolenic acid

(Lourenco *et al.*, 2007). Although, the effects cannot be attributed solely to the addition of lucerne in the diet, it may explain the differences observed in this investigation.

However, Kozłowska *et al.* (2021) reported lowered levels of C18:1 c9, C18:2 c9,12 and C18:3 c9,12,15 when dairy cows were supplemented with lucerne silage relative to grass silage. The cultivars of lucerne used in the study were Polish and German, and they were selected as they contained the greatest total saponin content of the available lucerne varieties. These inhibit rumen ciliate protozoa, hence the differences observed by Kozłowska *et al.* (2021), may be due to increased levels of saponin relative to New Zealand cultivars. Saponin levels are also said to increase during the ensilage process (Szumacher-Strabel *et al.*, 2019), whereas the involved deer in this study consumed fresh lucerne. Finally, studies examining the FA profile of milk from animals grazing solely lucerne are uncommon. Information could only be found regarding partial substitution, so it is difficult to make assumptions on the dietary effects.

Conjugated linoleic acid (CLA)

The lowered levels of CLA in deer milk relative to cows' milk may be explained by the increased levels of linoleic and linolenic acid. It was suggested that as CLA is an intermediate in the biohydrogenation of linoleic and linolenic acid, the amount of CLA present may be lower if less linoleic and linolenic acid is hydrogenated (Leiber *et al.*, 2005). Ström (2012) reported lower levels of CLA in milk from cows grazing diverse pastures, relative to grazing simple pastures. Leiber *et al.* (2005) also reported decreased levels of CLA in milk from cows fed botanically diverse pastures. Castillo *et al.* (2006) reported correlations between increasing CLA contents and increasing proportions of lucerne pasture in dairy cattle diets. In the present investigation milk from deer grazing lucerne had lower CLA content than milk from cow's grazing ryegrass and white clover. This may suggest that other factors such as the ones mentioned above, are primarily responsible for the decreased CLA content.

Polyunsaturated fatty acids (PUFA)

Deer milk in mid- and late-lactation had higher levels of PUFAs relative to cow's milk. The PUFA proportion of milk from grazing dairy cows ranges from around 3.3 to 4.5 g/100 g total FAs (Auld *et al.*, 1998; Mackle *et al.*, 1999; Schwendel *et al.*, 2015). In other dairy animals, such as sheep, the PUFA concentration has been reported to be 4 to 6.54 g/100g total FAs (De La Fuente *et al.*, 2009; Teng *et al.*, 2020). PUFA levels in milk was higher in ewes grazing pure legumes and grass-legume mixtures, relative to pure grass pastures (Cabiddu *et al.*, 2005).

The PUFAs are not synthesised by the ruminant, and their level in milk depends on outflow from the rumen. Pasture allowance could have effects on PUFA levels in milk from dairy cows. When cows are competitively grazed and 'forced' to eat further into the base of the sward, changes in FA profiles may

be observed due to increased dead material and lower quality herbage in the diet (Dewhurst *et al.*, 2006). Deer are preferential grazers (Semiadil *et al.*, 1995b) and due to less competition between the herd and them not being 'forced' to the bottom of the sward, it may be possible that the higher PUFA proportions observed were due to there being less dead material being consumed. Cabiddu *et al.* (2005) reported C18:2 c9,12 and C18:3 c9,12,15 contents in herbage DM tended to decrease between growth and reproductive phases of grass and legume species (*Lolium rigidum*, *Medicago polymorpha* L., *Hedysarum coronarium* L. and *Trifolium subterraneum* L.), meaning the PUFA content of the feed could also have had an influence on the deer milk.

Market potential

Deer meat, velvet and co-product markets are well developed. The potential to build on these markets through the introduction of a new high value niche product is high. Deer milk could be viewed as a valuable niche product, and the development of an exclusive product within the gourmet, cosmetic and pharmaceutical markets is possible. However, the success of commercial production will be dependent on achieving high prices, which will only come about if there is an emphasis on the qualities of the product and if there are willing buyers.

The use of some of these findings presented in this investigation, once supported by further research, may allow for the development of marketing techniques such as being able to highlight particular health benefits. For example, the elevated levels of omega-3 and omega-6 FAs relative to cows' milk could enable a novel market to develop.

The market potential of higher Omega-3 fatty acids levels

Omega-3 FAs are essential fats. Humans cannot synthesise them and therefore they must obtain adequate sources from their diet. Omega-3 fats are vital for cell membrane function, making them essential for many important processes within the body (Siriwardhana *et al.*, 2012).

The increased concentration of omega-3 in deer milk relative to cows' milk may provide a marketing advantage based on the benefit of including deer milk in the diet. Deer milk in both mid- and late-lactation had more than double the omega-3 concentration relative to the cow's milk investigated in this study. Typical omega-3 concentrations found in cow's milk range from 0.47 to 1.78 g/100g total FA (Kliem *et al.*, 2018; Capuano *et al.*, 2014), and sheep milk ranges from 0.65 to 2.76 g/100g total FA (Caroprese *et al.*, 2011; Mierlita *et al.*, 2018; Kasapidou *et al.*, 2021). Goats range from 0.51 to 0.92 g/100g total FA (Ceballos *et al.*, 2009; Novotna *et al.*, 2019).

Diet is a major contributor to the omega-3 content of milk, in particular the α -linolenic acid (C18:3; ALA) content (Gebreyowhans *et al.*, 2019). The fatty acid profile of lucerne reveals lower levels of

ALA in lucerne (24.8 mg/g DM) relative to perennial ryegrass (31.87 mg/g DM; Dierking *et al.*, 2010). This suggests the higher levels of omega-3 in deer milk may be due to other factors such as differences in the ruminal biohydrogenation of dietary UFAs. Deer may be more efficient at recovering forage ALA and converting this into fat in the milk, relative to other dairy species. Once again though, the effects of a predominantly lucerne-based diet on the FA profile of milk are not well known, hence the reasons for the differences in omega-3 contents of different milks are difficult to explain.

Fish are recognised as a good source of omega-3 PUFAs. The omega-3 contents of various fish species ranges from 4.62% (canned catfish) to 51.2% (cod fillet) of total FAME upon analysis (Strobel *et al.*, 2012). Another major source of omega-3 in the diet, is walnuts. The inclusion of 56 g/day of walnuts in the diet was found to increase the omega-3 content of Serbian residents over a four-week period. Serbian walnuts were found to contain 11.15% omega-3 (consisting primarily of ALA), upon total FAME analysis (Petrovic-Oggiano *et al.*, 2020), while flaxseed oil, another source of omega-3, has values reported to be as high as 55-58% (Harper *et al.*, 2006; Taylor *et al.*, 2010).

Although, deer milk omega-3 levels are lower than for the foods mentioned above, the deer milk in this investigation had higher levels than reported for other dairy species. This could provide a basis for marketing deer milk's unique properties.

General Conclusions

Limitations and future research

The lack of control of factors such as dietary influence is a limitation. This was specifically limiting when comparing the effects of stage of lactation on FA profiles. Changes in the FA profile may have been due to both changes in diet composition over lactation as well as stage of lactation effects, making it difficult to attribute different effects. There is an absence of research on the effects of grazing solely lucerne on FA profiles, accordingly further research is needed on both milk from animals grazing lucerne and from deer grazing different diets such as perennial ryegrass/white clover mixtures. The use of blood-based analyses to determine the FA available for milk fat synthesis may be utilised in future investigations to gain insight into the sources of FAs.

The number of deer investigated in this project was small. The second sampling in late- lactation resulted in only 22 of the original 43 hinds being sampled. This is a limitation as it meant the whole sample-population were unable to be compared over mid- and late-lactation. However, compared to other literature on FA profiles of deer containing even smaller numbers (n = 1-3) this investigation is still an improvement. Future investigations should aim to involve higher sample numbers, including deer that are able to be sampled throughout the whole lactation period.

The scarcity of other research on red deer milk, in particular FA profiles meant that it was difficult to compare and attribute effects to specific factors. This study aimed to increase the knowledge regarding deer milk FA profiles, but further work is required to confirm and expand on the present study's findings.

Conclusion

There was substantial variation within the FA proportions in the lactating hinds studied. Accordingly, the ability to select and breed for hinds with desirable FA profiles is a possibility for future breeding regimens.

Deer milk FA profiles change over mid- to late-lactation. The changes tend to be the opposite to that observed with cows' milk. It was suggested that in contrast to cows, deer may enter a period of negative energy balance towards the end of lactation.

The distribution of EFAs seen in the current investigation suggests that there may be a physiological baseline for the concentration of these in milk. The baseline concentrations of these may be more pronounced in deer milk due to them being less 'domesticated' and having had less selection for milk production. Deer milk may be closer to the 'natural state', where it serves as a food source for the neonate.

Deer milk is different to cows' milk for all the FAs studied in this investigation. The proportions of FAs also differed significantly from cows' milk. The reasons for this may be because deer are less domesticated, but it may also be because they were on a different diet to the cows.

Deer milk in this investigation had high levels of omega-3 FA, and this could be used as a marketing tool to advertise the benefits of the milk. However, the omega-3 contents of other foods are much higher.

From the above information, it can then be concluded that deer milk could succeed as a niche product. Further investigation into the FA profiles of deer milk, including study of deer grazing different pastures and early lactation studies, would provide further insight and allow confirmation of the present study's findings.

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Appendices

Appendix A: Raw deer milk sample data

Table A. 1. Excel spreadsheet containing the raw data for all FA (g/100 g total FA) in milk from deer collected in mid-lactation

Sample name date file fatty acid (in % of total fatty acid)	16-92	17-333	16-4645	17-2395	17-354	19-217	17-372	16-2448	18-4347	16-229	16-4109	17-2736	16-3508	0-2028	16-5425	16-220	0-2942	19-231	15-337	15-110	15-4178	16-2778	19-232	17-3290	19-225	17-2251	16-3441	18-1952	17-330	17-4084	17-286	17-4245	16-2645	17-627	16-3759	205	16-3762	16-3422	15-3907	19-207	16-3755	341	0-2092	
C6:0	2.399	2.429	2.559	2.527	2.408	2.550	2.480	2.390	2.516	2.416	2.500	2.046	3.072	2.565	3.009	2.710	2.935	2.356	2.432	2.557	2.522	2.469	2.532	2.737	2.890	2.527	2.097	2.351	2.591	2.494	2.992	2.725	2.701	2.893	2.376	2.602	2.399	2.616	2.259	2.562	2.599	2.283	2.814	
C6:1	1.979	1.679	1.457	1.684	1.592	1.644	1.585	1.512	1.479	1.585	1.763	1.489	1.470	1.592	1.569	1.607	1.476	1.513	1.464	1.658	1.417	1.521	1.512	1.469	1.462	1.609	1.813	1.349	1.414	1.375	1.276	1.379	1.311	1.359	1.816	1.698	1.375	1.164	1.789	1.163	1.325	1.245	1.237	
C8:0	1.455	1.242	0.967	1.238	1.151	1.063	1.038	1.057	1.044	1.134	1.167	1.003	0.924	1.009	1.032	1.049	1.078	0.946	0.936	0.928	0.795	0.993	0.970	0.939	0.836	1.058	1.446	0.806	1.001	0.876	0.695	0.831	0.822	0.792	1.225	1.258	0.933	0.743	0.444	0.676	0.762	0.959	0.727	
C10:0	2.724	2.545	1.707	2.575	2.213	1.875	1.942	1.991	2.125	2.195	2.135	1.609	1.560	1.870	1.521	1.937	1.876	1.662	1.944	1.454	1.826	2.015	1.723	1.421	1.966	3.117	1.514	1.796	1.572	1.221	1.399	1.491	1.303	2.237	2.422	1.711	1.267	0.909	1.200	1.355	1.573	1.207		
C10:1	0.204	0.179	0.127	0.197	0.151	0.214	0.160	0.163	0.204	0.179	0.165	0.128	0.107	0.160	0.149	0.123	0.142	0.114	0.114	0.146	0.135	0.146	0.124	0.135	0.144	0.142	0.128	0.156	0.122	0.164	0.142	0.159	0.125	0.160	0.200	0.116	0.124	0.076	0.121	0.087	0.146	0.115		
C11:0	0.027	0.025	0.000	0.023	0.030	0.000	0.000	0.018	0.022	0.000	0.018	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
C12:0	3.503	3.139	2.582	3.037	2.787	2.344	2.715	2.520	3.105	2.920	2.643	2.128	1.998	2.477	2.378	2.393	2.283	2.110	2.495	2.398	1.926	2.300	2.820	2.340	1.937	2.282	2.602	2.101	2.325	2.162	1.852	1.831	2.337	1.867	2.135	2.994	2.245	2.071	1.399	1.829	1.793	2.151	1.704	
C13:0 antineur	0.036	0.037	0.028	0.050	0.029	0.053	0.037	0.026	0.057	0.038	0.021	0.025	0.021	0.035	0.028	0.028	0.022	0.026	0.022	0.025	0.025	0.025	0.028	0.030	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	0.024	
C13:0	0.030	0.049	0.058	0.057	0.056	0.040	0.063	0.061	0.058	0.064	0.065	0.062	0.062	0.062	0.067	0.072	0.061	0.058	0.062	0.055	0.064	0.067	0.073	0.058	0.027	0.052	0.062	0.064	0.071	0.055	0.054	0.074	0.058	0.052	0.067	0.069	0.069	0.075	0.056	0.068	0.064	0.056	0.031	
C14:0	0.048	0.061	0.064	0.073	0.050	0.056	0.073	0.043	0.063	0.057	0.115	0.030	0.079	0.091	0.053	0.065	0.066	0.066	0.055	0.078	0.055	0.080	0.072	0.079	0.038	0.062	0.081	0.077	0.099	0.064	0.061	0.076	0.062	0.064	0.097	0.076	0.072	0.061	0.084	0.106	0.081	0.108	0.062	
C14:1	12.693	12.866	12.507	12.632	11.717	12.557	12.832	10.931	14.246	12.362	12.284	10.869	12.011	12.748	11.533	10.782	12.308	10.646	12.62	13.851	11.051	10.580	13.009	11.473	11.957	10.219	9.777	12.362	10.878	10.088	10.783	11.273	11.501	10.676	10.012	9.567	12.389	10.895	10.321	8.563	10.713	9.813	10.564	10.255
C15:0	0.311	0.333	0.374	0.381	0.333	0.271	0.383	0.242	0.346	0.340	0.409	0.448	0.419	0.408	0.339	0.366	0.352	0.448	0.344	0.348	0.285	0.362	0.411	0.401	0.306	0.427	0.402	0.369	0.455	0.433	0.410	0.401	0.402	0.420	0.473	0.344	0.376	0.401	0.452	0.426	0.421	0.485	0.411	
C16:0	0.245	0.299	0.225	0.215	0.226	0.489	0.298	0.213	0.405	0.235	0.240	0.248	0.213	0.233	0.229	0.231	0.217	0.208	0.206	0.180	0.265	0.226	0.217	0.245	0.258	0.199	0.290	0.358	0.190	0.273	0.248	0.204	0.456	0.237	0.193	0.315	0.204	0.243	0.196	0.300	0.151	0.271	0.249	
C15:0 antineur	0.390	0.402	0.423	0.293	0.423	0.325	0.490	0.532	0.403	0.399	0.511	0.462	0.424	0.473	0.377	0.405	0.370	0.525	0.352	0.595	0.476	0.474	0.500	0.454	0.279	0.396	0.463	0.490	0.500	0.462	0.425	0.543	0.490	0.407	0.503	0.451	0.480	0.413	0.551	0.511	0.499	0.605	0.423	
C16:0	0.972	0.880	0.908	0.925	1.020	0.740	0.979	0.998	0.912	0.821	1.087	1.026	1.089	1.106	0.907	0.980	0.903	1.067	0.939	1.059	1.090	1.007	1.051	0.926	0.759	0.962	0.999	1.085	1.122	1.132	0.941	1.190	1.023	0.973	1.064	1.023	1.062	0.947	1.087	1.063	1.057	1.276	1.040	
C16:1	0.192	0.192	0.206	0.222	0.203	0.174	0.252	0.209	0.194	0.194	0.287	0.245	0.204	0.249	0.191	0.201	0.213	0.283	0.186	0.243	0.238	0.249	0.219	0.242	0.157	0.214	0.251	0.230	0.238	0.258	0.215	0.206	0.245	0.233	0.225	0.233	0.248	0.224	0.251	0.278	0.255	0.291	0.193	
C18:0	27.216	30.204	28.569	29.120	29.974	35.751	30.500	29.018	31.764	30.795	28.316	30.499	31.845	28.376	29.555	28.001	34.963	27.204	33.513	25.166	30.439	28.947	27.791	24.252	33.992	28.162	27.526	33.325	28.013	27.967	24.784	29.039	30.621	30.312	31.818	27.559	32.047	27.836	30.245	29.280	28.430	32.398		
C18:1	0.134	0.103	0.122	0.116	0.106	0.067	0.122	0.119	0.109	0.112	0.131	0.127	0.114	0.112	0.121	0.104	0.119	0.098	0.103	0.110	0.129	0.144	0.101	0.099	0.107	0.160	0.169	0.166	0.112	0.116	0.127	0.111	0.091	0.105	0.123	0.141	0.115	0.104	0.172	0.152	0.174	0.124		
C18:1c	0.084	0.056	0.057	0.032	0.077	0.000	0.040	0.046	0.038	0.045	0.053	0.044	0.047	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044	0.044		
C18:1e	0.431	0.564	0.526	0.719	0.968	0.745	0.546	0.600	0.642	0.824	0.632	0.739	0.442	0.670	0.411	0.573	0.462	0.525	0.471	0.233	0.530	0.683	0.427	0.508	0.485	0.628	0.737	0.639	0.488	0.470	0.608	0.448	0.673	0.538	0.625	0.617	0.420	0.647	0.517	0.714	0.413	0.506	0.542	
C17:0	0.556	0.524	0.561	0.577	0.592	0.409	0.541	0.577	0.530	0.548	0.632	0.710	0.602	0.642	0.585	0.621	0.515	0.632	0.518	0.634	0.630	0.570	0.634	0.551	0.470	0.571	0.582	0.537	0.709	0.657	0.723	0.600	0.571	0.590	0.752	0.473	0.625	0.625	0.653	0.417	0.564	0.496	0.606	
C17:0 antineur	0.510	0.432	0.418	0.461	0.504	0.374	0.459	0.535	0.413	0.424	0.495	0.512	0.417	0.462	0.446	0.493	0.450	0.512	0.348	0.544	0.507	0.513	0.518	0.486	0.314	0.478	0.522	0.502	0.492	0.523	0.524	0.572	0.493	0.494	0.518	0.415	0.573	0.491	0.551	0.493	0.467	0.567	0.459	
C17:1	0.091	0.674	0.681	0.745	0.770	0.569	0.724	0.767	0.701	0.635	0.820	0.770	0.757	0.706	0.719	0.701	0.716	0.705	0.662	0.776	0.755	0.747	0.774	0.703	0.652	0.736	0.764	0.753	0.706	0.644	0.709	0.747	0.709	0.723	0.905	0.702	0.316	0.460	0.551	0.671	0.621	0.700	0.745	
C18:0	12.450	12.639	12.337	12.965	11.571	11.425	13.334	16.124	10.249	12.442	12.599	13.312	14.677	12.462	13.981	11.991	16.723	14.448	18.346	12.616	12.088	13.994	15.399	12.417	16.907	14.120	13.302	15.044	15.449	13.630	15.501	12.852	15.396	16.512	12.518	13.379	13.417	14.373	13.695	14.963	12.907	12.974		
C18:1c	0.229	0.205	0.247	0.135	0.204	0.085	0.180	0.193	0.159	0.181	0.190	0.200	0.171	0.157	0.189	0.221	0.175	0.221	0.155	0.324	0.220	0.222	0.242	0.200	0.228	0.186	0.163	0.202	0.232	0.185	0.195	0.296	0.178	0.197	0.229	0.143	0.226	0.182	0.216	0.182	0.185	0.208	0.201	
C18:1e	0.186	0.168	0.199	0.151	0.164	0.097	0.145	0.156	0.158	0.162	0.167	0.158	0.122	0.164	0.173	0.182	0.154	0.157	0.143	0.229	0.188	0.184	0.202	0.172	0.226	0.150	0.140	0.207	0.181	0.181	0.174	0.231	0.185	0.150	0.191	0.123	0.183	0.162	0.234	0.189	0			

Table A. 2. Excel spreadsheet containing the raw data for all FA (g/100 g total FA) in milk from deer collected in late-lactation

Sample name data file Fatty acid (in g/100g total fatty acids)	16-3441	17-3390	15-337	15-2907	17-2251	19-229	15-110	17-330	14-92	19-232	19-231	18-4297	16-4665	16-2645	16-2778	19-207	16-3762	17-4245	17-333	2503	16-2759	19-225
C4:0	1.925	2.426	2.296	2.216	2.183	1.933	2.345	2.475	2.354	2.490	2.062	2.366	2.540	2.457	2.550	2.544	2.126	2.944	2.267	2.885	2.263	2.871
C6:0	1.742	1.229	1.237	0.766	1.381	1.394	1.282	1.228	1.743	1.240	1.301	1.378	1.373	1.419	1.368	1.158	1.229	1.303	1.450	1.277	1.593	1.211
C8:0	1.403	0.775	0.803	0.444	0.910	0.948	0.787	0.720	1.215	0.759	0.816	0.951	0.907	0.968	0.915	0.675	0.856	0.742	1.126	0.799	1.140	0.656
C10:0	2.948	1.459	1.558	0.814	1.734	1.915	1.472	1.271	2.187	1.546	1.447	1.946	1.694	1.901	1.831	1.197	1.529	1.207	2.241	1.368	2.220	1.113
C10:1(?)	0.176	0.143	0.136	0.076	0.117	0.164	0.108	0.098	0.193	0.127	0.108	0.206	0.127	0.220	0.176	0.132	0.123	0.120	0.178	0.110	0.169	0.118
C11:0	0.029	0.020	0.020	0.021	0.021	0.021	0.021	0.021	0.024	0.020	0.020	0.022	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020	0.020
C12:0	2.361	2.063	2.224	1.249	2.071	2.522	1.957	1.699	2.740	2.259	1.777	2.907	2.264	2.725	2.712	1.719	1.999	1.659	2.927	1.759	2.679	1.617
C13:0 anteiso	0.049	0.032	0.033	0.022	0.021	0.036	0.023	0.023	0.031	0.029	0.023	0.048	0.027	0.050	0.041	0.032	0.024	0.025	0.049	0.022	0.043	0.028
C13:0	0.078	0.055	0.064	0.061	0.062	0.077	0.058	0.079	0.065	0.069	0.067	0.058	0.057	0.059	0.060	0.052	0.066	0.044	0.068	0.047	0.063	0.041
C14:0 iso	0.120	0.084	0.080	0.109	0.074	0.072	0.084	0.114	0.053	0.089	0.087	0.061	0.071	0.086	0.085	0.105	0.078	0.057	0.101	0.072	0.101	0.090
C14:0	11.157	10.442	11.154	7.521	8.385	11.552	8.451	8.026	9.977	9.811	7.828	12.978	11.295	13.495	12.169	10.263	8.899	10.546	11.639	11.128	8.500	10.038
C15:0 iso	0.486	0.486	0.484	0.534	0.468	0.386	0.468	0.537	0.416	0.473	0.506	0.393	0.411	0.416	0.423	0.447	0.415	0.425	0.445	0.426	0.514	0.480
C16:1 c9	0.227	0.294	0.263	0.164	0.167	0.240	0.179	0.171	0.226	0.228	0.190	0.301	0.233	0.484	0.231	0.311	0.239	0.263	0.296	0.214	0.217	0.236
C15:0 anteiso	0.520	0.454	0.456	0.593	0.423	0.373	0.537	0.599	0.374	0.495	0.474	0.357	0.406	0.423	0.452	0.469	0.465	0.390	0.397	0.422	0.503	0.425
C15:0	1.272	1.053	1.181	1.246	1.142	1.109	1.087	1.350	1.117	1.105	1.174	0.956	0.958	0.950	1.029	1.027	1.052	1.030	0.981	1.079	1.113	1.032
C16:0 iso	0.253	0.247	0.211	0.275	0.219	0.209	0.256	0.275	0.195	0.251	0.242	0.190	0.214	0.229	0.231	0.230	0.253	0.205	0.201	0.229	0.249	0.242
C16:0	24.661	28.092	28.304	26.176	26.318	29.766	24.251	26.547	25.591	26.814	25.399	29.040	27.691	28.401	26.626	28.841	25.127	28.259	27.414	30.941	23.290	28.358
C16:1 t9	0.188	0.141	0.152	0.198	0.143	0.150	0.157	0.199	0.150	0.150	0.166	0.100	0.136	0.108	0.179	0.130	0.306	0.120	0.117	0.112	0.234	0.111
C16:1 c7	0.030	0.000	0.000	0.052	0.032	0.000	0.025	0.032	0.065	0.000	0.000	0.000	0.027	0.000	0.032	0.025	0.049	0.035	0.030	0.031	0.036	0.000
C16:1 c9	0.546	0.772	0.512	0.452	0.332	0.459	0.456	0.571	0.459	0.498	0.492	0.593	0.524	0.724	0.440	0.767	0.823	0.677	0.645	0.517	0.687	0.576
C17:0 iso	0.571	0.520	0.572	0.753	0.495	0.535	0.711	0.777	0.216	0.517	0.716	0.940	0.501	0.571	0.531	0.460	0.492	0.676	0.645	0.504	0.511	0.685
C17:0 anteiso	0.496	0.481	0.439	0.526	0.457	0.427	0.497	0.520	0.451	0.531	0.460	0.367	0.435	0.469	0.470	0.445	0.547	0.451	0.427	0.469	0.454	0.475
C17:0	0.934	0.812	0.799	0.916	0.934	0.778	0.850	0.915	0.872	0.790	0.825	0.735	0.720	0.691	0.752	0.679	0.810	0.764	0.737	0.769	0.873	0.835
C18:0	17.205	15.326	16.326	16.315	19.959	13.785	20.928	18.281	16.040	16.639	18.860	12.917	16.563	13.331	13.642	15.176	14.223	17.015	15.709	16.132	17.126	16.137
C18:1 t5-8	0.159	0.157	0.135	0.240	0.188	0.165	0.277	0.227	0.232	0.223	0.229	0.159	0.226	0.175	0.211	0.203	0.253	0.166	0.155	0.179	0.197	0.197
C18:1 t9	0.157	0.164	0.153	0.246	0.158	0.126	0.205	0.178	0.195	0.193	0.182	0.164	0.183	0.155	0.209	0.175	0.403	0.213	0.149	0.120	0.201	0.174
C18:1 t10	0.170	0.164	0.145	0.296	0.212	0.158	0.231	0.240	0.252	0.209	0.204	0.186	0.234	0.215	0.240	0.210	0.503	0.267	0.157	0.180	0.199	0.194
C18:1 t11	1.395	1.247	1.500	2.111	1.445	1.276	1.574	2.079	1.216	1.517	1.716	0.940	1.257	0.571	1.475	1.824	2.163	1.051	1.201	1.811	1.045	1.685
C18:1 c5	0.409	0.384	0.315	0.634	0.462	0.367	0.455	0.504	0.491	0.497	0.445	0.268	0.473	0.359	0.496	0.296	0.621	0.565	0.418	0.440	0.472	0.343
C18:1 t5/c10 (?)	16.992	20.464	19.952	21.286	18.879	18.801	20.914	19.379	18.768	19.656	20.875	19.967	18.615	19.226	19.316	20.679	18.979	19.529	18.416	17.249	20.062	22.242
C18:1 c11	0.480	0.423	0.366	0.677	0.507	0.401	0.414	0.513	0.692	0.502	0.451	0.389	0.462	0.352	0.476	0.394	0.644	0.522	0.434	0.494	0.543	0.335
C18:1 c12	0.605	0.706	0.520	0.695	0.712	0.754	0.793	0.705	0.575	0.796	0.813	0.536	0.586	0.669	0.683	0.689	1.064	0.797	0.624	0.487	0.731	0.637
C18:1 c12	0.282	0.282	0.232	0.501	0.301	0.231	0.294	0.338	0.378	0.296	0.230	0.270	0.293	0.273	0.319	0.259	0.541	0.292	0.281	0.299	0.366	0.250
C18:1 c13	0.051	0.051	0.041	0.126	0.059	0.046	0.035	0.054	0.070	0.058	0.043	0.044	0.040	0.042	0.060	0.042	0.062	0.152	0.071	0.046	0.061	0.068
C18:1 c14/c16	0.512	0.563	0.509	0.844	0.599	0.522	0.335	0.672	0.859	0.656	0.617	0.484	0.516	0.439	0.692	0.723	0.692	0.561	0.654	0.718	0.415	0.415
C18:2 t4,12	0.095	0.067	0.085	0.101	0.081	0.067	0.058	0.092	0.070	0.072	0.071	0.026	0.059	0.025	0.082	0.072	0.128	0.046	0.055	0.070	0.099	0.102
C18:2 c9,t12	0.197	0.207	0.173	0.359	0.238	0.209	0.165	0.241	0.367	0.221	0.205	0.141	0.207	0.122	0.241	0.170	0.215	0.212	0.212	0.255	0.271	0.234
C18:2 c9,t12	0.292	0.310	0.276	0.464	0.292	0.291	0.234	0.324	0.438	0.329	0.278	0.315	0.294	0.296	0.398	0.313	0.458	0.373	0.288	0.334	0.379	0.087
C18:2 t9,c12	0.100	0.112	0.091	0.167	0.098	0.110	0.075	0.110	0.164	0.118	0.093	0.120	0.103	0.104	0.137	0.108	0.147	0.123	0.109	0.101	0.135	0.033
unknown	0.030	0.035	0.030	0.047	0.031	0.031	0.025	0.035	0.047	0.036	0.033	0.039	0.034	0.038	0.043	0.036	0.050	0.037	0.031	0.029	0.040	0.007
C19:0	0.138	0.118	0.108	0.110	0.157	0.125	0.147	0.113	0.129	0.112	0.121	0.096	0.112	0.106	0.089	0.107	0.129	0.124	0.110	0.115	0.123	0.099
unknown	0.402	0.399	0.453	0.474	0.331	0.277	0.391	0.465	0.223	0.334	0.360	0.301	0.290	0.245	0.407	0.263	0.540	0.203	0.278	0.353	0.475	0.305
C18:3 c6,9,12	2.249	2.210	1.755	3.285	2.391	2.752	2.343	2.546	2.895	2.654	2.286	2.311	2.374	2.313	2.403	2.243	3.413	2.176	2.420	2.109	2.686	2.281
C19:1	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
C18:3 c6,9,12	0.000	0.000	0.000	0.023	0.024	0.000	0.023	0.000	0.025	0.000	0.000	0.000	0.020	0.000	0.000	0.024	0.024	0.022	0.020	0.020	0.024	0.000
C18:3 c9,12,15	2.126	1.909	1.772	3.239	2.177	1.996	1.707	2.451	2.514	2.289	2.397	1.923	2.005	1.886	2.310	2.025	2.564	1.572	2.176	2.111	3.016	1.774
C20:0	0.222	0.269	0.212	0.252	0.239	0.183	0.254	0.251	0.204	0.246	0.226	0.212	0.205	0.220	0.207	0.234	0.243	0.243	0.250	0.219	0.199	0.266
CLA c9,t11	0.679	0.630	0.792	0.955	0.845	0.630	0.590	0.313	0.544	0.679	0.714	0.605	0.590	0.600	0.912	0.842	1.317	0.520	0.507	0.518	0.953	0.573
unknown	0.076	0.056	0.077	0.067	0.064	0.074	0.049	0.079	0.027	0.063	0.067	0.041	0.053	0.040	0.071	0.063	0.100	0.041	0.047	0.056	0.052	0.021
C20:1 c9	0.061	0.093	0.066	0.082	0.063	0.093	0.071	0.074	0.052	0.082	0.084	0.078	0.085	0.079	0.073	0.075	0.071	0.070	0.082	0.060	0.057	0.076
C20:1 c11	0.061	0.098	0.054	0.104	0.070	0.063	0.082	0.														

Appendix B: Average proportion of all detected FAs in mid- and late-lactation

Table A. 3. Table containing mean FA proportions of all FAs detected in deer milk samples in mid- and late-lactation

Fatty acid	mid-lactation (g/100 g total FA)	late-lactation (g/100 g total FA)
C4:0	2.59	2.39
C6:0	1.49	1.34
C8:0	0.98	0.88
C10:0	1.81	1.66
C10:1	0.14	0.14
C11:0	0.01	0.01
C12:0	2.41	2.23
C13:0 anteiso	0.03	0.03
C13:0	0.06	0.06
C14:0 iso	0.07	0.08
C14:0	11.61	10.24
C15:0 iso	0.39	0.46
C14:1 c9	0.27	0.26
C15:0 anteiso	0.45	0.45
C15:0	1.01	1.09
C16:0 iso	0.23	0.23
C16:0	29.73	27.09
C16:1 t9	0.13	0.16
C16:1 c7	0.05	0.02
C16:1 c9	0.58	0.64
C17:0 iso	0.60	0.66
C17:0 anteiso	0.48	0.47
C17:0	0.76	0.81
C18:0	13.69	16.29
C18:1 t5-8	0.20	0.22
C18:1 t9	0.17	0.19
C18:1 t10	0.21	0.23
C18:1 t11	1.24	1.55
C18:1 c6	0.68	0.47
C18:1 c9	16.85	19.56
C18:1 t15/c10	0.73	0.48
C18:1 c11	0.57	0.69
C18:1 c12	0.34	0.31
C18:1 c13	0.10	0.06
C18:1 c14/t16	0.89	0.62
C18:2 t9,12	0.07	0.07
C18:2 c9 t13	0.45	0.23
C18:2 c9 t12	0.48	0.32
C18:2 t9 c12	0.18	0.11
unknown	0.04	0.03
C19:0	0.10	0.12
unknown	0.44	0.35
C18:2 c9,12	2.17	2.51
C19:1	0.01	0.00
C18:3 c6,9,12	0.02	0.01
C18:3 c9,12,15	2.64	2.18
C20:0	0.20	0.23
CLA c9 t11	0.57	0.71
unknown	0.06	0.06
C20:1 c9	0.07	0.07
C20:1 c11	0.08	0.09
C20:2 c11,14	0.02	0.03
C20:3 c8,11,14	0.01	0.02
C20:4 c5,8,11,14	0.10	0.10
C20:3 c11,14,17	0.09	0.09
C22:0	0.08	0.10
C22:1 c13	0.01	0.02
C20:4 c8,11,14,17	0.02	0.01
C20:5 c5,8,11,14,17	0.07	0.07
C23:0	0.07	0.07
C24:0	0.04	0.05
C22:5 c7,10,13,16,19	0.29	0.23
C22:6 c4,7,10,13,16,19	0.05	0.05