Milk fatty acid and protein profiles of late-lactation dairy cows as affected by time of allocation of a fresh strip of ryegrass-based pasture

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Abstract
Eighty late-lactation dairy cows were used to examine the effects of allocating a new strip of a perennial ryegrass-based sward in the morning (AM) or in the afternoon (PM) on milk fatty acid (FA) and protein profiles. Milk total polyunsaturated FA (PUFA) were greater from cows on AM herbage, whereas total saturated and monounsaturated FA (MUFA) were similar. Time of allocation only affected the milk protein α-casein, which was greater in milk from cows on PM herbage, in turn affecting the casein:whey ratio. Under the current conditions, timing of allocation altered the herbage nutrient supply to cows; a greater concentration of precursor FA in AM herbage resulted in a greater concentration of beneficial FA in milk compared with cows on PM herbage. Quantifying the composition of FA in herbage could potentially aid in the design of grazing strategies to increase precursors of beneficial FA in dairy products.

Keywords: fatty acids, milk proteins, herbage composition, time of allocation

Introduction
Pasture-based diets offered to lactating dairy cows often result in improved nutritional quality of milk by shifting milk fatty acid (FA) composition towards less saturated FA and more polyunsaturated FA (PUFA) (Dewhurst *et al*. 2006; Elgersma 2015). Despite extensive ruminal biohydrogenation (BH), the amount and type of FA in feed and FA availability in the digestive tract and mammary gland, determine the FA profile in milk (Jenkins *et al*. 2008). In contrast, the composition of protein in milk from grazing dairy cows is largely unresponsive to variations in nutrition and management, unless large variations in intake of metabolisable energy (ME) and/or metabolisable protein occur (Walker *et al*. 2004).

Although extensive reviews on FA profiles of milk have been published (e.g. Elgersma 2015), the impact of time of allocation of a fresh strip of grass on the FA profile of milk has received limited attention. We hypothesised that a larger supply of PUFA in morning herbage (sole diet) offered to lactating dairy cows would lead to a greater PUFA concentration in milk, without affecting the milk protein composition, compared with afternoon herbage. Given the diurnal variation in FA profiles in herbage (Vibart *et al*. 2017) and in ruminal digesta (Sun & Gibbs 2012), the effects of time of milking on milk FA composition were also examined.

Methodology and analysis
Experimental site, cows, and pastures
The experiment was conducted at the Massey University No. 4 Dairy Farm (Palmerston North, New Zealand) during April and May 2010 (experimental period: 4 weeks), and was part of a larger study that examined the effects of timing of allocation of a fresh strip of a ryegrass (*Lolium perenne*) dominant pasture on milk production, N utilisation and grazing behaviour of late lactation dairy cows (Vibart *et al*. 2017). Eighty lactating cows (Holstein Friesian x Jersey crosses) averaging (mean ± standard deviation SD) 463 ± 46.8 kg body weight (BW), 224 ± 21 days in milk (DIM), 12.2 ± 2.1 kg initial milk yield, were used. Cows were blocked by milk yield, parity, and DIM, and randomly assigned to either a morning (AM; 0730 h, following the morning milking) or an afternoon (PM; 1530 h, following the afternoon milking) pasture strip allocation. Each allocation treatment included two herds of 20 cows (40 cows per treatment). Seven paddocks, comprising a total area of 14.5 ha, were used. Cows grazed the same strip for a 24 hours, and were offered the same daily dry matter (DM) allowance, with a targeted daily herbage DM intake (DMI) of 16 kg per cow (sole diet; calculated from clippings to a 4 cm height). Herbage DMI, calculated according to DMI [(kg DM/cow) = ((pre-grazing herbage mass (HM) – post-grazing HM)/cows per herd) × area offered (ha)], did not differ between treatments (Vibart *et al*. 2017).

Pasture chemical composition
Herbage samples were collected immediately before grazing, and every 30 minutes up to 4 hours after commencement of the main grazing events (n=64 samples). Samples were hand-plucked to mimic

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herbage grazed by cows. Chemical composition was obtained by near infrared reflectance spectroscopy (FeedTECH, Palmerston North, New Zealand). All herbage samples were analysed for ash, water-soluble carbohydrates (WSC), neutral-detergent fibre (NDF), acid-detergent fibre (ADF), crude protein (CP), and lipid concentrations, along with organic matter (OM) digestibility and metabolisable energy (ME; MJ/kg of DM) concentration (Corson *et al*. 1999). Also, subsamples collected during the first 60 minutes post-allocation (n=16) were analysed for soluble CP (non-protein N + soluble protein), degradable protein, neutral-detergent insoluble protein (NDICP) and acid-detergent insoluble protein (ADICP) by wet chemistry (Licitra *et al*. 1996) (Dairy One Forage Lab Services, Ithaca, NY, USA).

For herbage FA composition (expressed as g/100 g total FA), freeze-dried pasture subsamples collected during the first 60 minutes post-allocation were used (n=2 time points × 2 herds × 2 treatments × 2 periods = 16 samples). Fatty acids were extracted and methylated in a one-step incubation procedure with a mixture of toluene (Analar reagent), methanol and sulphuric acid (2.5% v/v sulphuric acid in methanol) (Sukhija & Palmquist 1988). The samples were analysed with a Shimadzu gas chromatograph (Shimadzu GC 2010, with a flame ionisation detector; Shimadzu, Kyoto, Japan). Nineteen FA methyl esters (FAME) were identified via retention time, quantified relative to internal standard tri-C11 (triundecanoin), and their corresponding response obtained from the concurrent analysis of an external standard (Supelco FAME Mix C4-C24, Sigma-Aldrich, St. Louis, MO, USA).

Milk composition

Milk samples were collected from each cow during the afternoon and following morning milking event three times (once during the adaptation period and twice during the data collection period; days 0, 16 and 23). Milk sample collection and analysis are further described in Vibart *et al*. (2017). Milk FA analysis (expressed as g/100 g total FA) was conducted as described by Schwendel *et al*. (2015). Briefly, forty-nine FAME were identified via retention time and their characteristic ion responses, quantified relative to the internal standard ([1,1,1-13C] trioctanoin), and their corresponding response obtained from the concurrent analysis of an external standard (Supelco FAME Mix C4-C24; Sigma-Aldrich, St. Louis, MO, USA). Milk protein analysis (expressed as g/kg milk) was conducted on the same samples according to Day *et al*. (2015). Sample analysis was performed using a Shimadzu LC10ADvp HPLC system equipped with a UV-VIS detector. Samples were injected into a Hi-Pore RP-318 column (Bio-Rad, New Zealand) and gradient elution was performed with a flow rate of 1 ml/min. Major milk

Table 1 Chemical composition of herbage allocated to cows either in the morning (AM) or in the afternoon (PM) (n = 64 herbage samples, unless specified otherwise).

Chemical composition (% DM unless stated otherwise)	Treatment		SE	P≤
	AM	PM		
DM (% of wet matter)	19.9	22.7	0.70	0.008
WSC	7.6	10.9	0.45	<0.001
NDF	50.4	48.8	0.45	0.02
ADF	25.7	25.8	0.26	0.93
Lipid	3.1	2.8	0.16	0.10
CP	22.2	20.5	0.25	<0.001
Protein fractions (% CP)				
Soluble protein	35.4	30.4	2.04	0.02
Degradable protein	73.6	74.4	2.16	0.50
Fatty acids (FA)	2.2	1.8	0.10	0.03
Individual FA (g/100 g FA)				
C12:0	2.39	2.94	0.12	0.006
C14:0	0.88	1.08	0.07	0.06
C16:0	25.25	29.43	1.65	0.09
C16:1	0.50	0.63	0.04	0.04
C18:0	2.79	3.31	0.17	0.05
C18:1 *c*9	2.72	3.50	0.19	0.01
C18:2 *c*9, *c*12	10.54	9.73	0.57	0.34
C18:3 *c*9, *c*12, *c*15	44.96	38.25	2.22	0.04
C20:0	0.75	0.94	0.05	0.02
C22:0	2.66	2.65	0.28	0.99
C24:0	2.41	2.60	0.24	0.59
Saturated FA (% total FA)	38.6	44.6	2.40	0.06
MUFA[1] (% total FA)	4.3	5.5	0.23	0.002
PUFA[2] (% total FA)	57.1	49.9	2.52	0.04

[1]Sum of monounsaturated FA. [2]Sum of polyunsaturated FA.

Table 2 Milk fatty acid (FA; g/100 g FA) composition from cows offered pasture either in the morning (AM) or in the afternoon (PM) and time of milking (afternoon or morning).

FA (g/100 g of FA)	Time of allocation (A)			Time of milking (M)[1]			P≤		
	AM	PM	SED[2]	Afternoon	Morning	SED	A	M	A x M
Even-chain saturated FA									
C10:0	2.43	2.35	0.135	2.33	2.46	0.058	0.64	0.03	0.10
C12:0	2.96	2.87	0.091	2.88	2.95	0.074	0.66	0.35	0.13
C14:0	10.77	10.59	0.278	10.70	10.67	0.151	0.58	0.83	0.009
C16:0	19.96	19.31	0.569	19.57	19.71	0.282	0.38	0.59	0.23
C18:0	8.76	9.08	0.244	9.22	8.62	0.244	0.18	0.02	0.55
C20:0	0.11	0.11	0.005	0.12	0.10	0.003	0.91	<0.001	0.24
Odd-chain saturated FA									
C15:0	2.58	2.48	0.031	2.59	2.48	0.026	0.08	<0.001	0.36
C17:0	1.14	1.12	0.026	1.16	1.10	0.016	0.45	<0.001	0.30
Branched-chain FA									
C15:0 *iso*	0.14	0.14	0.003	0.15	0.12	0.003	0.93	0.001	<0.001
C15:0 *anteiso*	0.82	0.78	0.027	0.82	0.78	0.013	0.30	<0.001	0.29
C16:0 *iso*	0.70	0.68	0.008	0.72	0.66	0.008	0.01	<0.001	0.001
C17:0 *iso*	0.73	0.72	0.009	0.76	0.70	0.009	0.37	<0.001	0.26
C17:0 *anteiso*	0.64	0.61	0.015	0.64	0.607	0.011	0.23	0.001	0.49
Monounsaturated FA (MUFA)									
C14:1	1.21	1.12	0.041	1.18	1.16	0.034	0.16	0.49	0.06
C16:1 *c9*	4.26	4.21	0.117	4.26	4.21	0.117	0.66	0.68	0.37
C16:1 *t9*	0.654	0.642	0.0191	0.667	0.629	0.0142	0.60	0.01	0.57
C17:1 *c9*	0.74	0.90	0.069	0.69	0.95	0.022	0.14	<0.001	<0.001
C18:1 *c9*	16.69	17.34	0.205	17.11	16.92	0.205	0.002	0.38	0.39
C18:1 *t9*	0.61	0.62	0.017	0.65	0.58	0.010	0.77	<0.001	0.38
C18:1 *c11*	2.15	2.26	0.027	2.29	2.11	0.028	<0.001	<0.001	0.35
C18:1 *t11*	7.14	6.67	0.403	7.30	6.51	0.212	0.35	<0.001	0.49
C19:1	0.35	0.36	0.005	0.37	0.34	0.005	0.11	<0.001	0.21
Polyunsaturated FA (PUFA)									
C18:2 *c9, c12*	1.51	1.54	0.060	1.59	1.46	0.022	0.67	<0.001	0.76
C18:2 *c9, t11*	3.30	2.87	0.131	3.30	2.88	0.130	0.001	0.001	0.74
C18:2 *t10, c12*	0.53	0.49	0.026	0.57	0.45	0.016	0.24	<0.001	0.87
C18:3	1.84	1.94	0.097	1.98	1.80	0.037	0.45	<0.001	0.03
C20:4 *n-6*	0.11	0.10	0.009	0.12	0.09	0.003	0.22	<0.001	0.97
C20:5 *n-3*	0.18	0.17	0.012	0.19	0.16	0.004	0.46	<0.001	0.35
Saturated FA	58.0	58.1	0.46	57.1	59.1	0.45	0.82	<0.001	0.72
OBCFA[3]	7.89	7.82	0.072	7.93	7.78	0.072	0.29	0.05	0.51
OBCFAm[4]	7.58	7.53	0.072	7.62	7.49	0.072	0.51	0.07	0.57
MUFA	34.4	34.7	0.35	35.0	33.9	0.34	0.30	0.001	0.72
PUFA	7.61	7.22	0.148	7.88	6.96	0.148	0.01	<0.001	0.79
FA (mg/mL)	33.4	39.2	3.51	39.5	33.0	1.43	0.24	<0.001	0.36

[1]Afternoon (at 1430 h) or morning (at 0630 h) milking. [2]SED = Standard error of the difference. [3]OBCFA = Odd- and branched-chain FA. [4]OBCFA of microbial origin (sum of *iso* C14:0, C15:0, *iso* C15:0, *anteiso* C15:0, *iso* C16:0, C17:0, *iso* C17:0, *anteiso* C17:0, and C17:1 *cis*-9) (Vlaeminck *et al*. 2006).

proteins were identified using external standards (α-, β-, and κ-casein, and α-lactalbumin and β-lactoglobulin; Sigma-Aldrich, Auckland).

Statistical analysis

Dependent variables (i.e. herbage chemical composition and milk composition) were analysed using SAS Analytics Software (SAS; version 9.3; SAS Institute Inc., Cary, NC, USA). Herbage chemical composition was analysed using a mixed model procedure; the model included fixed (time of allocation, day of sampling, and their interaction) and random (strip within paddock) effects. Milk composition was analysed using a mixed model procedure with repeated measurements over time. The models included fixed (time of allocation) and random (cow within herd) effects. Mean comparisons were performed using the Tukey test. Significance and trends were established at $P<0.05$ and $P<0.10$, respectively.

Results

Herbage Chemical Composition

Herbage composition differed between allocation treatments (Table 1). The PM herbage had greater DM ($P=0.008$) and WSC ($P<0.001$) concentrations, and lesser CP ($P<0.001$) and NDF ($P=0.02$) concentrations compared with AM herbage. Total FA, α-linolenic acid (ALA; C18:3 *cis*-9,12,15) and PUFA were greater ($P<0.05$) in AM herbage, whereas oleic acid (OA; C18:1 *cis*-9) and MUFA were greater ($P<0.05$) in PM herbage (Table 1). A trend ($P<0.10$) for greater total saturated FA in PM herbage was also observed.

Milk composition

Relative contributions of oleic acid and *cis*-vaccenic acid (*cis*-VA; C18:1 cis-11) were greater in milk from cows on PM herbage, whereas rumenic acid (RA; CLA isomer C18:2 *cis*-9, *trans*-11) was greater in milk from cows on AM herbage (Table 2). Total PUFA in milk were greater ($P=0.01$) from cows on AM herbage. Conversely, most of the milk FA analysed differed ($P<0.05$) in their relative contribution to total FA due to milking time, rather than herbage allocation time (Table 2). The relative contribution of saturated FA was greater ($P<0.001$) in morning-collected milk whereas the contribution of RA, MUFA, PUFA and total FA were greater ($P<0.001$) in afternoon-collected milk. Time of allocation of a fresh strip of pasture had lesser effects on milk protein composition (Table 3). Only α-casein was greater ($P=0.05$) in its relative contribution to total protein in milk from cows on PM herbage, in turn affecting ($P<0.001$) the casein:whey ratio.

Discussion

A greater concentration of total FA, ALA, and PUFA in AM herbage is consistent with values reported by Doreau *et al*. (2007). Perennial ryegrass manipulated for divergent soluble carbohydrate (SC):N ratios (+SC/-N versus -SC/+N) offered as sole diets showed that the -SC/+N ryegrass contained more FA (2.5 versus 1.8% of DM), a greater proportion of ALA (66 versus 61% of total FA) and a greater proportion of PUFA (78 versus 74% of total FA), often at the expense of saturated FA of 14-24 C, compared with +SC/-N ryegrass (Doreau *et al*. 2007). The greater concentrations of FA and ALA in -SC/+N ryegrass were attributed to the association between FA and N in chloroplasts, confirming the positive and overall linear relationship between ALA and N concentration in herbage (Elgersma 2015), also seen in this study.

Although allocation time altered a small number of milk FA, the greater contribution of RA and total PUFA in milk of cows on AM herbage is important, and most likely attributable to a greater contribution of C18 precursors in AM herbage, largely ALA. The concentration of PUFA in milk from grazing cows can be enhanced by increasing the amount and/or concentration of C18 precursors in the diet, by reducing the extent of ruminal BH, and/or by increasing the mammary gland enzymatic activity of the Δ-9 desaturase complex (i.e. the desaturation of VA into RA). Desaturase indices, acting as proxies for Δ-9 desaturase activity, were calculated for four pairs of FA characteristic of product and substrates of the complex (C14:1 *cis*-9/C14:0, C16:1 *cis*-9 /C16:0, C18:1 *cis*-9/C18:0, and C18:2 *cis*-9, *trans*-11/C18:1 *trans*-11), including an overall Δ-9 desaturase index. These indices did not differ between treatments ($P>0.05$; results not shown). Enhancing the amount and/or concentration of dietary precursors is most likely the contributing factor to greater RA and PUFA in milk from cows on AM herbage.

Table 3 Milk protein composition (g/kg of milk) from cows offered pasture either in the morning (AM) or in the afternoon (PM).

Protein composition (g/kg milk)	Treatment		SE	P ≤
	AM	PM		
Crude protein	40.6	41.7	0.69	0.34
True protein	35.9	37.1	0.64	0.33
Total casein	29.9	31.8	0.50	0.11
α-casein	13.8	15.3	0.23	0.05
β-casein	11.9	12.0	0.18	0.68
κ-casein	4.2	4.5	0.07	0.07
Total whey	6.1	5.3	0.16	0.08
α-lactalbumin	1.3	1.2	0.05	0.26
β-lactoglobulin	4.8	4.1	0.18	0.11
Casein:whey	5.1	6.1	0.12	<0.001

The greater concentrations of RA, MUFA and PUFA (largely at the expense of saturated FA), and total FA (mg/mL), were realised in milk collected following the AM grazing (i.e. afternoon milking) compared with milk collected following the PM allocation (i.e. morning milking). In agreement with current findings, a recent comparison between conventional and organic grazing systems showed greater concentrations of VA and RA, and lesser concentrations of saturated FA up to 16 C, in milk collected during the afternoon compared with milk collected during the morning milking (Schwendel *et al*. 2015).

Differences in protein composition due to allocation treatment were unexpected. Comparatively, supplementing pasture (as the sole diet) with either maize grain or maize grain plus pasture silage resulted in increased milk yield, but had negligible effects on milk protein composition from late lactation dairy cows (Mackle *et al*. 1999). In this study, the greater casein:whey ratio in milk from cows on PM herbage was largely due to a greater concentration of α-casein, and to a lesser extent, to κ-casein, since individual whey proteins were similar between treatments. The causes of these allocation treatment differences remain unknown. We can only speculate that differences in casein concentration could be attributed to differences in clover content of the pastures (not measured in our study) (Grandison *et al*. 1985), and to a certain extent, varying breed composition of the herds and the impact of genetic variants of the major milk proteins on the quantitative and qualitative milk traits.

A greater concentration of beneficial FA in AM herbage resulted in a greater concentration of RA and PUFA in milk from cows on AM herbage, compared with cows on PM herbage. These benefits, plus a lower concentration of saturated FA, were captured in milk collected following the AM (i.e. afternoon milking) compared with the PM allocation period (i.e. morning milking). Quantifying the composition of FA in herbage in response to grazing management could potentially aid in the design of grazing strategies to increase precursors of beneficial FA in dairy products.

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The implications of winter milk premiums for sustainable profitability of dairy systems

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Abstract

Matching seasonal pasture growth to cow demand has been the key to New Zealand's ability to produce milk competitively. However, driven by the need to process milk all year for value-add products like UHT milk, Fonterra has increased the incentive for producing milk in the winter. This has some farmers questioning their spring calving approach and considering calving outside spring to increase profitability of their system using the winter milk premium. In an attempt to answer farmer's questions, modelling was done using OVERSEER® for the environmental footprint and Farmax Dairy for the economic impact of changing the calving season. The objective of the modelling was to highlight key factors that need careful assessment for individual farm situations before deciding to change calving season. Whole farm system modelling was done for Ruakura and Pukekohe pasture growth profiles in the Waikato, and Te Hana and Maungatoroto growth profiles in Northland to consider the implications from both a profitability and environmental perspective. In the four districts modelled results suggest the key drivers for autumn calving profitability are: seasonal pasture growth profile, soil type, winter milk premium and cost of infrastructure/equipment upgrade. Regions with pasture growth profiles that remain profitable after changing from spring to autumn without the winter milk premium are the most ideal, as there is no guarantee the premium will stay at the current level.

Keywords: modelling, winter milk premium, pasture growth profile, profitability, nitrogen leaching

Introduction

The winter milk premium has been increased to encourage farmers to produce more milk during the winter months, driven by the increased demand for UHT cream in China (Fonterra 2017). As a result, there is a renewed interest among farmers as to whether or not they can take advantage of the increased premium. Autumn calving systems imply a less than ideal match between supply and demand for pasture, therefore, whether they are more profitable is likely to be dependent on the winter milk premium, their ability to source cheap supplements and the capital and transitional cost of changing to autumn calving. The inherent risk of changing calving season is that the winter milk premium is not guaranteed to remain at current levels. The objective of this modelling exercise was to reassess profitability of winter milking systems under the current milk price and newly announced milk premium and test profitability on different pasture growth profiles and winter milk premiums. Consequences for nitrogen (N) leaching were also assessed.

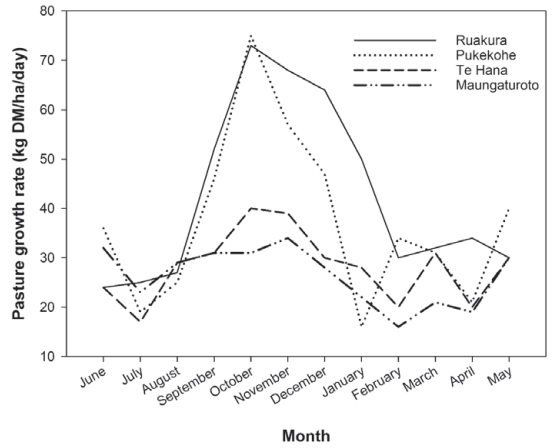

Figure 1 Average monthly pasture growth rates for the four districts.

Methods

Typical Waikato and Northland medium input spring calving systems were modelled using 2014/2015 Dairy Statistics (www.lic.co.nz), Economic Survey (www.dairynz.co.nz) and DairyNZ regional systems descriptor data. Medium input can be described as a farm that imports approximately 10-20% of total feed to the milking area to extend lactation (usually autumn feed), and to feed dry cows (Hedley *et al*. 2006). Two districts in the Waikato and two in Northland with different pasture growth profiles were chosen. In Waikato, pasture growth data from Ruakura (15.5 t DM/ha/year) and Pukekohe (13.6 t DM/ha/year) were used; in Northland, pasture growth data from Te Hana (10.3 t DM/ha/year) and Maungaturoto (9.6 t DM/ha/year) were used (DairyNZ 2010; Figure 1).

For each pasture growth profile, four autumn calving systems were modelled in Farmax Dairy (Bryant *et*

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