https://doi.org/10.17221/19/2020-CJAS

# Effects of dietary microalgae (*Schizochytrium* spp.) supplement on milk performance, blood parameters, and milk fatty acid composition in dairy cows

*Gaofei Liu[1], Xiong Yu[1], Shengli Li[2], Wei Shao[1]*, Nan Zhang[3]*

[1]*College of Animal Science, Xinjiang Agricultural University, Urumqi City, P.R. China*
[2]*College of Animal Science and Technology, China Agricultural University, Beijing, P.R. China*
[3]*Inner Mongolia Mengniu Dairy (Group) Co., Ltd, Inner Mongolia, P.R. China*

**Corresponding author: shaoweixjau@126.com*

**Citation:** Liu G., Yu X., Shengli L., Shao W., Zhang N. (2020): Effects of dietary microalgae (*Schizochytrium spp.*) supplement on milk performance, blood parameters, and milk fatty acid composition in dairy cows. Czech J. Anim. Sci, 65: 162–171.

**Abstract:** The objective of this study was to examine the effects of dietary inclusion of microalgae (*Schizochytrium* spp.) on milk yield, milk composition, and docosahexaenoic acid (DHA) transfer efficiency in dairy cows. Thirty-six lactating Chinese-Holstein dairy cows were randomly allocated to three treatment groups ($n$ = 12; 0, 170, and 255 g microalgae supplement per day) in a 60-day experimental period. No significant treatment effect was observed on DMI and milk performance. Similarly, there was no significant microalgae supplement effect on blood haematological and biochemical parameters, except for platelets ($P < 0.01$) and thrombocytosis ($P < 0.01$), suggesting that the inclusion of microalgae in dairy cow diets would not affect production performance and animal health. Compared to the control group, adding 170 and 255 g microalgae to diets significantly increased the proportion of linoleic acid, DHA, n-3 and n-3/n-6 ratio in the blood ($P < 0.05$). Consequently, DHA concentration and n-3/n-6 ratio were increased in milk, indicating that the milk fatty acid composition could be affected by nutritional manipulation. The overall DHA transfer efficiency was 10.1% and 11.3% for 170 and 255 g microalgae supplement, suggesting that the addition of microalgae to dairy cow diets is a feasible strategy to produce DHA enriched milk in practice.

**Keywords:** bovine; docosahexaenoic acid; microalgae; production performance

As the citizens' quality of life has improved rapidly during the last decades in the world, the demand for high quality milk with more added values and functions has increased (Lock and Bauman 2004). Currently, dairy companies are focusing on producing milk enriched with docosahexaenoic acid (DHA) as DHA is implicated in the development of cognitive function in infants (Birberg-Thornberg et al. 2006) and reducing susceptibility to cardiovascular diseases for elderly persons (Breslow 2006). Although DHA has a positive effect on human health (Grosso et al. 2014; Calder 2015), the level of original DHA in milk is quite low (Fougere et al. 2018). It is possible to produce milk enriched with DHA by directly adding DHA to dairy products during food processing. However, this non-natural DHA milk may bring some defects compared to natural DHA milk (Wright et al. 1998), such as off-flavour caused by oxidation of algae oil when the shelf life extends, or the non-natural property that may not be acceptable to consumers. Therefore, it is essential to find an economical and feasible approach to produce natural DHA milk.

Previous studies have demonstrated that natural DHA milk could be obtained from cows fed specific diets, such as feeding fish oil to cows, or offering algae to cow diets (Shingfield et al. 2003; Zachut et al. 2010; Moate et al. 2013). Moran et al.

https://doi.org/10.17221/19/2020-CJAS

(2017) reported that supplementing a dairy cow diet with *Aurantiochytrium limacinum* algae increased DHA concentrations to 0.37 g of total fatty acids per 100 g milk. Gagliostro et al. (2018) indicated that supplementation of soybean oil and microalgae could improve the quality of milk fatty acids by reducing saturated fatty acids and increasing conjugated linoleic acid, $\alpha$-linolenic and DHA contents in milk. However, the effects of microalgae supplement in diets on milk performance, animal health, and DHA transfer efficiency have not been well studied.

The objective of this study was to investigate the effects of microalgae (*Schizochytrium* spp.) addition to diets on milk yield, blood parameters, and milk fatty acid composition in dairy cows in order to provide guidance for farmers or dairy managers to produce natural DHA enriched milk. We hypothesized that including microalgae in the diets of dairy cow diets could alter the milk fatty acid composition without changing production performance and animal health.

## MATERIAL AND METHODS

### Ethics

All experimental procedures used in this research were accordance with the Regulation on the Administration of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee at Xinjiang Agricultural University.

### Animals and experimental design

Prior to the animal study, health examination was conducted following the protocol described by Thomsen and Baadsgaard (2006). Thirty-six healthy lactating Chinese-Holstein dairy cows were selected as experimental animals. The cows were distributed into blocks by parity, days in milk, and milk yield with three cows in each block (12 blocks in total) and then randomly assigned to 1 of the 3 dietary treatments with twelve cows in each treatment ($n$ = 12). Treatments included an addition of 0 (control), 170 g and 255 g of dried microalgae powder (*Schizochytrium*; Xiamen Huison Biotech Co., Ltd., Fujian, P.R. China) per head per day. To make sure each cow received the assigned amount, microalgae supplement was added to each cow's diet individually and then totally mixed by hand. Nutrient content and fatty acid composition of microalgae are listed in Table 1. The animals were housed in

Table 1. Nutrient content and fatty acid composition of microalgae (*Schizochytrium* spp.) supplement

| Item | % of DM |
|---|---|
| Nutrient content | |
| OM | 91.4 |
| Protein | 25 |
| Fat | 33.3 |
| Fiber | 16.0 |
| Fatty acid composition | |
| C8:0 | 3.51 |
| C10:0 | 2.22 |
| C12:0 | 0.02 |
| C14:0 | 0.13 |
| C15:0 | 0.05 |
| C16:0 | 4.15 |
| C16:1 | 0.09 |
| C17:0 | 0.03 |
| C18:0 | 0.21 |
| C18:1n9c | 0.22 |
| C18:2n6c | 0.1 |
| C18:3n3 | 0.09 |
| C20:0 | 0.03 |
| C20:3n3 | 0.05 |
| C20:4n6 | 0.05 |
| C22:0 | 0.03 |
| C22:6n3 | 17.64 |
| C24:0 | 0.02 |
| SFA[1] | 10.37 |
| MUFA[2] | 0.31 |
| PUFA[3] | 21.4 |
| n-3[4] | 18.02 |
| n-6[5] | 3.39 |

[1]SFAs (saturated fatty acids) = Σ(C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, C24:0); [2]MUFA (monounsaturated fatty acids) = Σ(C14:1c9, C15:1, C16:1, C17:1, C18:1n9c, C18:1t, C18:1c12, C20:1, C22:1n9, C24:1); [3]PUFA (polyunsaturated fatty acids) = Σ(C18:2n6c, C18:2n6t, C18:3n3, C18:3n6, C20:2, C20:3n3, C20:3n6, C20:4n6, C20:5n3, C22:2, C22:6 n3); [4]n-3 = Σ(C18:3n3, C20:3n3, C20:5n3 and C22:6n3); [5]n-6 = Σ(C18:2n6t, C18:2n6c, C18:3n6, C20:3n6 and C20:4n6)

https://doi.org/10.17221/19/2020-CJAS

a free stall barn with sand bedding. The cows had free access to water and were fed the total mixed ration (TMR) twice a day at 08:00 and 16:00. They were milked three times daily at 07:30, 15:00 and 23:00. The ingredients and nutrient composition of basal diet are presented in Table 2.

At the beginning of the experiment, the cows in 170 g and 255 g supplement groups were adapted to a stepwise improvement of microalgae feeding until the highest addition of microalgae on day 15. After that, the microalgae supplements to diets were applied for 60 days.

## Sampling and chemical analyses

Diets were sampled twice a week for DM assessment. Dry matter was analyzed by drying in a forced-draft oven at 105 °C for 24 h. The quantities of remained feeds on a feed table were weighed and recorded twice a week.

Table 2. Ingredients and composition (% of DM) of basal diet

| Item | % of DM |
|---|---|
| Ingredient | |
| Corn silage | 28.77 |
| Alfalfa hay | 9.67 |
| Whole cotton seed | 4.41 |
| Concentrate[1] | 47.67 |
| Brewers wet | 6.57 |
| Molasses cane | 2.54 |
| Bypass fat | 0.37 |
| Composition (%) | |
| DM | 52.55 |
| CP | 16.97 |
| EE | 5.44 |
| NDF | 33.07 |
| ADF | 20.6 |
| Ca | 0.71 |
| P | 0.50 |
| Ash | 6.71 |
| Nel (Mcal/kg) | 1.73 |

[1]The concentrate (dry matter basis) contained 345 g corn meal, 126 g steam-flaked corn, 153 g soybean meal, 155 g cotton meal, 81 g soy hulls, 56 g DDGS, 40 g corn gluten meal, and 44 g of premix per kg. The premix contained Mg ≥ 3.2%, K ≥ 3.7%, Zn ≥ 1 950 mg, Cu ≥ 450 mg, Mn ≥ 1 400 mg, Se ≥ 16 mg, Co ≥ 8 mg, I ≥ 26 mg, vitamin A ≥ 160 KIU, vitamin D ≥ 65 KIU, and vitamin E ≥ 1 200 IU

The milk yield was recorded through milking equipment meters (DeLaval milking machine, 2 × 12 parallel, Sweden). The milk samples from each cow were collected for each milking period, and totally mixed proportionally to corresponding yield (4 : 3 : 3 ratio, composite). Milk samples were collected twice a week and split into two aliquots. One was stored at 4 °C for milk component analysis (e.g. protein, fat, lactose) using FT-120 (FOSS, Denmark) and somatic cell counts using a DeLaval cell counter (DCC, Sweden). The other was analyzed for the fatty acid profile following the preparation of fatty acid methyl esters (Sukhija and Palmquist 1988) and detected by gas chromatograph (7890B, Agilent, USA) according to Shingfield et al. (2003).

Blood samples from individual cows were collected through the caudal vein puncture every 30 days. Blood samples were split into three aliquots. One was stored at 4 °C for haematological (2 ml, EDTAK2) analysis; one was centrifuged at 3 000 *g* at 4 °C for 10 min for biochemical (9 ml) analysis; the last one was centrifuged at 3 500 *g* at 4 °C for 10 min for fatty acid (3 ml) analysis. The haematological variables were measured and recorded using a haematology analyzer (BC-2800VET); they included white blood cells, monocytes, red blood cell distribution width, red blood cells, lymphocytes, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean platelet volume, haemoglobin, haematocrit, platelets, platelet distribution width, thrombocytosis, and neutrophils. The biochemical parameters which included lactate dehydrogenase, high-density lipoprotein cholesterol, total cholesterol, low-density lipoprotein cholesterol, calcium, triglyceride, creatinine, alkaline phosphatase, urea, uric acid, glucose, alanine aminotransferase, aspartate aminotransferase, and total bilirubin were measured using a clinical analyzer (71810, HITACHI, Japan). Blood fatty acids were analyzed using a gas chromatograph (GC) system (7890B, Agilent, USA) following the procedure described by Shingfield et al. (2003).

## Calculation and statistical analyses

The 3.5% fat-corrected milk yield (FCM) was calculated according to Maynard et al. (1979):

https://doi.org/10.17221/19/2020-CJAS

$$3.5\%FCM = 0.35 \times \text{milk yield (kg/day)} + 15 \times \text{fat yield (kg/day)} \quad (1)$$

where:
FCM – fat-corrected milk yield.

The transfer efficiency of DHA from diet to milk was calculated as follows:

$$TE\ (\%) = \frac{\text{DHA yield in milk (g/day)} \times 100\%}{\text{DHA of dietary intake (g/day)}} \quad (2)$$

where:
TE – transfer efficiency;
DHA – docosahexaenoic acid.

Statistical analysis was processed using SPSS v20.0 software to determine the effect of dietary microalgae addition on milk performance, fatty acid composition, and blood haematological and biochemical parameters, and blood fatty acid composition. Results were analyzed using a mixed model procedure with treatment as fixed effect, blocks as random effect, cows within treatments as random effect, and week as repeated measures.

For repeated measures, various covariance structures were tested and the first-order autoregression was selected based on the lowest value for the Akaike information criterion. Least squares means were compared using Tukey adjustment for multiple comparisons. $P < 0.05$ was declared as statistically significant; $P < 0.01$ was declared as highly significant, and $0.05 < P < 0.10$ was declared as a tendency.

## RESULTS

### Dry matter intake and milk performance

The effects of different treatments on dry matter intake (DMI), milk yield and composition are shown in Table 3. Overall, no significant difference was observed in DMI between the three treatments. Similarly, milk yield, 3.5% fat-corrected milk yield, milk fat percentage and yield, milk protein percentage and yield, milk lactose percentage and yield, and somatic cell counts were not affected by microalgae supplement to diets.

### Milk fatty acid composition

The effect of microalgae supplement on milk fatty acid composition is shown in Table 4. Compared to the control group, the addition of 170 g or 255 g microalgae to diets significantly decreased the proportions of C6:0, C13:0, C14:1, C15:0, C15:1, C16:1, C17:0, C17:1, C18:3n3, C18:3n6, C20:0, C20:2, C20:3n3, C20:3n6, C20:4n6, C20:5n3, C21:0, C22:0, C22:1n9, C22:2, C23:0, and C24:0, while the proportions of C16:0, C18:0, and C18:1t increased. Consequently, microalgae supplement increased the proportion of total saturated fatty acids ($P < 0.01$), but decreased total monounsaturated fatty acids, polyunsaturated fatty acids, and n-6 fatty acids proportions in milk ($P < 0.05$). However, no significant difference was observed between the treatments with 170 g and 255 g microalgae in diets.

Table 3. Effects of dietary microalgae (*Schizochytrium* spp.) supplement on DMI, milk yield and milk composition

| Item | Treatment | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | control | 170 g | 255 g | | |
| DMI (kg) | 24.14 | 24.9 | 24.05 | 1.02 | 0.24 |
| Milk yield (kg/day) | 35.95 | 36.5 | 36.1 | 2.83 | 0.99 |
| FCM (kg/day) | 31.56 | 29.68 | 29.85 | 1.83 | 0.54 |
| Fat (%) | 3.62 | 3.26 | 3.35 | 0.41 | 0.68 |
| Fat (kg/day) | 1.27 | 1.13 | 1.16 | 0.10 | 0.36 |
| Protein (%) | 3.59 | 3.42 | 3.57 | 0.15 | 0.48 |
| Protein (kg/day) | 1.27 | 1.21 | 1.25 | 0.07 | 0.68 |
| Lactose (%) | 5.13 | 5.14 | 5.08 | 0.05 | 0.47 |
| Lactose (kg/day) | 1.84 | 1.86 | 1.83 | 0.15 | 0.97 |
| SCC ($10^3$/ml) | 126.08 | 71.21 | 148.55 | 52.92 | 0.33 |

DMI = dry matter intake; FCM = 3.5% fat-corrected milk yield; SCC = somatic cell counts

https://doi.org/10.17221/19/2020-CJAS

Table 4. Effect of dietary microalgae (*Schizochytrium* spp.) supplement on milk fatty acid composition (%)[1]

| Item | Treatment | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | control | 170 g | 255 g | | |
| C4:0 | 3.75 | 3.92 | 3.68 | 0.37 | 0.79 |
| C6:0 | 2.73[a] | 2.27[b] | 2.35[b] | 0.19 | 0.05 |
| C8:0 | 2.01 | 1.64 | 1.74 | 0.22 | 0.25 |
| C10:0 | 3.93 | 3.25 | 3.72 | 0.38 | 0.20 |
| C11:0 | 3.39 | 3.98 | 3.56 | 0.12 | 0.29 |
| C12:0 | 4.09 | 3.61 | 3.78 | 0.38 | 0.45 |
| C13:0 | 1.67[a] | 0.61[b] | 0.48[b] | 0.34 | 0.002 |
| C14:0 | 12.09 | 11.57 | 13.66 | 0.83 | 0.04 |
| C14:1 | 2.41[a] | 1.56[b] | 1.27[b] | 0.36 | 0.01 |
| C15:0 | 2.41[a] | 1.41[b] | 1.34[b] | 0.21 | < 0.001 |
| C15:1 | 2.38[a] | 0.55[b] | 0.46[b] | 0.33 | < 0.001 |
| C16:0 | 16.53[b] | 27.30[a] | 24.85[a] | 1.33 | < 0.001 |
| C16:1 | 3.09[a] | 1.70[b] | 2.19[b] | 0.33 | 0.001 |
| C17:0 | 2.98[a] | 0.84[b] | 0.89[b] | 0.28 | < 0.001 |
| C17:1 | 1.16[a] | 0.35[b] | 0.25[b] | 0.21 | < 0.001 |
| C18:0 | 6.76[b] | 9.57[a] | 10.10[a] | 0.66 | < 0.001 |
| C18:1n9c | 15.31 | 15.62 | 14.87 | 0.81 | 0.64 |
| C18:1t | 2.20[a] | 3.77[b] | 4.14[b] | 0.47 | 0.001 |
| C18:2n6c | 3.19 | 3.04 | 3.16 | 0.25 | 0.82 |
| C18:2n6t | 1.33 | 1.07 | 1.05 | 0.18 | 0.25 |
| C18:3n3 | 0.42[a] | 0.18[b] | 0.19[b] | 0.07 | 0.003 |
| C18:3n6 | 0.70 [a] | 0.22[b] | 0.16[b] | 0.10 | < 0.001 |
| C20:0 | 1.51 [a] | 0.34[b] | 0.46[b] | 0.33 | 0.002 |
| C20:1 | 0.43 [a] | 0.33[a] | 0.20[b] | 0.09 | 0.06 |
| C20:2 | 0.26 [a] | 0.04[b] | 0.07[b] | 0.05 | < 0.001 |
| C20:3n3 | 0.20 [a] | 0.05[b] | 0.06[b] | 0.03 | < 0.001 |
| C20:3n6 | 0.35 [a] | 0.14[b] | 0.09[b] | 0.05 | < 0.001 |
| C20:4n6 | 0.40 [a] | 0.13[b] | 0.10[b] | 0.05 | < 0.001 |
| C20:5n3 | 0.12 [a] | 0.04[b] | 0.04[b] | 0.03 | 0.003 |
| C21:0 | 1.02 [a] | 0.11[b] | 0.16[b] | 0.05 | < 0.001 |
| C22:0 | 0.24 [a] | 0.08 [b] | 0.10[b] | 0.03 | < 0.001 |
| C22:1n9 | 0.31 [a] | 0.11[b] | 0.07[b] | 0.08 | 0.02 |
| C22:2 | 0.10[a] | 0.03[b] | 0.05[b] | 0.02 | 0.003 |
| C22:6n3 | 0.00[c] | 0.37[b] | 0.53[a] | 0.06 | < 0.001 |
| C23:0 | 0.21[a] | 0.06[b] | 0.05[b] | 0.03 | < 0.001 |
| C24:0 | 0.23[a] | 0.06[b] | 0.03[b] | 0.07 | 0.02 |
| C24:1 | 0.09 | 0.09 | 0.09 | 0.08 | 0.99 |
| SFA[2] | 65.54[b] | 70.62[a] | 70.95[a] | 1.66 | 0.003 |
| MUFA[3] | 27.37[a] | 24.08[b] | 23.54[b] | 1.48 | 0.03 |
| PUFA[4] | 34.46[a] | 29.38[b] | 29.05[b] | 1.62 | 0.003 |
| n-3[5] | 0.74 | 0.63 | 0.83 | 0.12 | 0.27 |
| n-6[6] | 5.96[a] | 4.60[b] | 4.56[b] | 0.36 | 0.001 |
| n-3/n-6 | 0.12[a] | 0.14[ab] | 0.18[b] | 0.03 | 0.04 |

[1]Means with different superscripts in the row differ significantly ($P < 0.05$); [2]SFAs (saturated fatty acids) = ∑(C4:0, C6:0, C8:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, C24:0); [3]MUFA (monounsaturated fatty acids) = ∑(C14:1 c9, C15:1, C16:1, C17:1, C18:1n9 c, C18:1t, C18:1 c12, C20:1, C22:1 n9, C24:1); [4]PUFA (polyunsaturated fatty acids) = ∑(C18:2n6c, C18:2n6t, C18:3n3, C18:3n6, C20:2, C20:3n3, C20:3n6, C20:4n6, C20:5n3, C22:2, C22:6 n3); [5]n-3 = ∑(C18:3n3, C20:3n3, C20:5n3 and C22:6n3); [6]n-6 = ∑(C18:2n6t, C18:2n6c, C18:3n6, C20:3n6 and C20:4n6)

https://doi.org/10.17221/19/2020-CJAS

## DHA transfer efficiency from feeding microalgae to milk

DHA transfer efficiency from feeding microalgae to milk is documented in Figure 1. The overall DHA transfer efficiency was 10.07% and 11.30% for 170 g and 255 g daily microalgae supplement, respectively. No significant difference was observed between 170 and 255 g microalgae supplement groups ($P$ = 0.54).

The highest transfer rate in 170 g and 255 g microalgae supplement groups was during the seventh and sixth week (Figure 1), which was 13.24% and 12.93%, respectively.

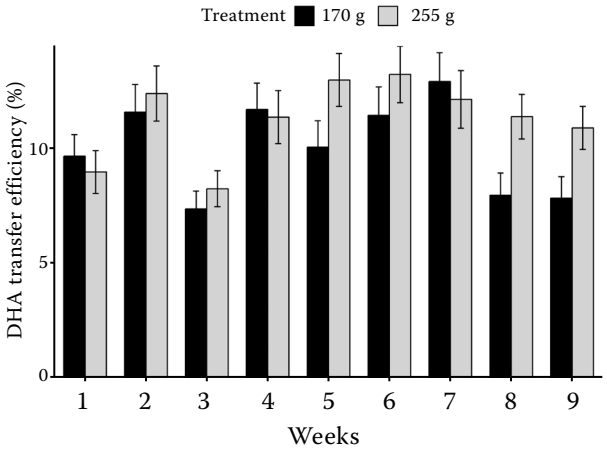


Figure 1. Docosahexaenoic acid (DHA) transfer efficiency from feeding 170 g or 255 g microalgae (*Schizochytrium* spp.) to milk. Data are shown as mean ± SEM

## Blood metabolic parameters

Blood haematological and biochemical parameters are presented in Table 5 and Table 6. There was no significant treatment effect on most of the blood haematological and biochemical parameters, except for platelets ($P$ < 0.01) and thrombocytosis ($P$ < 0.01). Adding 255 g microalgae per day to diets increased platelet and thrombocytosis proportions compared to the control and 170 g microalgae supplement.

However, no significant difference was observed between the control and 170 g supplement group. Compared with the control group, adding 170 g and 255 g microalgae to diets tended to increase the blood calcium concentration ($P$ = 0.07).

Table 5. Effect of dietary microalgae (*Schizochytrium* spp.) supplement on blood haematological parameters[1]

| Haematological parameters | Treatment | | | SEM | $P$-value |
|---|---|---|---|---|---|
| | control | 170 g | 255 g | | |
| White blood cells ($10^9$/l) | 8.27 | 9.01 | 8.55 | 0.92 | 0.72 |
| Monocytes ($10^9$/l) | 0.76 | 0.75 | 0.72 | 0.06 | 0.67 |
| Monocytes percentage (%) | 9.59 | 9.07 | 8.79 | 0.42 | 0.15 |
| Red blood cell distribution width | 17.0 | 17.7 | 17.4 | 0.49 | 0.40 |
| Red blood cells ($10^{12}$/l) | 6.32 | 6.4 | 6.32 | 0.19 | 0.89 |
| Lymphocyte ($10^9$/l) | 2.88 | 3.83 | 3.3 | 0.72 | 0.43 |
| Lymphocyte percentage (%) | 34.7 | 39.2 | 38 | 3.02 | 0.31 |
| Mean corpuscular volume (fl) | 51 | 49.2 | 50.4 | 1.45 | 0.47 |
| Mean corpuscular hemoglobin (pg) | 15.9 | 15.3 | 15.6 | 0.40 | 0.35 |
| Mean corpuscular hemoglobin concentration (g/l) | 313 | 312 | 311 | 2.16 | 0.78 |
| Mean platelet volume (fl) | 5.56 | 5.48 | 5.62 | 0.16 | 0.64 |
| Hemoglobin (g/l) | 100 | 98.1 | 98.9 | 2.68 | 0.69 |
| Hematocrit percentage (%) | 32 | 31.4 | 31.7 | 0.91 | 0.79 |
| Platelets ($10^9$/l) | 432[b] | 433[b] | 590[a] | 47.2 | 0.002 |
| Platelet distribution width | 16.4 | 16.4 | 16.4 | 0.10 | 0.69 |
| Thrombocytosis (%) | 0.24[b] | 0.24[b] | 0.30[a] | 0.02 | 0.001 |
| Neutrophils ($10^9$/l) | 4.62 | 4.42 | 4.53 | 0.40 | 0.88 |
| Neutrophils (%) | 55.6 | 51.8 | 53.3 | 2.87 | 0.42 |

[1]Means with different superscripts in the row differ significantly ($P$ < 0.05)

https://doi.org/10.17221/19/2020-CJAS

Table 6. Effect of dietary microalgae (*Schizochytrium* spp.) supplement on blood biochemical parameters

| Biochemical parameters | Treatment | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | control | 170 g | 255 g | | |
| Lactate dehydrogenase (IU/l) | 1 135.8 | 1 139.9 | 1 215.4 | 65.57 | 0.40 |
| High-density lipoprotein cholesterol (mmol/l) | 2.7 | 2.5 | 2.7 | 0.13 | 0.29 |
| Total cholesterol (mmol/l) | 5.5 | 5.5 | 6.1 | 0.35 | 0.20 |
| Low-density lipoprotein cholesterol (mmol/l) | 1.0 | 0.9 | 1.1 | 0.09 | 0.42 |
| Calcium (mmol/l) | 2.20 | 2.23 | 2.23 | 0.02 | 0.07 |
| Triglyceride (mmol/l) | 0.2 | 0.2 | 0.2 | 0.01 | 0.62 |
| Creatinine (umol/l) | 45.7 | 48.3 | 47.5 | 2.20 | 0.49 |
| Alkaline phosphatase (IU/l) | 53.6 | 55.9 | 54.4 | 6.79 | 0.94 |
| Urea (mmol/l) | 5.5 | 5.6 | 5.6 | 0.21 | 0.88 |
| Uric acid (umol/l) | 47.8 | 48.2 | 52 | 3.30 | 0.38 |
| Glucose (mmol/l) | 1.1 | 1.2 | 1.1 | 0.07 | 0.33 |
| Alanine aminotransferase (IU/l) | 31.4 | 33 | 34.7 | 1.61 | 0.14 |
| Aspartate aminotransferase (IU/l) | 119.9 | 117.9 | 131.8 | 15.98 | 0.44 |
| Total bilirubin (umol/l) | 1.0 | 0.9 | 1.0 | 0.12 | 0.79 |

Table 7. Effect of dietary microalgae (*Schizochytrium* spp.) supplement on blood fatty acid composition (%)[1]

| Item | Treatment | | | SEM | *P*-value |
|---|---|---|---|---|---|
| | control | 170 g | 255 g | | |
| C14:0 | 0.74 | 0.71 | 0.72 | 0.05 | 0.80 |
| C15:0 | 0.58 | 0.55 | 0.55 | 0.02 | 0.24 |
| C16:0 | 12.83 | 12.52 | 13.27 | 0.43 | 0.24 |
| C16:1 | 0.82 | 0.68 | 0.73 | 0.08 | 0.25 |
| C17:0 | 1.60$^{a}$ | 1.33$^{b}$ | 1.33$^{b}$ | 0.04 | < 0.001 |
| C18:0 | 16.31$^{a}$ | 15.11$^{b}$ | 15.02$^{b}$ | 0.50 | 0.03 |
| C18:1n9c | 6.58$^{a}$ | 5.23$^{b}$ | 5.44$^{b}$ | 0.36 | < 0.001 |
| C18:1t | 1.09$^{b}$ | 1.28$^{b}$ | 1.54$^{a}$ | 0.11 | < 0.001 |
| C18:2n6c | 46.54$^{b}$ | 50.15$^{a}$ | 48.76$^{b}$ | 1.17 | 0.01 |
| C18:2n6t | 2.94 | 2.76 | 2.51 | 0.29 | 0.35 |
| C18:3n3 | 2.64 | 2.79 | 2.64 | 0.16 | 0.54 |
| C20:3n6 | 2.73$^{a}$ | 1.48$^{b}$ | 1.27$^{b}$ | 0.11 | < 0.001 |
| C20:4n6 | 2.31$^{a}$ | 1.79$^{b}$ | 1.88$^{b}$ | 0.14 | < 0.001 |
| C20:5n3 | 0.14$^{b}$ | 0.24$^{b}$ | 0.54$^{a}$ | 0.08 | < 0.001 |
| C22:0 | 0.49 | 0.45 | 0.53 | 0.06 | 0.34 |
| C22:1n9 | 0.3 | 0.32 | 0.26 | 0.07 | 0.67 |
| C22:2 | 0.53 | 0.48 | 0.53 | 0.06 | 0.60 |
| C22:6n3 | 0.0$^{c}$ | 1.32$^{b}$ | 1.57$^{a}$ | 0.07 | < 0.001 |
| C24:0 | 0.63 | 0.64 | 0.7 | 0.08 | 0.57 |
| C24:1 | 0.1 | 0.09 | 0.16 | 0.04 | 0.21 |
| [2]SFA | 33.22 | 31.33 | 32.16 | 0.98 | 0.17 |
| [3]MUFA | 8.89$^{a}$ | 7.61$^{b}$ | 8.13$^{ab}$ | 0.51 | 0.05 |
| [4]PUFA | 57.89$^{b}$ | 61.04$^{a}$ | 59.71$^{ab}$ | 1.12 | 0.03 |
| [5]n-3 | 2.78$^{b}$ | 4.36$^{a}$ | 4.75$^{a}$ | 0.25 | < 0.001 |
| [6]n-6 | 54.58 | 56.18 | 54.42 | 1.08 | 0.21 |
| n-3/n-6 | 0.05$^{b}$ | 0.08$^{a}$ | 0.09$^{a}$ | 0.01 | < 0.001 |

[1]Means with different superscripts in the row differ significantly ($P < 0.05$); [2]SFAs (saturated fatty acids) = ∑(C4:0, C6:0, C8:0, C10:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C21:0, C22:0, C23:0, C24:0); [3]MUFA (monounsaturated fatty acids) = ∑(C14:1, C15:1, C16:1, C17:1, C18:1n9 c, C18:1t, C20:1, C22:1 n9, C24:1); [4]PUFA (polyunsaturated fatty acids) = ∑(C18:2n6c, C18:2n6t, C18:3n3, C18:3n6, C20:2, C20:3n3, C20:3n6, C20:4n6, C20:5n3, C22:2, C22:6n3); [5]n-3 = ∑(C18:3n3, C20:3n3, C20:5n3 and C22:6n3); [6]n-6 = ∑(C18:2n6t, C18:2n6c, C18:3n6, C20:3n6 and C20:4n6)

https://doi.org/10.17221/19/2020-CJAS

### Blood fatty acid composition

Blood fatty acid composition is presented in Table 7. Compared to the control group, both 170 g and 255 g microalgae supplements to diets significantly increased the proportion of C18:2n6c, C22:6n3, n-3, and n-3/n-6 in the blood ($P < 0.05$). However, the addition of 170 g and 255 g microalgae to diets decreased the proportion of C17:0, C18:0, C20:3n6, and C20:4n6 in the blood ($P < 0.05$). The 255 g microalgae supplement group showed increased proportions of C18:1t, C20:5n3 and C22:6n3 when compared to the control and 170 g microalgae supplement group ($P < 0.01$). The 170 g microalgae supplement group had a lower proportion of monounsaturated fatty acids ($P < 0.05$), but a higher proportion of polyunsaturated fatty acids than the control group ($P < 0.05$). However, no significant difference was observed between 255 g microalgae supplement and control in monounsaturated fatty acids and polyunsaturated fatty acids in the blood.

## DISCUSSION

In the present study, no significant treatment effect on DMI was found between the three treatments, which was consistent with previous studies (Stamey et al. 2012; Moate et al. 2013; Moran et al. 2017; Fougere et al. 2018). However, some studies reported that the addition of microalgae to the diets decreased DMI (Franklin et al. 1999; Vanbergue et al. 2018), which was attributed to negative effects of unsaturated fatty acid on rumen fermentation especially in relation to fibre degradation. The rumen archaea and cellulolytic activity may be hampered by an increase of dietary fat proportion, and hence DMI may be impaired (Moallem 2018). However, this phenomenon was not observed in this study, implying that the varied effects of microalgae supplement on DMI are not directly related to the presence of unsaturated fatty acids in microalgae, but to the general effects of dietary nutrient composition, processing techniques, and characters of microalgae products.

Since DMI was not affected by the microalgae supplement, no significant effect was observed on milk yield or 3.5% fat-corrected milk yield for the 170 g and 255 g microalgae supplement groups relative to the control. These results were consistent with Franklin et al. (1999) and Moate et al. (2013). A positive effect of adding microalgae to diets on milk yield was reported by Moran et al. (2017), but the fat-corrected milk yield was not affected. However, milk protein percentage, protein yield, lactose percentage, and lactose yield were not affected by microalgae supplementation. Similar results have been reported in the previous studies (Stamey et al. 2012; Moate et al. 2013; Moran et al. 2017; Fougere et al. 2018). In addition, the milk fat percentage and fat yield were similar in different treatments, which was consistent with results of Stamey et al. (2012) and Moran et al. (2017).

Certain intermediates, such as trans-10, cis-12-conjugated linoleic acid, from the rumen biohydrogenation of long-chain polyunsaturated fatty acids could inhibit *de novo* fatty acid synthesis in the mammary gland (Moallem 2018). Therefore, milk fat depression is often reported in diets containing polyunsaturated fatty acids (Shingfield et al. 2003; Moate et al. 2013). However, in the present study, proportions of short- or medium-chain fatty acids were not affected by microalgae addition except for C6:0. A dose response of marine algae supplement on fatty acid profiles was reported in previous studies (Zachut et al. 2010; Moate et al. 2013; Moran et al. 2018), suggesting that the addition of up to 255 g microalgae to dairy cow diets is durable without causing milk fat depression.

In the present study we found that microalgae supplements increased saturated fatty acids in milk compared to the control group. The increase was primarily due to increased proportions of palmitic acid (C16:0) and stearic acid (C18:0), though C16:0 and SFA in blood were not affected by microalgae supplements. Franklin et al. (1999) reported that microalgae addition increased milk C16:0 in dairy cows. Zachut et al. (2010) demonstrated that the proportions of C16:0 and C18:0 increased in the milk fat of cows fed encapsulated flaxseed oil. Our results were consistent with these studies. However, Moate et al. (2013) found that both C16:0 and saturated fatty acids were decreased in milk by the addition of microalgae. Different results might be caused by different dietary fat concentration and diet management strategy, which were critical factors to affect milk fatty acid profiles (Moallem 2018).

Although higher proportions of monounsaturated and polyunsaturated fatty acids were increased in the blood of cows fed microalgae than in the

https://doi.org/10.17221/19/2020-CJAS

control, monounsaturated and polyunsaturated fatty acids were reduced in milk. The decrease of monounsaturated fatty acids and polyunsaturated fatty acids in milk was mainly a result of higher saturated fatty acids, as these proportions were relative values. However, Franklin et al. (1999) indicated that the concentration of polyunsaturated fatty acids was increased in the milk fat of cows fed algae compared to the control. Similar results were also reported by Moallem (2018) and Moate et al. (2013). Clearly, an opposite result was observed in the present study. Our result implies a detrimental effect of microalgae addition on monounsaturated and polyunsaturated fatty acids in milk. It is unknown why such varied results were observed. One possible explanation is that unsaturated fatty acids were primarily stored in the body fat instead of being transferred to the mammary gland for milk fat synthesis. Further studies regarding microalgae supplement on fatty acid distribution (milk fat or body fat) in dairy cows need to be conducted in future.

The increased proportions of DHA in milk represent opportunities motivated by consumers for high quality milk. Increased consumption of DHA has been associated with beneficial effects on a variety of illnesses, including cardiovascular diseases (Russo 2009), depressive disorders (Grosso et al. 2014), and inflammatory processes (Calder 2015). However, it seems to be a challenge to generate polyunsaturated fatty acids in milk by dairy cows (Lanier and Corl 2015). In the present study, the DHA percentage was increased significantly. Hence adding microalgae to diets might be an applicable method to produce DHA enriched milk. In this study, we used a kind of fermented and dried algae powder from *Schizochytrium*. *Schizochytrium* spp. supplement to diets increased a DHA proportion in the blood, which was consistent with the result reported by Moran et al. (2017). The blood DHA percentage of total fatty acids was 1.99% for 255 g microalgae supplement group and 1.61% for 170 g microalgae supplement group on day 60 in our study. These results were similar to those reported by Moran et al. (2017), who observed 1.96% of DHA on day 56.

The highest DHA transfer rate was 13.24% in 255 g microalgae supplement group with an average level of 11.30%. This result was higher than the transfer rate of 3.4% and 8.9% reported in the previous studies (Stamey et al. 2012; Moate et al. 2013). However, it was consistent with the transfer rate in Franklin et al. (1999) and Moran et al. (2018).

The n-3/n-6 ratio is known to be important in the body for eicosanoid synthesis (Steffens 1997). For optimal infant nutrition, the ratio of n-3/n-6 must not be lower than 0.1 (Gerster 1998). Simopoulos (2002) suggested that a higher n-3/n-6 ratio was needed in western countries to decrease chronic diseases. In the present study, the n-3/n-6 ratio in blood was higher in microalgae supplement groups than in the control group. Consequently, the n-3/n-6 ratio in the 255 g microalgae supplement group was higher than in the control group, which implies that it could generate beneficial effects on human health.

Microalgae supplement to diets did not affect most of the blood haematological and biochemical parameters, except for platelets and thrombocytosis. However, platelets and thrombocytosis were still within reference ranges (George et al. 2010), suggesting that the addition of microalgae to diets would not affect health performance in dairy cows. Moran et al. (2017) reported that blood parameters in dairy cows fed 100 g algae were all within a regular range, though very few parameters were higher in the treatment group, which was consistent with our results.

## CONCLUSION

Supplementing diets with microalgae (*Schizochytrium* spp.) did not affect DMI, milk production, blood haematological and biochemical parameters of dairy cows, suggesting that the addition of microalgae to dairy cow diets would not affect production performance and animal health. However, both 170 g and 255 g microalgae supplements increased the DHA concentration and n-3/n-6 ratio in the blood and consequently caused an accumulation of DHA and n-3/n-6 ratio in milk, which supports our hypothesis that the inclusion of microalgae to dairy cow diets could alter milk fatty acid composition.

The overall DHA transfer efficiency was 10.07% and 11.30% for 170 g and 255 g microalgae supplement, implying that supplementing microalgae to diets is a feasible strategy to produce DHA enriched milk in practice.

### Conflict of interest

The authors declare no conflict of interest.

https://doi.org/10.17221/19/2020-CJAS

## REFERENCES

Birberg-Thornberg U, Karlsson T, Gustafsson PA, Duchen K. Nutrition and theory of mind – The role of polyunsaturated fatty acids (PUFA) in the development of theory of mind. Prostaglandins Leukot Essent Fatty Acids. 2006 Jul;75(1):33-41.

Breslow JL. n– 3 Fatty acids and cardiovascular disease. Am J Clin Nutr. 2006 Jun 1;83(6):1477S-82S.

Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta. 2015 Apr;1851(4):469-84.

Fougere H, Delavaud C, Bernard L. Diets supplemented with starch and corn oil, marine algae, or hydrogenated palm oil differentially modulate milk fat secretion and composition in cows and goats: A comparative study. J Dairy Sci. 2018 Sep;101(9):8429-45.

Franklin ST, Martin KR, Baer RJ, Schingoethe DJ, Hippen AR. Dietary marine algae (Schizochytrium sp.) increases concentrations of conjugated linoleic, docosahexaenoic and transvaccenic acids in milk of dairy cows. J Nutr. 1999 Nov;129(11):2048-54.

Gagliostro GA, Antonacci LE, Perez CD, Rossetti L, Carabajal A. Improving the quality of milk fatty acid in dairy cows supplemented with soybean oil and DHA-micro algae in a confined production system. Agric Sci. 2018 Sep 13;9(09):1115.

George JW, Snipes J, Lane VM. Comparison of bovine hematology reference intervals from 1957 to 2006. Vet Clin Pathol. 2010 Jun;39(2):138-48.

Gerster H. Can adults adequately convert a-linolenic acid (18: 3n-3) to eicosapentaenoic acid (20: 5n-3) and docosahexaenoic acid (22: 6n-3)? Int J Vitam Nutr Res. 1998; 68(3):159-73.

Grosso G, Pajak A, Marventano S, Castellano S, Galvano F, Bucolo C, Drago F, Caraci F. Role of omega-3 fatty acids in the treatment of depressive disorders: a comprehensive meta-analysis of randomized clinical trials. PloS One. 2014;9(5):e96905.

Lanier JS, Corl BA. Challenges in enriching milk fat with polyunsaturated fatty acids. J Anim Sci Biotechnol. 2015 Dec 1;6(1):26.

Lock AL, Bauman DE. Modifying milk fat composition of dairy cows to enhance fatty acids beneficial to human health. Lipids. 2004 Dec;39(12):1197-206.

Maynard LA, Loosli JK, Hintz HF, Warner RG. Animal nutrition. 7th ed. New York, NY, USA: McGraw-Hill Book Company; 1979.

Moallem U. Invited review: Roles of dietary n-3 fatty acids in performance, milk fat composition, and reproductive and immune systems in dairy cattle. J Dairy Sci. 2018 Oct 1;101(10):8641-61.

Moate PJ, Williams SR, Hannah MC, Eckard RJ, Auldist MJ, Ribaux BE, Jacobs JL, Wales WJ. Effects of feeding algal meal high in docosahexaenoic acid on feed intake, milk production, and methane emissions in dairy cows. J Dairy Sci. 2013 May 1;96(5):3177-88.

Moran CA, Morlacchini M, Fusconi G. Enhancing the DHA content in milk from dairy cows by feeding ALL-G-RICH™. J Appl Anim Nutr. 2017;5:1-9.

Moran CA, Morlacchini M, Keegan JD, Fusconi G. The effect of dietary supplementation with Aurantiochytrium limacinum on lactating dairy cows in terms of animal health, productivity and milk composition. J Anim Physiol Anim Nutr. 2018 Apr;102(2):576-90.

Russo GL. Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. Biochem Pharmacol. 2009 Mar 15;77 (6):937-46.

Shingfield KJ, Ahvenjarvi S, Toivonen V, Arola A, Nurmela KV, Huhtanen P, Griinari JM. Effect of dietary fish oil on biohydrogenation of fatty acids and milk fatty acid content in cows. Anim Sc. 2003 Apr;77(1):165-79.

Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 2002 Oct 1;56(8):365-79.

Stamey JA, Shepherd DM, De Veth MJ, Corl BA. Use of algae or algal oil rich in n-3 fatty acids as a feed supplement for dairy cattle. J Dairy Sci. 2012 Sep 1;95(9):5269-75.

Steffens W. Effects of variation in essential fatty acids in fish feeds on nutritive value of freshwater fish for humans. Aquaculture. 1997 May 15;151(1-4):97-119.

Sukhija PS, Palmquist DL. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J Agric Food Chem. 1988 Nov;36(6):1202-6.

Thomsen PT, Baadsgaard NP. Intra- and inter-observer agreement of a protocol for clinical examination of dairy cows. Prev Vet Med. 2006 Jul 17;75(1-2):133-9.

Vanbergue E, Peyraud JL, Hurtaud C. Effects of new n-3 fatty acid sources on milk fatty acid profile and milk fat properties in dairy cows. J Dairy Res. 2018 Aug;85(3):265-72.

Wright T, McBride B, Holub B. Docosahexaenoic acid-enriched milk. World Rev Nutr Diet. 1998 Jan 1;83(1):160-5.

Zachut M, Arieli A, Lehrer H, Livshitz L, Yakoby S, Moallem U. Effects of increased supplementation of n-3 fatty acids to transition dairy cows on performance and fatty acid profile in plasma, adipose tissue, and milk fat. J Dairy Sci. 2010 Dec 1;93(12):5877-89.

Received: February 2, 2020
Accepted: May 10, 2020