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Dairy cow feeding system alters the characteristics of low-heat skim milk powder and processability of reconstituted skim milk

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ABSTRACT

Low-heat skim milk powder (LHSMP) was manufactured on 3 separate occasions in mid lactation (ML, July 4–20) and late lactation (LL, September 27 to October 7) from bulk milk of 3 spring-calving dairy herds on different feeding systems: grazing on perennial ryegrass (*Lolium perenne* L.) pasture (GRO), grazing on perennial ryegrass and white clover (*Trifolium repens* L.) pasture (GRC), and housed indoors and offered total mixed ration (TMR). The resultant powders (GRO-SMP, GRC-SMP, and TMR-SMP) were evaluated for composition and color and for the compositional, physicochemical, and processing characteristics of the reconstituted skim milk (RSM) prepared by dispersing the powders to 10% (wt/wt) in water. Feeding system significantly affected the contents of protein and lactose, the elemental composition, and the color of the LHSMP, as well as the rennet gelation properties of the RSM. The GRO and GRC powders had a higher protein content; lower levels of lactose, iodine, and selenium; and a more yellow-green color (lower a* and higher b* color coordinates) than TMR powder. On reconstitution, the GRO-RSM had higher concentrations of protein, casein, and ionic calcium, and lower concentrations of lactose and nonprotein nitrogen (% of total N). It also produced rennet gels with a higher storage modulus (G′) than the corresponding TMR-RSM. These effects were observed over the combined ML and LL period but varied somewhat during the separate ML and LL periods. Otherwise, feeding system had little or no effect on proportions of individual caseins, concentration of serum casein, casein micelle size, casein hydration, heat coagulation time, or ethanol stability of the RSM at pH 6.2 to 7.2, or on the water-holding capacity, viscosity, and flow behavior of stirred yogurt prepared by starter-induced acidification of RSM. The differences in the functionality of the LHSMP may be of greater or lesser importance depending on the application and the conditions applied during the processing of the RSM.
Key words: pasture, total mixed ration, skim milk powder, processability

INTRODUCTION

Skim milk powder (**SMP**), also referred to as nonfat dry milk, is used extensively as an ingredient in the manufacture of dairy-based beverages and formulated food products (e.g., coffee creamers, ice cream, dairy-based desserts, sauces, soups, processed cheese products, bakery products). Depending on the application and functionalities required, the SMP may be low, medium, or high heat, based on the heat treatment of the skim milk before evaporation and drying. Typical heat treatments for low-, medium-, and high-heat SMP are 70 to 72°C for 15 s, 85°C for 60 s, and 120°C for 60 to 120 s or 90°C for 100 to 300 s, respectively (Patel et al., 2007). Low-heat SMP (**LHSMP**) is preferred for preparing recombined milk for cheese manufacture or for standardizing the content of milk protein or solids in products such as cheese milk, yogurt, and fermented milk products (Kelly and Fox, 2016).

Because of seasonal changes in milk composition (Mehra et al., 1999; O'Brien et al., 1999; Auldist et al., 2000), the composition and functionality (e.g., rennet gelation, heat stability) of LHSMP is likely to vary across the production season. The extent of seasonal variation in milk composition (e.g., concentrations and relative proportions of protein, casein, lactose, Ca) depends on many factors, including stage of lactation, herd calving pattern, diet, health, and weather. The changes are amplified when the milk supply is largely from spring-calved pasture-fed herds (O'Brien et al., 1999; O'Callaghan et al., 2016), for which the effect of stage of lactation on animal physiology and milk biosynthesis is most pronounced. Most notably, the

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concentrations of casein and protein increase during lactation, whereas that of lactose decreases (Holt and Jenness, 1984). Based on recent data from O'Callaghan et al. (2016) for a spring-calved herd milk, the concentrations of total protein and casein increased from 3.4 and 2.7% (wt/wt), respectively, in March to 4.1 and 3.5% (wt/wt), respectively, in October. Simultaneously, the concentration of lactose decreased from 5.02 to 4.70% (wt/wt) over the same period (O'Callaghan et al., 2016).

Dehydration of skim milk to a powder with specified moisture content (i.e., ≤5% wt/wt; FAO/WHO, 1999; FDA, 2018) removes the effect of seasonality-associated differences in the TS content of milk on the TS content of the powder or the reconstituted skim milk (**RSM**) prepared by dispersing and dissolving the powder on a given weight basis (e.g., 10% wt/wt). Nevertheless, seasonal variations in the ratios of individual constituents (e.g., lactose, protein, calcium phosphate, urea, Ca^{2+}) in skim milk are not affected by drying; hence, they also occur in the RSM. Such variations influence the processing behavior of RSM owing to their effects on buffering capacity, degree of heat-induced acidity, or susceptibility of protein to aggregation (Pouliot and Boulet, 1991; Rattray and Jelen, 1996; Sikand et al., 2010). Much information is available on the effects of season and cow diet on the processing characteristics of milk, such as rennet coagulability (O'Brien et al., 1999; Guinee et al., 2001), heat stability (Holt et al., 1978; Kelly, 1982; Banks et al., 1984), and ethanol stability (**ES**; O'Brien et al., 1997; Horne et al., 1986; Chen et al., 2014). In contrast, fewer studies have investigated seasonal changes in the composition of SMP or its functionality or processing behavior upon reconstitution to RSM. Most of the published work thus far has focused on the heat stability of RSM concentrates (which are also standardized to a fixed TS content) because of the large-scale use of SMP in the formulation of heat-treated beverages.

Kelly (1982) found that the maximum heat coagulation time ($\mathbf{HCT_{max}}$) of RSM concentrate (20% TS) from medium-heat SMP at 120°C increased from approximately 22 min in February to approximately 70 to 80 min in May, remained relatively constant between May and October, and decreased thereafter to approximately 5 to 10 min in December. The low HCT at the extremes of lactation (November–February) may be due to the incidence of subclinical mastitis in the dairy herds and prolonged storage at low temperature. In contrast, Pouliot and Boulet (1991) observed that the HCT of RSM concentrates (~31% TS) at native pH and 121°C varied little (7–10 min) over the year, and no distinct period of instability was evident. Differences in the quantities of Na_2HPO_4 required to attain maximum HCT at pH 6.7 varied due to differences in natural pH and buffering capacity. Cheng et al. (2002) reported significant seasonal variations in the consistency and syneresis of set and stirred yogurts made from RSM (10–14% wt/wt TS), prepared by dissolving SMP to a constant TS content. Viscosity of stirred yogurt and gel strength of set yogurt was positively correlated with protein concentration (3.4–5.7% wt/wt) and negatively correlated with the concentration of inorganic phosphate in the RSM, while syneresis decreased with protein content of the RSM. However, the relationship between viscosity and casein content differed for the RSM with 10, 12, and 14% (wt/wt) TS (Cheng et al., 2002), suggesting that the role of protein functionality on yogurt viscosity was modulated by other components in the milk. We are unaware of any studies on the effect of diet/feeding system or lactation on the rennet coagulability or ES of LHSMP.

The objective of the current study was to compare pasture- and TMR-based feeding systems for their effects on composition of LHSMP manufactured in mid lactation (**ML**) or late lactation (**LL**). We also investigated the HCT, ES, and rennet coagulability on reconstitution to 10% (wt/wt), and the properties of reduced-fat (2.3% wt/wt) stirred yogurt on reconstitution to 12.7% (wt/wt).

MATERIALS AND METHODS

Feeding Systems and Milk Collection

Sixty spring-calved dairy cows from the institute's herd, with a mean calving date of February 19, 2016, were allocated to 1 of 3 feeding systems: grazing on perennial ryegrass (*Lolium perenne* L.) pasture (**GRO**), grazing on perennial ryegrass and white clover (*Trifolium repens* L.) pasture (**GRC**), or housed indoors and offered a TMR . The details of the management of the dairy herds and feeding systems have previously been described (O'Callaghan et al., 2016; Gulati et al., 2018a,b). Each herd comprised 20 cows and was balanced for breed (16 Holstein-Friesian, 4 Holstein-Friesian × Jersey), lactation number (4 primiparous, 16 multiparous), calving date, and 2-wk pre-experimental milk yield and milk solids yield. The daily feed DM allocation for the GRO, GRC, and TMR herds was 18, 18, and 22.6 kg/cow, respectively.

Evening and morning milks from each of the 3 herds on the GRO, GRC, or TMR feeding systems were collected separately in designated refrigerated tanks. Following sufficient mixing of morning and evening milks (~1.5 h), a representative 150-L sample of milk from each herd was collected for analysis and manufacture of LHSMP. Low-heat SMP was manufactured on 3

separate occasions from each of the herd-milks in ML (July 4 to July 20, 137–153 d in lactation, **DIL**) and LL (September 27 to October 7, when cows were 222–232 DIL).

Manufacture of LHSMP

Low-heat SMP were produced in the Bio-functional Food Engineering pilot plant (Teagasc, Moorepark, Fermoy, Co. Cork). Milk was separated at 55°C (Westfalia model MM1254 separator, Westphalia, Germany), pasteurized at 72°C for 15 s using a pilot-scale tubular heat-exchanger (MicroThermics UHT/HTST Lab-25 EHVH, Raleigh, NC), cooled to 45°C, and concentrated to approximately 45 to 46% TS in a Falling Film Evaporator (Anhydro, Type F, SPX Flow Technology Denmark A/S, Søborg, Denmark). The concentrate was spray dried (Anhydro spray dryer, SPX Flow Technology Denmark A/S) using centrifugal disc atomization with inlet and outlet air temperatures of 180°C and 85°C, respectively. Powders were packed in silver aluminum bags and stored at 15°C until they were used for further analysis. Low-heat SMP produced from GRO, GRC, or TMR milks are denoted GRO, GRC, and TMR powders, respectively.

Composition of Skim Milk and LHSMP

Skim milk was analyzed for TS, lactose, and protein using the FOSS MilkoScan FT+ analyzer (Foss Electric A/S, Hillerød, Denmark). Low-heat SMP was analyzed for TS and fat using CEM SMART Trac II (CEM, Matthews, NC), protein by the Kjeldahl method (International Organization for Standardization, 2014), and whey protein nitrogen index (American Dairy Products Institute, 2016). Lactose, casein, and whey protein were measured in RSM prepared by dispersing the LHSMP to 10% (wt/wt) in distilled water, as described below. Samples (~0.2 g) were assayed for macroelements (Ca, P, K, Na, and Mg) and trace elements (S, Zn, Fe, I, Mn, Cu, Mo, and Se) using inductively coupled plasma mass spectrometry, as described by Gulati et al. (2018 a, b).

Color of LHSMP

The color characteristics of the LHSMP were evaluated by measuring the color-space coordinates (L*, lightness; a*, red-green color; and b*, yellow-blue color values), using a CR-400 Chroma Meter (Konica Minolta, Osaka, Japan) that had been calibrated using the Minolta calibration plate. Powder samples (15 g) were placed in a Petri dish and leveled before measurement in quadruplicate. The L* value varying from 0 (black) to 100 (white) was an index of lightness, whereas a* and b* values represented the variation and intensity in color from green (− values) to red (+ values) and from blue (− values) to yellow (+ values), respectively.

Preparation of RSM and Milk Serum

Reconstituted skim milk (10% wt/wt) was prepared by dispersing LHSMP in distilled water at 50°C and holding in a water bath (50°C), with stirring at 400 rpm for 2 h. The milk was then dispersed in 1-L glass containers (DURAN, Mainz, Germany) and stored at 4°C for 18 h to allow hydration of the proteins. Before all analyses, the RSM was heated to 40°C in a thermostatically controlled water bath (Grant, Cambridgeshire, UK) with stirring (Variomag-USA, Port Orange, FL), held for 30 min to reverse the cold-aging, and cooled to 21°C for analysis. Reconstituted skim milk prepared from GRO, GRC, or TMR powders are denoted GRO-, GRC-, and TMR-RSM, respectively.

Milk serum was prepared by ultracentrifugation at 100,000 × g at 25°C for 1 h (Sorvall Discovery 90SE ultracentrifuge, Kendro Laboratory Products, Asheville, NC). The supernatant was filtered through superfine glass wool (11-μm pore size; VWR International, Dublin, Ireland) to obtain fat-free serum, which was preserved with sodium azide (0.02% wt/wt) and stored at 4°C.

Compositional Analysis of RSM and Milk Serum

Reconstituted skim milk was analyzed for TS, lactose, and urea using the FOSS MilkoScanTM FT+ analyzer (N. Foss Electric A/S). Total nitrogen (**TN**), NPN, and noncasein nitrogen (**NCN**; including whey proteins and NPN) in the skim milk were measured using standard ISO methods (ISO, 2001, 2004, 2014). Casein number, which corresponds to casein N as a percentage of TN, was calculated as follows: Casein number = 100 − NCN (% total N). The concentrations of total protein and noncasein protein were calculated from TN and NCN by multiplying them by the nitrogen-to-protein conversion factor, 6.38. The concentrations of whey protein and casein in RSM were calculated using the following relationships: whey protein = 6.38(NCN − NPN); casein = 6.38(TN − NCN). Milk serum was assayed for TN and NCN, as described above, to obtain serum (soluble) casein.

The proportions of individual casein (α_{S1}-, α_{S2}-, β-, and κ-CN) and whey proteins (β-LG, α-LA) in milk were measured using reverse-phase (**RP**)_HPLC (Agilent 1200 series, Agilent Technologies, Santa Clara, CA) using a 300 SB-CIS RP poroshell column (Agilent Technologies), as described by Lin et al. (2016). The samples were diluted in buffer containing 7 M urea,

0.02 *M* bis-tris propane, and 0.5% (vol/vol) 2-mercaptoethanol before injection. The volume ratio of RSM to the dissociating buffer was 1:20, and the sample volume injected onto the column was 10 μL. Samples were analyzed in duplicate. Protein standards used for calibration of the RP-HPLC assay included κ-CN (0.5–2.5 μg), α_{S1}-CN (0.5–2.5 μg), α_{S2}-CN (0.072–0.288 μg), β-CN (0.5–2.5 μg), α-LA (0.1–0.5 μg), β-LG a (0.250–1.250 μg), and β-LG b (0.250–1.250 μg).

The concentration of ionic Ca ($[Ca^{2+}]$) in RSM was measured in triplicate at room temperature using a Ca ion–selective electrode (sensION+ 9660C, Hach Co., Loveland, CO), as described by Chen et al. (2014). The electrode was calibrated using $CaCl_2$ solutions (0–5 m*M*), and a logarithmic relationship existed between the electrical output (mV) from the electrode and $[Ca^{2+}]$. Potassium chloride (3 *M*) was added to the RSM and to the standard solutions at a level of 1% (vol/vol) to attain similar ionic strength.

Physicochemical Characteristics of RSM

The mean casein micelle size (**CMS**) of RSM diluted (1:100, vol/vol) in simulated skim milk ultrafiltrate (Jenness and Knoops, 1962) to give a protein concentration of approximately 0.035 to 0.050% (wt/wt), expressed as z-average hydrodynamic diameter (nm), was determined using a Malvern Zetasizer Nanoseries Nano-ZS (Malvern Instruments Ltd., Malvern, UK), as described by Lin et al. (2016).

Casein micelle hydration was measured by lyophilization of the pellet (FreeZone Freeze Dry Systems, Labconco, Kansas City, MO) obtained on ultracentrifugation of the milk at 100,000 × *g* for 1 h at 25°C, and expressed as grams of water per gram of sedimented casein (Lin et al., 2016).

Heat Coagulation Time and Ethanol Stability of RSM

Subsamples of RSM were adjusted to pH values in the range 6.2 to 7.2, at increments of 0.1 pH unit, at room temperature. The HCT of the pH-adjusted samples and a sample at natural pH were measured at 140°C, as described by Lin et al. (2016). The following heat coagulation 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}, and HCT at second inflection point.

Ethanol stability was measured for skim milk samples, adjusted to pH values in the range of 6.2 to 7.0 at 0.2 incremental pH units at 21°C. The pH-adjusted samples were blended with aqueous ethanol solutions ranging in concentration from 30 to 98% (vol/vol) and keeping the ethanol-to-protein ratio constant, based on milk-to-ethanol volume ratio of 1:2 for milk with 3.4% protein. The blend was agitated for 30 s (Whirlimixer, Fisons, Holmes Chapel, UK) and observed for the formation of visible flocs. The ES was recorded as the minimum concentration of aqueous ethanol solution required to induce flocculation.

Rennet Gelation of RSM

Skim milk was adjusted to pH 6.55 at room temperature using 0.1 *M* HCl, heated to 31°C, and inoculated with chymosin (ChyMax plus, 200 IMCU/mL; Chr. Hansen, Hørsholm, Denmark) at a rate of 10.6 IMCU per gram of protein. The chymosin was diluted 20-fold in distilled water immediately before inoculation and mixed with the skim milk for 60 s. The rennet-treated sample was placed in the cell of a controlled stress rheometer (CSL^2_{500} Carri-Med; TA Instruments, Inc., New Castle, DE) and the storage modulus G′ (index of gel stiffness) was measured as a function of time at a strain of 0.025 and frequency of 1 Hz (Lin et al., 2016). The following parameters were calculated from the resultant G′/time curve: rennet coagulation time, defined as the time required for G′ to reach a threshold value of 0.2 Pa; GFR_{max}, maximum gel firming rate, calculated as the maximum slope of the G′/time curve; and G'_{40}, G′ at 40 min from rennet addition.

Model Stirred Yogurt Preparation and Gel Formation from RSM

Low-heat SMP was dispersed to 12.7% (wt/wt) TS in distilled water at 50°C, with continual stirring at 5,000 rpm (Silverson model L4RT, Silverson, Chesham, UK) for 15 min, stored at 4°C overnight, and heated to 50°C. The constituted skim milk (10 L) was then blended with heated-treated (90°C) anhydrous milk fat (Glanbia, Kilkenny, Ireland), added at a level required to give 2.3% (wt/wt), and agitated at 5,000 rpm for 2 min (Silverson model L4RT, Silverson). The recombined milk was heat treated at 95°C for 5 min, homogenized at first- and second-stage pressures of 15 and 5 MPa, respectively, and cooled to 43°C (MicroThermics UHT/HTSTLab-25 EHVH). A portion (2 L) of the homogenized, heated milk was inoculated with direct-vat starter culture from Chr. Hansen Ireland Ltd. (Little Island, Co. Cork, Ireland), composed of a blend of YC380 YoFlex (*Streptococcus thermophilus*) and CH1 YoFlex (*Lactobacillus delbrueckii* ssp. *bulgaricus*) at a weight ratio of 1:3. The weight of starter culture inoculum was standardized to a level 0.01% (wt/wt) for milk with 5% protein and was varied accordingly as the protein content of the milk varied with treatment

or lactation period. The inoculated milk was incubated at 42°C (Heratherm Advance Protocol Microbiological Incubators, Thermo Scientific, Waltham, MA) until the pH reached 4.6 and the milk gelled. After starter culture inoculation, a well-mixed subsample (10 mL) of the dispersion was immediately withdrawn and monitored for changes in storage modulus (G′), loss modulus (G″), and loss tangent (tan δ = G″/G′) at 42°C, using low-amplitude strain oscillation rheometry as described for rennet gelation, until pH dropped to 4.6 (Lin et al., 2018b). Moisture evaporation during measurement was prevented by placing a thin layer of tetradecane (Sigma-Aldrich, St. Louis, MO) on the surface and covering the sample with an evaporation blocker.

The gelation-onset pH ($\mathbf{GO_{pH}}$) was defined as the pH at which tan δ decreased to 1. When the pH decreased to 4.6, the yogurt gel was placed in ice water, cooled to ~8°C and stirred at 70 rpm (model RW16; IKA-Werke GmbH & Co., Staufen im Breisgau, Germany), and stored at 4°C for 36 h before analysis.

Rheological Properties of Yogurt. Yogurt was stirred at 70 rpm for 1 min at room temperature (model RW16; IKA-Werke GmbH & Co.) to ensure sample homogeneity before rheological measurement. A 10-g sample was placed in the measuring cell of a controlled-stress rheometer (CSL^2_{500} Carri-Med; TA Instruments, Inc.). The sample was equilibrated at 8°C for 5 min and then subjected to a shear rate ($\dot{\gamma}$) sweep, in which the shear rate was increased from 10 to 120 s^{-1}. Shear stress (σ; Pa) and viscosity (Pa·s) were measured as a function of shear rate, as described by Lin et al. (2018b). The resultant shear rate versus shear stress data were fitted to the Herschel–Bulkley model using TA Rheology Advance Data Analysis software (version V5.7.0; TA Instruments):

$$\sigma = \sigma_o + K\dot{\gamma}^n,$$

where σ_o is the yield stress (Pa), K is the consistency coefficient (Pa/s), and n represents the flow behavior index.

Water-Holding Capacity of Yogurt. Immediately after the yogurt samples were cooled to 8°C, 6 subsamples of each yogurt were immediately poured into 50-mL screw-cap centrifuge tubes, held at 4°C for 36 h, and centrifuged at 300 × g at 8°C for 30 min. The expressed serum was decanted and weighed. The water-holding capacity (**WHC**) of the yogurt was calculated as the difference between total serum in yogurt (moisture, fat, lactose, NPN expressed as protein, undenatured whey protein) and the serum expressed on centrifugation per 100 g of yogurt.

Statistical Analysis

Low-heat SMP was manufactured on 3 separate occasions in both ML (137–153 DIL) and LL (222–232 DIL) from milk obtained from each of the feeding systems (GRO, GRC, and TMR). The data were classified according to feeding system and lactation period, as a factorial design, and analyzed using ANOVA for the effects of feeding system, lactation period, and their interaction. The data for ML and LL were also analyzed using one-way ANOVA. Analysis of variance was performed using the general linear model (GLM) procedure of SAS 9.3 (SAS Institute Inc., Cary, NC), and Tukey's multiple-comparison test was used for paired comparison of treatment means; the level of significance was determined at $P < 0.05$.

In the analysis for the effect of lactation period, ML milk refers to the composite of the milks from the herds on the GRO, GRC, and TMR feeding systems in mid lactation, and LL milk to the composite of the corresponding milks in late lactation.

The R-3.2.2 software (R Core Team, 2014) was used to compute Pearson correlation between different compositional parameters, where significance difference was determined at $P < 0.05$, $P < 0.01$, and $P < 0.001$ according to Student's t-test.

RESULTS AND DISCUSSION

Composition of Skim Milk

The gross composition of the nonpasteurized skim milk used for LHSMP manufacture was affected by feeding system and lactation period (Table 1). Compared with GRO and GRC milks, TMR milk had a significantly lower mean concentration of protein and higher concentration of lactose during overall lactation (ML+LL; $P < 0.01$); nevertheless, the effect of feeding system on composition was influenced to a greater, or lesser, degree by lactation period.

Lactation period had a significant effect on skim milk composition, with LL milk having higher protein and lower lactose concentrations than ML milk. The overall effects of feeding system and lactation period on gross composition of raw milk are similar to those reported previously for milk from spring-calved herds (Auldist et al., 2000; O'Callaghan et al., 2016; Gulati et al., 2018a,b).

Composition of LHSMP

The total protein and lactose contents in LHSMP were affected by feeding system and to an extent depen-

Table 1. Composition of skim milk from dairy herds on different feeding systems in mid- and late lactation[1]

Item	Feeding systems[2,3]							Probability values for overall effects[4]		
	Mid lactation (ML)				Late lactation (LL)					
	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation period (LP)	Interaction FS × LP
Total solids (%, wt/wt)	9.43[a]	9.35[a]	9.17[b]	0.017	9.47	9.35	9.34	0.048	0.022	0.205	0.375
Lactose (%, wt/wt)	5.02[b]	5.07[b]	5.11[a]	0.016	4.72[b]	4.77[ab]	4.91[a]	0.027	0.040	<0.0001	0.540
Protein (%, wt/wt)	3.66[a]	3.66[a]	3.40[b]	0.022	4.05[a]	3.94[b]	3.78[c]	0.016	<0.0001	<0.0001	0.125

[a–c]Values within a row relating to mid- or late lactation and not sharing a common lowercase superscripted letter differed significantly ($P < 0.05$) for the effect of feeding system, whereas values without a superscript did not ($P > 0.05$).

[1]Presented data for the different feeding systems are the means of 3 replicate trials in mid- and late lactation; SED = standard error of difference between means.

[2]GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration.

[3]Mid lactation = July 4–20 (137–153 DIL); late lactation = September 27 to October 7 (222–232 DIL). DIL = days in lactation.

[4]*P*-values for the effects of feeding system in overall lactation (ML+LL), lactation period (ML or LL) across the different feeding systems, and their interaction.

dent on lactation period (Table 2). The protein content in ML, LL, and overall lactation (ML+LL) decreased in the following order, GRO > GRC > TMR. The lactose content of TMR powder was higher than of GRO or GRC powder in LL and ML+LL. Linear regression analysis of the data for all powders in ML and LL indicated a significant inverse relationship between lactose and protein content (df = 16; r = 0.93), with lactose decreasing by approximately 1% (wt/wt) for every increase in 0.6% (wt/wt) protein content. Otherwise, feeding system did not affect the contents of TS or fat or the level of undenatured whey protein, as evidenced by the similar whey protein nitrogen index values. The results generally concur with those of studies using the same feeding systems, which found that TMR-based milk has lower protein and higher lactose content than GRO-based milk (Auldist et al., 2000; O'Callaghan et al., 2016; Gulati et al., 2018a,b). Apart from increasing the content of nonfat substances, the evaporation and drying processes during manufacture of LHSMP have little or no effect on the relative proportions of the different components of milk or denaturation of whey protein (Lin et al., 2018a).

Lactation period had a significant effect on powder composition, with LL powders having higher protein and lower lactose contents than ML powders. The trends for protein and lactose are consistent with those of previous studies for the effects of lactation period on the composition of milk from pasture-fed spring-calved herds over a period of approximately 15 to 250 DIL (Auldist et al., 2000; O'Callaghan et al., 2016; Gulati et al., 2018b); however, in more advanced lactation (e.g., ≫250 DIL), the protein and casein contents of milk have been found to decrease (Mehra et al., 1999; Guinee et al., 2007) to an extent dependent on management practice and the type and level of dietary supplementation (O'Brien et al., 1996).

Elemental Composition of LHSMP

Considering that the solids in skim milk underwent a 10.3-fold concentration during the manufacture of LHSMP, the contents of individual elements in all powders (Table 2) were consistent with the range of values reported for milk or skim milk from pasture-fed spring-calved herds (O'Brien et al., 2013; Gulati et al., 2018a, b). The levels of macroelements (K, Na, S) and trace elements (Zn, Fe, I, Cu, Mo, and Se) were affected to a degree dependent on feeding system and lactation period. Most notably, the quantities of I, Cu, and Se were significantly higher in TMR powder than in GRO or GRC powders in ML and ML+LL. The results concur with earlier work (Gulati et al., 2018a, b) that showed that milk from the TMR feeding system

Table 2. Composition of low-heat skim milk powders from milks of dairy herds on different feeding systems in mid- and late lactation[1]

	Feeding system[2,3]										
	Mid lactation (ML)				Late lactation (LL)				Probability values for overall effects[4]		
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation period (LP)	Interaction FS × LP
Gross composition											
TS (%, wt/wt)	95.9	95.9	95.9	0.026	96.3	96.6	96.5	0.253	0.921	0.138	0.950
Fat (%, wt/wt)	0.93	0.88	0.85	0.034	1.01	0.95	0.89	0.030	0.112	0.068	0.859
Lactose (%, wt/wt)	52.3	53.0	53.6	0.040	45.9^{b}	47.4^{b}	50.4^{a}	0.579	<0.001	<0.0001	0.020
Protein (%, wt/wt)	38.9^{a}	38.1^{b}	37.0^{c}	0.075	42.1^{a}	41.0^{b}	39.2^{c}	0.144	<0.0001	<0.0001	0.408
Whey protein nitrogen index (mg of N/g)	8.81	8.99	8.46	0.194	9.94	9.82	8.91	0.328	0.067	0.008	0.562
Elements											
Ca (mg/100 g)	1,253	1,213	1,281	22.21	1,297	1,269	1,265	12.59	0.526	0.314	0.520
P (mg/100 g)	1,006	1,106	1,123	39.5	1,048	1,101	1,122	54.47	0.752	0.635	0.729
K (mg/100 g)	1,595	1,512	1,652	55.1	1,329^{c}	1,396^{b}	1,470^{a}	4.92	0.026	<0.0001	0.178
Na (mg/100 g)	359.3	333.0	360.1	16.1	425.5^{a}	381.8^{b}	374.5^{b}	7.57	0.075	0.003	0.219
Mg (mg/100 g)	116	110	113	4.01	121	121	117	1.29	0.538	0.043	0.599
S (mg/100 g)	293	275	285	15.5	304^{a}	300^{a}	278^{b}	3.81	0.345	0.315	0.405
Zn (μg/kg)	45,333	41,667	51,000	4,528	43,000^{b}	43,667^{b}	51,000^{a}	1,387	0.032	0.964	0.763
Fe (μg/kg)	2,777	2,000	2,097	267.1	2,793^{b}	3,923^{a}	2,777^{b}	217.4	0.029	0.014	0.008
I (μg/kg)	376.7^{b}	230.0^{b}	6,390.0^{a}	1,016	616.7^{b}	383.3^{b}	4,746.7^{a}	451.7	<0.0001	0.537	0.441
Mn (μg/kg)	642	727	487	237.1	295	406	296	64.7	0.593	0.059	0.884
Cu (μg/kg)	750.0^{ab}	660.0^{b}	960.0^{a}	78.1	696.7	546.7	913.3	104.1	<0.01	0.657	0.982
Mo (μg/kg)	360.0	216.7	366.7	39.9	286.7	280.0	313.3	12.47	0.024	0.412	0.091
Se (μg/kg)	203.3^{b}	173.3^{b}	383.3^{a}	11.54	223.3^{b}	210.0^{b}	363.3^{a}	14.27	<0.0001	0.234	0.089
Color											
L*	92.3^{b}	93.0^{a}	92.6^{b}	0.067	92.4	92.8	92.9	0.214	0.116	0.784	0.609
a*	−3.55^{b}	−3.60^{b}	−2.95^{a}	0.104	−3.73^{b}	−3.61^{b}	−2.93^{a}	0.127	<0.0001	0.580	0.651
b*	13.7^{a}	13.2^{ab}	11.4^{b}	0.376	15.0^{a}	13.8^{ab}	12.3^{b}	0.483	0.0001	0.031	0.728

$^{a–c}$Values within a row relating to mid- or late lactation and not sharing a common lowercase superscripted letter differed significantly ($P < 0.05$) for the effect of feeding system, whereas values without a superscript did not ($P > 0.05$).

[1]Presented data for the different feeding systems are the means of 3 replicate trials in mid- and late-lactation; SED = standard error of difference between means.

[2]GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration.

[3]Mid lactation = July 4–20 (137–153 DIL); late lactation = September 27 to October 7 (222–232 DIL). DIL = days in lactation.

[4]*P*-values for the effects of feeding system in overall lactation (ML+LL), lactation period (ML or LL) across the different feeding systems, and their interaction.

had higher concentrations of I and Se than milk from pasture-feeding systems. Apart from higher contents of I, Cu, and Se, TMR powder had higher quantities of the trace elements Zn and Mo and a lower quantity of Fe than GRC powder in overall lactation (ML+LL).

Lactation period had a significant effect on elemental composition, with LL powders having higher mean quantities of Na, Mg, and Fe and a lower quantity of K than ML powders. Surprisingly, the quantities of Ca and P in powders were not significantly affected by lactation period despite the slightly higher mean casein content (~1.8–2.7%) of LL powder. Bijl et al. (2013) found that the concentrations of Ca and P in milk increased linearly with concentrations of protein and casein, at a rate of approximately 42 and 16 mg/g protein in the range 3.0 to 4.7% (wt/wt).

Color of LHSMP

The color coordinates (L*, a*, b*) of the LHSMP are shown in Table 2; the values are of similar magnitude to those previously reported for a range of commercial SMP on the US market, that is, L* = 94.0 to 96.3; a* = −3.4 to −2.1; b* = 12.4 to 17.9 (Abdalla et al., 2017).

The TMR powders had significantly higher mean a* values and lower b* values than the corresponding GRO and GRC powders in ML, LL, and ML+LL. Hence, on visual observation, the color/hue of LHSMP from pasture-based milks (GRO, GRC) was more green-yellow than that from the TMR-based milk. The higher intensities of green (a*) and yellow (b*) color of the GRO and GRC powders may reflect higher contents of riboflavin (Dufossé and Galaup, 2009; Božanić et al., 2014) and β-carotene (Nozière et al., 2006), respectively. Analogously, Cheddar and mozzarella cheeses from pasture milks have been found to be more straw-yellow colored and have higher b* values than cheeses from TMR milk (O'Callaghan et al., 2017; Gulati et al., 2018a).The TMR powder from LL milk had a slightly, but significantly ($P < 0.05$) higher b* than that from the ML milk.

Composition of RSM

Low-heat SMP was reconstituted to 10% (wt/wt). As expected, the effects of feeding system and lactation period on concentrations of lactose and protein in the RSM were similar to those for the LHSMP. Similar to the trend for protein concentration, the mean casein content of the GRO-RSM was higher than that of TMR-RSM in ML and ML+LL, but not in LL. Otherwise, feeding system did not influence the mean concentrations of whey protein (~0.60% wt/wt) or urea in the RSM. The mean concentration of urea for all RSM samples (~35–58 mg/100 g) was comparable to that previously reported in the literature for bovine milk (Mehra et al., 1999). Urea N, as a proportion of NPN, was 54 to 70%, which was comparable in magnitude to that (50–59%) reported by Mehra et al. (1999).

Nonprotein N, as a proportion of total N, was slightly but significantly influenced by feeding system, as indicated by the higher value in the TMR-RSM relative to GRO-RSM in LL and ML+LL (Table 3). Feeding system did not significantly affect the mean proportions of casein or whey protein (as % of total protein), soluble casein (as % of total casein), or individual caseins (as % of total casein), the mean values of which were typical of those previously reported for bovine milk (Bernabucci et al., 2015; Auldist et al., 2016; Lin et al., 2017);that is, α_S-CN (39–54% of total CN), β-CN (33–46% total CN), and κ-CN (9–15% total N). The overall trends for effect of feeding system on the composition of RSM are similar to those reported previously for the comparative effects of indoor feeding on TMR versus pasture grazing on the composition of milk during lactation; that is, from about 15 to 240 DIL (Auldist et al., 2000; O'Callaghan et al., 2016; Gulati et al., 2018b).

Lactation period had a significant effect on composition, with LL RSM having higher mean concentrations of protein, casein, soluble casein (% of total casein), NPN, and urea, and a lower lactose content. Nevertheless, casein, whey protein, NPN, and urea as proportions of total N, or individual caseins as a proportion of total casein, were unaffected by lactation period.

Physicochemical Characteristics of RSM

The values for pH, CMS, and casein micelle hydration (**CMH**) for RSM are typical of those previously reported in the literature for bovine milk (Table 3), that is, pH 6.73 to 6.87 (Grimley et al., 2009; Chen et al., 2014); CMS, ~160 to 210 nm (Glantz et al., 2010; Bijl et al., 2014; Chen et al., 2014); and CMH, ~2.8 to 3.4 g water/g casein at 20 to 25°C (Lin et al., 2017; Huppertz et al., 2017). The CMS in RSM samples in the current study is likely to have been somewhat higher than that in the native skim milk owing to some denaturation of whey protein (during heating, evaporation, and drying) and its complexation with κ-CN at the micelle surface (Devold et al., 2000; Lin et al., 2018a). The $[Ca^{2+}]$ (i.e., 1.13–1.45 m*M*) was relatively low compared with the published values for unheated milk (i.e., ~1.7–3.5 m*M*; Tsioulpas et al., 2007; Bijl et al., 2013; Chen et al., 2014). This finding may reflect the heat treatment (40°C × 30 min) applied to reverse the cold aging effect of holding the RSM at 8°C for approximately 15 to 18 h. Chandrapala et al. (2010) found that the calcium ion activity of milk decreased on heating milk to 60°C

Table 3. Composition of reconstituted skim milk (10% wt/wt) powders, from milks of dairy herds on different feeding systems in mid and late lactation[1]

	Feeding system[2,3]										
	Mid lactation (ML)				Late lactation (LL)				Probability values for overall effects[4]		
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation period (LP)	Interaction FS × LP
Composition											
TS (%, wt/wt)	9.59	9.59	9.59	0.002	9.63	9.66	9.65	0.025	0.921	0.138	0.950
Lactose (%, wt/wt)	5.23	5.30	5.36	0.004	4.59^{c}	4.74^{c}	5.04^{b}	0.057	0.000	<0.0001	0.020
Total protein (%, wt/wt)	3.90^{a}	3.81^{b}	3.70^{c}	0.007	4.21^{a}	4.10^{a}	3.92^{b}	0.014	<0.0001	<0.0001	0.410
Casein (%, wt/wt)	3.09^{a}	2.98^{b}	2.94^{b}	0.016	3.27	3.25	3.12	0.0379	0.023	<0.0001	0.516
Casein number	79.2	78.1	79.5	0.622	77.8	79.3	79.6	0.852	0.409	0.927	0.308
Soluble casein (% of total milk casein)	5.91	6.72	4.77	1.37	8.91	9.53	12.39	2.20	0.900	0.048	0.580
Individual caseins (% of milk casein)											
α_{S1} + α_{S2}-CN	47.8	47.5	47.6	0.247	48.8	49.7	48.7	0.481	0.116	0.462	0.975
β-CN	42.0	43.5	42.5	0.398	41.4	40.7	41.4	0.577	0.796	0.004	0.132
κ-CN	10.2	9.0	9.9	0.692	9.8	9.7	9.9	0.335	0.914	0.575	0.976
Whey protein (%, wt/wt)	0.62	0.62	0.57	0.015	0.69	0.56	0.59	0.029	0.030	0.721	0.089
α-LA:β-LG	0.192	0.196	0.195	0.003	0.188	0.190	0.193	0.005	0.914	0.575	0.976
NPN (% of total N)	4.96^{b}	5.54^{a}	5.23^{ab}	0.073	4.96^{b}	5.13^{ab}	5.45^{a}	0.076	0.036	0.601	0.138
Urea (mg/100 g)	34.7	40.3	35.6	2.00	58.4	54.6	53.3	9.70	0.899	0.005	0.775
Physicochemical characteristics											
pH	6.70	6.69	6.72	0.011	6.71	6.70	6.68	0.012	0.843	0.578	0.230
Ionic calcium (mg/100 g)	5.08	4.94	4.65	0.131	5.84^{a}	4.55^{b}	4.85^{b}	0.097	0.001	0.180	0.015
Ionic calcium-to-casein	1.65	1.66	1.58	0.042	1.79^{a}	1.40^{b}	1.55^{ab}	0.034	0.044	0.410	0.051
Casein micelle size (nm)	181	187	172	3.58	182	182	180	4.68	0.235	0.719	0.359
Casein hydration (g of water/g of casein)	3.20	3.04	3.05	0.087	2.97	3.01	3.16	0.161	0.588	0.261	0.140

[a–c]Values within a row relating to mid or late lactation and not sharing a common lowercase superscripted letter differed significantly ($P < 0.05$) for the effect of feeding system, whereas values without a superscript did not ($P > 0.05$).

[1]Presented data for the different feeding systems are the means of 3 replicate trials in mid and late lactation; SED = standard error of difference between means.

[2]GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration.

[3]Mid lactation = July 4–20 (137–153 DIL); late lactation = September 27 to October 7 (222–232 DIL). DIL = days in lactation.

[4]*P*-values for the effects of feeding system in overall lactation (ML+LL), lactation period (ML or LL) across the different feeding systems, and their interaction.

(i.e., by ~30% at pH 6.65), to an extent dependent on pH, but was essentially fully restored after overnight holding at 4°C. In the current study, the time of holding (6 h) at room temperature (~20°C), following heating at 40°C, may have been insufficient to allow restoration of equilibrium between insoluble and soluble Ca.

The mean [Ca^{2+}] of GRO-RSM was higher than that of GRC- or TMR-RSM in LL and ML+LL. Otherwise, feeding system had no effect on the mean pH, CMS, or CMH. The absence of an effect of feeding system on CMS corresponds with the results of Auldist et al. (2016), showing no effect of the type and level of feed supplement (milled wheat grain, crushed corn grain, or canola meal) on CMS (159–172 nm) of bulk herd milk from Holstein-Friesian cows. Analogously, Grimley et al. (2009) found no significant difference in the CMS of bulk herd milks before, during, or after the turnout of commercial dairy herds to pasture, when the supply and composition of pasture are likely to vary significantly (McCarthy et al., 2013). However, Devold et al. (2000) found that the type of supplement offered to grazing dairy herds during mid lactation affected the CMS of milk, as shown by the different values in milk from herds fed rolled barley supplement (191 nm) or commercial concentrate (175 nm). Interstudy differences on the effect of diet/feeding system on CMS may relate to several factors, including differences in the response of herds with cows of different breed and genetic merit, milk protein polymorphism, and degree of glycosylation (Glantz et al., 2010; Bijl et al., 2014). We are unaware of any published studies on how diet or feeding system affects CMH.

Lactation period did not affect CMS, CMH, or [Ca^{2+}]. Other studies (Grimley et al., 2009; Glantz et al., 2010; Chen et al., 2014) have also reported little or no effect of season or lactation period on CMS, CMH, or [Ca^{2+}] of herd milks; however, distinguishing between lactation period and season in the latter studies is not possible because the calving pattern of the cows (i.e., spring, autumn, or year-round calving) was not stated.

Processing Characteristics of RSM

Data on the processing characteristics of the RSM are shown in Figure 1 and Table 4. The HCT/pH and ES/pH profiles were typical of those reported for milk, that is, a type A HCT/pH profile with a maximum HCT (HCT_{max}) at pH ~6.7 and a minimum HCT (HCT_{min}) at pH ~6.9 (Huppertz, 2016), and an ES/pH profile that increased curvilinearly with pH (Horne, 2016).

Heat Coagulation Time. Overall, feeding system had little or no effect on HCT in the pH range 6.2 to 7.2 (Table 4). The absence of an effect of feeding system on the HCT profile of the RSM is consistent with the relatively small differences in compositional parameters (Table 3) identified as having a strong influence, for example, concentrations of lactose, protein, urea, and [Ca^{2+}], and the interactive effects of these parameters (Huppertz, 2016). The current results suggest that the potential HCT-enhancing effects of the slightly lower lactose content in the GRO-RSM relative to TMR-RSM was most likely offset by its higher contents of protein and ionic calcium-to-casein ratio (Shalabi and Fox, 1982).

Lactation period significantly affected HCT (Figure 1; Table 4). Most notably, LL RSM had higher mean values of HCT than ML RSM at all pH values, apart from pH 6.2 and 6.3. The higher HCT of LL RSM, suggests that the negative impact of higher concentrations of protein and casein on HCT (Rattray and Jelen, 1996; Meena et al., 2016) is mitigated by the lower and higher concentrations of lactose and urea, respectively (Singh, 2004; Sikand et al., 2010). Hence, regression analysis of the entire data set for all milks showed that HCT at natural pH and HCT_{max} correlated negatively with lactose content and lactose-to-protein ratio ($P < 0.01$) and positively with urea content ($P < 0.05$; Table 5). Other studies have also observed a positive correlation between the HCT_{max} and urea concentration of seasonal milks with 25 to 55 mg urea/100 mL (Holt et al., 1978; Kelly et al., 1982), and individual cow milks with 35 to 60 mg urea/100 mL (Banks et al., 1984). Lactose undergoes thermal-induced degradation to organic acids (e.g., formic) upon heating at temperatures of 140°C, and thereby it reduces the pH of the milk during the HCT assay. Heat-induced degradation of urea results in the production of ammonia, which buffers the pH decrease associated with thermal decomposition of lactose and precipitation of calcium phosphate, and thereby enhances HCT (Singh, 2004). Native pH and concentration of salts (Ca, Mg, P, K, Ca^{2+}) have also been identified as important factors affecting the HCT of milk and RSM (Newstead et al., 1977; Faka et al., 2009; Sikand et al., 2010); however, these parameters were scarcely affected by lactation period (Tables 2 and 3).

Ethanol Stability. As for HCT, feeding system had little impact on ES of the RSM in the pH range 6.2 to 7.0. Although ES at pH 6.8 and 7.0 differed somewhat with feeding system (Table 3), the magnitude of the differences (4–5% vol/vol) was relatively small and unlikely to be of practical significance (Figure 1). Analogously, O'Brien et al. (1997) reported no effect of altering the daily herbage allowance from 16 to 24 kg DM/cow on ES at natural pH, despite an increase in casein content of 0.2% (wt/wt). The lack of an effect of feeding system on ES may be attributed to the similarity in composition and casein profile of the GRO-, GRC-,

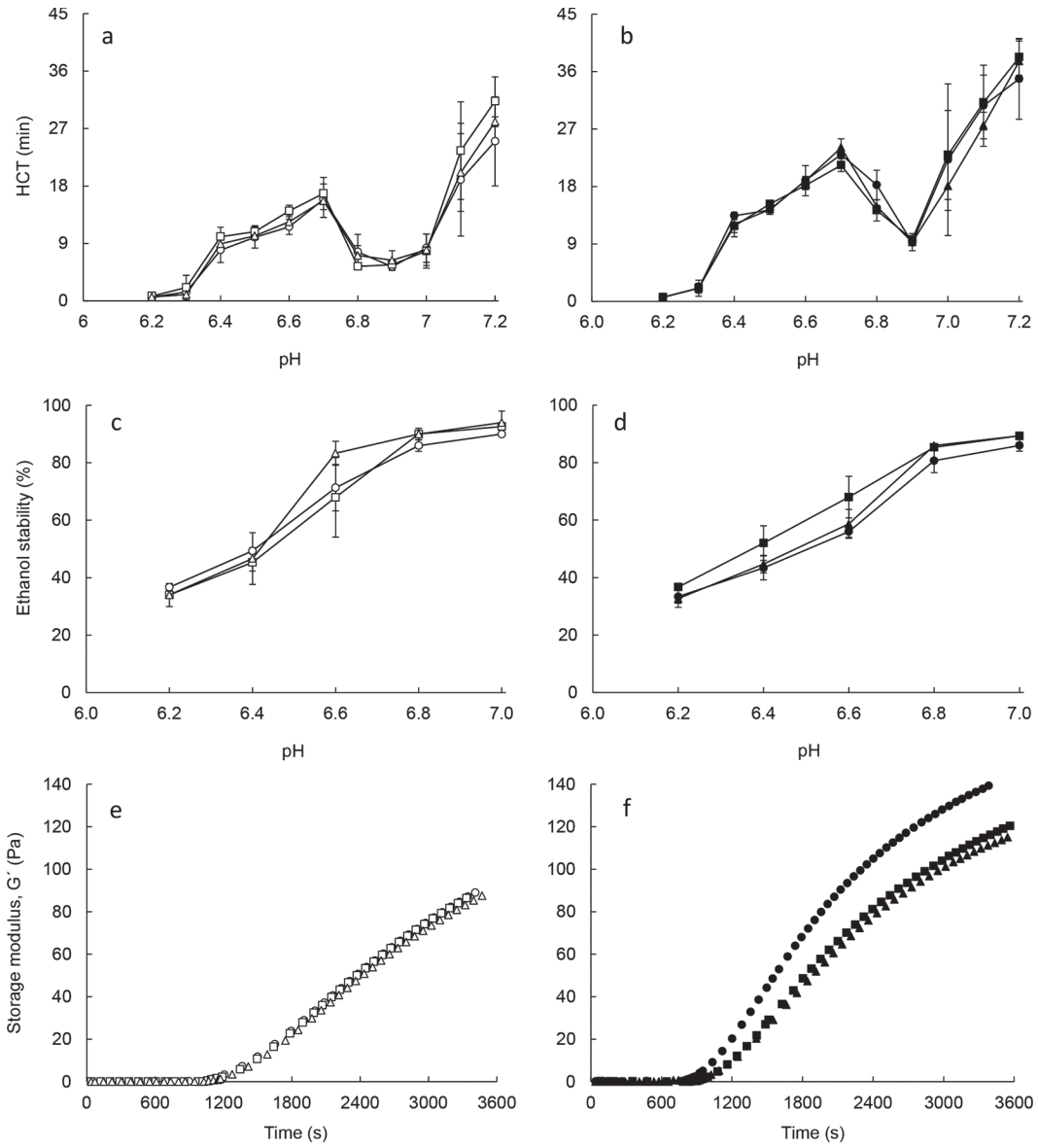

Figure 1. Processing characteristics of reconstituted skim milk (RSM, 10% wt/wt), prepared from low-heat skim milk powder produced in mid lactation (open symbols; a, c, e) and late lactation (closed symbols; b, d, f) from the milks of dairy herds on different feeding systems: grazing on perennial ryegrass pasture (GRO; ○,●), grazing on perennial ryegrass and white clover pasture (GRC; □,■), or housed indoors and offered total mixed ration (TMR; △,▲). Presented values for heat and ethanol stability over pH range 6.2 to 7.2 are the means of 3 replicate trials in both mid and late lactation; error bars represent standard deviation of the mean. A representative rennet gelation profile (G′ vs. time) for one trial in mid- and late-lactation milks is shown in e and f, respectively. HCT = heat coagulation time.

and TMR-RSM. Nevertheless, the slightly lower ES of GRO-RSM compared with the GRC- or TMR-RSM at pH 6.8 or 7.0 (Table 4) is compatible with its higher ratio of Ca^{2+} to casein (Table 3; Tsioulpas et al., 2007).

Lactation period had a significant effect on ES at pH 6.8 and 7.0, with the mean values of the ML milk (across all feeding treatments) being higher than that the corresponding LL milk. However, the magnitude of the difference in ES at these values (4–5% ethanol) was relatively small. A similar trend was noted at pH 6.6, but the magnitude of the difference in ES between the ML milk (75%, vol/vol) and LL milk (61%, vol/vol) was much larger. The results concur with those of Horne et al. (1986) who reported that the asymptotic maximum of ES (in pH range ~6.6–7.5) increased rapidly during the first 5 to 100 d of lactation and thereafter showed no further lactational trend.

Rennet Gelation. Rennet gelation was significantly affected by feeding system and lactation period. The GRO milk had higher gel strength, G'_{40}, than TMR

Table 4. Processing characteristics of reconstituted skim milk (10% wt/wt) powders, from milks of dairy herds on different feeding systems in mid and late lactation[1]

	Feeding system[2,3]								Probability values for overall effects[4]		
	Mid lactation (ML)				Late lactation (LL)						
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation period (LP)	Interaction FS × LP
Heat coagulation time (HCT, min)[5]											
HCT_{npH}	17.1	16.5	16.8	1.14	23.3	23.6	24.1	1.04	0.774	<0.0001	0.925
HCT_{max}	15.9	16.8	15.7	0.946	23.0	22.6	24.6	0.650	0.929	<0.0001	0.285
HCT_{min}	5.3	5.3	5.6	0.304	9.0	8.9	9.8	0.560	0.394	<0.0001	0.961
Ethanol stability (ES, %)[6]											
$ES_{6.2}$	36.7	34.0	34.0	1.38	33.3	36.7	32.7	1.53	0.536	0.659	0.255
$ES_{6.4}$	49.3	45.3	46.7	3.73	43.3	52.0	44.7	2.98	0.143	0.586	0.054
$ES_{6.6}$	71.3	72.0	83.3	3.32	56.0	68.0	58.7	3.54	0.240	0.003	0.115
$ES_{6.8}$	86.0[b]	90.0[a]	90.0[a]	0.666	80.7	85.3	86.0	1.24	0.006	0.001	0.968
$ES_{7.0}$	90.0	92.7	94.0	1.21	86.0[b]	89.3[a]	89.3[a]	0.384	0.001	<0.0001	0.204
ES_{npH}	82.0	84.0	84.7	0.902	77.3[b]	84.0[a]	80.7[ab]	0.942	0.026	0.027	0.225
Rennet-induced gelation											
Rennet coagulation time (min)	18.7	18.6	17.1	0.536	14.3	16.0	14.7	1.44	0.128	0.009	0.354
Gel firming rate (GFR_{max}; Pa/s)	0.057	0.055	0.053	0.001	0.090	0.069	0.060	0.008	0.172	0.021	0.286
Gel firmness at 40 min (G'_{40}; Pa)	56.6	54.0	55.3	1.63	109.7[a]	79.5[ab]	71.1[b]	8.89	0.033	0.023	0.592

[a,b]Values within a row relating to mid or late lactation and not sharing a common lowercase superscripted letter differed significantly ($P < 0.05$) for the effect of feeding system, whereas values without a superscript did not ($P > 0.05$).

[1]Presented data for the different feeding systems are the means of 3 replicate trials in mid and late lactation; SED = standard error of difference between means.

[2]GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration.

[3]Mid lactation = July 4–20 (137–153 DIL); late lactation = September 27 to October 7 (222–232 DIL). DIL = days in lactation.

[4]*P*-values for the effects of feeding system in overall lactation (ML+LL), lactation period (ML or LL) across the different feeding systems, and their interaction.

[5]HCT_{npH} = HCT at natural pH; HCT_{max} = maximum HCT; HCT_{min} = minimum HCT.

[6]Subscript numbers are pH values.

Table 5. Significant relationships between composition and processing characteristics of reconstituted skim milk (10% wt/wt) powders[1]

Processing characteristic[2]	Compositional parameter	Correlation coefficient (r)
Heat stability		
HCT_{max}, HCT_{npH}	Lactose (%, wt/wt)	−0.753,*** −0.797***
	Lactose-to-protein	−0.724,*** −0.788***
	Protein (%, wt/wt)	0.636,** 0.723***
	Casein (%, wt/wt)	0.553,* 0.660**
	NPN (%, wt/wt)	0.549,* 0.659**
	Urea (mg/100 g)	0.563,* 0.635**
Ethanol stability		
$ES_{6.8}$, $ES_{7.0}$	$[Ca^{2+}]$	−0.571,* −0.568*
Rennet gelation		
RCT	Protein (%, wt/wt)	−0.525*
	Casein (%, wt/wt)	−0.597**
GFR_{max}, G'_{40}	Protein (%, wt/wt)	0.718,*** 0.803***
	Casein (%, wt/wt)	0.658,** 0.789**

[1]The data set comprised 18 reconstituted skim milks from low-heat skim milk powders obtained from 3 different feeding systems (GRO, GRC, and TMR) in mid and late lactation in triplicate. GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration.

[2]HCT_{max} = maximum heat coagulation time; HCT_{npH} = heat coagulation time at natural pH; $ES_{6.8}$ = ethanol stability at pH 6.8; $ES_{7.0}$ = ethanol stability at pH 7.0; RCT = rennet coagulation time; GFR_{max} = maximum gel firming rate; G'_{40} = gel firmness at 40 min.

***$P < 0.001$; **$P < 0.01$; *$P < 0.05$: correlations were obtained using simple linear regression analysis; only statistically significant relationships are shown.

milk in LL and ML+LL, but not in ML; G'_{40} for GRC RSM was intermediate between that of GRO- and TMR-RSM. The relatively high G'_{40} of GRO-RSM is most likely due to its protein content (Guinee et al., 1996), as supported by the exponential increase in G′40 with protein and casein (Figure 2). Other studies have found similar relationships between milk protein content and gel strength (Guinee et al., 1996) in the range 3.0 to 7.5% (wt/wt). However, when protein differences between treatment milks are relatively small (e.g., <0.25% wt/wt), as between the GRO and TMR milks in ML, the effect of protein content may not be sufficiently large to manifest statistically. Hence, O'Brien et al. (1997) and Auldist et al. (2016) reported that differences of 0.1 to 0.3% (wt/wt) in casein, associated with alteration of diet (i.e., daily herbage allowance or type of dietary supplement), had no effect on the rennet gelation properties of milk.

Late-lactation RSM had a lower mean RGT and higher values of GFR_{max} and G'_{40} than the corresponding ML milk. The stronger rennet coagulability of LL is consistent with its higher mean concentration of protein (0.27% wt/wt) and casein (0.21% wt/wt), which correlated inversely with RGT and positively with GFR_{max} and G'_{40} (Table 5 or Figure 2).

Stirred-Yogurt Forming Properties. The changes in pH and G′ during acidification are shown in Figure 3 for GRO- and TMR-RSM in one of the trials in ML and LL; similar changes were observed for GRC-RSM in trials 1 and 2 (data not shown). G′ remained relatively constant until the GO_{pH} (5.56–5.38, Table 6) and then increased sigmoidally; simultaneously, tan δ, the ratio of the viscous or loss modulus (G″) to storage modulus (G′), decreased. The changes mark the gradual aggregation of the dispersed particles (casein micelles, casein micelle–denatured whey protein complexes, and protein-covered fat globules) into a gel network as the pH decreases from the pH at the onset of gelation (GO_{pH}) toward the casein isoelectric point, pH 4.6 (Lucey, 2016). Upon shearing of the resultant yogurt, shear stress decreased less than proportionally with shear rate. The shear stress versus shear rate data for all yogurts fitted to the Herschel–Bulkley model ($R > 0.99$). All yogurts exhibited a yield stress, σ_o (4–10 Pa) at low shear rate and thereafter shear thinned on increasing shear rate to 120 s^{-1} (Figure 3). The trend reflects the presence of a particulate protein network that was disrupted during shearing. The viscosity at 120 s^{-1} for all yogurts, 200 to 220 mPa·s, was of similar magnitude to that previously reported for yogurt with similar protein content and made under similar conditions (Guinee et al., 1995; Lin et al., 2018b).

Feeding system did not influence GO_{pH}, fermentation time (to pH 4.6), storage modulus of the gel at pH 4.6 before cooling and stirring ($G'_{pH4.6}$), or the consistency properties [σ_o, K, n, or $\eta 120s^{-1}$ (viscosity at shear rate of 120 s^{-1}, mPa·s)] and WHC of the final yogurt (Table 6). The absence of an effect on feeding system on GO_{pH} and fermentation time, despite the difference in the protein content between the milks from the dif-

ferent feeding systems (e.g., 0.19% protein in ML and 0.39% in LL; Table 6), most likely reflects the standardization of the starter culture inoculum pro rata with milk protein content. The rate of pH reduction during bacterial-induced lactic fermentation of milk is controlled primarily by the buffering capacity of the milk, which is determined by its protein content, especially casein and colloidal calcium phosphate attached to the casein (Lucey et al., 1993). Previous studies have shown an increase in the G′ of model acid-induced milk gels as a function of casein concentration in the range of 1 to 5% (wt/wt) (Roefs, 1986) and the viscosity of yogurt on fortification of milk with SMP (1.8% wt/wt; ~0.63% wt/wt, protein) or sodium caseinate (1.8, % wt/wt; ~1.6% protein) (Tamime and Deeth, 1980). The absence of an effect of feeding system on $G'_{pH4.6}$, σ_o, K, or $\eta 120s^{-1}$ suggests that the difference in the mean protein concentration of the yogurt milk between the feeding systems was insufficient to override the natural intertrial variation in factors such as the protein and mineral composition of LHSMP and starter culture activity. Likewise, Jasińska et al. (2010) found that the hardness of set yogurt made from nonstandardized milk from dairy herds fed on grass (supplemented with concentrates) or on total milk ration varied with month of year, with no evidence of a consistent effect of feeding system.

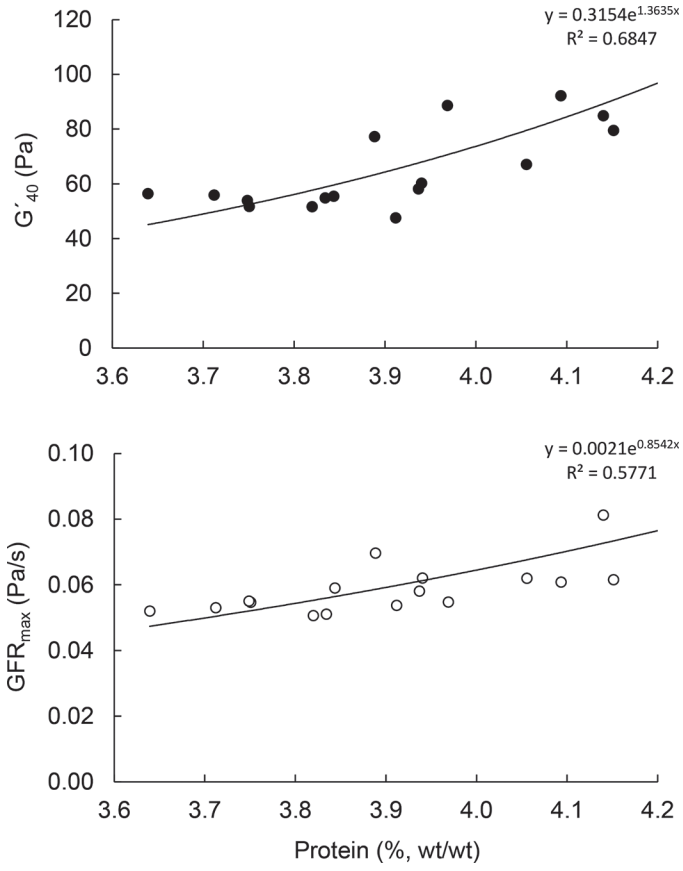

Figure 2. Relationship between gel firmness at 40 min, G'_{40} (●), or maximum gel firming rate, GFR_{max} (○), and the protein content of reconstituted skim milk (10% wt/wt) prepared from low-heat skim milk powder made from the milks of dairy herds on different feeding systems in mid and late lactation. Regression lines (—) were fitted to the entire data set, comprising 3 replicate trials for 3 feeding systems in mid and late lactation. Both relationships were statistically significant ($P < 0.001$).

Lactation period had no effect on yogurt properties (GO_{pH}, $G'_{pH4.6}$, σ_o, K, or $\eta 120s^{-1}$), apart from fermentation time, which was ~70 min longer for ML milk than LL milk on average (Table 6). Given that the starter inoculum was standardized relative to the casein content of the milk, the trend may reflect the slightly higher mean phosphorous-to-casein ratio in ML (36 mg/g casein) milk compared with LL milk (34 mg/g casein) (data not shown), which in turn would favor a higher buffering capacity and resistance to pH decrease (Lucey et al., 1993). In contrast, Muir and Tamime (1993) found a significant effect of season on the viscosity of stirred yogurt from homogenized ovine milk, which varied in concentrations of protein (~5.0–7.8% wt/wt), fat (~5.6–9.5% wt/wt), and Ca (~37–53 mM) over the period from March to September. Similarly, Cheng et al. (2002) reported that the viscosity of stirred-yogurt correlated positively with protein concentration (4.1–4.9% wt/wt, for RSM with 12% TS). The interstudy discrepancy on the effect of seasonality may reflect many factors, including differences in duration of lactation period and the range of protein in the yogurt (i.e., 0.32% wt/wt in the current study versus 0.9–2.8% wt/wt in the latter studies).

CONCLUSIONS

We investigated 3 different dairy cow feeding systems (GRO, GRC, or TMR) in ML and LL for their effects on composition and color of LHSMP, and the biochemical and processing characteristics on RSM prepared by dispersing the powder to 10% (wt/wt). Powder from the GRO or GRC feeding systems had higher mean content of protein (by ~2.5% wt/wt); lower contents of lactose (by ~3.5% wt/wt), I, Cu, and Se; and a more green-yellow color than the corresponding powder from TMR milk. The GRO-RSM had higher mean concentrations of protein (0.27% wt/wt) and casein, lower concentrations of lactose (~0.4% wt/wt) and NPN (% total N), and higher rennet gel strength than TMR-RSM. These effects were observed for the combined ML+ LL period, but varied in the separate ML and LL periods, depending on the parameter. The levels of protein and NPN (% TN) and rennet gel strength of GRC-RSM were intermediate between those of the corresponding

GRO-RSM and TMR-RSM. Feeding system had little or no effect on the physicochemical characteristics, heat coagulation time, or ES of the RSM or on the consistency characteristics of stirred yogurt prepared from the RSM. The lower lactose-to-protein ratio of the GRO and GRC powders may be more desirable from a nutritional and functional perspective in many applications, for example, in recombined milks that are used for cheese manufacture or subjected to high-heat treatment. The difference in the elemental composition of the powders from the different feeding systems is of relevance when formulating dairy-based nutritional beverages (e.g., infant milk formula) with target levels of minerals.

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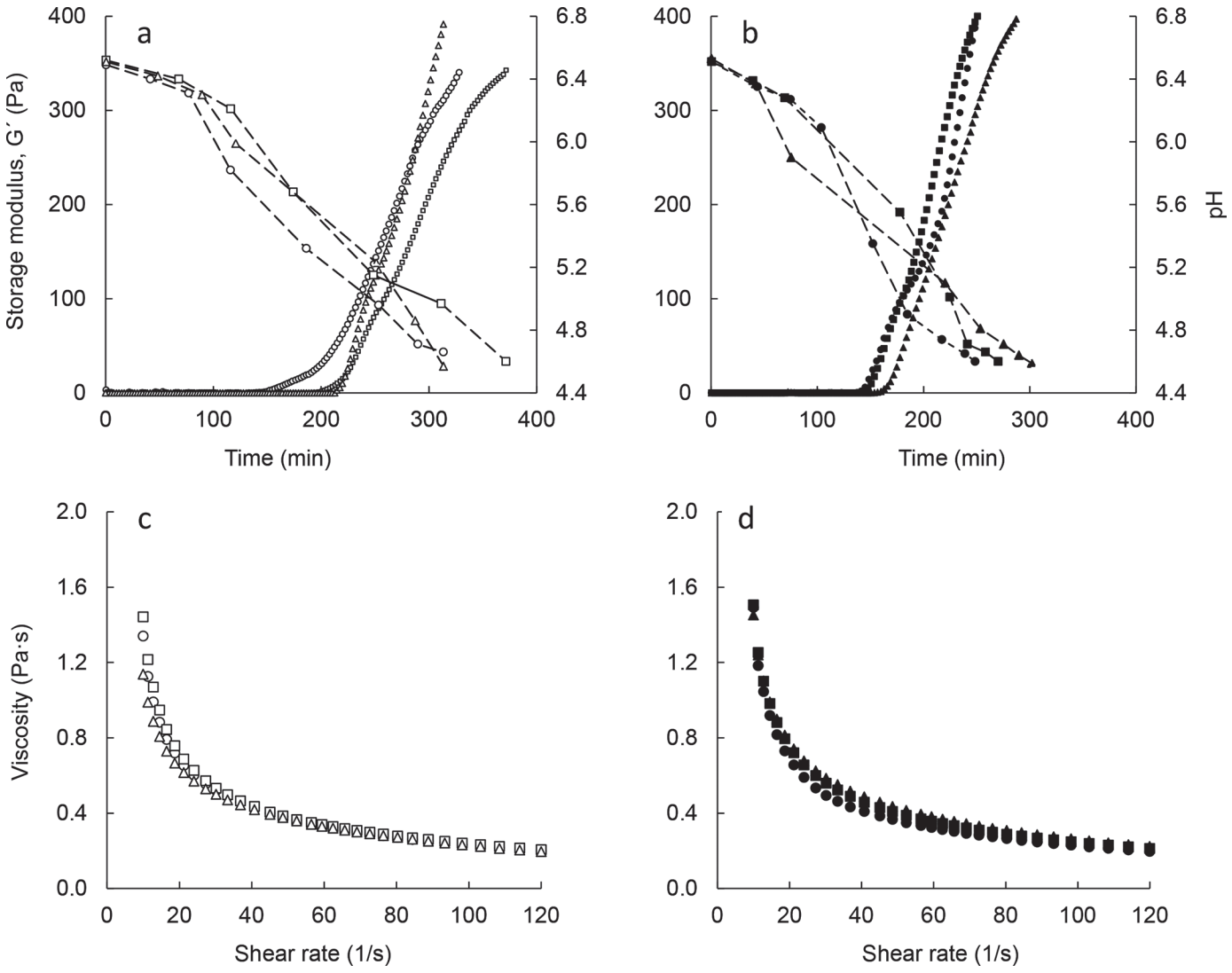

Figure 3. Effect of dairy cow feeding system on the properties of model yogurt prepared in mid lactation (a, c) and late lactation (b, d) using skim milk powder from the milks of dairy herds on different feeding systems: grazing on perennial ryegrass pasture (GRO; ○,●), perennial ryegrass and white clover pasture (GRC; □,■), or housed indoors and offered total mixed ration (TMR; △,▲). Changes in storage modulus, G′ (no line) and pH (broken line) during fermentation of milk at 42°C (a, b); viscosity of final yogurt on shearing at 8°C (c, d) are shown. The presented data and trends shown for one trial in mid and late lactation are representative of those in replicate trials.

Table 6. Characteristics of reduced-fat yogurt prepared using anhydrous milk fat (2.3% wt/wt) and reconstituted skim milk powder (12.7% wt/wt), from milks of dairy herds on different feeding systems in mid and late lactation[1]

	Feeding system[2,3]								Probability values for overall effects[4]		
	Mid lactation (ML)				Late lactation (LL)						
Item	GRO	GRC	TMR	SED	GRO	GRC	TMR	SED	Feeding system (FS)	Lactation period (LP)	Interaction FS × LP
Yogurt milk composition											
TS (%, wt/wt)	14.5	14.3	14.6	0.267	14.5	14.4	14.5	0.233	0.479	0.297	0.561
Fat (%, wt/wt)	2.27	2.27	2.21	0.042	2.33	2.32	2.29	0.037	0.546	0.121	0.944
Lactose (%, wt/wt)	6.66	6.68	6.83	0.056	5.64^{b}	5.99^{b}	6.54^{a}	0.159	0.018	0.001	0.082
Protein (%, wt/wt)	5.17^{a}	5.08^{ab}	4.98^{b}	0.020	5.60^{a}	5.38^{b}	5.21^{b}	0.023	0.019	0.021	0.349
Denatured whey protein (% of total)	78.9	79.4	79.0	0.401	78.3	80.4	80.1	0.741	0.561	0.434	0.556
Gelation during yogurt manufacture											
Gelation onset pH (GO_{pH})	5.56	5.40	5.40	0.100	5.47	5.55	5.38	0.111	0.597	0.906	0.620
Storage modulus at pH 4.6 ($G'_{pH4.6}$; Pa)	330.1	367.8	383.0	23.27	372.1	395.2	340.1	27.99	0.683	0.762	0.454
Fermentation time (min)	337.8	326.1	310.1	35.1	235.1	280.0	256.1	16.5	0.676	0.007	0.462
Yogurt properties											
Yield stress (σ_o; Pa)	7.92	10.40	11.08	0.794	8.18	8.14	4.95	1.93	0.701	0.076	0.208
Consistency coefficient (K; Pa/s^{n})	1.16	0.82	2.87	1.003	1.49	1.76	3.11	1.109	0.196	0.742	0.857
Flow behavior index (n, unitless)	0.66	0.62	0.45	0.122	0.69	0.53	0.48	0.093	0.194	0.927	0.817
Viscosity at shear rate of 10 s^{-1} (mPa·s)	1,317	1,538	1,469	180.6	1,282	1,530	1,354	128.2	0.363	0.683	0.932
Viscosity at shear rate of 120 s^{-1} (mPa·s)	202	209	206	4.16	216	222	210	8.45	0.754	0.261	0.887
WHC[5] at 300 × g (g of serum retained/100 g of yogurt)	71.2	83.0	78.9	2.35	76.8	80.9	80.0	2.42	0.103	0.591	0.528

[a,b]Values within a row relating to mid or late lactation and not sharing a common lowercase superscripted letter differed significantly ($P < 0.05$) for the effect of feeding system, whereas values without a superscript did not ($P > 0.05$).

[1]Presented data for the different feeding systems are the means of 3 replicate trials in mid and late lactation; SED = standard error of difference between means.

[2]GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration.

[3]Mid lactation = July 4–20 [137–153 d in lactation (DIL)]; late lactation = September 27 to October 7 (222–232 DIL).

[4]P-values for the effects of feeding system in overall lactation (ML+LL), lactation period (ML or LL) across the different feeding systems, and their interaction.

[5]WHC = water-holding capacity of yogurt.

for management of the dairy herds and assistance in milk collections and skim milk powder manufacture.

REFERENCES

Abdalla, A. K., K. Smith, and J. Lucey. 2017. Physical properties of nonfat dry milk and skim milk powder. Int. J. Dairy Sci. 12:149–154.

American Dairy Products Institute. 2016. Determination of undenatured whey protein nitrogen in nonfat dry milk. Pages 96–98 in ADPI Dairy Ingredient Standards. American Dairy Product Institute, Elmhurst, IL.

Auldist, M. J., J. S. Greenwood, M. M. Wright, M. Hannah, R. P. W. Williams, P. J. Moate, and W. J. Wales. 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. J. Dairy Sci. 99:4196–4205.

Auldist, M. J., A. R. Napper, and E. S. Kolver. 2000. Contribution of nutrition to seasonal variation of milk composition in New Zealand Friesian and US Holstein dairy cows. Asian-Australas. J. Anim. Sci. 13(Suppl. A):513–516.

Banks, W., J. L. Clapperton, D. D. Muir, A. K. Powell, and A. M. Sweetsur. 1984. The effect of dietary-induced changes in milk urea levels on the heat stability of milk. J. Sci. Food Agric. 35:165–172.

Bernabucci, U., L. Basiricò, P. Morera, D. Dipasquale, A. Vitali, F. P. Cappelli, and L. Calamari. 2015. Effect of summer season on milk protein fractions in Holstein cows. J. Dairy Sci. 98:1815–1827.

Bijl, E., R. de Vries, H. van Valenberg, T. Huppertz, and T. Van Hooijdonk. 2014. Factors influencing casein micelle size in milk of individual cows: Genetic variants and glycosylation of κ-casein. Int. Dairy J. 34:135–141.

Bijl, E., H. J. F. Van Valenberg, T. Huppertz, and A. C. M. Van Hooijdonk. 2013. Protein, casein, and micellar salts in milk: Current content and historical perspectives. J. Dairy Sci. 96:5455–5464.

Božanić, R., I. Barukčić, and K. Lisak. 2014. Possibilities of whey utilisation. Austin J. Nutr. Food Sci. 2:7.

Chandrapala, J., I. McKinnon, M. A. Augustin, and P. Udabage. 2010. The influence of milk composition on pH and calcium activity measured in situ during heat treatment of reconstituted skim milk. J. Dairy Res. 77:257–264.

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

Cheng, L. J., P. T. Clarke, and M. A. Augustin. 2002. Seasonal variation in yogurt properties. Aust. J. Dairy Technol. 57:187–191.

Devold, T. G., M. J. Brovold, T. Langsrud, and G. E. Vegarud. 2000. Size of native and heated casein micelles, content of protein and minerals in milk from Norwegian Red Cattle—Effect of milk protein polymorphism and different feeding regimes. Int. Dairy J. 10:313–323.

Dufossé, L., and P. Galaup. 2009. Color. Pages 585–605 in Handbook of Dairy Foods Analysis. L. M. L. Nollet and F. Toldrá, ed. CRC Press, Boca Raton, FL.

Faka, M., M. J. Lewis, A. S. Grandison, and H. Deeth. 2009. The effect of free Ca^{2+} on the heat stability and other characteristics of low-heat skim milk powder. Int. Dairy J. 19:386–392.

FAO/WHO. 1999. CODEX standard for milk powders and cream powders: CODEX STAN 207–1999. Pages 1–6 in Codex Alimentarius Milk and Milk Products. 2nd ed. Secretariat of the Codex Alimentarius Commission, Rome. Accessed Oct. 7, 2018. http://www.google.ie/url?sa=t&rct=j&q=&esrc=s&source=web&cd=1&cad=rja&uact=8&ved=2ahUKEwip9revrbzjAhXFoVwKHdazCjcQFjAAegQIARAC&url=http%3A%2F%2Fwww.fao.org%2Finput%2Fdownload%2Fstandards%2F333%2FCXS_207e.pdf&usg=AOvVaw2PGoCsRLxp0Q-bts8cBGiS.

FDA. 2018. Nonfat dry milk. Electronic Code of Federal Regulations 21CFR131.125. Food and Drugs (Title 21), Food for drug administration, Department of Health and Human Services (Chapter 1), Food for human consumption (Subchapter B), Milk and cream (Part 131.125). US Government Publishing Office, Washington, DC. Accessed Oct. 7, 2018. https://www.ecfr.gov/cgi-bin/text-idx?SID=1b80c81438ebed48137e67771dbd4d0f&mc=true&node=se21.2.131_1125&rgn=div8.

Glantz, M., T. G. Devold, G. E. Vegarud, H. L. Månsson, H. Stålhammar, and M. Paulsson. 2010. Importance of casein micelle size and milk composition for milk gelation. J. Dairy Sci. 93:1444–1451.

Grimley, H., A. Grandison, and M. Lewis. 2009. Changes in milk composition and processing properties during the spring flush period. Dairy Sci. Technol. 89:405–416.

Guinee, T. P., E. O. Mulholland, B. O'Brien, and J. J. Murphy. 2001. Effect of diet quality on the suitability of mid-lactation bovine milk for cheddar cheese manufacture. Aust. J. Dairy Technol. 56:3–8.

Guinee, T. P., C. G. Mullins, W. J. Reville, and M. P. Cotter. 1995. Physical properties of stirred-curd unsweetened yoghurts stabilised with different dairy ingredients. Milchwissenschaft 50:196–200.

Guinee, T. P., B. O'Brien, and E. O. Mulholland. 2007. The suitability of milk from a spring-calved dairy herd during the transition from normal to very late lactation for the manufacture of low-moisture Mozzarella cheese. Int. Dairy J. 17:133–142.

Guinee, T. P., D. J. O'Callaghan, P. D. Pudja, and N. O'Brien. 1996. Rennet coagulation properties of retentates obtained by ultrafiltration of skim milks heated to different temperatures. Int. Dairy J. 6:581–596.

Gulati, A., N. Galvin, D. Hennessy, S. McAuliffe, M. O'Donovan, J. J. McManus, M. A. Fenelon, and T. P. Guinee. 2018a. Grazing of dairy cows on pasture versus indoor feeding on total mixed ration: Effects on low-moisture part-skim Mozzarella cheese yield and quality characteristics in mid and late lactation. J. Dairy Sci. 101:8737–8756.

Gulati, A., N. Galvin, E. Lewis, D. Hennessy, M. O'Donovan, J. J. McManus, M. A. Fenelon, and T. P. Guinee. 2018b. 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. J. Dairy Sci. 101:2710–2723.

Holt, C., and R. Jenness. 1984. Interrelationships of constituents and partition of salts in milk samples from eight species. Comp. Biochem. Physiol. A Comp. Physiol. 77:275–282.

Holt, C., D. D. Muir, and A. W. M. Sweetsur. 1978. Seasonal changes in the heat stability of milk from creamery silos in south-west Scotland. J. Dairy Res. 45:183–190.

Horne, D. S. 2016. Ethanol stability and milk composition. Pages 225–246 in Advanced Dairy Chemistry: Vol. 1B. Proteins, Applied Aspects. P. L. H. McSweeney and S. A. O'Mahony, ed. Springer Science+Business Media LLC, New York, NY.

Horne, D. S., T. G. Parker, W. J. Donnelly, and D. T. Davies. 1986. Factors affecting the ethanol stability of bovine skim milk: VII. Lactational and compositional effects. J. Dairy Res. 53:407–417.

Huppertz, T. 2016. Heat stability of milk. Pages 179–196 in Advanced Dairy Chemistry: Vol. 1B. Proteins, Applied Aspects. P. L. H. McSweeney and S. A. O'Mahony, ed. Springer Science+Business Media LLC, New York, NY.

Huppertz, T., I. Gazi, H. Luyten, H. Nieuwenhuijse, A. Alting, and E. Schokker. 2017. Hydration of casein micelles and caseinates: Implications for casein micelle structure. Int. Dairy J. 74:1–11.

ISO (International Organization for Standardization). 2001. Milk—Determination of nitrogen content. Part 4 in Determination of non-protein-nitrogen content. ISO 8968-4:2001 (IDF 20-4:2001). ISO, Geneva, Switzerland.

ISO (International Organization for Standardization). 2004. Milk—Determination of casein-nitrogen content. Part 1 in Indirect Method. ISO 17997-1:2004 (IDF 29-1:2004). ISO, Geneva, Switzerland.

ISO (International Organization for Standardization). 2014. Milk and milk products—Determination of nitrogen content. Part 1 in Kjeldahl Principle and Crude Protein Calculation. ISO 8968-1: 2014 (IDF 20–1:2014). ISO, Geneva, Switzerland.

Jasińska, M., I. Dmytrów, A. Mituniewicz-Małek, and K. Wąsik. 2010. Cow feeding system versus milk utility for yoghurt manufacture. Acta Sci. Pol. Technol. Aliment. 9:189–199.

Jenness, R., and J. Koops. 1962. Preparation and properties of a salt solution which simulates milk ultrafiltrate. Neth. Milk Dairy J. 16:153–164.

Kelly, P. M. 1982. Effect of seasonal variation, urea addition and ultrafiltration on the heat stability of skim-milk powder. J. Dairy Res. 49:119–126.

Kelly, A. L., and P. F. Fox. 2016. Manufacture and properties of dairy powders. Pages 1–34 in Advanced Dairy Chemistry: Vol. 1B. Proteins, Applied Aspects. P. L. H. McSweeney and S. A. O'Mahony, ed. Springer Science+Business Media LLC, New York, NY.

Kelly, P. M., A. M. O'Keeffe, M. K. Keogh, and J. A. Phelan. 1982. Studies of milk composition and its relationship to some processing criteria: III: Seasonal variation in heat stability of milk. Irish. J. Food Sci. Technol. 6:29–38.

Lin, Y., A. L. Kelly, J. A. O'Mahony, and T. P. Guinee. 2016. Fortification of milk protein content with different dairy protein powders alters its compositional, rennet gelation, heat stability and ethanol stability characteristics. Int. Dairy J. 61:220–227.

Lin, Y., A. L. Kelly, J. A. O'Mahony, and T. P. Guinee. 2018a. 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. Int. Dairy J. 78:53–64.

Lin, Y., A. L. Kelly, J. A. O'Mahony, and T. P. Guinee. 2018b. Effects of milk heat treatment and solvent composition on physicochemical and selected functional characteristics of milk protein concentrate. J. Dairy Sci. 101:6799–6813.

Lin, Y., J. A. O'Mahony, A. L. Kelly, and T. P. Guinee. 2017. Seasonal variation in the composition and processing characteristics of herd milk with varying proportions of milk from spring-calving and autumn-calving cows. J. Dairy Res. 84:444–452.

Lucey, J. A. 2016. Acid coagulation of milk. Pages 309–328 in Advanced Dairy Chemistry: Vol. 1B. Proteins, Applied Aspects. P. L. H. McSweeney and S. A. O'Mahony, ed. Springer Science+Business Media LLC, New York, NY.

Lucey, J. A., B. Hauth, C. Gorry, and P. F. Fox. 1993. The acid-base buffering properties of milk. Milchwissenschaft 48:268–272.

McCarthy, B., K. M. Pierce, L. Delaby, A. Brennan, C. Fleming, and B. Horan. 2013. The effect of stocking rate and calving date on grass production, utilization and nutritive value of the sward during the grazing season. Grass Forage Sci. 68:364–377.

Meena, G. S., A. K. Singh, S. Borad, and N. R. Panjagari. 2016. Effect of concentration, homogenization and stabilizing salts on heat stability and rheological properties of cow skim milk ultrafiltered retentate. J. Food Sci. Technol. 53:3960–3968.

Mehra, R., B. O'Brien, J. F. Connolly, and D. Harrington. 1999. Seasonal variation in the composition of Irish manufacturing and retail milks: 2. Nitrogen fractions. Irish J. Agric. Food Res. 38:65–74.

Muir, D., and A. Tamime. 1993. Ovine milk. 3. Effect of seasonal variations on properties of set and stirred yogurts. Milchwissenschaft 48:509–513.

Newstead, D. F., W. B. Sanderson, and E. F. Conaghan. 1977. Effects of whey protein concentrations and heat treatment on the heat stability of concentrated and unconcentrated milk. N. Z. J. Dairy Sci. Technol. 12:29–36.

Nozière, P., B. Graulet, A. Lucas, B. Martin, P. Grolier, and M. Doreau. 2006. Carotenoids for ruminants: From forages to dairy products. Anim. Feed Sci. Technol. 131:418–450.

O'Brien, B., S. Crosse, and P. Dillon. 1996. Effects of offering a concentrate or silage supplement to grazing dairy cows in late lactation on animal performance and on milk processability. Irish J. Agric. Food Res. 35:113–125.

O'Brien, B., D. Gleeson, and K. Jordan. 2013. Iodine concentrations in milk. Irish J. Agric. Food Res. 52:209–216.

O'Brien, B., R. Mehra, J. F. Connolly, and D. Harrington. 1999. Seasonal variation in the composition of Irish manufacturing and retail milks: 1. Chemical composition and renneting properties. Irish J. Agric. Food Res. 38:53–64.

O'Brien, B., J. J. Murphy, J. F. Connolly, R. Mehra, T. P. Guinee, and G. Stakelum. 1997. Effect of altering the daily herbage allowance in mid lactation on the composition and processing characteristics of bovine milk. J. Dairy Res. 64:621–626.

O'Callaghan, T. F., H. Faulkner, S. McAuliffe, M. G. O'Sullivan, D. Hennessy, P. Dillon, K. N. Kilcawley, C. Stanton, and R. P. Ross. 2016. Quality characteristics, chemical composition, and sensory properties of butter from cows on pasture versus indoor feeding systems. J. Dairy Sci. 99:9441–9460.

O'Callaghan, T. F., D. T. Mannion, D. Hennessy, S. McAuliffe, M. G. O'Sullivan, N. Leeuwendaal, T. P. Beresford, P. Dillon, K. N. Kilcawley, and J. J. Sheehan. 2017. Effect of pasture versus indoor feeding systems on quality characteristics, nutritional composition, and sensory and volatile properties of full-fat Cheddar cheese. J. Dairy Sci. 100:6053–6073.

Patel, H. A., S. G. Anema, S. E. Holroyd, H. Singh, and L. K. Creamer. 2007. Methods to determine denaturation and aggregation of proteins in low-, medium- and high-heat skim milk powders. Lait 87:251–268.

Pouliot, Y., and M. Boulet. 1991. Seasonal variations in the heat stability of concentrated milk: Effect of added phosphates and pH adjustment. J. Dairy Sci. 74:1157–1162.

R Core Team. 2014. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org/.

Rattray, W., and P. Jelen. 1996. Thermal stability of skim milk with protein content standardized by the addition of ultrafiltration permeates. Int. Dairy J. 6:157–170.

Roefs, S. P. F. M. 1986. Structure of acid casein gels—A study of gels formed after acidification in the cold. PhD Thesis. Wageningen Agricultural University, the Netherlands.

Shalabi, S. I., and P. F. Fox. 1982. Heat stability of milk: Synergic action of urea and carbonyl compounds. J. Dairy Res. 49:197–207.

Sikand, V., P. S. Tong, and J. Walker. 2010. Heat stability of reconstituted, protein-standardized skim milk powders. J. Dairy Sci. 93:5561–5571.

Singh, H. 2004. Heat stability of milk. Int. J. Dairy Technol. 57:111–119.

Tamime, A. Y., and H. C. Deeth. 1980. Yogurt: Technology and biochemistry. J. Food Prot. 43:939–977.

Tsioulpas, A., A. S. Grandison, and M. J. Lewis. 2007. Changes in physical properties of bovine milk from the colostrum period to early lactation. J. Dairy Sci. 90:5012–5017.