J. Dairy Sci. 102:10934–10946
https://doi.org/10.3168/jds.2019-16391

Influence of rate of inclusion of microalgae on the sensory characteristics and fatty acid composition of cheese and performance of dairy cows

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ABSTRACT

Modification of milk and cheese fat to contain long-chain n-3 fatty acids (FA) by feeding microalgae (ALG) to dairy cows has the potential to improve human health, but the subsequent effect on the sensory attributes of dairy products is unclear. The objective was to determine the effect of feeding dairy cows different amounts of ALG that was rich in docosahexaenoic acid (DHA) on milk and cheese FA profile, cheese sensory attributes, and cow performance. Twenty Holstein dairy cows were randomly allocated to 1 of 4 dietary treatments in a 4 × 4 row and column design, with 4 periods of 28 d, with cheddar cheese production and animal performance measurements undertaken during the final 7 d of each period. Cows were fed a basal diet that was supplemented with ALG (*Schizochytrium limancinum*) at 4 rates: 0 (control, C), 50 (LA), 100 (MA), or 150 g (HA) of ALG per cow per day. We found that both milk and cheese fat content of DHA increased linearly with ALG feed rate and was 0.29 g/100 g FA higher in milk and cheese from cows fed HA compared with C. Supplementation with ALG linearly reduced the content of saturated FA and the ratio of n-6:n-3 FA in milk and cheese. Supplementation with ALG altered 20 out of the 32 sensory attributes, with a linear increase in cheese air holes, nutty flavor, and dry mouth aftertaste with ALG inclusion. Creaminess of cheese decreased with ALG inclusion rate and was positively correlated with saturated FA content. We also observed a quadratic effect on fruity odor, which was highest in cheese from cows fed HA and lowest in LA, and firmness and crumbliness texture, being highest in MA and lowest in HA. Supplementation with ALG had no effect on the dry matter intake, milk yield, or live weight change of the cows, with mean values of 23.1, 38.5, and 0.34 kg/d respectively, but milk fat content decreased linearly, and energy-corrected milk yield tended to decrease linearly with rate of ALG inclusion (mean values of 39.6, 38.4, 37.1, and 35.9 g/kg, and 41.3, 41.3, 40.5, and 39.4 kg/d for C, LA, MA, and HA, respectively). We conclude that feeding ALG to high-yielding dairy cows improved milk and cheese content of DHA and altered cheese taste but not cow performance, although milk fat content reduced as inclusion rate increased.
Key words: cheese, dairy cow, fatty acid, microalgae, sensory profile

INTRODUCTION

A considerable body of research exists on the benefits of long-chain (**LC**) n-3 fatty acid (**FA**) on human health (Calder, 2014; Kliem and Shingfield, 2016). Two important LC n-3 PUFA are eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**), which, when provided in small quantities, can significantly decrease the likelihood of developing coronary heart disease via their role in modulating prostaglandin metabolism and decreasing blood triglycerides (Marventano et al., 2015). At high doses these LC n-3 PUFA can lower blood cholesterol and have antithrombotic and anti-inflammatory properties (Calder, 2014; Marventano et al., 2015). These LC n-3 PUFA are also important for growth, development, immunity, and insulin activity (Calder, 2014). In addition to the direct health benefits of PUFA, intermediates in the biohydrogenation of unsaturated FA in the rumen of cattle, such as CLA, have been shown to have health benefits including anticarcinogenic properties in both animal models and human cancer cells (Lock et al., 2005; Gebauer et al., 2011).

Ruminant products such as milk, cheese, and beef have been criticized for their low content of LC n-3 PUFA and high content of SFA (Kliem and Shingfield, 2016; Rodriguez-Herrera et al., 2017). Despite this, one of the most effective means of increasing the content of LC n-3 PUFA in the human diet is via dairy products, particularly cheese (Givens and Gibbs, 2006). In the

Received February 11, 2019.
Accepted August 7, 2019.
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majority of studies that have attempted to improve the health attributes of milk and cheese, the main dietary source of LC n-3 PUFA has been fish oil (**FO**; Chilliard et al., 2001; Palmquist and Grinari, 2006). However, the primary producer of LC n-3 PUFA at the base of the food chain is microalgae (**ALG**; Givens and Gibbs, 2006). Feeding ALG has therefore been proposed as a more effective means of manipulating the FA composition of ruminant products, partly due to its high concentration of LC n-3 PUFA, but also due to its lower extent of biohydrogenation in the rumen compared with FO (Sinclair et al., 2005), although the transfer efficiency into milk may not always be improved (Vahmani et al., 2013).

When evaluating the manipulation of the FA content of food products, it is important to determine the resultant effect on the organoleptic properties of the product. Most studies that have investigated the influence of LC n-3 PUFA on the sensory attributes of cheese or other dairy products have either fed FO to dairy cows (Allred et al., 2006; Vargas-Bello-Pérez et al., 2015) or directly fortified dairy products with sources of FO (Martini et al., 2009; Bermúdez-Aguirre and Barbosa-Cánovas, 2011). Such studies have reported varying effects on color, aroma, and flavor, with acceptance generally being lower at higher levels of FO inclusion (Allred et al., 2006; Martini et al., 2009; Bermúdez-Aguirre and Barbosa-Cánovas, 2011). Studies that have evaluated the effect of ALG on the sensory attributes of cheese are, however, limited and do not cover the range of inclusion of ALG that may be encountered in commercial practice (Vanbergue et al., 2018b). Those that have been conducted rated the cheese lower for color and firmness, higher for graininess, and higher for spicy flavor, attributes associated with a higher content of unsaturated alcohols and ketones, as well as the sulfur compound 2,4-dithiapentane, a product of methionine catabolism (Vanbergue et al., 2018b).

The inclusion of LC n-3 PUFA sources such as ALG has often been associated with negative effects on performance and milk composition, particularly when included at high levels. For example, a substantial decline in milk fat content has been reported in some studies (Boeckaert et al., 2008; Bichi et al., 2013; Vanbergue et al., 2018a), which has been linked to the production of *trans* isomers such as *trans*-10,*cis*-12 CLA in the rumen (Bauman and Griinari, 2003). Additionally ALG may reduce whole-tract digestibility, as unsaturated FA have been suggested to be toxic to fiber-digesting bacteria (Maia et al., 2007).

There is a lack of literature on the effects of ALG on milk and cheese FA profile and cheese sensory attributes in studies that have fed ALG at a range of levels that do not affect animal performance. The objectives of this study were to determine the effect of rate of inclusion of DHA-enriched ALG on milk and cheese FA profiles, cheddar cheese sensory attributes, and cow performance.

MATERIALS AND METHODS

This study was conducted in accordance with the requirements of the United Kingdom Animals (Scientific Procedures) Act 1986 (amended 2012) and received local ethical approval.

Animals and Treatments

Twenty early-lactation (77 ± 17.0 DIM) Holstein-Friesian dairy cows yielding 44 (±1.9) kg/d of milk, with a live weight of 654 (±42.4) kg, and BCS (Ferguson et al., 1994) of 3.0 (±0.2) at the beginning of the study were used. The study design was a 4 × 20 row and column design (Mead et al., 1993), with each of the 4 periods consisting of a 21-d adaptation period followed by 7 d of sampling. All cows were fed the same basal ration (Table 1), which was supplemented with one of 4 inclusion levels of ALG (*Schizochytrium limancinum*; Alltech, Nicholasville, KY) during each period. Treatment diets were as follows: no algae inclusion (control, **C**), 50 g of microalgae per cow per day (**LA**), 100 g of microalgae per cow per day (**MA**), and 150 g of microalgae per cow per day (**HA**). A 50:50 (DM basis) wheat/dried sugar beet feed mix replaced ALG in C, LA, and MA, and was fed at 150, 100, and 50 g per cow per day, respectively. The ALG contained 135 g/kg CP, 580 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, C20:5 n-3, and C22:6 n-3, respectively. The diets were formulated to produce approximately 37 kg/d (Thomas, 2014) and contain approximately 200 g starch/kg of DM, and were fed as a TMR once daily at 1.05 of the intake measured in the previous 24 h, with feed refusals collected 3 times per week. The forages and straight feeds were mixed with ALG (or wheat and sugar beet feed) using a mixer wagon (HiSpec, County Carlow, Ireland), calibrated to ± 1 kg, and fed through roughage intake feeders (Insentec B.V., Marknesse, the Netherlands) fitted with an automatic animal identification and weighing system calibrated to ± 0.1 kg. Cows were housed together in the same portion of a building containing freestalls fitted with foam mats, which were bedded twice weekly with sawdust, limed weekly, and scraped every 2 h by automatic scrapers. Cows were milked twice daily at approximately 0615 and 1600 h, and had free access to fresh water.

Cheese Production

Milk was collected for cheesemaking during each sampling week from 4 cows per treatment at consecutive p.m. and a.m. milkings, into 50-L buckets. The cows were selected from the highest- and lowest-yielding animals to be representative of the group, with their mean performance over the study provided in Supplemental Table S1 (https://doi.org/10.3168/jds.2019-16391). The p.m. milk was bulked, rapidly cooled to 4°C, and stored overnight in a mini bulk milk tank (Frigomilk milk cooler G1, FIC S.p.A., Via Trivulzia, Italy), and stirred continuously. Milk from the morning was mixed with p.m. milk for 30 min before being transferred to a 50-L cheese vat (Jongia, Solihull, UK). Cheese was made following a cheddar recipe as described by Scott et al. (1998). The milk was pasteurized by heat-treating to 63°C for 30 min, with temperature and titratable acidity percentage measured every 15 min by titration with 0.1 *N* NaOH. When the milk had cooled to 29.5°C, 3 g of a starter culture of mixed lactic acid bacteria (single shot culture OV26, Orchard Valley Dairy Supplies, Worcestershire, UK) was added. Ripening continued until the titratable acidity reached 0.20 to 0.22% (up to 1 h), and vegetarian marzyme rennet (Orchard Valley Dairy Supplies, Worcestershire, UK) was added as a clotting agent at a rate of 25 mL diluted in 175 mL of water per 100 L of milk, and the temperature was held at 29.5°C. The curd was then allowed to set over 50 min before being cut into 3- to 5-mm cubes. The temperature was then raised to 40°C over 40 min with stirring, the whey drained off, and the curd cut and blocked every 20 min until dry. The curd was then milled by chopping into finger-sized pieces and cooled to 25.5°C. Salt was then mixed into the curd (100 g per 5 kg of curd) before being transferred into 3 cheese molds and pressed overnight at 75 kN/m^2. The cheese was turned the following day in the molds and re-pressed at 200 kN/m^2 for 24 h. The cheese wheels were then vacuum packed in individual embossed vacuum bags, and stored at 4°C for 120 d to mature before analysis.

Table 1. Composition (kg/kg of DM) of the basal diet and chemical composition (g/kg of DM) of TMR that contained no microalgae (C), 50 g of microalgae/cow per day (LA), 100 g of microalgae/cow per day (MA), or 150 g of microalgae/cow per day (HA)

Item	Basal diet	Treatment			
		C	LA	MA	HA
Ingredient, kg/kg of DM					
Corn silage	0.436				
Grass silage	0.118				
Rapeseed meal	0.077				
Wheat distillers grains and solubles	0.077				
Soybean meal	0.045				
Palm kernel meal	0.022				
Molasses	0.006				
Molassed sugar beet feed	0.051				
Wheat	0.051				
Soy hulls	0.094				
Megalac[1]	0.015				
Urea	0.003				
Minerals and vitamins[2]	0.005				
Chemical composition					
DM, g/kg		372	374	369	371
Ash		64	73	66	70
OM		936	927	934	930
CP		166	170	165	164
NDF		452	455	452	460
Fatty acid, g/kg of DM					
C16:0		10.1	11.2	12.5	13.0
C18:0		0.8	0.8	0.9	0.9
C18:1 *cis*-9		7.6	7.8	7.9	7.6
C18:2 *cis*-9,*cis*-12		9.5	10.0	9.4	9.3
C18:3 *cis*-9,*cis*-12,*cis*-15		1.4	1.6	1.6	1.6
C20:5 n-3		0.000	0.004	0.007	0.009
C22:6 n-3		0.00	0.33	0.68	1.00

[1]Protected fat; Volac International Ltd., Royston, UK.

[2]Mineral and vitamin premix (KW Feeds, Sherburn-in-Elmet, Leeds, Yorkshire, UK). Major minerals (g/kg): Ca 220, P 30, Mg 80, Na 80; trace minerals (mg/kg): Cu 760, Se 30.3, I 200, Co 70, Mn 5,000, Zn 6,350; vitamins (mg/kg): retinol 300, cholecalciferol 7.5, all *rac* α-tochopherol acetate 2,000, vitamin B_{12} 2.50, biotin 135.

Sensory Evaluation of Cheese

For the assessment of cheese sensory quality, a generic descriptive sensory analysis was applied. The sensory methodology provided sensorial quantitative descriptions (sensory profiles) of food products, which were obtained from the perceptions and evaluations of qualified panelists. Eight skilled panelists were selected from a base group recruited and screened in accordance with best practice BS EN ISO 8586:2014 (BSI, 2014); panelists had previous experience with sensory profiling of food products. The selection criteria for the cheese panel were based on the ability to detect differences among various cheese, to identify cereal and feed-like odors, and to correctly identify the maturity of cheese on the basis of a ranking test (BSI, 2009).

The panelists were then trained with a cheese sample range over a total of 40 h, developing a sensory lexicon to establish descriptive terms and the sequence of attribute testing based on odor (sniffing), appearance (looking), flavor and aftertaste (tasting), and texture (looking, touching, and tasting; Supplemental Table S2, https://doi.org/10.3168/jds.2019-16391). The cheese lexicon of 32 sensory attributes was generated and calibrated with reference products for cheese profiling, in accordance with guidelines for sensory analysis in milk and milk products (BSI, 2009) and sensory profiling in cheese research (Drake, 2007; Drake et al., 2010; Rogers et al., 2009). References were used to aid panelists in training and attribute identification and scale usage. A 15-cm unstructured line scale with end anchor words was used for the descriptive analysis for each attribute. Panelist performances were tested for individual repeatability and discriminability on cheese samples, to ensure that the panel was qualified before the sensory profiling test.

Three cheese samples per test session were monadically evaluated on all the sensory attributes at a time, to minimize panelists' fatigue, with a 30-min break between sessions. Water crackers, cucumber sticks, and drinking water were used as cleansing materials. Each panelist was provided with 2 cubes per sample per replication, resulting in 32 samples to evaluate (4 treatments × 4 periods × 2 cubes). Surplus cubes were available if required. The mature cheese samples were trimmed of all external surfaces and cut into cubes measuring 2 × 3 cm and maintained at 12°C (Brown et al., 2003) for evaluation. Samples were presented in lidded plastic sample pots, and evaluation sessions took place in individual booths equipped with Compusense Five software (Compusense Inc., Guelph, Ontario, Canada), using a random and balanced order serving plan.

Animal Performance

Feed intake was recorded daily during the sampling week of each period, and subsamples of each TMR were collected daily and stored at −20°C for subsequent analysis. Forage samples were collected weekly and oven-dried at 105°C, and the ratio of corn to grass silage was adjusted to the desired level on a DM basis. Milk yield was recorded daily and samples collected on 4 occasions during the sampling week of each period, a preservative added (Microtabs II, Advanced Instruments, Inc., Norwood, MA), and stored at 4°C before subsequent analysis. Additional samples were collected on successive milkings for FA analysis. Cows were weighed and BCS recorded at 1100 h before the start of the study and on the final day of each period. Blood samples were collected from the jugular vein from 3 cows per treatment per period (resulting in n = 12 cows per treatment). Cows were selected from the highest-, mid-, and lowest-yielding animals in the group, with their performance over the study provided in Supplemental Table S1 (https://doi.org/10.3168/jds.2019-16391). Blood samples were collected over 2 d at 0700, 1000, and 1300 h (to assess diurnal fluctuations) into evacuated tubes containing sodium heparin for subsequent determination of BHB, or potassium oxalate for determination of glucose and nonesterified fatty acids (**NEFA**). Samples were centrifuged at 1,000 × *g* for 15 min and the plasma separated and stored at −20°C before subsequent analysis.

Chemical Analysis

Milk compositional analysis was conducted using a Milkoscan Minor (Foss Electric, Hillerød, Denmark), calibrated using standards according to AOAC (2012). Milk FA analysis followed the method described by Hara and Radin (1978) for lipid extraction and Chouinard et al. (1999) for methylation. Cheese FA analysis was as described by Coakley et al. (2007) for lipid extraction and followed the same method as the milk fat for methylation, and TMR FA was determined as described by Jenkins (2010). Fatty acids were identified using a GC (model 6890, Agilent, Waldbronn, Germany) fitted with an automatic sampler, flame ionization detector and 100-m column (CPSil88, Agilent) as described by Lock et al. (2006). The oven temperature started at 70°C and was held for 2 min, followed by an increase of 8°C/min until it reached 110°C, held for 4 min, then increased 5°C/min to reach 170°C, held for 10 min, and finally increased at 4°C/min to 225°C and held for 15 min. Each sample had a run time of 61.8 min and a post run time of 1 min at 70°C. Peaks were identified

by comparison of the retention time with individual FAME standards (Sigma-Aldrich, St. Louis, MO).

The TMR samples for each diet were bulked within each period and a subsample analyzed according to AOAC (2012) for DM (934.01), CP (988.05), and ash (924.05), and NDF was analyzed according to Van Soest et al. (1991). Plasma samples were analyzed for glucose, BHB, and NEFA, using kits (catalog numbers RB1008, GU611, and FA115, respectively; Randox Laboratories, Crumlin, UK) and a Cobas Mira Plus autoanalyzer (ABX Diagnostics, Shefford, Bedfordshire, UK).

Calculations and Statistical Analysis

The atherogenic (**AI**) and thrombogenic indices (**TI**) in cheese were calculated as described by Ulbricht and Southgate (1991). Data were analyzed via XLSTAT software (Addinsoft, 2018; https://www.xlstat.com/en/), using the analyzing data/principal component analysis (**PCA**) option to gain an overview of both sensory and FA profiles of all treatment combinations. We used PCA to investigate and visualize correlations between the attributes and to obtain noncorrelated factors. Milk and cheese FA, sensory, and performance data were analyzed by ANOVA using Genstat 17th edition (VSN Ltd., Oxford, UK) as a row and column design (Mead et al., 1993) using the following model:

$$Y_{ijk} = \mu + T_i + P_j + A_k + \varepsilon_{ijk},$$

where Y_{ijk} is the observation, μ is the overall mean, T_i is treatment, P_j is period, A_k is animal, and ε_{ijk} is the residual error. Treatment effects were split into orthogonal polynomial contrasts (linear, quadratic, and cubic). Blood metabolites were analyzed as repeated measures ANOVA using Genstat 17th edition (VSN Ltd.). Results are presented as treatment means with the standard error of the mean (SEM).

RESULTS

Feed Fatty Acid and Proximate Analysis

The content of C18:0, C18:1n-9, C18:2n-6, and C18:3n-3 were similar in all 4 diets, with mean values of 0.9, 7.7, 9.6, and 1.6 g/kg of DM respectively. We detected no DHA in C, with the content of DHA and C16:0 increasing as dietary inclusion of ALG increased. All diets had a similar DM content, with a mean of 372 g/kg (Table 1). The OM content was also similar across all diets (mean of 932 g/kg of DM), whereas the LA diet had a CP content 6 g/kg of DM higher than the HA diet, which had the lowest value, and C and MA being intermediate. The NDF content was similar between treatments, with a mean of 455 g/kg of DM.

Milk and Cheese Fatty Acid Profile

We observed no effect ($P > 0.05$) of dietary treatment on milk fat content of C4:0, C14:0 to C17:1, C20:0, or C22:5 n-3 (Table 2). In contrast, we observed a linear decrease ($P < 0.05$) in milk fat content of C6:0, C8:0, C10:0, C12:0, C18:0, C18:1*cis*-9, and C22:0, as the inclusion level of ALG increased in the diet. The milk fat concentration of C18:1*trans*-8 to C18:1 *trans*-12, C18:2 *cis*-9,*cis*-12, C18:3 *cis*-9,*cis*-12,*cis*-15, C18:2 *cis*-9,*trans*-11 CLA, C18:2 *trans*-10,*cis*-12 CLA, C20:3n -6, and C20:3n-3 increased linearly ($P < 0.05$) as the inclusion level of ALG increased in the diet. Milk fat DHA content also increased linearly ($P < 0.001$) from 0.08 g/100 g in cows fed C diet to 0.37 g/100 g FA when fed HA.

We observed a linear decrease ($P = 0.02$) in the proportion of milk FA of chain length less than C16, but we found no effect of treatment on the proportion of C16:0 plus C16:1 or longer than C16 ($P > 0.05$). Increasing the inclusion level of ALG had a linear effect ($P < 0.001$) on milk fat content of saturated FA, being highest in cows when offered C and lowest in those offered HA. In contrast, both the MUFA and PUFA contents in milk fat increased linearly ($P < 0.001$) as dietary inclusion of ALG increased. We also observed a linear increase ($P < 0.001$) in total n-3 and n-6 FA in milk fat as ALG inclusion increased, and a linear decrease ($P < 0.001$) in the ratio of n-6 to n-3, being highest in cows offered C and lowest in those offered HA.

We observed a linear decrease ($P < 0.05$) in cheese C6:0, C18:0, C18:1*cis*-9, and C22:0 as ALG increased in the diet, but we discovered no effect ($P > 0.05$) on any of the other FA below C18:0 or on C18:2 *cis*-9,*cis*-12, C20:0, C18:2 *trans*-10,*cis*-12 CLA, or C20:3n -3 (Table 3). Cheese FA content of C18:1 *trans* 10, 11, and 12, C18:3 *cis*-9,*cis*-12,*cis*15, C18:2 *cis*-9,*trans*-11 CLA, and C20:3n-6 increased linearly ($P < 0.05$) as supplementation of ALG increased. Cheese content of DHA increased quadratically with dietary inclusion of ALG ($P < 0.001$), being highest in cheese from cows fed HA. There was a small but linear increase ($P < 0.05$) in the content of EPA in cheese with ALG inclusion, from 0.05 g/100g in C to 0.06 g/100g in HA. We found no effect ($P > 0.05$) of treatment on the sum of cheese FA of chain length less than C16:0 or more than C16:0, MUFA, or total n-6. However increasing dietary supplementation of ALG had an effect ($P < 0.05$) on the total SFA in cheese, which decreased linearly from 67.9 in C to 66.2 g/100 g FA in HA, and on total PUFA, which

increased from 3.92 in C to 4.61 g/100 g in HA. We also saw a linear change ($P < 0.001$) in the ratio of n-6:n-3 in cheese as the inclusion level of ALG increased in the diet, being lowest in cheese from cows fed HA and highest in those fed C. Both AI and TI also decreased linearly with ALG inclusion rate.

Cheese Composition and Sensory Analysis

Cheese moisture content increased linearly ($P < 0.001$) with dietary inclusion rate of ALG, whereas fat content decreased linearly ($P < 0.05$; Table 3). Supplementation with ALG altered 20 out of the 32 sensory attributes ($P < 0.05$; Table 4). We observed a linear increase ($P < 0.05$) in the appearance of air holes, sweetness, nutty flavor, acidity, and dry throat aftertaste, and a linear decrease ($P < 0.05$) in the creamy flavor of the cheese as the level of ALG increased in the diet. Creamy flavor was positively and highly correlated with the percentage of SFA (r = 0.601), AI (r = 0.603), and TI (r = 0.560) in the cheese. We also observed quadratic effects ($P < 0.05$) on fruity odor, which was highest in cheese from cows fed HA and lowest in those receiving LA; edge cut appearance ($P < 0.001$), which

Table 2. Milk fatty acid (FA) composition (g/100 g of FA) of dairy cows fed no microalgae (C), 50 g of microalgae/cow per day (LA), 100 g of microalgae/cow per day (MA), or 150 g of microalgae/cow per day (HA)

	Treatment					*P*-value		
FA, g/100 g of FA	C	LA	MA	HA	SEM	Linear	Quadratic	Cubic
C4:0	1.43	1.44	1.39	1.39	0.025	0.20	0.82	0.25
C6:0	1.24	1.27	1.19	1.17	0.023	0.01	0.31	0.12
C8:0	0.90	0.90	0.84	0.82	0.018	<0.001	0.42	0.21
C10:0	2.23	2.24	2.09	2.04	0.047	<0.001	0.55	0.23
C12:0	3.11	3.03	2.96	2.90	0.063	0.02	0.81	0.97
C14:0	11.2	11.1	11.0	10.9	0.13	0.14	0.62	0.70
C14:1 *cis*-9	0.95	0.93	1.02	0.99	0.030	0.16	0.79	0.08
C15:0	1.03	0.98	0.97	0.98	0.023	0.18	0.23	0.94
C16:0	37.5	36.9	37.5	36.9	0.28	0.38	0.87	0.07
C16:1 *cis*-9	1.59	1.51	1.44	1.62	0.078	1.00	0.10	0.49
C17:0	0.40	0.39	0.39	0.40	0.005	0.65	0.05	0.23
C17:1 *cis*-9	0.22	0.24	0.23	0.24	0.008	0.21	0.56	0.46
C18:0	9.70	9.60	8.58	8.73	0.169	<0.001	0.47	0.01
C18:1 *trans*-8	0.33	0.39	0.39	0.49	0.035	0.003	0.57	0.27
C18:1 *trans*-9	0.29	0.37	0.56	0.54	0.031	<0.001	0.17	0.02
C18:1 *trans*-10	0.61	0.78	0.83	0.87	0.064	0.01	0.35	0.69
C18:1 *trans*-11	1.15	1.28	1.63	1.84	0.122	<0.001	0.85	0.18
C18:1 *trans*-12	0.46	0.54	0.90	0.82	0.075	<0.001	0.29	0.03
C18:1 *cis*-9	21.3	21.2	20.6	20.7	0.20	0.01	0.58	0.09
C18:2 *cis*-9,*cis*-12	2.61	2.66	2.75	2.78	0.033	<0.001	0.90	0.50
C20:0	0.07	0.07	0.07	0.07	0.001	0.92	0.98	0.05
C18:3 *cis*-9,*cis*-12,*cis*-15	0.45	0.46	0.49	0.50	0.006	<0.001	0.72	0.07
C18:2 *cis*-9,*trans*-11 CLA	0.61	0.76	0.86	0.90	0.022	<0.001	0.02	0.96
C18:2 *trans*-10,*cis*-12 CLA	0.03	0.03	0.04	0.05	0.004	<0.001	0.35	0.17
C22:0	0.04	0.04	0.03	0.03	0.001	0.01	0.52	0.31
C20:3n-6	0.05	0.06	0.06	0.06	0.001	0.01	0.52	0.31
C20:3n-3	0.13	0.14	0.14	0.16	0.004	<0.001	0.01	0.07
C20:5n-3	0.07	0.07	0.06	0.07	0.004	0.24	0.40	0.38
C22:6n-3	0.08	0.15	0.25	0.37	0.012	<0.001	0.05	0.86
Indices								
<C16:0	22.0	21.9	21.5	21.2	0.27	0.02	0.64	0.56
16:0 + C16:1	39.1	38.4	38.9	38.6	0.30	0.42	0.56	0.14
>C16:0	38.5	37.6	37.4	38.0	1.38	0.78	0.60	0.97
Σ SFA[1]	68.7	68.0	67.0	66.7	0.31	<0.001	0.85	0.62
Σ MUFA[2]	26.5	27.1	27.9	27.9	0.28	<0.001	0.3	0.52
Σ PUFA[3]	4.03	4.32	4.67	4.89	0.054	<0.001	0.50	0.41
Σ n-3[4]	0.73	0.82	0.94	1.10	0.018	<0.001	0.06	0.79
Σ n-6[5]	2.67	2.71	2.80	2.84	0.033	<0.001	0.91	0.55
n-6:n-3	3.66	3.31	3.01	2.62	0.051	<0.001	0.71	0.51

[1]Sum of SFA: C4:0, C6:0; C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, and C22:0.

[2]Sum of MUFA: C14:1, C16:1, C17:1, C18:1 *trans*-8, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, C18:1 *trans*-12, and C18:1 *cis*-9.

[3]Sum of PUFA: C18:2 *cis*-9,*cis*-12, C18:3 *cis*-9,*cis*-12,*cis*-15, C18:2 *cis*-9,*trans*-11 CLA, C18:2 *trans*-10,*cis*-12 CLA, C20:3n-6, C20:3n-3, C20:5 n-3, and C22:6n-3.

[4]Sum of n-3 FA: C18:3 *cis*-9,*cis*-12,*cis*-15, C20:3n-3, C20:5n-3, and C22:6n-3.

[5]Sum of n-6 FA: C18:2 *cis*-9,*cis*-12 and C20:3n-6.

was highest in HA and lowest in cheese made from cows fed MA; and firmness and crumbly texture ($P < 0.05$), highest in cheese from cows fed MA, with HA cows producing crumblier and less firm cheese. There were also cubic effects of treatment ($P < 0.05$) on farmyard odor, stickiness, acid flavor, bitterness, and dry mouth aftertaste.

The PCA biplot (Figure 1a) highlights the main sensory attributes in relation to the cheese FA. The PCA accounted for 67.4% of the data variance, with savory and nutty flavors being major sensory attributes contributing to dimensions 1 and 2. Nutty flavor was higher in samples from MA and HA, and was correlated with DHA, C10, C12, C14, and C < 16 (r = 0.521,

Table 3. Cheese composition, yield, and fatty acid (FA) composition in dairy cows fed no microalgae (C), 50 g of microalgae/cow per day (LA), 100 g of microalgae/cow per day (MA), or 150 g of microalgae/cow per day (HA)

	Treatment					*P*-value		
Cheese composition	C	LA	MA	HA	SEM	Linear	Quadratic	Cubic
Moisture, g/kg	414	415	429	429	3.3	<0.001	0.75	0.08
Fat, g/kg	246	237	208	213	9.3	0.005	0.51	0.20
FA, g/100 g of FA								
C4:0	0.49	0.47	0.46	0.47	0.010	0.18	0.31	0.80
C6:0	1.72	1.68	1.63	1.59	0.045	0.05	0.95	0.99
C8:0	0.82	0.80	0.78	0.75	0.025	0.06	0.9	0.98
C10:0	2.27	2.26	2.18	2.12	0.080	0.16	0.76	0.81
C12:0	3.32	3.32	3.27	3.20	0.095	0.35	0.71	0.95
C14:0	11.7	11.8	11.9	11.8	0.13	0.58	0.49	0.86
C14:1 *cis*-9	1.11	1.15	1.21	1.09	0.065	0.98	0.24	0.50
C15:0	1.06	1.10	1.12	1.06	0.025	0.85	0.05	0.56
C16:0	37.4	37.1	36.8	36.8	0.41	0.22	0.76	0.96
C16:1 *cis*-9	1.84	1.79	1.95	1.86	0.062	0.49	0.72	0.10
C17:0	0.37	0.38	0.38	0.38	0.006	0.42	0.40	0.78
C17:1 *cis*-9	0.26	0.24	0.24	0.24	0.006	0.07	0.32	0.13
C18:0	8.61	8.67	7.9	7.98	0.107	<0.001	0.94	0.002
C18:1 *trans*-9	0.36	0.52	0.64	0.63	0.025	<0.001	0.004	0.53
C18:1 *trans*-10	0.27	0.31	0.41	0.46	0.041	0.002	0.88	0.54
C18:1 *trans*-11	0.68	1.06	1.51	1.75	0.223	0.001	0.77	0.79
C18:1 *trans*-12	0.91	1.19	1.33	1.48	0.063	<0.001	0.35	0.59
C18:1 *cis*-9	22.7	21.9	21.8	21.8	0.32	0.05	0.21	0.77
C18:2 *cis*-9,*cis*-12	2.62	2.63	2.67	2.70	0.058	0.28	0.88	0.83
C20:0	0.07	0.07	0.07	0.07	0.001	0.08	0.95	0.01
C18:3 *cis*-9,*cis*-12,*cis*-15	0.44	0.43	0.46	0.47	0.011	0.03	0.44	0.39
C18:2 *cis*-9,*trans*-11 CLA	0.60	0.70	0.83	0.87	0.023	<0.001	0.12	0.22
C18:2 *trans*-10,*cis*-12 CLA	0.02	0.03	0.03	0.02	0.004	0.17	0.18	0.82
C22:0	0.04	0.03	0.03	0.03	0.003	0.03	0.91	0.61
C20:3n-6	0.04	0.06	0.06	0.06	0.004	0.02	0.17	0.46
C20:3n-3	0.09	0.10	0.09	0.10	0.007	0.79	0.62	0.33
C20:5n-3	0.05	0.05	0.05	0.06	0.001	0.03	0.06	0.36
C22:6n-3	0.06	0.13	0.23	0.35	0.007	<0.001	<.001	0.59
Indices								
<C16:0	22.5	22.6	22.5	22.1	0.34	0.41	0.43	0.87
16:0 + C16:1	39.3	38.9	38.8	38.6	0.43	0.28	0.81	0.85
>C16:0	38.2	38.5	38.7	39.3	0.53	0.17	0.75	0.80
Σ SFA[1]	67.9	67.7	66.6	66.2	0.57	0.02	0.91	0.5
Σ MUFA[2]	28.2	28.2	29.0	29.2	0.53	0.11	0.89	0.52
Σ PUFA[3]	3.92	4.12	4.42	4.61	0.094	<0.001	0.96	0.65
Σ n-3[4]	0.64	0.71	0.83	0.97	0.020	<0.001	0.09	0.75
Σ n-6[5]	2.66	2.68	2.73	2.75	0.058	0.21	0.97	0.87
n-6:n-3	4.16	3.77	3.31	2.85	0.040	<0.001	0.41	0.70
AI[6]	2.75	2.73	2.63	2.6	0.089	0.07	0.96	0.58
TI[7]	3.3	3.24	3.04	2.96	0.104	<0.001	0.89	0.42

[1]Sum of SFA: C4:0, C6:0; C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, and C22:0.

[2]Sum of MUFA: C14:1, C16:1, C17:1, C18:1 *trans*-8, C18:1 *trans*-9, C18:1 *trans*-10, C18:1 *trans*-11, C18:1 *trans*-12, and C18:1 *cis*-9.

[3]Sum of PUFA: C18:2 *cis*-9,*cis*-12, C18:3 *cis*-9,*cis*-12,*cis*-15, C18:2 *cis*-9,*trans*-11 CLA, C18:2 *trans*-10,*cis*-12 CLA, C20:3n-6, C20:3n-3, C20:5n-3, and C22:6n-3.

[4]Sum of n-3 FA: C18:3 *cis*-9,*cis*-12,*cis*-15, C20:3n-3, C20:5n-3, and C22:6n-3.

[5]Sum of n-6 FA: C18:2 *cis*-9,*cis*-12 and C20:3n-6.

[6]Atherogenicity index = [C12:0 + 4(C14:0) + C16:0]/(MUFA + PUFA).

[7]Thrombogenicity index = (C14:0 + C16:0 + C18:0)/[0.5(MUFA) + 0.5(n-6) + 3(n-3) + (n-3/n-6)].

0.579, 0.640, 0.717, and 0.620, respectively). Textural attributes such as air holes contributed to dimension 3 (Figure 1b) and were positively correlated with EPA, PUFA, and *cis*-9,*trans*-11 CLA, and negatively correlated with TI (r = 0.501, 0.585, 0.558 and −0.515, respectively). We also found a correlation in dimension 3 between color and several FA; the higher the C14:1 *cis*-9, C15, and AI, the more intense the yellow shade in the cheese (r = 0.537, 0.692, and 0.681, respectively), whereas the color was paler when C14, C > 16, C18:2 n-6, and C18:1 *cis*-9 increased (r = −0.503, −0.566, −0.611, and −0.592, respectively).

Animal Performance

We observed no effect ($P > 0.05$) of dietary treatment on DMI or milk yield, with mean values of 23.4 and 38.5 kg/d, respectively (Table 5). We observed a linear decrease ($P < 0.001$) in milk fat content and yield with increasing dietary inclusion of ALG, with cows fed HA producing 3.7 g/kg and 0.15 kg/d less than those receiving C. Milk protein content and yield and lactose yield were not affected by dietary treatment ($P > 0.05$), with mean values of 32.4 g/kg, 1.24 kg/d, and 1.78 kg/d, respectively. In contrast, milk lactose concentration decreased linearly ($P = 0.007$) with increasing dietary inclusion of ALG, from 46.5 g/kg in cows receiving C to 45.8 g/kg in HA, and we discovered a trend ($P = 0.06$) for ECM to decrease linearly with ALG inclusion. We also observed no effect ($P > 0.05$) of dietary treatment on mean live weight, live weight change, or BCS, with mean values of 667 kg, 0.34 kg/d, and 2.94 units respectively. We observed no effect ($P > 0.05$) of dietary treatment on the mean plasma concentration of glucose, BHB, or NEFA, but we did find an effect of time ($P < 0.001$), with concentrations of BHB increasing and NEFA and glucose decreasing across the 3 time points.

Table 4. Sensory attribute ratings of cheese made from dairy cows fed no microalgae (C), 50 g of microalgae/cow per day (LA), 100 g of microalgae/cow per day (MA), or 150 g of microalgae/cow per day (HA)

	Treatment					*P*-value		
Item	C	LA	MA	HA	SEM	Linear	Quadratic	Cubic
Odor								
Fruity	4.71	3.43	4.52	4.76	0.331	0.27	0.02	0.03
Sweet	3.94	3.31	3.71	3.83	0.262	0.83	0.15	0.25
Acidic	4.12	4.95	3.73	5.60	0.283	0.001	0.04	<0.001
Farmyardy	1.09	1.36	0.84	1.48	0.153	0.18	0.17	0.01
Creamy	3.16	3.50	3.35	2.81	0.245	0.15	0.06	0.91
Appearance								
Edge cut	7.08	6.38	6.15	7.81	0.324	0.04	<0.001	0.33
Air holes	1.78	1.69	2.05	2.39	0.192	0.004	0.25	0.57
Color	1.59	1.86	1.76	1.69	0.057	0.59	0.002	0.11
Glossy	5.19	5.76	6.10	5.64	0.260	0.20	0.04	0.63
Flavor								
Sweet	1.16	1.47	1.56	1.83	0.211	0.02	0.93	0.67
Fruity	1.25	1.45	1.63	1.64	0.188	0.09	0.60	0.86
Tangy	5.62	5.78	5.89	5.96	0.290	0.35	0.87	1.00
Acidic	6.49	6.83	5.66	7.11	0.351	0.40	0.08	0.01
Creamy	2.52	2.45	2.44	1.87	0.203	0.01	0.19	0.49
Salty	2.15	2.47	2.23	2.31	0.136	0.66	0.38	0.14
Nutty	0.91	1.37	1.06	2.04	0.245	0.001	0.23	0.06
Savory	0.68	0.78	0.81	0.82	0.069	0.11	0.52	0.86
Bitter	4.10	4.74	3.70	5.25	0.381	0.06	0.18	0.01
Metallic	0.70	0.98	0.65	0.94	0.137	0.41	0.93	0.05
Aftertaste								
Salty	1.97	2.21	2.05	2.22	0.148	0.34	0.84	0.28
Acidic	5.09	5.57	5.09	6.25	0.328	0.01	0.25	0.07
Bitter	5.24	5.61	5.51	6.91	0.387	<.001	0.16	0.25
Dry mouth	5.55	6.12	5.49	6.63	0.245	0.02	0.28	0.03
Dry throat	3.37	3.70	3.56	4.46	0.264	0.002	0.25	0.19
Metallic	1.25	1.65	1.17	1.60	0.206	0.41	0.88	0.05
Creamy	1.58	1.55	1.75	1.33	0.180	0.33	0.24	0.29
Texture								
Firm	5.05	5.67	5.92	3.98	0.226	<0.001	<0.001	0.07
Dry	6.35	6.31	5.81	6.41	0.278	0.98	0.21	0.21
Crumbly	5.20	5.43	5.58	4.14	0.223	<0.001	<0.001	0.14
Gritty	1.05	0.98	0.85	1.62	0.193	0.02	0.02	0.26
Sticky	9.34	10.3	9.47	9.56	0.252	0.84	0.11	0.02
Emulsifying	11.2	11.1	10.7	11.2	0.29	0.83	0.22	0.25

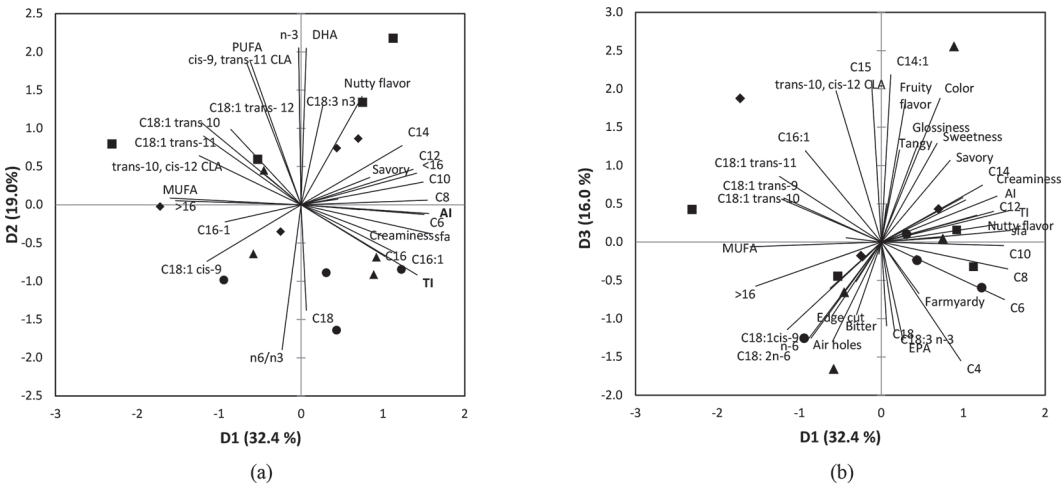

Figure 1. Principal component analysis (PCA) of sensory attributes and fatty acids shown in biplots of samples: (a) biplot between dimensions (D) 1 and 2; (b) biplot between D1 and D3. Cows were fed no microalgae (●), 50 g of microalgae/cow per day (▲), 100 g of microalgae/cow per day (◆), or 150 g of microalgae/cow per day (■). DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; AI = atherogenic index; TI = thrombogenic index.

DISCUSSION

Milk and Cheese Fatty Acid Profiles

The primary objective of our study was to increase milk fat and cheese concentrations of DHA and to determine the subsequent effect on the sensory attributes of cheddar cheese. The dietary levels of ALG used here were chosen because previous studies that have evaluated the effect of ALG on cheese sensory attributes (e.g., Vanbergue et al., 2018b) have used very high levels that were associated with major disturbance to rumen function and reduced animal performance (Vanbergue et al., 2018a).

The similarity between the milk and cheese FA profile across treatments in this study indicates that cheese manufacturing and packaging had little effect on the FA profile, a finding in agreement with Chilliard and Ferlay (2004). We found that DHA increased linearly with the addition of ALG in the diet, a finding in accordance with Stamey et al. (2012), Vahmani et al. (2013), and Boeckaert et al. (2008). However, the DHA content of the cheese from cows fed HA in the current study was lower than when Martini et al. (2009)

Table 5. Milk performance and blood metabolites in dairy cows fed no microalgae (C), 50 g of microalgae/cow per day (LA), 100 g of microalgae/cow per day (MA), or 150 g of microalgae/cow per day (HA)

	Treatment					*P*-value		
Item	C	LA	MA	HA	SEM	Linear	Quadratic	Cubic
DMI, kg/d	23.7	23.3	23.1	23.3	0.32	0.16	0.28	0.93
Milk yield, kg/d	38.1	38.8	38.6	38.4	0.50	0.77	0.36	0.63
ECM,[1] kg/d	41.3	41.3	40.5	39.4	0.52	0.06	0.44	0.90
Milk fat, g/kg	39.6	38.4	37.1	35.9	0.78	<0.001	0.97	0.97
Fat yield, kg/d	1.50	1.47	1.41	1.35	0.039	0.01	0.65	0.85
Milk protein, g/kg	32.2	32.2	32.8	32.2	0.28	0.62	0.24	0.14
Protein yield, kg/d	1.22	1.24	1.26	1.22	0.021	0.97	0.18	0.67
Milk lactose, g/kg	46.5	46.6	45.9	45.8	0.22	0.01	0.44	0.16
Lactose yield, kg/d	1.77	1.81	1.77	1.78	0.025	0.82	0.55	0.28
Live weight, kg	668	663	667	669	2.9	0.60	0.24	0.35
Live weight change, kg/d	0.56	0.06	0.37	0.37	0.157	0.73	0.12	0.12
Body condition	2.91	2.94	2.92	2.99	0.035	0.17	0.56	0.43
Blood metabolites								
Glucose, mmol/L	3.11	3.18	3.07	3.06	0.079	0.49	0.60	0.41
BHB, mmol/L	0.57	0.52	0.55	0.57	0.024	0.35	0.59	0.21
NEFA,[2] mmol/L	0.142	0.168	0.120	0.130	0.0241	0.28	0.63	0.10

[1]Calculated as (0.327 × milk kg/d) + (12.95 × fat kg/d) + (7.65 × protein kg/d).

[2]Nonesterified fatty acid.

fortified reduced-fat cheese with FO. Opportunities for fortification of dairy products with FO are limited, however, as oxidative deterioration causes off-flavors, and Kolanowski and Weissbrodt (2007) reported that cheese stability was limited to only 4 weeks, restricting its commercial use.

As a consequence of the significant increase in DHA and to a lesser extent C18:3 *cis*-9,*cis*-12,*cis* 15, and EPA in milk from cows supplemented with ALG, we found that the n-6:n-3 ratio in milk and cheese decreased from approximately 3.9 in cows fed C to 2.7 at the HA level. The recommended daily ratio of n-6:n-3 FA in the human diet is 2.3:1 (Kris-Etherton et al., 2000), but this ratio is often higher in most Western-style diets. This is principally due to a high consumption of n-6 FA, and therefore a reduction is attractive for human health (Allred et al., 2006), although the usefulness of the dietary n-6:n-3 ratio in reducing cardiovascular disease has recently been questioned (Salter, 2013). The content of SFA, AI, and TI in the cheese in our study also decreased with increasing dietary inclusion of ALG, whereas content of MUFA and PUFA increased. This altered FA profile is in agreement with previously reported responses to ALG (Glover et al., 2012; Boeckaert et al., 2008). The European Food Safety Authority (2012) suggested that people should consume at least 250 mg LC n-3 FA daily, although a higher intake is required for the prevention of cardiovascular diseases (Marventano et al., 2015). In the European Union, consumption of cheese averages 50 g/d, whereas in the United States it is reported to be 43 g/d (Canadian Dairy Information Centre, 2015). In our study, 50 g of cheese made from cows fed HA would supply a daily intake of 43.5 mg of DHA + EPA, a 2.5-fold increase compared with the 13.8 mg of DHA + EPA in cheese made from cows fed C, and would contribute approximately 17% of the daily recommendation of LC n-3 PUFA.

Cheese Composition and Sensory Evaluation

Sensory analysis is the ultimate measure of product quality and success, and is often the final step in many experiments or applications (Drake, 2007). Improvements in the LC n-3 PUFA content of cheese will therefore only have a meaningful effect on the farmer and customer if consumer perception is not adversely affected. Previous studies have reported that high dietary inclusion of ALG resulted in cheese that was less colored, which was attributed to smaller milk fat globule diameters (Vanbergue et al., 2018b). At the lower levels of dietary ALG fed in our study, there was no consistent effect on cheese color, although we found a strong relationship with individual FA: cheese containing C14:1 *cis*-9 and C15 was more yellow, and cheeses were paler when C18:2 n-6 and C18:1 *cis*-9 were increased.

It is well established that a high content of LC n-3 PUFA can predispose dairy products to oxidation and can significantly decrease the sensory qualities of cheese due to the development of fishy off-flavors (Kolanowski and Weissbrodt, 2007; Damodaran and Parkin, 2017). Fortification of cheese with FO was reported to result in significant off-flavors in the study by Martini et al. (2009), but only at the highest rates of inclusion, whereas the fishy flavor decreased as a function of age and became nonsignificant after 3 mo of age (Martini et al., 2009). In our study, the cheese was matured for 120 d, which may explain the lack of an effect of treatment on a fishy flavor, even at the highest rate of inclusion of ALG. Allred et al. (2006) and Vargas-Bello-Pérez et al. (2015) also reported no detectable fish flavors in cheese made from cows fed FO alone or in combination with soybean products, although the concentrations of LC n-3 PUFA in milk were considerably lower than those reported here. Feeding ALG to dairy cows at a higher level than those used here was also reported to have no major effect on the flavor of cheese (Vanbergue et al., 2018b).

We did detect a slight linear increase in acidic and bitter aftertaste in our cheese. Bitterness in cheese has predominantly been associated with hydrophobic peptides from proteolytic reactions, with several amino acids such as aspartate and glutamate contributing (McSweeney, 2007; Baptista et al., 2017). Bitterness in aged cheddar cheese has also been reported to be higher when milk was inoculated with a blend of *Lactococcus lactis* strains that had a low level of autolysis (Hannon et al., 2007). Our cheese processing conditions and recipe were based on published standards using a commercially available starter culture comprising mixed lactic acid bacteria not previously associated with bitterness. A bitter aftertaste could also be due to taste interactions and masking effects of salty-sour and bitter tastes. Thomas-Danguin et al. (2016) reviewed taste interactions in cheese models and reported that perceived intensity of sourness could be enhanced by the concentration of salt, although we did not measure final salt concentrations in our cheese. In contrast to our findings, Vanbergue et al. (2018b) reported no effect on acidic or bitter taste in cheese made from cows fed ALG, and it would therefore appear that, unless inclusion rates are very high or cheese maturation short, feeding ALG may not have a major effect on acidic or bitter tastes.

Food structure can play a major role in the release of flavor compounds, as structure can affect the release of volatiles and the taste release profile (Lamichhane

et al., 2018), with a higher release of flavor compounds when the product has a more porous structure. In our study, the number of air holes increased linearly with the inclusion of ALG and was positively correlated with the EPA, PUFA, and *cis*-9,*trans*-11 CLA of the cheese. These changes were associated with an increase in acid notes, initial sweetness, bitterness, and pleasant nutty flavor and inversely associated with creaminess. A softer structure has been reported in some studies when cheese was made from milk from cows fed diets rich in PUFA (Chen et al., 2004). Similarly, cheese made from our cows fed HA was less firm and more crumbly, and may therefore be used to produce dairy products for markets that prefer a softer structure. We also found a linear decrease in the creamy flavor of the cheese as the level of PUFA increased, a finding consistent with Chen et al. (2004), who stated that PUFA can inhibit lipases that are important for the generation of a cultured dairy product flavor by releasing free FA. Others have reported an increase in a pleasant nutty flavor, which was related to content of linoleic acid (Stuchlík and Zak, 2002), although in our study the relationship was stronger with DHA, with a linear increase in nutty flavor with ALG inclusion rate.

Animal Performance

All of the diets used in our study had similar DM, CP, and NDF contents that were comparable to the mean dietary composition reported in a recent survey of UK dairy rations (Tayyab et al., 2018). As the inclusion rate of ALG in our study was increased, the supply of DHA increased to provide approximately 0, 8, 16, and 24 g/cow per day in C, LA, MA, and HA, respectively. These dietary inclusion levels were selected because higher amounts have been associated with decreases in animal performance and milk fat content (Boeckaert et al., 2008; Vanbergue et al., 2018a). In the current study we observed no effect of treatment on DMI, which averaged 23.4 kg/d, a finding in accordance with Stamey et al. (2012) and Vahmani et al. (2013), who reported no effect of feeding 200 g/d of ALG or FO to Holstein cows. However, at a higher inclusion level of 50 g DHA/cow per day, Moate et al., (2013) reported a 6% decrease in DMI, with an 11% decrease at an inclusion level of 75 g/cow per day, and it would therefore appear that supplying DHA from marine algae at up to 25g/d can be achieved without a negative effect on intake.

We found no effect of dietary treatment on milk yield, although ECM tended to decrease linearly with increasing rate of ALG inclusion, principally due to a reduction in milk fat content. Our results are in agreement with Moate et al. (2013), who also reported a linear decrease in ECM (but not milk yield) with increasing inclusion of algal meal. In contrast, ALG inclusion was associated with a reduction in milk yield in the study of Vanbergue et al. (2018a), which was also associated with a decrease in milk fat content. Milk fat depression induced by ALG supplementation has been reported in both dairy cows (Moate et al., 2013; Vahmani et al., 2013) and sheep (Bichi et al., 2013). However, the precise mechanism behind milk fat depression following supplementation with marine oils such as ALG or FO is unclear (Bichi et al., 2013). Bauman and Griinari (2003) described how unique FA intermediates that are produced through the biohydrogenation of PUFA can cause an inhibitory effect on milk fat synthesis, with *trans*-10,*cis*-12 CLA being identified as a potent inhibitor (Peterson et al., 2003; Sinclair et al., 2007; Hussein et al., 2013), although other intermediaries may also be involved (Chilliard et al., 2001). Supplementation of oil mixtures rich in PUFA or intermediaries of biohydrogenation in the rumen can strongly inhibit de novo synthesis and uptake of circulating FA by the mammary gland (Hussein et al., 2013), and may therefore explain our results. For example Vahmani et al., (2013) reported a 15% reduction in the expression of sterol regulatory element binding protein in the mammary tissue of cows fed FO or ALG compared with the control diet. The antilipogenic effects of *trans*-10,*cis*-12 CLA has been well demonstrated (Bauman and Griinari, 2003; Lock et al., 2006), and in the current study we also observed a linear increase in *trans*-10,*cis*-12 CLA, as daily milk fat content and yield decreased with the addition of ALG in the diet, although the inhibition of milk fat synthesis is often accompanied by little or no change in this isomer in animals fed marine lipids, suggesting a role for other isomers or FA.

Mattos et al. (2004) reported a decrease in plasma glucose concentration when FO was fed to cattle, which was associated with a decrease in DMI, but in our study DM intake and plasma glucose concentration were unaffected by treatment. Overall, the lack of effect of dietary treatment on blood glucose, NEFA, or BHB in our study reflects the lack of difference in intake, weight change, and milk yield.

CONCLUSIONS

Feeding DHA-enriched ALG to dairy cows linearly increased milk and cheese concentration of DHA and PUFA, and decreased concentrations of SFA, which may have human health benefits. We observed an increase in crumbliness and decrease in firmness and creamy flavor of cheddar cheese as well as an increase in nutty flavor as the inclusion of ALG increased. The modified FA composition was associated with a linear decrease in milk fat content, but we found no effect on

DMI or milk yield, although ECM tended to be reduced as the inclusion rate of ALG increased. It is therefore recommended that cheese can be made from cows fed ALG, as this will improve milk and cheese fatty acid quality but will alter the sensory attributes of cheese and reduce milk fat content if fed at high levels.

ACKNOWLEDGMENTS

This study was funded by Alltech (Nicholasville, KY), which also provided the algae. The authors greatly appreciate the technical assistance of S. Williams, N. Blowey, H. Wright, G. Pearman, and J. Kelly, and the staff at Harper Adams University dairy farm, and sensory technical support from C. Hutchison and M. Pusey at Harper Adams University, Newport, Shropshire, UK.

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