Effect of altering the daily herbage allowance to cows in mid lactation on the composition, ripening and functionality of low-moisture, part-skim Mozzarella cheese

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Summary. Milk was collected from three spring-calving herds, on different daily herbage allowances (DHA) of perennial rye-grass (16, 20 or 24 kg dry matter (DM)/cow for a 17 week period. On five occasions, at weekly intervals in the middle of the period, the three different milks were converted into low-moisture part-skim Mozzarella cheese. Increasing the DHA resulted in significant increases in the concentrations of protein in the cheesemilk ($P < 0{\cdot}05$) and cheese whey ($P < 0{\cdot}02$). The moisture-adjusted cheese yield increased significantly ($P < 0{\cdot}01$) on raising the DHA from 16 to 24 kg grass DM/cow. DHA had no significant effects on any of the gross compositional values of the cheese (although moisture and fat-in-DM levels tended to decrease and increase respectively with increasing DHA). The hardness of the uncooked cheese and functionality of cooked cheese (i.e. melt time, flowability, stretch and viscosity) were not significantly influenced by DHA over the 115 d ripening period at 4 °C.

Marked changes occur in milk composition and the state of its components throughout the year, especially in Ireland, New Zealand and parts of Australia, where milk is largely from spring-calving herds fed predominantly on pasture grass (Donnelly & Barry, 1981; Phelan *et al*. 1982; Auldist *et al*. 1996). These changes are in turn associated with inconsistencies in rennet coagulation properties, cheese composition, cheese yield and quality (Lyall, 1969; Grandison *et al*. 1984; O'Keeffe, 1984; Banks & Tamime, 1987; Lucey & Fox, 1992; Lucey *et al*. 1992). It is generally recognized that the seasonal changes in composition of milk and its suitability for cheesemaking are attributable in large part to stage of lactation and changes in diet (O'Keeffe, 1984). The findings of Grandison *et al*. (1984) and Kefford *et al*. (1995), who attempted to segregate the effects of diet and lactation, suggest that diet may exert a marked influence on milk composition and its processability.

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The current study reports on the effect of altering the daily herbage allowance (DHA) to cows in the range 16–24 kg grass dry matter (DM), which is typical of the variation in pasture allowance in Ireland in mid lactation, on the composition of milk and its suitability for the manufacture of low-moisture Mozzarella cheese.

MATERIALS AND METHODS

Milk supply

Milk was collected from three spring-calving herds (16 cows each) on DHA of 16, 20 and 24 kg grass DM/cow i.e. DHA 16, 20 and 24. The estimated DM intakes for DHA 16, 20 and 24 were 15·2, 16·4 and 17·1 kg/cow, and the digestibility of the organic matter in the herbage was similar across the three diet treatments at ~ 820 g/kg organic matter (O'Brien *et al.* 1997). The herd selection criteria, herd management and dietary regimen were also described by O'Brien *et al.* (1997).

Milks from each of the herds were separately collected over 2 d between 113 and 148 d of lactation, bulked, stored at 4 °C, standardized on day 3 and made into cheese on day 4. Five replicate trials were undertaken.

Cheese manufacture

Milks were standardized to a casein:fat ratio of 0·9, pasteurized (72 °C, 15 s), cooled to 36 °C and inoculated at 15 g/kg with a starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus helveticus* (Chr. Hansen's Laboratory (Ireland) Ltd, Little Island, Cork, Irish Republic) at a ratio of 2:1. After a 30 min ripening period, the milk, at 36 °C, was set with chymosin (Double Strength Chymax, 50000 MCU/ml; Pfizer Inc., Milwaukee, WI 53214-4298, USA), which was added at 0·077 ml/kg milk. After cutting (~ 35 min later), the curd–whey mixture was cooked to 42 °C and pitched at pH 6·1. The curd was cheddared and milled, at pH ~ 5·15, directly into the stretching unit (Automatic Stretching Machine, Model f; CMT, S. Lorenzo di Peveragno (CN), Italy), heated to 60 °C (using water at 78 °C), kneaded and stretched until it had formed a cohesive molten elastic mass. The heating water was then drained completely from the stretch unit and the curd was dry salted at a rate of 10 g/kg; using the kneading device the added salt was mixed thoroughly with the curd. The salted curd was moulded into rectangular 2·3 kg blocks, cooled to 23 °C in water at 4 °C, brine salted (210 g NaCl/l) at 10 °C for 2 h, vacuum wrapped and stored at 4 °C.

Sampling and mass balance

Pasteurized cheesemilks and wheys (expressed up to the point of curd milling) were collected and weighed as described by Guinee *et al.* (1996). The curd was weighed just prior to milling, and 45 kg was heated, stretched and kneaded. The moulded cheese was weighed to the nearest 0·01 kg before cooling and after brining. The weights of the residual uncooked cheese and unmoulded cheese, in the moulding unit, were recorded and the weight of the final cheese was adjusted *pro rata* to account for these. The cheeses were stored at 4 °C, analysed for composition at 5, 25 and 115 d and for all other characteristics at 5, 15, 25, 35, 75 and 115 d.

Analyses of the uncooked cheese and whey

Cheese composition. Representative samples of cheese were finely grated and analysed in duplicate for salt, fat, protein, moisture, pH, calcium and phosphorus as described by Guinee *et al.* (1994). The levels of cheese nitrogen soluble in water at pH 4·6 and in phosphotungstic acid (50 g/l) were measured as described by Guinee *et al.* (1994).

Cheese rheology. The forces to compress cylindrical cheese samples were measured on a Model 112 Universal Instron Testing Machine (Instron Ltd, High Wycombe HP12 35Y, UK), as described by Guinee *et al.* (1996).

Cheese wheys. Cheese wheys were analysed for fat, protein and fines, as described by Guinee *et al.* (1996).

Evaluation of cheese functionality on cooking

Melt time. Freshly grated cheese was applied on to a polished stainless steel tray at 1·73 kg/m^2 and placed in an interior lit, electric fan oven at 280 °C. The time required for all cheese shreds to disappear completely and form a uniform molten mass was assessed by viewing the samples through the glass oven door.

Flowability. The flowability of the cheese on cooking was determined by the Schreiber test (Park *et al.* 1984). A disc of cheese (diam. 44·5 mm, height 4·0 mm) was removed from the freshly cut surface of the block on to a pyrex glass disc and placed in a thermostatically controlled electric fan oven at 280 °C for 4 min. The flowability was defined as the increase in the diameter of the cheese disc on melting, expressed as a percentage of the diameter of the unmelted cheese disc.

Stretch. The stretchability of the molten cheese on baked pizza pie was measured by uniaxial extension at a velocity of 0·066 m/s, as described by Guinee & O'Callaghan (1997).

Apparent viscosity. The maximum apparent viscosity of the molten cheese was measured as described by Guinee & O'Callaghan (1997).

Statistical analysis

Results were from five replicate trials undertaken over a 6 week period from 15 June to 26 July 1996. The measurements for the three different diet treatments were compared using analysis of variance to determine the significance of the treatment effects. The statistical model used was

$$y_{ij} = \mathrm{m} + d_i + r_j + E_{ij},$$

where y_{ij} is the response to the ith diet, jth replicate; m is a constant; d_i is the effect of the ith diet; r_j is the effect of the jth replicate and E_{ij} is the random error. It was assumed that there was no diet–replicate interaction. In the results and discussion that follow, effects described as significant were at least $P < 0{\cdot}05$.

RESULTS AND DISCUSSION

Milk composition

Increasing the DHA from 16 to 20–24 kg grass DM/cow, which corresponded with an increase in DM intake of 1·2–1·9 kg (O'Brien *et al.* 1997), resulted in significant increases in the levels of milk protein and casein, but had no effect on casein number or lactose concentration (Table 1).

Losses of fat, protein and fines in cheese whey

Increasing the DHA in the range 16–24 h had no significant effect on the levels of fat and curd fines in the bulk cheese wheys, though the levels of the latter decreased numerically with increasing DHA (Table 1). These results are in agreement with those of Kefford *et al.* (1995), who found no significant increase in fat recovery

Table 1. *Effect of daily herbage allowance on the composition of cheesemilk and its suitability for the production of low-moisture part-skim Mozzarella cheese*

(Values are means for five replicate trials)

Characteristic	Daily herbage allowance, kg dry matter/cow			SED
	16	20	24	
Milk composition				
Total protein, g/kg	31·6[a]	32·4[b]	32·5[b]	0·34
Casein no.	72·7[a]	72·7[a]	73·4[a]	0·55
Casein, g/kg	23·0[a]	23·6[b]	23·9[c]	0·25
Lactose, g/kg	45·6[a]	45·4[a]	45·3[a]	0·44
Whey composition				
Fat, g/kg	4·14[a]	4·00[a]	3·70[a]	0·27
Curd fines, mg/kg	240[a]	199[a]	145[a]	67
Total protein, g/kg	8·65[a]	9·40[b]	9·30[b]	0·21
Cheese composition				
Moisture, g/kg	481·7[a]	479·2[a]	477·6[a]	4·1
Moisture in non-fat substance, g/kg	609·5[a]	610·4[a]	610·3[a]	3·7
Fat in dry matter, g/kg	404·5[a]	412·8[a]	415·0[a]	4·7
Salt in moisture, g/kg	26·2[a]	27·3[a]	27·3[a]	1·2
Calcium, mg/g protein	25·2[a]	24·7[a]	25·5[a]	0·4
Phosphorus, mg/g protein	19·1[ab]	18·9[a]	19·7[b]	0·3
pH at 5 d	5·44[a]	5·41[a]	5·43[a]	0·01
Cheese yield				
Actual, kg/1000 kg	87·64[a]	87·84[a]	90·70[a]	1·99
MACY, kg/1000 kg†	83·64[a]	86·48[ab]	89·26[b]	1·60
MACYCFAM, kg/1000 kg‡	90·18[a]	90·76[ab]	93·40[b]	1·21
Recovery in cheese				
Milk fat, g/kg total	706[a]	720[ab]	749[b]	17·8
Milk protein, g/kg total	743[a]	734[a]	738[a]	11·8

[a, b, c] Values within a row without a common superscript were significantly different: $P < 0·05$.

† MACY, moisture-adjusted (to 470 g/kg) cheese yield (kg/1000 kg).

‡ MACYCFAM, moisture-adjusted (to 470 g/kg) cheese yield (kg/1000 kg milk adjusted to 25 g casein/kg and 28 g fat/kg).

in Cheddar cheese on raising the daily dietary DM intake from 11·3 to 14·7 kg/cow in mid lactation. Increasing the DHA from 16 to 20–24 resulted in a significant increase in the level of protein in the bulk cheese whey, a result expected because of the higher level of whey protein in the milk at the higher DHA (O'Brien *et al.* 1997).

Cheese composition

DHA had no significant effect on any of the gross compositional values apart from phosphorus, the concentration of which significantly increased on raising the DHA from 20 to 24 (Table 1). However, the levels of moisture, on a total weight basis, and fat in dry matter tended to decrease and increase respectively with increasing DHA. The trend toward lower moisture content with increasing DHA and milk casein level is in agreement with previous findings showing an inverse relationship between Cheddar cheese moisture and milk casein level in mid lactation (Guinee *et al.* 1996). In contrast to the current study, Kefford *et al.* (1995) found a significant reduction (12 g/kg) in moisture content of Cheddar cheese on increasing the daily dietary DM intake from 11·3 to 14·7 kg/cow in mid lactation. The discrepancies among this and previous studies with regard to the effect of dietary intake on cheese moisture may be attributable to a number of factors including the larger variation in DM intake, the higher quality of the diet at the higher DM intake, and the larger difference in somatic cell count (SCC) between the lower and higher dietary intakes in the study of Kefford *et al*. (1995). It is generally agreed that increases in SCC in milk result in higher plasmin activity, lower concentrations of β-casein and subsequent higher levels of γ-caseins (Donnelly & Barry, 1981; Politis & Ng-Kwai-Hang, 1988). Donnelly *et al*. (1984) found that trypsin treatment of milk, which resulted in a casein proteolysis pattern similar to that obtained by plasmin, resulted in higher moisture levels in Cheddar cheese. The authors concluded that β-casein plays a central role in curd contraction following cutting, and in syneresis.

Cheese yield

Although DHA had no significant effect on actual cheese yield, there was a tendency toward increasing yield with increasing herbage allowance (Table 1). The moisture-adjusted (to 470 g/kg) cheese yield (MACY) at DHA 24 was significantly higher than that at DHA 16, a difference expected because of the higher concentrations of casein and fat in the cheese milk at the higher DHA (Guinee *et al*. 1996). Similar to the changes reported by Kefford *et al*. (1995) for Cheddar cheese, an approximate increase in milk casein of 3·8% (i.e. 0·9 g/kg) on raising the DHA from 16 to 24 resulted in a 6·7% increase in moisture-adjusted Mozzarella cheese yield (i.e. 0·562 kg/100 kg milk). The inverse relationship between cheese moisture and milk casein level (Guinee *et al*. 1996) explains why increasing the DHA caused a significant increase in moisture-adjusted cheese yield but not in the actual yield. To eliminate the effect of variations in milk composition, i.e. casein and fat, and hence to observe the effects of diet other than compositional, the yield was also expressed as MACYCFAM: the moisture-adjusted cheese yield/1000 kg milk adjusted for casein (to 25 g/kg) and fat (to 28 g/kg) levels. Similar to the changes in MACY, the MACYCFAM at DHA 24 was significantly higher than that at DHA 16, an effect which may be attributed to higher fat recovery at the higher DHA (Table 1). The increases in fat recovery and MACYCFAM on raising the DHA from 16 to 24 suggest that factors (e.g. casein micelle structure or composition, levels of free fatty acids in milk) additional to the increases in concentrations of milk casein and fat contributed to the higher yield in the milks from the herd on the high DHA (O'Brien *et al*. 1997; Sapru *et al*. 1997).

Changes in soluble nitrogen and pH

As in previous studies (Kindstedt, 1995; Guinee & O'Callaghan, 1997), the levels of water-soluble nitrogen increased slowly with ripening time (Fig. 1) and were not significantly influenced by DHA, except on day 75. A similar trend was noted for phosphotungstic acid-soluble N with values (expressed as g/kg total N) increasing from ~ 3 at day 5 to ~ 15 at day 115 (results not shown). The relatively low levels of proteolysis in Mozzarella cheese compared with Cheddar (Wilkinson *et al*. 1992), despite its higher moisture and lower salt levels, may be attributed to the lower level of residual rennet activity in low-moisture part-skim Mozzarella (Singh & Creamer, 1990).

The pH of all cheeses increased from ~ 5·42 at day 5 to ~ 5·55 at day 35 and thereafter decreased to greater or lesser degrees during ripening (i.e. to ~ 5·50–5·42 at day 115). The relatively large increase in pH between curd milling (i.e. 5·15) and day 5, which we have noted consistently with low-moisture Mozzarella, could be due to losses of lactic acid and soluble calcium and phosphorus in the stretch water, during the heating–stretching process, and during subsequent cooling and brining. On cooling and storage of the cheese, slow resolubilization of micellar calcium

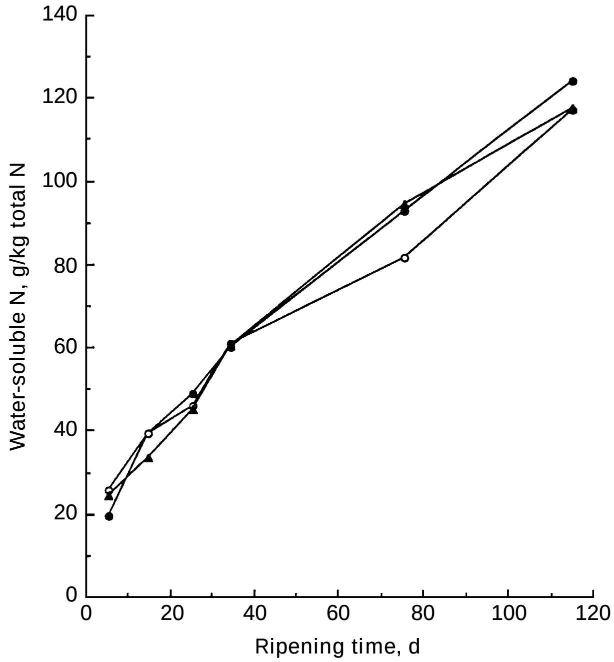

Fig. 1. Content of water-soluble N in low-moisture part-skim Mozzarella cheese during ripening at 4 °C as influenced by daily herbage allowances of ●, 16; ○, 20 or ▲, 24 kg grass dry matter/cow. Each value is the mean of five replicates.

Table 2. *Effect of daily herbage allowance on the textural and functional properties of low-moisture part-skim Mozzarella cheese ripened at 4 °C*

(Values are means for five replicate trials)

Property	Age, d	Daily herbage allowance, kg dry matter/cow 16	20	24	SED
Hardness, N	5	330[a]	325[a]	321[a]	34·9
	25	302[a]	310[a]	312[a]	24·0
	75	242[a]	261[a]	232[a]	23·4
Melt time, s	5	135·6[a]	141·0[a]	135·9[a]	4·4
	25	91·7[a]	90·9[a]	84·5[b]	1·6
	75	106·5[a]	107·6[a]	103·2[a]	3·8
Flowability, %	5	29·9[a]	31·0[a]	35·9[a]	4·2
	25	49·9[a]	48·9[a]	50·8[a]	2·0
	75	61·8[a]	59·1[a]	61·9[a]	2·5
Stretchability, mm	5	630[a]	704[a]	688[a]	96
	25	966[a]	1090[a]	1100[a]	86
	75	983[a]	943[a]	960[a]	81
Apparent viscosity, Pa s	5	1000[a]	990[a]	959[a]	30·1
	25	472[a]	535[a]	456[a]	54·0
	75	214[a]	239[a]	205[a]	21·1

[a, b] Values within a row without a common superscript were significantly different: $P < 0{\cdot}05$.

phosphate to restore equilibrium in the curd–serum mineral levels would be expected to result in the formation of phosphate anions, which reduce the H^+ activity. Increasing the DHA had no significant effect on pH at any time throughout ripening (results not shown).

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Changes in texture and functionality

The changes in the textural and functional characteristics of the uncooked and baked cheeses are summarized for days 5, 25 and 75 in Table 2. There was a notable improvement in functionality of the melted cheese in the first 3 weeks ripening, as reflected by the marked increases in flowability and stretch and reductions in melt time and apparent viscosity and a decrease in cheese hardness. The improved functionality may be attributed to a number of factors: the decrease in intact para-casein level, as reflected by the increase in primary proteolysis, the increase in pH over the first 35 d (Guinee *et al*. 1997) and the expected consequential increase in the ratio of bound:unbound moisture in the cheese during maturation (Creamer, 1985; Kindstedt, 1995; Guinee *et al*. 1997). Generally, varying DHA had no significant effect on any of these characteristics over the 115 d ripening period, a result expected because of the absence of differences in pH and water-soluble nitrogen between the different DHA treatments.

The increases in milk protein level and cheese solids/kg milk associated with higher DHA reported in this study suggest that it may prove worthwhile for farmers to put high emphasis on grazing dairy cows on high quality sward.

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