



## Physicochemical properties of whole milk powder derived from cows fed pasture or total mixed ration diets

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### ABSTRACT

This study examined the effect of dietary factors on compositional and functional properties of whole milk powder (WMP) produced from bovine milk. Raw milk samples were obtained from 3 groups of 18 Holstein Friesian spring-calving cows randomly assigned to diets based on perennial ryegrass (GRS), perennial ryegrass/white clover sward (CLV), and total mixed ration (TMR). Raw milks obtained in late lactation were subsequently standardized for fat, heat-treated (90°C for 30 s), evaporated, and homogenized before spray drying. The WMP produced from each diet were analyzed to determine differences in color, particle size distribution, heat coagulation time, yogurt gelation, texture profile, and protein profile due to each diet. Significant differences in heat coagulation time were observed between the CLV and TMR samples, whereas color values were significantly different between GRS and TMR samples. No significant differences in gross composition, protein profile, or whey protein nitrogen index were found between the 3 WMP samples. Average  $D_{90}$  values (the particle size at which 90% of the particles were smaller than the specified size) for fat globules were significantly lower in the TMR sample compared with the GRS and CLV samples. Yogurts produced from GRS- and CLV-derived WMP had significantly higher elastic moduli ( $G'$ ) than those produced from TMR-derived WMP. Similarly, texture profile analysis revealed significantly higher firmness values in yogurt samples derived from CLV compared with TMR samples. Our data characterize the effect of these diets on the composition and functional properties of fat-standardized WMP, suggesting better yogurt functionality and thermal stability in WMP derived from pasture-based bovine diets.

**Key words:** whole milk powder, pasture, total mixed ration, yogurt gelation, heat coagulation time

### INTRODUCTION

Whole milk powder (WMP) is a high-value dairy commodity produced through processing and spray drying of standardized whole milk. Although WMP retains the inherent fat composition of the whole milk from which it is produced, it typically undergoes the same centrifugal separation process as skim milk to yield skim and cream, which are subsequently recombined to standardize the fat content of the whole milk (Kelly et al., 2002). Whole milk powder is used extensively in the production of chocolate and ice cream, but it is also frequently rehydrated as a source of whole milk or for use as the base ingredient in yogurt production.

The temperate climate of countries such as Ireland and New Zealand is most suited to pasture-based milk production systems, the utilization of which has been shown to result in a range of compositional differences in milk compared with that from concentrate-based production systems. The latter systems are widely implemented in the Americas and parts of Europe (Chilliard et al., 2001; Couvreur et al., 2006). Previous studies have demonstrated greater levels of carotenoids (particularly  $\beta$ -carotene), greater unsaturated fatty acid content, most notably CLA (Kelly et al., 1998), and greater true protein content in pasture-derived milk compared with TMR-derived milk (O'Callaghan et al., 2016b). Because of the increased energy density of high-protein TMR diets, their application has been shown to result in an increase in milk yield but not milk protein content (Kolver et al., 2000). Indeed, over-feeding of dietary CP may not necessarily lead to an increase in milk protein content, because excess nitrogen is excreted as urinary nitrogen (Broderick, 2003). Similarly, increasing dietary oil supplements and reducing dietary fiber content can affect rumen biohydrogenation pathways, producing inhibitory fatty acid

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intermediates that limit milk fat synthesis (Bauman and Griinari, 2003). Conversely, pasture-based diets that are higher in roughage content tend to promote the action of cellulolytic rumen microflora, leading to increased milk fat biosynthesis and ultimately higher milk fat content (Bauman and Griinari, 2003).

Differences in milk composition due to dietary variation may have a significant effect on the thermal stability and processability of products derived from WMP. Gel strength, viscosity, and textural qualities of acidified reconstituted WMP are properties of significant importance in determining the behavior of set-style yogurts. Gelation can be influenced by various factors, such as milk fat and protein (particularly casein) content, heat treatment, and homogenization pressure (Lee and Lucey, 2010). Given the various heating processes involved in WMP production, thermal stability is an important functional parameter (Singh and Creamer, 1991). Heat-induced changes are primarily caused by the denaturation or aggregation of whey protein fractions (particularly  $\beta$ -LG) or the interaction of  $\kappa$ -casein and whey proteins (Anema and Li, 2003). The whey protein nitrogen index (WPNI) quantifies the level of undenatured whey protein in dairy commodities, indicating the level of heat treatment a dairy powder has received during processing. These WPNI values are of particular importance for predicting the gel strength of yogurts.

Although the effects of pasture-based and concentrate-based bovine feeding systems on the composition of raw milk have been reported, limited information exists to characterize their effect on the composition of standardized milk powder products. The objective of this study was to characterize the influence of perennial ryegrass (*Lolium perenne* L.), perennial ryegrass/white clover (*Trifolium repens* L.), and TMR-based feeding systems on the color, thermal stability, particle size distribution, and protein profile of standardized WMP produced from raw milk derived from each system and the subsequent gelation and textural properties of yogurt produced from the WMP.

## MATERIALS AND METHODS

### Materials

Raw milk was obtained from the Teagasc Animal and Grassland Research and Innovation Centre dairy unit (Moorepark, Fermoy, Co. Cork, Ireland). The mesophilic starter culture MO1, used for yogurt production (a mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*), was sourced from Chr. Hansen (Cork, Ireland).

### Experimental Design

The experimental design for the bovine feeding systems was similar to that described previously for butter (O'Callaghan et al., 2016a), Cheddar cheese (O'Callaghan et al., 2017), and raw milk (O'Callaghan et al., 2016b, 2018; Faulkner et al., 2018). Fifty-four spring-calving Friesian cows were allocated to 3 groups ( $n = 18$ ) at the Teagasc, Animal and Grassland Research and Innovation Centre (Moorepark, Fermoy, Co. Cork, Ireland). Groups were randomized based on milk yield, milk solids yield, calving date (mean calving date February 19, 2015), and lactation number. Group 1 was housed indoors and fed a TMR diet; group 2 was maintained outdoors on perennial ryegrass only pasture (GRS); and group 3 was maintained outdoors on a perennial ryegrass/white clover pasture (CLV). For further information on the chemical and nutritional values of each of the feeding systems, see O'Callaghan et al. (2016b). Briefly, the TMR system consisted of, on a DM basis, 7.15 kg of grass silage, 7.15 kg of maize silage, and 8.3 kg of concentrates. Cows within the TMR system were fed at 0830 h daily into electronically controlled individual feed bins (Mealmaster; Griffith Elder and Company Ltd., Suffolk, UK) and the TMR was available ad libitum. Both groups of pasture-based cows consumed  $\sim 18$  kg of DM/d, allocated using estimates of pregrazing herbage mass and daily postgrazing sward heights, as described by Egan et al. (2017). The CLV sward contained 20% (wt/wt) white clover. Milking took place at 0730 and 1530 h daily. To obtain a representative sample of milk, the cows in each of the 3 feeding systems were milked separately into designated 5,000-L refrigerated tanks. The evening milk was stored at 4°C overnight, to which the morning milk was then added. Tanks were maintained at 4°C and agitated before sample collection. Milk was collected from each of the groups in the trial for milk powder manufacture on 3 separate occasions over a 3-wk period in September 2015 to produce 3 batches of WMP from each feeding system, when cows were  $184 \pm 7$  d in lactation on their respective diets.

### WMP Production

All milk powder production was carried out at the Moorepark Technology Limited pilot-plant facility (Fermoy, Co. Cork, Ireland). Raw whole milk ( $\sim 1,000$  kg) was heated to 50°C in an APV plate heat-exchanger (SPX Flow Technology, Crawley, UK) before being separated into skim milk and cream in a centrifugal disk separator (GEA Westfalia, Oelde, Germany). The separate skim and cream fractions were then re-

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combined to produce a whole milk of standardized fat content (3.5%, wt/wt). This standardized whole milk was then pasteurized at 90°C for 30 s using the APV plate heat-exchanger, before being evaporated to ~52% TS in a Niro 3-effect falling film evaporator (GEA Niro A/S, Soeborg, Denmark). The pasteurized concentrate was heated to 65°C in the APV plate heat-exchanger and homogenized using a 2-stage homogenizer (APV Gaulin, Lake Mills, WI) at first- and second-stage pressures of 15,000 and 5,000 kPa, respectively. The homogenized whole milk concentrate was then dried using a Niro Tall-Form Anhydro 3-stage spray dryer (air inlet temperature 180°C; air outlet temperature 80°C). First and second fluid bed temperatures were set at 65°C and 25°C, respectively. Fines were returned from the second fluid bed and the cyclone to the top of the spray dryer to yield an agglomerated WMP of approximately 97% TS. The WMP production was carried out in triplicate from 3 independent raw milk collections.

### Compositional Analysis of Powder

#### *Determination of Nitrogen and Fat Contents.*

The total nitrogen content of each powder was determined using the Kjeldahl method, as described in ISO 8968-1 (ISO, 2001). A nitrogen-to-milk protein conversion factor of 6.38 was used. The fat content of each powder was determined using the Röse-Gottlieb gravimetric method, as described by the International Dairy Federation (1996). The ash content of each powder was determined by ashing approximately 3 g of each sample in a Carbolite muffle furnace (Carbolite Gero Ltd., Hope, Sheffield, UK) overnight. The moisture content of each powder was determined using a HR83 Halogen rapid moisture analyzer (Mettler Toledo, Columbus, OH). The lactose content of each powder was calculated by difference.

Noncasein nitrogen (NCN) content was determined by precipitation of the casein component of a whole milk sample. The sample was diluted with deionized water at 40°C and acidified to pH 4.6 by addition of acetic acid and sodium acetate. The mixture was cooled to 20°C and allowed to settle before filtration using Whatman No. 1 filter paper. A Kjeldahl determination was then carried out on the filtrate as described above. The NPN content was determined by precipitation of the protein component of a whole milk sample using trichloroacetic acid (15% wt/vol). The precipitate was removed from the mixture using Whatman No. 1 filter paper and a Kjeldahl determination was then carried out on the filtrate as described above.

**HPLC.** The protein profile of each WMP was determined using reversed-phase HPLC as described by

Mounsey and O’Kennedy (2009). The aqueous (phase A) and organic (phase B) phases consisted of acetonitrile, HPLC-grade water, and trifluoroacetic acid (TFA) in ratios of 100:900:0.1 (vol/vol/vol) and 900:100:0.1 (vol/vol/vol), respectively. Samples at a dilution factor of 1:20 sample:buffer were filtered through a 0.2- $\mu$ m filter and separated using an Agilent 300SB Poroshell (2.1  $\times$  75mm) column (Agilent Technologies, Santa Clara, CA) at 35°C. Detection wavelength was 214 nm, injection volume was 5  $\mu$ L, and flow rate was 0.5 mL/min.

### Color Measurements

The CIE L\*a\*b\* method was used to measure the color of each WMP. Lightness (L\*), red/green color (a\*), and yellow/blue color (b\*) values were determined using a Konica Minolta CR-400 Chroma Meter (Chiyoda, Tokyo, Japan). Powdered and reconstituted (13% wt/wt) samples of each WMP were placed into plastic cuvettes and measured in triplicate.

### Whey Protein Nitrogen Index

The WPNI was determined using the GEA Niro Method No. A21a, modified from the Harland-Ashworth method (Kuramoto et al., 1959).

### Heat Coagulation Time

The heat coagulation time (HCT) of raw whole milk and reconstituted powders was determined using an Elbanton Oil Bath (Hettich Benelux Laboratory Equipment, Geldermalsen, the Netherlands). Each WMP sample was reconstituted to 1.5% (wt/wt) protein using deionized water. Milk samples were divided into a further 13 aliquots (~30 mL each), the pH values of which were adjusted in pH increments of 0.1 between pH 6.2, and 7.4 using 0.1 N HCl or NaOH. Following approximately 2 h of equilibration and final pH adjustment, where necessary, ~3.4-g aliquots of each sample were pipetted into 4-mL glass tubes, stoppered, and inserted into the oil bath rack. The rack was then inserted into the temperature-controlled oil bath at 140°C and rocked at 8 oscillations/min. The time taken (in minutes) for visible coagulation of each sample was recorded.

### Particle Size of Rehydrated WMP

The particle size distribution of 15% (wt/wt) WMP dispersions was measured in triplicate by static light scattering using a Malvern Mastersizer laser-light dif-

fraction unit (Hydro MV, Mastersizer 3000, Malvern Instruments Ltd., Malvern, UK) equipped with a 300 RF lens. Refractive indices were set at 1.462 and 1.33 for the particle and dispersant (water), respectively. Size measurements were determined as the median ( $D_{50}$ ) and cumulative diameters  $D_{90}$  and  $D_{10}$ , in which 50, 90, and 10% of the volume of particles were smaller than the specified size, respectively. Size distributions were determined by polydisperse analysis, and measurements were taken when laser obscuration reached ~3%.

### Rheological Properties of Yogurt

**Yogurt Production.** Whole milk powder from each group of cows was reconstituted to 15% TS in deionized water and refrigerated overnight at 4°C to ensure complete hydration. Dispersions (1 L) were then tempered at 30°C before inoculation in a laminar flow hood. Freeze-dried pellets of the starter culture (0.2 g) were then added to ~20-mL aliquots of the tempered milk before being added back into the dispersions and mixed thoroughly. Sub-samples (100 mL) not intended for rheological measurements were added to sealed cups and placed in an incubator at 30°C (temperature chosen based on supplier recommendation). The pH of the inoculated milk was constantly monitored until a pH of 4.6 was reached. At pH 4.6, the incubated samples were immediately steeped in an ice bath to halt starter culture activity. Upon reaching a temperature of 10°C, these samples were refrigerated overnight.

Low-amplitude oscillation measurements were carried out using a Discovery HR-1 hybrid rheometer (TA Instruments, New Castle, DE), equipped with a concentric cylinder, maintained at 30°C. An aliquot (17 g) of the freshly inoculated milk was immediately weighed into the concentric cylinder. A time sweep was initiated using the following conditions: 5-s temperature equilibration at 30°C, 15-s pre-shear at a shear rate of 50 s<sup>-1</sup>, and 10-s equilibration. The sample was then oscillated at 1% strain and a frequency of 1 Hz over a 10-s sampling interval until pH 4.6 was reached. This was monitored by measuring the pH of parallel-incubated samples of inoculated milk, which were maintained at the same temperature as the sample in the rheometer for the same duration. Once the pH reached pH 4.6, the time sweep was stopped. This was followed immediately by a logarithmic frequency sweep from 1 to 63.1 Hz at a constant strain of 1%.

**Texture Profile Analysis.** Following overnight refrigerated storage, set yogurt samples (100 mL) were analyzed at 4°C using a 35-mm flat-disk backward extrusion rig on a Texture Expert Exceed system (Stable Microsystems, Godalming, UK). Probe force was cali-

brated using a 2-kg weight mounted on a 5-kg load cell. Trigger force was set at 2 g. The probe penetrated the sample to a depth of 25 mm and returned to the starting point. Pretest, test, and posttest probe speeds were set at 1 mm/s. The sample firmness, consistency, cohesiveness, and index of viscosity were recorded. Yogurt gels from each WMP sample were also tested at 30°C to determine the influence of variation in fatty acid melting points between the samples.

### Statistical Analysis

All analyses were carried out on WMP from 3 independent trials from each dietary treatment. Statistical analysis was performed using SPSS v18.0 (IBM Statistics Inc., Armonk, NY). Data sets were analyzed for normality using the Shapiro-Wilk test. Data were deemed normally distributed, and analysis was carried out using one-way ANOVA with post hoc Tukey test. *P*-values < 0.05 were considered significant.

## RESULTS AND DISCUSSION

### WMP Composition

Total nitrogen, total fat, lactose, NPN, and NCN contents of WMP samples are shown in Table 1. The highest mean protein content was present in the GRS sample (31.5%, wt/wt), followed by CLV (30.7%, wt/wt), and TMR (30.3%, wt/wt), although these values were not significantly different ( $P > 0.05$ ). Similarly, no significant difference in mean fat content was found between the powders, with mean fat contents of 25.8, 25.7, and 25.5% (wt/wt) for CLV, TMR, and GRS milk samples, respectively. No significant differences in lactose, ash, or free moisture contents were found between the samples. However, NPN and NCN values for CLV were significantly higher ( $P < 0.05$ ) than those from TMR. Increased NPN content may be attributed to increased urea content arising from the inclusion of white clover in the CLV feeding system. Significantly higher levels of urea have previously been reported in milk from this system, compared with milk from a TMR system (O'Callaghan et al., 2018). Significant differences in protein and fat content in pasture-derived raw unstandardized whole milk, relative to concentrate-derived raw whole milk, have been observed previously (O'Callaghan et al., 2016b); however, no information exists on the effect of these feeding systems on the composition of standardized WMP. Another study that used these feeding systems recorded significant differences in total protein and casein content between pasture-derived and concentrate-derived raw whole milks,

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**Table 1.** Compositional analysis (% wt/wt) data for whole milk powders (mean values  $\pm$  SD)

Sample <sup>1</sup>	Total protein	Total fat	Lactose	NPN	Noncasein N	Ash	Free moisture
GRS	31.5 $\pm$ 0.99 <sup>a</sup>	25.5 $\pm$ 0.78 <sup>a</sup>	35.6 $\pm$ 0.52 <sup>a</sup>	0.32 $\pm$ 0.05 <sup>ab</sup>	0.99 $\pm$ 0.02 <sup>ab</sup>	5.42 $\pm$ 0.54 <sup>a</sup>	1.94 $\pm$ 0.29 <sup>a</sup>
CLV	30.7 $\pm$ 0.53 <sup>a</sup>	25.8 $\pm$ 0.63 <sup>a</sup>	36.2 $\pm$ 0.79 <sup>a</sup>	0.37 $\pm$ 0.02 <sup>a</sup>	1.03 $\pm$ 0.02 <sup>a</sup>	5.57 $\pm$ 0.13 <sup>a</sup>	1.83 $\pm$ 0.02 <sup>a</sup>
TMR	30.3 $\pm$ 1.35 <sup>a</sup>	25.7 $\pm$ 0.48 <sup>a</sup>	36.4 $\pm$ 1.22 <sup>a</sup>	0.29 $\pm$ 0.01 <sup>b</sup>	0.97 $\pm$ 0.02 <sup>b</sup>	5.72 $\pm$ 0.03 <sup>a</sup>	1.84 $\pm$ 0.12 <sup>a</sup>

<sup>a,b</sup>Values within a column not sharing a common superscript differed significantly ( $P < 0.05$ ).

<sup>1</sup>Whole milk powders were from cows fed perennial ryegrass only (GRS), perennial ryegrass/20% white clover sward (CLV), or an indoor TMR ad libitum (TMR).

although a significant difference in total fat was only observed between the 2 pasture-derived diets (Gulati et al., 2018a).

The mass of protein fractions present in each WMP dispersion (13% wt/wt) are shown in Table 2. No significant differences ( $P > 0.05$ ) were observed in protein profile between the WMP samples. Slightly higher amounts of  $\kappa$ -CN,  $\alpha_{S2}$ -CN,  $\alpha_{S1}$ -CN,  $\beta$ -CN,  $\beta$ -LG-a, and  $\beta$ -LG-b were observed in the GRS sample than in the CLV and TMR samples. A previous study by Mackle et al. (1999) investigated the effect of feeding cows on ryegrass-white clover pasture, pasture supplemented with maize grain, or pasture supplemented with a combination of maize grain and pasture silage. The above study, using SDS-PAGE, identified a significantly lower proportion of  $\beta$ -CN in milk from cows supplemented with maize grain only and higher  $\beta$ -CN content in milk from cows fed on pasture only. This trend for  $\beta$ -CN appears to be similar to that in the present study; however, the lack of significance ( $P > 0.05$ ) could be related to reduced sample size ( $n = 3$ ) in the present study.

Average WPNI values for the CLV, GRS, and TMR powder samples were  $4.2 \pm 0.19$ ,  $3.8 \pm 0.11$ , and  $4.0 \pm 0.05$  mg of undenatured whey protein per gram of powder, respectively. Excessive heat treatment will lead to increased whey protein denaturation, as indicated by a low WPNI value of  $<1.5$  (Harland and Ashworth, 1947); in contrast, a high WPNI value ( $>6.0$ ) indicates a high level of native whey protein in dairy-based powders (Sikand et al., 2008). All WMP samples exhibited WPNI values in the medium heat treatment range ( $>1.5$  to  $<6.0$ ), indicating a consistent heat treatment process and no significant difference in the level of denatured whey protein content between the samples.

**Table 2.** Mass of protein fractions (mg/mL) present in whole milk powder dispersions (13% wt/wt), determined by reversed-phase HPLC (mean values  $\pm$  SD)

Sample <sup>1</sup>	$\kappa$ -CN	$\alpha_{S2}$ -CN	$\alpha_{S1}$ -CN	$\beta$ -CN	$\alpha$ -LA	$\beta$ -LGa	$\beta$ -LGB
GRS	3.4 $\pm$ 0.32	2.9 $\pm$ 0.08	12.4 $\pm$ 0.20	12.1 $\pm$ 0.48	0.4 $\pm$ 0.10	1.5 $\pm$ 0.26	1.4 $\pm$ 0.51
CLV	3.1 $\pm$ 0.44	2.5 $\pm$ 0.26	11.4 $\pm$ 0.44	11.3 $\pm$ 0.49	0.5 $\pm$ 0.15	1.4 $\pm$ 0.09	1.3 $\pm$ 0.39
TMR	2.6 $\pm$ 0.69	2.5 $\pm$ 0.56	10.9 $\pm$ 1.47	10.8 $\pm$ 1.23	0.6 $\pm$ 0.15	1.4 $\pm$ 0.43	1.4 $\pm$ 0.30

<sup>1</sup>Whole milk powder dispersions were from cows fed perennial ryegrass only (GRS), perennial ryegrass/20% white clover sward (CLV), or an indoor TMR ad libitum (TMR).

**Color Analysis of WMP**

Color measurements of WMP samples are shown in Table 3. Lightness (**L\***) indicates the degree of whiteness of a sample, ranging from 0 (black) to 100 (white). Positive values on the red/green (**a\***) component indicate redness and negative values indicate greenness. Similarly, positive values on the yellow/blue (**b\***) component indicate sample yellowness and negative values indicate blueness.

Differences in **a\*** and **L\*** values were not significant between milk powders from each of the diets, although significant differences ( $P < 0.05$ ) were observed in **b\*** values. These differences were apparent between the GRS and CLV samples and the TMR sample. The powder samples exhibited higher overall color intensity and **L\*** values than the rehydrated powder samples. Maximum powder **L\*** values were observed in the TMR sample, followed by CLV and GRS. The highest **L\*** value of the rehydrated WMP samples was observed in the CLV sample, followed by TMR and GRS. The highest **a\*** values in milk powder and rehydrated WMP samples were observed in the GRS sample, followed by CLV and TMR, respectively. However, there were no significant differences in **L\*** or **a\*** values between the milk powders or between the rehydrated samples. The GRS powder **b\*** values were significantly higher ( $P < 0.05$ ) than those of the TMR sample. The CLV **b\*** values were also higher than TMR **b\*** values, albeit not significantly ( $P > 0.05$ ). Table 3 includes  $\Delta E^*$  values for each sample, denoting the difference between each sample that can be visibly determined. A difference of 3.21 in  $\Delta E^*$  value between the TMR and GRS samples indicates that these samples were visibly distinct. Dif-

**Table 3.** Average lightness ( $L^*$ ), red/green color ( $a^*$ ), and yellow/blue color ( $b^*$ ) values for whole milk powders and whole milk powder dispersions (15% wt/wt; mean values  $\pm$  SD)

Sample <sup>1</sup>	Powder			Dispersion			$\Delta E^*$
	$L^*$	$a^*$	$b^*$	$L^*$	$a^*$	$b^*$	
GRS	93.0 $\pm$ 0.71 <sup>a</sup>	-5.44 $\pm$ 0.56 <sup>a</sup>	18.5 $\pm$ 1.40 <sup>a</sup>	85.0 $\pm$ 1.64 <sup>a</sup>	-4.58 $\pm$ 0.86 <sup>a</sup>	11.0 $\pm$ 1.67 <sup>a</sup>	2.78 <sup>(GRS-CLV)</sup>
CLV	93.3 $\pm$ 0.75 <sup>a</sup>	-5.24 $\pm$ 0.63 <sup>a</sup>	17.5 $\pm$ 1.13 <sup>ab</sup>	86.2 $\pm$ 0.27 <sup>a</sup>	-3.69 $\pm$ 0.10 <sup>a</sup>	8.66 $\pm$ 0.49 <sup>a</sup>	0.86 <sup>(CLV-TMR)</sup>
TMR	93.5 $\pm$ 0.85 <sup>a</sup>	-4.65 $\pm$ 0.70 <sup>a</sup>	15.0 $\pm$ 1.15 <sup>b</sup>	85.7 $\pm$ 1.53 <sup>a</sup>	-3.84 $\pm$ 1.00 <sup>a</sup>	7.96 $\pm$ 1.72 <sup>a</sup>	3.21 <sup>(GRS-TMR)</sup>

<sup>a,b</sup>Values within a column not sharing a common superscript differed significantly ( $P < 0.05$ ).

<sup>1</sup>Whole milk powders and dispersions were from cows fed perennial ryegrass only (GRS), perennial ryegrass/20% white clover sward (CLV), or an indoor TMR ad libitum (TMR).

<sup>2</sup> $\Delta E^*$  denotes total color difference between samples that can be visibly determined. Values  $<1$  are imperceptible; values between 1 and 3 are visibly perceptible; values  $>3$  are visually distinct.

ferences between GRS and CLV were visibly perceptible, whereas differences between the TMR and CLV samples were imperceptible.

Similar differences in the color of milk and milk products derived from TMR or pasture-based diets have been identified previously (Hurtaud et al., 2002). The slightly lower  $L^*$  and more negative  $a^*$  values exhibited by the GRS sample, along with the more positive  $b^*$  values exhibited by the GRS and CLV samples, may be attributed to increased  $\beta$ -carotene content in both pasture-based samples (Nozière et al., 2006; O'Callaghan et al., 2016a). Present in high concentrations in grass,  $\beta$ -carotene imparts a red/orange pigmentation and acts as a precursor compound to retinol (vitamin A) synthesis in the liver (Darwish et al., 2016). As vitamin A is fat-soluble, the quantity of  $\beta$ -carotene in various dietary fat sources has been widely investigated. Studies that investigated the effect of pasture and concentrate-based feeding systems on the composition of beef muscle (Duckett et al., 2009), butter (O'Callaghan et al., 2016a), and Cheddar cheese (O'Callaghan et al., 2017) have identified significantly higher concentrations of  $\beta$ -carotene in pasture-derived samples than in concentrate-derived samples.

### HCT of Reconstituted WMP

All raw whole milk samples exhibited typical “type A” HCT–pH profiles, characterized by clear HCT maxima and minima and a sharp decline in HCT to a local minimum at pH 6.9 (O'Sullivan et al., 2001). Figure 1A shows typical HCT–pH profiles for raw milk samples from each feeding system. The trend of HCT increasing as a function of pH to a local maximum at pH 6.7 and then decreasing before further increasing is typically attributed to heat-induced dissociation of  $\kappa$ -CN from the casein micelle and subsequent complex formation with  $\beta$ -LG at pH  $>6.6$  (Singh and Fox, 1985; Anema, 2008). The  $\kappa$ -CN-depleted casein micelle is then susceptible to calcium-induced aggregation, leading to protein precipitation (McSweeney et al., 2004). This is followed by increased HCT above pH 6.9, as the charge of the casein micelle increases due to the loss of  $\kappa$ -CN (Panda, 2011).

In contrast, all WMP samples exhibited unusual HCT–pH profiles, whereby HCT increased as a function of pH (type B profile); however, a slight decrease in HCT was observed at pH 7.1 for both the TMR and CLV samples and at 7.2 for the GRS sample, followed by HCT maxima at pH 7.4 in all samples (Figure 1B). The highest overall HCT was observed in the CLV sample, followed by GRS and TMR samples, respectively. Visible coagulation occurred after approximately 26, 19, and 18 min at pH 7.4 and after 19, 15, and 14

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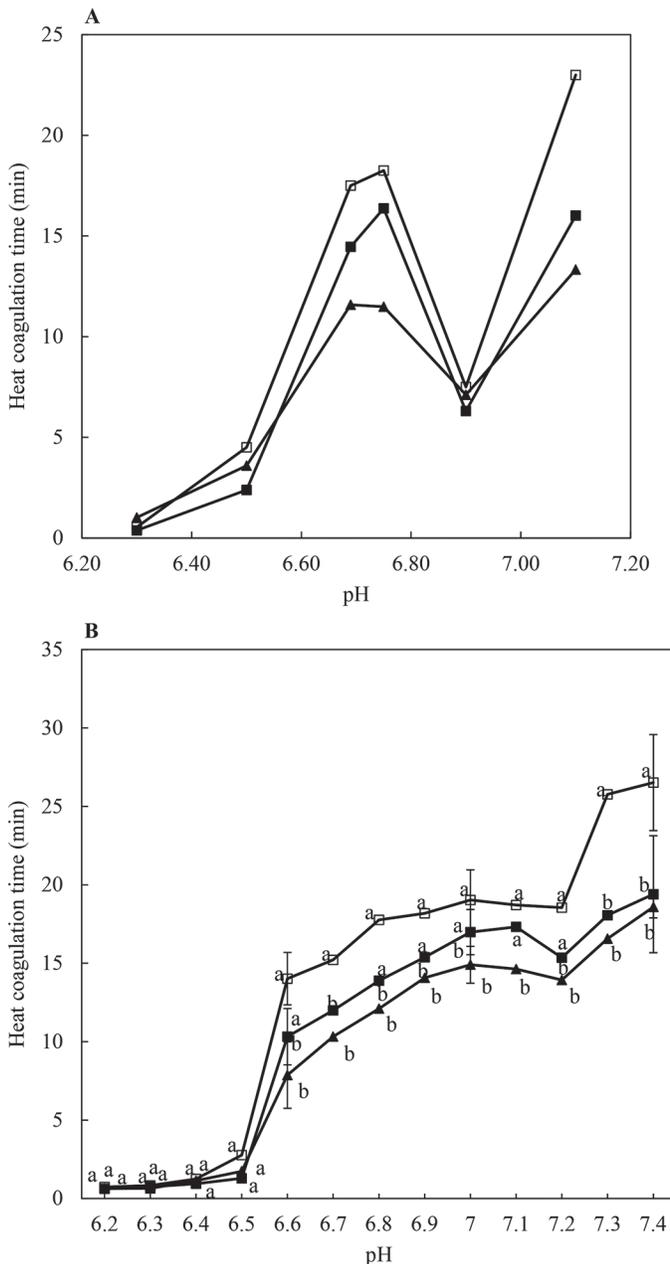
min at pH 7.2 for the CLV, GRS, and TMR samples, respectively. We detected significant differences ( $P < 0.05$ ) between the CLV and TMR samples at each pH increment between pH 6.6 and 7.4. The HCT of the GRS sample was significantly higher than that of the

TMR sample at pH 7.1 ( $P < 0.05$ ), whereas the HCT of the CLV sample was significantly higher than that of the GRS sample at pH 6.7, 7.3, and 7.4 ( $P < 0.05$ ). These samples, however, did not exhibit a decrease in HCT between pH 6.7 and 6.9.

Similar type-B HCT–pH profiles have previously been observed in low-heat ( $72^{\circ}\text{C} \times 15\text{ s}$ ) skim milk concentrates heated at  $120^{\circ}\text{C}$  (Lin et al., 2018). Skim milk powder samples reconstituted at low concentration (9.4% TS) showed trends of steadily increasing HCT with increasing pH, followed by a slight decline at  $\sim\text{pH } 7.0$ , whereas HCT decreased significantly at higher pH values in samples reconstituted at higher TS concentrations (Lin et al., 2018). In the present study, standardization of reconstituted WMP samples at 1.5% total protein content resulted in a TS content of approximately 5%. This reduced concentration may contribute to the type-B HCT–pH profile and lack of decline in HCT at pH 6.9 observed in all 3 samples. The significant differences in HCT between feeding systems is notable because a previous study on mid-lactation skim milk produced from these same feeding systems did not show a similar trend of significant variation of HCT (Gulati et al., 2018b).

Results for calcium ion activity ( $\text{Ca}^{2+}$ ) showed a higher average concentration of ionic calcium in TMR-based WMP (2.26 mM), compared with the GRS (2.21 mM) and CLV (2.12 mM) samples, although these did not differ significantly ( $P > 0.05$ ). Although  $\text{Ca}^{2+}$  level decreases with increasing pH (Tsioulpas et al., 2007), milk HCT decreases with increasing  $\text{Ca}^{2+}$  (Sievanen et al., 2008). The significant differences in HCT observed between the CLV and TMR samples may be attributable to increased NPN content in the CLV sample, arising from increased urea levels (Huppertz, 2016) because of the white clover content of the diet (Harris et al., 1998). The addition of high concentrations of urea to unconcentrated milk has been shown to increase milk HCT (Muir and Sweetsur, 1976). Heat-induced decomposition of urea to ammonia reduces the susceptibility of milk to heat-induced acidification, leading to increased heat-induced dissociation of  $\kappa\text{-CN}$  and a decrease in  $\text{Ca}^{2+}$  level (Huppertz, 2016). As previously discussed, significantly higher concentrations of urea have been detected in milk derived from the CLV feeding system compared with milk derived from the TMR system (O’Callaghan et al., 2018).

The lower HCT values in the TMR sample may also have been due to reduced fat particle size. The  $D_{90}$  values for TMR WMP dispersions were significantly lower than those for both GRS and CLV dispersions (Figure 4). Decreases in HCT due to homogenization and consequently reduced fat particle size have been previously described (McCrae, 1999).



**Figure 1.** Heat coagulation time of (A) raw whole milk, and (B) whole milk powder dispersions (1.5%, wt/wt, protein) heated at  $140^{\circ}\text{C}$  at 8 oscillations per minute obtained from cows fed grass (■), grass/clover (□), or TMR (▲). Values in A represent a single measurement, and values in B are the means of data from triplicate trials and duplicate analysis. Error bars represent standard deviations. Data points with different letters (a, b) differed significantly ( $P < 0.05$ ).

### Rheological Properties of Yogurt

Rheological values showing the onset of gelation of yogurts produced from each WMP sample are shown in Table 4 and Figures 2 and 3. We found no significant differences in the time and pH values at which the elastic modulus ( $G'$ ) values of each sample exceeded 1 Pa or the time at which  $G'$  values exceeded viscous modulus ( $G''$ ) values. At pH 4.6, the TMR sample yielded an average maximum  $G'$  of 51.5 Pa after 514 min. In contrast, the GRS and CLV samples yielded average  $G'$  maxima of 92.6 and 94.3 Pa after 525 and 541 min, respectively. The  $G'$  and  $G''$  values were significantly ( $P < 0.05$ ) higher in the CLV and GRS samples compared with the TMR sample. A previous study investigating characteristics of mid-lactation milk from these feeding systems reported significantly higher  $G'$  values and gel-firming rates in rennet gels from GRS-derived milk than in those from TMR-derived milk, although no significant differences were found between CLV and TMR rennet gels (Gulati et al., 2018b). No significant differences were found between the other gelation properties in the present study (Table 4).

Logarithmic frequency sweeps recorded typical viscoelastic behavior in each set yogurt sample up to a frequency of 63.1 Hz (Figure 3). At this frequency, the highest average  $G'$  value was observed in the GRS sample (236 Pa), followed by CLV (152 Pa) and TMR (105 Pa). The highest average  $G'$  values for the CLV (165 Pa) and TMR (109 Pa) samples were observed at 25.1 and 39.8 Hz, respectively. All samples exhibited overall thixotropic (time-dependent shear-thinning) behavior, characterized by a substantial decrease in complex viscosity ( $\eta^*$ ) as a function of increasing frequency, along with a decrease in  $G'$  and concomitant increase in  $G''$  at 63.1 Hz (Figure 3). The GRS sample, however, did not exhibit a decrease in  $G'$  at this frequency, despite an increase in  $G''$ . The highest average  $\eta^*$  value at 1 Hz was observed in the GRS sample ( $18.2 \text{ Pa}\cdot\text{s}^{-1}$ ), followed by CLV ( $16.0 \text{ Pa}\cdot\text{s}^{-1}$ ) and TMR ( $9.37 \text{ Pa}\cdot\text{s}^{-1}$ ). The  $\eta^*$  value of the GRS sample was significantly higher ( $P < 0.05$ ) than that of the TMR sample. The higher  $G'$ ,  $G''$ ,

and  $\eta^*$  values observed in the GRS and CLV samples, compared with those from the TMR sample, indicate the formation of stronger, more cohesive gel matrices, which are more resistant to deformation.

Standardization of yogurt samples at 4% true protein content did not result in significant differences in gel strength in yogurt samples from the same dietary treatments standardized at 15% TS. This implies that variations in the gel strength of yogurts between dietary treatments were not attributable to variations in their true protein content. The significantly higher  $G'$  values observed in both pasture-based samples relative to the TMR sample may be attributable, therefore, to variation in the composition and structure of their fat components.

The fat particle size distribution results indicated the relative abundance of fat globules of varying size in WMP dispersions. No significant difference in volume mean diameter ( $D_{[4,3]}$ ) was observed among the samples (Figure 4); however, the TMR sample had a significantly lower ( $P < 0.05$ )  $D_{90}$  value compared with both the GRS and CLV samples. This indicated a lower abundance of large fat globules in the TMR sample, which may be caused by increased fat globule flocculation or coalescence in the GRS and CLV samples due to the presence of lower-melting-point fatty acids (O'Callaghan et al., 2016a). The degree of electrostatic repulsion between protein-adsorbed fat globules in milk at the onset of gelation may be an influencing factor in the gel strength of the final gel matrix.

### Texture Profile Analysis

Average texture profile data for each yogurt gel are shown in Table 5 and Figure 5. Firmness, cohesiveness, and consistency are defined as the force required to penetrate the gel structure, the degree of deformation the gel matrix can withstand before it ruptures (Rawson and Marshall, 1997), and the resistance of the gel matrix to deformation (do Espírito Santo et al., 2012), respectively. The highest gel firmness (192.8 g) and consistency ( $4,218 \text{ g}\cdot\text{s}^{-1}$ ) were exhibited by the CLV

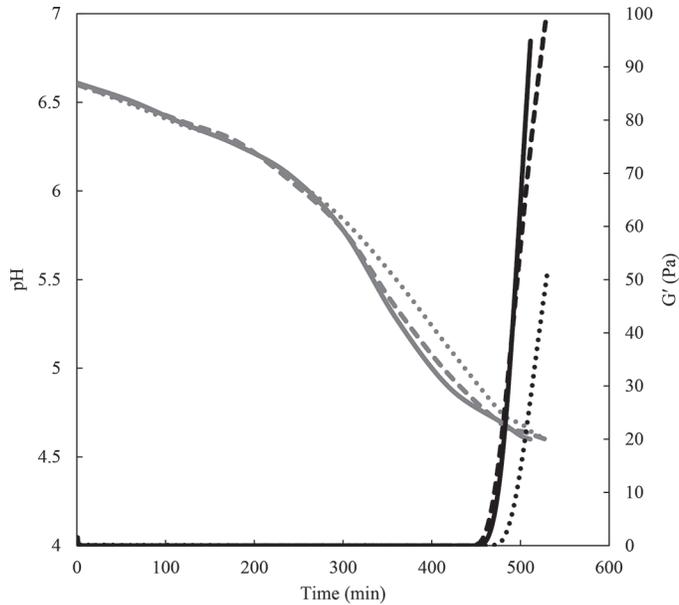
**Table 4.** Gelation properties (elastic modulus,  $G'$ ; and viscous modulus,  $G''$ ) of yogurt gels produced from whole milk powder dispersions (15%, wt/wt, TS) inoculated with 0.2 g of mesophilic starter culture (mean values  $\pm$  SD)

Sample <sup>1</sup>	Time at which $G' = 1 \text{ Pa}$ (min)	pH at which $G' = 1 \text{ Pa}$	Time at which $G' > G''$ (min)	Time at which pH = 4.6 (min)	Final $G'$ value at pH 4.6 (Pa)
GRS	$469 \pm 10^a$	$4.77 \pm 0.09^a$	$462 \pm 10^a$	$525 \pm 13^a$	$92.6 \pm 10^a$
CLV	$473 \pm 20^a$	$4.76 \pm 0.04^a$	$471 \pm 20^a$	$541 \pm 23^a$	$94.3 \pm 5^a$
TMR	$467 \pm 31^a$	$4.77 \pm 0.02^a$	$463 \pm 27^a$	$514 \pm 29^a$	$51.5 \pm 12^b$

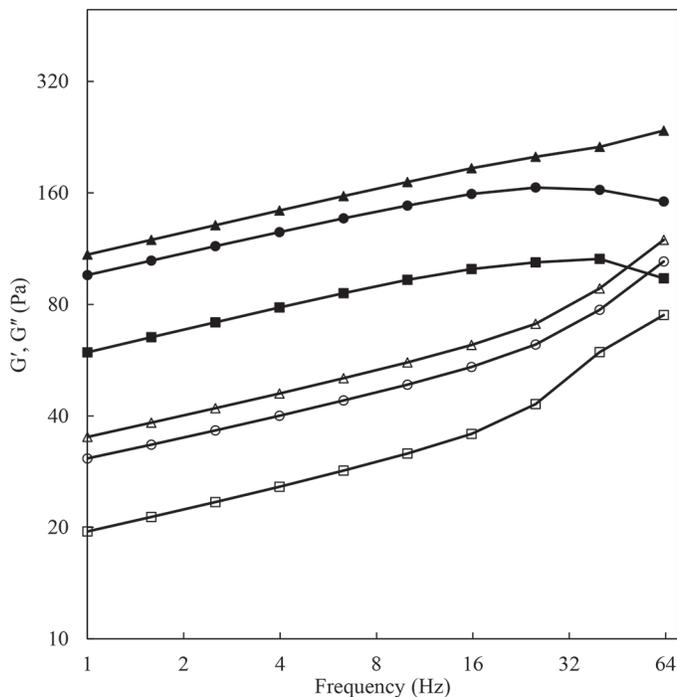
<sup>a,b</sup>Values within a column not sharing a common superscript differed significantly ( $P < 0.05$ ).

<sup>1</sup>Whole milk powder dispersions were from cows fed perennial ryegrass only (GRS), perennial ryegrass/20% white clover sward (CLV), or an indoor TMR ad libitum (TMR).

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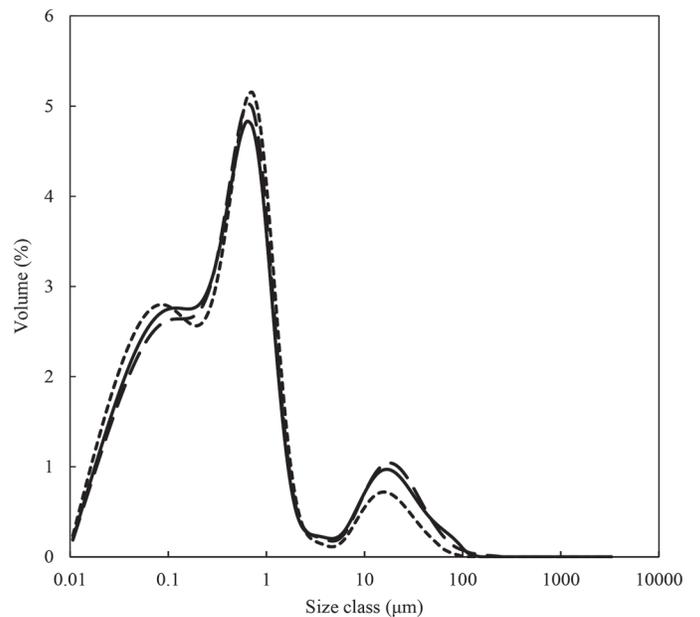
**Figure 2.** Typical elastic modulus,  $G'$  (black; Pa) and pH profile (gray) of yogurt gels produced from whole milk powder dispersions (15% wt/wt) for grass (solid line), grass/clover (dashed line), and TMR (dotted line) diets as a function of incubation time at 30°C. Measurements were ceased at pH 4.6.



**Figure 3.** Average elastic ( $G'$ , filled symbols; Pa) and viscous ( $G''$ , open symbols; Pa) moduli of yogurt gels produced from whole milk powder dispersions (15% wt/wt) for grass (▲, △), grass/clover (●, ○), and TMR (■, □) diets as a function of oscillation frequency (1–63.1 Hz).

yogurt sample. The CLV-derived yogurt gel yielded significantly higher ( $P < 0.05$ ) firmness values than did the TMR-derived sample. Maximum gel cohesiveness ( $-60.9$  g) and index of viscosity ( $-940$  g·s $^{-1}$ ) were exhibited by the GRS sample. Lower values were observed in the TMR sample for all texture profile components. The WMP from the CLV feeding system produced the thickest and firmest yogurt samples overall, whereas WMP from the GRS system produced a more cohesive, elastic gel.

Warming yogurt samples to 30°C before texture profile analysis resulted in an approximately 30% decrease in firmness and consistency and an approximately 50% decrease in cohesiveness and index of viscosity across all samples. This implies that the textural variations identified between yogurts produced from WMP from each diet were not attributable to variations in their fatty acid melting points. The effect of seasonality must be noted as a source of variation in WMP composition and functionality. The characteristic behavior of the late-lactation milk processed and analyzed in this study may differ substantially from that of milk produced at a different stage of lactation or in a separate lactation cycle. A previous study by Gulati et al. (2018a) noted higher concentrations of total protein, fat, and casein in late-lactation milk from these feeding systems compared with that of mid-lactation milk.



**Figure 4.** Particle size distribution of whole milk powder dispersions (15%, wt/wt, total solids), for grass (solid line), grass/clover (long-dashed line), and TMR (short-dashed line) diets represented as a function of triplicate trials.

**Table 5.** Textural properties of yogurts produced from whole milk powder dispersions (15%, wt/wt, TS) inoculated with 0.2 g of mesophilic starter culture (mean values  $\pm$  SD)

Sample <sup>1</sup>	Firmness (g)	Consistency (g·s <sup>-1</sup> )	Cohesiveness (g)	Index of viscosity (g·s <sup>-1</sup> )
GRS	175 $\pm$ 36.1 <sup>ab</sup>	4,089 $\pm$ 805 <sup>a</sup>	-60.9 $\pm$ 12.3 <sup>a</sup>	-940 $\pm$ 172 <sup>a</sup>
CLV	192 $\pm$ 20.9 <sup>a</sup>	4,218 $\pm$ 477 <sup>a</sup>	-57.3 $\pm$ 8.87 <sup>a</sup>	-878 $\pm$ 123 <sup>a</sup>
TMR	130 $\pm$ 6.96 <sup>b</sup>	3,095 $\pm$ 194 <sup>a</sup>	-40.4 $\pm$ 2.95 <sup>a</sup>	-644 $\pm$ 32.7 <sup>a</sup>

<sup>a,b</sup>Values within a column not sharing a common superscript differed significantly ( $P < 0.05$ ).

<sup>1</sup>Whole milk powder dispersions were from cows fed perennial ryegrass only (GRS), perennial ryegrass/20% white clover sward (CLV), or an indoor TMR ad libitum (TMR).

## CONCLUSIONS

Pasture-based feeding of cows compared with a TMR diet resulted in some significant differences in WMP functionality, characterized by increased thermal stability of WMP (particularly CLV) and significantly higher yogurt gel strength and firmness. However, the WMP samples were not found to differ significantly over a range of characteristics, including WPNI and gross composition (powders were standardized for fat content), although NPN values did differ between samples. The differences in minor compositional constituents and behavior of the WMP samples used in this study may be attributed to the feeding system used. The sig-

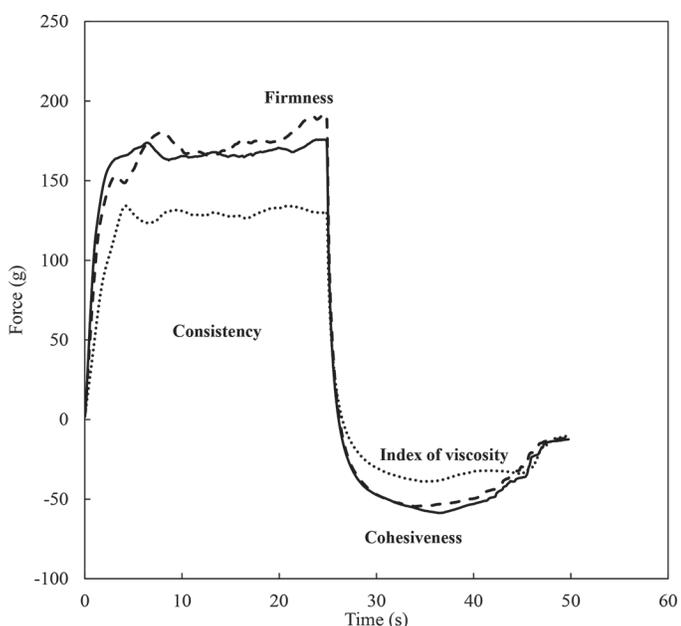
nificantly higher b\* values observed in both WMP and reconstituted WMP produced from the GRS feeding system compared with the CLV and TMR systems supports previous studies on the color profile of pasture-derived milk products. Overall, our results suggest that application of pasture-based dietary treatments confers increased thermal stability in WMP and increased gel strength in yogurt derived from these systems, indicating increased functionality when used as a base material for set-style yogurt manufacture.

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**Figure 5.** Average texture profile of yogurt gels produced from whole milk powder dispersions (15% wt/wt) for grass (solid line), grass/clover (dashed line), and TMR (dotted line) diets. Firmness indicates maximum force (g) recorded on initial probe stroke. Consistency (area) indicates the force per second (g·s<sup>-1</sup>) on initial probe stroke. Cohesiveness indicates maximum negative force (g) recorded on probe return stroke. Index of viscosity (area) indicates the force per second (g·s<sup>-1</sup>) on probe return stroke.

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