



Long term dietary supplementation with microalgae increases plasma docosahexaenoic acid in milk and plasma but does not affect plasma 13, 14-dihydro-15-keto PGF2 α concentration in dairy COWS

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1 **Long term dietary supplementation with microalgae increases plasma**
2 **docosahexaenoic acid in milk and plasma but does not affect plasma**
3 **13, 14-dihydro-15-keto PGF_{2α} concentration in dairy cows**

4

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24 The aims of the study were to determine the long-term effects of dietary supplementation with
25 microalgae (SCIM) on milk and blood fatty acid (FA) composition and reproductive hormones in
26 early lactation dairy cows. Sixty Holstein-Friesian dairy cows (30 per treatment) were
27 unsupplemented (Control) or supplemented with 100 g of SCIM (*Schizochytrium imancinum sp*)
28 per cow per day from 25 ± 0.5 days post-partum for 98 days. Intake and milk yield were recorded
29 daily, with milk samples collected at weeks 0, 1, 2, 4, 8 and 14, and blood samples collected
30 from 12 representative pairs per treatment at weeks 0, 2, 4, 8, and 14 for subsequent analysis
31 of FA, β -hydroxybutyrate, non-esterified fatty acids and glucose. At 33 ± 0.9 days postpartum
32 the oestrus cycle of 24 cows (12 per treatment) were synchronised and plasma 13,14-dihydro-
33 15-keto PGF_{2 α} (PGFM) concentrations determined following an oxytocin challenge. Data were
34 analysed by repeated measures analysis of variance. There was no effect of treatment on dry
35 matter intake, milk yield or milk fat content, with mean values across treatments of 22.1 and
36 40.6 kg/d, and 37.2 g/kg respectively. Milk fat concentration of C22:6 n-3 increased rapidly in
37 cows receiving SCIM, reaching a maximum of 0.38 g/100 g FA by week 14. Similarly, blood
38 concentration of C22:6 n-3 increased to 1.6 g/100 g FA by week 14 in cows fed SCIM. There
39 was no effect of treatment on plasma metabolites, but plasma glucose was lower in cows fed
40 SCIM compared to the Control at week 2, and higher in weeks 4 and 8. There was no effect of
41 treatment on peak plasma PGFM concentration or area under the curve. It is concluded that
42 feeding SCIM rapidly increases blood and milk concentrations of C22:6 n-3 which are
43 maintained over time, but does not improve plasma PGFM in dairy cows.

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45 **Keywords:** dairy cow, fatty acids, hormones, milk quality, microalgae

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50 Increasing the content of very long chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) such
51 as eicosapentaenoic acid (C20:5n-3) and docosahexaenoic acid (C22:6n-3) in food products is
52 of interest due to their role in the prevention of certain cancers, development of the retina and
53 brain tissue, anti-inflammatory properties, and their role in the modulation and prevention of
54 coronary heart disease (Zarate et al. 2017). Several studies have successfully increased the LC
55 n-3 PUFA content of dairy and meat products by supplementing with marine sources such as
56 fish oil (FO) or microalgae (Rodriguez-Herrera et al. 2017; Vanbegue et al. 2018). The transfer
57 efficiency of LC n-3 PUFA from marine sources to milk is, however, low (Chilliard et al. 2001)
58 as the majority of the PUFA are biohydrogenated in the rumen to saturated fatty acids (FA) or
59 their intermediaries (Sinclair et al. 2005). Additionally, a time dependent adaptation of the rumen
60 to supplementation with LC n-3 PUFA and production of intermediaries has also been reported
61 in some studies, further reducing the flow of PUFA to the small intestine (Shingfield et al. 2006).
62 Most studies that have examined the effect of feeding LC n-3 PUFA have however, been short-
63 term, and there is a lack of information on the long-term effects of supplementation on blood
64 and milk FA profiles.

65 The fertility of dairy cows in most Western countries has declined over the past five
66 decades, which has been associated with an intensification of production and higher milk yields
67 (Rodney et al. 2015). Polyunsaturated FA have a major role in the endocrine system,
68 metabolism and disease control, influencing the reproductive status of dairy cows in various
69 ways. For example, the series 1 and 2 prostaglandins are synthesised from n-6 PUFA and are
70 intimately involved in uterine involution and subsequent ovulation post-partum (Otto et al. 2014).
71 In contrast, the 3 series prostaglandins are synthesised from n-3 PUFA and are involved in
72 improving the environment for embryo implantation and survival by decreasing the secretion of
73 $\text{PGF}_{2\alpha}$, resulting in an increased lifespan of the corpus luteum (CL) (Dong Hyeon et al. 2016),
74 improvement in blastocyst cell numbers, and maintenance of pregnancy (Otto et al. 2016). The
75 objective of this study was to determine the effect of supplementation with microalgae that is
76 high in C22:6 n-3 on milk and blood LC n-3 PUFA concentrations over a 14 week period, and
77 to determine the effect on the synthesis of $\text{PGF}_{2\alpha}$.

78

79 **Material and methods**

80 The study was conducted in accordance with the requirement of the Animals (Scientific
81 Procedures) Act 1986 (amended 2013) and received local ethical approval (reference 0115).

82

83 *Animals, diets and experimental design*

84 Sixty Holstein-Friesian dairy cows (12 primiparous and 48 multiparous) were randomly allocated
85 into two homogenous groups at 25 ± 0.5 days post calving based on parity and milk yield in
86 the 7 days prior to the start of the study. Animals remained on treatment for 14 weeks and each
87 group received one total mixed ration (TMR) that was either unsupplemented (Control) or
88 supplemented with 100 g/day of dried *Schizochytrium imancinum* sp., (SCIM; Alltech, Kentucky,
89 USA; Table 1). Cows in the Control group received an additional 100 g per cow per day of a
90 rolled wheat/sugar beet feed mixture to provide a similar energy intake. Cows were fed the TMR
91 once daily at 0900 h at 1.05 of the previous days intake via roughage intake feeders (Insentec
92 B.V., Marknesse, The Netherlands) fitted with an automatic animal identification and weighing
93 system calibrated to ± 0.1 kg. Feed intake was recorded daily and the diets sampled weekly and
94 stored at -20 °C for subsequent analysis. The SCIM contained 135 g/kg DM crude protein, 580
95 g/kg oil and (g/100 g FA) 3.7, 1.5, 53.9, 1.7, 0.28, and 25.7 as C14:0, C14:1 *cis*-9, C16:0, C18:0,
96 C20:5 n-3, and C22:6 n-3, respectively. From calving to the start of the study the cows were fed
97 the same basal ration that did not contain SCIM. All cows had free access to salt blocks and
98 water throughout the study.

99 All cows were milked twice daily at 0615 and 1600 h. Milk yield was recorded daily and
100 cows were weighed and body condition scored (BCS; Ferguson et al. 1994) at approximately
101 1100 h at 1 week prior to the start of study, then every other week. Milk samples were collected
102 weekly at consecutive am and pm milkings for subsequent analysis. During weeks 0, 1, 2, 4, 8
103 and 14 of the study milk samples were collected at 2 consecutive am and pm milkings from 16
104 representative pairs of cows per group (based on their parity and milk yield in the week prior to
105 allocation) and pooled based on the respective am and pm milk yield for FA determination.

106

107 *Blood metabolites and reproductive hormones*

108 Blood samples were collected from the jugular vein from 12 representative pairs of cows (based
109 on their parity and milk yield in the week prior to allocation) at 1100 h during weeks 0, 2, 4, 8
110 and 14. Samples were centrifuged at 1000 g for 15 min, the plasma separated and stored at -
111 20°C prior to subsequent analysis. At day 33 (\pm 0.9) postpartum, 24 representative cows (12
112 per treatment group cows based on their parity and milk yield in the week prior to allocation)
113 were synchronized in pairs using progesterone releasing intra-vaginal devices (PRID; Ceva
114 Prid®Delta, Ceva Animal Health Ltd., Amersham, UK). The PRID's were removed after 10 d,
115 and on day 17 of the synchronised oestrous cycle (Robinson et al., 2002), a catheter was
116 inserted into the jugular vein following sedation with Sedaxylan (20 mg/ml xylazine solution at
117 0.5 ml/100 kg; Dechra Pharmaceuticals PLC, Northwich, UK) injected into the coccygeal vein.
118 Blood samples were collected via the jugular catheter at 15 min intervals for 1 h prior to the
119 administration of oxytocin (100 IU; MSD Animal Health, Milton Keynes, UK), and at 15 min
120 intervals for a further 3 h, and then at 30 min intervals until 4 h post oxytocin infusion to monitor
121 uterine secretion of 13,14-dihydro-15-keto PGF_{2 α} metabolite (PGFM). The blood was
122 centrifuged at 1000 g for 15 min and the plasma frozen at -20°C prior to subsequent analysis.

123

124 *Chemical analysis*

125 The TMR samples were bulked within each month and a sub-sample analysed according to
126 AOAC (2012) for DM (934.01), CP (988.05) and ash (924.05), whilst NDF was analysed
127 according to Van Soest et al. (1991). Feed, milk and plasma fatty acid extraction and analysis
128 are provided in the Supplementary Material. Milk fat, protein and somatic cell count (SCC) was
129 determined at the National Milk Laboratories (Four Ashes, UK). Plasma samples were analysed
130 for, 3-OHB, glucose and non-esterified fatty acids (NEFA) (kit catalogue no; RB1008; GU611
131 and FA115, respectively Randox Laboratories, County Antrim, UK), using a Cobas Mira Plus
132 autoanalyser (ABX Diagnostics, Bedfordshire, UK). Plasma concentration of PGFM, was

133 assayed using an ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) with an inter- and intra-
134 assay coefficient of variation of 13.0 and 9.9 % respectively.

135

136 *Calculations and statistical analysis*

137 All data were checked for normal distribution and analysed using Genstat 17th edition (VSN.
138 Ltd, Oxford, UK). The SCC data were converted to their natural log prior to analysis. Daily live
139 weight and body condition change were calculated as the final minus the initial value divided
140 by the days on study. The PGFM area under the curve was calculated as described by
141 Robinson et al. (2002). Variables having more than one observation were analysed using
142 repeated measures ANOVA as: $Y_{ijk} = \mu + P_i + D_j + T_k + D.T_{jk} + \epsilon_{ijk}$, where Y_{ijk} = dependent
143 variable; μ = overall mean; P_i = fixed effect of pair; D_j = effect of diet, T_k = effect of time; $D.A_{jk}$
144 = interaction between diet and time and ϵ_{ijk} = residual error. Variables with one observation
145 were analysed by ANOVA using Genstat 18th edition (VSN Ltd., Oxford, UK).

146 **Results**

147 *Feed analysis*

148 The treatment diets had a similar chemical position with a mean DM of 378 g/kg, OM of 927
149 g/kg DM, CP of 162 g/kg DM and NDF of 419 g/kg DM, whereas the pre-study diet was higher
150 in CP and lower in NDF (Table 1). The pre-study diet had also a higher concentration of C14:0
151 and C16:0 compared to the treatment diets. The SCIM diet contained 0.01 g/kg DM of C20:5 n-
152 3 and 0.71 g/kg DM C22:6 n-3, whereas the pre-study and Control diets did not contain any
153 detectable levels.

154

155 *Animal performance and blood metabolites*

156 There was no effect ($P > 0.05$) of dietary treatment on DM intake, with a mean value of
157 22.1 kg/d (Table 2), but was affected by time ($P < 0.001$), increasing from 21.1 kg/d in week 1
158 of the study to 23.4 kg/d at week 3 before decreasing to 20.9 kg/d at week 14. Similarly, there
159 was no effect ($P > 0.05$) of treatment on daily milk yield with a mean value of 40.6 kg/d, and a

160 peak yield of 42.2 kg/d occurring during week 3 of the study. Mean milk fat content was 37.2
161 g/kg and fat yield 1.49 kg/d, and were not affected by dietary treatment ($P > 0.05$), with both
162 decreasing over time ($P = 0.048$ and 0.013 respectively). Milk protein content and yield were
163 not affected ($P > 0.05$) by dietary treatment, and decreased with time ($P < 0.001$). There was
164 no effect ($P > 0.05$) of dietary treatment on live weight, which increased by 0.23 kg/d over the
165 14 week ($P < 0.001$). Body condition score was unaffected ($P > 0.05$) by treatment or time.

166 There was no effect ($P > 0.05$) of dietary treatment on the mean concentration of plasma
167 3-OHB, glucose or NEFA (Table 2). Plasma NEFA tended to decrease ($P = 0.06$) from week 2
168 to week 14 of the study, whilst plasma glucose was lower in cows receiving SCIM compared to
169 the Control at week 2, and higher in weeks 4 and 8 ($P < 0.05$).

170

171 *Milk and plasma fatty acid profile*

172 There was no effect ($P > 0.05$) of dietary treatment on milk fat content of C4:0 to C18:0,
173 C18:1 t-12, C18:1 n-9, C18:2 n-6, C20:0, C18:2 t-10, cis-12 CLA, C20:3 n-3 and C20:5 n-3,
174 SFA, MUFA or total n-6 FA (Table 3). Milk fat content of C18:1 t 10, and c-9, t-11 CLA were
175 similar at week 0 in cows fed either treatment, and increased ($P < 0.05$) in SCIM fed cows from
176 week 2 onwards (Fig. 1a, b). There was also a higher milk fat content of C22:6n-3 in cows fed
177 SCIM from week 2 onwards ($P < 0.001$), with the maximum difference between treatments of
178 0.34 g/100g FA occurring at week 14 of the study (Fig. 1c). Milk fat content of total PUFA and
179 total n-3 PUFA increased and the n-6 to n-3 PUFA ratio decreased in SCIM fed cows from week
180 2 of the study ($P < 0.05$; Fig. 1d, e and f respectively), whilst C18:3 n-3 was lower at week 2 and
181 C22:0 higher at weeks 8 and 14 in SCIM fed cows (Supplementary Fig. 1a and b). Milk fat
182 content of C18:1 t-8, t-9, t-11 and C20:3 n-6 were higher in cows fed SCIM than the Control. The
183 content of C10:0, C12:0, C14:0, C14:1 n-5, C16:1 n-7, C18:1 t-8, C18:2 n-6, C20:0, and Σ n-6
184 FA increased with time, whilst C4:0, C6:0, C15:0, C17:0, C17:1, C18:1 t-12, C18:1 c-9, C18:2
185 t-10, c-12 CLA, C20:3 n-6, C20:5 n-3 and MUFA decreased over the study period.

186 There was no effect ($P > 0.05$) of dietary treatment on blood plasma fat content of
187 C14:0 to C17:0, C18:1 t9, 12, or 15, C18:1 c-9, C20:5 n-3, total MUFA, PUFA or n-6 FA (Table

188 4). There was an interaction between time ($P < 0.001$) on plasma C22:6 n-3 concentration,
189 which was higher in SCIM fed cows from week two of the study, and remained high for the
190 remainder of the study. In contrast, the ratio of the total n-6 to n-3 PUFA in blood plasma was
191 lower ($P < 0.001$) from week 8 of the study in cows fed SCIM (Fig. 2b). Blood plasma C18:0,
192 C20:4 n-6, C20:0 and the sum of the saturated FA were similar at week 0 and decreased in
193 cows fed SCIM compared to the control (Supplementary Fig. 2 a,d,e,f), whilst C18 t-10, C18:3
194 n-3, and the sum of the total PUFA increased in cows fed SCIM (Supplementary Fig 2 b,c,g).

195

196 *Plasma PGFM concentrations*

197 Plasma PGFM concentrations increased to a peak at 15-30 min following the oxytocin challenge
198 (Fig. 3) before gradually returning to the basal level at 150 min for cows receiving either
199 treatment. There was no effect of treatment on mean plasma PGFM concentration, area under
200 the curve, or peak concentration ($P > 0.05$).

201

202 **Discussion**

203 *Animal performance and blood metabolites*

204 The primary objective of this study was to determine the long-term effects of feeding microalgae
205 that is high in C22:6 n-3 on milk and plasma fat concentration of LC n-3 PUFA and animal
206 performance. The cows were fed 100 g of microalgae per d as higher inclusion levels have been
207 shown to reduce DM intake and/or result in milk fat depression (Vanbergue et al. 2018; Marques
208 et al., 2019), with the consequence of a reduced milk and/or fat yield. In the current study there
209 was no effect of dietary treatment on DM intake, which averaged 22.1 kg/d over the 14 week
210 period. This finding is in accordance with Till et al. (2019) who reported no effect on intake when
211 cows were fed 100 g of SCIM/d. There is no clear consensus in the literature on the effects of
212 the addition of marine lipids on milk performance, with the level and composition of the
213 supplement, as well as the basal ration, having a major influence (Sinedino et al. 2017; Mattos
214 et al. 2004). In the current study there was no effect of treatment on milk fat content or yield.

215 Bauman & Griinari (2003) described how unique FA intermediates that are produced during the
216 biohydrogenation of PUFA can cause an inhibitory effect on sterol regulatory element binding
217 protein signaling on milk fat synthesis, with one intermediate identified as a potent inhibitor being
218 C18:2t-10, c-12 CLA. In the current study milk fat concentration of C18:2 t-10, c-12 CLA was
219 similar between treatments, and milk fat content was also unaffected by dietary treatment. Lock
220 et al. (2007) investigated the effect of abomasal infusion of C18:1 t-10 on milk fat content in
221 dairy cows and reported that it had no effect on milk fat synthesis. In the current study
222 concentrations of C18: t-10 were higher in both blood plasma and milk of SCIM fed cows
223 compared to the Control, yet milk fat content was unaffected, supporting that C18:1 t-10 is not
224 a major factor controlling milk fat synthesis in dairy cows.

225 In early lactation mobilisation of lipid reserves is required to compensate for the
226 imbalance between energy consumed, and energy secreted in milk (Cozzi et al. 2011), and is
227 generally associated with an elevation in plasma 3-OHB (McArt et al. 2013). In the current study
228 blood samples were collected at one time point and differences may have been detected had
229 samples been taken throughout the day, although previous studies have demonstrated no
230 interaction between feeding microalgae and time of sampling on plasma metabolites (Till et al.,
231 2019). The mean plasma concentration of 3-OHB was not affected by dietary treatment and
232 was within the accepted cut-point concentration of ≥ 1.2 mmol/l that is associated with sub-
233 clinical or clinical ketosis (McArt et al. 2013). The lack of a difference in milk energy output,
234 along with a similar DMI and live weight change in cows receiving the Control or SCIM
235 treatments may explain the similarity in the plasma metabolite concentrations between
236 treatments.

237

238 *Milk and plasma fatty acid profile*

239 Cows that received the SCIM supplement in the current study had higher milk and
240 plasma concentrations of C22:6 n-3 compared to the Control from week 2 onwards, with the
241 difference increasing until week 14 of the study. Most other studies that have fed microalgae to
242 dairy cows have used short-term, change over-studies or fed for a short period of time, and

243 reported increases in C22:6 n-3 in milk of up to 0.46 g/100g FA with unprotected microalgae or
244 0.76 g/100g FA with rumen protected microalgae (Till et al. 2019; Vanbergue et al. 2018).
245 Studies that have fed microalgae for longer periods have reported an increase in the
246 concentration of C22:6 n-3 in milk, but only measured milk FA at single time points and did not
247 monitor the change over time (Sinedino et al. 2017). In contrast to the current findings, Shingfield
248 et al. (2006) reported a temporal pattern in milk C22:6 n-3 concentration when FO was fed,
249 reaching a maximum 5 days after FO introduction before declining. This response was
250 suggested to be due to an adaptation of the rumen microbiome and progressive increase in the
251 extent of biohydrogenation, or a shift in the incorporation of these FA from blood triacylglycerides
252 toward phospholipids (Shingfield et al. 2006). In the current study milk samples were collected
253 1 week after SCIM was introduced, and it is not possible to determine changes in milk fat
254 C22:6n-3 concentration before this. However, the persistent increase in plasma and milk
255 concentration over time does not support a significant ruminal adaptation or reduction in
256 mammary uptake.

257 The inclusion of LC n-3 PUFA in the diet of ruminants typically lowers short and medium
258 chained FA concentration in milk due to their inhibitory effects on mammary *de novo* FA
259 synthesis (Shingfield et al. 2006), but in the current study the concentration of FA with a chain
260 length < 16 was unchanged. A reduction in the milk fat concentration of C18:0 was observed by
261 Till et al. (2019) when cows were fed SCIM, but in the current study there was only a trend for
262 a reduction in milk C18:0 in SCIM fed cows which may be attributed to the inhibitory effect of
263 SCIM on the biohydrogenation of C18-unsaturated FA to C18:0 in the rumen. It was also
264 suggested by Shingfield et al. (2006) that mammary synthesis of C18:1 *cis*-9 from C18:0 via Δ^9 -
265 desaturase was required for the maintenance of the fluidity of milk fat (Bichi et al. 2013). This
266 is difficult to conclude from the current study as milk concentrations of C18:1 *cis*-9 were similar
267 between dietary treatments.

268

269 *Plasma PGFM concentration*

270 The second objective of the current study was to investigate the effect of feeding SCIM
271 on the plasma concentration of PGFM. Diets high in n-3 FA may reduce $\text{PGF}_{2\alpha}$ synthesis and
272 consequently prevent the regression of the corpus luteum (CL), allowing continued secretion of
273 progesterone that can help improve embryo survival (Gulliver et al. 2012). The effects of added
274 PUFA on reproductive function have however, not always been consistent. To date only two
275 other studies have reported the effects of feeding LC n-3 PUFA from microalga on reproduction
276 in dairy cows, with Sinedino et al. (2017) reporting that microalgae fed cows had an increase in
277 conception rate, and upregulation of the interferon-stimulated gene RTP4 which is associated
278 with placental development, immunomodulation and conceptus elongation (Riberio et al., 2016).
279 In contrast, Vlcek et al. (2017) reported no effect on the size of the pre-ovulatory follicle at first
280 or second synchronised oestrus, although the size of the corpus luteum was larger in cows fed
281 microalgae. However, neither Sinedino et al. (2017) or Vlcek et al. (2017) determined the
282 concentration of plasma $\text{PGF}_{2\alpha}$. In the current study SCIM supplementation had no effect on
283 mean, peak or area under the curve of plasma PGFM. In contrast, Dirandeh et al. (2013)
284 investigated the effect of feeding linseed oil as a source of n-3 on plasma concentration of PGFM
285 from calving to 70 days post calving and reported a reduced plasma PGFM concentration.
286 Similarly, Mattos et al. (2004), fed FO to dairy cows from 21 days pre-partum until 21 days post-
287 partum, and reported a significant decrease in plasma PGFM concentrations at days 0, 0.5, 2
288 and 2.5 post-partum in cows fed FO. By day 17 of the synchronized oestrus cycle, the cows
289 selected for PGFM analysis in the current study had received the SCIM supplement for 39 ± 0.9
290 days. Results from other studies suggest that this period of feeding was sufficient to affect the
291 size of the corpus luteum and alter PGFM synthesis (Mattos et al. 2004; Petit et al. 2002).

292

293 **Conclusion**

294 The increase in milk and blood plasma C22:6 n-3 content over the 14 week study period
295 suggest that the rumen microbial ecosystem did not adapt over time to the dietary
296 supplementation of 100 g/d of SCIM. The increase in milk C22:6n-3 and *cis*-9, *trans*-11 CLA

297 improves milk quality for human consumption without affecting milk performance.
298 Supplementing dairy cows with SCIM at the rate and length of time in the current study did not
299 affect plasma PGFM concentrations.

300

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424

Table 1. Diet composition (kg/kg DM) of the pre-study and study diets that contained no SCIM (Control) or 100 g of microalgae per cow per day (SCIM)

Ingredient	Pre-study	Basal diet	
Maize silage	0.350	0.413	
Grass silage	-	0.130	
Lucerne silage	0.152	-	
Chopped wheat straw	0.019	-	
Rapeseed meal	0.059	0.065	
Wheat distillers dark grains	0.071	0.078	
Soya bean meal	0.030	0.065	
Palm kernel meal	0.020	0.022	
Molasses	0.006	0.007	
Caustic wheat	0.114	0.109	
Distillery syrup ¹	0.040	-	
Soya hulls	0.060	0.078	
Food industry by product ²	0.039	-	
Rumen protected fat ³	0.007	0.013	
Rumen protected fat ⁴	0.007	-	
Minerals and vitamins	0.015	0.006	
Chemical composition		Control ⁵	SCIM ⁵
Organic matter	930	928	928
Crude protein	166	163	161
NDF	375	419	419
Fatty acid (g/kg DM)			
C14:0	1.4	0.9	1.0
C16:0	15.8	10.0	12.6
C18:0	1.3	0.9	0.9
C18:1 n-9	8.6	7.9	8.3
C18:2 n-6	10.4	10.6	10.0
C18:3 n-3	1.7	1.7	1.3
C22:5 n-3	0.00	0.00	0.01
C22:6 n-3	0.00	0.00	0.7

¹Spey syrup; KW Feeds, Ternhill, UK. ²Sweetstarch: a by-product from the bakery, confectionary, pastry and breakfast cereal industries; KW Feeds, Ternhill, UK. ³Megalac, a calcium salt of palm fatty acids, Volac, Royston, UK). ⁴Butterfat Extra, a calcium salt of palm fatty acids fatty acids, Trident Feeds, Peterborough, UK. ⁵The SCIM group received the basal ration with an additional 100 g/cow/day of microalgae, and the Control group received the basal ratio with an additional 100 g/cow/day of an equal mixture of a rolled wheat/sugarbeet feed mixture.

Table 2. Animal performance and blood metabolites in dairy cows fed no SCIM (Control) or 100 g of microalgae per cow per day (SCIM)

	Treatment		SED	D	T
	Control	SCIM			
DM intake (kg/d)	22.1	22.0	0.70	0.905	<0.001
Milk yield (kg/d)	39.6	39.9	0.78	0.980	<0.001
Milk fat (g/kg)	37.5	36.9	1.59	0.702	0.048
Fat yield (kg/d)	1.52	1.46	0.063	0.401	0.013
Milk protein (g/kg)	31.3	31.5	0.55	0.670	<0.001
Protein yield (g/kg)	1.27	1.25	0.034	0.584	<0.001
Live weight (kg)	653	651	12.0	0.895	<0.001
Live weight change, kg/d	0.29	0.17	0.084	0.179	--
Milk SCC (log _e)	3.97	3.87	0.217	0.617	0.436
Body condition	2.69	2.81	0.071	0.115	0.837
Body condition change	0.04	-0.08	0.090	0.191	--
3-OHB (mmol/L)	1.07	1.12	0.087	0.550	0.457
Glucose (mmol/L)	2.82	2.83	0.075	0.814	<0.001
NEFA (mmol/L)	0.18	0.21	0.031	0.399	0.061

¹Main effects of diet (D), time (T), and their interaction (D x T). There was no diet x treatment interaction except for plasma glucose, which was approximately 0.1 mmol/L higher in week 2 and 0.1 mmol/L lower in week 8 ($P < 0.05$) in cows fed the Control compared to SCIM.

Table 3. Milk fatty acid composition (g/100g of FA) of dairy cows fed no SCIM (Control) or 100 g of microalgae per cow per day (SCIM)

Fatty acid (g/100 g)	Treatment			P value ¹		
	Control	SCIM	SED	D	T	D x T
C4:0	2.37	2.37	0.083	0.969	<0.001	0.727
C6:0	1.70	1.67	0.070	0.877	0.002	0.737
C8:0	1.18	1.15	0.045	0.624	0.052	0.355
C10:0	2.58	2.48	0.137	0.449	0.009	0.299
C12:0	3.33	3.08	0.176	0.174	<0.001	0.540
C14:0	10.4	9.90	0.309	0.164	<0.001	0.217
C14:1 n-5	0.91	0.83	0.053	0.132	<0.001	0.430
C15:0	1.04	0.98	0.040	0.146	0.002	0.345
C16:0	31.0	30.6	0.616	0.507	0.124	0.250
C16:1 n-7	0.51	0.52	0.020	0.816	0.013	0.361
C17:0	0.51	0.52	0.017	0.845	<0.001	0.228
C17:1	0.26	0.26	0.020	0.924	<0.001	0.488
C18:0	8.38	7.90	0.233	0.058	0.131	0.215
C18:1 t-8	0.26	0.44	0.034	0.002	<0.001	0.130
C18:1 t-9	0.24	0.34	0.019	<.001	0.506	0.176
C18:1 t-10	0.55	0.94	0.166	0.034	0.026	0.033
C18:1 t-11	0.84	1.22	0.101	0.002	0.109	0.356
C18:1 t-12	0.48	0.56	0.046	0.088	0.009	0.152
C18:1 n-9	21.1	20.4	0.87	0.456	<0.001	0.069
C18:2 n-6	2.93	2.99	0.102	0.620	0.009	0.205
C20:0	0.13	0.13	0.009	0.876	0.023	0.680
C18:3 n-3	0.48	0.47	0.023	0.789	0.109	0.012
C18:2 c-9, t-11 CLA	0.57	0.75	0.054	<.001	0.002	0.049
C18:2 t-10, c-12 CLA	0.05	0.04	0.005	0.958	<0.001	0.947
C22:0	0.12	0.08	0.011	0.002	0.260	<0.001
C20:3 n-6	0.05	0.07	0.006	0.034	0.008	0.062
C20:3 n-3	0.18	0.17	0.012	0.648	0.129	0.216
C20:5 n-3	0.08	0.09	0.009	0.376	<0.001	0.242
C22:6 n-3	0.04	0.22	0.015	<.001	<0.001	<.001
Indices						
ΣSFA	62.9	60.7	1.06	0.059	0.150	0.423
ΣMUFA	26.1	26.7	0.97	0.570	<0.001	0.272
ΣPUFA	4.37	4.80	0.151	0.012	<0.001	0.002
Σn-3	0.83	0.97	0.039	0.002	0.121	0.023
Σn-6	2.96	3.03	0.100	0.505	<0.001	0.092
n-6:n-3	3.75	3.24	0.184	0.003	0.817	0.032

¹Main effects of diet (D), time (T), and their interaction (D x T)

Table 4. Total plasma lipid fatty acid composition (g/100g of FA) of dairy cows fed no SCIM (Control) or 100 g of microalgae/cow per day (SCIM)

Fatty acid (g/100 g)	Treatment			<i>P</i> value ¹		
	Control	SCIM	SED	D	T	D x T
C14:0	0.82	0.66	0.103	0.141	<0.001	0.084
C14:1 n-5	0.18	0.13	0.021	0.112	<0.008	0.482
C15:0	0.40	0.42	0.015	0.241	<0.001	0.436
C16:0	12.1	12.4	0.36	0.363	<0.001	0.845
C16:1 n-7	0.78	0.77	0.074	0.854	<0.001	0.859
C17:0	0.63	0.62	0.027	0.682	<0.001	0.497
C18:0	15.3	14.3	0.33	0.016	0.532	0.027
C18:1 t 6-8	0.10	0.14	0.012	0.014	0.003	0.291
C18:1 t-9	0.13	0.16	0.022	0.151	0.038	0.388
C18:1 t-10	0.17	0.31	0.057	0.029	0.189	0.012
C18:1 t-11	0.44	0.64	0.045	<0.001	<0.001	0.056
C18:1 t-12	0.37	0.39	0.020	0.317	<0.001	0.349
C18:1 t-15	0.13	0.13	0.008	0.902	0.004	0.489
C18:1 c-9	8.45	7.67	0.387	0.070	<0.001	0.577
C18:2 n-6	44.2	45.6	0.70	0.067	<0.001	0.259
C20:0	0.64	0.41	0.039	<0.001	<0.001	<0.001
C18:3 n-3	3.34	3.60	0.157	0.120	<0.001	0.035
ΣCLA	0.10	0.12	0.009	0.053	<0.001	0.333
C20:4 n-6	1.75	1.56	0.073	0.022	<0.001	<0.001
C20:5 n-3	0.47	0.50	0.040	0.388	<0.001	0.065
C22:5 n-6	0.29	0.17	0.042	0.014	0.033	0.093
C22:5 n-3	0.71	0.53	0.041	0.001	<0.001	0.065
C22:6 n-3	0.13	1.11	0.028	<0.001	<0.001	<0.001
Indices						
ΣSFA	31.0	29.7	0.48	0.017	<0.001	0.014
ΣMUFA	12.7	12.3	0.45	0.318	<0.001	0.722
ΣPUFA	52.6	54.3	0.85	0.077	<0.001	0.076
Σn-3	4.65	5.74	0.283	0.005	<0.001	<0.001
Σn-6	47.8	48.5	0.66	0.331	<0.001	0.492
n-6:n-3	10.35	8.61	0.821	0.132	<0.001	<0.001

¹Main effects of diet (D), time (T), and their interaction (D x T)

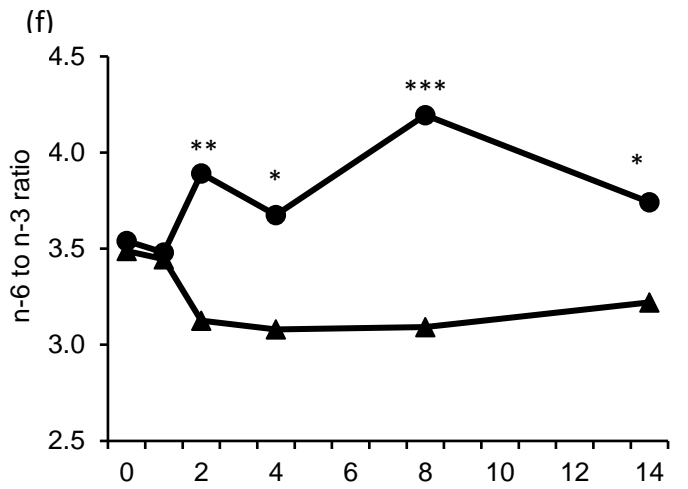
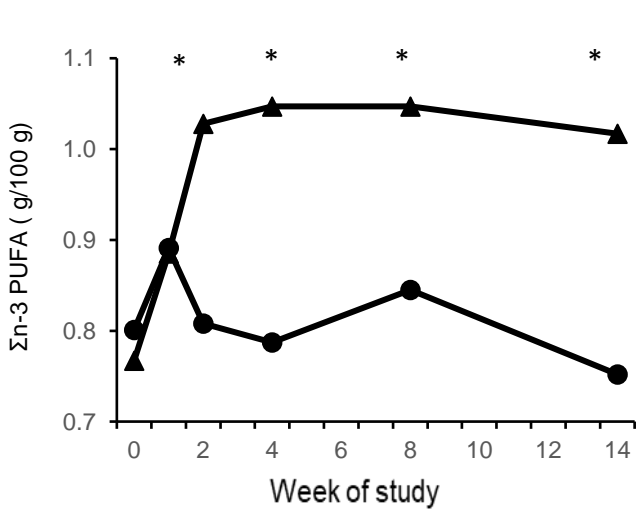
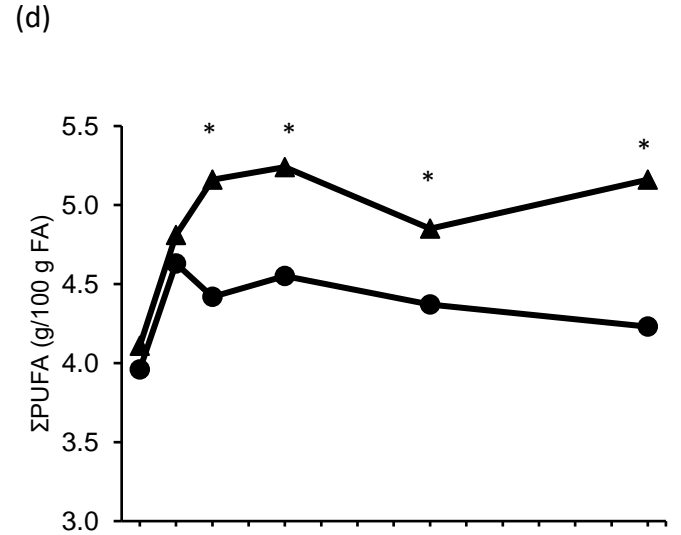
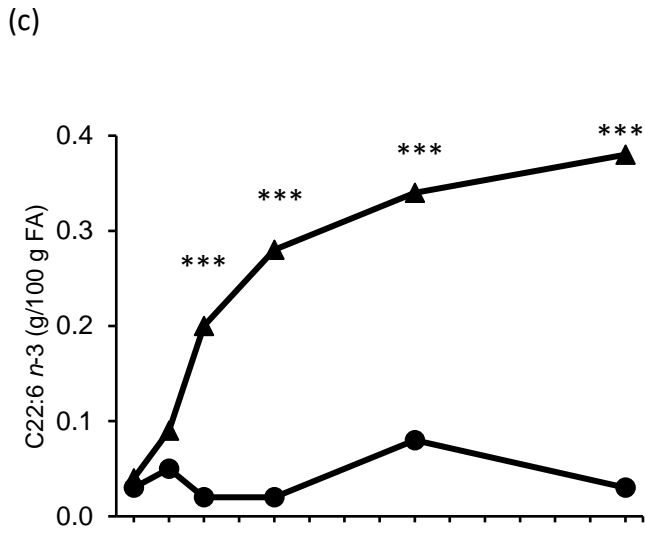
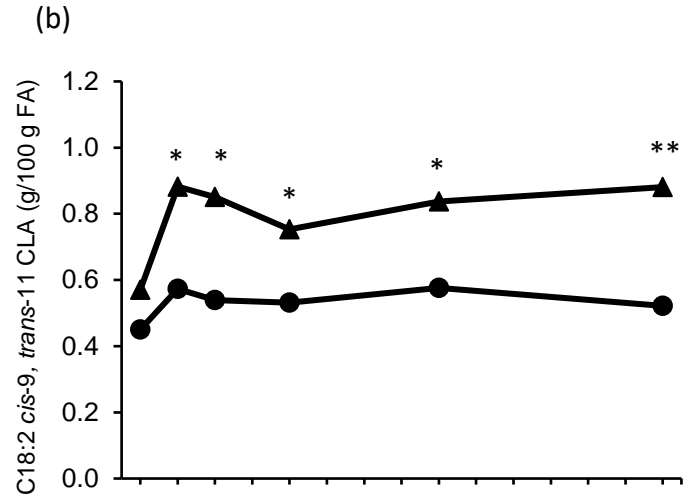
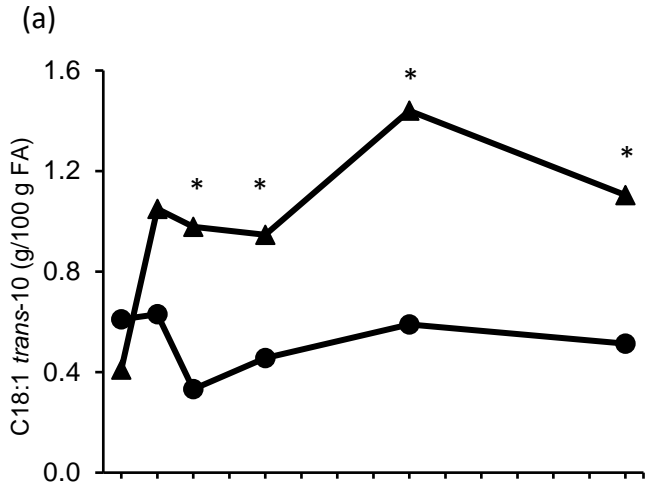


Fig. 1. Milk fat concentration of (a) C18:1 *trans*-10 (b) C18:2 *cis*-9 *trans*-11 CLA (c) C22:6 n-3, (d) sum of PUFA (e) sum of n-3 PUFA and (f) n-6 to n-3 ratio in dairy cows fed no SCIM (Control ●) or 100 g per cow per day of microalgae (SCIM ▲). SED = 0.25, 0.071, 0.030, 0.22 0.070 g/100g and 0.28 respectively. Within time points, treatments differ at $P < 0.05$, $P < 0.01$ or $P < 0.001$ are denoted by *, ** or *** respectively.

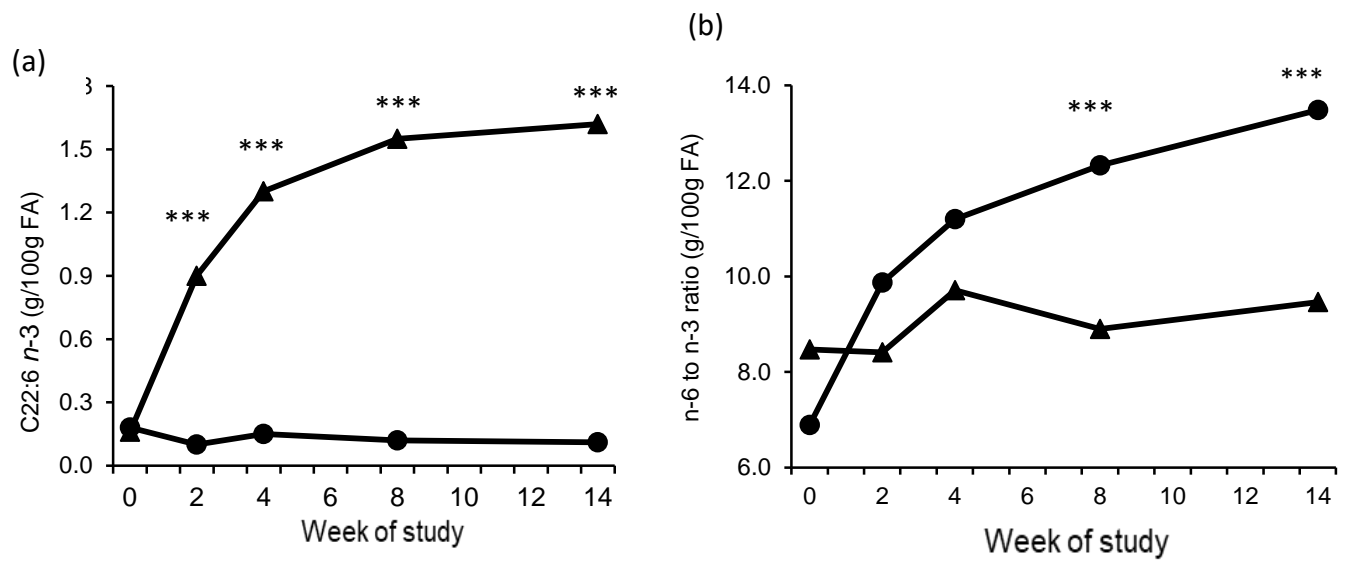


Fig. 2. Blood plasma fat concentration of (a) C22:6 n-3 (b) n-6 to n-3 PUFA ratio in dairy cows fed no SCIM (Control ●) or 100 g per cow per day of microalgae (SCIM ▲). SED = 0.045 g/100g and 1.07 respectively. Within time points, treatments differ at $P < 0.05$, $P < 0.01$ or $P < 0.001$ are denoted by *, **, or *** respectively.

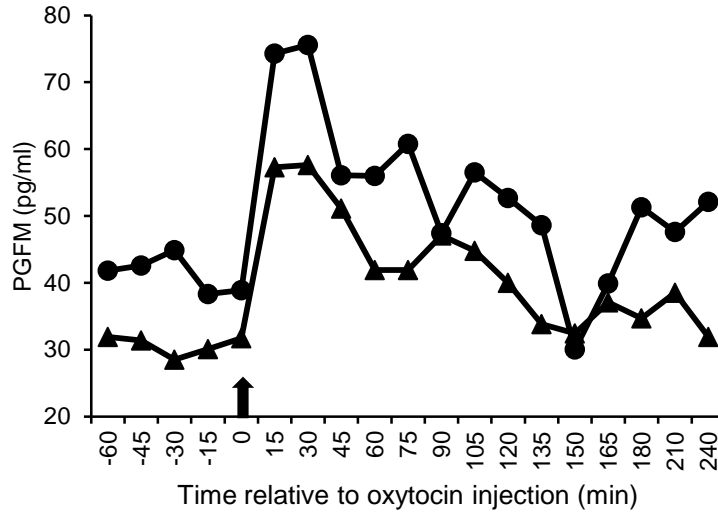


Fig. 3. Plasma 13,14-dihydro-15-keto PGF_{2α} metabolite (PGFM) concentration after an oxytocin challenge (time = 0) in cows fed no SCIM (Control ●) or 100 g per cow per day of microalgae (SCIM ▲). Arrow represents when oxytocin was administered. SED = 11.3 pg/ml. Significance for Diet, Time and D x T = 0.307, 0.003 and 0.351 respectively. For the Control and SCIM, peak value = 67.5 and 73.9 pg/ml (SED 17.61; *P* = 0.731) and area under curve = 2236 and 4046 (SED = 987.0; *P* = 0.126) respectively.