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

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DOI: https://doi.org/10.1017/S002202991900102X

Till, B.E., Huntington, J.A., Kliem, K.E., Taylor-Pickard, J. and Sinclair, L.A. 2019. Long term dietary supplementation with microalgae increases plasma docosahexaenoic acid in milk and plasma but does not affect plasma 13, 14-dihydro-15-keto PGF 2α concentration in dairy cows. *Journal of Dairy Research*.

12 February 2020

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

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Short title: Microalgae and milk fatty acids

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

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

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

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

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 (± 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 $PGF_{2\alpha}$ 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 Antrium, UK), using a Cobas Mira Plus
132 autoanalyser (ABX Diagnostics, Bedfordshire, UK). Plasma concentration of PGFM, was

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

Calculations and statistical analysis

All data were checked for normal distribution and analysed using Genstat 17th edition (VSN. Ltd, Oxford, UK). The SCC data were converted to their natural log prior to analysis. Daily live weight and body condition change were calculated as the final minus the initial value divided by the days on study. The PGFM area under the curve was calculated as described by Robinson et al. (2002). Variables having more than one observation were analysed using repeated measures ANOVA as: $Yijk = \mu + Pi + Dj + Tk + D.Tjk + \varepsilon ijk$, where Yijk = dependent variable; μ = overall mean; Pi = fixed effect of pair; Dj = effect of diet, Tk = effect of time; D.Ajk = interaction between diet and time and εijk = residual error. Variables with one observation were analysed by ANOVA using Genstat 18th edition (VSN Ltd., Oxford, UK).

Results

Feed analysis

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

Animal performance and blood metabolites

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

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

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

Milk and plasma fatty acid profile

There was no effect ($P > 0.05$) of dietary treatment on milk fat content of C4:0 to C18:0, 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, SFA, MUFA or total n-6 FA (Table 3). Milk fat content of C18:1 t 10, and c-9, t-11 CLA were similar at week 0 in cows fed either treatment, and increased ($P < 0.05$) in SCIM fed cows from week 2 onwards (Fig. 1a, b). There was also a higher milk fat content of C22:6n-3 in cows fed SCIM from week 2 onwards ($P < 0.001$), with the maximum difference between treatments of 0.34 g/100g FA occurring at week 14 of the study (Fig. 1c). Milk fat content of total PUFA and total n-3 PUFA increased and the n-6 to n-3 PUFA ratio decreased in SCIM fed cows from week 2 of the study ($P < 0.05$; Fig. 1d, e and f respectively), whilst C18:3 n-3 was lower at week 2 and C22:0 higher at weeks 8 and 14 in SCIM fed cows (Supplementary Fig. 1a and b). Milk fat content of C18:1 t-8, t-9, t-11and C20:3 n-6 were higher in cows fed SCIM than the Control. The 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 Σn-6 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 t-10, c-12 CLA, C20:3 n-6, C20:5 n-3 and MUFA decreased over the study period.

There was no effect ($P > 0.05$) of dietary treatment on blood plasma fat content of 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

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

Plasma PGFM concentrations

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

Discussion

Animal performance and blood metabolites

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

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

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

Milk and plasma fatty acid profile

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

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

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

Plasma PGFM concentration

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

Conclusion

The increase in milk and blood plasma C22:6 n-3 content over the 14 week study period suggest that the rumen microbial ecosystem did not adapt over time to the dietary 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
301 **Acknowledgements**
302 The authors would like to acknowledge the technical support from Sarah Williams, Claire
303 Cianchi, Ginny Whiston, Richard Beattie, Francesca Metherell and Keara Helen.
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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

[1]Spey syrup; KW Feeds, Ternhill, UK. [2]Sweetstarch: a by-product from the bakery, confectionary, pastry and breakfast cereal industries; KW Feeds, Ternhill, UK. [3]Megalac, a calcium salt of palm fatty acids, Volac, Royston, UK). [4]Butterfat Extra, a calcium salt of palm fatty acids fatty acids, Trident Feeds, Peterborough, UK.[5]The 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				
	Control	SCIM	SED	D	T
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

[1]Main 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)

	Treatment			*P* value[1]		
Fatty acid (g/100 g)	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

[1]Main 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)

	Treatment			*P* value[1]		
Fatty acid (g/100 g)	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

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

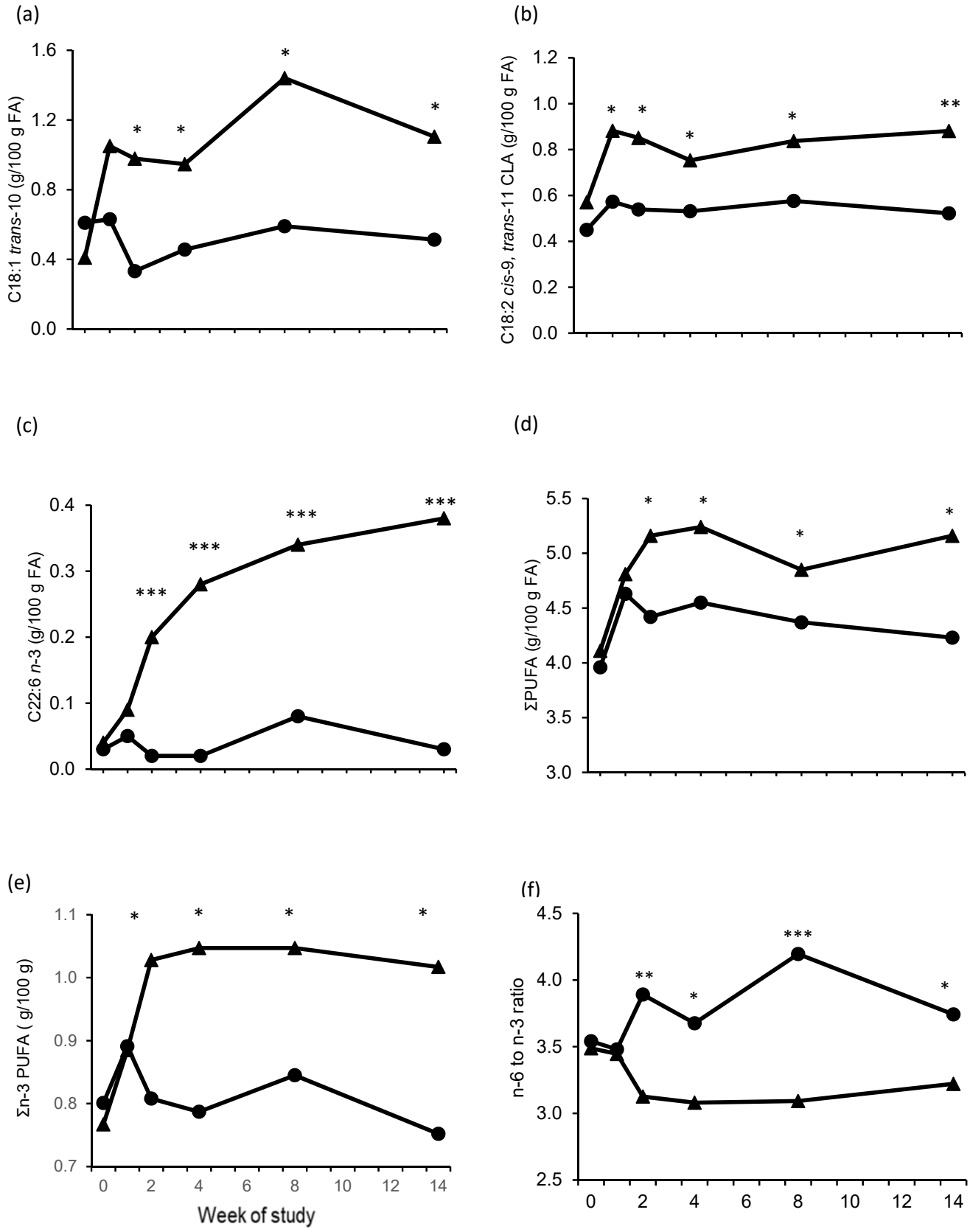
(a)
C18:1 trans-10 (g/100 g FA)
1.6
1.2
0.8
0.4
0.0
(b)
C18:2 cis-9, trans-11 CLA (g/100 g FA)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
(c)
C22:6 n-3 (g/100 g FA)
0.4
0.3
0.2
0.1
0.0
(d)
ΣPUFA (g/100 g FA)
5.5
5.0
4.5
4.0
3.5
3.0
(e)
Σn-3 PUFA (g/100 g)
1.1
1.0
0.9
0.8
0.7
0 2 4 6 8 10 12 14
Week of study
(f)
n-6 to n-3 ratio
4.5
4.0
3.5
3.0
2.5
0 2 4 6 8 10 12 14

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 tp 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.

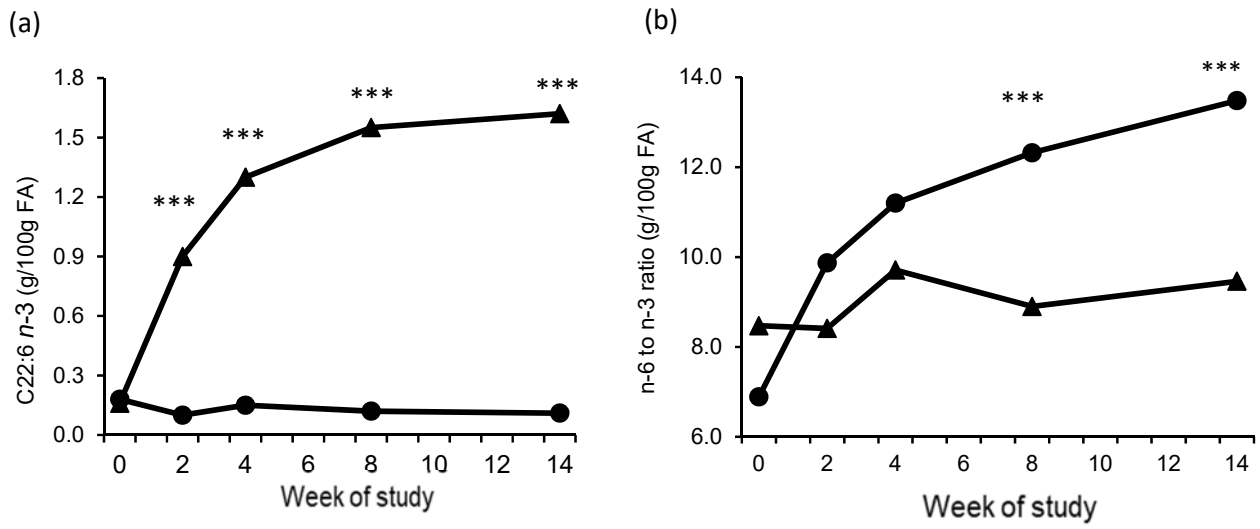

Fig. 2. Blood plasma fat concentration of (a) C22:6 n-3 (b) n-6 to n-3 PUFA ratio in dairy cows fed no SCIM (Control ●) or 100 g per cow per day of microalgae (SCIM ▲). SED = 0.045 g/100g and 1.07 respectively. Within time points, treatments differ at $P < 0.05$, $P<0.01$ or $P < 0.001$ are denoted by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

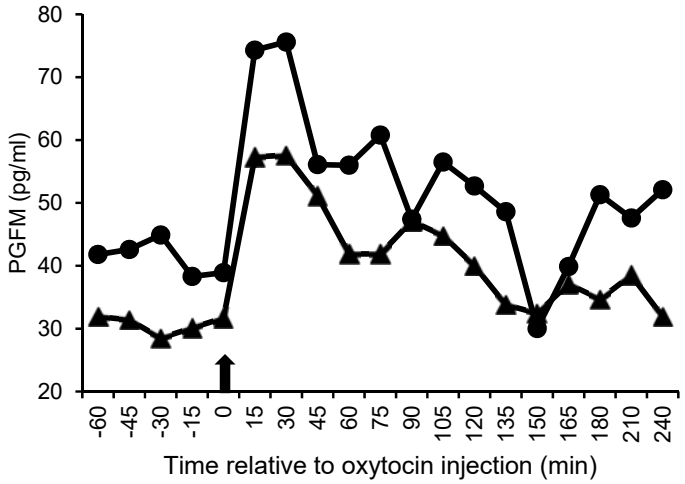

Fig. 3. Plasma 13,14-dihydro-15-keto $PGF_{2\alpha}$ metabolite (PGFM) concentration after an oxytocin challenge (time = 0) in cows fed no SCIM (Control ●) or 100 g per cow per day of microalgae (SCIM ▲). Arrow represents when oxytocin was administered. SED = 11.3 pg/ml. Significance for Diet, Time and 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.