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Original Research

Enhancing the DHA content in milk from dairy cows by feeding ALL-G-RICH™

C. A. Moran[1]*, M. Morlacchini[2] and G. Fusconi[2]

[1]*Regulatory Affairs Department, Alltech SARL, Rue Charles Amand, 14500 Vire, France*
[2]*CERZOO S.r.l, San Bonica, 29122 Piacenza, Italy*

Summary

The objective of this study was to evaluate the effect of the dietary inclusion of 6 g/kg dry matter intake of an unextracted *Aurantiochytrium limacinum* algae (AURA) in mid-lactation Italian Friesian cows under commercial conditions on milk yield, milk composition and docosahexaenoic acid (DHA) content. Cows were allocated to two groups ($n = 18$; 108.2 ± 66.1 and 104.4 ± 54.6 days in milk, control and treated groups, respectively). Feeding AURA for 84 d had no effect on dry matter intake, body condition score or weight gain, but did improve milk yield by 1.9 kg/cow/d (+5.4%; $P < 0.1$) over the course of the experiment. Milk fat concentration declined by 12% ($P < 0.0001$) without any significant change in 4% fat corrected milk, protein or lactose. Supplementing AURA for 12 weeks substantially altered the fatty acid profile of milk compared with milk from CON-fed cows such that the proportion of unsaturated fatty acids increased, omega-3 fatty acid content increased by 73.1% ($P < 0.0001$) and was accompanied by a favourable increase in the omega-3:6 fatty acid ratio by 75.0% ($P < 0.0001$). The AURA supplement, during day 7–84, increased the DHA concentration to 0.37 g /100 g milk total fatty acids ($P < 0.0001$) with a mean transfer efficiency of 18.1% from feed to milk. Together these results indicated that supplementing a dairy cow diet with DHA-rich microalgae is a feasible and efficient means for creating DHA-enriched milk for human consumption.

Keywords: **DHA: DMI: Milk production: Milk fat: milk DHA: Algae: PUFA**

Introduction

Increased reliance on intensive animal production systems that utilise high-energy, vegetable-based feed ingredients has caused a general shift in the fatty acid composition of food animals and food-animal products. Modern intensive production systems result in foods that typically contain lesser amounts of omega-3 polyunsaturated fatty acids (PUFAs) and greater amounts of saturated fatty acids compared with foods derived from free-range and wild-caught animals (Raper *et al.*, 1992). For several reasons this shift has raised significant public health concerns. A general decline in omega-3 fatty acid consumption is of concern because of the crucial roles these PUFAs play in human growth, development, physiologic function (Connor and Neuringer, 1988, Das, 2006a) and because of their role in suppressing or preventing inflammation linked with cancer, cardiovascular diseases, and type-2 diabetes (Das, 2008, Azrad *et al.*, 2013). A general rise in the consumption of medium-chain, saturated fatty acids and trans fatty acids is similarly of concern because of their strong association with the development of obesity (Neal *et al.*, 2013) and diseases such as cancer, cardiovascular diseases (Mente *et al.*, 2009, Siri-Tarino *et al.*, 2010, Santos *et al.*, 2013), and type-2 diabetes (Ney, 1991, Hu *et al.*, 2001, Bauman and Griinari, 2003, Das, 2006b, Riserus *et al.*, 2009).

The essential PUFAs linoleic acid (LA) and α-linolenic acid (ALA) are used in the body to synthesise their longer chain derivatives required by humans; the omega-6 fatty acid arachidonic acid (ARA) is derived from LA, whereas the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are synthesised from ALA. Each of these PUFA can also be obtained directly through diet. The EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) have proposed that an adequate daily intake of DHA and EPA of 250 mg for

* **Corresponding author:** cmoran@alltech.com

adults and 100 mg for infants and young children (6 months – 24 months old; EFSA, 2010). In pregnant and nursing women, a DHA intake of 300 mg/d is recommended (Simopoulos *et al.*, 1999) and in children (2–18 years old) a DHA intake of 300 to 400 mg/d is regarded as nutritionally desirable (Schuchardt *et al.*, 2010). However, based on a National Health and Nutrition Examination Survey between 2003–2008, mean dietary intake of DHA from foods in the United States was estimated to average only 51 and 75 mg/d for women and men, respectively (Papanikolaou *et al.*, 2014). To address these nutritional shortfalls, research has been conducted to develop DHA-enriched foods to help bolster intake levels without necessitating major changes in eating habits.

Supplementation using DHA-rich microalgae sources has been used successfully in monogastric livestock such as pigs and poultry to produce DHA-enriched meat and eggs (Bourre, 2005, Rymer and Givens, 2005, Cheng *et al.*, 2006, Sardi *et al.*, 2006, Meadus *et al.*, 2010, Fraeye *et al.*, 2012, Moran *et al.*, 2017a,b). Similarly, attention has been directed to producing DHA-enriched foods from ruminants, however, the extensive lipolysis and subsequent biohydrogenation of unsaturated fatty acids that occurs in the rumen presents unique challenges in achieving this goal (Jenkins *et al.*, 2008, Lourenco *et al.*, 2010, Shingfield *et al.*, 2013).

Numerous studies have examined the effects of algae-derived supplements on ruminant feed intake, enteric methane production, and milk composition and yield (Franklin *et al.*, 1999, Papadopoulos *et al.*, 2002, Reynolds *et al.*, 2006, Boeckaert *et al.*, 2008a, Boeckaert *et al.*, 2008b, Or-Rashid *et al.*, 2008, AbuGhazaleh *et al.*, 2009, Angulo *et al.*, 2012, Glover *et al.*, 2012, Stamey *et al.*, 2012, Moate *et al.*, 2013). Researchers have found that feeding algae may depress milk fat concentration (Franklin *et al.*, 1999, Papadopoulos *et al.*, 2002, Boeckaert *et al.*, 2008b, Moate *et al.*, 2013), increase milk omega-3 fatty acid concentration (Franklin *et al.*, 1999, Papadopoulos *et al.*, 2002, Boeckaert *et al.*, 2008b, Stamey *et al.*, 2012), indirectly increase milk conjugated linoleic acid (CLA) concentration (Franklin *et al.*, 1999, Boeckaert *et al.*, 2008b, Or-Rashid *et al.*, 2008, AbuGhazaleh *et al.*, 2009), and inhibit voluntary dry matter intake (Franklin *et al.*, 1999).

The objective of this study was to evaluate the efficacy of an unextracted algal (*Aurantiochytrium limacinum*) supplement ALL-G-RICH®, high in DHA and produced under heterotrophic and low sodium conditions, when fed to mid-lactation dairy cattle under commercial conditions on feed intake, milk yield and milk composition.

Material and methods

Animals and diets

The research protocol and animal care were in accordance with guidelines on the protection of animals used for scientific purposes (European Parliament and the Council of the European Union, 2010). After a 10-day pre-experimental adaptation period, 36 multiparous ($n = 2.5 \pm 0.92$) Italian Friesian cows (622.3 ± 71.7 kg) were allocated based on parity, milk yield, and days in milk (DIM) into two similar groups (18 cows per group). In a randomised complete block design, 12-week experiment, groups received one of two dietary treatments: control (CON) total mixed ration (TMR) (108.2 ± 66.1 DIM) or TMR supplemented with microalgae (AURA) at 6.0 g/kg dry matter intake (DMI) (104.4 ± 54.6 DIM). The microalgae (AURA) product was provided by Alltech Inc. (ALL-G-RICH®, Nicholasville, KY, USA) and consisted of a heterotrophically grown, unextracted *Aurantiochytrium limacinum* (CCAP 4087/2), with a guaranteed minimum of 160 mg DHA/g and not more than 0.3% sodium.

The TMR (as-fed basis) contained corn silage (24.0 kg), concentrate + cotton seed mix (70:30; 5.5 kg), corn meal + barley flake mix (60:40; 1.5 kg), corn meal + sorghum meal mix (70:30; 4.5 kg), water (7.0 kg), ryegrass hay (1.8 kg), and dehydrated alfalfa hay (4.5 kg). The composition of the concentrate was as follows: soy protein, wheat bran, dehulled sunflower meal (34%), limestone, sodium bicarbonate, salt, corn meal, cane molasses, dicalcium phosphate, magnesium oxide, soluble molasses concentrate and corn germ meal. Moreover, each kg of concentrate contained vitamin A (47,640 IU), vitamin D3 (4,368 IU), vitamin E (85.68 mg), Ca (13.47 mg), P (6.02 mg), Mg (4.45 mg), Na (12.65 mg), Cu (47.35 mg), Fe (261.16 mg), Zn (135.90 mg), I (1.55 mg), Co (16.67 mg), Mn (138.65 mg), and Se (0.47 mg). AURA was pre-diluted with corn meal 50:50 and each cow received either 300 g corn meal (CON) or the AURA: corn meal mixture as top-dressing in the morning during the TMR administration. Cows were housed at the CERZOO Research Centre farm (Piacenza, Italy) in three pens with six cows per treatment. Housing management, feeding, and husbandry conditions were considered representative of modern, commercial, European dairy operations.

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Sampling, measurement and analyses

The analytical composition of AURA was determined prior to the start of the study: crude protein (AOAC 990.03), crude fat (AOAC 954.02), fatty acid composition (AOAC 996.06), moisture (AOAC 930.15) and ash (AOAC 942.05). The nutrient composition of fresh TMR samples were analysed four times (every 28 days): crude protein (ISO 5983-1), ADF (ISO 13906), NDF (ISO 16472), starch (ISO 10520:1997E), crude fat (ISO 6492) and predicted metabolisable energy (Gallo *et al.*, 2013). Dry matter was calculated weekly by force drying TMR samples at 103°C to a constant weight (ISO 6496). DHA in the corn meal: AURA mixture was quantified following fat extraction by the Folch method (Folch *et al.*, 1957), esterification of extracted fat, separation of individual fatty acid methyl esters by gas chromatography and quantification against known standards (Bannon *et al.* 1985).

Performance data were collected as follows: live weight (per cow daily), TMR intake (per pen daily), and body condition score (BCS) (per cow weekly). Daily milk production (per cow) was reported as the sum of morning and evening milk yields. Milk was sampled from each cow in the morning and afternoon of d 0, 7, 14, 21, 28, 56, and 84. The morning and afternoon samples were combined for each cow, according to their milk production, and divided into two aliquots of not less than 50 ml each. The first aliquot was analysed for milk components (*i.e.*, fat, protein, lactose, somatic cell, and urea). Fat corrected milk (FCM, 4%) was calculated as per Gaine's formula (1923): FCM (kg) = 0.4M + 15 F where: M = milk yield (kg). F = M x fat content (%). The second milk aliquot was analysed for individual fatty acid composition following an *in situ* preparation of fatty acid methyl esters, separation by gas chromatography and quantification against known standards (Park and Goins, 1994, Loor and Herbein, 2001). Milk fatty acid (FA) content was assumed to be 93.3% of total milk fat (Glasser *et al.*, 2007; Moates *et al.,* 2013). DHA in milk yield (g/d) was calculated as:

$$0.933 \times \text{mean daily milk fat yield}(100\text{g/day}) \times \text{DHA concentration in milk FA}(\text{g}/100\text{gFA}).$$

DHA transfer efficiency (%) from diet to milk was calculated (Moates *et al.*, 2013) as:

$$\text{DHA in milk yield}(\text{g/d})/\text{DHA intake}(\text{g/d}) \times 100.$$

Statistical analyses

All statistical analyses were performed using SAS 9.3 software (SAS Institute Inc., 2011). Milk yield, fat content and DHA content were analysed using linear mixed models for repeated measures using proc MIXED procedure. For each the model included the fixed effects for treatment (CON vs AURA), day, and their interactions; for fat content and DHA, the model also included the random effect of cows. For milk yield, each model was subjected to two covariance structures (i.e., compound symmetry and autoregressive); the model with the smallest Akaike information criterion was then used. Because of the irregular sampling intervals used to determine milk fat and DHA content each of these models was subjected to compound symmetric and spatial power covariance structure. Two-sample t-tests were used to determine between-treatment differences. Mean body condition score was compared between treatments using the Mann Whitney U test (Sawilowsky, 2007).

Results and discussion

Ingredient and diet analyses

The microalgae, AURA, used in the study primarily consisted of 66.9 g crude fat /100 g DM biomass composed of a significant level of palmitic acid and docosahexaenoic acid (DHA), 36.66 g and 16.12 g / 100 g DM biomass respectively. Additionally, AURA contained 12% crude protein, 3.2% ash and 2.2% moisture. No presence of conjugated linoleic acid (C18:2 c9, t11) was found in the fatty acid analysis of the test article.

The raw materials were analysed for each new lot of production of TMR during the study. The analytical characteristics of the raw materials (% of dry matter) used in the preparation of the TMR and the analysis of the complete TMR are provided in Table 1.

Cow health and performance

Cows maintained good health status throughout the study and no veterinary treatments were required. Body condition score, an indicator of cow energy status, was similar between treatments over the 12-week experiment (Table 2). These results are consistent with other studies that showed dairy cow BCS was unaffected by microalgae supplementation (Franklin *et al.*, 1999, Glover *et al.*, 2012). All SCC counts were less than 400,000 CFU/ml of milk, the regulatory limit in Italy.

Average body weight gain of cows fed AURA was numerically higher (+10.7 kg) compared with CON (*P*

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Table 1. Analytical characteristics (% of dry matter) of the raw materials used in TMR preparation and the complete TMR mix

	Corn Silage[1]	Concentrate + cotton seed mix (70:30)[2]	Corn meal + barley flake mix. (60:40)[3]	Corn meal + Sorghum meal mix. (70:30)[3]	Rye grass Hay[4]	Dehydrated alfalfa hay[4]	Complete TMR mix[1]
Dry matter	36.85	79.44	84.43	87.29	90.20	96.95	54.13
Crude protein	8.54	27.29	9.56	12.44	10.74	16.92	14.65
Fat	2.51	6.66	2.84	3.82	1.09	1.15	3.35
Non-fibre carbohydrates	34.66	6.61	78.34	68.36	24.96	33.30	40.29
ADF	26.00	36.31	3.18	3.88	31.31	30.80	21.83
NDF	47.21	52.06	9.63	12.64	57.05	41.91	36.77
Metabolisable energy	2.25	2.61	3.18	3.10	1.94	2.03	2.61
Net Energy Lactation	1.39	1.66	2.05	2.00	1.17	1.24	1.64

Note: [1] Produced in the facility, [2] Provided by Consorzio Agrario of Cremona (CR, Italy), [3] Provided by Consorzio Agrario of Piacenza (PC, Italy); [4] Provided by ALIVERDE (RA, Italy)

= 0.592) (Table 2). Dry matter intake was unaffected by treatment in agreement with a study involving dairy cow supplementation with algal oil (Stamey *et al.*, 2012), but contrary to reports of dry matter intake depression in dairy cows in response to algae meal (Franklin *et al.*, 1999, Moate *et al.*, 2013) or fat-protected algae (Glover *et al.*, 2012) and in ewes in response to algal oil supplementation (Reynolds *et al.*, 2006).

Milk yield

At the start of the experiment, milk yield was similar between treatment groups. As the study progressed, cows in the AURA group had slightly greater yield. This difference became most pronounced in the final three weeks of the experiment (Table 3). For the total 12-week feeding period there was a trend in productivity, with milk yield 1.9 kg/cow/d (5.4%) greater in cows that consumed the AURA (37.3 *versus* 35.4 kg/cow/d; $P = 0.0948$). Previous investigators have reported that dairy cow milk yield was unaffected by algae biomass or algal oil supplementation (Stamey *et al.*, 2012) or whole algal cell supplementation (Moate *et al.*, 2013), however, these studies were restricted to supplementation periods of much shorter duration (*i.e.* 7 and 16 d, respectively). These data may suggest that feeding AURA over an extended period may lead to better persistency in the lactation curve when fed around mid–lactation; this needs to be verified in further studies.

Milk fat

The AURA treated cows consistently produced milk with lower fat content (3.19%) compared with milk from CON cows (3.66%) (Table 4), a reduction of 12%. Fat production was also lower ($P = 0.0538$) in milk from the AURA treated cows, but the 4% fat corrected milk production did not appreciably decline. These findings

Table 2. Health and performance indicators for mid-lactation cows fed a control (CON) diet or CON supplemented with a docosahexaenoic acid (DHA) rich microalgae

	Treatments[1]			
Parameter	CON	AURA	s.e.m.[2]	*P*-value
Body condition score [3, 4]	2.11	2.16	0.07	0.6005
SCC (x1000 CFU/ml) [4]	99.96	100.16	15.09	0.9928
TMR intake (kg DM) [5,6]	23.64	23.73	0.04	0.1314
Body weight (kg) [5]	634.50	645.20	11.89	0.5292

SCC = somatic cell count
[1] Cows were fed a control (CON) total mixed ration or TMR supplemented with unextracted *Aurantiochytrium limacinum* algae (AURA, Alltech Inc.) at 6 g/kg DM. [2] Standard error of the mean for $n = 18$. [3] Body condition score: 1 = Emaciated; 2 = Thin; 3 = Moderate; 4 = Stout; 5 = Obese. [4] Mean of day 7–84, data analysed using an ANOVA with repeated measures. [5] Mean of day 1–84 data analysed using an ANOVA with repeated measures. [6] Daily TMR intake on a dry matter (DM) basis

Table 3. Milk production from mid-lactation cows fed a control (CON) diet or CON supplemented with a docosahexaenoic acid (DHA) rich microalgae

	Treatments[1]			
Days[2]	CON	AURA	s.e.m.[3]	*P*-value
	Mean milk production (kg/cow/d)			
1–7	37.3	38.1	1.4	0.6992
8–14	36.5	37.8	1.3	0.4981
15–21	37.4	39.3	1.6	0.3939
22–28	36.3	38.4	1.5	0.3039
29–35	37.2	39.0	1.4	0.3453
36–42	36.7	38.2	1.3	0.4208
43–49	36.2	36.8	1.3	0.7616
50–56	36.5	37.9	1.4	0.4993
57–63	35.7	37.3	1.3	0.3930
64–70	33.0	35.8	1.3	0.1393
71–77	30.9[a]	34.8[b]	1.2	0.0238
78–84	31.5[a]	34.6[b]	1.3	0.0880
1–84	35.4[a]	37.3[b]	0.8	0.0948

[1] Cows were fed a control (CON) total mixed ration or TMR supplemented with unextracted *Aurantiochytrium limacinum* algae (AURA, Alltech Inc.) at 6 g/kg DM. [2] Data analysed using an ANOVA with repeated measures for each period and the entire study (days 1–84). [3] Standard error of the mean for $n = 18$. a,b Values within a row with different superscripts differ significantly at $P < 0.10$.

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Table 4. Components and component output of milk from mid-lactation cows fed a control (CON) diet or CON supplemented with a docosahexaenoic acid (DHA) rich microalgae

Parameter	Treatments[1]		s.e.m.[3]	*P*-value
	CON[2]	AURA[2]		
Fat (%)	3.66[a]	3.19[b]	0.07	0.0001
Fat (kg/d)	1.33[a]	1.20[b]	0.05	0.0538
Fat 4% corrected milk (kg/d)	34.41	33.22	1.19	0.4833
Protein (%)	3.37	3.39	0.06	0.8570
Protein (kg/d)	1.22	1.28	0.04	0.3401
Lactose (%)	5.11	5.07	0.05	0.5768
Lactose (kg/d)	1.85	1.93	0.07	0.4437
Urea (mg/dl)	20.46	19.31	0.08	0.2907

[1] Cows were fed a control (CON) total mixed ration or TMR supplemented with unextracted *Aurantiochytrium limacinum* algae (AURA, Alltech Inc.) at 6 g/kg DM. [2] Mean for days 7–84, data analysed as an ANOVA with repeated measures. [3] Standard error of the mean for $n = 18$. a,b Values within a row with different superscripts differ significantly at $P < 0.10$.

are consistent with those of previous investigators (Franklin *et al.*, 1999, Boeckaert *et al.*, 2008b, Moate *et al.*, 2013) who reported milk fat depression in response to algae supplementation of dairy cow rations. Conjugated linoleic acid biohydrogenation intermediates, especially the CLA c12t10 isomer, have been implicated in inhibiting mammary lipid metabolism (Shingfield *et al.*, 2013) resulting in milk fat depression. Although the CLA isomer profile underwent changes in the current study, the CLA c12t10 isomer was not measured so we are unable to explain the milk fat depression observed based on our fatty acid profile data.

Milk protein, lactose and urea

Milk protein content (%) and protein production (kg/d) were unaffected by AURA supplementation (Table 4). Other investigators have similarly reported no effects on milk protein in response to supplementation with algae biomass, algal oil (Stamey *et al.*, 2012) or algae meal (Moate *et al.*, 2013). Franklin and colleagues (1999) reported a tendency ($P = 0.08$) for the percentage of protein in milk to be lower in cows supplemented with algae, but that the total protein yield remained unaffected ($P < 0.53$) (Franklin *et al.*, 1999). In the current study, lactose content and total production and urea content were unaffected by AURA treatment (Table 4).

Milk fatty acid profile

Supplementation with AURA significantly altered the fatty acid profile of milk (Table 5) to increase the percentages of unsaturated fatty acids ($P = 0.0011$), PUFAs ($P = 0.0001$), omega-3 fatty acids ($P = 0.0001$), DHA ($P = 0.0001$), and conjugated linoleic acid CLA (C18:2 c9, t11) ($P = 0.0001$). The omega-3 : omega-6 fatty acid ratio also increased from 0.20 to 0.35 (% of sum (Σ) total fatty acid) ($P = 0.0001$). Concomitant reductions

Table 5. Repeated measures for fatty acid composition of milk (% of the Σ) from mid-lactation cows fed a control (CON) diet or CON supplemented with a docosahexaenoic acid (DHA) rich microalgae

Fatty acid	Treatments[1]		s.e.m.[3]	*P*-value
	CON[2]	AURA[2]		
Butyric acid (C4:0)	4.40	4.45	0.0865	0.6507
Caproic acid (C6:0)	2.57	2.53	0.0648	0.6756
Caprylic acid (C8:0)	1.35	1.31	0.0424	0.5037
Capric acid (C10:0)	3.00	2.81	0.1145	0.2626
Undecanoic acid (C11:0)	0.07	0.06	0.0046	0.0095
Lauric acid (C12:0)	3.46	3.31	0.1322	0.4247
Tridecanoic acid (C13:0)	0.12	0.11	0.0062	0.1660
Myristic acid (C14:0)	11.98	11.53	0.2663	0.2418
Myristoleic acid (C14:1)	0.85	0.92	0.0554	0.3677
Pentadecanoic acid (C15:0)	1.11	1.05	0.0375	0.2330
cis-10-pentadecenoic acid (C15:1)	–	–	–	–
Palmitic acid (C16:0)	32.16	30.11	0.4426	0.0024
Palmitoleic acid (C16:1)	1.57	1.57	0.1033	0.9704
Heptadecanoic acid (C17:0)	0.54	0.56	0.0146	0.4926
cis-10-heptadecenoic (C17:1)	0.05	0.05	0.0040	0.8529
Stearic acid (C18:0)	10.92	9.74	0.4389	0.0653
Vaccenic acid (C18:1trans)	1.99	7.03	0.7201	0.0001
Oleic acid (C18:1n9cis)	19.61	17.60	0.6240	0.0287
C18:1cis11	0.59	0.63	0.0304	0.4749
Linolelaidic acid (C18:2n6t)	–	–	–	–
Linoleic acid (C18:2n6c)	2.35	2.42	0.0618	0.4330
Rumenic acid (CLA, C18:2c9,t11)	0.34	0.86	0.0467	0.0001
α-Linolenic acid (C18:3n3)	0.30	0.32	0.0178	0.4695
γ-Linolenic acid (C18:3n6)	0.05	0.03	0.0020	0.0001
Nonadecanoic acid (C19:0)	–	–	–	–
Arachidic acid (C20:0)	0.12	0.13	0.0026	0.2279
cis-11-Eicosenoic acid (C20:1)	0.03	0.04	0.0032	0.0208
cis-11,14-Eicosadienoic acid (C20:2)	0.02	0.03	0.0007	0.0001
cis-11,14,17-Eicosatrienoic acid (C20:3n3)	0.18	0.15	0.0040	0.0001
cis-8,11,14-Eicosatrienoic acid (C20:3n6)	0.13	0.09	0.0038	0.0001
Arachidonic acid (C20:4n6)	0.005	0.009	0.0004	0.0001
Eicosapentaenoic (EPA, C20:5n3)	0.03	0.05	0.0034	0.0001
Henicosanoic acid (C21:0)	0.02	0.03	0.0006	0.0001
Behenic acid (C22:0)	0.04	0.05	0.0018	0.0001
Erucic acid (C22:1n9)	0.01	0.02	0.0009	0.0001
cis-13,16-Docosadienoic acid (C22:2)	0.001	0.0002	0.0002	0.0042
Docosahexaenoic acid (DHA, C22:6n3)	–	0.37	0.0600	0.0001
Lignoceric acid (C24:0)	0.03	0.03	0.0010	0.0001
Nervonic (C24:1)	–	0.002	0.0007	0.1535
Σ Short chain	8.32	8.29	0.1690	0.9225
Σ Medium Chain	20.59	19.78	0.5120	0.2730
Σ Long Chain	71.09	71.92	0.5656	0.3064
Σ Saturated	71.89	67.82	0.8095	0.0011
Σ Unsaturated	28.11	32.18	0.8095	0.0011
Σ Monounsaturated	24.69	27.86	0.7691	0.0061
Σ Polyunsaturated	3.41	4.32	0.0825	0.0001
Σ Omega 3	0.52	0.90	0.0188	0.0001
Σ Omega 6	2.54	2.54	0.0627	0.9649
Omega 3/ Omega 6[4]	0.20	0.35	0.0080	0.0001

[1] Cows were fed a control (CON) total mixed ration or TMR supplemented with unextracted *Aurantiochytrium limacinum* algae (AURA, Alltech Inc.) 6 g/kg DM. [2] Mean for days 7–84, data analysed using an ANOVA with repeated measures. [3] Standard error of the mean for $n = 18$. [4] Ratio of omega 3 / omega 6 concentrations of total fatty acid content of milk

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occurred in the percentages of saturated fatty acids (P= 0.0011), γ-linolenic acid (C18:3n6) (P = 0.0001), oleic acid (P = 0.0287), stearic acid (P = 0.0653), palmitic acid (P = 0.0024), and undecanoic acid (P = 0.0095). These alterations are generally consistent with those of other investigators (Franklin *et al.*, 1999, Boeckaert *et al.*, 2008b, Stamey *et al.*, 2012, Moate *et al.*, 2013).

DHA was not found in the unsupplemented CON milk samples at any time point in the study. Incorporation of DHA in milk increased (P < 0.0001) over the course of the experiment, increasing from the start of trial until about d 28, where the concentration plateaued until the end of the study period, d 84 (Figure 1). AURA supplementation enriched (P = 0.0001) milk fat in DHA (% of sum (Σ) total fatty acid) to 0.37 ± 0.06% and 0.46 ± 0.020% following d 7–84 and d 28–84 days of continuous feeding respectively. Therefore, supplementation of dairy cows with approximately 146 g AURA/h/d under the conditions of this trial, for a minimum of 28 days, resulted in 13 mg DHA per 100 ml milk. A pattern of DHA concentration rise and plateau, in response to dietary enrichment using a rumen protected algae, was similarly observed by investigators in a six week trial (Franklin *et al.*, 1999).

In cows fed the AURA treatment there was a transfer of DHA from algae to milk with an efficiency of 18.1% (d 7–84; Table 6). A broad range of transfer efficiencies have been reported in the literature, from 1.0% - 16.7% (Franklin *et al.*, 1999; Chilliard *et al.*, 2001; Boeckaert *et al.*, 2008; Stamey *et al.*, 2012; Moate *et al.*, 2013).

Stamey and colleagues reported DHA transfer efficiencies from algae to milk fat ranging from 1.0 to 3.4% over a seven day feeding period and hypothesised that by feeding earlier in lactation the transfer efficiency may have improved (Stamey *et al.*, 2012). However, the cows on this current study were all in mid- to late- lactation and yet had a very high transfer efficiency. Albeit, the longer feeding period in the current study played a significant role in the increase in DHA yield in milk (Figure 1).

Two independent studies reported transfer efficiencies of 8.4% and 8.9% when feeding unprotected sources of algal biomass to dairy cows (Franklin et al., 1999; Moate et al., 2013). However, the DHA transfer efficiency found in the current study compared similarly with the transfer efficiency when feeding a protected algal biomass as reported by Franklin and colleagues (1999); 18.1 *versus* 16.7% respectively. This transfer efficiency exceeds those reported for the transfer of DHA from fish oil to milk

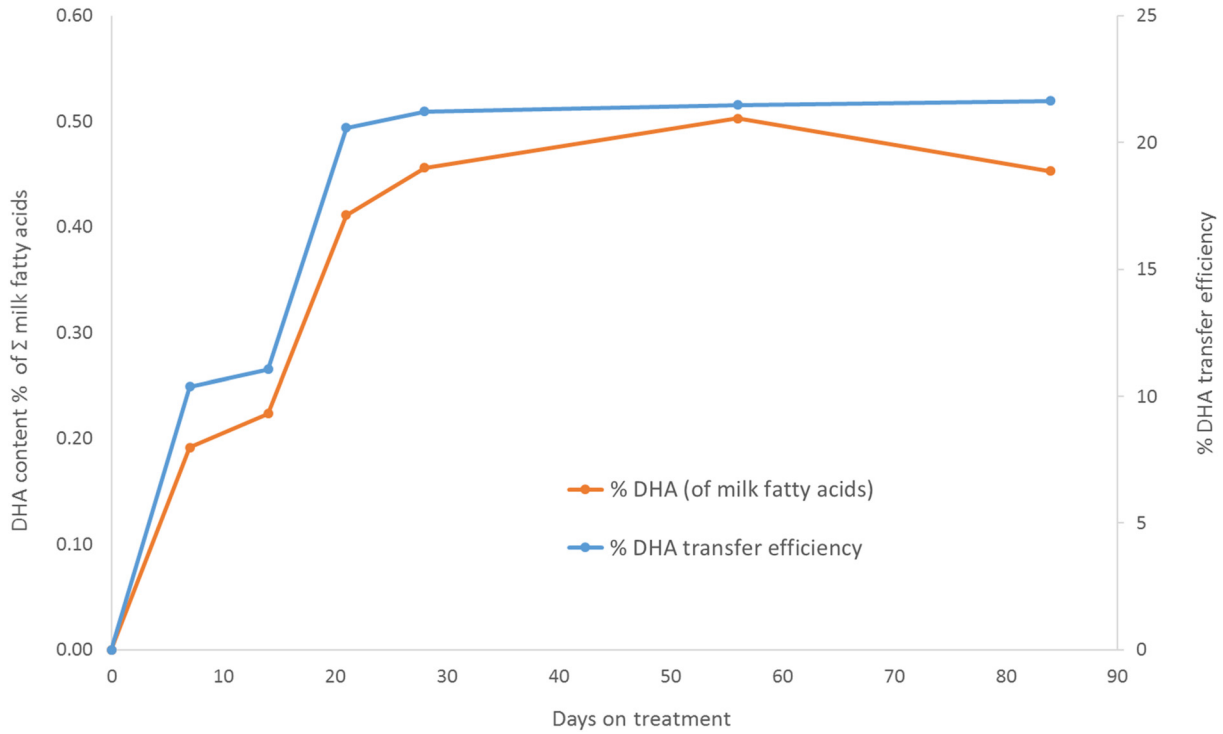

Figure 1. Temporal pattern of docosahexaenoic acid (DHA) incorporation (% of total) and transfer efficiency (%) into milk fatty acids in response to AURA supplementation of dairy cow TMR

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Table 6. Transfer efficiency (%) of docosahexaenoic acid (DHA) to milk from feeding AURA to dairy cows

Days on study	Fat (100 g/d)	DHA (g/100 g fatty acid)	DHA in milk yield (g/d)[1]	TMR intake (kg/d)[2]	DHA intake (g/d)[3]	DHA transfer efficiency (%)[4]
D0	14.6	0.00	0.00	–	–	–
D7	13.1	0.19	2.32	23.15	22.36	10.4
D14	12.4	0.22	2.55	23.87	23.06	11.1
D21	12.8	0.41	4.90	24.65	23.81	20.6
D28	11.6	0.46	4.98	24.28	23.45	21.2
D56	11.2	0.50	5.22	25.16	24.30	21.5
D84	10.9	0.45	4.58	21.91	21.17	21.6
D7–84[5]	12.0	0.37	4.14	23.73	22.92	18.1

[1] DHA in milk yield (g/d) = 0.933 x mean daily milk fat yield (100 g/day) x DHA concentration in milk fatty acid (g/100 g fatty acid). [2] TMR intake based on previous weekly average intake. [3] DHA intake based on 161.2 mg DHA/g AURA = 0.966 g DHA/ kg TMR DMI. [4] DHA transfer efficiency (%) from diet to milk = DHA in milk yield (g/d) / DHA intake (g/d) x 100. [5] Mean of d 7–84, data analysed using an ANOVA with repeated measures

fat, which has been reported as generally having a transfer efficiency of < 4% (Chilliard *et al.*, 2001, Lock and Bauman, 2004, Palmquist, 2009). The current study used an unextracted algal biomass which was produced under heterotrophic and low sodium conditions (non-marine) which may allow a degree of protection to the algal cell membrane during drying. Apajalahti *et al.* (unpublished) found in rumen simulation tests that the algal cell protects the DHA with no significant loss to leaching and oxidation over eight hours. This may explain the low degree of biohydrogenation products in the rumen and high transfer efficiency of DHA in this study.

A significant increase in the CLA content was observed in milk of cows receiving the AURA supplement compared to cows receiving the control diet, 0.86 *versus* 0.34% of sum of total fatty acid, respectively ($P = 0.0001$). The AURA supplement contained no CLA. However, the DHA content of the algae is thought to promote the accumulation of vaccenic acid in the rumen by inhibiting C18 biohydrogenation (Chow *et al.*, 2004, Boeckaert *et al.*, 2008b). Vaccenic acid is the primary of precursor for CLA synthesis in the mammary gland via Δ^9-desaturase (Griinari *et al.*, 2000, AbuGhazaleh and Jenkins, 2004, Mosley *et al.*, 2006, AbuGhazaleh *et al.*, 2009). In the current study, vaccenic acid increased ($P = 0.0001$) from 1.99 to 7.03% in the fatty acid profile in response to the AURA supplementation, possibly explaining the observed increase ($P = 0.0001$) in CLA in milk from AURA-treated cows. This increase is consistent with increases in CLA (and C18:1 isomers) reported by other investigators (Franklin *et al.*, 1999, Boeckaert *et al.*, 2008b, Stamey *et al.*, 2012, Moate *et al.*, 2013). The secondary enhancement of CLA potentially confers additional health benefits to DHA-enriched milk, since CLA has been shown to inhibit carcinogenesis (Kelley *et al.*, 2007, Amaru and Field, 2009, Donnelly *et al.*, 2009) and to increase lean body mass in humans (Steck *et al.*, 2007).

Conclusions

Supplementing dairy cow TMR with the algae treatment ALL-G-RICH® (AURA) at levels of 6 g/kg DMI for 12 weeks altered the fatty acid profile of milk compared with milk from unsupplemented cows such that the proportion of unsaturated fatty acids increased and the proportion of saturated fatty acid content declined. Omega-3 fatty acid content increased and was accompanied by a favourable increase in the omega-3: omega-6 fatty acid ratio. A high transfer efficiency of DHA from feed to milk was observed and hypothesised to be related to the manufacturing method of the heterotrophic algal biomass.

Milk yield tended to be greater (+5.4%) in cows fed the algae supplement, whereas milk fat content and fat production significantly declined without a significant change in (4%) fat corrected milk. Together these results indicate that supplementing dairy cow diet with DHA-rich microalgae is a feasible means for creating DHA-enriched milk for human consumption.

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Declaration of Interest

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