MASSEY
UNIVERSITY

Phenotypic Relationship between Milk Fatty Acid Profile and Live Weight Change in Early Lactation in New Zealand Dairy Cattle

A thesis presented in partial fulfilment of the requirements for the degree of

Master of Science

in

Animal Science

Institute of Veterinary, Animal and Biomedical Sciences

Palmerstone North, New Zealand

Vedastus Mkunda

2016

ABSTRACT

The phenotypic relationship between milk fatty acid (FA) profile and live weight (LW) change in early lactation in grazing Friesian x Jersey (FxJ) cows was investigated in this study. Data used in this study compromised of 73,040 daily milk yields, 5,936 fortnightly herd-tests for fat, protein and lactose, 41,981 daily live weights, and 882 determinations of FA profiles from 300 second-lactation FxJ crossbred cows recorded during the production season 2003-04. Cows were classified based on the magnitude of LW change from calving to peak lactation into three groups: cows with low live weight loss (L; below -0.012kg), medium live weight loss (M; below -0.174kg and high live weight loss (H; below -0.340kg). LW change was considered as a proxy for energy balance. Correlations between LW change and individual FAs or group of FAs were estimated at the three stages of lactation (early, mid and late). Stage of lactation affected significantly ($P>0.05$) the concentration of all FAs considered in this study, except the concentration of C20:0. Higher concentration of C18:0 and C18:1 cis-9 was observed in early lactation relative to other lactation stages. Compared to the L and M cows, the H cows had higher concentration of C18:1 cis-9 in early lactation. Live weight loss in early lactation was significantly associated with higher concentrations of unsaturated ($r = -0.19$), long-chain FA ($r = -0.17$), C17:0 ($r = -0.14$), C18:1 cis-9 ($r = -0.20$) and C18:3 cis-9, cis-12, cis-15 ($r = -0.21$), but live weight loss was significantly associated with lower concentrations of saturated FA ($r = 0.18$), medium-chain FA ($r = 0.16$), C12:0 ($r = 0.24$), C14:0 ($r = 0.17$) and C15:0 ($r = 0.22$). The association between LW changes in early lactation and most of the FAs were not significant in mid and late lactation. If determination of FA can be implemented using mid-infrared spectroscopy, a conclusion from this study is that concentration C18:1 cis-9 in early lactation can be used as indicator of live weight change (energy balance). Further studies are required to evaluate the inclusion of concentrations of FAs in breeding programs to improve fertility in seasonal grazing dairy cattle.

DEDICATION

I would like to dedicate this thesis to my kids, Abednego and Abigail, and I am grateful to have such wonderful loving kids, to my beloved wife, Beatrice Senyagwa.

ACKNOWLEDGEMENTS

First, I would like to express my sincerest thanks to the Ministry of Foreign Affairs and Trade of New Zealand for the scholarship to study my Master's degree at Massey University. Special thanks to my chief supervisor, Professor Nicolas Lopez-Villalobos, for guiding me throughout my study and thesis writing period. I appreciated all of his suggestions on theoretical and mathematical aspects that enriched this thesis. Nicolas, I thank you very much for your unconditional support and sharing with me ideas from your very clear mind – muchas gracias Nicolas.

I express further thanks to the New Zealand Livestock Improvement Corporation (LIC) for providing the data used for this research. Thanks to my colleagues, postgraduate students (Charlotte Gray, Felipe Lembeye and Robin Harris) and others. Simple acknowledgment can't express my thanks to you all. I wouldn't have finished this thesis without your bright ideas. Special thanks goes to the International Student Office team – Sylvia Hooker, Jamie Hooper, Broderick Dave and among others – for their kindness and support. Thank you to the government of United Republic of Tanzania through the President's office public service management team for allowing me to pursue my study in New Zealand.

Special thanks to my wife, Beatrice John Senyagwa, for her persistent encouragement and motivation during the period of my study. Very special thanks go to my son and daughter, Abednego and Abigail, for their patience when I was not with them.

Last, but not least, acknowledgement goes to my mother, Angeline Mkunda and uncle Ishumalia Mkunda, for raising me in a good way, and I appreciate their love and support throughout my study life. Above all, I thank GOD for bestowing on me good health and the necessary patience for me to complete my study in New Zealand.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ΔLW	Live weight change
ALA	α-Linolenic acid
CLA	Conjugated linoleic acid
EB	Energy balance
F	Friesian
FA	Fatty acids
FxJ	Friesian x Jersey
J	Jersey
LA	Linoleic acid
LCFA	Long-chain fatty acids
LW	Live weight
MCFA	Medium-chain fatty acids
MIR	Mid-infrared spectroscopy
MUFA	Monounsaturated fatty acid
NEB	Negative energy balance
PUFA	Polyunsaturated fatty acids
SCFA	Short-chain fatty acids
SFA	Saturated fatty acids
UFA	Unsaturated fatty acids
VA	Vaccenic acid

CHAPTER ONE

GENERAL INTRODUCTION

Milk fat is the main energy component in milk and the most easily digested animal fat in the human diet. Milk fat contains 400 to 500 fatty acids (FA) (Nogalski et al., 2012) and 75% of them are saturated FA (SFA) with even carbons between C4:0 to C20:0 (Ducháček et al., 2013). The short-chain saturated FA (SCFA) are the typical acids found in bovine milk. Monounsaturated FA (MUFA) from C14:1 to C18:1 comprises 25% of the total FA, with the most abundant MUFA being oleic acid (C18:1 cis-9), whereas polyunsaturated FA (PUFA) with carbon between C16 to C18 comprise 5% of the total FA (Bastin et al., 2012). The low proportion of PUFA in bovine milk fat is due to biohydrogenation of FA in the rumen (Welch et al., 1997). However, in dairy cows about half of the FA (C4:0 to C14:0 and half of C16:0) are synthesised by de-novo processes in the mammary gland from SCFA. The remaining half of FA (half of C16 and C18 and longer-chain FA) is transported to the mammary gland by blood, especially through its high lipoprotein fraction, in the form of non-esterified fatty acids originating from either diet or adipose tissue (Bauman and Griinari, 2003). Due to the high content of SFA and the presence of cholesterol, milk fat is considered a risk factor for atherosclerosis by some doctors and dieticians, although it remains a rich source of many FA known for their health-promoting properties (Nogalski et al., 2012). However, changes in milk fat composition during the lactation imply shifts in the activity of FA pathways and are related to changes in the energy status of the cow (Gross et al., 2011). A study by Van Knegsel et al. (2005) indicated that during negative energy balance (NEB), de-novo synthesis of milk FA (C6:0 to C14:0) was reduced and body fat reserves used. Stage of lactation and energy balance significantly contributes to variation in milk fat composition and alter the activity of different FA pathways.

In recent years questions have been raised about the causes of variation in the milk FA profile with respect to changes in energy balance during lactation (Ducháček et al., 2013; Gross et al., 2011; Bastin et al., 2016). Increased milk yield in peak lactation results in changes to energy balance and FA profiles, which correspond to increased times to first ovulation and first breeding in dairy cows, but if investigated thoroughly can be prevented by proper feeding regimes prior to calving (Colazo and Mapletoft, 2014). At the present time, there is limited information about the relationship between milk FA composition and live weight change during lactation. Previous research with Friesian dairy cows under New Zealand conditions (Payne et al., 1979) suggested that the ratio between oleic acid (C18:1 cis-9) and C10:0 could be a useful biochemical indicator of energy balance. Similarly, Thomson et al. (2002) also working with Friesian cows, revealed that severe loss in live weight would be associated with a change in the pattern of FA profiles, especially an increased proportion of MUFA in particular C18:1 cis-9 and a decreased proportion of SFA. Since live weight change in the 4 weeks after calving has considerable influence on fertility (Alawneh et al., 2012), knowledge on the relationship between LW change and FA profile could be of practical significance to dairy farmers. Therefore, the objective of this study was to determine the phenotypic relationship between milk FA profile and live weight change in pasture grazed New Zealand dairy cattle.

CHAPTER TWO

REVIEW OF LITERATURE

2.1. Typical versus ideal milk fat acids composition

The FA profile of bovine milk is different from the proposed ideal FA profile of milk from a human health point of view (Grummer, 1991) (Figure 2.1). Bovine milk contains large proportions of UFA, especially medium-chain FA (MCFA; C14:0 and C16:0), while UFA are found in small proportions. The MCFA, mainly C16:0 has a major role in increasing the level of bad cholesterol (LDL cholesterol) in blood that affects human health negatively.

The long-chain FA (LCFA), such as C18:0, C18:1 cis-9 or higher, are commonly considered to increase the level of good cholesterol (HDL cholesterol) in blood and they have a neutral effect or affect human health positively (Mensink et al., 2003). However, increase of C18:1 cis-9 in milk contents would be associated with higher mobilization of body fatty reserves which results to negative energy balance in grazing cattle during early lactation (Nogalski et al., 2012).

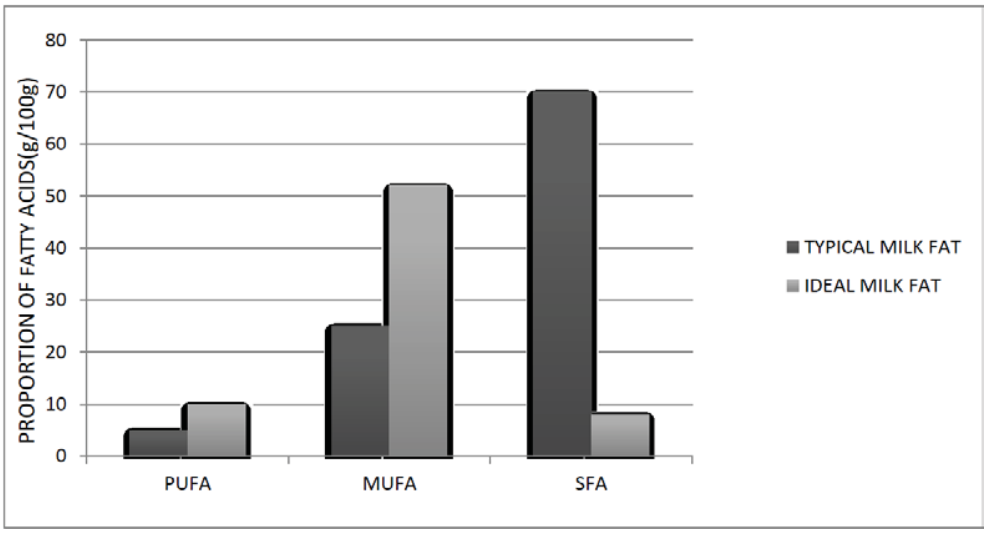

Figure 2.1. Typical versus ideal fatty acid profile for bovine milk proposed by Wisconsin Milk Board 1988 Milk Fat Roundtable, modified from O'Donnell (1989) (PUFA=Polyunsaturated fatty acids; MUFA = Monounsaturated fatty acids; SFA = Saturated fatty acids).

2.2. Factors affecting the fatty acid profile of bovine milk

In the last decade, several authors outlined many factors which could alter the FA profile in bovine milk but in this review only some factors are discussed.

2.2.1. Effect of breed and individual variation of fatty acid profile

Silva-Villacorta et al. (2012) reported that milk fat from Holstein-Friesian cows had higher concentrations of UFA than the milk fat from Jersey cows under grazing conditions. The same differences between these breeds were reported by Back and Thomson (2005) who found an intermediate value for the concentration of UFA in milk fat of crossbred cows in late lactation. Differences in FA composition between breeds have been reported in many overseas studies (Palladino et al., 2010), but differences between strains within breed have been also reported (Wales et al., 2009). An explanation of breed differences for FA profile was provided by Soyeurt and Gengler (2008). These authors showed that activity of the enzyme Stearoyl-CoA desaturase-1 (SCD1) can vary during lactation and it was found to be lower in Jersey than in Holstein-Friesian cows. Evidence was presented on how the presence of SCD1 plays an important role in the desaturation of FA and could partly explain the variability in the concentration of UFA in milk fat between breeds. A recent New Zealand study (Littlejohn et al., 2014) found that genes controlling the synthesis of the enzyme 1-acylglycerol-3-phosphate-O-acyltransferase 6 (AGPAT6) are causally involved in differential synthesis of FA.

Fernandez and Rodriguez (2002) reported cow breed effects contributed about 1% of milk FA variation. This implies that variation of FA can be as result of cow breed in very small proportion and transmissible to next generation. Furthermore, the differences in LCFA, especially CLA in Jersey and Holstein-Friesian cows and their variability, could be either due to Δ9 desaturase enzyme activity or the influence of vaccenic acid production in the rumen (Samková et al., 2012).

Moreover, Givens et al. (2006) found a relationship between cow genotype and the FA profile, suggesting that animal genotypes may trigger variation in the milk FA profile. Breed differences in the milk FA profile have been reported by several studies summarized by Lopez-Villalobos (2012) and presented in Table 2.1.

2.2.2. Effect of feed type on fatty acid profile

Since diet plays a major role on the composition of milk FA, it has received the most attention in the scientific literature (Beaulieu and Palmquist, 1995; Chilliard et al., 2001; Dewhurst et al., 2003). According to Croissant et al. (2007), grazing cows would have a higher concentration of CLA in milk when shifting from feeding concentrates to pasture grazing. This indicates that cows grazing on pasture should have a higher concentration of CLA compared to cows feed concentrates (Daley et al., 2010). Heck et al. (2009) found that FA composition in milk from grazing cows may be influenced by the season of the year due to the effect of season on pasture quality. Pasture in winter compared to pasture in summer, contains less concentration of PUFA, especially α-linolenic acid (ALA), which is essential FA in the formation of CLA. Low concentration of PUFA in pasture would be due to the lipolysis and oxidation that occurs during the wilting and maturation of pasture. Hence, pasture harvesting and composition should be taken into consideration during diet formulation due to its association with FA composition (Samková et al., 2012). However, an increase in CLA formation results in the inhibition of de-novo synthesis for FA (Chilliard et al., 2001).

Table 2.1. Differences in the fatty acid profile of milk fat from Holstein (or Holstein–Friesian) and Jersey cows obtained in various studies, adapted from Lopez-Villalobos (2012).

	DePeters *et al.* [50] (total mixed ration)		Beaulieu and Palmquist [51] (total mixed ration)		Palladino *et al.* [53] (grazing ryegrass)		Auldist *et al.* [52] (grazing ryegrass)	
	(*N*=16)	Jersey (*N*=23)	Holstein (*N*=8)	Jersey (*N*=8)	Holstein–Friesian (*N*=27)	Jersey (*N*=27)	Holstein–Friesian (*N*=29)	Jersey (*N*=29)
Milk yield (kg)	30.3	17.6**	36.1	24.0**	21.10	14.50**		
Composition (%)								
Fat	3.74	4.81**	2.97	3.99**	3.79	5.09**	4.47	5.82**
Protein	3.16	3.95**	2.97	3.61**	3.43	4.01**	3.55	3.98**
Fatty acid (g/100 g of fat)								
C4:0 (butyric)	4.08	3.88	4.10	4.00	6.45	6.17	4.92	5.07*
C6:0 (caproic)	2.71	2.80*	2.40	2.80*	3.88	3.92	3.00	3.14**
C8:0 (caprylic)	1.59	1.71**	1.30	1.80	2.20	2.21	1.73	1.81**
C10:0 (capric)	3.68	4.18**	3.00	4.30**	4.20	4.46	3.62	3.86**
C10:1							0.32	0.28
C12:0 (lauric)	4.26	4.98**	3.50	5.00**	4.29	4.44	3.79	3.99
C12:1							0.08	0.05**
C14:0 (myrsitic)	13.56	13.88	11.60	12.60*	11.67	11.63	11.03	11.42*
C14:1					1.18	0.97**	0.86	0.73*
C15:0					1.27	1.17*	1.97	1.58**
C16:0 (palmitic)	35.33	34.89	32.40	30.20*	23.75	25.53**	25.50	26.69
C16:1					1.38	1.34	1.17	0.99**
C16:1 *trans*	0.44	0.41						
C16:1 *cis*	1.99	1.80						
C17:0					0.45	0.42	1.44	1.32**
C17:1							0.22	0.17
C18:0 (stearic)	8.93	9.52**	7.20	8.10	7.97	8.30	10.96	12.62**
C18:1							23.83	21.63**
C18:1 *cis*-9 (oleic)	17.88	16.18**	17.30	15.10**	15.81	13.93*		
C18:1 *trans*-9	0.21	0.23						
C18:1 *trans*-11 (vaccenic)	1.51	1.54			4.34	4.50		
C18:2 *cis*-9,*cis*-12 (linoleic)	2.53	2.57	2.20	2.20	0.59	0.47**	1.69	1.54**
C18:2 *cis*-9, *trans*-11 (conjugated linoleic acid)					1.72	1.56	1.53	1.08**
C18:3 *n*-3 (linolenic)	1.29	1.49	0.48	0.40	0.66	0.60	0.77	0.69**
C18:1/C18:0	2.06	1.72**	2.54	2.02*				
C20:0 (arachidic)							0.09	0.10**
UFA[1]	25.86	24.18**						
UFA[2]					27.36	24.60*		
SFA[3]					65.88	68.90*		
UFA[2]:SFA[3]					0.05	0.04**		
SCFA[4]	8.38	8.38			12.46	12.41		
MCFA[5]	21.50	23.04*						
MCFA[6]					48.56	50.43		
LCFA[7]	32.35	31.48						
LCFA[8]					32.18	30.71		

[1]Unsaturated fatty acids; sum of C16:1 trans, C16:1cis, C18:1 cis 9, Cl8:1 trans-9, C18:1 trans-11, C18:2 and C18:3.
[2]Unsaturated fatty acids; sum of C10:1, C14:1, C16:1, C17:1, C18:1, vaccenic acid, linoleic acid, conjugated linoleic acid and linolenic acid.
[3]Saturated fatty acids; sum of C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0 and C24:0.
[4]Short-chain fatty acids; sum from C4:0toC8:0.
[5]Medium-chain fatty acids; sum of C10:0, C12:0 and C14:0
[6]Medium-chain fatty acids; sum of C10:0, C12:0, C14:0, C15:0 C16:0 and C16:1.
[7]Long-chain fatty acids; sum of C18:0, C18:1 trans-11, Cl8:1cis-9, C18:2 and C18:3.
[8]Long-chain fatty acids; sum from C17:0, C18:0, C18:1cis-9, C18:1 trans-11, vaccenic acid, linoleic acid, conjugated linoleic acid, C20:0, C20:3 n-6, C20:4 n-6, and C22:6 n-3.
*P<0.05, **P<0.01.

Recent studies have shown that pasture during summer limits de-novo FA synthesis, while in winter limits blood pre-formed FA (Palladino et al., 2010). Therefore, further studies are needed to address the link between LW change and CLA and their relationship to ruminant energy balance as the increase of CLA in pasture-grazing cows depend on the provision of ALA substrate which is converted into CLA (Collomb et al., 2006).

2.2.3. Parity number and age on fatty acid profile

The information on the effects of physiological factors such as parity and age at calving on FA composition of bovine milk is limited and mainly based on previous research conducted with limited data sets (Bilal et al., 2014) and sometimes it is contradictory.

In Canadian Holstein cows, Kgwatalala et al. (2009) found that, the FA composition of bovine milk did not differ across parities, whereas the recent studies of Bilal et al. (2014) reported that first-parity cows had a relatively lower value of C14 index (14:1 cis-9/[14:0 + 14:1 cis-9]) compared with later parity cows, suggesting that the rate of unsaturation of C14:0 was lower for first-parity cows as compared with cows in later parities.

Secchiari et al. (2003) with pasture grazed cattle observed that parity did not affect the concentration of major FA in 22 Italian Friesian cows, but Kelsey et al. (2003) reported parity affects on the concentration of most FAs in milk of US dairy cows. Rani et al. (2011), in a study with 44 Nguni cows in South Africa, reported a significant parity effect on milk FA composition. The effect of age at calving on FA composition of cow's milk is not known. In a study on 190 cows belonging to four different breeds in the Netherlands, Maurice-Van Eijndhoven et al. (2011) included fixed effect of age at calving nested within parity in their model for FA analysis, and reported that age at calving effect was not significant on FA composition.

2.2.4. Stage of lactation on fatty acid profile

Milk FA composition seems to be influenced by the stage of lactation (Palmquist et al., 1993; Kelsey et al., 2003; Kay et al., 2005; Garnsworthy et al., 2006; Craninx et al., 2007; Stoop et al., 2009; Kgwatalala et al., 2009; Samková et al., 2012; Nantapo et al., 2014). At the start of lactation, cows are most often mobilising adipose FA, which are partially incorporated into milk fat (Palmquist et al., 1993). The greater uptake of LCFA during this period decreases the proportion of SCFA and MCFA in milk fat, due to both a dilution effect and inhibition of de novo synthesis of FA (Chilliard et al., 2001). Therefore, proportions of SCFA and MCFA are relatively low in the beginning of the lactation and increase until at least 8 to 10 weeks after calving (Palmquist et al., 1993; Chilliard et al., 2001), whereas the proportions of LCFA progressively decrease in the early lactation period. For example, Kgwatalala et al. (2009) observed that milk in early lactation (<100 DIM) had significantly higher concentrations of C18:1 cis-9, trans-vaccenic acid, C18:2, total MUFA and PUFA compared to either milk in mid (100-200 DIM) or late (<200 DIM) lactation (see Table 2.2). The authors indicated that an increase in C18:1 cis-9 would be associated with cow energy status in high yielding cows. This association between FA profile and stage of lactation imply that cows producing more milk during early lactation could face negative energy status due to mobilization of more fatty reserves, which also increases the proportion of C18:1 cis-9.

Similar results were reported by Auldist et al. (1998) in grazing dairy cows. The authors found lower concentration of C6:0 to C12:0 and higher concentration of pre-formed FAs, i.e. C18:0 and C18:1 cis-9 from milk produced in early lactation compared with milk produced in mid and late lactation.

2.2.5. Energy status of the cow in early lactation on fatty acid profile

Energy status of the cow is one of the main factor driving health disorders in the transition from dry to the early stage of lactation. Due to the increased milk yield and inability of cows to get sufficient amount of energy through diet, NEB commonly appears in early lactation (Berry et al., 2006; Heuer et al., 2001) (Figure 2.2). Although glycogen and body proteins participate in compensation of energy deficit, the major source of energy in this period is body fat reserves, i.e. lipomobilisation.

Table 2.2. Effect of stage of lactation (early, mid, and late) on the fatty acid profile (g/100 g of total fatty acids) of Canadian Jersey cows, modified from Kgwatalala et al. (2009).

Fatty acid[1]	Early (<100 DIM)	Mid (100-200 DIM)	Late (> 200 DIM)	P-value
C4:0	$4.40^{a} \pm 0.07$	$4.18^{b} \pm 0.06$	$4.16^{b} \pm 0.06$	0.0007
C6:0	$3.13^{a} \pm 0.04$	$3.04^{ab} \pm 0.04$	$3.02^{b} \pm 0.03$	0.0325
C8:0	0.136 ± 0.004	0.131 ± 0.003	0.128 ± 0.003	0.117
C10:0	6.72 ± 0.01	6.76 ± 0.09	6.69 ± 0.09	0.2079
C10:1	$0.58^{a} \pm 0.01$	$0.68^{b} \pm 0.01$	$0.70^{b} \pm 0.01$	<0.0001
C12:0	$6.10^{a} \pm 0.11$	$6.52^{b} \pm 0.10$	$6.40^{b} \pm 0.10$	0.0002
C12:1	$0.14^{a} \pm 0.01$	$0.16^{b} \pm 0.01$	$0.17^{b} \pm 0.01$	<0.0001
C14:0	$14.9^{a} \pm 0.16$	$15.7^{b} \pm 0.15$	$15.7^{b} \pm 0.14$	<0.0001
C14:1	$0.94^{a} \pm 0.03$	$1.05^{b} \pm 0.03$	$1.10^{b} \pm 0.03$	<0.0001
C16:0	$25.6^{a} \pm 0.27$	$26.9^{b} \pm 0.25$	$25.9^{a} \pm 0.22$	<0.0001
C16:1	1.21 ± 0.03	1.22 ± 0.02	1.21 ± 0.02	0.8514
C18:0	8.37 ± 0.30	8.10 ± 0.28	7.72 ± 0.25	0.0888
C18:1 cis-9	$18.7^{a} \pm 0.29$	$16.2^{b} \pm 0.27$	$17.5^{c} \pm 0.24$	<0.0001
C18:1 trans-11	0.81 ± 0.03	0.76 ± 0.02	0.78 ± 0.02	0.3257
CLA	$0.22^{a} \pm 0.01$	$0.23^{ab} \pm 0.01$	$0.25^{b} \pm 0.01$	0.0038
C18:2	1.43 ± 0.04	1.33 ± 0.04	1.35 ± 0.03	0.0868
C18:3	0.44 ± 0.02	0.45 ± 0.02	0.47 ± 0.01	0.3456
SFA	$69.3^{a} \pm 0.35$	$71.5^{b} \pm 0.33$	$69.7^{a} \pm 0.29$	<0.0001
MUFA	$21.6^{a} \pm 0.28$	$19.3^{b} \pm 0.26$	$20.7^{c} \pm 0.24$	<0.0001
PUFA	2.89 ± 0.07	2.77 ± 0.06	2.84 ± 0.06	0.2813

[a–c] Means within a row with different superscripts differ ($P \leq 0.05$).
[1] CLA = conjugated linoleic acid.
SFA = saturated fatty acids.
MUFA = monounsaturated fatty acids.
PUFA = polyunsaturated fatty acids.

Lipomobilisation is mainly manifested in cows that are not adequately prepared to start lactation or those cows that enter lactation in obese condition (Goff, 2006). Fats mobilized from body depots are passing through the liver, thus limiting its synthetic and detoxifying capacity, which, together with NEB, can adversely affect the cow's productive and reproductive performance (Horvat et al., 2009). Based the above mentioned relation, the period of early lactation not only represents the most important period in the production but also reproductive cycle of high yielding dairy cows, so animal scientist and other dairy stakeholder should pay special attention to diagnose and eliminate the health disorders occurring in early lactation. This applies particularly to nutrition related disorders. The most important indicators of the metabolic status of cows in early lactation are metabolically active hormones, body condition score, concentration of biochemical blood parameters, and the concentration and ratio of organic milk ingredients (Kampl, 2005).

Estimation of energy status of cows on the basis of determination of the concentration and the ratios of organic milk ingredients is a method which, has the advantage over others because of its simplicity, reliability and economy and is widely used in practice. The parameters for the evaluation in this method are the concentrations of fat, protein and urea in milk, as well as the ratio of fat and protein (fat to protein ratio) A more detailed description of this method, as well as the characteristics of the physiological processes that underlie has been frequently discussed in previous studies (Heuer, 2000). Further studies should target cows for energy status assessment between 10 and 60 days of lactation, which is the most critical period for future productive and reproductive performance of the cows.

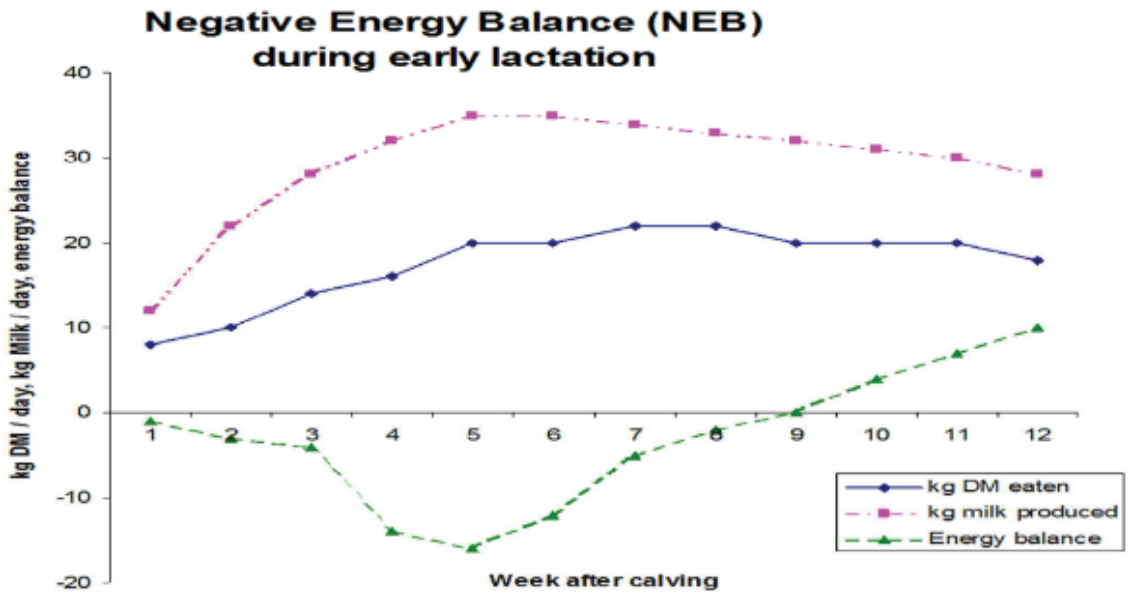

Figure 2.2. The relationship between milk yield, dry matter intake and energy balance in first 12 weeks of lactation in dairy cattle, modified from Heuer (2000).

2.2.5.1. Effect of live weight change on fatty acid profile in early lactation

Knowledge about the relationship between LW change and the FA profile is limited. Previous studies investigated the relationships between live weight, milk yield and dry matter intake during early lactation (Figure 2.3; Heuer, 2001). Thomson et al. (2002) reported a negative relationship between the concentrations of C18:1 cis-9 and live weight change; the higher the live weight loss the higher the concentration of C18:1 cis-9. Similarly, Palmquist et al. (1993) found that when the body reserves were mobilised resulted in a higher proportion of unsaturated long-chain FA in milk (especially C18:1 cis-9), de-novo synthesized FAs decreased, resulting in lower concentration of short and medium-chain FA. Schroeder et al. (2005) observed a relationship between live weight loss and increases in unsaturated long-chain FA in milk.

A more recent study (Stoop et al., 2009) showed that changes in energy balance were related with shifts in the synthesis of FA and changes in the milk fat composition. Cows with NEB in early lactation had an increased concentration of C18:1 cis-9 and a decreased concentration in odd-chain FA C5:0 to C15:0, reflecting the reduced allocation of glucogenic components to milk fat synthesis.

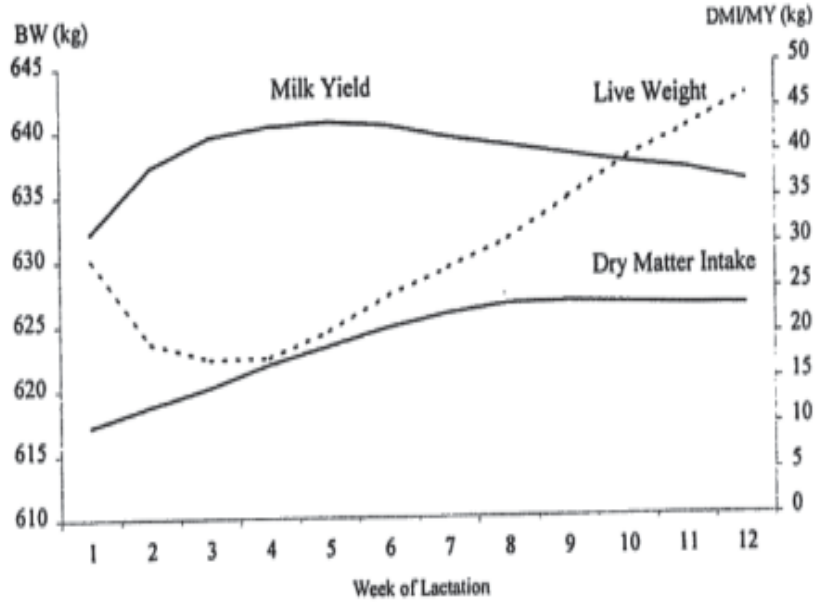

Figure 2.3. Daily dry matter intake, live weight and milk yield during the first 12 weeks of lactation in dairy cattle, from Heuer (2001).

2.2.5.2. Effect of energy balance in early lactation

The demand of energy during early lactation typically exceeds the dietary energy intake, which often results in a negative energy balance (Patton et al., 2006). High producing dairy cows mobilize their body fat, and to some extent, protein reserves in order to sustain their milk production which leads animals to enter a state of NEB until energy intake meets the output

requirements (Knop and Cernescu, 2009). Loss of energy in feed reduces the ability of rumen microbes to digest plant proteins and synthesize animal proteins, thus reducing the protein percentage in milk. The mobilization of body fat increases the concentration of non-esterified fatty acids in the liver and consequently the percentage of fat in milk; the resulting effect is that the fat to protein ratio in milk increases. The resulting NEB and metabolic demands influence the postpartum interval to first ovulation, thereby affect the interval to conception (Butler, 2003) and the reproductive potential of the affected cows. Conception rates are thought to be low in animals that experienced NEB pre-partum and early post-partum because of the poor quality of oocytes generated during this stage (Knop and Cernescu, 2009). Many studies have shown that while feed intake and milk production both increase during early lactation, maximum feed intake is only achieved some weeks after maximum milk yield (Garnsworthy, 1988). A relationship that involves three components, dry matter intake, live weight and milk production comes into play throughout lactation (Figure 2.3). Most of the cows have a peak of milk production after calving that is impaired by a decrease in dry matter intake resulting in a loss of live weight, leading to a NEB during the first 6 to 8 weeks of lactation (Heuer, 2000). It would therefore be a challenging task to prevent NEB in cows that are naturally high producers because of the interplay of these factors.

2.3. Hormonal linkage between energy balance early lactation and reproductive performance

During periods of NEB in early lactation, blood concentrations of glucose, insulin and insulin-like growth factor 1 (IGF-1) are low as are the pulse frequencies of GnRH and LH (Kadokawa and Martin, 2006; Fenwick et al., 2008). Plasma progesterone concentrations are also affected by the energy balance of dairy cows. These metabolites and hormones have been shown to affect

folliculogenesis, ovulation, and steroid production in vitro and in vivo (Kadokawa and Martin, 2006). The exact mechanism by which energy affects secretion of releasing hormones and gonadotropins is not well defined; but it is clear that lower levels of blood glucose, IGF-I, and insulin may mediate this process (Wathes et al., 2011). It has been suggested that NEB influences reproduction of dairy cows by impacting the quality and viability of the oocyte and the corpus luteum resultant of the ovulation of that follicle (Lucy et al., 1991; Thatcher et al., 2006; Roche et al., 2009). Because there is substantial evidence that metabolic factors can influence early follicular development, it is conceivable that changes in metabolism during periods of NEB could influence preantral follicles destined to ovulate weeks later during the breeding period. In seasonal calving systems, New Zealand studies have emphasised the role of the cows' negative energy status around calving on the resumption of follicular activity, the duration of the postpartum anovulatory period (Burke and Roche, 2007; McDougall et al., 1995) and its detrimental effect on cow fertility.

2.4. Determining fatty acid profile using mid-infrared spectroscopy

The importance of knowing the FA composition of milk is increasing, however, the accurate measurement of FA requires gas-liquid chromatography. Although this technique is suitable, it is a time-consuming procedure that requires expensive reagents and qualified staff. On the other side, prediction of concentration of individual FA in cow milk using mid-infrared spectroscopy is robust, precise and cost effective (Soyeurt et al., 2006; Rutten et al., 2009; De Marchi et al., 2011; Gottardo et al., 2016), and has been applied to large data sets to predict the FA profiles of milk samples using routine herd-testing for fat, protein and lactose (Lopez-Villalobos et al., 2014). Considering that changes in milk composition over the lactation reflects the physiological status

of the cow, Bastin et al. (2016) showed that MIR analysis of milk could be used to predict a wide range of milk components that can be used as potential indicator traits of fertility and health. The authors showed MIR prediction of C18:1 cis-9 and C10:0 concentrations can be used as indicators of energy balance of cows in early lactation and therefore used as indicators of fertility.

2.5. Improving fatty acid composition of bovine milk

The negative perception of milk fat associated products is a major concern of the dairy sector and there is a growing interest on the milk fat composition of bovine milk around the world (Nantapo et al., 2014). The FA composition could be changed through either dietary manipulation or genetic selection. The use of pasture (fresh leafy grass) to change the FA profile in a desirable direction is an important option, although the influence of pasture and forages in changing FA composition is significantly smaller compared to feeding concentrates (Dewhurst et al., 2006).

Presently, there is no genetic selection program in animal breeding anywhere in the world which considers the proportion of FA as genetic traits. In recent years, the dairy sector has become more focused on improving health aspects of milk products, especially changing bovine milk fat composition by increasing the content of MUFA and PUFA and decreasing the proportions of undesirable SFA and trans fatty acids in milk. Stoop et al. (2008) further indicated that the marked demand for healthy milk and dairy products at large has increased in recent years and this may result in large adaptations to the present dairy production and breeding programs if it aims to breed cows for changes in FA composition rather than increased milk yield.

The use of genetic selection to improve fat content and the FA composition of milk by breeding will only be efficient and operative when there is enough additive genetic variation among dairy cows in FA composition (Soyeurt et al., 2007). Several studies have reported estimates of genetic

parameters of individual FA and found that genetic variation exists amongst dairy cows with respect to individual FA (Bastin et al., 2011; Garnsworthy et al., 2010; Soyeurt et al., 2007; Stoop et al., 2008; Lopez-Villalobos et al., 2014).

2.6. Summary of review of literature

The FA profile can be influenced by several factors including breed, stage of lactation and nutrition. The present review identified that there is no clear relationship between the FA profile and live weight change for New Zealand pasture based dairy cattle. Live weight records are becoming available and live weight changes can be used as a proxy of energy balance, especially at early lactation, and can be related to the FA profile and reproductive performance. Knowledge about the association between live weight changes and the FA profile in early lactation would help in providing an approach that could improve cow fertility.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Data

The data used in this study was obtained from a QTL experiment conducted by Livestock Improvement Corporation (LIC) in Hamilton (Spelman et al., 2004). The experiment consisted of an F_2 design, with a half-sibling family structure (Spelman et al., 2001). Reciprocal crosses of Holstein-Friesian and Jersey cows were conducted to produce six F_1 bulls. Eight hundred and fifty F_2 female progeny were produced through mating high genetic-merit F_1 cows from New Zealand's national herd with these F1 bulls. There were two cohorts of cows. The first cohort comprised 354 cows milked during the season 2002-03 and the second cohort comprised 456 cows milked during the season 2003-04. The herd was managed as a conventional spring calving herd grazing on rye grass/white clover pastures, milking twice a day in a 60 bale rotary milk harvesting system. Dry-off date of cows was determined by condition score (less than 4.0) and level of production (less than 5 litres per day for 2 consecutive weeks) of individual cows and pastures availability to ensure that the cows will start the next lactation with a condition score of 5.5. Data used in this thesis comes from the first cohort of cows when they were in second lactation. The average calving date for the cohort was 8 August 2003.

Animal ethics approval was granted by the Ruakura Animal Ethics Committee in Hamilton, New Zealand.

3.1.1. Live weight measurements

Live weights of individual cows were obtained using an automatic weighing system, which was installed in the milking shed to ensure that on average over 80% of the cows had two live weights recorded each day i.e. morning and evening live weight (Spelman et al. 2004). There

was no 100% rate of recording animal weights twice a day due to errors that occurred in the weighing process or due to other unknown factors (Lopez-Villalobos et al., 2014), for example, if a cow was not correctly on the weighing platform an over or under estimation or the weight would have occurred and the measurement was not entered into the database. After editing data 41,981 daily live weight records were analysed.

3.1.2. Milk yield and composition

Daily milk volumes (am and pm) are collected on the research farm. This near continuous collection of milk production data enabled the lactation curve of each cow to be modelled. Fortnightly herd-tests for each cow were taken from each cow for the determination of concentration of fat, protein, lactose and somatic cell counts. The concentrations of fat, protein and lactose were assessed by mid-infrared spectroscopy using a MilkoScan FT6000 (Foss, 2006; Foss, Hillerød, Denmark) under standard herd-testing procedures by Livestock Improvement in Hamilton, New Zealand. After editing of data 73,040 daily milk yields and 5,936 fortnightly herd-tests for fat, protein and lactose were analysed.

3.1.3. Fatty acids

Each of 300 cows was sampled three times during the lactation for FA determination, peak (September/October 2003), mid (November 2003) and late lactation (February 2004). Concentrations of FA in 882 milk samples were determined by fatty acid methyl ester analysis using gas chromatography (MacGibbon and Reynolds 2011). The results were expressed as percentage FA of total FA. Individual FAs were grouped as shown in Table 3.1.

Table 3.1. Groups of fatty acids analysed in the study.

Group	Fatty acids involved
Short chain fatty acids (SCFA)	C4:0 to C8:0
Medium chain fatty acids (MCFA)	C10:0 to C16:1
Long chain fatty acids (LCFA)	C17:0 to C22:6
Saturated fatty acids (SFA)	Sum of C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C18:0, C20:0, C21:0, C22:0, and C24:0
Monounsaturated fatty acids (MUFA)	C16:1, C17:1, C18:1n-7 , C18:1n-9
Polyunsaturated fatty acids (PUFA)	Sum of polyunsaturated fatty acids; Omega-6 fatty acids, Omega-3 fatty acids and conjugated fatty acids

3.2. Statistical analysis

All data were analysed using the statistical analysis system (SAS) version 9.3 (SAS Institute Inc., Cary, NC, USA, 2012). Descriptive statistics for all variables considered in this study were obtained using the MEANS procedure.

The lactation period was defined as: peak lactation (<90 days), mid-lactation (91-180 days) and late lactation (>181 days). Least squares means and standard errors of milk, fat and protein yields, live weight and concentrations of fat, protein and each of the FA for each stage of lactation were obtained with the MIXED procedure. The model included fixed effect of stage of lactation and the random effect of the cow to account for a repeated measure on the same cows. Multiple means comparisons between stages of lactation were performed using the LSD test as

implemented in the MIXED procedure. Differences between means were considered significant when $P<0.05$.

As it was of interest to determine the relationship between LW change and concentration of FA at different stages of lactation, lactation curves for live weight were modelled using a random regression Legendre polynomial order 5 represented with the following model,

$$y_{it} = (\beta_0 P_{0t} + \beta_1 P_{1t} + \beta_2 P_{2t} + \beta_3 P_{3t} + \beta_4 P_{4t} + \beta_5 P_{5t}) + (\alpha_0 P_{0it} + \alpha_1 P_{1it} + \alpha_2 P_2 + \alpha_3 P_{3it} + \alpha_4 P_{4it} + \alpha_5 P_{5it}) + e_{it}$$

where y_{it} is live weight of animal i recorded at day t after calving, βs are the fixed regression coefficients of the population, αs are the random regression coefficients for animal i, and e_{it} is the random error associated with each observation y_{it}. The P coefficients of the Legendre polynomial's function were calculated as

$P_0 = 1$

$P_1 = x$

$P_2 = 1/2(3x^2-1)$

$P_3 = 1/2(5x^3-3x)$

$P_4 = 1/8(35x^4-30x^2+3)$

$P_5 = 1/8(63x^5-70x^3+15x)$

where x represents the standardised unit of time from -1 to 1 calculated as

$$x = 2 [(t - t_{min})/(t_{max} - t_{min})] - 1$$

and where t_{min} and t_{max} are minimum and maximum days in milk from parturition. In this thesis 1 and 365 days in milk were used as t_{min} and t_{max}, respectively.

Estimates of βs and αs were obtained using the MIXED procedure. Then, the random regression coefficients were obtained for each cow and for each of them the live weight was predicted from day 1 to day 365 postpartum. Using the individual curves of live weight cows were classified

into three groups according to their LW change in early lactation: cows with low (L) live weight loss -0.012kg/d, medium (M) live weight loss -0.174kg/d and high (H) live weight loss -0.340kg/d. Least squares means and standard errors for each of these groups for each of the variables were obtained using the MIXED procedure and multiple means comparisons were performed using the LSD test. Differences between means were considered significant when $P<0.05$.

Lactation curves for milk yield, live weight, fat and protein percentages in milk for each of the live weights loss groups were modelled with Legendre polynomials of order 5^{th}, whereas lactation curves for concentrations of SFA, UFA, C12:0 and C18:1 cis-9 for each of the live weights loss groups were modelled with Legendre polynomials of 3^{rd} order.

Estimates of Pearson correlation coefficients between LW change and each of the FA in early lactation were obtained using the CORR procedure.

CHAPTER FOUR

RESULTS

4.1. Descriptive statistics

The descriptive statistics for milk yield and composition, live weight and the FA profile for FxJ crossbred cows are presented in Table 4.1. Short-chain FA (C4:0 to C8:0) averaged about 8.11% of total FA while medium-chain FA (C10:0 to C16:1) averaged 51.97%, making them the highest contribution to the total FA analysed in the present study. The long-chain FA (>C17:0) had the second highest concentration comprising 38.77% of the total FA. The SFA represented 71.23% of the total FA and the remaining 28.77% was represented by the UFA. The C18:2 cis-9, trans-11; C18:2n-6 cis-9, cis-12; and C18:3 cis-9, cis-12, cis-15 occurred only in small proportions.

4.2. Changes in milk composition with stages of lactation

Stage of lactation had a significant effect ($P<0.001$) on all the variables considered in this study except on C20:0 (Table 4.2). Daily milk yield increased in early lactation and decreased subsequently in mid and late lactation (Table 4.2; Figure 4.1). Lactation curves for concentration of fat and protein had an inverse pattern of the lactation curve for daily milk yield (Table 4.2; Figure 4.1).

The concentration of SFA was 68.93g/100g FA in early lactation (90 days), increased to 74.51g/100g in mid lactation and then decreased in late lactation to 70.14g/100g, whereas UFA and PUFA exhibited an opposite trend with minimum values (4.32 and 31.07g/100g) at around 90 days in milk. The concentration of SCFA was almost constant in early (8.40g/100g) and mid-lactation (8.38g/100g) with a decrease in late lactation (7.54g/100g). The concentration of

MCFA in early lactation was 46.88g/100g FA, increased to 55.89g/100g FA and then decreased to 53.09g/100g FA. The LCFA had the opposite trend to the MCFA.

The variation in the concentration of C12:0 and C14:0 across the lactation resembled that of SFA with increasing concentrations between calving and 90 day in milk and a decreased plateau thereafter (Figure 4.2). Similarly the concentration of C18:1 cis-9 followed the same pattern to the concentration of UFA, decreasing values from calving to 90 days in milk and an increase thereafter until a plateau in late lactation with another a further decrease at the end of the lactation.

Table 4.1. Descriptive statistics for milk components, fatty acids and live weight for grazing Friesian x Jersey crossbred cows.

Variable[1]	N	Mean	SD[3]	Minimum	Maximum
Live weight, (kg/d)	41,981	423.3	39.3	306	670
Milk yield (l/d)	73,040	13.8	4.71	0.10	31.2
Fat yield (kg/d)	5,936	0.81	0.02	0.03	1.66
Protein yield (kg/d)	5,936	0.56	0.15	0.02	1.19
Fat (%)	6,663	5.65	0.86	2.83	14.97
Protein (%)	6,663	3.91	0.41	2.71	6.22
Fatty acid[1] (% of the total FA)					
C4:0	882	3.95	0.33	3.00	5.17
C6:0	882	2.57	0.22	1.90	3.21
C8:0	882	1.58	0.19	0.94	2.25
C10:0	882	3.62	0.63	1.74	2.25
C10:1	882	0.31	0.08	0.09	0.59
C12:0	882	4.02	0.76	1.88	6.75
C12:1	882	0.12	0.04	0.00	0.24
C13:0	882	0.09	0.04	0.00	0.25
C14:0	882	11.52	1.27	6.91	15.90
C14:1	882	0.75	0.24	0.24	1.72
C15:0	882	1.14	0.17	0.69	1.77
C16:0	882	27.71	3.59	18.92	39.20
C16:1	882	1.53	0.26	0.96	2.56
C17:0	882	0.67	0.12	0.18	0.98
C18:0	882	12.07	2.27	4.49	19.74
C18:1 cis-9	882	16.82	2.70	10.32	26.19
C18:1 cis-7	882	4.59	0.87	2.30	26.19
LA	882	1.19	0.14	0.68	1.85
ALA	882	0.82	0.14	0.48	1.38
CLA	882	0.09	0.32	0.31	2.55
C20:0	882	0.12	0.04	0.00	0.35
SCFA	882	8.11	0.66	6.32	10.12
MCFA	882	51.97	5.09	35.60	67.96
LCFA	882	38.77	5.01	24.02	55.60
SFA	882	71.23	3.44	60.29	80.12
PUFA	882	4.08	0.53	2.50	5.97
UFA	882	28.77	3.44	19.88	39.70

[1]LA= linoleic acid (C18:2n-6 cis-9, cis-12), ALA= α-linolenic acid (C18:3 cis-9, cis-12, cis-15)
CLA= conjugated linoleic acids (C18:2 cis-9, trans-11)
SCFA = short-chain fatty acids: sum from C4:0 to C8:0
MCFA = medium-chain fatty acids: sum from C10:0 to C16:1
LCFA = long-chain fatty acids: sum from C17:0 to C22:6
SFA = saturated fatty acids: sum of C4:0 to C24:0
PUFA = polyunsaturated fatty acids: include ALA, LA, CLA, oleic acids
UFA = unsaturated fatty acids: sum of C10:1, C14:1, C16:1, C17:1, LA, CLA, ALA, vaccenic acid

Table 4.2. Least squares means of milk components, fatty acids (g/100g) and live weight for three stages of lactation in Friesian x Jersey crossbred cows.

	Stage of lactation				
Variable[1]	Early	Mid	Late	SEM[2]	P-Value
Live weight (kg/d)	403.78[a]	415.45[b]	426.29[c]	2.065	<0.0001
Milk yield (l//d)	19.3[c]	17.6[b]	11.8[a]	0.150	<0.0001
Fat yield (kg/d)	1.01[c]	0.97[b]	0.65[a]	0.029	<0.0001
Protein yield (kg/d)	0.69[a]	0.68[b]	0.44[c]	0.020	<0.0001
Fat (%)	5.09[b]	5.42[a]	5.91[a]	0.109	<0.0001
Protein (%)	3.51[a]	3.79[b]	4.07[c]	0.040	<0.0001
Fatty acid1 (% of the total FA)					
C4:0	4.12[c]	3.99[b]	3.74[a]	0.017	<0.0001
C6:0	2.63[b]	2.69[c]	2.39[a]	0.010	<0.0001
C8:0	1.65[b]	1.68[c]	1.41[a]	0.009	<0.0001
C10:0	3.67[b]	4.04[c]	3.14[a]	0.087	<0.0001
C10:1	0.26[a]	0.34[c]	0.32[a]	0.003	<0.0001
C12:0	3.85[b]	4.61[c]	3.59[a]	0.036	<0.0001
C12:1	0.11[a]	0.15[c]	0.11[b]	0.002	<0.0001
C13:0	0.07[a]	0.12[c]	0.08[b]	0.002	<0.0001
C14:0	10.65[a]	12.69[c]	11.22[b]	0.054	<0.0001
C14:1	0.54[a]	0.81[b]	0.88[c]	0.011	<0.0001
C15:0	0.99[a]	1.21[b]	1.23[c]	0.008	<0.0001
C16:0	24.24[a]	29.28[b]	29.59[c]	0.153	<0.0001
C16:1	1.44[b]	1.44[b]	1.73[a]	0.013	<0.0001
C17:0	0.73[c]	0.70[b]	0.56[a]	0.006	<0.0001
C18:0	14.18[b]	11.08[a]	10.96[a]	0.099	<0.0001
C18:1 cis-9	18.72[c]	14.28[a]	17.51[b]	0.113	<0.0001
C18:1 cis-7	5.07[c]	4.23[a]	4.46[b]	0.046	<0.0001
C18:2 cis-9, 12 (LA)	1.38[c]	1.06[a]	1.15[b]	0.007	<0.0001
ALA	0.96[b]	0.75[a]	0.75[a]	0.006	<0.0001
CLA	0.75[a]	0.78[b]	1.07[c]	0.016	<0.0001
C20:0	0.12[a]	0.11[a]	0.12[a]	0.002	0.8989
SCFA	8.40[b]	8.38[b]	7.54[a]	0.030	<0.0001
MCFA	46.88[b]	55.89[c]	53.09[a]	0.201	<0.0001
LCFA	43.48[b]	34.59[c]	38.28[a]	0.123	<0.0001
SFA	68.93[a]	74.51[c]	70.14[b]	0.144	<0.0001
PUFA	4.32[c]	3.74[a]	4.20[b]	0.027	<0.0001
UFA	31.07[c]	25.48[a]	29.85[b]	0.143	<0.0001

[a,b,c] Least squares mean values with different superscript differ (P<0.05)

[1] LA = linoleic acid (C18:2n-6 cis-9, cis-12)

ALA= α-linolenic acid (C18:3 cis-9, cis-12, cis-15)

CLA= conjugated linoleic acids (C18:2 cis-9, trans-11)

SCFA = short-chain fatty acids: sum from C4:0 to C8:0

MCFA = medium-chain fatty acids: sum from C10:0 to C16:1

LCFA = long-chain fatty acids: sum from C17:0 to C22:6

SFA = saturated fatty acids: sum of C4:0 to C24:0

PUFA = polyunsaturated fatty acids: include ALA, LA, CLA, oleic acids

UFA = unsaturated fatty acids: sum of C10:1, C14:1, C16:1, C17:1, LA, CLA, ALA, vaccenic acid

[2]SEM= standard error of the means.

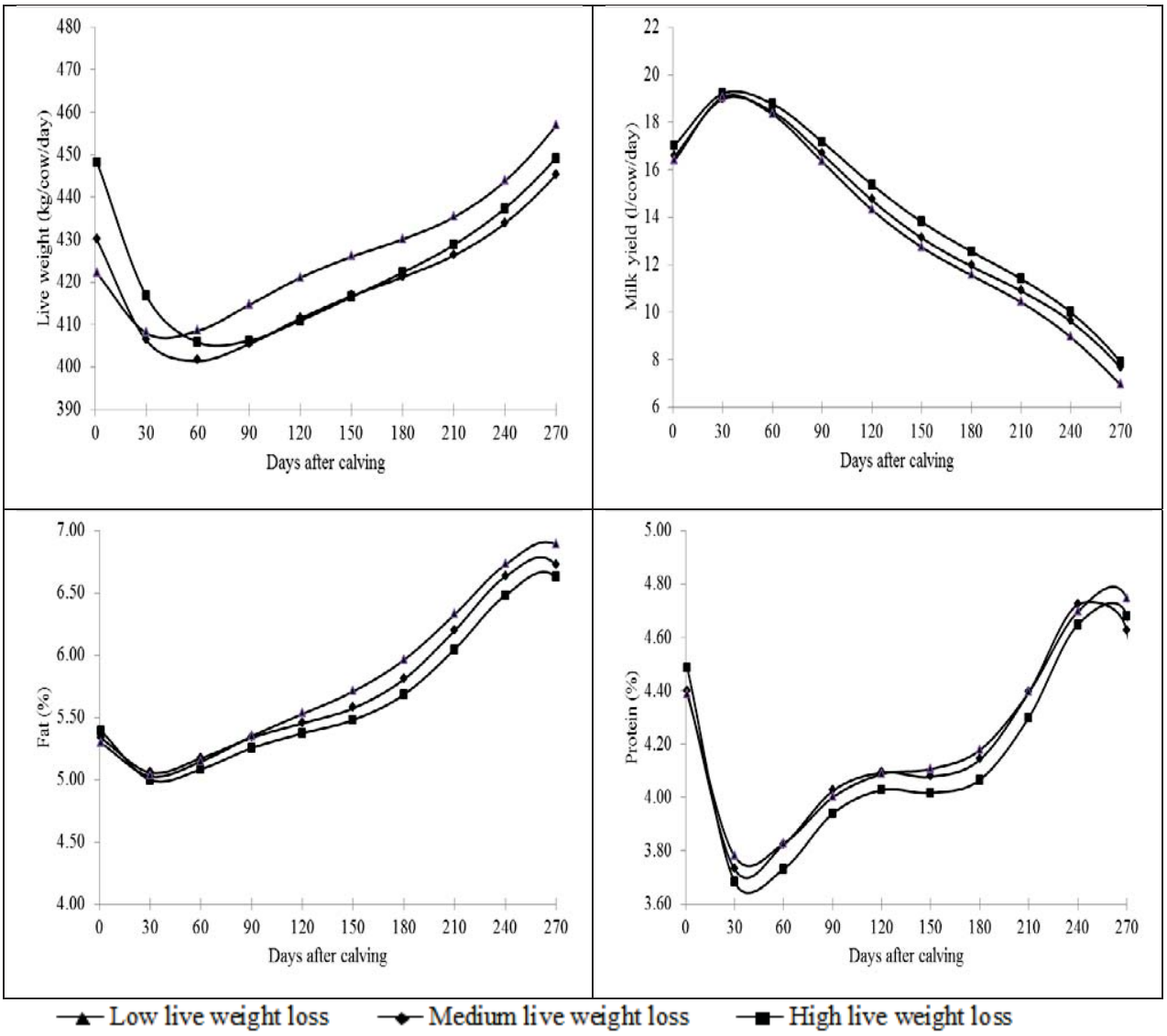

Figure 4.1. Changes in milk yield and composition across lactation in Friesian x Jersey crossbred cows classified according to the live weight change in early lactation.

Table 4.3. Least squares means of live weight change, milk yield, milk composition and concentrations of fatty acids (g/100g) in Friesian x Jersey crossbred cows classified according to their live weight loss in early lactation.

	Live weight loss group[1]				
Variable	L	M	H	SEM[2]	P-Value
Live weight(kg/d)	412.6	408.4	416.4	5.2	0.2479
Δ LW (kg/d)	-0.012[a]	-0.174[b]	-0.340[c]	0.006	<0.0001
Milk yield (l/d)	19.19	19.13	19.78	0.325	0.3536
Fat yield (kg/d)	1.00	1.00	1.04	0.018	0.1925
Protein yield (kg/d)	0.70	0.69	0.71	0.012	0.6518
Fat (%)	5.06	5.10	5.13	0.054	0.7162
Protein (%)	3.75	3.75	3.71	0.084	0.4020
Fatty acid[1] (% of the total FA)					
C4:0	4.12	4.14	4.09	0.036	0.6003
C6:0	2.65[b]	2.67[c]	2.56[a]	0.046	0.0150
C8:0	1.68[c]	1.66[b]	1.59[a]	0.021	0.0087
C10:0	3.79[c]	3.68[b]	3.46[a]	0.064	0.0040
C10:1	0.28[c]	0.27[b]	0.26[a]	0.006	0.2254
C12:0	4.0[c]	3.85[b]	3.62[a]	0.075	0.0063
C12:1	0.12	0.11	0.11	0.005	0.2090
C13:0	0.08	0.07	0.07	0.004	0.0652
C14:0	10.82	10.62	10.45	0.112	0.0897
C14:1	0.54	0.55	0.54	0.016	0.8740
C15:0	1.03	0.98	0.96	0.014	0.0048
C16:0	24.14	24.34	24.26	0.232	0.8098
C16:1	1.39[c]	1.46[a]	1.48[b]	0.018	0.0032
C17:0	0.72[a]	0.73[b]	0.75[c]	0.008	0.0240
C18:0	14.07	14.15	14.36	0.184	0.5816
Cis-9 C18:1	18.37[a]	18.65[a]	19.39[b]	0.251	0.0298
Cis-7 C18:1	5.37	5.07	4.98	0.087	0.6126
Cis-9,12 C18:2 (LA)	1.37	1.38	1.39	0.013	0.4714
ALA	0.93[a]	0.96[ab]	0.98[b]	0.013	0.0316
CLA	0.79	0.73	0.73	0.026	0.1976
C20:0	0.12[a]	0.11[b]	0.12[ab]	0.002	0.1453
SCFA	8.51	8.38	8.34	0.070	0.1032
MCFA	47.61	46.80	46.31	0.379	0.0572
LCFA	42.67[b]	43.58[ab]	44.11[a]	0.386	0.0328
SFA	69.32[b]	68.96[ab]	68.31[a]	0.289	0.0774
PUFA	4.32	4.32	4.33	0.046	0.9839
UFA	30.68[a]	31.03[ab]	31.69[b]	0.289	0.1976

[a,b,c] Least squares mean values within a row with different superscript are significantly different (P<0.05)

[1] L = low live weight loss, M = medium live weight loss and H = high live weight loss in early lactation

[2]SEM = standard error of the means ,[3] **Δ** LW = live weight changes; ALA = α-linolenic acid (C18:3 cis-9, cis-12, cis-15), CLA = conjugated linoleic acids (C18:2 cis-9, trans-11) , LA = linoleic acid (C18:2n-6 cis-9, cis-12); LW = live weight, SCFA = short-chain fatty acids: sum from C4:0 to C8:0; MCFA = medium chain fatty acids: sum from C10:0 to C16:1, LCFA = long-chain fatty acids: sum from C17:0 to C22:6

SFA = saturated fatty acids: sum of C4:0 to C24:0, PUFA = polyunsaturated fatty acids: include ALA, LA, CLA, oleic acids; UFA = unsaturated fatty acids: sum of C10:1, C14:1, C16:1, C17:1, LA, CLA, ALA, vaccenic acid.

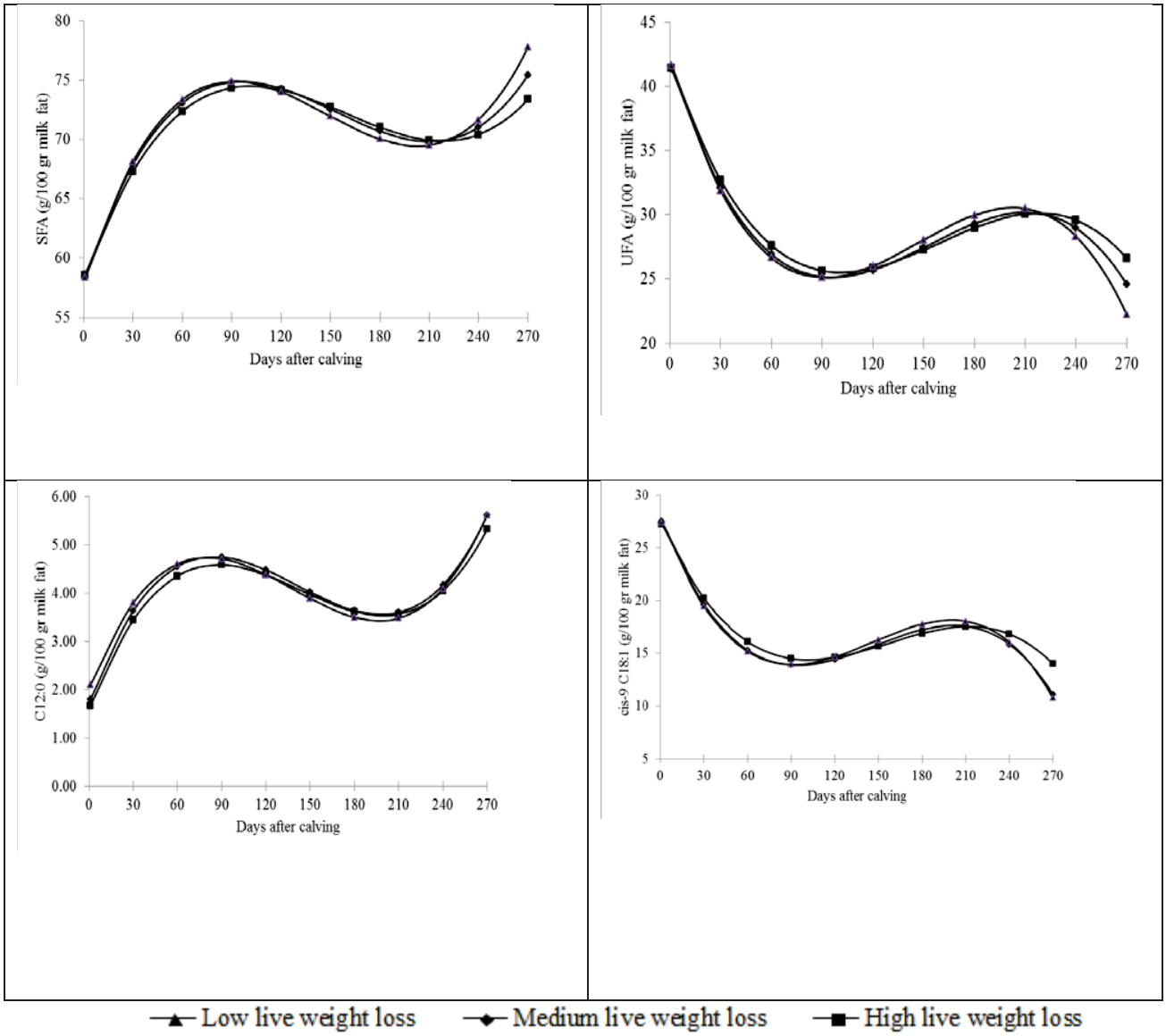

Figure 4.2. Changes in concentration of individual fatty acids in Friesian x Jersey crossbred cows classified according to the live weight change in early lactation.

4.3. Changes in milk fat composition with live weight change

Milk FA profiles in early lactation of three groups of cows classified according to the live weight loss in early lactation are presented in Table 4.3. Cows with high live weight loss had significantly ($P<0.05$) lower concentrations of SCFA and MCFA, mainly C6:0, C8:0, C10:0,

C12:0 and C15:0, and higher concentrations of C16:1, C17:0, cis-9, C18:1, cis-9, 12 C18:2 and ALA.

4.4. Relationship between live weight change and fatty acids

Tables 4.4, 4.5 and 4.6 show the estimates of Pearson correlations coefficients between the concentration of FA and live weight changes in early, mid and late lactation, respectively. There were some significant correlations between live weight changes with some FA. Live weight loss at early lactation was associated with higher concentrations of UFA (r = -0.19), LCFA (r = -0.17), C17:0 (r = -0.14) and pre-formed FA; C18:1 cis-9 (r = -0.20), C18:3 cis-9, cis-12, cis-15 (r = -0.21) ($P<0.05$; Table 4.5) whereas, live weight loss in early lactation was associated with lower concentrations of SFA (r = 0.18), MCFA (r = 0.16) and de-novo synthesis FA; C12:0 (r = 0.24), C14:0 (r = 0.17) and C15:0 (r = 0.22).

The association between LW changes and most of the FAs were not significant in mid and late lactation (Table 4.5 and 4.6), only a few weak correlations were significant between live weight change and C10:0 (r = 0.10), C16:0 (r = -0.13) and C18:0 (r = 0.12) in mid lactation.

Table 4.4. Correlations between live weight changes (ΔLW), individual and grouped fatty acids [1]in early lactation for pasture-based Friesian x Jersey crossbred cows.

	SFA	PUFA	UFA	SC	MC	LC	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	OA	VA	LA	ALA	CLA
ΔLW	0.19	-0.03	-0.19	0.13	0.16	-0.17	0.00	0.16	0.21	0.24	0.24	0.17	0.22	0.01	-0.18	-0.08	-0.21	0.05	-0.06	-0.18	0.07
SFA	1.00	-0.55	-1.00	0.26	0.64	0.78	-0.10	0.43	0.50	0.62	0.64	0.75	0.24	0.57	-0.32	-0.17	-0.88	-0.32	-0.49	-0.33	-0.40
PUFA		1.00	0.55	-0.01	-0.07	-0.65	0.01	-0.03	0.00	-0.07	-0.08	-0.22	0.29	-0.48	-0.12	-0.17	0.17	0.70	0.67	0.45	0.62
UFA			1.00	-0.26	-0.63	-0.78	0.10	-0.43	-0.50	-0.62	-0.64	-0.75	-0.23	-0.57	0.32	0.17	0.88	0.32	0.49	0.33	0.40
SC				1.00	0.41	-0.21	0.81	0.94	0.74	0.48	0.35	0.22	-0.06	-0.25	-0.49	-0.03	-0.25	-0.06	-0.01	0.18	-0.15
MC					1.00	-0.98	-0.17	0.62	0.88	0.99	0.99	0.81	0.55	-0.03	-0.54	-0.43	-0.76	0.15	-0.36	-0.28	0.12
LC						1.00	-0.25	-0.12	-0.14	0.02	0.09	0.37	-0.03	0.80	0.08	0.07	-0.55	-0.48	-0.39	-0.29	-0.54
C4:0							1.00	0.60	0.23	-0.09	-0.23	-0.24	-0.40	-0.25	-0.19	0.24	0.19	-0.16	0.18	0.35	-0.24
C6:0								1.00	0.89	0.70	0.58	0.43	0.13	-0.16	-0.59	-0.18	-0.47	0.00	-0.13	0.06	-0.09
C8:0									1.00	0.92	0.85	0.64	0.37	-0.20	-0.60	-0.30	-0.62	0.13	-0.23	-0.10	0.06
C10:0										1.00	0.98	0.79	0.50	-0.08	-0.55	-0.37	-0.74	0.16	-0.35	-0.25	0.10
C12:0											1.00	0.84	0.58	0.02	-0.54	-0.46	-0.78	0.15	-0.38	-0.31	0.13
C14:0												1.00	0.51	0.33	-0.51	-0.57	-0.82	-0.03	-0.48	-0.36	0.06
C15:0													1.00	0.06	-0.38	-0.57	-0.56	0.55	-0.20	-0.42	0.62
C16:0														1.00	-0.08	-0.41	-0.43	-0.44	-0.38	-0.26	-0.31
C17:0															1.00	0.49	0.48	-0.16	0.13	0.03	-0.21
C18:0																1.00	0.37	-0.15	0.28	0.23	-0.46
OA																	1.00	-0.14	0.37	0.33	0.00
VA																		1.00	0.24	-0.07	0.81
LA																			1.00	0.66	-0.04
ALA																				1.00	-0.22
CLA																					1.00

Estimates of correlation coefficients greater than |0.1| are significantly different from 0 ($P<0.05$)

[1]SFA = saturated fatty acids, PUFA = polyunsaturated fatty acids, UFA = unsaturated fatty acids, SC = short-chain fatty acids: sum from C4:0 to C8:0, MC = medium chain fatty acids: sum from C10:0 to C16:1, LC = long-chain fatty acids: sum from C17:0 to C22:6, OA = oleic acid (C18:1 cis-9), VA = vaccenic acid (C18:1 cis-7), LA= linoleic acid (C18:2n-6 cis-9, cis-12), ALA= α-linolenic acid (C18:3 cis-9, cis-12, cis-15).

Table 4.5. Correlations between live weight changes (ΔLW), individual and grouped fatty acids [1]in mid lactation for pasture-based Friesian x Jersey crossbred cows.

	SFA	PUFA	UFA	SC	MC	LC	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	OA	VA	LA	ALA	CLA
ΔLW	-0.02	0.04	0.02	-0.02	0.09	-0.06	-0.06	-0.01	0.07	0.10	0.09	0.01	0.02	-0.13	0.05	0.12	0.01	0.08	0.05	-0.04	0.03
SFA	1.00	-0.80	-1.00	0.22	0.32	0.86	0.10	0.31	0.20	0.32	0.33	0.47	-0.20	0.66	-0.21	-0.11	-0.81	-0.68	-0.54	-0.28	-0.66
PUFA		1.00	0.80	-0.17	-0.02	-0.80	-0.20	-0.18	0.03	-0.03	-0.04	-0.19	0.40	-0.55	-0.09	-0.15	0.36	0.82	0.61	0.39	0.77
UFA			1.00	-0.22	-0.32	-0.86	-0.10	-0.31	-0.20	-0.32	-0.33	-0.47	0.20	-0.66	0.21	0.11	0.81	0.68	0.54	0.28	0.66
SC				1.00	0.08	-0.03	0.88	0.95	0.64	0.21	0.01	0.01	-0.38	-0.15	-0.03	0.27	-0.12	-0.09	0.12	0.21	-0.26
MC					1.00	-0.17	-0.34	0.27	0.76	0.98	0.99	0.76	0.23	-0.24	-0.26	-0.23	-0.46	0.03	-0.16	-0.10	0.02
LC						1.00	0.08	-0.01	-0.30	-0.18	-0.13	0.13	-0.24	0.85	-0.10	-0.06	-0.61	-0.71	-0.52	-0.29	-0.66
C4:0							1.00	0.72	0.21	-0.23	-0.40	-0.27	-0.45	0.00	0.08	0.32	0.07	-0.14	0.14	0.19	-0.28
C6:0								1.00	0.77	0.39	0.20	0.14	-0.32	-0.14	-0.12	0.20	-0.26	-0.10	0.06	0.19	-0.26
C8:0									1.00	0.84	0.70	0.47	-0.03	-0.41	-0.15	0.06	-0.29	0.10	0.08	0.12	-0.04
C10:0										1.00	0.96	0.74	0.14	-0.31	-0.21	-0.11	-0.44	0.05	-0.11	-0.04	-0.01
C12:0											1.00	0.77	0.24	-0.19	-0.28	-0.28	-0.47	0.00	-0.20	-0.13	0.02
C14:0												1.00	0.06	0.02	-0.28	-0.36	-0.51	-0.21	-0.31	-0.17	-0.10
C15:0													1.00	-0.03	-0.16	-0.45	-0.17	0.42	-0.09	-0.25	0.54
C16:0														1.00	-0.31	-0.51	-0.58	-0.58	-0.53	-0.30	-0.36
C17:0															1.00	0.52	0.47	-0.03	0.13	0.06	-0.16
C18:0																1.00	0.40	0.03	0.35	0.24	-0.34
OA																	1.00	0.19	0.47	0.28	0.18
VA																		1.00	0.37	0.11	0.84
LA																			1.00	0.71	0.03
ALA																				1.00	-0.11
CLA																					1.00

Estimates of correlation coefficients greater than |0.1| are significantly different from 0 (P<0.05)

[1]SFA = saturated fatty acids, PUFA = polyunsaturated fatty acids, UFA = unsaturated fatty acids, SC = short-chain fatty acids: sum from C4:0 to C8:0, MC = medium chain fatty acids: sum from C10:0 to C16:1, LC = long-chain fatty acids: sum from C17:0 to C22:6, OA = oleic acid (C18:1 cis-9), VA = vaccenic acid (C18:1 cis-7), LA= linoleic acid (C18:2n-6 cis-9, cis-12), ALA= α-linolenic acid (C18:3 cis-9, cis-12, cis-15).

Table 4.6. Correlations between live weight changes (ΔLW), individual and grouped fatty acids[1] in late lactation for pasture-based Friesian x Jersey crossbred cows.

	SFA	PUFA	UFA	SC	MC	LC	C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	OA	VA	LA	ALA	CLA
ΔLW	0.03	-0.06	-0.03	-0.04	0.06	0.02	-0.09	-0.01	0.06	0.07	0.06	0.02	-0.01	-0.02	0.09	0.03	0.00	-0.04	-0.07	-0.09	-0.04
SFA	1.00	-0.73	-1.00	0.37	0.48	0.86	0.15	0.45	0.42	0.47	0.49	0.50	-0.25	0.66	-0.08	-0.24	-0.86	-0.56	-0.64	-0.37	-0.61
PUFA		1.00	0.73	-0.23	-0.05	-0.80	-0.25	-0.20	-0.02	-0.05	-0.06	-0.18	0.44	-0.54	0.14	-0.05	0.33	0.80	0.70	0.61	0.80
UFA			1.00	-0.37	-0.48	-0.86	-0.15	-0.45	-0.42	-0.47	-0.49	-0.50	0.25	-0.66	0.08	0.24	0.86	0.56	0.64	0.37	0.61
SC				1.00	0.23	0.11	0.82	0.92	0.55	0.29	0.20	0.15	-0.35	0.07	-0.25	0.03	-0.31	-0.10	-0.05	-0.03	-0.30
MC					1.00	0.01	-0.32	0.48	0.89	0.99	1.00	0.76	0.24	-0.08	-0.04	-0.25	-0.61	0.01	-0.17	0.01	-0.04
LC						1.00	0.16	0.09	-0.07	-0.01	0.03	0.17	-0.35	0.81	-0.02	-0.17	-0.64	-0.65	-0.66	-0.43	-0.64
C4:0							1.00	0.56	-0.01	-0.26	-0.34	-0.20	-0.50	0.15	-0.24	0.14	0.01	-0.15	0.00	-0.08	-0.30
C6:0								1.00	0.74	0.53	0.45	0.30	-0.20	0.04	-0.25	-0.05	-0.45	-0.07	-0.10	-0.02	-0.26
C8:0									1.00	0.91	0.86	0.59	0.12	-0.14	-0.03	-0.15	-0.55	0.06	-0.06	0.09	-0.08
C10:0										1.00	0.98	0.75	0.20	-0.14	-0.03	-0.17	-0.59	0.05	-0.13	0.02	-0.07
C12:0											1.00	0.77	0.24	-0.04	-0.05	-0.29	-0.62	-0.02	-0.19	0.00	-0.04
C14:0												1.00	0.09	0.09	-0.14	-0.43	-0.53	-0.19	-0.35	-0.17	-0.03
C15:0													1.00	-0.22	0.01	-0.21	-0.07	0.47	0.04	0.01	0.60
C16:0														1.00	-0.16	-0.63	-0.60	-0.62	-0.65	-0.33	-0.28
C17:0															1.00	0.14	0.09	0.05	0.06	0.05	-0.08
C18:0																1.00	0.46	0.25	0.42	0.10	-0.34
OA																	1.00	0.17	0.49	0.19	0.19
VA																		1.00	0.60	0.35	0.69
LA																			1.00	0.74	0.24
ALA																				1.00	0.20
CLA																					1.00

Estimates of correlation coefficients greater than |0.1| are significantly different from 0 ($P<0.05$)

[1]SFA = saturated fatty acids, PUFA = polyunsaturated fatty acids, UFA = unsaturated fatty acids, SC = short-chain fatty acids: sum from C4:0 to C8:0, MC = medium chain fatty acids: sum from C10:0 to C16:1, LC = long-chain fatty acids: sum from C17:0 to C22:6, OA = oleic acid (C18:1 cis-9), VA = vaccenic acid (C18:1 cis-7), LA= linoleic acid (C18:2n-6 cis-9, cis-12), ALA= α-linolenic acid (C18:3 cis-9, cis-12, cis-15).

CHAPTER FIVE

DISCUSSION

5.1. Overview of milk yield and live weight changes

Lactation curves for daily milk yield and live weight presented in Figure 4.1 were similar to other reported in New Zealand studies (Lopez-Villalobos et al., 2001). Milk production increased from calving until a peak at day 30 and then declined linearly.

In this study, live weight change was considered as a proxy of energy balance. Cows that experience high live weight loss in early lactation were considered to be experiencing negative energy balance. This approach was taken because energy intake per cow was not available and direct calculation of energy balance was not possible.

Cows were classified into one of three groups according to the live weight change in early lactation, i.e. high, medium and low live weight loss groups. Least squares means for daily milk yield and composition in early lactation for each of the live weight loss groups showed no significant differences. However cows in the H group had higher average daily milk yield during the whole lactation than those in the other two groups (data not presented but see Figure 4.1) and cows that did not lose weight in early lactation were heavier at the end of the lactation (see Figure 4.1).

5.2. Changes in milk fat acids with lactation

Previous research has shown that stages of lactation have been closely linked to the composition of milk (Bansal, 2009). Milk fat yield increased and was at its highest in early lactation. It decreases in later stages inversely to milk fat percentages. Similar trends were found in the present study. Protein yield has been reported also to be associated with stages of lactation. In previous research undertaken with grazing cows under New Zealand's seasonal grazing system, protein yields were different across all stages of lactation, with the highest yield in peak lactation

(Lopez-Villalobos et al., 2001), which agrees with the present study. Lactation curves for fat and protein percentages found in this study agree well lactation curves found in cows from commercial cows (Sneddon et al, 2016).

The concentration of many FA in bovine milk fat changed with the stage of lactation. However, the concentration of C20:0 did not change as lactation continued, contrary to studies of Bilal et al. (2014) who reported that amongst other FA the concentration of C13:0 remained unchanged as lactation progressed. The concentration of C14:0 was lower in early lactation, but increased during mid-lactation and thereafter decreased in later lactation. Similar results have been reported, in which C14:0 increased approximately to day 100 postpartum and thereafter remained constant for the rest of lactation (Bilal et al., 2014). Similar trends were also observed for C6:0, C8:0, C10:0, C10:1, C11:0, C12:0, C12:1, C13:0 and C14:0, although they occurred in different concentrations. The concentration of C14:1 had a similar increasing trend, with C15:0 and C16:0 showing a high concentration in late lactation.

The concentration of C18:1 cis-9 was high in early lactation, and decreased to a minimum at day 90 postpartum, and then showed a slight increase during later lactation which was similar to Canadian Holstein cows (Bilal et al., 2014) and New Zealand Friesian cows (Thomson and Vander Poel, 2000). To a certain extent, a similar trend to C18:1 cis-9 was reported for C17:0 and C18:1 cis-7. In accordance with the present study, Kgwatalala et al. (2009) reported a significantly higher concentration of C18:1 cis-9 in early lactation compared with mid and late lactation milk fat. Stoop et al. (2009) reported the effect of stage of lactation on the FA composition of milk in first parity Dutch Holstein cows and concluded that the concentration of de-novo synthesis FA (C12:0, C14:0 and C16:0) increased during the first three months of lactation (i.e mid-lactation) and thereafter decreased, whereas the concentration of C18:0 and

longer FA followed an opposite trend. Other previous studies (Palmquist et al., 1993; Kay et al., 2005; Garnsworthy et al., 2006) also reported similar findings to the present study. The concentration of C4:0 was higher in early lactation in contrast with the other de-novo synthesised FA (C6:0 to C14:0), possibly due to its unique origin from two different pathways (β-hydroxy butyrate pathway and β-reduction pathway), which do not depend on the acetyl-coenzyme A carboxylase pathway, and thus the synthesis of C4:0 is not affected by the inhibitory effects of FA originating from dietary lipids (pre-formed FA) on de-novo synthesis during early lactation (Palmquist et al., 1993).

The concentration of ALA, which belongs to the polyunsaturated omega-3 fatty acid group, was observed to be higher in early lactation and then decreased and remained constant in mid and late lactation. This trend was different from that found by Bilal et al. (2014) who reported a higher concentration of ALA during the early part of lactation and it decreased in mid-lactation and increased in late lactation. The difference in the trends probably can be due to differences in quality of pasture over the milking period. The concentration of C18:1 cis-7 and CLA was significantly affected by the stage of lactation. In general, CLA increased with the advancing stage of lactation, and the findings were in accordance with Bilal et al. (2014) who reported a higher concentration of CLA during late lactation for pasture-grazed Canadian Holstein cows.

Stoop et al. (2009) reported an increase in CLA as the stage of lactation progressed. Similar results were also reported by Auldist et al. (1998) in a study of 80 New Zealand pasture-grazed dairy cows. In contrast to the present study, Kelsey et al. (2003) reported no significant effect of the stage of lactation on concentration of CLA. Kgwatalala et al. (2009) also observed that the concentration of CLA in milk fat was not affected by stage of lactation. Generally, milk fat produced from a grazing cow has more variability in cis-9 C18:1.

5.3. Milk fat acids and live weight change

In dairy cows, milk FA originate from four main pathways: directly from dietary intake, de-novo synthesis in the mammary gland, synthesis in the rumen by biohydrogenation, and body fat mobilisation (Bastin et al., 2012). The milk FA profile can vary due to the changes in one or more of these major pathways (Stoop et al., 2009). During early lactation, the occurrence of a negative energy balance is common in dairy cows. Gross et al. (2011) showed that the concentration of short and medium-chain FA in milk fat increased, while the concentration of LCFA (particularly C18:1 cis-9) decreased as negative energy balance in early lactation improved, which agrees with the present study. The LCFA may cause an inhibitory effect to the synthesis of SCFA and MCFA in the mammary gland during early lactation (Palmquist et al., 1993) when cows are in negative energy balance.

In this study, the concentration of C12:0, C14:0 and C15:0 was significantly higher in the L group than in the M and H groups. The elevated concentration of C12:0 and C14:0 partly explains how a cow with less live weight loss would have a relatively higher concentration of de-novo FA compared to a cow with high live weight loss. Similar results have been reported in which C14:0 de-novo synthesised FA decreased with severe negative energy balance (Gross et al., 2011). However it is difficult to compare findings of the present study with other studies due to differences in pasture composition and the influence of strain within breeds on milk fat composition (Wales et al., 2009).

The concentrations of C6:0, C8:0, C10:0, C10: and C12:0 in early lactation in the H group were lower than in the L group, but in the opposite direction, the concentration of C16:1, C17 and C18:1 cis-9 were higher in the H than in the L group. The overall effect was that H cows had significantly higher concentration of LCFA than L cows. These trends agree with the study by

Nogalski et al. (2012) who reported that severe live weight loss lead to a decrease in the concentration of short and medium-chain FA and an increase in long-chain FA.

The concentration of C18:1 cis-9 was significantly lower ($P<0.05$) in the L cow group than in M and H cow groups. A similar trend was noted for C17:0 and ALA, which increased concentration as live weight loss became severe. This trend from the present study agrees with Gross et al. (2011), who found a significant higher concentration of pre-formed FA (including C17:0 and ALA) in cows with severe negative energy balance (i.e. severe live weight loss) compared with cows with a positive energy balance. Nogalski et al. (2012) demonstrated the effect of negative energy balance on FA composition of milk in different Polish Holstein-Friesian cows and concluded that the high mobilisation of body fat reserves during negative energy balance in the early part of lactation lead to a significant increase in the concentration of C18:1 cis-9 and a significant decrease in the concentrations of C12:0, C14:0 and C16:0. Similar results were found in the present study.

Other previous works (Rukkwamsuk et al., 2000; Stoop et al., 2009; Ducháček et al., 2014; Bilal et al., 2014) have also reported a higher concentration of C18 and C18:1 cis-9 in early lactation, probably due to lipolysis as result of most energy stores in the form of adipose tissue triglycerides are mobilised during times of negative energy balance.

The concentration of C4:0 has been reported to increase with severe negative energy balance during the early stage of lactation (Stoop et al., 2009). This study did not find significant differences between live weight change groups and not significant correlation between the concentration of C4:0 and live weight change in early lactation (Table 4.4).

Therefore, the notable finding from the present study is the increase in the concentration of C18:1 cis-9 for the H cow group and a decreased concentration of this FA for the low and

medium LW loss cow groups. The increase of C18:1 cis-9 during severe LW loss may be used as an indicator of the level of mobilisation for body tissue occurring in early lactation which agrees with the study of Payne et al. (1979) under New Zealand pastoral system.

The most conclusive finding in this study agrees well with the most well-established relationship that cows experiencing severe negative energy balance have a higher concentration of C18:1 cis-9 (Grummer, 1991; Rukkwamsuk et al., 2000; Stoop et al., 2009; Ducháček et al., 2014; Nogalski et al., 2012; Bilal et al., 2014). If a rapid and cost-effective method to determine the concentration of C18:1 cis-9 in early lactation can be implemented using mid-infrared spectroscopy (Lopez-Villalobos et al., 2014), then the concentration of this FA can be used as a rapid indicator of energy balance (McParland et al., 2011) of the cows. This can be a useful tool to design feeding and management strategies that reduce severe NEB in early lactation and reduce the risk of infertility, especially, in seasonal grazing systems.

In the long term, Bastin et al. (2016) showed that milk FA traits, especially contents in milk of C8:0 to C14:0 and C18:1 cis-9, can be used in a genetic selection index to improve fertility. Bastin et al. (2014) investigated the usefulness of FA traits in fertility breeding programs. They calculated the accuracy of a fertility index, including days open as direct trait, the content in milk of C10:0 at 5 days in milk, the content in milk of C18:1 cis-9 at 5 days in milk, or a combination of these. The authors concluded that although direct selection for days open provided the best accuracy for the fertility index, using FA traits in a fertility index would allow faster, indirect selection on reproduction performance; and that an index including the two FA traits had higher accuracy than an index including only one trait, thereby substantiating the opportunity to combine FAs related to various aspects of energy balance status and body fat mobilization.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The present study found that stage of lactation affects FA profile and that live weight change is associated with changes in concentration of milk FAs in early lactation. Live weight change was chosen as a proxy of energy balance. Cows losing live weight in early lactation can be considered to be in negative energy balance. The most relevant conclusion is that the concentration of C18:1 cis-9 could be used as an indicator of the mobilisation of body reserves in early lactation. The present study also agreed with previous findings that de-novo synthesised FA increases with improved energy balance, while pre-formed FA, especially C18:0 and C18:1 cis-9, increases as the mobilisation of body fat reserves increases (severe live weight loss).

6.2. Recommendations

This thesis has contributed to a better understanding of milk FA composition and change in live weight as indicators of energy balance. The following areas of research need to be investigated in New Zealand grazing dairy cattle:

- Estimation of phenotypic and genetic correlations among live weight change, milk FA and fertility traits, within- and across the main breed groups.
- Development of a selection index for the genetic improvement of fertility in seasonal calving cows; this index should include concentration of the most relevant FA identified in this study and traits related to energy balance and body fat mobilization.

REFERENCES

Auldist, M.J., Johnston, K.A., White, N.J., Fitzsimons, W.P. and Boland, M.J. 2004. A comparison of the composition, coagulation characteristics and cheese making capacity of milk from Friesian and Jersey dairy cows. Journal of Dairy Research 71:51–7.

Alawneh, J. I., Stevenson, M. A., Williamson, N. B., Lopez-Villalobos, N. and Otley, T. 2012. The effect of liveweight change on reproductive performance in a seasonally calving, pasture fed dairy herd. Livestock Science 145: 131-139.

Auldist, M.J., Walsh, B.J. and Thomson, N.A. 1998. Seasonal and lactational influences on bovine milk composition in New Zealand. Journal of Dairy Research 65: 401-411.

Auldist, M.J., Walsh, B.J. and Thomson, N.A. 1997. Seasonal and lactational influences on milk composition in New Zealand. Proceedings of the New Zealand Society of Animal Production 57: 205-206.

Back, P. and Thomson, N.A. 2005. Exploiting cow genotype to increase milk value through production of minor milk components. Proceedings of the New Zealand Society of Animal Production 65: 53-58.

Bansal, B., Habib, B., Rebmann, H. and Chen, X.D. 2009. Effect of seasonal variation in milk composition on dairy fouling. Proceeding of International Conference on Heat Exchanger and Cleaning Peer Review 165-166.

Bastin, C., Berry, D.P., Soyeurt, H. and Gengler, N. 2012. Genetic correlations of days open with production traits and contents in milk of major fatty acids predicted by mid-infrared spectrometry. Journal of Dairy Science 95: 6113-6121.

Bastin, C., Gengler, N. and Soyeurt, H. 2011. Phenotypic and genetic variability of production traits and milk fatty acid contents across days in milk for Walloon Holstein first-parity cows. Journal of Dairy Science 94: 4152-4163.

Bastin, C., Théron, L., Lainé, A. and Gengler, N. 2016. On the role of mid-infrared predicted phenotypes in fertility and health dairy breeding programs. Journal of Dairy Science 99: 4080-4094.

Bastin, C., Vandenplas, J. and Gengler, N. 2014. Improving dairy cow fertility using milk-based indicator traits. Abstract number 155 in Proceedings of the 10th World Congress on Genetics Applied to Livestock Production. Vancouver, BC, Canada.

Bauman, D.E. and Griinari, J.M. 2003. Nutritional regulation of milk fat synthesis. Annual Review of Nutrition 23: 203-227.

Beaulieu, A.D. and Palmquist, D.L. 1995. Differential effects of high fat diets on fatty acid composition in milk of Jersey and Holstein cows. Journal of Dairy Science 78:1336–44.

Berry, D.P., Veerkamp, R. and Dillon, P. 2006. Phenotypic profiles for body weight, body condition score, energy intake, and energy balance across different parities and concentrate feeding levels. Livestock Science 104: 1-12.

Bilal, G., Cue, R., Mustafa, A. and Hayes, J. 2014. Effects of parity, age at calving and stage of lactation on fatty acid composition of milk in Canadian Holsteins. Canadian Journal of Animal Science 94: 401-410.

Burke, C.R. and Roche, J.R. 2007. Effects of pasture feeding during the periparturient period on postpartum anovulation in grazed dairy cows. Journal of Dairy Science 90: 4304–4312.

Butler, W. R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. Livestock Production Science 83: 211-218.

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

Colazo, M. G. and Mapletoft, R. J. 2014. A review of current timed-AI (TAI) programs for beef and dairy cattle. The Canadian Veterinary Journal 55:772–780.

Craninx, M., Steen, A., Van Laar, H., Van Nespen, T., Martín-Tereso, J., De-Baets, B. and Fievez, V. 2007. Effect of lactation stage on the odd- and branched-chain milk fatty acids of dairy cattle under grazing and indoor conditions. Journal of Dairy Science 91: 2662-2677.

Croissant, A. E., Washburn, S., Dean, L. and Drake, M. 2007. Chemical properties and consumer perception of fluid milk from conventional and pasture-based production systems. Journal of Dairy Science 90: 4942-4953.

Daley, C. A., Abbott, A., Doyle, P. S., Nader, G. A. and Larson, S. 2010. A review of fatty acid profiles and antioxidant content in grass-fed and grain-fed beef. Nutrition Journal 9: 10.

De Marchi, M., Penasa, M., Cecchinato, A., Mele, M., Secchiari, P. and Bittante, G. 2011. Effectiveness of mid-infrared spectroscopy to predict fatty acid composition of Brown Swiss bovine milk. Animal 5:1653-1658.

Dewhurst, R.J., Shingfield, K. J., Lee, M.R.F. and Scollan, N. D. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Journal of Animal Feed Science and Technology 131: 168-206.

Dewhurst, R. J., Evans, R.T., Scollan, N.D., Moorby, J.M., Merry, R.J. and Wilkins, R.J. 2003. Comparison of grass and legume silages for milk production. 2. In vivo and in sacco-evaluations of rumen function. Journal of Dairy Science 86: 2612-2621.

DePeters, E.J., Medrano, J.F. and Reed, B.A. 1995. Fatty acid composition of milk fat from three breeds of dairy cattle. Canadian Journal of Animal Science 75: 267–9.

Ducháček, J., Stádník, L., Ptáček, M., Beran, J., Okrouhlá, M., Čítek, J. and Stupka, R. 2014. Effect of cow energy status on the hypercholesterolaemic fatty acid proportion in raw milk. Czech Journal of Food Science 32: 273-279.

Ducháček, J., Vacek, M., Stádník, L., Beran, J. and Okrouhlá, M. 2013. Changes in milk fatty acid composition in relation to indicators of energy balance in Holstein cows. Acta Universitatis Agriculturaeet Silviculturae Mendelianae Brunensis 60: 29-38.

Fenwick, M.A., Llewellyn, S., Fitzpatrick, R., Kenny, D.A., Murphy, J J., Patton, J. and Wathes, D. C. 2008. Negative energy balance in dairy cows is associated with specific changes in IGF-binding protein expression in the oviduct. Journal of Reproduction and Fertility 135: 63-75.

Fernandez, R. and Rodriguez, G. 2012. Effect of dietary and animal factors on milk fatty composition of grazing dairy cows: A review. Iranian Journal of Applied Animal Science 2: 97-109.

Garnsworthy, P.C. 1988. The effect of energy reserves at calving on performance of dairy cows. In “Nutrition and lactation in the dairy cow” (edited by P.C. Garnsworthy) pp. 157–170. Proceedings of the 46th University of Nottingham Easter School in Agricultural Science.

Garnsworthy, P., Feng, S., Lock, A. L. and Royal, M.D. 2010. Short communication: Heritability of milk fatty acid composition and stearoyl-CoA desaturase indices in dairy cows. Journal of Dairy Science 93: 1743-1748.

Garnsworthy, P., Masson, L., Lock, A. and Mottram, T. 2006. Variation of milk citrate with stage of lactation and de novo fatty acid synthesis in dairy cows. Journal of Dairy Science 89: 1604-1612.

Givens, D. I., Shingfield, K., Williams, C. and Buttriss, J. 2006. Optimising dairy milk fatty acid composition. Review of improving the Fat Content of Foods 252-280.

Gottardo, P., Penasa, M., Lopez-Villalobos, N. and De Marchi, M. 2016. Variable selection procedures before partial least squares regression enhance the accuracy of milk fatty acid composition predicted by mid-infrared spectroscopy. Journal of Dairy Science 99: 7782-7790.

Grimard, B., Humblot, P., Ponter, A., Mialot, J., Sauvant, D. and Thibier, M. 1995. Influence of postpartum energy restriction on energy status, plasma LH and oestradiol secretion and follicular development in suckled beef cows. Journal of Reproduction and Fertility 104: 173-179.

Goff, J.P. 2006. Major advances in our understanding of nutritional influences on bovine health. Journal of Dairy Science 89:1291-1301.

Gross, J., van Dorland, H. A., Bruckmaier, R.M. and Schwarz, F.J. 2011. Milk fatty acid profile related to energy balance in dairy cows. Journal of Dairy Research 78: 479-488.

Grummer, R.R. 1991. Effect of feed on the composition of milk fat. Journal of Dairy Science 74: 3244-3257.

Heck, J., Van Valenberg, H., Dijkstra, J. and Van Hooijdonk, A. 2009. Seasonal variation in the Dutch bovine raw milk composition. Journal of Dairy Science 92: 4745-4755.

Heuer, C., Van Straalen, W.M., Schukken, Y.H., Dirkzwager, A. and Noordhuizen, J.P. 2001. Prediction of energy balance in high yielding dairy cows with test day information. Journal of Dairy Science 84: 471-81.

Kadokawa, H. and Martin, B.G. 2006. A new perspective on management of reproduction in dairy cows: the need for detailed metabolic information, an improved selection index and extended lactation. Journal of Reproduction Development 52: 161-168.

Kay, J., Weber, W., Moore, C., Bauman, D., Hansen, L., Chester-Jones, H. and Baumgard, L. 2005. Effects of week of lactation and genetic selection for milk yield on milk fatty acid composition in Holstein cows. Journal of Dairy Science 88: 3886-3893.

Kelsey, J. A., Corl, B.A., Collier, R. J. and Bauman, D.E. 2003. The effect of breed, parity, and stage of lactation on conjugated linoleic acid (CLA) in milk fat from dairy cows. Journal of Dairy Science 86: 2588-2597.

Kgwatalala, P., Ibeagha-Awemu, E., Mustafa, A. and Zhao, X. 2009. Influence of stearoyl-coenzyme adesaturase 1 genotype and stage of lactation on fatty acid composition of Canadian Jersey cows. Journal of Dairy Science 92: 1220-1228.

Knop, R. and H. Cernescu 2009. Effects of negative energy balance on reproduction in dairy cows. Lucrări Stiinłifice Medicină Veterinară, 12: 198- 205.

Littlejohn, M.D., Tiplady, K., Lopdell, T., Law, T.A., Scott, A., Harland, C., Sherlock, R., Henty, K., Obolonkin, V., Lehnert, K., MacGibbon, A., Spelman, R.J., Davis, S.R. and Snell, R.G. 2014. Expression variants of the lipogenic AGPAT6 gene affect diverse milk composition phenotypes in Bos taurus. PLoS ONE 9, e85757.

Lopez-Villalobos, N., Lemus-Ramirez, V., Holmes, C.W. and Garrick, D.J. 2001. Lactation curves for milk traits, live weight and body condition score for heavy and light Holstein-Friesian cows. Proceedings of the New Zealand Society of Animal Production 61: 217-220.

Lopez-Villalobos, N., Spelman, R.J., Melis, J., Davis, S.R., Berry, S.D., Lehnert, K., Holroyd, R.E., MacGibbon, A.K.H. and Snell, R.G. 2014. Estimation of genetic and crossbreeding parameters of fatty acid concentrations in milk fat predicted by mid-infrared spectroscopy in New Zealand dairy cattle. Journal of Dairy Research 81: 340-349.

Lucy, M.C., Staples, C.R., Michel, F.M. and Thatcher, W.W. 1991. Energy balance and size and number of ovarian follicles detected by ultrasonography in early postpartum dairy cows. Journal of Dairy Science 74: 473-482.

Maurice-Van Eijndhoven, M., Soyeurt, H., Dehareng, F. and Calus, M. 2013. Validation of fatty acid predictions in milk using mid-infrared spectrometry across cattle breeds. Animal 7: 348-354.

MacGibbon, A.K.H. and Reynolds, M.A. 2011. Milk lipids: Analytical methods. In: Fuquay, J.W., Fox, P.F. and McSweeney, P.L.H. (eds.) Encyclopedia of dairy sciences, second edition No. 3. p 698–703. Academic Press, San Diego, USA.

McDougall, S., Burke, C.R., Williamson, N.B. and Macmillan, K.L. 1995. The effect of stocking rate and breed on the period of postpartum anoestrum in grazing dairy cattle. Proceedings of the New Zealand Society of Animal Production 55: 236–238.

McParland, S., Banos, G., Wall, E., Coffey, M., Soyeurt, H., Veerkamp, R. and Berry, D. 2011. The use of mid-infrared spectrometry to predict body energy status of Holstein cows. Journal of Dairy Science 94: 3651-3661.

Mensink, R.P., Zock, P.L., Kester, A.D. and Katan, M.B. 2003. Effects of dietary fatty acids and carbohydrates on the ratio of seru m total to HDL cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. The American Journal of Clinical Nutrition. 77: 1146-1155.

Nantapo, C., Muchenje,V. and Hugo, A. 2014. Atherogenicity index and health-related fatty acids in different stages of lactation from Friesian, Jersey and Friesian × Jersey cross cow milk under a pasture-based dairy system. Journal of Food Chemistry 146: 127-133.

Nogalski, Z., Wroński, M., Sobczuk-Szul, M., Mochol, M. and Pogorzelska, P. 2012. The effect of body energy reserve mobilization on the fatty acid profile of milk in high-yielding cows. Asian-Australasian Journal of Animal Sciences 25: 1712-1720.

O'Donnell, J.A. 1989. Milk fat technologies and markets: A summary of the Wisconsin Milk Marketing Board 1988 Milk Fat Roundtable 1. Journal of Dairy Science 72: 3109-3115.

Palladino, R., Buckley, F., Prendiville, R., Murphy, J., Callan, J. and Kenny, D. 2010. A comparison between Holstein-Friesian and Jersey dairy cows and their F1 hybrid on milk fatty acid composition under grazing conditions. Journal of Dairy Science 93: 2176-2184.

Palmquist, D.L., Denise Beaulieu, A. and Barbano, D.M. 1993. Feed and animal factors influencing milk fat composition. Journal of Dairy Science 76: 1753-1771.

Payne, E., Trigg, T.E., Rattray, P.V., Nicoll, G.B. and Smeaton, D.C. 1979. The biochemical assessment of energy status in the grazing lactating ruminant. Proceedings of the New Zealand Society of Animal Production 39:233-241.

Rani, Z.T., Chimonyo, M., Hugo, A., Marume U. and Muchenje,V. 2011. Effect of parity on the proximate composition and fatty acid profile of milk from Nguni cattle grazing on natural pastures. African. Journal of Biotechnology 10: 8647-8653.

Roche, J.R., Friggens, N.C., Kay, J.K., Fisher, M.W., Stafford, K.J. and Berry, D.P. 2009. Invited review: body condition score and its association with dairy cow productivity, health, and welfare. Journal of Dairy Science 92: 5769–5801.

Rukkwamsuk, T., Geelen, M., Kruip, T. and Wensing, T. 2000. Interrelation of fatty acid composition in adipose tissue, serum, and liver of dairy cows during the development of fatty liver postpartum. Journal of Dairy Science 83: 52-59.

Rutten, M., Bovenhuis, H., Hettinga, K., van Valenberg, H. and Van Arendonk, J. 2009. Predicting bovine milk fat composition using infrared spectroscopy based on milk samples collected in winter and summer. Journal of Dairy Science 92: 6202-6209.

Samková, E., Spicka, J., Pesek, M., Pelikánová, T. and Hanus, O. 2012. Animal factors affecting fatty acid composition of cow milk fat: A review. South African Journal of Animal Science 42: 83-100.

Secchiari, P., Mele, M. Serra, A. Buccioni, A. Paoletti, F. and. Antongiovanni, M. 2003. Effect of breed, parity and stage of lactation on milk conjugated linoleic acid content in Italian Friesian and Reggiana cows. Italian Journal of Animal Science 2: 269-271.

Schroeder, G., Couderc, J., Bargo, F. and Rearte, D. 2005. Milk production and fatty acid profile of milkfat by dairy cows fed a winter oats (Avena sativa L.) pasture only or a total mixed ration. New Zealand Journal of Agricultural Research 48: 187-195.

Silva-Villacorta, D., Lopez-Villalobos, N., Blair, H., Hickson, R., Spelman, R., Melis, J. and Snell, R. 2011. Effect of lactation stage and breed on the concentration of unsaturated fatty acids in milk fat of New Zealand dairy cattle. Proceedings of the New Zealand Society of Animal Production 71: 203-207.

Sneddon, N.W., Lopez-Villalobos, N., Davis, S.R., Hickson, R.E., Shalloo, L. and Garrick, D.J. 2016. Supply curves for yields of dairy products from first-lactation Holstein Friesian, Jersey and Holstein Friesian-Jersey crossbred cows accounting for seasonality of milk composition and production. Proceedings of the New Zealand Society of Animal Production 76: 139-143.

Soyeurt, H., Gillon, A., Vanderick, S., Mayeres, P., Bertozzi, C. and Gengler. N. 2007. Estimation of heritability and genetic correlations for the major fatty acids in bovine milk. Journal of Dairy Science 90: 4435-4442.

Soyeurt, H., Dardenne, P., Dehareng, F., Lognay, G., Veselko, D., Marlier, M., Bertozzi, C., Mayeres, P. and Gengler, N. 2006. Estimating fatty acid content in cow milk using mid-infrared spectrometry. Journal of Dairy Science 89:3690-3695.

Spelman, R., Miller, F., Hooper, J., Thielen, M. and Garrick, D. 2001. Experimental design for QTL trial involving New Zealand Friesian and Jersey breeds. Proceedings of the New Zealand Society of Animal Production 64: 92-95.

Spelman, R. J., Hooper, J. D., Stanley, G., Kayis, S. A. and Harcourt, S. 2004. Friesian Jersey crossbreed trial: Generating phenotypes for the discovery of quantitative trait loci. Proceedings of the New Zealand Society of Animal Production 64: 92-95.

Stoop, W., Bovenhuis, H., Heck, J. and Van Arendonk, J. 2009. Effect of lactation stage and energy status on milk fat composition of Holstein-Friesian cows. Journal of Dairy Science 92: 1469-1478.

Stoop, W.M., van Arendonk, J.A., Heck, J. M., van Valenberg, H. J. Bovenhuis, H. 2008. Genetic parameters for major milk fatty acids and milk production traits of Dutch Holstein-Friesians. Journal of Dairy Science 9: 385-394.

Thatcher, W.W., Bilby, T.R., Bartolome, J.A., Silvestre, F., Staples, C.R. and Santos, J.E.P. 2006. Strategies for improving fertility in the modern dairy cow. Theriogenology 65: 30-44.

Thomson, N., Bryant, M., Chand, A., MacGibbon, A. and Chagas, L. 2002. Effect of body condition at calving of milk fat composition of Friesian heifers during the first ten weeks of lactation. Proceedings of the New Zealand Society of Animal Production 62: 38-41.

Thomson, N. and Vander Poel, W.C. 2000. Seasonal variation of the fatty acid composition of milk fat from Friesian cows grazing pasture. Proceedings of the New Zealand Society of Animal Production 60: 314-317.

Van Knegsel, A.T., Van Den Brand, H., Dijkstra, J., Tamminga, S. and Kemp, B. 2005. Effect of dietary energy source on energy balance, production, metabolic disorders and reproduction in lactating dairy cattle. Journal of Reproduction Nutrition Development 45: 665-688.

Wales, W.J., Kolver, E.S., Egan, A.R. and Roche, J.R. 2009. Effects of strain of Holstein-Friesian and concentrate supplementation on the fatty acid composition of milk fat of dairy cows grazing pasture in early lactation. Journal of Dairy Science 92: 247-255.

Wathes, D.C., Cheng, Z., Fenwick, M.A., Fitzpatrick, R. and Patton, J. 2011. Influence of energy balance on the somatotrophic axis and matrix metalloproteinase expression in the endometrium of the postpartum dairy cow. Reproduction 141**:** 269-281

Welch, R.A., Burns, D. J., Davis, S. R., Popay, A. I. and Prosser, C.G. 1997. Milk composition, production and biotechnology. Cab International.

Vita

Vedastus Mkunda was born in Chamtui Village, Kilindi District, Tanga Region, Tanzania on 15 April 1982. He completed his primary education at Chamtui primary school in 1998,ordinary secondary education at Handeni secondary school in Tanzania in 2002, and high school at Kwiro High School in Mahenge-Ulanga, Morogoro, Tanzania in 2005. He then went to the Sokoine University of Agriculture (SUA) in 2006. In November 2009, Vedastus received his Bachelor of Science degree in Animal Science, and began his Master of Science (Animal Science) at Massey University in New Zealand in 2014 under the supervision of Professor Nicolas-Lopez Villalobos. In the summer of 2016, Vedastus completed his Master's degree and went back to Tanzania to continue with his career as an Animal Scientist.