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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

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ABSTRACT

This study investigated the effects of 3 dairy cow feeding systems on the composition, yield, and biochemical and physical properties of low-moisture part-skim Mozzarella cheese in mid (ML; May–June) and late (LL; October–November) lactation. Sixty spring-calving cows were assigned to 3 herds, each consisting of 20 cows, and balanced on parity, calving date, and pre-experimental milk yield and milk solids yield. Each herd was allocated to 1 of the following 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 total mixed ration (TMR). Mozzarella cheese was manufactured on 3 separate occasions in ML and 4 in LL in 2016. Feeding system had significant effects on milk composition, cheese yield, the elemental composition of cheese, cheese color (green to red and blue to yellow color coordinates), the extent of flow on heating, and the fluidity of the melted cheese. Compared with TMR milk, GRO and GRC milks had higher concentrations of protein and casein and lower concentrations of I, Cu, and Se, higher cheese-yielding capacity, and produced cheese with lower concentrations of the trace elements I, Cu, and Se and higher yellowness value. Cheese from GRO milk had higher heat-induced flow and fluidity than cheese from TMR milk. These effects were observed over the entire lactation period (ML + LL), but varied somewhat in ML and LL. Feeding system had little, or no, effect on gross composition of the cheese, the proportions of milk protein or fat lost to cheese whey, the texture of the unheated cheese, or the energy required to extend the molten cheese. The differences in color and melt characteristics of cheeses obtained from milks with the different feeding systems may provide a basis for creating points of differentiation suited to different markets.
Key words: pasture, total mixed ration, milk, Mozzarella

INTRODUCTION

Milk composition is a key factor affecting cheese yield, the recoveries of fat and protein from milk to cheese, and, hence, the profitability of manufacturing plants (Fox et al., 2017). Consequently, the effects of differences in the concentration of milk constituents, especially fat and protein, on cheese yield and component recoveries have been investigated extensively (Fox et al., 2017). In many of these studies, the concentrations of protein and fat in milk have been altered by process intervention, for example by low-concentration factor membrane filtration (Govindasamy-Lucey et al., 2005, 2007; Ong et al., 2013; Soodam et al., 2014), addition of low-heat skim milk powder or buttermilk powder, or standardization to different protein-to-fat ratios in the manufacturing of reduced-fat cheese variants (Fenelon and Guinee, 1999). The focus of many of these studies was to simulate the potential effects of seasonal changes in milk protein concentration, especially in milk from dairy herds composed of spring-calving cows grazed on pasture, as opposed to milk from herds of year-round calving of cows fed indoors on preserved forages supplemented with concentrates. As milk for cheese manufacturing is generally standardized to a fixed protein-to-fat ratio to ensure compliance to compositional specifications and consistent quality, variation of fat content in raw milk is of little relevance in large-scale modern cheese manufacture.

Increasing milk protein in the range of 3.0 to 4.5% when maintaining a standard protein-to-fat ratio gener-

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ally results in higher cheese yield, but has little effect on protein recovery or cheese composition (Soodam and Guinee, 2018). The magnitude of the effect depends on the degree to which the protein concentration is increased and cheesemaking conditions (Soodam and Guinee, 2018). Reducing protein-to-fat ratio of milk, by changing fat content in the range of 0.1 to 3.5% (wt/wt), has pronounced effects, the most notable being an increase in cheese firmness and fracture stress, impairment of cooking properties, and a deterioration in sensory qualities (e.g., a loss of typical cheese flavor and creaminess). As for protein, the effects of altering fat content depend on the degree of fat reduction and manufacturing procedure (Rudan et al., 1999; Fenelon and Guinee, 2000; Henneberry et al., 2015, 2016; McCarthy et al., 2016).

Auldist et al. (2016) investigated the effect of varying the type and quantity of supplement (wheat grain, corn grain, canola meal, alfalfa hay) to cows grazed on perennial ryegrass. Altering the diet affected milk fat content, fatty acid profile, and cheese yield, but not milk protein concentration, protein profile, or rennet gelation properties. More recently, O'Callaghan et al. (2016, 2017) reported on the effect of feeding system on milk composition and Cheddar cheese, where cows were grazed on pasture, either perennial ryegrass or perennial ryegrass with white clover, or offered a TMR indoors. Significant effects of feeding system were observed for milk composition, fatty acid profile, color, hardness, and sensory characteristics of the cheese. Cheese from milk produced by the pasture-feeding systems had higher concentrations of β-carotene, lower weight proportions (g/100 g of milk fat) of palmitic (C16:0) and linoleic (C18:2c) acids, a higher proportion of linolelaidic acid (C18:2-*trans*), and were softer at 20°C and more yellow in color (O'Callaghan et al., 2017).

Mozzarella and Cheddar represent the cheese varieties produced in the largest quantities in the United States (USDA, 2018), primarily because of their use as an ingredient in foods such as sandwiches and pizza. In these applications, the physical characteristics of the unheated and heated cheese are key determinants of quality. Auldist et al. (2010) compared the properties of Cheddar cheese from milk from cows on extended lactation [up to 670 d in lactation (**DIL**)] and fed indoors on TMR or grazed on pasture grass supplemented with grain (barley and triticale) and alfalfa silage and hay. Apart from milk from TMR-fed cows having a slightly, but significantly, lower proportion of α_{S1}-CN and concentration of phosphorous, feeding system had no effect on milk composition, cheese composition, cheese yield, recovery of milk fat or protein to cheese, or grading scores received for flavor and texture. We are unaware of any studies on the comparative effects of TMR and pasture-based feeding systems on Mozzarella cheese. The current study compared pasture- and TMR-based feeding systems for their effects on composition, yield, color, texture and thermophysical properties of low-moisture part-skim Mozzarella (**LMPS**) cheese manufactured in mid lactation or late lactation. Milk was obtained from 3 spring-calving herds, each assigned to 1 of 3 feeding systems.

MATERIALS AND METHODS

Feeding Systems and Milk Collection

Sixty spring-calving dairy cows from the Teagasc Moorepark herd with a mean calving date of February 19, 2015, were allocated to 1 of 3 different 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, as described by O'Callaghan et al. (2016) and Gulati et al. (2018). The average sward clover content across the year was 23.8% of herbage DM. The herds were each composed of 20 cows and were 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 cows were placed on the different feeding systems 1 wk after calving, and individual cows were maintained on the treatments until the milk yield dropped to <8 L/d or until November 29, 2016.

As described previously (Gulati et al., 2018), the grazing treatments (GRO, GRC) were stocked at 2.75 livestock units/ha and were rotationally grazed at a frequency of 8.3 grazing rotations per season. Cows were retained on pasture (grass or grass with white clover) paddocks until a minimum postgrazing sward height of 4 cm. Cows on the GRO or GRC pastures had a daily DMI of 18 kg/cow. The TMR diet has been described in detail by O'Callaghan et al. (2016). It comprised grass silage, maize silage, and concentrates, including beet pulp, soybean meal, maize distillers grains, rolled barley, rapeseed meal, Megalac, acidbuf, and mineral balancer (McDonnell Bros. Agricultural Suppliers Ltd., Fermoy, Co. Cork, Ireland). The daily DMI of TMR-fed cows was 7.15 kg of grass silage, 7.15 kg of maize silage, and 8.3 kg of concentrate. The concentrate portion of the TMR feed was fortified with a commercial mineral balancer (Dairy Hi-Phos; McDonnell Bros. Agricultural Suppliers Ltd., Fermoy, Co. Cork, Ireland), giving added levels of Ca, Na, P, Zn, Cu, Mn, I, Co, and Se of 3,340, 2,000, 1,200, 140, 100, 70, 10, 2, and 0.8 mg/kg, respectively. Cows on all feeding systems were offered water fortified with a liquid mineral supplement

(Terra Liquid Minerals, Moone Lodge, Moone, Athy, Co. Kildare, Ireland); the daily mean intake of Na, Mg, Zn, Cu, Se, and Co from water was 5.0, 1.2, 219, 106, 3.8, and 3.0 mg/cow, respectively.

Milk from each of the 3 herds on the GRO, GRC or TMR feeding systems was collected separately in designated refrigerated bulk tanks and denoted as GRO, GRC or TMR milk, respectively. Cows were milked twice daily, at 0730 and 1530 h. On 7 different occasions in 2016, 3 in mid lactation (**ML**; May 23 to June 8, when cows were 94–110 DIL) and 4 in late lactation (**LL**; October 10 to November 5, when cows were 234–260 DIL), ~800 to 1,000 kg of milk from combined a.m. and p.m. milkings were collected from each herd over a period of 2 to 3 d.

Standardization and Pasteurization of Milks

Milk from each feeding system was standardized to a protein-to-fat ratio of 1.15 in ML or 1.20 in LL, held overnight in separate tanks at 4°C, pasteurized at 72°C for 15 s, cooled to 36°C, and pumped to the cheese vats (500-L; APV Schweiz AG, Worb, Switzerland).

Cheese Manufacture

Milk (~460 kg) maintained at 36°C was inoculated with direct vat cultures TH4, consisting of *Streptococcus thermophilus*, and LHB02, consisting of *Lactobacillus helveticus*, at levels of 10 and 5 g per 100 kg of milk with 3.4% (wt/wt) protein, respectively, as recommended by the supplier (Chr. Hansen, Little Island, Cork, Ireland). After a 40-min inoculation period when the milk pH was 6.50–6.55, chymosin (single strength Chy-Max plus, 200 international milk clotting units (IMCU; Chr. Hansen, Hørsholm, Denmark), diluted 1 in 10 in distilled water, was added at 36 IMCU/kg of milk with 3.4% (wt/wt) protein. Culture inoculum and rennet dosage were increased pro rata with milk protein concentration to ensure similar acidification rates and rennet-to-casein ratio, respectively, in ML and LL, despite the difference in milk protein concentration. Added chymosin was thoroughly mixed for 90 s with the milk and immediately a sample (40 mL) of the rennet-treated cheese milk was taken from the cheese vat and monitored for changes in storage modulus (**G′**) at 36°C by subjecting it to a strain of 0.025 at a frequency of 1 Hz at 36°C in a controlled stress rheometer (CSL2 500 Carri-Med, TA Instruments Inc., New Castle, DE; Hou et al., 2017).

Cheese manufacture was as previously described by Guinee et al. (2002). In brief, the rennet-treated milk was cut at a gel strength (G′) of 30 Pa, and the curd-whey mixture was cooked to 42°C at a rate of 0.2°C/min. The curd-whey mixture was pumped to a draining vat when the curd reached a pH value of 6.1, and the resultant curd was cheddared, milled, and salted at rate of 4.6% (wt/wt) when the pH reached 5.2. The salted curds were held for 20 min and mixed at 5-min intervals (mellowed) to ensure uniform salt distribution. The curd was kneaded in hot water (78–80°C) and heated to 58 to 59.5°C (Automatic Stretching Machine, model d; CMT, S. Lorenzo di Peveragno CN, Italy), and the plasticized curd was molded into 2.3-kg rectangular blocks, which were cooled in dilute brine (10% wt/wt NaCl, 0.2% wt/wt Ca, pH 5.1, 4–8°C) for 30 min, allowed to drip-dry for 10 min, vacuum-packed, and stored at 4°C.

Sampling and Mass Balance

All inputs (cheese milk, diluted rennet, salt) and outputs (whey, stretch water, curd, cheese) were collected and weighed, as described previously (Fenelon and Guinee, 1999). Cheese milk refers to milk from each cheese vat following pasteurization and cooling; bulk whey to the composite of whey collected during whey drainage and curd cheddaring; salty whey to the whey expressed during salting and mellowing; and stretch water to the a mixture of the hot water added during plasticization and the curd serum released during curd plasticization.

Compositional Analysis of Milk and Whey

Milk samples were analyzed for fat, total N, and casein using standard International Dairy Federation methods. The determination of macro (Ca, P, Na, Mg) and trace elements (Zn, Cu, Mo, and Se) involved acid extraction (with nitric acid, hydrochloric acid, and hydrogen peroxide) of weighed samples (~1 g) and analysis of the extract using inductively coupled plasma mass spectrometry (**ICPMS**; Agilent ICPMS 7700x, with ASX-500 series auto-sampler and MassHunter software A.01.02 Patch 4; Agilent, Santa Clara, CA), as described by Gulati et al. (2018). The measurement of I involved alkaline extraction of a 0.5-g sample using tetramethylammonium hydroxide (**TMAH**; Inorganic Ventures, Christiansburg, VA) and analysis of the extract with ICPMS, as described by British Standard Institution (2007). Samples (0.5 g) and Standard Reference Material 1849a (0.5 g; LGC Standards, London, UK) were diluted with 5 mL of 5% (vol/vol) TMAH, digested by holding at 90°C for 3 h, and cooled to room temperature. Tellurium (1,000 μg/mL; Reagecon, Shannon, Ireland) was added to the

cooled sample digest at a level 0.5 g and the sample digest was diluted to 50 g with 1% (vol/vol) TMAH solution to give a final dilution factor of 100. Iodine standards for calibration were prepared from stock iodide solution (500 μg/L; Inorganic Ventures). Serial dilutions of iodide solution with 1% (vol/vol) TMAH solution and Tellurium at a concentration of 10 μg/g were prepared to give I concentrations ranging from 0 to 50 μg/L. Whey streams (bulk whey, salty whey) and stretch water were analyzed for protein (IDF 2001) and fat (IDF 1987); all samples were heated to 40°C before fat analysis to ensure uniform distribution of fat.

Cheese Yield and Component Losses

Cheese yield was expressed as actual yield ($\mathbf{Y_a}$), defined as kilograms of cheese per 100 kg of cheese milk, and normalized yield ($\mathbf{Y_n}$) defined as kilograms of cheese per 100 kg of reference milk, with fat and protein of 2.89% (wt/wt) and 3.40% (wt/wt), respectively. The percentages of total milk fat or protein lost in the different whey or stretch water streams were calculated from the percentages of fat and protein in, and weight of, the milk and individual streams, as described previously (Guinee et al., 2006).

Analysis of Unheated Cheese

Composition. Grated cheese samples were analyzed in duplicate at 1 d for protein by the Kjeldahl method (IDF 2001), for moisture by oven drying at 102°C for 5 h (IDF, 1982), for fat by the Röse-Gottlieb method (IDF, 1996), for salt using the potentiometric method (IDF, 1981), and for elements using ICPMS, as described for milk, except that the sample weight was 0.2 g. The pH of grated cheese slurry obtained from 20 g of cheese and 12 g of distilled water was measured at all sampling points using a pH meter (British Standards Institution, 1976).

Proteolysis. A mixture of cheese and water (45°C), at a weight ratio of 1:2, was homogenized for 5 min (Stomacher, Lab-Blender 400; Seward Medical, London, UK), held at 40°C for 1 h, and centrifuged at 3,000 × *g* for 30 min at 4°C (Sorvall LYNX 6000 superspeed centrifuge, Thermo Scientific, Dublin, Ireland). The supernatant was filtered through glass wool, adjusted to pH 4.6 using 0.1 *N* HCl, and recentrifuged as described above and filtered. The resultant centrifugate was assayed for N concentration using the macro-Kjeldahl method (IDF 2001), which was expressed as pH 4.6 soluble N (**SN**) as a percentage of total N in cheese.

Water-Holding Capacity. Grated cheese (120 g) was centrifuged at 12,500 × *g* for 75 min at 25°C to obtain expressible cheese serum as described by Guo and Kindstedt (1995). The water-holding capacity (**WHC**) was calculated by subtracting the weight of expressible serum per 100 g of cheese from the weight of moisture per 100 g and expressed as grams per gram of protein. It was used as an indicator of the WHC of cheese matrix.

Texture Profile Analysis. Six cheese cubes (25 mm each side) were obtained from a block of cheese using cheese blocker (Bos Kaasgereedschap, Boven graven, Postbus, the Netherlands). The samples were tightly wrapped in tin foil and equilibrated at 4°C overnight. Cheese cubes were taken from the fridge and immediately compressed to 70% of original height in 2 successive strokes (bites) using a TAHDi texture analyzer (Stable Micro Systems, Goldalming, UK) at a rate of 1 mm/s. The following parameters were obtained from the resultant force-time curve: firmness, the force at full compression in bite 1; cohesiveness, the ratio of the compression area during bite 2 to that during bite 1; springiness, the ratio of sample compression distance in bite 2 to that in bite 1; and chewiness, the product of firmness by cohesiveness by springiness (Guinee et al., 2015).

Color. The color space coordinates, namely the L*, a*, and b* values, were measured on cheese discs (47.5 mm diameter) using the CR-400 Chroma Meter (Konica Minolta, Osaka, Japan), which had been calibrated using the Minolta calibration plate. Four disc-shaped samples were taken from each cheese, wrapped tightly in tin foil, equilibrated at 4°C overnight, withdrawn, and immediately assayed in quadruplicate. The L* value varying from 0 (black) to 100 (white) is an index of lightness, whereas a* and b* values represent the variation and intensity in color from green (– values) to red (+ values) and of blue (– values) to yellow (+ values), respectively.

Thermophysical Properties of Cheese

Flow. The flow or spread of a cheese disc (47.5 mm diameter), placed on circular glass dish, was measured in quadruplicate after heating in a convection oven at 280°C for 4 min (Binder FD 35, Binder GmbH, Tuttlingen, Germany). The melted cheese disc was withdrawn, cooled to room temperature, and measured for diameter on 4 equally spaced locations (spokes); flow was expressed as the percentage increase in disc diameter.

Stretchability. Stretchability of cheese was analyzed on quadruplicate samples by uniaxial extension of the hot molten cheese (95°C) to a distance of 380 mm at 10 mm/s using a TAHDi Texture Analyzer (Stable Micro Systems; Guinee et al., 2015). The extension work

($\mathbf{E_w}$) was calculated as the area of resultant force (F) time curve at full extension, where $E_w = F \times$ extension distance.

Viscoelastic Changes during Heating and Cooling. Changes in G′, loss modulus (**G″**), and loss tangent (G″/G′) on heating of cheese discs (40 mm in diameter, 2 mm thick) at at a rate of 3.25°C/min from 25 to 90°C and immediately recooling at a rate of 3.25°C/min to 25°C were measured using low-amplitude strain oscillation rheometry (Anton Paar Rheometer MCR50, Anton Paar GmbH, Graz, Austria), as described previously (Guinee et al., 2015). The cheese discs were placed between 2 parallel, serrated plates (40 mm in diameter) of the rheometer cell, tempered at 25°C for 15 min, and subjected to a low-amplitude shear strain (γ) of 0.0063 at an angular frequency of 1 Hz during heating and recooling. The following parameters were calculated from the resultant G′- and G″-temperature curves: crossover or melting temperature (**COTh**) during heating, the temperature at which G″ attains to a value equal to that of G′ and the cheese changes from a viscoelastic solid to a viscoelastic fluid; the maximum value of loss tangent ($\mathbf{LT_{max}}$), an index of the maximum fluidity attained by the cheese during heating; and crossover or congealing temperature (**COTc**) during cooling, the temperature at which G″ and G′ become equal and the cheese transitions from a viscoelastic fluid to a viscoelastic solid.

Statistical Analysis

Cheese was made from milk from each feeding system (GRO, GRC, and TMR) on 3 separate occasions in ML (94–110 DIL) and 4 in LL (234–260 DIL). The data were classified according to feeding system and lactation period and analyzed using ANOVA as a factorial design. The effects of lactation period, feeding system, and their interaction 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 $P < 0.05$. Mid-lactation milk refers to the composite of the milk samples collected from the herds on the GRO, GRC, and TMR feeding systems in mid lactation; the composite of the LL milk samples was similarly denoted as LL milk. Whey, stretch water, and cheese from milks in ML and LL were similarly denoted.

A split-plot design was used to evaluate the effects of feeding system, storage time, and their interaction on the biochemical and physical characteristics of cheese measured during storage. The data were analyzed using the PROC MIXED procedure of SAS (SAS Institute, 2011) with Tukey's multiple-comparison test for paired comparison of means at a significance level of $P < 0.05$. Similarly, the overall effects of feeding system and lactation period were determined using the PROC MIXED procedure of SAS.

RESULTS AND DISCUSSION

Milk Composition

Raw Milk. The composition of the raw milk was affected by feeding system, lactation period, and their interaction to an extent depending on constituent (Table 1). The concentration of protein in raw milks from the different feeding systems across the overall lactation period (ML + LL) decreased in the order of GRO > GRC > TMR ($P < 0.001$). The concentration of protein and casein of the pasture-based raw milks (GRO or GRC) was significantly higher (~0.3–0.4%, wt/wt, and 0.20–0.27%, wt/wt) than that of the TMR milk in ML and LL. Although we found no difference in the protein concentration between the GRO and GRC raw milk in ML, that of the GRO was significantly higher (~0.12%, wt/wt) in LL. Feeding system did not affect the mean fat content or lactose concentration of milk in ML or ML + LL, but did in LL when the values of fat and lactose in TMR milk were lower and higher, respectively, than in GRO milk.

Lactation period significantly affected composition, with LL milk having higher protein and fat and a lower concentration of lactose than ML milk. The overall effects of feeding system and lactation period on gross composition of raw milk are similar to those reported previously (Auldist et al., 2000; O'Callaghan et al., 2016; Gulati et al., 2018).

Standardized Cheese Milk. The effect of feeding system and lactation period on the concentrations of protein and lactose in the cheese milk were generally similar to those observed for raw milk. The concentration of protein in GRO and GRC milk was higher than that in TMR milk in ML, LL, and ML + LL. The fat content of GRO or GRC cheese milk was significantly higher than that of the corresponding TMR milk in ML, LL, and ML+LL, owing to the standardization to a fixed protein-to-fat ratio of all milks for cheese manufacture.

The concentrations of individual elements in the cheese milk from all feeding systems were within the ranges previously reported in bovine milk (O'Brien et al., 1999; Rodríguez Rodríguez et al., 2001; Bijl et al., 2013; Gulati et al., 2018). The concentration of I was relatively high in TMR milk and low in the pasture-based milks compared with that reported in other studies; for example, ~217 or 450 μg/kg in milk from cows with no dietary I supplement and with or without post-

Table 1. Composition of raw milk and standardized pasteurized cheese milk, from cows on different feeding systems (GRO, GRC, TMR) in mid and late lactation[1,2,3]

	Mid lactation (ML)				Late lactation (LL)				Overall effects (P-value) throughout lactation (ML+LL)		
Item	GRO	GRC	TMR	SED[4]	GRO	GRC	TMR	SED[4]	Feeding system (FS)	Lactation period (LP)	Interaction FS × LP
Raw milk											
Fat (%, wt/wt)	4.03^{c}	4.00^{c}	4.03^{c}	0.031	4.82^{a}	4.78^{ab}	4.66^{b}	0.028	0.081	<0.001	0.044
Protein (%, wt/wt)	3.50^{d}	3.51^{d}	3.20^{e}	0.022	4.08^{a}	3.96^{b}	3.65^{c}	0.019	<0.001	<0.001	0.010
Casein (%, wt/wt)	2.76^{c}	2.74^{c}	2.54^{d}	0.017	3.17^{a}	3.13^{a}	2.90^{b}	0.015	<0.001	<0.001	0.337
Lactose (%, wt/wt)	4.75^{a}	4.74^{a}	4.80^{a}	0.069	4.57^{b}	4.59^{b}	4.66^{a}	0.060	0.477	0.021	0.896
Cheese milk											
Fat (%, wt/wt)	3.00^{b}	3.01^{b}	2.71^{c}	0.036	3.39^{a}	3.29^{a}	3.02^{b}	0.031	<0.001	<0.001	0.297
Protein (%, wt/wt)	3.46^{c}	3.46^{c}	3.12^{d}	0.031	4.07^{a}	3.95^{a}	3.64^{b}	0.026	<0.001	<0.001	0.141
Lactose (%, wt/wt)	4.70^{a}	4.70^{a}	4.74^{a}	0.067	4.51^{b}	4.54^{b}	4.61^{a}	0.058	0.476	0.010	0.925
Macroelements (mg/100 g)											
Ca	108.3^{bc}	114.3^{abc}	101.8^{c}	4.90	136.4^{a}	132.8^{a}	130.2^{ab}	4.25	0.254	<0.001	0.339
P	82.2^{a}	93.3^{a}	90.1^{a}	4.69	99.3^{a}	96.3^{a}	93.1^{a}	4.06	0.640	0.053	0.211
Na	39.5^{a}	44.1^{a}	46.9^{a}	7.56	51.7^{a}	51.9^{a}	51.3^{a}	6.54	0.877	0.190	0.866
Mg	9.2^{c}	9.9^{bc}	9.8^{bc}	0.61	13.5^{a}	12.7^{a}	12.2^{ab}	0.53	0.815	<0.001	0.266
Trace elements (µg/kg)											
Zn	3,796^{b}	3,855^{ab}	3,540^{ab}	248	4,257^{a}	4,114^{ab}	4,302^{a}	214	0.600	<0.001	0.303
I	177^{c}	67.1^{c}	640^{b}	133	190^{c}	133^{c}	1,245^{a}	115	<0.001	0.041	0.059
Cu	59.1^{bc}	54.2^{c}	94.1^{a}	5.34	42.7^{c}	39.3^{c}	72.0^{ab}	4.62	<0.001	0.001	0.767
Mo	33.3^{a}	34.9^{a}	45.7^{a}	3.57	47.3^{a}	42.0^{a}	47.4^{a}	3.09	0.068	0.013	0.215
Se	17.4^{c}	18.2^{c}	32.8^{a}	1.32	25.1^{b}	22.3^{bc}	32.1^{a}	1.14	<0.001	0.002	0.015

[a–e]Values within a row relating to mid lactation and late lactation and not sharing a common lowercase superscripted letter differ significantly ($P < 0.05$) for the effect of feeding system.

[1]Presented data are the mean values of 3 replicate trials in mid lactation and 4 in late lactation.

[2]Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration.

[3]Lactation period (LP): mid lactation [May 23–June 8; 94–110 d in lactation (DIL)] and late lactation (October 10–November 5; 234–260 DIL).

[4]SED = standard error of difference between means.

milking teat dipping treatment containing I (O'Brien et al., 2013), or 200 to 530 μg/kg in creamery milk over the year (O'Brien et al., 1999; O'Kane et al., 2016).

The concentrations of macroelements (Ca, P, Mg, and Na) were not affected by feeding system in ML, LL, or ML + LL. The TMR milk had higher concentrations of I, Cu, and Se than GRO or GRC milks in ML, LL, and ML + LL. The higher concentrations of I and Se in TMR milk is likely to reflect higher concentrations in the TMR compared with pasture (Castro et al., 2012; O'Brien et al., 2013); the concentrations of the latter elements in bovine milk have been found to increase linearly with quantity in the diet (Juniper et al., 2006; Heard et al., 2007). Feeding system did not affect the concentrations of Zn and Mo.

Lactation period had a significant effect on the concentrations of most elements, with LL milk having higher concentrations of Ca, Mg, Zn, I, Mo, and Se than ML milk. In contrast, the concentration of Cu in ML was higher than in LL; otherwise, lactation period did not influence the concentrations of P or Na. The generally higher concentrations of Ca, Mg, Zn, and Se in LL milks are consistent with the increase in protein and, hence, casein concentration in the milk; these elements are predominantly associated with casein, as evidenced by their sedimentation with the casein during ultracentrifugation (Vegarud et al., 2000; Gulati et al., 2018).

Cheese Composition

Gross Composition and pH. The compositions of the GRO, GRC, and TMR cheeses in ML and LL (Table 2) were within the range previously reported for LMPS Mozzarella cheese (Kindstedt et al., 1995; Guinee et al., 2000; Feeney et al., 2001) and comply with the Codex Alimentarius Standard for low-moisture Mozzarella cheese (WHO/FAO, 2011) and the Code of Federal Regulations for LMPS Mozzarella (CFR, 2016). All compositional parameters and pH at 1 d were unaffected by feeding system in ML, LL, or ML + LL. The absence of an effect of feeding system on cheese composition is consistent with the results of previous studies showing little effect of plane of cow nutrition or breed on LMPS Mozzarella (Guinee et al., 1998) or Cheddar cheese (Auldist et al., 2004, 2016; O'Callaghan et al., 2017). Lactation period had a significant effect on composition, with cheeses from LL milk having significantly higher contents of moisture, salt-in-moisture, and moisture-in-nonfat substances, and lower contents of protein, fat and fat-in-DM. The higher moisture in the LL cheeses was most likely associated with the standardization of the LL milk to a higher protein-to-fat ratio than that of the ML milk (i.e., 1.20 vs. 1.15). Standardization of LL milk to a higher protein-to-fat ratio was undertaken to counteract the anticipated reduction in moisture of LL cheeses to a content below that of the corresponding cheese from ML milk, owing to the higher protein concentration (~0.5 to 0.6%, wt/wt) of all LL milk samples (Soodam and Guinee, 2018). Generally, all conditions being equal, increasing the protein of milk results in a reduction in moisture content (i.e., ~0.29% per 0.1% increase in milk protein in the range 3.0–4.5%; Guinee et al., 2006).

Elements. The contents of Ca, P, and Na in the cheese were similar to those reported previously for low-moisture Mozzarella (USDA, 1976; Guinee et al., 2000; Feeney et al., 2001; Govindasamy-Lucey et al., 2007). The contents of Mg, Zn, and Se were comparable to those reported previously for LMPS Mozzarella (USDA, 1976) or Mozzarella (Gaucheron, 2013); that is, ~21 to 26 mg/100 g, 24,600 to 31,300 μg/kg, and 161 μg/kg, respectively. In contrast, the Cu content was generally higher than that (~220 μg/kg) given by Gaucheron (2013) for Mozzarella. Interstudy differences in mineral content may relate to differences in milk as influenced by diet and season (Nantapo and Muchenje, 2013; Gulati et al., 2018), cheesemaking conditions that alter the extent of moisture loss and mineral solubilization at whey drainage, and elements present in the dry salt added to the curd or in the water used for curd plasticization. We are unaware of any previous studies on the concentrations of I and Mo in LMPS Mozzarella cheese.

The TMR cheese had higher mean concentrations of I, Cu, and Se than the corresponding GRC or GRO cheeses in the overall lactation period, ML + LL; however, the specific effect of feeding system on the concentrations of these elements in ML cheese or LL cheese depended on the element (Table 2). The high concentrations of I, Cu, and Se in TMR cheese are consistent with their relatively high concentration in TMR milk (Table 1). No significant differences were observed between the cheeses for concentrations of Ca, P, Na, Mg, Zn, and Mo. Lactation period did not affect the concentration of elements in cheese, apart from Zn, Cu, and Mo, the contents of which were higher in ML cheeses than LL cheeses.

The recovery of elements, expressed as the weight of an element in 100 g of cheese as a percentage of the weight of the element in milk required to produce 100 g of cheese, were 58 to 77% Ca, 55 to 67% P, 25 to 37% Mg, 96 to 109% Zn, 74 to 93% Cu, 19 to 33% Mo, 46 to 63% Se, and 11 to 29% I. The relatively high recovery value for Zn and low values for Mg and Mo are consistent with the high proportion of sedimentable Zn and low proportions of sedimentable Mg and Mo in milk (Gaucheron, 2013; Gulati et al., 2018). We found

Table 2. Composition of low-moisture part-skim Mozzarella cheese from milk obtained using different feeding systems in mid and late lactation[1,2,3]

	Mid lactation (ML)				Late lactation (LL)				Overall effects (P-values) throughout lactation (ML+LL)		
Item	GRO	GRC	TMR	SED[4]	GRO	GRC	TMR	SED[4]	Feeding system (FS)	Lactation period (LP)	Interaction FS × LP
Moisture (%, wt/wt)	46.4^{b}	46.4^{b}	46.3^{b}	0.481	48.4^{a}	47.8^{ab}	47.1^{ab}	0.416	0.302	0.001	0.408
Protein (%, wt/wt)	30.4^{a}	29.0^{ab}	29.2^{ab}	0.574	26.6^{b}	26.5^{b}	27.0^{b}	0.497	0.257	<0.001	0.104
Fat (%, wt/wt)	21.4^{abc}	21.6^{a}	21.5^{ab}	0.293	20.1^{c}	20.3^{bc}	20.5^{abc}	0.253	0.686	<0.001	0.793
SM[5] (%, wt/wt)	3.47^{a}	3.50^{a}	3.60^{a}	0.099	3.64^{a}	3.87^{a}	3.73^{a}	0.086	0.372	0.010	0.413
MNFS[5] (%, wt/wt)	59.0^{a}	59.2^{a}	58.9^{a}	0.518	60.6^{a}	60.0^{a}	59.2^{a}	0.449	0.322	0.029	0.399
FDM[5] (%, wt/wt)	39.9^{a}	40.3^{a}	39.9^{a}	0.472	39.0^{a}	38.9^{a}	38.7^{a}	0.409	0.815	0.006	0.779
pH at d 1	5.36^{a}	5.40^{a}	5.39^{a}	0.022	5.42^{a}	5.42^{a}	5.39^{a}	0.019	0.136	0.241	0.826
Macroelements (mg/100 g)											
Ca	899^{a}	845^{a}	838^{a}	51.2	810^{a}	726^{a}	816^{a}	36.2	0.343	0.053	0.529
P	578^{a}	557^{a}	561^{a}	24.6	549^{a}	489^{a}	542^{a}	21.3	0.125	0.476	0.812
Na	689^{a}	706^{a}	744^{a}	40.5	773^{a}	688^{a}	747^{a}	35.1	0.435	0.469	0.388
Mg	36^{a}	34^{a}	38^{a}	4.26	32^{a}	28^{a}	30^{a}	3.69	0.625	0.074	0.839
Trace elements (μg/kg)											
Zn (×10^{3})	40.7^{a}	43.4^{a}	43.0^{a}	3.20	40.2^{a}	35.9^{a}	42.7^{a}	2.8	0.080	0.005	0.991
I	537^{bc}	123^{c}	1840^{ab}	325	175^{c}	150^{c}	2665^{a}	281	<0.001	0.521	0.173
Cu	580^{ab}	502^{ab}	914^{a}	108.6	338^{b}	298^{b}	517^{ab}	94.1	0.002	0.015	0.890
Mo	108^{ab}	95^{ab}	129^{a}	13.4	84^{ab}	71^{b}	91^{ab}	11.6	0.138	0.013	0.809
Se	114^{bc}	88^{c}	237^{a}	18.3	114^{bc}	93^{c}	171^{ab}	15.9	<0.001	0.170	0.107

$^{a–c}$Values within a row relating to mid lactation and late lactation and not sharing a common lowercase superscripted letter differ significantly ($P < 0.05$) for the effect of feeding system.

[1]Presented data are the mean values of 3 replicate trials in mid lactation and 4 in late lactation.

[2]Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration.

[3]Lactation period (LP): mid lactation [May 23–June 8; 94–110 d in lactation (DIL)] and late lactation (October 10–November 5; 234–260 DIL).

[4]SED = standard error of difference between means.

[5]SM = salt-in-moisture; MNFS = moisture-in-nonfat substances; FDM = fat-in-DM.

no effect of feeding system or lactation period on the recoveries of different elements.

Composition of Whey and Stretch Water

Whey Streams. The protein concentration and fat content in bulk whey and salty whey are shown in Table 3. Protein in the bulk whey (0.78 to 0.91%, wt/wt) in ML was comparable in magnitude to that reported previously for LMPS Mozzarella (Guinee et al., 1998, 2000).

Feeding system and lactation period affected the protein concentration of bulk whey. The concentration of protein in GRC bulk whey was higher than that in TMR bulk whey during ML, LL, and ML + LL, whereas the concentration in GRO bulk whey was higher than that in TMR bulk whey in LL only. The mean protein concentration of bulk whey in LL was higher than that in ML. The higher protein concentration in LL is consistent with the increase in protein concentration of the cheese milk (Table 1). Previous studies reported a linear increase in the protein concentration of bulk whey as protein in the cheese milk was increased from 3.3 to 8.0% (wt/wt) using low-concentration factor ultrafiltration (Soodam and Guinee, 2018). The increase in bulk whey protein with milk protein is consistent with the relatively high rate of whey expulsion during the early stages of stirring (following cutting), when the differences in protein content between gels (from milks with different protein concentrations) still persist and manifest in the whey. As stirring time progresses, the outwork migration of whey and, most likely, the diffusivity of whey components (e.g., protein) also diminish (Everard et al., 2008; Mateo et al., 2009; Silva et al., 2015), concomitant with dehydration and concentration of paracasein within the cheese curd particle matrix.

The protein concentration of salty whey was unaffected by feeding system or lactation period, despite the difference in protein between TMR and pasture-based milks, and between the ML and LL milks. A similar trend was observed by Ong et al. (2013), namely no change in the protein concentration of salty whey from Cheddar cheese curd on increasing milk protein concentration from 3.5 to 6.0% (wt/wt). Such a trend is expected, owing to the impedance of the concentrated cheese matrix to the passage and diffusion of relatively large macromolecular solutes such as lactose and whey proteins (Silva et al., 2015; Czárán et al., 2018).

Fat in bulk whey and salty whey ranged from 0.37 to 0.53% (wt/wt) and 3.56 to 5.68% (wt/wt), respectively. These values for bulk whey are within the range (0.25 to 0.56%, wt/wt) previously reported for LMPS Mozzarella-style cheese (Guinee et al., 2000; Govindasamy-Lucey et al., 2005, 2007); little, or no, information is available on the fat content of salty whey for conventionally manufactured LMPS Mozzarella. Feeding system had no effect on the fat content of bulk whey or salty whey in ML, LL, or ML + LL. Lactation period affected the fat content of bulk whey and salty whey, both of which were higher in LL than in ML. Hence, unlike the positive relationship between milk protein and bulk whey protein, we observed no consistent trend between the concentration of protein in milk and fat content of whey samples in the current study. Variable results have been reported for the effect of milk protein concentration on fat content of bulk whey or salty whey. Ong et al. (2013) reported no change in the fat content of bulk whey from Cheddar cheese curd when increasing milk protein from 3.0 to 4.0% (wt/wt). In contrast, Govindasamy-Lucey et al. (2007) found an increase in fat content of bulk whey (0.25% to 0.31%, wt/wt) from non-pasta filata LMPS pizza cheese when increasing milk protein from 3.1 to 4.0% (wt/wt). The interstudy discrepancy may reflect differences in gel firmness at cutting, cut program, and the firming rate of curd particles after cutting. The latter factors are likely to influence the rate of the paracasein concentration and contraction of the paracasein network and the ability of the network to retain occluded fat, especially during the early stages of stirring and cooking.

Stretch Water. Protein and fat in the stretch water ranged from ~0.13 to 0.24% (wt/wt) and 2.13 to 3.45% (wt/wt), respectively. Feeding system affected the values of protein and fat in stretch water, both of which were higher in stretch water from GRO milk than from GRC milk in ML + LL. In contrast to the trend for bulk whey, the mean fat content of stretch water was higher in ML than in LL.

Component Losses and Cheese Yield. The percentage of milk protein and fat lost in the combined whey and stretch water streams [i.e., ~23.0–24.4% (wt/wt) and ~26.0–28.2% (wt/wt) respectively; Table 4] are of similar magnitude to those reported previously by Guinee et al. [2000; i.e., ~24–27% (wt/wt) protein and ~20–24% (wt/wt) fat]. Though the protein loss is typical of that reported for other rennet curd cheeses, the proportion of fat lost is markedly higher compared with that (~10–13%, wt/wt, of total fat) for cheeses such as Cheddar, Edam, and Emmental (Antila et al., 1982; Fenelon and Guinee, 1999). The higher fat loss during the manufacture of LMPS Mozzarella has been attributed to the kneading and stretching of the curd in hot (~80°C) water (Fox et al., 2017), which is conducive to shearing of the fat globule membrane, coalescence of fat into large pools (McMahon and Oberg, 2017), and leaching of free fat into the stretch water. Hence, the current results show that ~13 to 15% (wt/wt) of the total milk fat was lost in the stretch water (Table 4).

Table 3. Composition of bulk whey, salty whey, and stretch water, produced during cheesemaking from milk obtained using different feeding systems in mid and late lactation[1,2,3]

	Mid lactation (ML)				Late lactation (LL)				Overall effects (P-values) throughout lactation (ML+LL)		
Item	GRO	GRC	TMR	SED[4]	GRO	GRC	TMR	SED[4]	Feeding system (FS)	Lactation period (LP)	Interaction FS × LP
Bulk whey											
Weight (kg/100 kg of milk)	88.6^{ab}	88.4^{abc}	88.9^{a}	0.256	86.7^{d}	87.4^{cd}	87.7^{bc}	0.222	0.048	<0.001	0.140
Protein (%, wt/wt)	0.86^{bc}	0.91^{b}	0.78^{c}	0.018	1.10^{a}	1.04^{a}	0.90^{b}	0.016	<0.001	<0.001	0.008
Fat (%, wt/wt)	0.37^{a}	0.38^{a}	0.40^{a}	0.037	0.53^{a}	0.51^{a}	0.43^{a}	0.032	0.298	0.001	0.451
Salty whey											
Weight (kg/100 kg of milk)	0.87^{a}	0.89^{a}	0.72^{a}	0.122	1.22^{a}	0.93^{a}	0.83^{a}	0.106	0.090	0.090	0.387
Protein (%, wt/wt)	0.91^{a}	0.87^{a}	0.81^{a}	0.072	1.03^{a}	0.94^{a}	0.92^{a}	0.063	0.308	0.098	0.926
Fat (%, wt/wt)	3.56^{a}	3.60^{a}	4.15^{a}	0.597	4.19^{a}	5.68^{a}	4.65^{a}	0.517	0.478	0.029	0.331
Stretch water											
Weight (kg/100 kg of milk)	13.2^{b}	15.9^{ab}	13.2^{b}	1.08	17.0^{ab}	20.1^{a}	15.6^{ab}	0.943	0.008	0.001	0.688
Protein (%, wt/wt)	0.24^{a}	0.16^{bc}	0.18^{abc}	0.014	0.20^{ab}	0.13^{c}	0.16^{bc}	0.014	<0.001	0.045	0.769
Fat (%, wt/wt)	3.45^{a}	2.38^{ab}	2.75^{ab}	0.257	2.70^{ab}	2.13^{b}	2.30^{b}	0.223	0.011	0.026	0.589

[a–d]Values within a row relating to mid lactation and late lactation and not sharing a common lowercase superscripted letter differ significantly ($P < 0.05$) for the effect of feeding system.

[1]Presented data are the mean values of 3 replicate trials in mid lactation and 4 in late lactation.

[2]Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration.

[3]Lactation period (LP): mid lactation [May 23–June 8; 94–110 d in lactation (DIL)] and late lactation (October 10–November 5; 234–260 DIL).

[4]SED = standard error of difference between means.

Table 4. Effect of different feeding systems and lactation period on the losses of milk fat and protein to cheese whey and stretch water during the manufacture of low-moisture part-skim Mozzarella cheese in mid and late lacation[1,2,3]

	Mid lactation (ML)				Late lactation (LL)				Overall effects (P-values) throughout lactation (ML+LL)		
Item	GRO	GRC	TMR	SED[4]	GRO	GRC	TMR	SED[4]	Feeding system (FS)	Lactation period (LP)	Interaction FS × LP
Protein losses (% of total milk protein)											
Bulk whey	22.0^{ab}	23.2^{a}	22.2^{ab}	0.364	23.3^{a}	23.1^{ab}	21.6^{b}	0.315	0.007	0.467	0.036
Salty whey	0.22^{ab}	0.22^{ab}	0.18^{b}	0.027	0.31^{a}	0.22^{ab}	0.21^{ab}	0.023	0.042	0.071	0.252
Stretch water	0.92^{a}	0.76^{a}	0.74^{a}	0.057	0.83^{a}	0.70^{a}	0.77^{a}	0.051	0.271	0.649	0.385
Total losses	23.1^{ab}	24.2^{ab}	23.1^{ab}	0.370	24.4^{a}	23.9^{ab}	22.6^{b}	0.324	0.012	0.576	0.037
Fat losses (% of total milk fat)											
Bulk whey	10.9^{a}	11.2^{a}	12.1^{a}	1.07	13.6^{a}	13.7^{a}	12.5^{a}	0.929	0.982	0.041	0.553
Salty whey	0.90^{a}	1.06^{a}	1.03^{a}	0.194	1.47^{a}	1.74^{a}	1.27^{a}	0.168	0.395	0.008	0.488
Stretch water	15.2^{a}	12.7^{a}	13.1^{a}	0.871	13.4^{a}	13.0^{a}	12.9^{a}	0.754	0.175	0.439	0.457
Total losses	27.1^{a}	25.8^{a}	24.9^{a}	1.41	28.4^{a}	29.1^{a}	26.2^{a}	1.22	0.525	0.112	0.360
Cheese yield[5] (kg/100 kg of milk)											
Y_a	9.30^{cd}	9.72^{bc}	8.68^{d}	0.166	11.48^{a}	10.96^{a}	10.11^{b}	0.144	<0.001	<0.001	0.020
Y_n	9.14^{b}	9.54^{ab}	9.46^{ab}	0.124	9.59^{a}	9.44^{ab}	9.46^{ab}	0.107	0.574	0.007	0.071

[a-d]Values within a row relating to mid lactation and late lactation and not sharing a common lower-case superscripted letter differ significantly ($P < 0.05$) for the effect of feeding system.

[1]Presented data are the mean values of three replicate trials in mid lactation and four in late lactation.

[2]Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration.

[3]Lactation period (LP): mid lactation [May 23–June 8; 94–110 d in lactation (DIL)] and late lactation (October 10–November 5; 234–260 DIL).

[4]SED = standard error of difference between means.

[5]Y_a = actual cheese yield; Y_n = normalized cheese yield per 100 kg of milk normalized to reference fat (2.89%, wt/wt) and protein (3.40%, wt/wt) levels.

The percentage of milk protein lost in the whey plus stretch water from TMR milk was 1 to 2% lower than that of the corresponding whey from the GRO milk in LL and in ML + LL; such an effect was not observed in ML. The percentage of milk fat lost in the combined whey and stretch water streams was unaffected by feeding system in ML, LL, or ML + LL. Lactation period had no effect on the overall losses of fat or protein from milk to whey and stretch water.

Actual cheese yield (Y_a) varied from 8.7 to 11.5 kg/100 kg of milk, which is typical of that reported for LMPS Mozzarella cheese (Guinee et al., 1998, 2000; Lilbæk et al., 2006). The Y_a from GRO or GRC milk were higher than that from TMR milk ($P < 0.05$) in LL and ML+LL; however, Y_a from GRO or TMR were similar in ML. The generally lower Y_a of TMR cheese milk coincides with its lower protein concentration and fat content (Table 1), which are major determinants of cheese yield (Fox et al., 2017). The effect of differences in protein concentration and fat content of milk on cheese yield was confirmed by the absence of a significant effect of feeding system on Y_n in ML, LL or ML+LL, and by the similar Y_n for corresponding GRC and TMR milks in ML and LL. Normalizing yield to a reference milk with defined percentages of fat and protein and standardized to a fixed protein-to-fat ratio mitigates the effects of differences in milk composition on cheese yield.

Proteolysis

The pH 4.6 SN, as a percentage of total N, increased in all cheeses during storage ($P < 0.05$), from ~2 to 3% at 1 d to ~5% at 50 d. The relatively low percentage of pH 4.6 SN compared with other hard cheeses, such as Cheddar (McCarthy et al., 2016), was typical for LMPS Mozzarella (Yun et al., 1993) and reflects the heat-induced denaturation of the coagulant at high temperature (58–62°C) during the plasticization stage of manufacture (Feeney et al., 2001).

Feeding system had no affect proteolysis in ML, LL, or in overall lactation (ML + LL). Such a trend is consistent with the similar composition of cheeses from all 3 feedings systems (Table 2) and concurs with the findings of O'Callaghan et al. (2017) for Cheddar cheese. Lactation period also had an effect, with the overall mean percentage of pH 4.6 SN in LL cheeses from the different feeding systems being significantly higher than that of the corresponding ML cheeses.

WHC. The WHC has been used as an index of the serum immobilized by the calcium phosphate paracasein network (Guinee et al., 2000). It increased progressively from ~1.4 to 1.6–1.8 g/g of protein between 1 and 20 d, after which it remained constant (as no further serum was expressed). The increase during early storage is consistent with the results of previous studies (Guinee et al., 2002) and reflects the hydration and swelling of the paracasein network (McMahon and Oberg, 2017), concomitant with proteolysis and calcium solubilization (Guo and Kindstedt, 1995; O'Mahony et al., 2005).

The mean WHC of GRO cheese during storage was higher than that in TMR cheese in LL; otherwise, feeding system had no effect in ML, LL, or ML + LL. The mean WHC of LL cheeses from the GRO, GRC, and TMR milks was higher than that of the corresponding ML cheeses.

Texture Profile Analysis

The changes in textural parameters during storage are shown in Figure 1. The range of firmness (250–450 *N*) was comparable to that (i.e., ~320–420 *N*) previously reported for LMPS Mozzarella cheese, compressed under similar conditions (Guinee et al., 2001). The values of cohesiveness (~0.27–0.52), springiness (0.64–0.78), and chewiness (72–145 *N*), all of which are indices of the resistance of the cheese to fracture and size reduction, were within the range previously reported for Mozzarella and Kachkaval-type cheeses (Chevanan et al., 2006; Guinee et al., 2015; Henneberry et al., 2016); none of the cheeses fractured on compression by 75% (data not shown).

The firmness of all cheeses from ML milk decreased significantly during storage, from ~430 *N* at 1 d to ~230 to 320 *N* after 50 d ($P < 0.05$; Table 5). Despite a similar downward trend in the firmness of all cheeses in LL (Figure 1), the reduction was not significant. Similar trends have been previously reported for low-moisture LMPS Mozzarella (Yun et al., 1993; Moynihan et al., 2016) and have been attributed to the increases in proteolysis and protein hydration. In contrast, storage resulted in an increase in the cohesiveness of all LL cheeses ($P < 0.05$) but not ML cheeses. Otherwise, we found no significant change in the chewiness or springiness during storage.

The mean values of firmness, cohesiveness, or springiness during storage were not significantly affected by feeding system in ML, LL, or ML + LL. Chewiness was influenced by feeding system in ML, with the mean value of the GRO cheese over the storage period being higher than that of GRC cheese. The current results differ from those of Combs et al. (2007), who stated that Cheddar cheese made from milk of cows grazed on pasture (low-endophyte tall fescue with kura clover) was consistently softer than that from milk of cows fed on TMR (grain-based feed with alfalfa silage as the sole forage); no details were given on the conditions of ripening or texture measurement. O'Callaghan et al.

(2017) evaluated the effect of feeding system on Cheddar cheese, using milk from the same herds (GRO, GRC, and TMR) as evaluated in the current study. Those authors found that the mean firmness, cohesiveness, or chewiness of Cheddar cheese after maturation for 3 or 9 mo was not affected by feeding system when the cheeses were tempered to 4°C before rheological evaluation. Nevertheless, the 9-mo-old cheese from TMR milk was significantly firmer than that of cheese from the GRO or GRC milk when the cheeses were equilibrated to room temperature (20°C for 3 h) before measurement. The latter trend was attributed to the higher proportion of palmitic acid in fat from TMR milk (O'Callaghan et al., 2016). Palmitic acid is the major fatty acid in milk fat and has a relatively high melting point (~63°C) compared with oleic acid (~14°C), the second most abundant fatty acid in milk fat (Huppertz et al., 2009; Knothe and Dunn, 2009).

Lactation period had a significant effect on firmness (Table 5), with the mean value for the ML cheeses being higher than that of the LL cheeses. Such an effect is consistent with the lower moisture content and pH 4.6 SN as a percentage of total N in the former (Visser, 1991; Watkinson et al., 2001).

Color. The color coordinates (L*, a*, b*) are shown in Figure 2. They are indices of color dimensions, green to red (a*), blue to yellow (b*), and lightness (L*), which constitutes the balance of green, red, and blue. On storage, a* did not change, b* increased in GRO and GRC cheeses, and L* decreased in all cheeses. Visually, the GRO and GRC cheeses became more yellow and the TMR cheese less white during storage. The decrease in L* during aging has also been reported for reduced-fat Mozzarella (Rudan et al., 1998; Sheehan et al., 2005). This may be may be attributed partly to the reduction in free moisture pockets (droplets) and light scattering, as the water binding of the casein increases (Paulson et al., 1998), and to the change in the relative intensities of redness (a*) to yellowness (b*). In contrast to the current results, O'Callaghan et al. (2017) found that the L* and a* values of Cheddar cheese increased and b* values decreased during maturation (90 and 270 d); it was suggested that the reduction in L* may have been due to the light-induced degradation of carotenoids and riboflavin in the cheese (Juric et al., 2003). However, cheese variety may also affect the changes in color during ripening because of differences in fat content and age-related transitions in the distributions of moisture, fat, and protein (Auty et al., 2001; McMahon and Oberg, 2017). β-Carotene has been found to be quite stable during cheese maturation (Nozière et al., 2006).

The GRO and GRC cheeses had a significantly lower mean a* values and higher mean b* values compared with TMR cheeses in ML, LL, and ML + LL. The higher b* value in the GRO and GRC cheeses agrees with the general observation that milk from pasture-fed cows is more yellow than that of cows fed indoors on concentrates, owing to its higher concentration of β-carotene (Nozière et al., 2006). The L* value was unaffected by feeding system. Visually, the TMR cheese was notably whiter that the GRO and GRC cheeses at all storage times; the latter were typically had a pale butter-yellow color. The current results concur with those of O'Callaghan et al. (2016), who showed that Cheddar cheese from GRO or GRC milk had higher b* values and were more yellow than cheese from TMR milk. However, our results differ with respect to L*

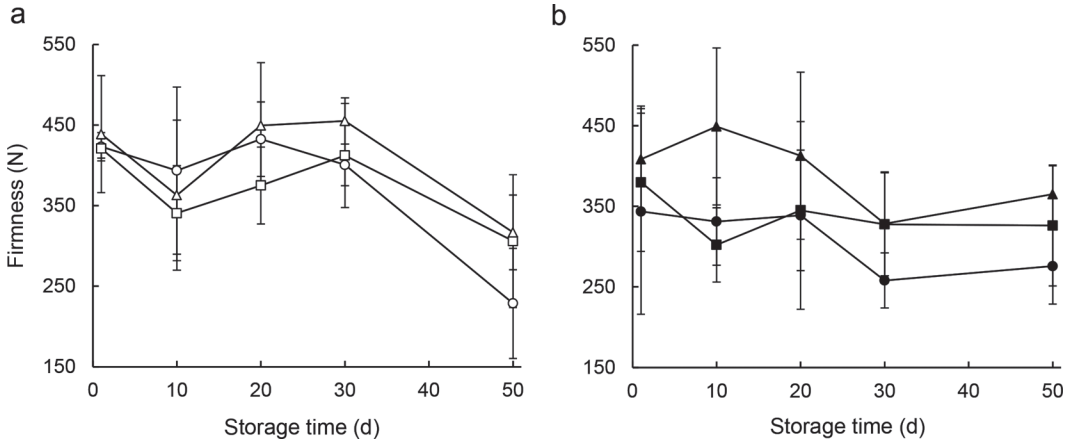

Figure 1. Storage-related changes in the firmness of low-moisture part-skim Mozzarella cheese in mid lactation (open symbols; a) or late lactation (closed symbols; b) from milk produced using different dairy cow feeding systems: grazing on perennial ryegrass pasture (GRO; ○,●), grazing on perennial ryegrass and white clover pasture (GRC; □,■), or housed indoors and offered total mixed ration (TMR; △,▲). Presented values are the means of 3 replicate trials in mid lactation and 4 in late lactation; error bars represent SD of the mean.

Table 5. Statistical significances (P-values) for changes in physicochemical properties, color coordinates, texture, and thermophysical properties of low-moisture part-skim Mozzarella cheeses from mid- and late-lactation milks from cows on different feeding systems[1,2,3]

	Effects in mid lactation (ML)			Effects in late lactation (LL)			Overall effects throughout lactation (ML + LL)	
Item	Feeding system (FS)	Storage time (ST)	Interaction FS × ST	Feeding system (FS)	Storage time (ST)	Interaction FS × ST	Feeding system (FS)	Lactation period (LP)
Physicochemical properties								
pH	—	***	—	—	***	—	—	—
WHC (g of water/g of protein)	—	***	—	*	***	—	—	***
pH4.6 SN/TN (g/100 g)	—	***	—	—	***	—	—	***
Color coordinates								
L* value	—	***	—	—	**	—	—	—
a* value	*	—	—	**	—	—	*	—
b* value	***	*	—	***	***	—	***	—
Texture								
Firmness (N)	—	***	—	—	—	—	—	*
Cohesiveness (-)	—	—	—	—	***	—	—	—
Springiness (-)	—	—	—	—	—	—	—	—
Chewiness (N)	*	—	—	—	—	—	—	—
Thermophysical properties								
Flowability (%)	***	***	—	**	***	—	***	—
Extension work, E_W (mJ)	—	***	—	—	***	—	—	***
CoTh (°C)	—	***	—	**	***	***	**	*
LT_{max} (-)	**	***	**	*	***	—	***	***
COTc (°C)	—	***	—	—	***	*	—	***

[1]Degree of freedom: 2 for feeding system, 4 for 50-d storage time of cheese, 8 for interaction of feeding system and storage time in mid or late lactation, 2 for effect of feeding system during overall lactation (ML+LL), and 1 for the effect of lactation period across the different feeding systems.

[2]Feeding system (FS): GRO = grazing on perennial ryegrass pasture; GRC = grazing on perennial ryegrass and white clover pasture; TMR = housed indoors and offered total mixed ration.

[3]Lactation period (LP): mid lactation [May 23–June 8; 94–110 d in lactation (DIL)] and late lactation (October 10–November 5; 234–260 DIL).

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; — = $P > 0.05$.

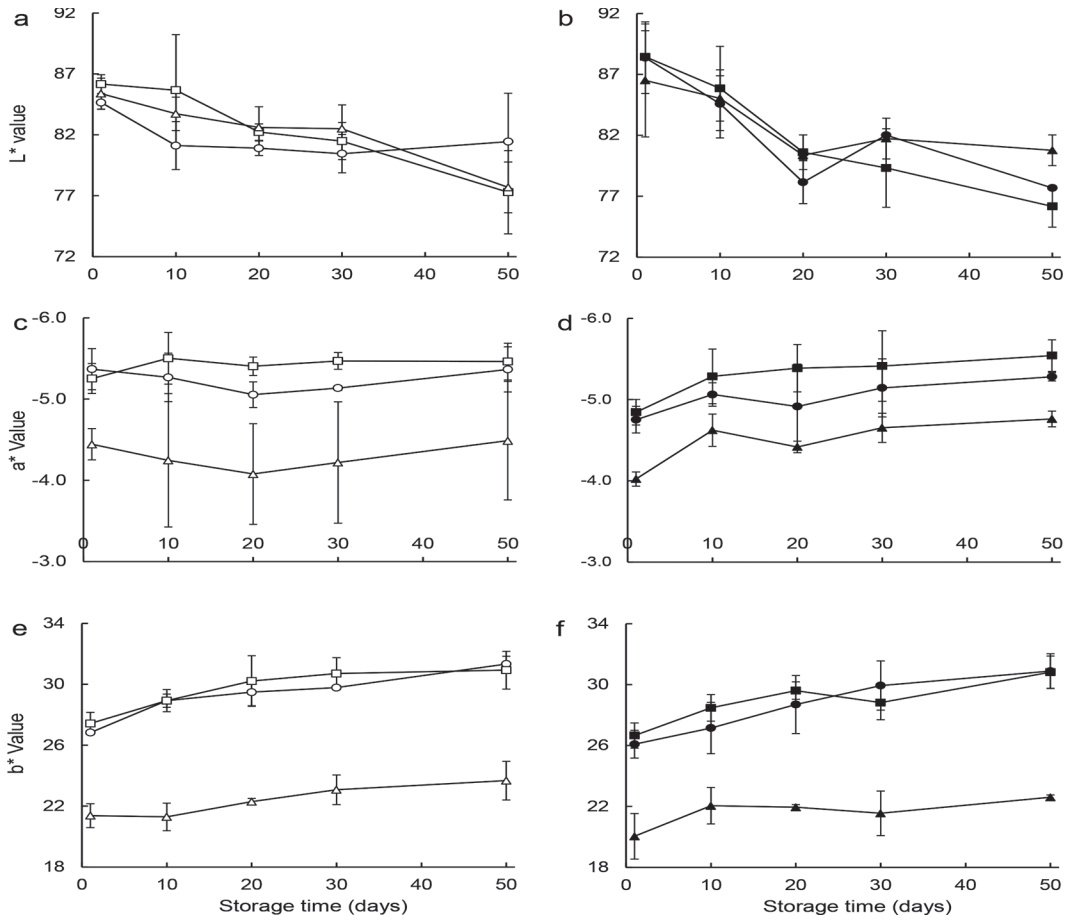

Figure 2. Storage-related changes in the color coordinates, lightness (L*) (a, b), green to red (a*) (c, d), and blue to yellow (b*) (e, f), of low-moisture part-skim Mozzarella cheese in mid lactation (open symbols) and late lactation (closed symbols) from milk produced using different dairy cow feeding 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 are the means of 3 replicate trials in mid lactation and 4 in late lactation; error bars represent SD of the mean.

value, which for the current Mozzarella cheeses did not significantly differ with feeding system. The difference between the current study and that of O'Callaghan et al. (2017), with respect to the effect of feeding system on L* value (lightness), may, as discussed above, arise from differences in fat content and microstructure between Cheddar and Mozzarella. The color differences between the TMR and GRO or GRC cheeses could influence consumer preference, to a degree depending on the fat-in-DM content of the cheese (Wadhwani and McMahon, 2012). Lactation period had no effect on the mean values of L*, a*, or b*.

Thermophysical Properties of Cheese

Flow and Extension Work. The heat-induced flowability increased in all cheeses during maturation (Figure 3); simultaneously, the E_w for hot molten cheese decreased ($P < 0.05$). These changes concur with the increases in proteolysis and water-binding capacity. The ensuing increases in hydrolysis of the calcium phosphate paracasein network and moisture retention are expected to facilitate the relative displacement of adjoining planes of the cheese mass during heating and subsequent extension (Lefevere et al., 2000; Guinee, 2016).

Feeding system had a significant effect on flow of the heated cheese but not on E_w (Table 5). The mean flow of the GRO cheese during storage was significantly higher than that of the GRC or TMR cheese in both ML, LL, and ML + LL. The reason for the relatively higher flow of the GRO cheese is unclear considering the similar composition and extent of proteolysis in all cheeses. In contrast to feeding system, lactation period

influenced E_w but not flow. The higher mean E_w of the ML cheeses, relative to LL cheeses, accords with their lower moisture content and degree of proteolysis.

Viscoelastic Changes During Heating and Cooling. On heating from 25 to 90°C, G′ and G″ decreased curvilinearly, and the loss tangent (**LT**), representing the ratio G″:G′, increased simultaneously (Figure 4a, b). The changes in LT, which represent a transition from a largely elastic cheese at 25°C (LT $\ll$ 1) to a more viscous molten cheese mass at 70 to 90°C (LT $\gg$ 1) are typical of those reported for various cheese types, including LMPS Mozzarella (Guinee et al., 2015; Moynihan et al., 2016). The physicochemical changes contributing to the changes in LT have been attributed to heat-induced fat liquefaction and coalescence, microphase separation of serum, and aggregation of serum-soluble proteins (Guinee et al., 2015). On recooling, the molten cheese congealed and LT decreased to <1 (data not shown). The cooling-induced solidification of the molten cheese has been ascribed to reabsorption of free serum and rehydration of the paracasein network and solidification of fat (Dave et al., 2001; Pastorino et al., 2002; Guinee et al., 2015).

Most of the 1-d-old cheeses scarcely melted, with LT_{max} remaining $\leq$1.0. The LT_{max} increased significantly in all cheeses during storage (Figure 4), indicating that the cheeses became more fluid on heating. This trend is similar to that for flowability; hence, linear regression of the data for all ML and LL cheeses during storage indicated a significant linear correlation between the latter parameters, where LT_{max} = 0.0541 flow + 0.64 (R^2 = 0.82). In contrast, the COTc decreased significantly during storage from ~68°C at 10 d to 61°C at 50 d (data not shown).

The mean LT_{max} for the GRO cheese over the 50-d storage period was slightly, but significantly, higher than that of TMR cheese in ML, LL, and ML + LL (Figure 4). The GRC cheese had the lowest LT_{max} in ML and a value intermediate between that of GRO and TMR cheeses in LL. The relatively high LT_{max} of

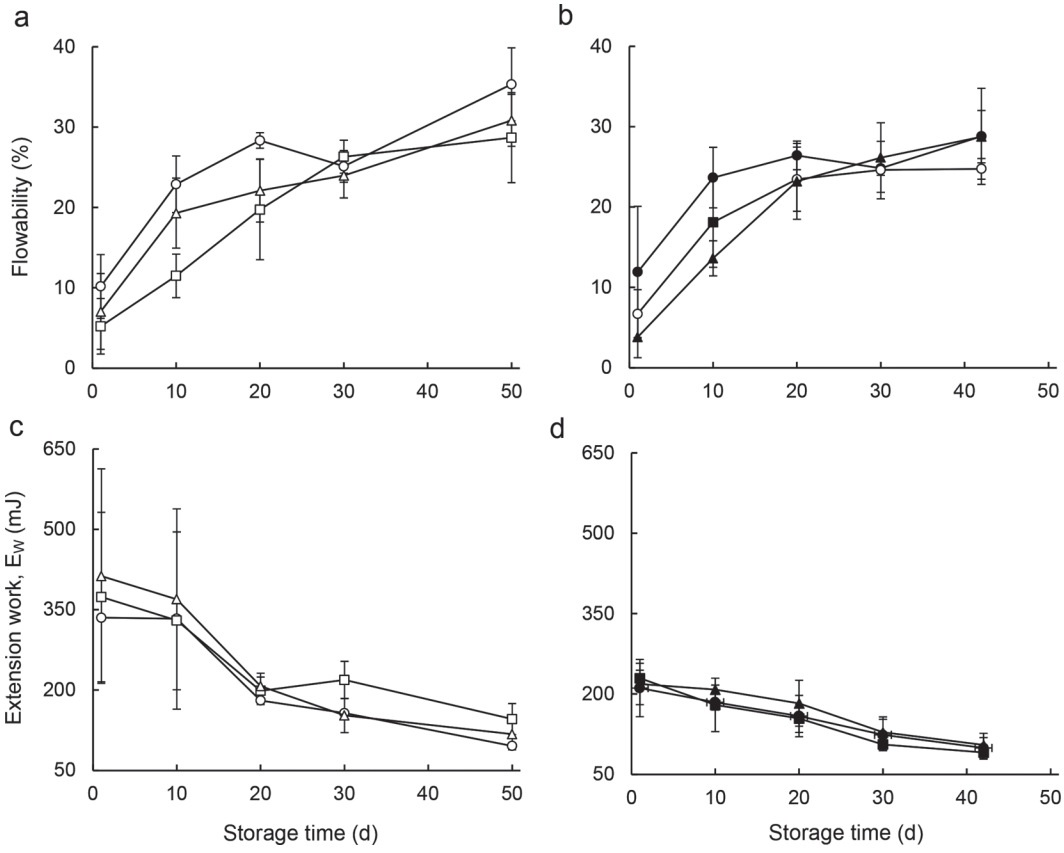

Figure 3. Storage-related changes in the flowability (a, b) and extension work (c, d) of low-moisture part-skim Mozzarella cheese in mid lactation (open symbols) and late lactation (closed symbols) lactation from milk produced using different dairy cow feeding systems: grazing on perennial ryegrass pasture (GRO; ○, ●), grazing on perennial ryegrass and white clover pasture (GRC; □, ■), or housed indoors and offered total mixed ration (TMR; △, ▲). Presented values are the means of 3 replicate trials in mid lactation and 4 in late lactation; error bars represent SD of the mean.

GRO cheese is consistent with its higher flowability, as a more-fluid cheese is expected to flow and spread to a higher degree. The GRO cheese also had a slightly lower mean COTh than TMR cheese in LL and ML+LL ($P < 0.05$), indicating that it melted more quickly. The COTc was not influenced by feeding system in ML, LL, or ML + LL. Lactation period had a significant effect LT_{max}, COTh, and COTc, with the former higher in LL cheese than ML cheese, whereas COTh and COTc were higher in ML cheese. From a practical viewpoint, our results indicate that the GRO cheese melts at a lower temperature and becomes more fluid and flowable

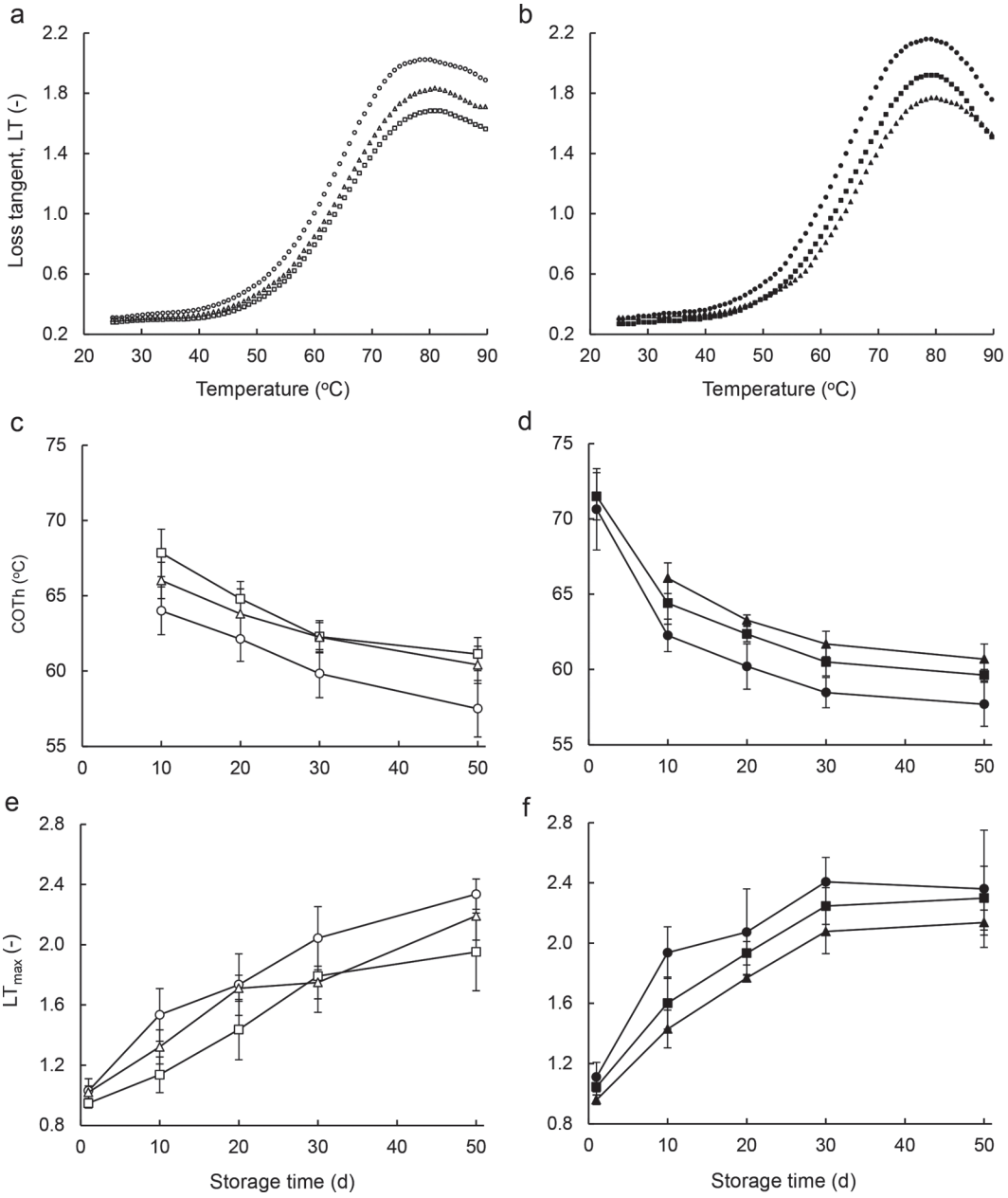

Figure 4. Changes in loss tangent during heating (a, b) and storage-related changes in cross over temperature during heating (COTh; c, d) and maximum loss tangent (LT_{max}; e, f) of low-moisture part-skim Mozzarella cheese in mid lactation (open symbols) and late lactation (closed symbols) from milk produced using different dairy cow 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 data: (a) and (b) are for 20-d-old cheeses from 1 of the replicate trials in mid and late lactation, respectively; c through f are the means of 3 replicate trials in mid lactation and 4 in late lactation. Error bars represent SD of the mean.

than the TMR cheese, but that feeding system does not affect the temperature or time at which the molten cheese congeals on cooling.

CONCLUSIONS

We investigated the effect of 3 different feeding systems (GRO, GRC, or TMR) on the properties of milk and LMPS Mozzarella cheese in mid and late lactation. Milk from pasture-based systems had a higher concentration of protein, a higher cheese-yielding capacity, and produced cheese that had a more yellow color. Moreover, cheese from GRO milk was more flowable and fluid on heating to 90 to 95°C. The TMR milk, and cheese from TMR milk, had higher concentrations of I, Cu, and Se. Otherwise, feeding systems had little, or no, effect on losses of milk fat and protein to whey, cheese composition, or texture. From a manufacturer's perspective, the higher yield, and greater heat-induced flow and fluidity of cheese from GRO milk may prove attractive; varied thermophysical properties are a means of providing customized cheese ingredient solutions. Nevertheless, the more yellow color of LMPS Mozzarella cheese from pasture-based milk may be less acceptable in some markets that are more accustomed to eating white-colored cheese varieties.

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