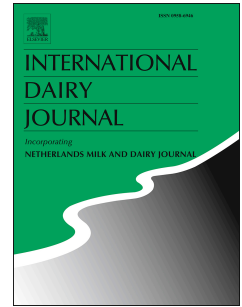


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Effect of reducing daily herbage allowance during early lactation on composition and processing characteristics of milk from spring-calved herds

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

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ABSTRACT

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

47 1. Introduction

48

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

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

70 level of herbage reduction do effects become significant?', and 'are effects of a shortage of
71 herbage in early lactation carried into mid- and late-lactation when herbage again becomes
72 plentiful?'

73 Lowering DHA in the range 13–19 kg DM per cow in early lactation (15–95 days in
74 milk, DIM) has been generally found to coincide with reductions in milk yield and protein
75 concentration, but to have no effect on the concentration of fat or lactose (Kennedy, O'Donovan,
76 O'Mara, Murphy, & Delaby, 2007; McEvoy et al., 2008). A similar trend for effect of DHA on
77 milk yield and concentrations of protein and casein was reported by Auldish, Thomson, Mackle,
78 Hill, and Prosser (2000b) when DHA was limited to ~40% of ad libitum allowance in early
79 lactation (~60-68 DIM). Similarly, Bargo, Muller, Delahoy, and Cassidy (2002) found that a
80 decrease in DHA from 40 to 25 kg DM per cow during four different periods across the year
81 (93–113, 114–134, 209–229, and 230–250 DIM) reduced milk yield, but had no effect on the
82 mean concentrations of milk protein, fat or milk urea nitrogen.

83 Variations in the concentrations of the major milk constituents (e.g., casein, whey
84 protein, lactose) are of relevance in dairy processing as they affect manufacturing efficiency
85 dairy products such as cheese, casein and milk powder, and processing characteristics such as
86 rennet gelation (Guinee et al., 1997; Lin, O'Mahony, Kelly, & Guinee, 2017b), heat stability
87 (Huppertz, 2016) and ethanol stability (Chen, Lewis, & Grandison, 2014; Lin et al., 2017b).
88 However, other composition-related factors are also likely to affect the processing behaviour of
89 milk, e.g., concentration of different elements (Tsioulpas, Lewis, & Grandison, 2007),
90 proportions of different caseins (Jõudu, Henno, Kaart, Püssa & Kärt 2008), and the partitioning
91 of components (e.g., casein, calcium) between the serum and casein micelle (Lin, Kelly,
92 O'Mahony, & Guinee, 2018). Wedholm, Larsen, Lindmark-Månsson, Karlsson, and André

93 (2006) reported that a low concentration of κ -casein and a low proportion of κ -casein (% total
94 casein) in individual cow milk samples collected from different breeds (Swedish Red and White,
95 Swedish Holstein and Danish Holstein-Friesian) correlated with poor rennet-coagulating
96 properties. Little information is available on how variation in DHA in early lactation affects
97 these other compositional parameters and milk processability, or whether such effects are carried
98 over into mid- and late-lactation.

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

103

104 **2. Materials and methods**

105

106 *2.1. Herd treatments and paddock management*

107

108 Thirty-six spring-calved cows from the Teagasc Animal and Grassland Research and
109 Innovation Centre (Moorepark, Fermoy, Co. Cork, Ireland) were made available for the study.
110 The cows had a mean calving date of February 9, 2015 \pm 8.4 d. Following calving, the cows had
111 access to pasture by day and housed by night until 22 February. During this period of partial
112 turnout, the cows received a daily herbage allowance of 7 kg DM per cow while grazing to a post
113 grazing height of >3.5 cm, and had ad-libitum access to grass silage by night. Cows were let out
114 to grass full-time on February 23 and received a DHA of 13 kg DM per cow until March 2, one
115 week prior to dividing of the cows into three separate herds. During the period February 9 –

116 March 2, cows received a concentrate supplement that was gradually reduced from 5 to 0 kg per
117 cow per day. After this period, cows grazed on pasture without any further concentrate
118 supplementation.

119 The 36 cows were assigned to three different herds which were placed on different DHA
120 on March 9. The herds were balanced with respect to calving date (9 February 2015, ± 8.4 d),
121 breed (16 Holstein Friesian (HF), 13 HF \times Jersey, 7 HF \times Norwegian Red) lactation number
122 (2.56 ± 1.4), bodyweight (523 ± 53.2 kg), body condition score (BCS, 3.17 ± 0.142), pre-
123 experimental daily milk yield (25.3 ± 3.70 kg) and milk solids yield (2.20 ± 0.33 kg). Each herd
124 was assigned to a different DHA treatment for 6 weeks from March 9 – April 19 (29–70, DIM)
125 within the same paddocks, which were divided into separate areas using electric wires. The three
126 DHA were 11.8, 14.4 and 15.0 kg DM per cow, respectively; these are denoted as low (L-DHA),
127 medium (M-DHA) and high (H-DHA), respectively. Fresh pasture areas were offered after each
128 milking while the DHA treatments were being imposed and on a 24-h basis thereafter.

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

137 Following the 6 week experimental period, all 36 cows grazed once more as a single
138 herd, receiving a common DHA of 18 kg DM per cow for the remainder of the lactation period

139 (November 23, DIM 288). Individual cows were dried off when daily milk yield decreased to 8
140 kg per cow, BCS dropped to 2.5, or when the interval from next calving date was 8 weeks.

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

146

147 2.2. *Milk sampling*

148

149 Milk was sampled at 10 day intervals during the period March 9-April 19 (29–70 DIM),
150 when the herds were on different DHA; this period was denoted early lactation (EL). Thereafter,
151 milk was sampled at 1–3 week intervals for the remainder of the lactation period, which was
152 divided into two sub-periods, namely mid lactation (ML, April 27–August31) when cows were
153 78-183 DIM, and late lactation (LL, September 20 –November2) when cows were 205–267
154 DIM.

155 Cows were milked daily at 07:00 and 15:30. The milk from all 36 cows was collected
156 separately after evening and morning milkings; evening milk samples were stored at 4 °C
157 overnight prior to blending with morning milk samples. A composite sample for each of the
158 treatment herds was generated by blending the milk from individual cows in the herd, in
159 quantities proportional to the total milk yield of each cow in both evening and morning milkings.
160 The three composite herd milk samples were preserved with sodium azide (0.2%, w/w) and

161 stored overnight at 4 °C until required for analysis, which was completed with 3-48 h after
162 collection.

163

164 2.3. *Preparation of skim milk and milk serum*

165

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

170 The preparation of milk serum involved heating the cold skim milk samples at 40 °C for
171 30 min (to reverse cold ageing effects, including solubilisation of casein and calcium phosphate;
172 Dalgleish & Law, 1988) and ultracentrifugation at 100,000 × g at 25 °C for 1 h (Sorvall
173 Discovery 90SE ultracentrifuge, Kendro Laboratory Products, Asheville, NC, USA). The
174 supernatant was filtered through glass wool to obtain fat-free serum which was stored at 4 °C
175 and assayed for nitrogen (N) content within 48 h of sample collection; subsamples of the fat-free
176 serum (2 mL) were taken in Eppendorf tubes (Thermo Fisher Scientific, Ireland) which were
177 stored at -20 °C and assayed later for protein profile as described below.

178

179 2.4. *Compositional analysis of milk, skim milk and serum*

180

181 Milk was assayed in triplicate for: total solids, fat, lactose and urea using the FOSS
182 MilkoScan FT+ analyser (N. Foss Electric A/S, Hillerød, Denmark), protein, non-casein nitrogen
183 (NCN) and non-protein nitrogen (NPN) using standard International Dairy Federation methods

184 (Lin et al., 2017b). Skim milk was analysed for individual proteins by reversed phase HPLC, as
185 described by Lin et al. (2017b). Minor whey proteins, including bovine serum albumin,
186 lactoferrin, and immunoglobulins were not detected by the RP-HPLC protocol used. Fresh serum
187 was analysed for protein, as described above, and frozen samples of serum were thawed at 4 °C
188 for ~1 h and analysed for individual proteins and soluble casein, which is defined as follows:

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

190

191 2.5. *Element analysis*

192

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

199

200 2.6. *Rennet gelation*

201

202 Cold skim milk samples were heated at 40 °C for 30 min, cooled to 21 °C, adjusted to pH
203 6.55 at 21 °C and heated to 31 °C. Chymosin (Chy-Max® plus, 200 International milk clotting
204 units (IMCU) mL⁻¹; Chr. Hansen, Hørsholm, Denmark) was diluted 20-fold in distilled water,
205 was added to the milk at a level of 10.6 IMCU g⁻¹ protein. Immediately, the sample was assayed
206 for changes in storage modulus, G' (a measure of system elasticity), as a function of time from

207 rennet addition, using dynamic low amplitude strain oscillation rheometry in a controlled stress
208 rheometer (Carri-Med, type CSL2500, TA instruments, New Castle, USA), as described by Lin
209 et al. (2017b).

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

214

215 2.7. Heat coagulation time

216

217 Skim milk samples were adjusted to pH values in the range 6.2 to 7.2 with 0.1 M
218 HCl/NaOH, at 0.1 pH unit intervals, at 21 °C. The pH-adjusted samples and a sample at natural
219 pH (HCT_{npH}) were assayed for heat coagulation time (HCT) at 140 °C in a temperature
220 controlled oil bath (Hettich ESP oil-heating bath; Hettich Benelux BV, Geldermalsen,
221 Netherland), as described by Lin et al. (2017b). The following parameters were obtained from
222 the resultant pH/HCT curves, all of which had a typical type A HCT/pH profile (Huppertz,
223 2016): HCT_{max} , HCT at the first inflection point; HCT_{min} , HCT at second inflection point.

224

225 2.8. Statistical analysis

226

227 The data set relating to the bulk milk from each of three DHA treatments (L-DHA, M-
228 DHA and H-DHA) in the individual lactation periods (EL, ML and LL) was analysed using
229 analysis of variance (ANOVA), to determine the effect of DHA in each lactation period, the

230 effect of DHA in overall lactation, and the effect of lactation period across all the DHA
231 treatments. The experimental unit was herd milk, while the replication unit was the sampling
232 time. The effects of DHA and lactation period were determined using the general linear model
233 (GLM) procedure of SAS 9.3 (SAS Institute Inc., Cary, NC). Tukey's multiple-comparison test
234 was used for paired comparison of means and the level of significance was determined at $P <$
235 0.05.

236 R-3.2.2 software (R Core Team, 2014) was used to compute a Pearson correlation
237 between the different compositional variables, where significance was determined at $P < 0.05$, P
238 < 0.01 , and $P < 0.001$, according to Students t-test.

239

240 **3. Results and discussion**

241

242 *3.1. Gross composition and pH of milk*

243

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

247 Reducing DHA from 15.0 (H-DHA) to 11.8 (L-DHA) kg DM per cow during the six-
248 week EL period resulted in lower yields of milk and milk solids, and concentrations of total
249 protein, true protein and casein in EL, but had no effect on the concentrations of total solids,
250 lactose, fat, whey protein, NPN and urea, casein number, or proportions of individual caseins in
251 EL. The results concur with those of previous studies (O'Brien et al., 1997; Kennedy et al.,
252 2007; McEvoy et al., 2008) which found that reducing DHA in the range 19.0 to 13.0 kg DM per

253 cow in early- (15–95 DIM) or mid- (88–177 DIM) lactation led to lower milk yield and
254 concentrations of protein and casein, but did not affect the concentrations of fat and lactose.
255 Likewise, the absence of an effect of DHA on the proportions of different caseins (α_{S1} -, α_{S2} -, β -
256 or κ -caseins) agrees with the results of Auld et al. (2000b) on restricting DHA to ~40% of
257 ad libitum intake in early lactation (~60–68 DIM).

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

261 This trend confirms the results of Kennedy et al. (2007), which showed that differences in
262 milk yield and composition as a result of DHA variation (13–19 kg DM per cow) in early
263 lactation (15–91 DIM) disappeared on normalisation of DHA to 20 kg DM per cow in mid
264 lactation (92–119). Conversely, Roche (2007) found that restricting DHA from 13.5 to 8.6 kg
265 DM per cow in early lactation (1–35 DIM) coincided with lower yields of milk, fat and protein,
266 and concentrations of fat and protein during later lactation (36–105 DIM). McEvoy et al. (2008)
267 also found that lowering DHA (from 17 to 13 kg DM per cow) in early lactation (19–95 DIM)
268 resulted in a significant reduction in milk protein (0.13%, w/w) in mid-lactation (96–181 DIM).
269 Roche (2007) concluded that the most plausible reason for this carryover effect is a negative
270 effect of energy restriction on mammary secretory cell number and activity, and potentially a
271 reduced uptake of nutrients by the mammary gland. The inter-study discrepancy (Kennedy et al.,
272 2007; McEvoy et al., 2008; Roche, 2007) on the effects of DHA restriction in early lactation on
273 the composition of milk as lactation advances may relate to factors such as extent and duration of
274 feed restriction, and level of nutrient intake in the pre-calving and post-restriction periods.

275 Despite its effects in EL, reducing DHA in EL had no effect on the mean values for milk yield or
276 different compositional parameters over the entire lactation.

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

291

292 3.2. *Macro- and trace-elements*

293

294 The elemental content of milk affects its processing behaviour, and the nutritional value
295 and stability of dairy products (Gaucheron, 2013). The mean concentrations of macroelements
296 (Ca, P, Na and Mg) and trace elements (Zn, Fe, Cu, Mo, Mn, Se and Co) are shown in Table 2.
297 Overall, reducing DHA in EL had little influence on the concentrations of most elements, apart

298 from giving higher and lower concentrations of Mg and Fe, respectively, in EL and a higher
299 concentration of Zn in LL. The results concur with O'Brien et al. (1997) who reported that
300 alteration of DHA in mid-lactation (88–177 DIM) did not significantly alter the concentrations of
301 Ca or P. The absence of an effect of DHA on the concentrations of Ca and P might be explained
302 on the basis that the animal skeleton acts as a reservoir for these minerals where mineral intake
303 in the diet is deficient (Fox, Uniacke-Lowe, McSweeney, & O'Mahony, 2015).

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

312

313 3.3. *Composition of milk serum*

314

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

319 The concentration of protein and soluble casein in serum increased over lactation,
320 concomitant with the increase in the concentrations of total protein, casein and whey protein in

321 milk. Casein in serum, as a proportion of total casein in milk, increased significantly from a
322 mean value of 5.3 % in EL to 12.9% in EL; the range of values (3.4–13.9%) over lactation was
323 broader than that (3.6–10.5 %) reported by Lin et al. (2017b) for a mixed mixed-herd of spring-
324 and autumn-calving cows over the year, but narrower than that (7–25% of total casein) found by
325 Rose (1968) for fresh milk from individual cows and equilibrated at 35 °C prior to
326 ultracentrifugation. The overall mean proportions of α_S -, β - and κ -caseins, as percentages of
327 casein in serum, were ~26, 41 and 33, respectively, and were not influenced by DHA or lactation
328 period (data not shown); the values are of similar magnitude to those reported by Lin et al.
329 (2017b). A tentative explanation for the relatively high proportion of soluble casein in the LL
330 milk is the reduction in ratio of Ca and P-to-casein (Fig. 1b). Rose (1968) investigated the effects
331 of incremental reduction in the colloidal calcium content of milk from 18.6 to 0.6 mM, and
332 concluded that the calcium phosphate content of micelles and the polymerisation of temperature-
333 sensitive caseins (Dalglish & Law, 1988), especially β -casein, is the major factor controlling the
334 level of intact casein in serum. High levels of casein in serum (\gg 15% of total casein) are
335 undesirable as they impair rennet gelation and curd syneresis, and reduce cheese yield (Ali,
336 Andrews, & Cheeseman, 1980).

337

338 3.4. *Rennet gelation*

339

340 Rennet gelation is a key functional parameter of milk used for cheesemaking as it
341 determines the rate at which the milk sets and the changes in gel strength (storage modulus, G')
342 and gel firming rate as a function of time from rennet addition. It influences the ability of the gel
343 to withstand fracture during cutting and to synerese, and consequently the moisture content and

344 quality of the cheese, the recovery of fat, and cheesemaking efficiency (Fox, Guinee, Cogan, &
345 McSweeney, 2017). Alteration of DHA in EL had no effect on rennet coagulation time, (RCT),
346 maximum gel firming rate (GFR_{max}) or gel firmness at 40 min (G'_{40}) during EL, ML or LL
347 (Table 3). The lack of an effect of DHA on rennet coagulation characteristics is consistent with
348 the results of O'Brien et al. (1997) and is scarcely surprising based on the relatively small, or
349 lack of, difference between the DHA treatments with respect to casein concentration in EL
350 (maximum difference of 0.13%, w/w, in EL) (Auldist, Johnston, White, Fitzsimons, & Boland,
351 2004; O'Brien et al., 1997), individual caseins, soluble casein, and ratios of Ca- and P- to casein
352 (Guinee et al., 1997; Horne & Lucey, 2017).

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

367

368 3.5. *Heat coagulation time*

369

370 Heat coagulation time determines the stability of milk to high temperature treatment, as
371 applied for example during the preparation of milk-based beverages (e.g., UHT milk, infant milk
372 formula, and nutritional drinks), condensed milk and recombined milks (Sharma, Jana, &
373 Chavan, 2012). Commercially, beverages are frequently prepared from skim milk concentrates
374 which have a lower pH than native milk which increases the susceptibility to heat-induced
375 aggregation and destabilisation (Lin et al., 2018). Reducing DHA in EL had no effect on the heat
376 stability characteristics of milk at 140 °C (HCT_{min} , HCT_{max} and HCT_{npH}) in EL, ML or LL. The
377 absence of an effect of DHA is consistent with the similar values of lactose, urea, Ca, P and pH
378 for each of the DHA treatments in EL, ML and LL (Holt, Muir, & Sweetsur; 1978; Huppertz,
379 2016); and the relatively small difference in protein content (0.22%, w/w) (Rattray & Jelen,
380 1996) between the treatments in EL.

381 While stage of lactation also had no effect on HCT_{min} or HCT_{npH} , the mean HCT_{max}
382 across the three DHA treatments in EL was lower than that in ML or LL (Table 4). The results
383 suggest that changes in the various compositional parameters during lactation have interactive
384 effects on HCT, to an extent dependent on the magnitude of the change (Huppertz, 2016). Hence,
385 while the relatively low concentration of lactose and high concentration of urea might be
386 expected to enhance the HCT_{npH} of LL milk compared with ML- or EL- milk, such an increase
387 may be offset by the higher protein concentration and lower pH of LL milk (Meena, Singh,
388 Borad, & Panjagari, 2016). Regression analysis indicated that HCT_{max} and HCT_{npH} correlated
389 positively with concentrations of urea, NPN, total protein and soluble casein, and proportions of

390 κ - and α_{S1} - or α_{S2} -caseins, and negatively with lactose (Table 4). The positive effect of higher
391 soluble casein on HCT_{max} and HCT_{pH} is analogous to the increase observed on increasing the
392 proportion of soluble casein by addition of NaCl (1.2%, w/w) or addition of sodium caseinate (\geq
393 0.3%, w/w) (Lin, Kelly, O'Mahony, & Guinee 2017a; Tessier & Rose, 1964).

394

395 **4. Conclusions**

396

397 Reducing DHA of a spring-calved herd from 15 to 11.8 kg DM per cow in EL (9 March –
398 19 April; 29–70 DIM) led to lower milk yield and concentrations of total protein and casein, but
399 had little, or no, effect on other aspects of composition (e.g., concentrations of fat, lactose, non-
400 protein N, urea, elements or proportions of individual caseins), rennet gelation or heat stability at
401 pH values 6.2–7.2. Moreover, there was little, or no, impact of reducing DHA in EL on milk
402 composition, rennet gelation or heat stability in ML (27 April – 31 August; 85–190 DIM), LL
403 (20 September – 2 November; 210–275 DIM) or overall lactation (EL + ML + LL). The absence
404 of an effect of lowering DHA in EL on most compositional parameters and processability
405 characteristics (rennet gelation and heat stability) in EL, ML or LL suggests that restricted
406 grazing without concentrate supplementation can, within limit, be applied in early lactation with
407 little consequence apart from the lower yields of milk and milk solids during that period. This of
408 relevance to farm management where adverse weather in spring can reduce grass availability to
409 cows, especially where stocking rate is high.

410

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417

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1 **Figure legend**

2

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

9

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

Item	Effect of DHA treatment in different lactation periods															Overall effects throughout lactation	
	Early-lactation (EL)					Mid-lactation (ML)					Late-lactation (LL)					DHA	LP
	H-DHA	M-DHA	L-DHA	SED	<i>P</i>	H-DHA	M-DHA	L-DHA	SED	<i>P</i>	H-DHA	M-DHA	L-DHA	SED	<i>P</i>	<i>P</i>	<i>P</i>
Milk yield (kg per cow per day)	26.1 ^a	24.4 ^b	23.0 ^b	0.34	**	20.8	20.4	21.4	0.03	-	12.4 ^{ab}	12.2 ^b	13.5 ^a	1.14	**	-	***
Milk solids yield (kg per cow per day)	3.43 ^a	3.23 ^{ab}	3.01 ^b	0.06	**	2.67	2.72	2.82	0.04	-	1.78	1.83	1.91	0.16	-	-	***
Total solids (% w/w)	13.1	13.2	13.1	0.16	-	12.9	13.3	13.2	0.13	-	14.3	14.6	14.4	0.18	-	-	***
Lactose (% w/w)	4.88	4.83	4.85	0.02	-	4.88	4.84	4.85	0.01	-	4.6 ^a	4.48 ^b	4.52 ^{ab}	0.05	*	-	***
Fat (% w/w)	4.01	4.20	4.16	0.02	-	3.74	4.19	4.05	0.14	-	4.78	4.9	4.77	0.10	-	-	***
Total protein (% w/w)	3.37 ^a	3.29 ^{ab}	3.15 ^b	0.05	*	3.46	3.52	3.45	0.02	-	4.08	4.31	4.17	0.11	-	-	***
True protein (% w/w)	3.17 ^a	3.09 ^{ab}	2.95 ^b	0.04	*	3.26	3.31	3.26	0.02	-	3.78	4.00	3.90	0.13	-	-	***
Casein (% w/w)	2.59 ^a	2.56 ^a	2.46 ^b	0.02	*	2.61	2.68	2.62	0.02	-	3.03	3.21	3.10	0.08	-	-	***
Individual caseins (% milk casein)																	
α_{s1} -casein	42.3	42.7	40.3	1.1	-	37.4	37	39.4	2.2	-	39.3	39.1	41.0	0.69	-	-	*
α_{s2} -casein	8.54	8.74	8.51	0.72	-	11.1	9.83	9.58	3.2	-	10.24	11.49	8.56	0.61	-	-	-
β -casein	32.4	32.2	33.9	1.1	-	31.8	33.2	33.8	3.3	-	32.4	28.9	31.2	1.14	-	-	-
κ -casein	16.8	16.3	17.3	1.1	-	19.7	20.0	17.2	2.8	-	18.1	20.5	19.2	1.42	-	-	-
Casein number	76.8	77.8	78.0	0.81	-	75.4	76.2	75.9	0.35	-	74.2	74.5	74.2	0.46	-	-	**
Whey protein (% w/w)	0.58	0.53	0.50	0.02	-	0.65	0.64	0.65	0.01	-	0.76	0.79	0.80	0.06	-	-	***
α -Lac: β -Lg	0.21	0.22	0.22	0.01	-	0.21	0.22	0.23	0.38	-	0.23	0.20	0.25	0.07	-	-	-
Casein:whey protein	4.47	4.83	4.92	0.20	-	4.02	4.19	4.03	0.17	-	3.99	4.06	3.88	0.14	-	-	**
NPN (% total N)	5.92	6.04	6.17	0.20	-	5.79	5.72	5.50	0.09	-	7.15	7.28	6.49	0.81	-	-	-
Urea (mg 100 g ⁻¹)	28.5	27.2	28.2	1.2	-	28.5 ^{ab}	29.6 ^a	27.8 ^b	0.40	*	32.5	34.5	34.7	2.30	-	-	-
pH	6.70	6.74	6.72	0.02	-	6.69	6.65	6.67	0.01	-	6.64	6.61	6.63	0.01	-	-	***
Soluble protein (% w/w)	0.91 ^{ab}	0.97 ^a	0.84 ^b	0.03	*	1.06	1.07	1.01	0.02	-	1.53	1.6	1.49	0.08	-	-	***
Soluble casein (% milk casein)	3.4	7.9	4.6	1.44	-	6.4	7.1	5.1	0.71	-	13.2	13.9	11.7	1.58	-	-	***

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

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

Item	Effect of DHA treatment in different lactation periods															Overall effects throughout lactation	
	Early-lactation (EL)					Mid-lactation (ML)					Late-lactation (LL)					DHA	LP
	H-DHA	M-DHA	L-DHA	SED	<i>P</i>	H-DHA	M-DHA	L-DHA	SED	<i>P</i>	H-DHA	M-DHA	L-DHA	SED	<i>P</i>	<i>P</i>	<i>P</i>
Ca (mg 100 g ⁻¹)	121	128	122	3.1	-	126	130	124	1.7	-	143	142	144	2.1	-	-	***
P (mg 100 g ⁻¹)	92.7	98.3	92.7	3.0	-	91.6	92.6	91.4	1.1	-	98.7	102	101.7	1.7	-	-	*
Na (mg 100 g ⁻¹)	36.6	39.5	36.8	1.1	-	41.3	41.6	40.5	0.36	-	55.0	58.2	56.9	2.2	-	-	***
Mg (mg 100 g ⁻¹)	10.5 ^b	11.4 ^a	11.4 ^a	0.11	**	10.8	11.2	11.0	0.15	-	13.4	13.6	14.0	0.18	-	-	***
Zn (µg kg ⁻¹)	4086	4212	4467	171	-	3957	4066	3859	93.9	-	4300 ^b	4521 ^{ab}	4603 ^a	119	*	-	*
Fe (µg kg ⁻¹)	332 ^{ab}	371 ^a	276 ^b	19.1	*	304	375	323	50.7	-	295	262	274	24.9	-	-	-
Cu (µg kg ⁻¹)	98.3	117.9	101	7.6	-	72.1	78.6	81.3	4.9	-	42.3	48.9	48.7	7.2	-	-	***
Mo (µg kg ⁻¹)	29.6	31.2	33.9	2.67	-	45.4	46.2	45.0	1.4	-	44.5	44.8	45.8	3.5	-	-	**
Mn (µg kg ⁻¹)	30.9	33.9	29.1	2.1	-	34.5	37.3	35.9	3.2	-	33.8	34.3	32.0	1.5	-	-	-
Se (µg kg ⁻¹)	8.9	9.2	9.2	0.29	-	14.7	15.7	15.1	0.39	-	16.2	17.8	17.4	1.2	-	-	***
Co (µg kg ⁻¹)	0.75	0.71	0.83	0.03	-	0.52	0.71	0.60	0.01	-	0.88	0.74	0.80	0.02	-	-	*

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

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

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

^a H-DHA, M-DHA and L-DHA denote high-, medium- and low- DHA, i.e., 15.0, 14.4 and 11.8 kg dry matter per cow, respectively. Early (EL)-, mid (ML)- and late (LL)-lactation correspond to March 16-April 19, April 27-August 10, and September 1-November 2, when cows were 29-70, 78-183, and 205-267 days in lactation, respectively. Values within a row relating to effect of DHA treatment in EL, ML or LL and not sharing a common lower-case superscripted letter differ significantly for effect of DHA; values within a row without a superscript do not differ for effect of DHA ($P > 0.05$). SED = standard error of difference between means; *P* values denote statistical significance, where ***, **, * and - denote $P < 0.001$, < 0.01 , < 0.05 and > 0.5 , respectively. The statistical significance (*P*) for the effects of DHA in overall lactation, and lactation period (LP) across all DHA treatments are also shown. Abbreviations: RCT, rennet coagulation time; GFR_{max}, maximum gel firming rate; G'₄₀, gel firmness at 40 min; HCT_{npH}, HCT at natural pH; and HCT_{max} and HCT_{min} are the maximum and minimum heat coagulation times, respectively, of the HCT/pH (6.2–7.2) curve.

Table 4

Significant relationships between milk composition and rennet gelation or heat stability characteristics. ^a

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

^a The data set comprised 45 milk samples collected on 15 different occasions throughout the year from three spring-calving herds on different daily herbage allowance in early lactation. Correlations were obtained using simple linear regression analysis; only relationships found to be statistically significant are shown: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$. Positive and negative correlations between two parameters are indicated by a positive sign (+) and a negative sign (-), respectively.

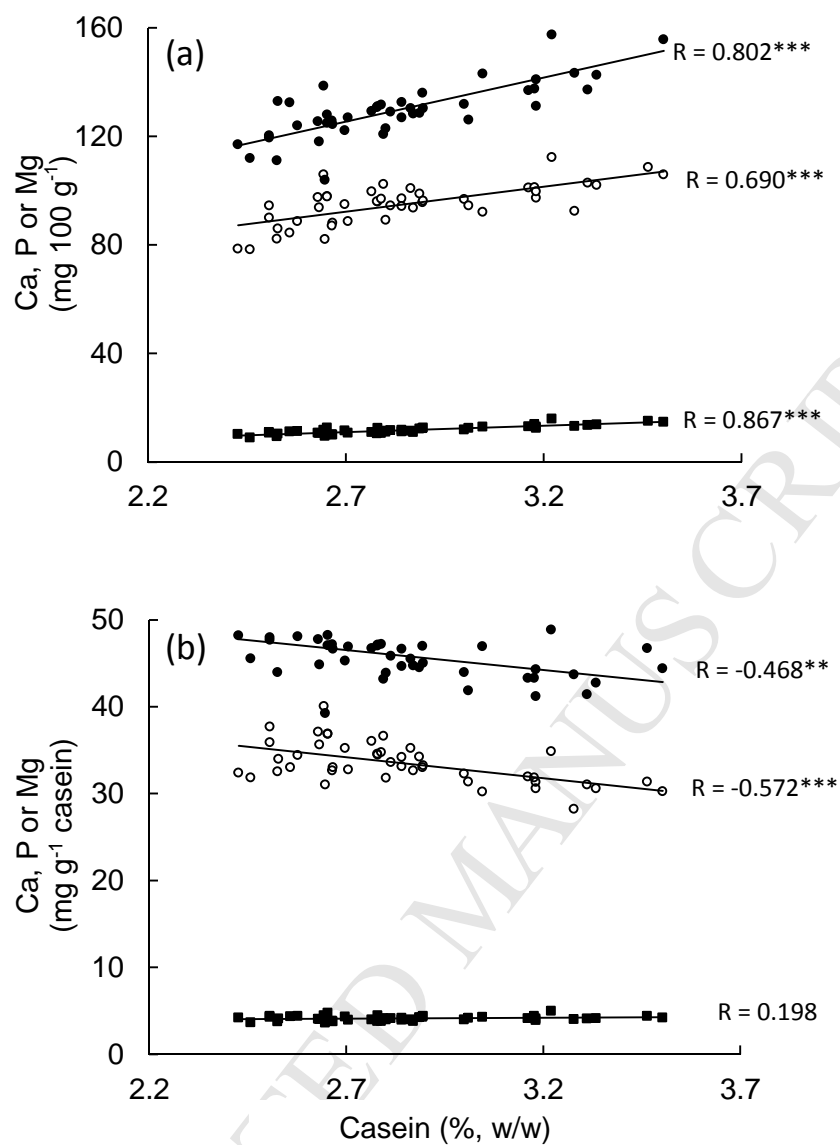


Figure 1.