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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MILK AND CHEESE FATTY ACIDS

INTERPRETIVE SUMMARY

Influence of rate of inclusion of microalgae on the sensory characteristics and fatty acid composition of cheese and performance of dairy cows *by Till* et al. Long chain omega-3 PUFA such as docosahexaenoic acid (DHA) have human health benefits and are naturally high in microalgae. We fed different amounts of microalgae to dairy cows and found that milk and cheese content of DHA increased with the rate of inclusion, whilst the saturated fat content decreased. Feeding microalgae increased the air holes in cheese and the nutty flavor, and decreased the creaminess. Cow performance was unaffected except milk fat content which was reduced as the feeding level of microalgae increased.

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 one of four dietary treatments in a 4 x 4 row and column design, with four periods of 28 days, with cheddar cheese production and animal performance measurements undertaken during the final 7 days of each period. Cows were fed a basal diet that was supplemented with ALG (*Schizochytrium limancinum sp)* at four rates; 0 (Control; C); 50 g (LA); 100 g (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 when fed HA compared to 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 the cheese decreased with ALG inclusion rate and was positively correlated to the saturated FA content. We also observed a quadratic effect on the fruity odor, which was highest in cheese from cows when 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

There has been a considerably body of research 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 (Marventano et al., 2015; Calder, 2014). 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 conjugated linoleic acids (CLA) have been shown to have health benefits including anti-carcinogenic 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., 2018). 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 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 Grinnari, 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 the lower extent of biohydrogenation in the rumen compared to 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 (Bermúdez-Aguirre and Barbosa-Cánovas, 2011; Martini et al., 2009). 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., 2009; Bermúdez-Aguirre and Barbosa-Cánovas, 2011; Martini et al., 2009). 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., 2018a). Those that have been conducted rated the cheese lower for color and firmness, more grainy, and a higher spicy flavor, attributes that were 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., 2018a).

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., 2018b), which has been linked to the production of *trans* isomers such as *tran*-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 effect 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 impact on 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 profile, cheddar cheese sensory attributes, and cow performance.

MATERIALS AND METHODS

The study was conducted in accordance with the requirement 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 d in milk) Holstein-Friesian dairy cows yielding 44 (±1.9) kg/d of milk, with a live weight of 654 (± 42.4) kg, and body condition score (Ferguson et al., 1994) of 3.0 (± 0.2) at the beginning of the study were used. The study design was a 4 x 20 row and column design (Mead et al., 1993), with each of the 4 periods consisting of a 21 d adaption period followed by 7 d of sampling. All cows were fed the same basal ration (Table 1) which was supplemented with one of four inclusion levels of ALG (*Schizochytrium limancinum sp.,* Alltech, Kentucky, USA) during each period. Treatment diets were; control (C) no algae inclusion, 50 g microalgae/cow per day (LA), 100 g microalgae/ cow per day (MA) and 150 g microalgae/cow per day (HA). A 50:50 (DM basis) wheat/dried sugar beet feed mix replaced the ALG in C, LA and MA and was fed at 150, 100 and 50 g/cow per day respectively. The ALG contained 135 g/kg crude protein, 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, 2004) and contain approximately 200 g starch/kg 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 along with the ALG (or wheat/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 free stalls 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 cheese making during each sampling week from 4 cows per treatment at consecutive pm and am 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 Supplementary Table 1. The pm milk was bulked, rapidly cooled to 4°C and stored overnight in a mini bulk milk tank (Frigomilk milk cooler G1, Via Trivulzia, Italy), and stirred continuously. Milk from the morning was mixed with the pm milk for 30 min before being transferred to a 50 L cheese vat (Jongia, UK). Cheese was made following a cheddar recipe as described by Robinson and Wilbey (1998). The milk was pasteurized by heat-treating to 63°C for 30 min, with temperature and titratable acidity % (TA) 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 bacteria (single shot culture OV26, Orchard Valley Dairy Supplies, Worcestershire, UK) was added. Ripening continued until the TA reached 0.20-0.22 % (up to 1 h), and vegetarian marzyme rennet (Orchard Valley dairy supplies, Worcestershire, UK) 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 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 size 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 prior to analysis.

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), and had previous experience with sensory profiling of food products. The selection criteria for the cheese panel was based on the ability to detect differences among various cheese, 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; Supplementary Table 1). 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). The 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 prior to the sensory profiling test.

Three cheese samples per test session were monadically evaluated on all the sensory attributes at a time to minimize the panelists' fatigue, with a 30-minute break between sessions. Water crackers, cucumber sticks and drinking water were used as cleansing materials. Each panelist was provided with two cubes per sample per replication resulting in 32 samples to evaluate (4 treatments x 4 periods x 2 cubes). Surplus cubes were available if required. The mature cheese samples were trimmed of all external surfaces and cut into 2 x 3 cm cubes and maintained at 12°C (Brown et al., 2003) for evaluation. The samples were then presented in lidded plastic sample pots, and the 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 sub-samples of each TMR were collected daily and stored at -20°C for subsequent analysis. Forage samples were collected weekly, oven dried at 105°C and the ratio of corn:grass silage adjusted to the desired level on a DM basis. Milk yield was recorded daily and samples collected on four occasions during the sampling week of each period, a preservative added (Microtabs II, Advanced Instruments, Inc., Massachusetts, USA) and stored at 4°C prior to subsequent analysis. Additional samples were collected on successive milkings for FA analysis. Cows were weighed and body condition score recorded at 1100 h prior to 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). The cows were selected from the highest, mid and lowest yielding animals in the group, with their performance over the study provided in Supplementary Table 1. The blood samples were collected over two days at 0700, 1000 and 1300 h (to assess diurnal fluctuations) into vacutainers containing sodium heparin for the subsequent determination of β-hydroxybutyrate (3-OHB), or potassium oxalate for the determination of glucose and non-esterified fatty acids (NEFA). Samples were centrifuged at 1000 x g for 15 min, the plasma separated and stored at -20°C prior to subsequent analysis.

Chemical analysis

Milk compositional analysis was conducted using a Milkoscan Minor (Foss Electric, 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, whilst the TMR FA was determined as described by Jenkins (2010). Fatty acids were identified using a GC (model 6890, Agilent, Germany) fitted with an automatic sampler, flame ionization detector and 100 m column (CPSil88, Agilent Technologies, UK) as described by Lock et al. (2006). The oven temperature started at 70 °C, 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, UK).

The TMR samples for each diet were bulked within each period and a sub-sample analyzed according to AOAC (2012) for DM (934.01), CP (988.05) and ash (924.05), whilst NDF was analyzed according to Van Soest et al. (1991). Plasma samples were analyzed for glucose, 3-OHB and NEFA, using kits (catalogue no’s RB1008; GU611 and FA115, respectively, Randox Laboratories, County Antrium, UK) and a Cobas Mira Plus autoanalyzer (ABX Diagnostics, Bedfordshire, UK).

Calculations and statistical analysis

The atherogenic (AI) and thrombogenic indices (TI) in cheese were calculated as described by Ulbright and Southgate (1991). Sensory data were analyzed using XLSTAT software (Addinsoft, 2018), using the analyzing data/principal component analysis option to gain an overview of both sensory and FA profiles of all treatment combinations. Principal Component Analysis (PCA) was used to investigate and visualize correlations between the attributes and to obtain non-correlated 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 analysis of variance using Genstat 17th edition (VSN. Ltd, Oxford, UK). 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, C18:1*n*-9, C18:2*n*-6 and C18:3*n*-3 were similar in all four diets, with mean values of 0.9, 7.7, 9.6 and 1.6 g/kg DM respectively. We detected no DHA in C, with the content of DHA and C16:0 increasing as the 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 DM respectively), whereas the LA diet had a CP content that was 6 g/kg DM higher than the HA diet, which had the lowest value, with C and MA being intermediate. The NDF content was similar between treatments with a mean value of 455 g/kg 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 the milk fat content of C6:0, C8:0, C10:0, 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, C20:3*n*-6 and C20:3*n*-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, and increase in FA more than C16 as the dietary inclusion rate of ALG increased, but there was no effect of treatment on the proportion of C16:0 plus C16:1 ($P > 0.05$). 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 when offered HA. In contrast both the MUFA and PUFA content in milk fat increased linearly ($P < 0.001$) as the dietary inclusion level 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 the inclusion level of ALG increased in the diet, but there was 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 and C20:3*n*-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 C20:3*n*-6 increased linearly (P < 0.05) as the supplementation of ALG increased. Cheese content of DHA increased quadratically with dietary inclusion of ALG (P < 0.001), being highest in cheese made 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 chain length more than C16:0, MUFA or total *n*-6. However increasing the 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 cubic 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 LA and highest in those fed C. In contrast, both the atherogenicity (AI) and thrombogenicity index (TI) 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 the 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, acidic, and dry throat aftertaste, and a linear decrease (P < 0.05) in the creamy flavor of the cheese as the inclusion level of ALG increased in the diet. The creamy flavor was positively and highly correlated to the percentage of SFA (r = 0.601), Al (r = 0.603) and TI (r = 0.560) in the cheese. We also observed a cubic effect (P < 0.05) on the fruity odor, which was highest in cheese from cows when fed HA and lowest in those receiving LA; edge cut appearance (P < 0.001) which was highest in HA and lowest in cheese made from cows fed MA; and firmness and crumbliness texture (P < 0.05), being highest in cheese from cows when fed MA, with HA fed cows producing crumblier and less firm cheese. There were also cubic effects of treatment (P <0.05) on farm-yardy 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 the flavors of savory and nutty being major sensory attributes contributing to Dimensions (D) 1 and 2. The nutty flavor was higher in samples from MA and HA, and were correlated to DHA, C10, C12, C14 and C<16 (r = 0.521, 0.579, 0.640, 0.717, and 0.620 respectively). Textural attributes such as air holes contributed to D3 (Figure 1b), and was positively correlated to EPA, PUFA, and *cis*-9, *trans*-11 CLA, and negatively correlated to TI (r = 0.501, 0.585, 0.558 and -0.515 respectively). We also found a correlation in D3 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 rate 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 there was a trend ($P = 0.06$) for energy corrected milk yield (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 body condition score, 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, 3-OHB or NEFA, but there was an effect of time ($P < 0.001$), with concentrations of 3-OHB increasing and NEFA and glucose decreasing across the 3 time points.

DISCUSSION

Milk and cheese fatty acid profile

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 as previous studies that have evaluated the effect of ALG on cheese sensory attributes (e.g. Vanbergue et al., 2018a) have used very high levels that were associated with a major perturbance to rumen function and reduced animal performance (Vanbergue et al., 2018b).

The similarity between the milk and cheese FA profile across treatments 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). The DHA content of the cheese from cows fed HA in the current study was however, lower than when Martini et al. (2009) fortified reduced-fat cheese with FO. The opportunities for fortification of dairy products with FO is 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 0.81 in cows fed the Control to 0.76 at the highest dietary addition of ALG. 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 however, 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, whilst the content of MUFA and PUFA increased. This altered FA profile is in agreement with previously reported responses to ALG (Glover et al., 2010; Boeckaert et al., 2008). The European Food Safety Authority (2012) suggested that people should consume at least 250 mg LC *n*-3 FA /d, although a higher intake is required for the prevention of cardiovascular diseases (Marventano et al., 2015). In the European Union (EU) consumption of cheese averages 50 g/d, whereas in the United States it is reported to be 43 g/d (Canadian Dairy Information Centre, 2016). 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 to 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 impact on the farmer and customer if consumer perception is not adversely affected. Previous studies have reported that a high dietary inclusion of ALG resulted in cheese that was less colored, which was attributed to a smaller milk fat globule diameter (Vanbergue et al., 2018a). At the lower levels of dietary ALG fed in our study there was no consistent effect on cheese color, although there was a strong relationship with individual FA, with cheese containing C14:1 *cis*-9, and C15 being more yellow, and 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 quality 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 of Martini et al., (2009), but only at the highest rates of inclusion, whilst the fishy flavor decreased as a function of age and became non-significant 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 that reported here. Feeding ALG to dairy cows at a higher level than used here was also reported to have no major effect on the flavor of cheese (Vanbergue et al., 2018a).

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 (Baptista et al. 2017; McSweeny, 2007). 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 comprised of mixed lactic bacteria that has not previously been 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 NaCl, although we did not measure final NaCl concentrations in our cheese. In contrast to our findings, Vanbergue et al., (2018a) 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, that feeding ALG may not have a major effect on acidic and bitter taste.

Food structure can play a major role in the release of flavor compounds as this 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 contains a more porous structure. In our study air holes increased linearly with the inclusion of ALG, and were positively correlated to the EPA, PUFA, and *cis*-9, *trans*-11 CLA of the cheese. These changes were associated with an increase in an acid note, 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. There was also 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, (Stuchlik and Zak, 2002), although in our study the relationship was stronger with DHA with a linear increase in a nutty flavor with ALG inclusion rate.

Animal performance

All of the diets used in our study had a similar DM, CP and NDF content that was 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 d in C, LA, MA and HA respectively. These dietary inclusion levels were selected as higher amounts have been associated with a decrease in animal performance and milk fat content (Boeckaert et al., 2008; Vanbergue et al., 2018b). In the current study we observed no effect of treatment on DMI, which averaged 23.3 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 d in the study of Moate et al., (2013) there was 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 impact 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 Vanergue et al., (2018), 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). The precise mechanism behind milk fat depression following supplementation with marine oils such as ALG or FO is however, 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 (Hussein et al., 2013;

Peterson et al., 2003; Sinclair et al., 2007), 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 to the control diet. The antilipogenic effects of *trans*-10 *cis*-12 CLA has been well demonstrated (Bauman and Chillard, 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 an effect of dietary treatment on blood glucose, NEFA or 3-OHB in our study reflects the lack of a 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 there was no effect on DMI or milk yield, although energy corrected milk yield 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.

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REFERENCES

Allred, S. L., T. R. Dhiman, R. C. Brennand, D. J. McMahon, and N. D. Luchini. 2006. Milk and cheese from cows fed calcium salts of palm and FO alone or in combination with soyabean products. J. Dairy Sci, 89: 234 - 248.

Association of Official Analytical Chemists (2012). Official methods of analysis. Association of Official Analytical Chemists, 19th Edition. Washington, DC, USA.

Baptista, D. P., F. D. da Silva Araújo, M. N. Eberlin, and M. L. Gigante. 2017. Reduction of 25% salt in Prato cheese does not affect proteolysis and sensory acceptance. Int. Dairy J. 75: 101-110.

Bauman, D. E., and J. M. Griinari. 2003. Nutritional regulation of milk fat synthesis. Annu. Rev. Nutr, 23: 203 - 227.

Bermúdez-Aguirre, D., and G. V. Barbosa-Cánovas. 2011. Quality of selected cheese fortified with vegetable and animal sources of omega-3. LWT-Food Sci. Technol. 44: 1577-1584.

Bichi, E., G. Hervas, P. G. Toral, J. J. Loor, and P. Frutos. 2013. Milk fat depression induced by dietary marine algae in dairy ewes: Persistency of milk fatty acid composition and animal performance responses. J. Dairy Sci, 96: 524 - 532.

Boeckaert, C., B. Vlaeminck, J. Dijkstra, A. Issa-Zacharia, T. Van Nespen, W. Van Straalen, and V. Fievez. 2008. Effect of dietary starch or micro algae supplementation on rumen fermentation and milk fatty acid composition on dairy cows. J. Dairy Sci, 91: 4714 - 4727.

Brown, J. A., E. A. Foegeding, C. R. Daubert, M. A. Drake, and M. Gomperts. 2003. Relationships among rheological and sensorial properties of young cheeses. J. Dairy Sci. 86: 3054 - 3067.

BSI. 2014. BS EN ISO 8586:2014. Sensory Analysis: General Guidelines for the Selection, Training and Monitoring of Selected Assessors and Expert Sensory Assessors. Geneva, Switzerland

BSI. 2009. BS ISO 22935-1:2009 (IDF 99-1:2009). Milk and milk products, Sensory analysis, Part 1: General guidance for the recruitment, selection, training and monitoring of assessors

Calder, P. C. 2014. Very long chain omega-3 (*n*-3) fatty acids and human health. *Eur. J.* Lipid Sci. Technol, 116: 1280 - 1300.

Canadian Dairy Information Centre. 2016. Global cheese consumption (kg per capita). Available at http://www.dairyinfo.gc.ca/index_e.php?s1=dff-fcil&s2=cons&s3=consglo&s4=tc-ft&ncid=txtlnkusaolp00000618 [Accessed 01/21/2019].

Chen, S., G. Bobe, S. Zimmerman, E. G. Hammond, C. M. Luhman, T. D. Boylston, A. E. Freeman, and D. C. Beitz. 2004. Physical and sensory properties of dairy products from cows with various milk fatty acid composition. J. Agric. Food Chem, 52: 3422 - 3428.

Chilliard, Y., A. Ferlay, and M. Doreau. 2001. Effect of different types of forages, animal fat or marine oils in cow's diet on milk fat secretion and composition, especially conjugated linoleic acid (CLA) and polyunsaturated fatty acids. Livest. Prod. Sci, 70: 31 - 48

Chilliard, Y., and A. Ferlay. 2004. Dietary lipids and forages interactions on cow and goat milk fatty acid composition and sensory properties. Reprod. Nutr. Dev, 44: 467 - 492.

Chouinard, P. Y., L. Corneau, A. Saebo, and D. E. Bauman. 1999. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci, 82: 2737 - 2745.

Coakley, M., E. Barrett, J. J. Murphy, R. P. Ross, R. Devery, and C. Stanton. 2007. Cheese manufacture with milk elevated conjugated linoleic acid levels caused by dietary manipulation. J. Dairy Sci, 90: 2919 - 2927.

Damodaran, S., and K. L. Parkin. 2017. Fennema's Food Chemistry, 5th Edition, Florida, CRC Press Taylor and Francis group.

Drake, M.A. 2007. Invited Review: Sensory analysis of dairy foods. J. Dairy Sci, 90: 4925 - 4937.

Drake, S. L., Yates, M.D. and Drake, M.A. 2010. Development of a flavor lexicon for processed and imitation cheeses. J. Sensory Studies 25: 720–739.

European Food Safety Authority. 2012. EFAS assesses safety of long-chain omega-3 fatty acids. Available at: https://www.efsa.europa.eu/en/press/news/120727 [Accessed 05/07/2017].

Ferguson, J. D., D. T. Galligan, and N. Thomsen. 1994. Principal descriptors of body condition score in Holstein cows. J. Dairy Sci, 77: 2695 - 2703.

Gebauer, S. K., J. Cardiginy, M. U. Jakobsen, B. Lamarche, A. L. Lock, S. D. Proctor, and D. Baer. 2011. Effects of ruminant trans fatty acids on cardiovascular disease and cancer:

a comprehensive review of epidemiological, clinical, and mechanistic studies. Adv. Nutr, 2: 332 – 354.

Givens, D. I., and R. A. Gibbs. 2006. Very long chain *n*-3 polyunsaturated fatty acids in the food chain in the UK and the potential of animal derived foods to increase intake. Nutr Bull, 31: 104 - 110.

Glover, K. E., S. Budge, M. Rose, H. P. Rupasinghe, L. Maclaren, J. Green-Johnson, and A. H. Fredeen. 2012. Effects of feeding fresh forages and marine algae on the fatty acid composition and oxidation of milk and butter. J. Dairy Sci, 95: 2797 - 2809.

Hannon, J. A., K. N. Kilcawley, M. G. Wilkinson, C. M. Delahunty, and T. P. Beresford, T.P. 2007. Flavour precursor development in cheddar cheese due to lactococcal starters and the presence and lysis of *Lactobacillus helveticus*. Int. Dairy J. 17: 316-327.

Harra, A., and N. S. Radin. 1978. Lipid extraction of tissues with a low toxicity solvent. Anim. Biochem, 90: 420 - 426.

Hussein, M., K. H. Harvatine, W. M. P. B. Weerasinghe, L. A. Sinclair, and D. E. Bauman. 2013. Conjugated linoleic acid-induced milk fat depression in lactating ewes is accompanied by reduced expression of mammary genes involved in lipid synthesis. J. Dairy Sci, 96**:** 3825 - 3834.

Jenkins, T. C. 2010. *Technical note:* Common analytical errors yielding inaccurate results during analysis of fatty acids in feed and digesta samples. J. Dairy Sci. 93, pp. 1170-1174.

Kris-Etherton, P. M., D. S. Taylor, S. Yu-Poth, P. Huth, K. Moriarty, V. Fishell, R. L. Hargrove, G. Zhao, and T. D. Etherton. 2000. Polyunsaturated fatty acids in the food chain in the United States. J. Clin Nutr. 71: 179S - 88S.

Kliem, K. E., and K. J. Shingfield. 2016. Manipulation of milk fatty acid composition in lactating cows: opportunities and challenges. Eur. J. Lipid Sci. Technol. 118: 1661 – 1683.

Kolanowski, W., and J. Weissbrodt. 2007. Sensory quality of dairy products fortified with FO. Int. Dairy J. 17: 1248 - 1253.

Lamichhane, P., A. L. Kelly., and J. J. Sheehan. 2018. Symposium Review: Structure-function relationships in cheese. J. Dairy Sci. 101: 2692-2709.

Lock, A. L., C. A. M. Horne, D. E. Bauman, and A. M. Salter. 2005. Butter naturally enriched in conjugated linoleic acid and vaccenic acid alters tissue fatty acids and improves the plasma lipoprotein profile in cholesterol-fed hamsters. J. Nutr. 135: 1934-1939.

Lock, A. L., B. N. Teles, I. I. Perfield, D. E. Bauman, and L. A. Sinclair. 2006. A conjugated linoleic acid supplement containing *trans*-10, *cis*-12 reduces milk fat synthesis in lactating sheep. J. Dairy Sci. 89: 1525- 1532.

Maia, M. R. G., L. C. Chaudhary, L. Figueres, and R. J. Wallace. 2007. Metabolism of polyunsaturated fatty acids and their toxicity to the microflora of the rumen. Antonie van leeuwenhoek. 91: 303-314.

Marventano, S., P. Kolacz, S. Castellano, F. Galvano, S. Buscemi, A. Mistretta, and G. Grosso. 2015. A review of recent evidence in human studies of *n*-3 and n-6 PUFA intake on cardiovascular disease, cancer, and depressive disorders: does the ratio really matter? Int J Food Sci Nutr. 66: 611 – 622.

Martini, S., J. E. Thurgood, C. Brotherse, R. Ward, and D. J. McMahon. 2009. Fortification of reduced fat cheddar cheese with *n*-3 fatty acids: Effect on off-flavor generation. J. Dairy Sci. 92: 1876 - 1884.

Mattos, R., C. R. Staples, A. Arteche, M. C. Wiltbank, F. J. Diaz, T. C. Jenkins, and W. W. Thatcher. 2004. The effects of feeding FO on uterine secretions of PGF2α, milk composition, and metabolic status of periparturient Holstein cows. J. Dairy Sci. 87: 921 - 932.

McSweeney, P. L. H. 2007. Cheese manufacture and ripening and their influence on cheese flavor. Pages 1-25 in Improving the Flavour of Cheese. B.C. Weimer, ed. Woodhead Publishing Limited, Cambridge, UK.

Mead, R., R.N. Curnow, and A. M. Hasted. 1993. Statistical methods in agriculture and experimental biology (2nd edition). Chapman and & Hall, New York.

Moate, P. J., S. R. O. Williams, M. C. Hannah, R. J. Eckard, B. E. Suldist, J. L. Jacobs, and W. J. Wales. 2013. Effects of feeding algal meal high in docosahexaenoic acid on feed intake, milk production, and methane emissions in dairy cows. J. Dairy Sci. 96: 3177 - 3188.

Palmquist, D. L., and J. M. Griinari. 2006. Milk fatty acid composition in response to reciprocal combinations of sunflower and FOs in the diet. Anim. Feed Sci. Technol. 131: 358 - 369.

Peterson, D. G., E. A. Matitashvili, and D. E. Bauman. 2003. Diet-induced milk fat depression in dairy cows results in increased trans-10, cis-12 CLA in milk fat and coordinate suppression of mRNA abundance for mammary enzymes involved in milk fat synthesis. J. Nutr. 10: 3098 - 3102.

Robinson, R. K., and R. A. Wilbey. 1998. Cheesemaking Practise, R.Scott, 3rd Edition, Reading, Aspen Publishers, inc.

Rodriguez-Herrera, M., Y. Khatri, S. P. Marsh, W. Posri, and L. A. Sinclair. 2017. Feeding microalgae at a high level to finishing heifers increases the long-chain n-3 fatty acid composition of beef with only small effects on the sensory quality. Int J Food Sci Technol. 53: 1405-1413.

Rogers, N.R., Drake, M.A., Daubert, C.R., Mcmahon, D.J., Bletsch, T.K. and Foegeding, E.A. 2009. The effect of aging on low-fat, reduced-fat, and full-fat Cheddar cheese texture. J. Dairy Sci. 92 :4756–4772.

Salter, A.M. 2013. Dietary fatty acids and cardiovascular disease. Animal 7: 163-171.

Sinclair, L.A., A. L. Lock, R. Early, and D. E. Bauman. 2007. Effects of *trans*-10, *cis*-12 conjugated linoleic acid on ovine milk fat synthesis and cheese properties. J. Dairy Sci. 90: 3326 - 3335.

Sinclair, L.A., S. L. Cooper, S. Chikunya, R. G. Wilkinson, K. G. Hallett, M. Enser, and J. D. Wood. 2005. Biohydrogenation of n-3 polyunsaturated fatty acids in the rumen and their effects on microbial metabolism and plasma fatty acid concentration in sheep. Anim. Sci. 81: 239-248.

Stamey, J.A., D. M. Shepherd, M. J. de Veth, and B. A. Corl. 2012. Use of algae or algal oil rich in *n*-3 fatty acids as a feed supplement for dairy cattle. J. Dairy Sci. 95: 5269 - 5275.

Stuchlik, M., and S. Zak. 2002. Vegetable lipids as components of functional foods. Biomed Papers. 146: 3-10.

Tayyab, U., R. G. Wilkinson, C. K. Reynolds, and L. A. Sinclair. 2018. Particle size distribution of forages and mixed rations, and their relationship with ration variability and performance of UK dairy herds. Livest. Sci. 217; 108-115.

Thomas, C. 2014. Feed into milk. Nottingham University Press, Nottingham, UK.

Thomas-Danguin, T., C. Barba, C. Salles, and Guichard, E. 2016. Perception of mixtures of odorants and tastants: sensory and analytical points of view. Pages 319-340 in Flavour: From Food to Perception. E. Guichard, C. Salles, M. Morzel and A. M. Le Bon ed. Wiley Blackwell, Oxford, UK.

Ulbricht, T. L. V. and D. A. T Southgate. 1991. Coronary heart disease: Seven dietary factors. The Lancet 338: 985-992.

Vahmani, P., A. H. Fredeen, and K. E. Glover. 2013. Effect of supplementation with FO or microalgae on fatty acid composition of milk from cows managed in confinement or pasture systems. J. Dairy Sci. 96: 6660 - 6670.

Vanbergue, E., C. Hurtaud, J. Peyraud, E. Beuvier, G. Duboz., and S. Buchin. 2018a. Effects of n-3 fatty acids sources on butter and hard cooked cheese; technological properties and sensory quality. Int. Dairy J. 82: 35-44.

Vanbergue, E., C. Hurtaud., and J. Peyraud. 2018b . Effects of new n-3 fatty acid sources on milk fatty acid profile and milk fat properties in dairy cows. J. Dairy. Res. 85: 265-272.

Van Soest, P. J., J. B. Robertson, and B. A. Lewis. 1991. Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74: 3583 - 3597.

Vargas-Bello-Pérez, E., G. Íñiguez-González, K. Fehrmann-Cartes, P. Toro-Mujica., and P.C. Garnsworthy. 2015. Influence of fish oil alone or in combination with hydrogenated palm oil on sensory characteristics and fatty acid composition of bovine cheese. Anim. Feed Sci. and Technol. 205: 60-68.

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

	Treatment			
Ingredient, kg/kg DM	C	LA	MA	HA
Corn silage	0.436			
Grass silage	0.118			
Rape seed meal	0.077			
Wheat distillers grains and soluble	0.077			
Hipro 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 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, UK

[2]Mineral/vitamin premix. 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 5000; Zn 6350; vitamins (mg/kg) retinol 300; cholecalciferol 7.5; all *rac* α-tochopherol acetate 2000; vitamin B_{12} 2.50; biotin 135.

[3]Not detected

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

	Treatment					*P* value		
Fatty acids, g/100 g	CA	LA	MA	HA	SEM	Lin	Quad	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	<.001	0.42	0.21
C10:0	2.23	2.24	2.09	2.04	0.047	<.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	<.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	<.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	<.001	0.85	0.18
C18:1 *trans*-12	0.46	0.54	0.90	0.82	0.075	<.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	<.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	<.001	0.72	0.07
C18:2 *cis*-9, *trans*-11 CLA	0.61	0.76	0.86	0.90	0.022	<.001	0.02	0.96
C18:2 *trans*-10, *cis*-12 CLA	0.03	0.03	0.04	0.05	0.004	<.001	0.35	0.17
C22:0	0.04	0.04	0.03	0.03	0.001	0.01	0.52	0.31
C20:3*n*-6	0.05	0.06	0.06	0.06	0.001	0.01	0.52	0.31
C20:3*n*-3	0.13	0.14	0.14	0.16	0.004	<.001	0.01	0.07
C20:5*n*-3	0.07	0.07	0.06	0.07	0.004	0.24	0.40	0.38
C22:6*n*-3	0.08	0.15	0.25	0.37	0.012	<.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	40.5	41.2	41.1	41.5	0.35	0.03	0.84	0.37
ΣSFA[1]	68.7	68.0	67.0	66.7	0.31	<.001	0.85	0.62
ΣMUFA[2]	26.5	27.1	27.9	27.9	0.28	<.001	0.3	0.52
ΣPUFA[3]	4.48	4.79	5.21	5.43	0.059	<.001	0.54	0.22
Σ*n*-3[4]	0.73	0.82	0.94	1.10	0.018	<.001	0.06	0.79
Σ*n*-6[5]	3.12	3.18	3.34	3.39	0.036	<.001	0.92	0.20
n-6:*n*-3	0.81	0.79	0.78	0.76	0.003	<.001	0.14	0.30

[1]Sum of saturated fatty acids; C4:0, C6:0; C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0

[2]Sum of monounsaturated fatty acids; 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, C18:1*cis*-9

[3]Sum of polyunsaturated fatty acids; 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, C20:3*n*-6, C20:3*n*-3, C20:5 *n*-3, C22:6 *n*-3
[4]Sum of omega-3 fatty acids; C18:3 *cis*-9, *cis*-12, *cis*-15, C20:3 *n*-3, C20:5 *n*-3, C22:6 *n*-3
[5]Sum of omega-6 fatty acids; C18:2 *cis*-9, *cis*-12, C20:3 *n*-6

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

	Treatment					*P* value		
Cheese composition	C	LA	MA	HA	SEM	Lin	Quad	Cubic
Moisture, g/kg	414	415	429	429	3.3	<.001	0.75	0.08
Fat, g/kg	246	237	208	213	9.3	0.005	0.51	0.20
Fatty acids, g/100 g								
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	<.001	0.94	0.002
C18:1 *trans*-9	0.36	0.52	0.64	0.63	0.025	<.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	<.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	<.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
C20:3*n*-6	0.04	0.06	0.06	0.06	0.004	0.02	0.17	0.46
C20:3*n*-3	0.09	0.10	0.09	0.10	0.007	0.79	0.62	0.33
C20:5*n*-3	0.05	0.05	0.05	0.06	0.001	0.03	0.06	0.36
C22:6*n*-3	0.06	0.13	0.23	0.35	0.007	<.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	40.1	40.3	40.6	41.2	0.54	0.15	0.78	0.95
ΣSFA[1]	67.9	67.7	66.6	66.2	0.57	0.02	0.91	0.5
ΣMUFA[2]	28.2	28.2	29.0	29.2	0.53	0.11	0.89	0.52
ΣPUFA[3]	3.92	4.12	4.42	4.61	0.094	<.001	0.96	0.65
Σ*n*-3[4]	0.64	0.71	0.83	0.97	0.020	<.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	0.81	0.74	0.79	0.77	0.002	<.001	<.001	<.001
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	<.001	0.89	0.42

[1]Sum of saturated fatty acids; C4:0, C6:0; C10:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0
[2]Sum of monounsaturated fatty acids; 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, C18:1*cis*-9
[3]Sum of polyunsaturated fatty acids; 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, C20:3*n*-6, C20:3*n*-3, C20:5*n*-3, C22:6*n*-3
[4]Sum of omega-3 fatty acids; C18:3 *cis*-9, *cis*-12, *cis*-15, C20:3*n*-3, C20:5*n*-3, C22:6*n*-3
[5]Sum of omega-6 fatty acids; C18:2 *cis*-9, *cis*-12, C20:3*n*-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)

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

	Treatment					P value		
Item	C	LA	MA	HA	SEM	Lin	Quad	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	<.001
Farm-yardy	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	<.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	<.001	<.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	<.001	<.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

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

	Treatment					*P*-value		
	C	LA	MA	HA	SEM	Lin	Quad	Cub
DM intake, 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	<.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
3-OHB, mmol/L	0.57	0.52	0.55	0.57	0.024	0.35	0.59	0.21
NEFA, mmol/L	0.142	0.168	0.120	0.130	0.0241	0.28	0.63	0.10

[1]Energy corrected milk calculated as:(0.327 x milk kg/d) + (12.95 x fat kg/d) + (7.65 x protein kg/d)

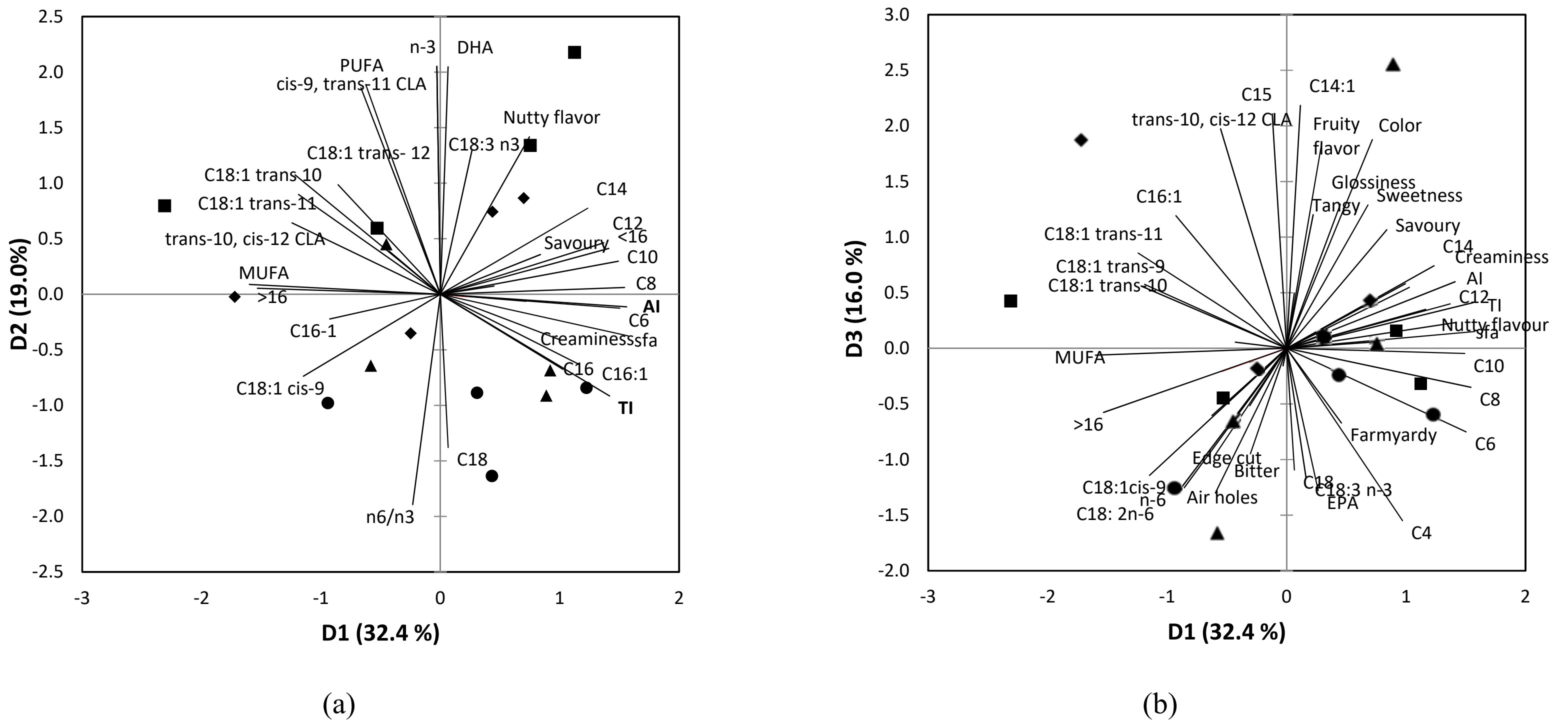

Figure 1. Principal Component Analysis (PCA) on sensory attributes and fatty acids shown in biplots of samples (a) biplot between Dimensions 1 and 2; (b) biplot between Dimensions 1 and 3. Cows were fed no microalgae (●), 50 g/microalgae per cow per day (▲); 100 g/microalgae per cow per day (♦), or 150 g/microalgae per cow per day (■)

Supplementary Table 1. Intake and milk performance of the sub-set of cows that were used for blood sampling or cheese production and fed no microalgae (C), 50 g/microalgae per cow per day (LA); 100 g/microalgae per cow per day (MA), or 150 g/microalgae per cow per day (HA)

	Treatment					*P*-value		
	C	LA	MA	HA	SEM	Lin	Quad	Cub
Cows that were blood sampled (n=12)								
DM intake, kg/ d	23.7	22.9	22.7	23.3	0.48	0.58	0.80	0.94
Milk yield, kg/ d	38.4	38.4	38.7	39.1	0.60	0.37	0.98	0.93
Milk fat, g/ kg	40.4	39.2	38.2	36.2	0.94	0.004	0.70	0.77
Fat yield, kg/d	1.53	1.48	1.46	1.39	0.042	0.03	0.76	0.67
Milk protein, g/kg	31.6	31.5	32.1	31.8	0.25	0.32	0.61	0.22
Protein yield, kg/d	1.20	1.20	1.23	1.23	0.023	0.16	0.90	0.56
Live weight, kg	650	648	653	652	4.0	0.51	0.67	0.37
Cows used for cheese production (n=16)								
DM intake, kg/ d	23.5	23.1	22.9	22.9	0.38	0.18	0.72	0.95
Milk yield, kg/ d	38.3	38.8	38.5	38.9	0.52	0.49	0.85	0.52
Milk fat, g/ kg	40.8	39.2	38.2	36.8	0.93	0.004	0.89	0.82
Fat yield, kg/d	1.55	1.50	1.46	1.41	0.044	0.02	0.96	0.95
Milk protein, g/kg	32.1	32.3	32.4	31.9	0.33	0.93	0.55	0.18
Protein yield, kg/d	1.23	1.24	1.25	1.23	0.023	0.82	0.30	0.67
Live weight, kg	657	653	659	658	3.1	0.44	0.57	0.23
Milk FA, g/100g								
C16:0	38.1	37.3	37.8	36.9	0.29	0.29	0.96	0.06
C18:0	9.80	9.83	8.72	9.01	0.193	<.001	0.50	0.01
C18:1 *cis*-9	20.8	20.9	20.5	21.0	0.27	0.004	0.44	0.19
C20:5 *n*-3	0.07	0.08	0.06	0.07	0.006	0.21	0.82	0.18
C22:6 *n*-3	0.07	0.15	0.25	0.36	0.001	<.001	0.03	0.99

Supplementary Table 2. Definitions and scaling magnitudes used for the sensory evaluation of the experimental cheese

Attribute	Description	0	15
Odor			
Fruity	Smell associated with fruits (especially pineapple)	None	Extreme
Sweet	Overall sweet smell	None	Extreme
Acidic	Smell associated with acids	None	Extreme
Farm-yardy	Smell associated with hay and dairy farm	None	Extreme
Creamy	Smell associated with dairy richness	None	Extreme
Appearance			
Edge cut	How clean/smooth is the knife cut.	Firm	Crumbly
Air holes	Number of round holes on the surface	None	Extreme
Color	Color in white to yellow shade	White	Dark yellow
Glossy	Shiny appearance	Dull	Shiny
Flavor			
Sweet	Taste associated with sucrose solutions, initially perceived as first note	None	Extreme
Fruity	Combinations of tastes and aromas	None	Extreme
Tangy	Sensations in mouth with sharp, clean and acidic notes	None	Extreme
Acidic	Taste associated with acids, mainly sour	None	Extreme
Creamy	Amount of dairy richness in mouth	None	Extreme
Nutty	Distinctive flavor with pleasant nutty note	None	Extreme
Savory	Umami taste, presence of glutamates	None	Extreme
Bitter	Taste resembles from caffeine solutions, including r pungent sensation	None	Extreme
Metallic	Taste associated with ion solutions	None	Extreme
Salty	Taste associated with NaCl solutions	None	Extreme
Aftertaste (Residual)			
Salty	Taste left after swallowing NaCl solutions	None	Extreme
Acidic	Taste associated with acids, including citric acid solutions	None	Extreme
Bitter	Taste left after swallowing caffeine solutions including pungent sensation	None	Extreme
Dry mouth	Left-over dry sensation in oral cavity	Moist	Dry
Dry throat	Left-over dry sensation in throat	Moist	Dry
Metallic	Taste associated with ion solutions	None	Extreme
Creamy	Dairy richness associated with both texture and flavor dimensions	None	Extreme
Texture			
Firm	Fore required to bite through sample using front teeth	Soft	Firm
Dry	Perceived degree of water in sample during chewing	Moist	Dry
Crumbly	Ease sample breaks into small crumbs	Cohesive	Very crumbly
Gritty	Amount of small crystals in the sample	None	Extreme
Sticky	Sticks to the roof of the mouth	None	Extreme
Emulsifying	The presence of fat lumps	Lumpy	Dissolved