International Dairy Journal 92 (2019) 69–76

Contents lists available at ScienceDirect

International Dairy Journal

journal homepage: www.elsevier.com/locate/idairyj

Effect of reducing daily herbage allowance during early lactation on composition and processing characteristics of milk from spring-calved herds

Arunima Gulati [a, c], Norann Galvin [b], Emer Kennedy [b], Eva Lewis [b], Jennifer J. McManus [c], Mark A. Fenelon [a], Timothy P. Guinee [a, *]

[a] Teagasc Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland
[b] Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland
[c] Department of Chemistry, Maynooth University, Maynooth, Co. Kildare, Ireland

ARTICLE INFO

Article history:
Received 13 July 2018
Received in revised form
2 January 2019
Accepted 12 January 2019
Available online 30 January 2019

ABSTRACT

The study investigated the effects of reducing daily herbage allowance (DHA) from 15.0 to 11.8 kg dry matter per cow (>3.5 cm post grazing sward height) to a spring-calved herd during early lactation on the composition, rennet coagulability and heat stability characteristics of milk during early lactation (EL, 29–70 days in milk, DIM), mid lactation (ML, 78–183 DIM), and late lactation (LL, 205–267 DIM). Samples of milk were taken at approximate 10 d intervals during EL and at 1–3 week intervals during ML and LL. Reducing DHA led to reductions in milk yield, milk solids yield, and concentrations of protein (~0.22%, w/w) and casein (0.13%, w/w) during EL. Otherwise, it had little effect on milk composition or on the selected processing characteristics in ML, LL or overall lactation. Stage of lactation resulted in comparatively large changes in most compositional parameters, rennet gelation and heat stability.

1. Introduction

Grazing of dairy cows on pasture grass features prominently in temperate regions, such as Ireland and New Zealand, where grass growth occurs over most of the year. Grazing with compact calving of cows in early spring is often the most cost-effective approach for milk production, as the maximum milk production volume coincides with maximum grass growth (O'Brien & Hennessy, 2017). The diet of pasture-grazed dairy herds may be supplemented with a low quantity of concentrate supplementation offered at the extremes of the pasture growing season, i.e., in early and late lactation.

Commercial milk primarily from spring-calved pasture-grazed dairy herds has a lactational supply pattern, with peak supply occurring in late spring-early summer when cows are in early lactation and grass growth is high (O'Brien & Hennessy, 2017). Moreover, the milk also displays variation in composition and yield over the year to an extent dependent on stage of lactation (Auldist, Napper, & Kolver, 2000a), quality/allowance of feed (Auldist et al., 2016; Mackle, Bryant, Petch, Hooper, & Auldist, 1999), animal health, weather, and husbandry practices (Chen, Grandison, & Lewis, 2017). Variation in the DHA of pasture-based spring-calved herds can vary according to pedoclimatic conditions and local changes in weather conditions. Data on DHA of cows in Ireland indicates that the DHA varies from ~12 to 16 kg dry matter (DM) per cow during the initial 12 weeks of lactation (Lewis, O'Donovan, Kennedy, O'Neill, & Shalloo, 2011). Cold wet weather in spring (March–April) can significantly reduce grass growth and, hence, the DHA available to grazing herds in early lactation especially where stocking rate is high (Kennedy, Galvin, & Lewis, 2015). The following questions arise: 'does a shortage of herbage in early lactation affect composition and processability of milk?', 'at what level of herbage reduction do effects become significant?', and 'are effects of a shortage of herbage in early lactation carried into mid- and late-lactation when herbage again becomes plentiful?'

Lowering DHA in the range 13–19 kg DM per cow in early lactation (15–95 days in milk, DIM) has been generally found to coincide with reductions in milk yield and protein concentration,

* Corresponding author. Tel.: + 353 25 42204.
E-mail address: tim.guinee@teagasc.ie (T.P. Guinee).

https://doi.org/10.1016/j.idairyj.2019.01.008
0958-6946/

but to have no effect on the concentration of fat or lactose (Kennedy, O'Donovan, O'Mara, Murphy, & Delaby, 2007; McEvoy et al., 2008). A similar trend for effect of DHA on milk yield and concentrations of protein and casein was reported by Auldist, Thomson, Mackle, Hill, and Prosser (2000b) when DHA was limited to ~40% of ad libitum allowance in early lactation (~60–68 DIM). Similarly, Bargo, Muller, Delahoy, and Cassidy (2002) found that a decrease in DHA from 40 to 25 kg DM per cow during four different periods across the year (93–113, 114–134, 209–229, and 230–250 DIM) reduced milk yield, but had no effect on the mean concentrations of milk protein, fat or milk urea nitrogen.

Variations in the concentrations of the major milk constituents (e.g., casein, whey protein, lactose) are of relevance in dairy processing as they affect manufacturing efficiency of dairy products such as cheese, casein and milk powder, and processing characteristics such as rennet gelation (Guinee et al., 1997; Lin, O'Mahony, Kelly, & Guinee, 2017b), heat stability (Huppertz, 2016) and ethanol stability (Chen, Lewis, & Grandison, 2014; Lin et al., 2017b). However, other composition-related factors are also likely to affect the processing behaviour of milk, e.g., concentration of different elements (Tsioulpas, Lewis, & Grandison, 2007), proportions of different caseins (Jõudu, Henno, Kaart, Püssa, & Kärt, 2008), and the partitioning of components (e.g., casein, calcium) between the serum and casein micelle (Lin, Kelly, O'Mahony, & Guinee, 2018). Wedholm, Larsen, Lindmark-Månsson, Karlsson, and Andrén (2006) reported that a low concentration of κ-casein and a low proportion of κ-casein (% total casein) in individual cow milk samples collected from different breeds (Swedish Red and White, Swedish Holstein and Danish Holstein-Friesian) correlated with poor rennet-coagulating properties. Little information is available on how variation in DHA in early lactation affects these other compositional parameters and milk processability, or whether such effects are carried over into mid- and late-lactation.

The current study investigated the effect of altering DHA (11.8–15.0 kg DM per cow) in early lactation (29–70 DIM) on the composition, rennet gelation and heat stability of cows' milk during early-, mid-, and late-lactation. The typical DHA required for spring-calved cows in early lactation in Ireland is ~15 kg DM per cow (Lewis et al., 2011).

2. Materials and methods

2.1. Herd treatments and paddock management

Thirty-six spring-calved cows from the Teagasc Animal and Grassland Research and Innovation Centre (Moorepark, Fermoy, Co. Cork, Ireland) were made available for the study. The cows had a mean calving date of February 9, 2015 ± 8.4 d. Following calving, the cows had access to pasture by day and housed by night until 22 February. During this period of partial turnout, the cows received a daily herbage allowance of 7 kg DM per cow while grazing to a post grazing height of >3.5 cm, and had ad-libitum access to grass silage by night. Cows were let out to grass full-time on February 23 and received a DHA of 13 kg DM per cow until March 2, one week prior to dividing of the cows into three separate herds. During the period February 9–March 2, cows received a concentrate supplement that was gradually reduced from 5 to 0 kg per cow per day. After this period, cows grazed on pasture without any further concentrate supplementation.

The 36 cows were assigned to three different herds which were placed on different DHA on March 9. The herds were balanced with respect to calving date (9 February 2015, ±8.4 d), breed (16 Holstein Friesian (HF), 13 HF × Jersey, 7 HF × Norwegian Red) lactation number (2.56 ± 1.4), bodyweight (523 ± 53.2 kg), body condition score (BCS, 3.17 ± 0.142), pre-experimental daily milk yield (25.3 ± 3.70 kg) and milk solids yield (2.20 ± 0.33 kg). Each herd was assigned to a different DHA treatment for 6 weeks from March 9 – April 19 (29–70, DIM) within the same paddocks, which were divided into separate areas using electric wires. The three DHA were 11.8, 14.4 and 15.0 kg DM per cow, respectively; these are denoted as low (L-DHA), medium (M-DHA) and high (H-DHA), respectively. Fresh pasture areas were offered after each milking while the DHA treatments were being imposed and on a 24-h basis thereafter.

Paddocks were dusted with calcined magnesite (Inform Nutrition, Cork, Ireland) to prevent grass tetany. Herbage mass for each treatment paddock was calculated at a sward height of >3.5 cm by mowing six strips (120 m^2) with a motorised harvester (Etesia UK Ltd., Warwick, UK) twice weekly. All mown herbage from each strip was collected, weighed, sub-sampled, and analysed for DM by drying for 16 h at 90 °C (Kennedy et al., 2007). The pre- and post-grazing sward height was measured on a daily basis at 40 locations across the two diagonals of each paddock, using a folding pasture plate meter with a steel plate (diameter 355 mm and 3.2 kg m^{-2}; Jenquip, Fielding, New Zealand).

Following the 6 week experimental period, all 36 cows grazed once more as a single herd, receiving a common DHA of 18 kg DM per cow for the remainder of the lactation period (November 23, DIM 288). Individual cows were dried off when daily milk yield decreased to 8 kg per cow, BCS dropped to 2.5, or when the interval from next calving date was 8 weeks.

All experimental procedures involving cows were approved by the Teagasc Animal Ethics Committee (TAEC69/2014) and authorised by the Health Products Regulatory Authority (Project licence No.: AE19132/P017), which is the competent authority in Ireland responsible for the implementation of European Union legislation for the protection of animals used for scientific purposes.

2.2. Milk sampling

Milk was sampled at 10 day intervals during the period March 9–April 19 (29–70 DIM), when the herds were on different DHA; this period was denoted early lactation (EL). Thereafter, milk was sampled at 1–3 week intervals for the remainder of the lactation period, which was divided into two sub-periods, namely mid lactation (ML, April 27–August 31) when cows were 78–183 DIM, and late lactation (LL, September 20–November 2) when cows were 205–267 DIM.

Cows were milked daily at 07:00 and 15:30. The milk from all 36 cows was collected separately after evening and morning milkings; evening milk samples were stored at 4 °C overnight prior to blending with morning milk samples. A composite sample for each of the treatment herds was generated by blending the milk from individual cows in the herd, in quantities proportional to the total milk yield of each cow in both evening and morning milkings. The three composite herd milk samples were preserved with sodium azide (0.02%, w/w) and stored overnight at 4 °C until required for analysis, which was completed with 3–48 h after collection.

2.3. Preparation of skim milk and milk serum

Milk was skimmed to a fat concentration of <0.1% (w/w) fat using a disc bowl centrifuge (FT15 Disc Bowl Centrifuge, Armfield Limited, Ringwood, UK), and stored at 4 °C prior to analysis (within 24–48 h). Representative sub-samples (~10 mL) of the skim milk for analysis of protein profile and concentrations of elements were stored at −20 °C until required.

The preparation of milk serum involved heating the cold skim milk samples at 40 °C for 30 min (to reverse cold ageing effects, including solubilisation of casein and calcium phosphate; Dalgleish & Law, 1988) and ultracentrifugation at 100,000×*g* at 25 °C for 1 h (Sorvall Discovery 90SE ultracentrifuge, Kendro Laboratory Products, Asheville, NC, USA). The supernatant was filtered through glass wool to obtain fat-free serum which was stored at 4 °C and assayed for nitrogen (N) content within 48 h of sample collection; subsamples of the fat-free serum (2 mL) were taken in Eppendorf tubes (Thermo Fisher Scientific, Ireland) which were stored at −20 °C and assayed later for protein profile as described below.

2.4. Compositional analysis of milk, skim milk and serum

Milk was assayed in triplicate for: total solids, fat, lactose and urea using the FOSS MilkoScan FT+ analyser (N. Foss Electric A/S, Hillerød, Denmark), protein, non-casein nitrogen (NCN) and non-protein nitrogen (NPN) using standard International Dairy Federation methods (Lin et al., 2017b). Skim milk was analysed for individual proteins by reversed phase HPLC, as described by Lin et al. (2017b). Minor whey proteins, including bovine serum albumin, lactoferrin, and immunoglobulins were not detected by the RP-HPLC protocol used. Fresh serum was analysed for protein, as described above, and frozen samples of serum were thawed at 4 °C for ~1 h and analysed for individual proteins and soluble casein, which is defined as follows:

Soluble casein = total protein in serum - whey protein − NPN (expressed as protein).

2.5. Element analysis

The concentrations of macro- (Ca, P, Na, Mg) and trace- (Zn, Fe, Cu, Mo, Mn, Se, Co) elements in skim milk were measured in acid-extracted samples using inductively coupled plasma mass spectrometry (Agilent ICPMS 7700×, with ASX-500 series auto-sampler and MassHunter software A.01.02 Patch 4), as described by Gulati et al. (2018). The method involved acid extraction (with nitric acid, hydrochloric acid and hydrogen peroxide) of milk samples (~1 g) and appropriately dilution of the extract and its analysis on ICPMS.

2.6. Rennet gelation

Cold skim milk samples were heated at 40 °C for 30 min, cooled to 21 °C, adjusted to pH 6.55 at 21 °C and heated to 31 °C. Chymosin (Chy-Max® plus, 200 International milk clotting units (IMCU) mL^{-1}; Chr. Hansen, Hørsholm, Denmark) was diluted 20-fold in distilled water, was added to the milk at a level of 10.6 IMCU g^{-1} protein. Immediately, the sample was assayed for changes in storage modulus, G′ (a measure of system elasticity), as a function of time from rennet addition, using dynamic low amplitude strain oscillation rheometry in a controlled stress rheometer (Carri-Med, type CSL2500, TA instruments, New Castle, USA), as described by Lin et al. (2017b).

The following parameters were calculated from the resultant G′/time curve: rennet coagulation time (RCT), defined as the time required for G′ to increase to a value of ≥0.2 Pa; maximum gel firming rate (GFR_{max}), calculated as the maximum slope of the G′/time curve; and, G′ at 40 min from rennet addition (G'_{40}).

2.7. Heat coagulation time

Skim milk samples were adjusted to pH values in the range 6.2–7.2 with 0.1 M HCl/NaOH, at 0.1 pH unit intervals, at 21 °C. The pH-adjusted samples and a sample at natural pH (HCT_{npH}) were assayed for heat coagulation time (HCT) at 140 °C in a temperature controlled oil bath (Hettich ESP oil-heating bath; Hettich Benelux BV, Geldermalsen, Netherland), as described by Lin et al. (2017b). The following parameters were obtained from the resultant pH/HCT curves, all of which had a typical type A HCT/pH profile (Huppertz, 2016): HCT_{max}, HCT at the first inflection point; HCT_{min}, HCT at second inflection point.

2.8. Statistical analysis

The data set relating to the bulk milk from each of three DHA treatments (L-DHA, M-DHA and H-DHA) in the individual lactation periods (EL, ML and LL) was analysed using analysis of variance (ANOVA), to determine the effect of DHA in each lactation period, the effect of DHA in overall lactation, and the effect of lactation period across all the DHA treatments. The experimental unit was herd milk, while the replication unit was the sampling time. The effects of DHA and lactation period were determined using the general linear model (GLM) procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). Tukey's multiple-comparison test was used for paired comparison of means and the level of significance was determined at $P < 0.05$.

R-3.2.2 software (R Core Team, 2014) was used to compute a Pearson correlation between the different compositional variables, where significance was determined at $P < 0.05$, $P < 0.01$, and $P < 0.001$, according to Students t-test.

3. Results and discussion

3.1. Gross composition and pH of milk

The yield and composition of milk from the different DHA treatments, applied during early lactation, are shown in Table 1. DHA had significant effects on milk yield and composition, the extent of which depended on compositional parameters and lactation period.

Reducing DHA from 15.0 (H-DHA) to 11.8 (L-DHA) kg DM per cow during the six-week EL period resulted in lower yields of milk and milk solids, and concentrations of total protein, true protein and casein in EL, but had no effect on the concentrations of total solids, lactose, fat, whey protein, NPN and urea, casein number, or proportions of individual caseins in EL. The results concur with those of previous studies (Kennedy et al., 2007; McEvoy et al., 2008; O'Brien et al., 1997) which found that reducing DHA in the range 19.0 to 13.0 kg DM per cow in early- (15–95 DIM) or mid- (88–177 DIM) lactation led to lower milk yield and concentrations of protein and casein, but did not affect the concentrations of fat and lactose. Likewise, the absence of an effect of DHA on the proportions of different caseins (α_{S1}-, α_{S2}-, β- or κ-caseins) agrees with the results of Auldist et al. (2000b) on restricting DHA to ~40% of ad libitum intake in early lactation (~60–68 DIM).

Apart from a quite small, but significant, change in urea in ML, reducing DHA in EL had no significant carry-over effects on yield of milk solids or milk composition in ML or LL (Table 1), as evidenced by the similar values for the latter variables in ML and LL.

This trend confirms the results of Kennedy et al. (2007), which showed that differences in milk yield and composition as a result of DHA variation (13–19 kg DM per cow) in early lactation (15–91 DIM) disappeared on normalisation of DHA to 20 kg DM per cow in mid lactation (92–119). Conversely, Roche (2007) found that restricting DHA from 13.5 to 8.6 kg DM per cow in early lactation (1–35 DIM) coincided with lower yields of milk, fat and protein, and concentrations of fat and protein during later lactation (36–105 DIM). McEvoy et al. (2008) also found that lowering DHA

Table 1
Effect of reducing daily herbage allowance (DHA) in early lactation on the composition of whole milk in early-, mid- and late-lactation.[a]

Item	Effect of DHA treatment in different lactation periods															Overall effects throughout lactation	
	Early-lactation (EL)					Mid-lactation (ML)					Late-lactation (LL)					DHA	LP
	H- DHA	M-DHA	L-DHA	SED	*P*	H- DHA	M-DHA	L-DHA	SED	*P*	H- DHA	M-DHA	L-DHA	SED	*P*	*P*	*P*
Milk yield (kg per cow per day)	26.1^{a}	24.4^{b}	23.0^{b}	0.34	**	20.8	20.4	21.4	0.03	–	12.4^{ab}	12.2^{b}	13.5^{a}	1.14	**	–	***
Milk solids yield (kg per cow per day)	3.43^{a}	3.23^{ab}	3.01^{b}	0.06	**	2.67	2.72	2.82	0.04	–	1.78	1.83	1.91	0.16	–	–	***
Total solids (%, w/w)	13.1	13.2	13.1	0.16	–	12.9	13.3	13.2	0.13	–	14.3	14.6	14.4	0.18	–	–	***
Lactose (%, w/w)	4.88	4.83	4.85	0.02	–	4.88	4.84	4.85	0.01	–	4.6^{a}	4.48^{b}	4.52^{ab}	0.05	*	–	***
Fat (%, w/w)	4.01	4.20	4.16	0.02	–	3.74	4.19	4.05	0.14	–	4.78	4.9	4.77	0.10	–	–	***
Total protein (%, w/w)	3.37^{a}	3.29^{ab}	3.15^{b}	0.05	*	3.46	3.52	3.45	0.02	–	4.08	4.31	4.17	0.11	–	–	***
True protein (%, w/w)	3.17^{a}	3.09^{ab}	2.95^{b}	0.04	*	3.26	3.31	3.26	0.02	–	3.78	4.00	3.90	0.13	–	–	***
Casein (%, w/w)	2.59^{a}	2.56^{a}	2.46^{b}	0.02	*	2.61	2.68	2.62	0.02	–	3.03	3.21	3.10	0.08	–	–	***
Individual caseins (% milk casein)																	
α_{S1}-casein	42.3	42.7	40.3	1.1	–	37.4	37.0	39.4	2.2	–	39.3	39.1	41.0	0.7	–	–	*
α_{S2}-casein	8.5	8.7	8.5	0.7	–	11.1	9.8	9.6	3.2	–	10.2	11.5	8.6	0.6	–	–	–
β-casein	32.4	32.2	33.9	1.1	–	31.8	33.2	33.8	3.3	–	32.4	28.9	31.2	1.14	–	–	–
κ-casein	16.8	16.3	17.3	1.1	–	19.7	20.0	17.2	2.8	–	18.1	20.5	19.2	1.42	–	–	–
Casein number	76.8	77.8	78.0	0.8	–	75.4	76.2	75.9	0.4	–	74.2	74.5	74.2	0.5	–	–	**
Whey protein (%, w/w)	0.58	0.53	0.50	0.02	–	0.65	0.64	0.65	0.01	–	0.76	0.79	0.80	0.06	–	–	***
α-Lac:β-Lg	0.21	0.22	0.22	0.01	–	0.21	0.22	0.23	0.38	–	0.23	0.20	0.25	0.07	–	–	–
Casein:whey protein	4.47	4.83	4.92	0.20	–	4.02	4.19	4.03	0.17	–	3.99	4.06	3.88	0.14	–	–	**
NPN (% total N)	5.92	6.04	6.17	0.20	–	5.79	5.72	5.50	0.09	–	7.15	7.28	6.49	0.81	–	–	–
Urea (mg 100 g^{-1})	28.5	27.2	28.2	1.2	–	28.5^{ab}	29.6^{a}	27.8^{b}	0.4	*	32.5	34.5	34.7	2.3	–	–	–
pH	6.70	6.74	6.72	0.02	–	6.69	6.65	6.67	0.01	–	6.64	6.61	6.63	0.01	–	–	***
Soluble protein (%, w/w)	0.91^{ab}	0.97^{a}	0.84^{b}	0.03	*	1.06	1.07	1.01	0.02	–	1.53	1.6	1.49	0.08	–	–	***
Soluble casein (% milk casein)	3.4	7.9	4.6	1.44	–	6.4	7.1	5.1	0.71	–	13.2	13.9	11.7	1.58	–	–	***

[a] H-DHA, M-DHA and L-DHA denote high-, medium- and low- DHA, i.e., 15.0, 14.4 and 11.8 kg dry matter per cow, respectively. Early (EL)-, mid (ML)- and late (LL)-lactation correspond to March 16-April 19, April 27-August 10, and September 1-November 2, when cows were 29–70, 78–183, and 205–267 days in lactation, respectively. Values within a row relating to effect of DHA treatment in EL, ML or LL and not sharing a common lower-case superscripted letter differ significantly for effect of DHA; values within a row without a superscript do not differ for effect of DHA ($P > 0.05$). SED = standard error of difference between means; *P* values denote statistical significance, where ***, **, * and - denote $P < 0.001$, <0.01, <0.05 and >0.5, respectively. The statistical significance (*P*) for the effects of DHA in overall lactation, and lactation period (LP) across all DHA treatments are also shown.

(from 17 to 13 kg DM per cow) in early lactation (19–95 DIM) resulted in a significant reduction in milk protein (0.13%, w/w) in mid-lactation (96–181 DIM). Roche (2007) concluded that the most plausible reason for this carryover effect is a negative effect of energy restriction on mammary secretory cell number and activity, and potentially a reduced uptake of nutrients by the mammary gland. The inter-study discrepancy (Kennedy et al., 2007; McEvoy et al., 2008; Roche, 2007) on the effects of DHA restriction in early lactation on the composition of milk as lactation advances may relate to factors such as extent and duration of feed restriction, and level of nutrient intake in the pre-calving and post-restriction periods. Despite its effects in EL, reducing DHA in EL had no effect on the mean values for milk yield or different compositional parameters over the entire lactation.

The mean daily milk yield decreased progressively with stage of lactation, from ~24.5 kg in EL per cow to 12.7 kg per cow in LL. Lactation period also had a significant effect on milk composition (Table 1), with LL milk having higher mean concentrations of fat, protein, casein, whey protein and NPN, a lower concentration of lactose, and a lower pH value. However, casein as a proportion of total casein (casein number) decreased, while whey protein as a proportion of total protein increased with advance in lactation. It has been suggested that the reduction in the casein:whey protein ratio in LL milk is due to an influx of blood components (including albumin and immunoglobulins) into the milk, concomitant with an increase in the permeability of the alveolar epithelium as involution approaches (Auldist & Hubble, 1998; Bobbo et al., 2017). The mean proportion of α_{S1}-casein in ML milk (37.9%) was lower than that in EL milk (41.8%); otherwise lactation period did not affect the proportions of κ-, β- and α_{S2}-caseins, or the ratio of α-lactalbumin to β-lactoglobulin. The trends in protein and lactose with lactation period are similar to those reported previously for milk from spring-calved herds (Auldist et al., 2000a; Gulati et al., 2018).

3.2. Macro- and trace-elements

The elemental content of milk affects its processing behaviour, and the nutritional value and stability of dairy products (Gaucheron, 2013). The mean concentrations of macroelements (Ca, P, Na and Mg) and trace elements (Zn, Fe, Cu, Mo, Mn, Se and Co) are shown in Table 2. Overall, reducing DHA in EL had little influence on the concentrations of most elements, apart from giving higher and lower concentrations of Mg and Fe, respectively, in EL and a higher concentration of Zn in LL. The results concur with O'Brien et al. (1997) who reported that alteration of DHA in mid-lactation (88–177 DIM) did not significantly alter the concentrations of Ca or P. The absence of an effect of DHA on the concentrations of Ca and P might be explained on the basis that the animal skeleton acts as a reservoir for these minerals where mineral intake in the diet is deficient (Fox, Uniacke-Lowe, McSweeney, & O'Mahony, 2015).

Lactation period significantly affected the mean concentration of most elements, apart from Fe, Mn and Co (Table 2). LL milk had higher mean concentrations of Ca, Mg, P, Na, Se, Zn and Mo, and a lower concentration of Cu, than EL or ML milk. The trend aligns with the lactational increase in casein, with which a relatively high proportion of many of the latter elements (Ca, P, Mg, Zn) associate (Vegarud, Langsrud, & Svenning, 2000) in the formation of the casein micelles (Lucey & Horne, 2018). Hence, the concentrations of Ca, P and Mg correlated positively with casein content (Fig. 1a). Nevertheless, the ratio of total Ca or P to casein decreased slightly, but significantly, as casein increased (Fig. 1b) from 120 DIM onwards.

Table 2
Effect of reducing daily herbage allowance (DHA) during early lactation on the elemental composition of milk in early-, mid- and late-lactation.[a]

Item	Effect of DHA treatment in different lactation periods															Overall effects throughout lactation	
	Early-lactation (EL)					Mid-lactation (ML)					Late-lactation (LL)					DHA	LP
	H- DHA	M-DHA	L-DHA	SED	*P*	H- DHA	M-DHA	L-DHA	SED	*P*	H- DHA	M-DHA	L-DHA	SED	*P*	*P*	*P*
Ca (mg 100 g^{-1})	121	128	122	3.1	–	126	130	124	1.7	–	143	142	144	2.1	–	–	***
P (mg 100 g^{-1})	92.7	98.3	92.7	3.0	–	91.6	92.6	91.4	1.1	–	98.7	102.0	101.7	1.7	–	–	*
Na (mg 100 g^{-1})	36.6	39.5	36.8	1.1	–	41.3	41.6	40.5	0.36	–	55.0	58.2	56.9	2.2	–	–	***
Mg (mg 100 g^{-1})	10.5[b]	11.4[a]	11.4[a]	0.11	**	10.8	11.2	11.0	0.15	–	13.4	13.6	14.0	0.18	–	–	***
Zn (μg kg^{-1})	4086	4212	4467	171	–	3957	4066	3859	93.9	–	4300[b]	4521[ab]	4603[a]	119	*	–	*
Fe (μg kg^{-1})	332[ab]	371[a]	276[b]	19.1	*	304	375	323	50.7	–	295	262	274	24.9	–	–	–
Cu (μg kg^{-1})	98.3	117.9	101.0	7.6	–	72.1	78.6	81.3	4.9	–	42.3	48.9	48.7	7.2	–	–	***
Mo (μg kg^{-1})	29.6	31.2	33.9	2.67	–	45.4	46.2	45.0	1.4	–	44.5	44.8	45.8	3.5	–	–	**
Mn (μg kg^{-1})	30.9	33.9	29.1	2.1	–	34.5	37.3	35.9	3.2	–	33.8	34.3	32.0	1.5	–	–	–
Se (μg kg^{-1})	8.9	9.2	9.2	0.29	–	14.7	15.7	15.1	0.39	–	16.2	17.8	17.4	1.2	–	–	***
Co (μg kg^{-1})	0.75	0.71	0.83	0.03	–	0.52	0.71	0.60	0.01	–	0.88	0.74	0.80	0.02	–	–	*

[a] H-DHA, M-DHA and L-DHA denote high-, medium- and low- DHA, i.e., 15.0, 14.4 and 11.8 kg dry matter per cow, respectively. Early (EL)-, mid (ML)- and late (LL)-lactation correspond to March 16-April 19, April 27-August 10, and September 1-November 2, when cows were 29–70, 78–183, and 205–267 days in lactation, respectively. Values within a row relating to effect of DHA treatment in EL, ML or LL and not sharing a common lower-case superscripted letter differ significantly for effect of DHA; values within a row without a superscript do not differ for effect of DHA ($P > 0.05$). SED = standard error of difference between means; *P* values denote statistical significance, where ***, **, * and - denote $P < 0.001$, <0.01, <0.05 and >0.5, respectively. The statistical significance (*P*) for the effects of DHA in overall lactation, and lactation period (LP) across all DHA treatments are also shown.

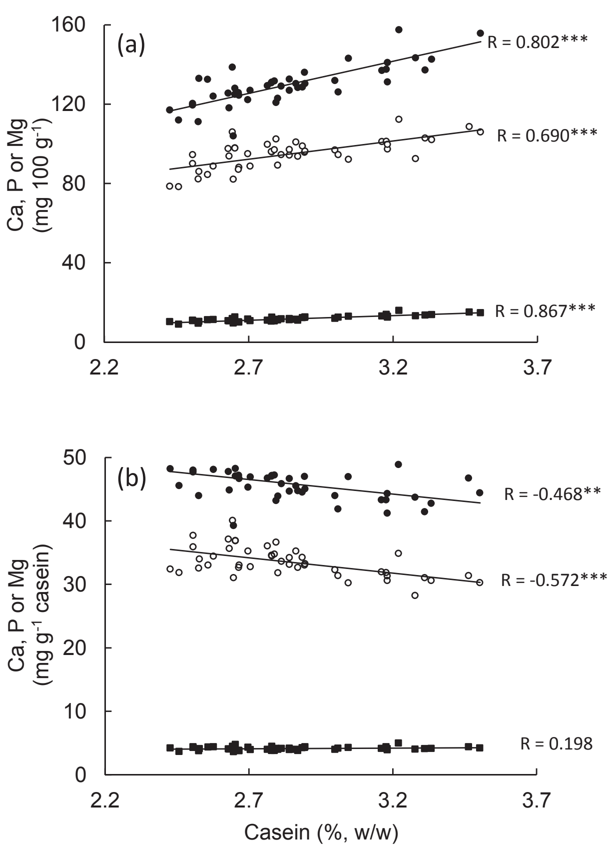

Fig. 1. Concentrations of: Ca (●), P (○) and Mg (■) (a), and the ratio of Ca- (●), P- (○) and Mg- (■) to casein (b), as a function of casein content of milk. The data are from 45 milk samples collected on 15 different occasions throughout the year from three spring-calved herds on different daily herbage allowance in early lactation. Linear regression lines (——) were fitted to the experimental data points. The regression coefficient (R) and significance of correlations are shown, where statistical probability is denoted by: ***, $P < 0.001$; **, $P < 0.01$.

3.3. Composition of milk serum

Reducing DHA from 14.4 (M-DHA) to 11.8 (L-DHA) kg DM per cow in EL led to a significant reduction in the concentration of protein in the serum (~0.13%, w/w) in EL milk, but otherwise had no effect on serum composition (soluble casein, casein profile) in EL, ML or LL milks (Table 1).

The concentration of protein and soluble casein in serum increased over lactation, concomitant with the increase in the concentrations of total protein, casein and whey protein in milk. Casein in serum, as a proportion of total casein in milk, increased significantly from a mean value of 5.3% in EL to 12.9% in EL; the range of values (3.4–13.9%) over lactation was broader than that (3.6–10.5%) reported by Lin et al. (2017b) for a mixed mixed-herd of spring- and autumn-calving cows over the year, but narrower than that (7–25% of total casein) found by Rose (1968) for fresh milk from individual cows and equilibrated at 35 °C prior to ultracentrifugation. The overall mean proportions of α_S-, β- and κ-caseins, as percentages of casein in serum, were ~26, 41 and 33, respectively, and were not influenced by DHA or lactation period (data not shown); the values are of similar magnitude to those reported by Lin et al. (2017b). A tentative explanation for the relatively high proportion of soluble casein in the LL milk is the reduction in ratio of Ca- and P-to-casein (Fig. 1b). Rose (1968) investigated the effects of incremental reduction in the colloidal calcium content of milk from 18.6 to 0.6 mM, and concluded that the calcium phosphate content of micelles and the polymerisation of temperature-sensitive caseins (Dalgleish & Law, 1988), especially β-casein, is the major factor controlling the level of intact casein in serum. High levels of casein in serum (>> 15% of total casein) are undesirable as they impair rennet gelation and curd syneresis, and reduce cheese yield (Ali, Andrews, & Cheeseman, 1980).

3.4. Rennet gelation

Rennet gelation is a key functional parameter of milk used for cheesemaking as it determines the rate at which the milk sets and the changes in gel strength (storage modulus, G′) and gel firming rate as a function of time from rennet addition. It influences the ability of the gel to withstand fracture during cutting and to synerese, and consequently the moisture content and quality of the cheese, the recovery of fat, and cheesemaking efficiency (Fox, Guinee, Cogan, & McSweeney, 2017). Alteration of DHA in EL had no effect on rennet coagulation time, (RCT), maximum gel firming rate (GFR_{max}) or gel firmness at 40 min (G'_{40}) during EL, ML or LL (Table 3). The lack of an effect of DHA on rennet coagulation

Table 3
Effect of reducing daily herbage allowance (DHA) during early lactation on rennet gelation and heat stability of skim milk in early-, mid- and late-lactation.[a]

Item	Effect of DHA treatment in different lactation periods															Overall effects throughout lactation	
	Early-lactation (EL)					Mid-lactation (ML)					Late-lactation (LL)					DHA	LP
	H-DHA	M-DHA	L-DHA	SED	*P*	H-DHA	M-DHA	L-DHA	SED	*P*	H-DHA	M-DHA	L-DHA	SED	*P*	*P*	*P*
Rennet gelation																	
RCT (min)	13.0	13.3	14.0	1.5	–	15.3	15.5	15.4	0.63	–	12.4	11.7	12.3	0.10	–	–	*
GFR_{max} (Pa s^{-1})	0.08	0.09	0.07	0.01	–	0.08	0.10	0.08	0.01	–	0.12	0.16	0.16	0.01	–	–	***
G'_{40} (Pa)	100.4	103.7	88.9	12.1	–	91.1	106.8	89.3	6.49	–	151.9	190.5	187.1	13.7	–	–	***
Heat coagulation time (HCT)																	
HCT_{npH}	13.1	13.7	13.4	0.54	–	17.0	16.3	18.2	1.57	–	14.0	15.2	20.3	1.4	–	–	–
HCT_{max}	13.8	14.9	13.5	0.22	–	14.0	15.4	18.1	0.46		14.6	16.2	17.0	0.50	–	–	–
HCT_{min}	4.7	4.9	4.7	0.62	–	5.3	6.6	5.5	1.1	–	5.2	5.7	4.4	0.79	–	–	**

[a] H-DHA, M-DHA and L-DHA denote high-, medium- and low- DHA, i.e., 15.0, 14.4 and 11.8 kg dry matter per cow, respectively. Early (EL)-, mid (ML)- and late (LL)-lactation correspond to March 16-April 19, April 27-August 10, and September 1-November 2, when cows were 29–70, 78–183, and 205–267 days in lactation, respectively. Values within a row relating to effect of DHA treatment in EL, ML or LL and not sharing a common lower-case superscripted letter differ significantly for effect of DHA; values within a row without a superscript do not differ for effect of DHA ($P > 0.05$). SED = standard error of difference between means; *P* values denote statistical significance, where ***, **, * and - denote $P < 0.001$, <0.01, <0.05 and >0.5, respectively. The statistical significance (*P*) for the effects of DHA in overall lactation, and lactation period (LP) across all DHA treatments are also shown. Abbreviations: RCT, rennet coagulation time; GFR_{max}, maximum gel firming rate; G'_{40}, gel firmness at 40 min; HCT_{npH}, HCT at natural pH; and HCT_{max} and HCT_{min} are the maximum and minimum heat coagulation times, respectively, of the HCT/pH (6·2–7·2) curve.

characteristics is consistent with the results of O'Brien et al. (1997) and is scarcely surprising based on the relatively small, or lack of, difference between the DHA treatments with respect to casein concentration in EL (maximum difference of 0.13%, w/w, in EL) (Auldist, Johnston, White, Fitzsimons, & Boland, 2004; O'Brien et al., 1997), individual caseins, soluble casein, and ratios of Ca- and P- to casein (Guinee et al., 1997; Horne & Lucey, 2017).

LL milk had enhanced coagulability, as evidenced by the lower value of RCT and higher values of GFR_{max} and G'_{40} relative to EL or ML milks (Table 3). The improved gelation characteristics of LL milk were most likely associated with the higher casein concentration, which correlated positively with GFR_{max} and G'_{40} (Table 4). Hence, the increase in casein concentration over lactation was sufficiently large to outweigh the slight, but significant, reductions in the ratios of Ca- and P- to casein and increase in the proportion of soluble casein, which are expected to impair rennet gelation (Ali et al., 1980; Fox et al., 2017). The levels of α_{S1}-, α_{S2}-, β-, and κ-caseins (as proportions of total casein) had no effect on rennet gelation properties. Conversely, Jõudu et al. (2008) found significant effects of the proportions of α_{S1}-, α_{S2}-, β-, and κ-caseins on the rennet gelation characteristics of individual milk samples from different breeds (Estonian Red, Red-and-White Holstein, Estonian Holstein) over a period of 1.5 year period. The inter-study discrepancy may relate to the magnitude of the changes in the proportions of individual caseins over the investigation periods, which were relatively small in current study (Table 1); these data were not presented by Jõudu et al. (2008).

3.5. Heat coagulation time

Heat coagulation time determines the stability of milk to high temperature treatment, as applied for example during the preparation of milk-based beverages (e.g., UHT milk, infant milk formula, and nutritional drinks), condensed milk and recombined milks (Sharma, Jana, & Chavan, 2012). Commercially, beverages are frequently prepared from skim milk concentrates which have a

Table 4
Significant relationships between milk composition and rennet gelation or heat stability characteristics.[a]

Processing characteristic	Compositional parameter	Correlation coefficient (r)
Rennet gelation		
RCT, rennet coagulation time	Casein (%, w/w)	−0.41**
GFR_{max}, maximum gel firming rate	Casein (%, w/w)	+0.84***
	Ca (mg 100 g^{-1})	+0.70***
	P (mg 100 g^{-1})	+0.62***
G'_{40}, gel firmness at 40 min	Casein (%, w/w)	+0.82***
	Ca (mg 100 g^{-1})	+0.72***
	P (mg 100 g^{-1})	+0.65***
Heat stability		
Maximum heat coagulation time, HCT_{max}	Lactose (%, w/w)	−0.44**
	Protein (%, w/w)	+0.55***
	NPN (%, w/w)	+0.35*
	Urea (mg 100 g^{-1})	+0.50***
	Soluble casein (%, w/w)	+0.40**
	Soluble casein (% total casein)	+0.37*
Heat coagulation time at natural pH, HCT_{npH}	Lactose (%, w/w)	−0.31*
	Protein (%, w/w)	+0.35*
	NPN (%, w/w)	+0.38*
	Urea (mg 100 g^{-1})	+0.42**
	Soluble casein (%, w/w)	+0.36*
	Soluble casein (% total casein)	+0.33*

[a] The data set comprised 45 milk samples collected on 15 different occasions throughout the year from three spring-calved herds on different daily herbage allowance in early lactation. Correlations were obtained using simple linear regression analysis; only relationships found to be statistically significant are shown: ***, $P < 0.001$; **, $P < 0{\cdot}01$; *, $P < 0{\cdot}05$. Positive and negative correlations between two parameters are indicated by a positive sign (+) and a negative sign (−), respectively.

lower pH than native milk which increases the susceptibility to heat-induced aggregation and destabilisation (Lin et al., 2018). Reducing DHA in EL had no effect on the heat stability characteristics of milk at 140 °C (HCT_{min}, HCT_{max} and HCT_{npH}) in EL, ML or LL. The absence of an effect of DHA is consistent with the similar values of lactose, urea, Ca, P and pH for each of the DHA treatments in EL, ML and LL (Holt, Muir, & Sweetsur, 1978; Huppertz, 2016); and the relatively small difference in protein content (0.22%, w/w) (Rattray & Jelen, 1996) between the treatments in EL.

While stage of lactation also had no effect on HCT_{min} or HCT_{npH}, the mean HCT_{max} across the three DHA treatments in EL was lower than that in ML or LL (Table 4). The results suggest that changes in the various compositional parameters during lactation have interactive effects on HCT, to an extent dependent on the magnitude of the change (Huppertz, 2016). Hence, while the relatively low concentration of lactose and high concentration of urea might be expected to enhance the HCT_{npH} of LL milk compared with ML- or EL-milk, such an increase may be offset by the higher protein concentration and lower pH of LL milk (Meena, Singh, Borad, & Panjagari, 2016). Regression analysis indicated that HCT_{max} and HCT_{npH} correlated positively with concentrations of urea, NPN, total protein and soluble casein, and proportions of κ- and α_{S1}- or α_{S2}-caseins, and negatively with lactose (Table 4). The positive effect of higher soluble casein on HCT_{max} and HCT_{npH} is analogous to the increase in HCT_{max} observed on increasing the proportion of soluble casein in milk by addition of NaCl (1.2%, w/w) or sodium caseinate (≥0.3%, w/w) (Lin, Kelly, O'Mahony, & Guinee, 2017a; Tessier & Rose, 1964).

4. Conclusions

Reducing DHA of a spring-calved herd from 15 to 11.8 kg DM per cow in EL (9 March – 19 April; 29–70 DIM) led to lower milk yield and concentrations of total protein and casein, but had little, or no, effect on other aspects of composition (e.g., concentrations of fat, lactose, non-protein N, urea, elements or proportions of individual caseins), rennet gelation or heat stability at pH values 6.2–7.2. Moreover, there was little, or no, impact of reducing DHA in EL on milk composition, rennet gelation or heat stability in ML (27 April – 31 August; 85–190 DIM), LL (20 September – 2 November; 210–275 DIM) or overall lactation (EL + ML + LL). The absence of an effect of lowering DHA in EL on most compositional parameters and processability characteristics (rennet gelation and heat stability) in EL, ML or LL suggests that restricted grazing without concentrate supplementation can, within limit, be applied in early lactation with little consequence apart from the lower yields of milk and milk solids during that period. This is of relevance to farm management where adverse weather in spring can reduce grass availability to cows, especially where stocking rate is high.

Acknowledgements

This study was funded by the Department of Agriculture, Food and the Marine (Dublin, Ireland) Stimulus fund (11/sf/309), and additionally supported by funding from the Dairy Levy Trust Co-Operative Society Limited (Dublin, Ireland). The authors would also like to thank Paula Reid (Teagasc Food Research Centre, Ashtown, Dublin, Ireland) for her advice on the statistical analysis, and Teagasc farm staff for assistance in milk collections.

References

Ali, A. E., Andrews, A. T., & Cheeseman, G. C. (1980). Influence of storage of milk on casein distribution between the micellar and soluble phases and its relationship to cheese-making parameters. *Journal of Dairy Research, 47*, 371–382.

Auldist, M. J., Greenwood, J. S., Wright, M. M., Hannah, M., Williams, R. P. W., Moate, P. J., et al. (2016). Incorporating mixed rations and formulated grain mixes into the diet of grazing cows: Effects on milk composition and coagulation properties, and the yield and quality of Cheddar cheese. *Journal of Dairy Science, 99*, 4196–4205.

Auldist, M., & Hubble, I. (1998). Effects of mastitis on raw milk and dairy products. *Australian Journal of Dairy Technology, 53*, 28–36.

Auldist, M. J., Johnston, K. A., White, N. J., Fitzsimons, W. P., & Boland, M. J. (2004). A comparison of the composition, coagulation characteristics and cheesemaking capacity of milk from Friesian and Jersey dairy cows. *Journal of Dairy Research, 71*, 51–57.

Auldist, M. J., Napper, A. R., & Kolver, E. S. (2000a). Contribution of nutrition to seasonal variation of milk composition in New Zealand Friesian and US Holstein dairy cows. *Asian Australasian Journal of Animal Sciences, 13*, 513–516.

Auldist, M. J., Thomson, N. A., Mackle, T. R., Hill, J. P., & Prosser, C. G. (2000b). Effects of pasture allowance on the yield and composition of milk from cows of different β-lactoglobulin phenotypes. *Journal of Dairy Science, 83*, 2069–2074.

Bargo, F., Muller, L. D., Delahoy, J. E., & Cassidy, T. W. (2002). Milk response to concentrate supplementation of high producing dairy cows grazing at two pasture allowances. *Journal of Dairy Science, 85*, 1777–1792.

Bobbo, T., Fiore, E., Gianesella, M., Morgante, M., Gallo, L., Ruegg, P., et al. (2017). Variation in blood serum proteins and association with somatic cell count in dairy cattle from multi-breed herds. *Animal, 11*, 2309–2319.

Chen, B., Grandison, A. S., & Lewis, M. J. (2017). Best use for milk–A review. II–Effect of physiological, husbandry and seasonal factors on the physicochemical properties of bovine milk. *International Journal of Dairy Technology, 70*, 155–164.

Chen, B., Lewis, M. J., & Grandison, A. S. (2014). Effect of seasonal variation on the composition and properties of raw milk destined for processing in the UK. *Food Chemistry, 158*, 216–223.

Dalgleish, D. G., & Law, A. J. (1988). pH-induced dissociation of bovine casein micelles. I. Analysis of liberated caseins. *Journal of Dairy Research, 55*, 529–538.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2017). *Fundamentals of cheese science*. New York, NY, USA: Springer- Science+ Business Media.

Fox, P. F., Uniacke-Lowe, T., McSweeney, P. L. H., & O'Mahony, J. A. (2015). *Dairy chemistry and biochemistry*. Geneva, Switzerland: Springer- Science+ Business Media.

Gaucheron, F. (2013). Milk minerals, trace elements, and macroelements. In Y. W. Park, & G. F. W. Haenlein (Eds.), *Milk and dairy products in human nutrition: Production, composition and health* (pp. 172–199). Chichester, UK: Wiley-Blackwell.

Guinee, T. P., Gorry, C. B., O'Callaghan, D. J., O'Kennedy, B. T., O'Brien, B., & Fenelon, M. A. (1997). The effects of composition and some processing treatments on the rennet coagulation properties of milk. *International Journal of Dairy Technology, 50*, 99–106.

Gulati, A., Galvin, N., Lewis, E., Hennessy, D., O'Donovan, M., McManus, J. J., et al. (2018). Outdoor grazing of dairy cows on pasture versus indoor feeding on total mixed ration: Effects on gross composition and mineral content of milk during lactation. *Journal of Dairy Science, 101*, 2710–2723.

Holt, C., Muir, D. D., & Sweetsur, A. M. (1978). Seasonal changes in the heat stability of milk from creamery silos in south-west Scotland. *Journal of Dairy Research, 45*, 183–190.

Horne, D., & Lucey, J. A. (2017). Rennet-induced coagulation of milk. In P. L. H. McSweeney, P. F. Fox, P. D. Cotter, & D. W. Everett (Eds.), *Cheese chemistry, physics and microbiology – general aspects* (4th edn., Vol. 1, pp. 115–143). London, UK: Elsevier.

Huppertz, T. (2016). Heat stability of milk. In P. L. H. McSweeney, & J. A. O'Mahony (Eds.), *Advanced dairy chemistry. Vol 1B. Proteins: Applied aspects* (4th edn., pp. 179–196). New York, NY, USA: Springer- Science+ Business Media.

Jõudu, I., Henno, M., Kaart, T., Püssa, T., & Kärt, O. (2008). The effect of milk protein contents on the rennet coagulation properties of milk from individual dairy cows. *International Dairy Journal, 18*, 964–967.

Kennedy, E., Galvin, N., & Lewis, E. (2015). Early lactation challenge – feeding the dairy cow. In *Irish dairying – sustainable expansion (1 July, pp. 42–43)*. Moorepark, Fermoy, Ireland: Teagasc, Animal & Grassland Research and Innovation Centre.

Kennedy, E., O'Donovan, M., O'Mara, F., Murphy, J., & Delaby, L. (2007). The effect of early-lactation feeding strategy on the lactation performance of spring-calving dairy cows. *Journal of Dairy Science, 90*, 3060–3070.

Lewis, E., O'Donovan, M., Kennedy, E., O'Neill, B., & Shalloo, L. (2011). Feeding the dairy cow in spring: Supplementation requirements and responses. In *National dairy conference – the Irish dairy industry – to 2015 and beyond* (pp. 71–83). Last accessed on 13th July 2015. Available online at: https://www.teagasc.ie/media/website/publications/2011/Dairy_Conference_Proceedings_2011.pdf.

Lin, Y., Kelly, A. L., O'Mahony, J. A., & Guinee, T. P. (2017a). Addition of sodium caseinate to skim milk increases nonsedimentable casein and causes significant changes in rennet-induced gelation, heat stability, and ethanol stability. *Journal of Dairy Science, 100*, 908–918.

Lin, Y., Kelly, A. L., O'Mahony, J. A., & Guinee, T. P. (2018). Effect of heat treatment, evaporation and spray drying during skim milk powder manufacture on the compositional and processing characteristics of reconstituted skim milk and concentrate. *International Dairy Journal, 78*, 53–64.

Lin, Y., O'Mahony, J. A., Kelly, A. L., & Guinee, T. P. (2017b). Seasonal variation in the composition and processing characteristics of herd milk with varying proportions of milk from spring-calving and autumn-calving cows. *Journal of Dairy Research, 84*, 1–9.

Lucey, J. A., & Horne, D. S. (2018). Perspectives on casein interactions. *International Dairy Journal, 85*, 56–65.

Mackle, T. R., Bryant, A. M., Petch, S. F., Hooper, R. J., & Auldist, M. J. (1999). Variation in the composition of milk protein from pasture-fed dairy cows in late lactation and the effect of grain and silage supplementation. *New Zealand Journal of Agricultural Research, 42*, 147–154.
McEvoy, M., Kennedy, E., Murphy, J. P., Boland, T. M., Delaby, L., & O'Donovan, M. (2008). The effect of herbage allowance and concentrate supplementation on milk production performance and dry matter intake of spring-calving dairy cows in early lactation. *Journal of Dairy Science, 91*, 1258–1269.
Meena, G. S., Singh, A. K., Borad, S., & Panjagari, N. R. (2016). Effect of concentration, homogenization and stabilizing salts on heat stability and rheological properties of cow skim milk ultrafiltered retentate. *Journal of Food Science and Technology, 53*, 3960–3968.
O'Brien, B., & Hennessy, D. (2017). Scientific appraisal of the Irish grass-based milk production system as a sustainable source of premium quality milk and dairy products. *Irish Journal of Agricultural and Food Research, 56*, 120–129.
O'Brien, B., Murphy, J. J., Connolly, J. F., Mehra, R., Guinee, T. P., & Stakelum, G. (1997). Effect of altering the daily herbage allowance in mid lactation on the composition and processing characteristics of bovine milk. *Journal of Dairy Research, 64*, 621–626.
R Core Team. (2014). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.R-project.org/.
Rattray, W., & Jelen, P. (1996). Thermal stability of skim milk with protein content standardized by the addition of ultrafiltration permeates. *International Dairy Journal, 6*, 157–170.
Roche, J. R. (2007). Milk production responses to pre- and postclaving dry matter intake in grazing cows. *Livestock Science, 110*, 12–24.
Rose, D. (1968). Relation between micellar and serum casein in bovine milk. *Journal of Dairy Science, 51*, 1897–1902.
SAS Institute. (2011). *SAS user's guide: Statistics. Version 9* (3rd edn.). Cary, NC, USA: SAS Institute Inc.
Sharma, A., Jana, A. H., & Chavan, R. S. (2012). Functionality of milk powders and milk-based powders for end use applications-a review. *Comprehensive Reviews in Food Science and Food Safety, 11*, 518–528.
Tessier, H., & Rose, D. (1964). Influence of κ-casein and β-lactoglobulin on the heat stability of skimmilk1. *Journal of Dairy Science, 47*, 1047–1051.
Tsioulpas, A., Lewis, M. J., & Grandison, A. S. (2007). Effect of minerals on casein micelle stability of cows' milk. *Journal of Dairy Research, 74*, 167–173.
Vegarud, G. E., Langsrud, T., & Svenning, C. (2000). Mineral-binding milk proteins and peptides; occurrence, biochemical and technological characteristics. *British Journal of Nutrition, 84*, 91–98.
Wedholm, A., Larsen, L. B., Lindmark-Månsson, H., Karlsson, A. H., & Andrén, A. (2006). Effect of protein composition on the cheese-making properties of milk from individual dairy cows. *Journal of Dairy Science, 89*, 3296–3305.