Macromineral and trace element concentrations in milk from Finnish Ayrshire cows fed microalgae (Spirulina platensis) and rapeseed (Brassica napus)

J. Dairy Sci. 105:8866–8878
https://doi.org/10.3168/jds.2022-22050

Macromineral and trace element concentrations in milk from Finnish Ayrshire cows fed microalgae (*Spirulina platensis*) and rapeseed (*Brassica napus*)

Eric E. Newton,[1] Marjukka Lamminen,[2*] Partha Ray,[1,3] Alexander M. Mackenzie,[4] Christopher K. Reynolds,[1] Michael R. F. Lee,[4] Anni Halmemies-Beauchet-Filleau,[2] Aila Vanhatalo,[2] and Sokratis Stergiadis[1*]
[1]School of Agriculture, Policy, and Development, University of Reading, Earley Gate, PO Box 237, RG6 6EU, Reading, United Kingdom
[2]Department of Agricultural Sciences, University of Helsinki, PO Box 28, FI-00014 Helsinki, Finland
[3]The Nature Conservancy, 4245 North Fairfax Drive, Suite 100, Arlington, VA 22203
[4]Department of Agriculture and the Environment, Harper Adams University, Edgmond, Shropshire, TF10 8NB, Newport, United Kingdom

ABSTRACT

Given the lack of research regarding the effect of microalgal supplementation in dairy cows on milk mineral concentrations, this study investigated the effect of feeding different protein supplements in dairy cow diets on milk, feces, and blood plasma mineral concentrations, associated milk and blood plasma transfer efficiencies, and apparent digestibility. Lactating Finnish Ayrshire cows (n = 8) were allocated at the start of the trial to 4 diets used in a replicated 4 × 4 Latin square design experiment: (1) control diet (CON), (2) a pelleted rapeseed supplement (RSS; 2,550 g/d), (3) a mixture of rapeseed and *Spirulina platensis* (RSAL; 1,280 g of RSS + 570 g of *S. platensis* per day), and (4) *S. platensis* (ALG; 1,130 g of *S. platensis* per day). In each of the 4 experimental periods, a 2-wk adaptation to the experimental diets was followed by a 7-d sampling and measurement period. Feed samples were composited per measurement period, milk, and feed samples (4 consecutive days; d 17–20), and blood plasma samples (d 21) were composited for each cow period (n = 32). Data were statistically analyzed using a linear mixed effects model with diet, period within square, square and their interaction as fixed factors, and cow within square as a random factor. Cows fed ALG were not significantly different in their milk or blood plasma mineral concentrations compared with CON, although feeding ALG increased fecal concentrations of macrominerals (Ca and Mg) and trace elements (Co, Cu, Fe, I, Mn, and Zn), and reduced their apparent digestibility, compared with CON. When compared with CON and ALG, milk from cows fed RSAL and RSS had lower milk I concentrations (−69.6 and −102.7 μg/kg of milk, respectively), but total plasma I concentrations were not affected significantly. Feeding *S. platensis* to dairy cows did not affect mineral concentrations in cows' blood or milk, but care should be taken when rapeseed is fed to avoid reducing milk I concentrations which may in turn reduce consumers' I intake from milk and dairy products.

Key words: bovine, milk, minerals, microalgae, rapeseed

INTRODUCTION

Microalgae is often considered to be a possible partial solution to food security-related problems stemming from land scarcity and climate change within the agricultural sector (Ullmann and Grimm, 2021). The benefits from growing microalgae are numerous, as algal aquacultural systems can be located in nonarable land and use wastewater, reducing the cost of production and providing a more sustainable and eco-friendly solution to deliver biomass for animal feed (Dębowski et al., 2020). In 2019, an estimated 56,456 tons of microalgae were cultivated, the vast majority of which, 56,208 tons, were of *Spirulina* (Cai et al., 2021).

Given the applicability of microalgal farming across several disciplines, and the potential benefits, there has been increasing interest in feeding microalgae as a protein source to ruminants and the effect this might have on the quality of the milk from microalgal-fed cows (Halmemies-Beauchet-Filleau et al., 2018; Lamminen et al., 2019). Previous work has shown that microalgae has potential to be included with rapeseed in dairy diets as a source of protein (Lamminen et al., 2019). Some microalgae species, although CP-rich, have palatability issues that have been seen to reduce feed intake in ruminants when substituted for conventional feeds such as bromegrass (*Bromus madritensis*) hay, soybean meal (*Glycine max*), or corn (*Zea mays*), but this could be potentially overcome if microalgae was used as a pelleted supplement or deodorization with ethanol

Received March 4, 2022.
Accepted July 8, 2022.
*Corresponding authors: s.stergiadis@reading.ac.uk and marjukka.lamminen@helsinki.fi

(Van Emon et al., 2015; Cuellar-Bermúdez et al., 2017; Halmemies-Beauchet-Filleau et al., 2018; Lamminen et al., 2019).

Of the approximately 25,000 microalgal species currently identified, *Spirulina platensis* (also known as *Arthrospira platensis*) has been considered as a potential for animal feed (Vale et al., 2020). *Spirulina platensis* is the most cultivated photosynthetic prokaryote, given its array of use in food and animal feed, and estimates for production are ~56,000 tons, with the majority grown in China and the Asia-Pacific region (Cai et al., 2021). This phycological species contains large concentrations of minerals such as calcium (Ca; 6,000 mg/100 g), magnesium (Mg; 100 mg/100 g), phosphorus (P; 10,088 mg/100 g), potassium (K; 2,502 mg/100 g), and sodium (Na; 14,004 mg/100 g; Seghiri et al., 2019), and its production is projected to continue to increase in availability given the wide uses for the product (Lum et al., 2013).

Milk and dairy products are a rich source of I, Ca, P, Se, Mg, and Zn (Haug et al., 2007), which can have beneficial effects on human health such as increased bone health, a protective effect against certain cancers, and reduction in childhood obesity that can lead to the development of type 2 diabetes (Thorning et al., 2016). Published reports in Finland have shown that dairy products, including milk, provide 18% of K, 36% of P, 67% of Ca, 23% of Se, 27% of Zn, and 32% of I as a portion of total daily intake for women (Kaartinen et al., 2020). Milk products are estimated to provide between 13 and 64% of the recommended daily intake for I depending on the country (van der Reijden et al., 2017). This is even more important with the consideration that 1.9 billion individuals on the planet would be described as having inadequate I intake, with Europe making up the largest portion (59.9%; de Benoist et al., 2003). In 2017, it was estimated that 2 billion people still suffer from I deficiency, and this is realized in 50 million cases of clinical symptoms globally requiring intervention (Biban and Lichiardopol, 2017).

Previous studies have shown that a cows' diet is a major factor influencing milk mineral concentrations (Qin et al., 2021; Stergiadis et al., 2021). Notably, inclusion of macroalgae in a cows' diet has been found to increase milk concentration of I and decrease milk concentration of Cu (Newton et al., 2021). Certain protein sources (e.g., rapeseed, cassava, sorghum, soy, cruciferous vegetables), in particular those rich in goitrogens (e.g., thiocyanate, glucosinolates, flavonoids, goitrin), can also play an important role in milk I concentrations (Flachowsky et al., 2014; Bertinato, 2021). Goitrogens can be found in several animal feeds and some can inhibit the activity of the thyroid peroxidase enzyme (Babiker et al., 2020). Depending on the type of goitrogen, this results in a suppression of thyroid gland function, reduction of production of thyroid hormones, inability of the thyroid to properly uptake and process I, leading to lower I excretion into the milk, or all of these. (Petroski and Minich, 2020; Bertinato, 2021).

Given that dairy products are major suppliers of minerals in human diets, and the fact that changes in a cow's diet may affect resulting mineral concentrations, supplementing microalgae to dairy diets may well affect milk mineral concentrations and mineral supply to consumers. To the best of our knowledge, there are no published studies concerning the effect of feeding *S. platensis* on milk mineral concentrations. In the present study, we hypothesize that the inclusion of rapeseed or microalgae as a protein source in cows' diets will affect milk mineral concentrations. Therefore, the present study aimed to: (1) investigate the comparative effects of including *S. platensis* and rapeseed meal in dairy cow diets on milk, feces, and plasma concentrations of macro- and microminerals, and (2) estimate the effect that the consumption of the produced milk may have on consumers' mineral intakes, based on milk intakes and dietary guidelines from the Finnish Institute for Health and Welfare National FinDiet 2017 Survey (Kaartinen et al., 2020) and the 2012 Nordic Nutritional Recommendations (NNR, 2014).

MATERIALS AND METHODS

Experimental Design

The current study was conducted from May to July, 2014, at the University of Helsinki research farm, Helsinki, Finland. All animal procedures were approved by the National Animal Experiment Board in Finland according to the guidelines of the European Union Directive 2010/63/EU and the current Finnish legislation on animal experimentation (Act on the Protection of Animals Used for Scientific or Educational Purposes 497/2013). Lactating Finnish Ayrshire multiparous cows (n = 8) were selected based on similar lactation stage and milk yield; specifically, the means at the start of the experimental work are as follows: milk yield = 35.8 ± 3.08 kg/d; BW = 718 ± 54.4 kg; BCS = 2.89 ± 0.330. Cows were randomly allocated in a replicated balanced 4 × 4 Latin square study with 4 dietary treatments and four 21-d periods, which consisted of 2 wk of diet adaptation, followed by a 7-d sampling and measurement period. The dietary treatments were (1) control diet (**CON**), (2) pelleted rapeseed supplementation (**RSS**; 2,550 g/d), (3) supplementation of a mixture of RSS and *S. platensis* (**RSAL**; 1,280 g of RSS + 570 g of *S. platensis* per day), and (4) *S. platensis* supplementation (**ALG**; 1,130 g/d of *S. platensis*).

Rapeseed supplement consisted of 695 g/kg of rapeseed meal, 138 g/kg of turnip rape (*Brassica rapa*) cake, 117 g/kg of molassed sugar beet (*Beta vulgaris*) pulp, and 50 g/kg of molasses. The CON diet consisted of grass silage, cereal-sugar beet pulp, and mineral-vitamin supplement (53:47 forage to concentrate). All treatments that received protein supplementation (RSS, RSAL, ALG) were nearly iso-nitrogenous (120–127 g of N per day between rapeseed and *S. platensis*, 277–305 g of N per day for all concentrates). Crude protein coming from the concentrate feeds was based on cereals (CON), rapeseed (RSS), rapeseed and microalgae (RSAL), and microalgae (ALG). Diet ingredient profiles of the 4 treatments can be found in Table 2 of our previously published work (Lamminen et al., 2017). In short, all 4 experimental groups were fed cereal-sugar beet pulp (7.87–10.50 kg of DM per day), and a mineral-vitamin supplement (0.30 kg of DM per day). Algae-containing groups (RSAL and ALG) were fed molassed sugar beet pulp (0.09 and 0.18 kg of DM per day, respectively) and molasses (0.03 and 0.06 kg of DM per day, respectively), along with *S. platensis* at 0.57 kg of DM per day and 1.13 kg of DM per day, respectively. Rapeseed-containing treatments (RSS and RSAL) were a rapeseed supplement (2.55 and 1.28 kg of DM per day, respectively). *Spirulina platensis* was purchased from Duplaco B.V. The microalgae was produced in open raceway ponds, centrifuged after harvesting, and subsequently dried. The same grass silage was used across all treatments as the basal forage, which was preserved from the secondary growth of a mixed sward of timothy (*Phleum pratense*) and meadow fescue (*Festuca pratensis*). Pre-wilted grass silage was ensiled with formic acid-based additive, applied at a rate of 6 L/1,000 kg of fresh matter. Mineral compositions of individual feeds and mineral-vitamin supplement are shown in Table 1. Chemical composition of the feed ingredients (silage, cereal-sugar beet pulp, molassed sugar beet pulp, molasses, mineral-vitamin supplement, rapeseed supplement, and *S. platensis*) can be found in Table 2. The concentrate feeds were provided at 12 kg/d on fresh matter basis (4 times daily, at 0600, 1100, 1700, and 1930 h), and grass silage was offered ad libitum. Animals had ad libitum access to water. A mineral-vitamin supplement was provided (Pihatto-Melli Plus, Raisioagro Ltd.) by inclusion in a paste made of microalgae, molasses, and water, which was then mixed with the other concentrate components. Mineral composition of the 4 experimental diets is shown in Table 3. Mineral intakes from the 4 experimental diets are also presented in Table 3. The experimental diets were number coded, and the codes and group allocation were under the responsibility of the study coordinator. Research personnel knew the contents of the diets (as they were responsible to prepare them), but the interpretation of the codes was not revealed to other animal caretakers.

Sample Collection

Detailed sampling information can be found in Lamminen et al. (2017). In brief, feed samples (from every feed ingredient) were collected daily and composited per sample of feed per period, except for the mineral-vitamin supplement, which were composited to provide 1 sample for the entire experiment, and the molasses products, which were composited by combining periods 1 and 2, and then 3 and 4, resulting in 2 representative samples. Samples for each composite diet ingredient were stored at −20°C until further analysis. Milk samples were obtained daily for 4 consecutive days. Cows were milked twice daily (a.m. and p.m.), and samples taken from each milking were combined into 1 representative daily sample based on milk yield. Milk samples from 4 consecutive days were then composited based on milk yield to represent 1 sample per cow per period, and stored at −20°C. Feces were collected as rectal grab samples twice daily from d 17 to 20 of each period, and composited, which resulted in 1 fecal sample per cow per period. Representative composite samples were collected and frozen at −20°C for future analysis. Blood was taken and processed akin to Puhakka et al. (2016) by drawing from the coccygeal vein 3 times (0530, 0830, and 1130 h) on the 21st day of the period, and plasma was later composited to give 1 sample per cow per period. Samples were collected into 10-mL test tubes (Vacutainer; BD Medical) containing an anticoagulant agent (lithium heparin). After collection, samples were placed immediately on ice until centrifuged (2,220 × *g* for 10 min at room temperature, 21°C) to separate plasma. Plasma was stored in polypropylene tubes at −20°C. No samples were missing or excluded from the experiment.

Quantification of Mineral Concentrations in Feed, Milk, Feces, and Blood Plasma

Concentrations of I in feed, milk, and fecal samples were quantified according to British Standards Institution Publication (BS EN 17050:2017), using ICP-MS (Agilent 7000, Agilent) with slight modifications, as published in Newton et al. (2021). In brief, feed and feces were extracted using a tetramethylammonium hydroxide solution and heated, syringe filtered, and then diluted for analysis. Milk I was extracted with a 2% tetramethylammonium hydroxide solution, syringe filtered, and then diluted for analysis (Newton et al., 2021). Dried feed, feces, and milk concentrations of all other minerals were analyzed according to

Table 1. Mineral concentrations of feed components fed in experimental treatments[1] each day, including average (AVG), SD, and range for mineral values

	Grass silage			Cereal-sugar beet pulp			Rapeseed[2]			Algae[3]			Molassed sugar beet pulp[3]			Molasses[3]			Mineral-vitamin supplement[4]
Item	AVG	SD	Range	AVG	SD	Range	AVG	SD	Range	AVG	SD	Range	AVG	SD	Range	AVG	SD	Range	AVG
Macromineral (g/kg of DM)																			
Calcium	5.5	0.36	5.1–6.0	1.3	0.13	1.2–1.5	8.3	0.48	7.8–9.0	2.9	0.18	2.7–3.2	10.6	0.38	10.0–11.0	5.7	1.00	4.6–7.2	273
Magnesium	1.8	0.09	1.7–1.9	0.02	0.036	0.02–0.02	4.7	0.21	4.4–4.9	3.1	0.06	3.0–3.2	2.0	0.09	1.9–2.1	0.42	0.016	0.40–0.44	69
Phosphorus	2.1	0.10	2.0–2.2	3.1	0.06	3.0–3.2	10.0	0.51	9.2–10.4	10.7	0.20	10.4–10.9	1.0	0.06	0.9–1.1	1.4	0.33	1.0–1.9	0.3
Potassium	30	1.3	27–31	6.0	0.04	5.7–5.8	14	0.9	13–15	14	0.5	14–15	8.3	0.39	7.8–8.9	46	0.5	45–46	0.5
Sodium	0.09	0.007	0.08–0.10	0.54	0.027	0.51–0.58	0.80	0.029	0.76–0.83	7.3	0.57	6.7–8.2	6.4	0.17	6.2–6.6	16	0.4	16–17	80
Sulfur	3.1	0.29	2.7–6.5	1.9	0.15	1.7–2.1	8.0	0.37	7.3–8.3	7.9	0.90	6.9–9.2	7.0	0.24	6.6–7.2	4.7	0.61	4.1–5.7	12
Trace element (µg/kg of DM unless otherwise indicated)																			
Cobalt	31	19.1	0–48	15	9.7	5–28	108	11.3	92–123	291	2.6	288–295	408	182.2	267–716	503	30.0	480–553	0.02
Copper[5]	5.5	0.73	4.4–6.4	6.1	1.93	4.8–9.3	5.4	0.21	5.1–5.7	1.5	0.99	0.2–2.9	7.3	1.13	5.4–8.1	3.4	1.06	2.4–4.9	0.4
Iron[5]	334	12.4	323–355	114	5.5	106–120	181	7.1	170–187	902	30.0	875–949	394	25.5	360–421	252	25.4	228–290	7
Iodine	136	2.5	134–141	95	0.0	95–95	126	61.8	83–230	1,719	149.7	1,518–1,931	1,342	66.6	1,265–1,411	335	71.3	234–431	0.07
Manganese[5]	28	1.9	25–30	23	0.7	22–23	66	2.9	62–70	37	1.2	36–39	145	166.8	46–429	17	2.9	14–21	0.92
Zinc[5]	32	1.6	30–34	31	1.7	29–33	58	1.6	56–59	15	1.3	14–17	46	4.7	40–51	55	48.7	27–138	1.5

[1]Control = no microalgae; RSS = rapeseed supplement; RSAL = 570 g/d of rapeseed + microalgae supplement; ALG = 1,130 g/d of microalgae supplement.

[2]Only fed to RSS and RSAL treatments.

[3]Only fed to RSAL and ALG treatments.

[4]Expressed in g/kg of DM, the same mineral-vitamin supplement was used across the experiment; therefore, the values are constant.

[5]Expressed in mg/kg of DM.

Table 2. Chemical composition (g/kg of DM) of experimental feed[1]

Item	Silage	Cereal-sugar beet pulp	Molassed sugar beet pulp	Molasses	Mineral-vitamin supplement	Rapeseed supplement	*Spirulina platensis*
DM (g/kg)	288	899	878	710	992	866	946
Ash	81.7	31.6	67.8	103	918	66.1	71.7
CP	133	119	113	106		311	697
Crude fat		48.4	2.93			41.5	51.3
NDF[2]	480	363	338			272	0
Starch							66.2

[1]Blank entries were not analyzed.

[2]Results of silage analyzed without heat stable amylase and expressed inclusive of residual ash (NDF); results of concentrate components analyzed with heat-stable amylase and expressed inclusive of residual ash (aNDF).

previous publications (Cope et al., 2009; McCaughern et al., 2020). In brief, approximately 0.5 g of previously dried and milled sample or liquid was digested with 1 mL of concentrated trace element-grade HCl (Fisher Scientific) and 6 mL of concentrated trace element-grade nitric acid (HNO_3; Fisher Scientific) in DigiPREP tubes, and placed in a DigiPREP heating block (QMX Laboratories Ltd.). The temperature of the heating block was increased in a stepwise fashion to 100°C and maintained at this temperature for 45 min. Digested samples were then diluted to 50 mL using type-1 water. Once cooled, digested samples were then diluted 1:20 using an acidic diluent consisting of 0.50% trace element-grade HNO_3 (Fisher Scientific), 2.00% HPLC-grade methanol (Sigma-Aldrich), and 0.05% Triton X-100 (Fisher Scientific) in type-1 water. Concentrations of I in heparinized plasma samples were quantified according to previously published methods of human serum analysis by Yu et al. (2018), which, in brief, involved digestion of the composite samples with aqueous ammonia, isopropanol, and ultrapure water, and utilizing a rhenium internal standard for accuracy. Concentrations of all other plasma sample minerals analyzed used methods by McCaughern et al. (2020). Briefly, plasma samples were diluted 1:50 in an acidic diluent, which contained 0.50% concentrated HNO_3 (Fisher Scientific), 2.00% HPLC-grade methanol (Sigma-Aldrich), and 0.05% Triton X-100 (Fisher Scientific) in type-1 water.

Statistical Analysis

ANOVA was performed by linear mixed effects model in Minitab 18 (Minitab, 2019). Diet, period within square, square, and their interaction were used as fixed factors, and cow (experimental unit) within square as a random factor. Normality of residuals were evaluated visually, and no data showed deviation from normality. Tukey's least significance difference test ($P < 0.05$) was used for pairwise comparison of the means, where the mixed effects model showed a significant effect of diet or period. Mineral transfer efficiencies from feed to milk were calculated as follows: [100 × (milk mineral concentration, μg/kg) × milk output (kg/d)]/[(diet mineral concentration, μg/kg of DM) × DMI (kg/d)]. Apparent digestibility from feed to feces were calculated as follows: 100 − [(100 × (feces mineral concentration, μg/kg) × feces output (kg/d)]/[(diet mineral concentration, μg/kg of DM) × (DMI, kg/d)]. Acid insoluble ash was used as a marker to calculate fecal output.

RESULTS

Milk Mineral Concentrations and Transfer Efficiencies

We found a significant effect of dietary treatment on the concentrations of I in milk ($P < 0.001$; Table 4), with average CON and ALG milk both containing 273% more than RSS milk and 98% more than RSAL milk. Transfer efficiency of Ca ($P = 0.020$), Mg ($P = 0.003$), P ($P < 0.001$), Na ($P = 0.018$), and I ($P < 0.001$) from feed to milk were influenced by dietary treatment (Table 4). Transfer efficiency of Ca, Mg, and P was highest in CON, lowest in RSS, and had intermediate values in ALG and RSAL, although not all differences were statistically significant. The Na transfer efficiency was higher for CON than in ALG. Transfer efficiency of I was higher in CON and ALG than in RSS and RSAL.

Fecal Mineral Concentrations and Apparent Digestibility

Dietary treatments influenced fecal concentrations of Ca ($P < 0.001$), Mg ($P < 0.001$), P ($P < 0.001$), S ($P = 0.027$), Co ($P < 0.001$), Cu ($P < 0.001$), Fe ($P < 0.001$), I ($P < 0.001$), Mn ($P < 0.001$), Mo ($P = 0.008$), and Zn ($P < 0.001$; Table 5). Fecal concentrations of Ca, Mg, S, Mn, and Zn were highest in RSAL cows, lowest in CON, and intermediate in feces from RSS and ALG cows; in some cases, differences were not statistically significant. The group fed RSAL had

Table 3. Mineral concentrations of experimental diets, including the average (AVG), SD, and range for mineral concentrations of feed and mineral intakes, per experimental treatment[1]

	CON			RSS			RSAL			ALG		
Item	AVG	SD	Range	AVG	SD	Range	AVG	SD	Range	AVG	SD	Range
Macromineral concentration of experimental diet (g/kg of DM)												
Calcium	7.1	0.16	6.9–7.3	7.8	0.20	7.6–8.2	7.6	0.21	7.2–7.9	7.3	0.20	6.9–7.5
Magnesium	2.4	0.05	2.3–2.5	2.8	0.06	2.7–2.9	2.7	0.08	2.6–2.8	2.5	0.06	2.4–2.6
Phosphorus	2.5	0.04	2.5–2.6	3.3	0.05	3.2–3.3	3.1	0.10	3.0–3.3	2.9	0.06	2.8–3.0
Potassium	18.2	0.81	17.2–19.2	19.5	0.96	18.4–21.0	19.0	1.28	16.8–20.7	18.7	1.32	16.5–20.2
Sodium	1.3	0.06	1.3–1.5	1.3	0.06	1.3–1.4	1.6	0.14	1.4–1.9	1.8	0.10	1.6–1.9
Sulfur	2.7	0.17	2.5–3.0	3.3	0.14	3.2–3.5	3.2	0.18	3.0–3.5	3.0	0.22	2.8–3.4
Trace element concentration of experimental diet (mg/kg of DM unless otherwise indicated)												
Cobalt[2]	318	15.5	295–349	322	14.2	304–351	332	26.9	308–388	337	20.7	312–378
Copper	10.3	0.65	9.6–11.4	10.1	0.54	9.5–11.0	10.2	0.92	9.3–12.2	10.1	0.85	9.3–11.4
Iron	318	8.7	308–332	327	9.6	316–341	343	8.9	332–355	360	8.3	348–370
Iodine	1.06	0.045	1.01–1.15	1.04	0.047	0.97–1.11	1.10	0.094	1.01–1.31	1.15	0.076	1.06–1.28
Manganese	37.4	1.06	35.5–38.9	41.9	0.91	40.4–42.9	40.7	1.45	38.4–42.4	39.1	2.18	37.5–43.4
Zinc	51.3	1.50	49.6–54.4	53.8	0.96	52.1–54.9	52.5	2.33	50.1–57.1	50.7	1.43	48.2–52.4
Macromineral intake from experimental diet (g/d)												
Calcium	162	8.0	147–174	183	7.7	172–194	175	12.9	149–189	166	10.1	153–184
Magnesium	54.8	2.28	50.4–57.0	64.9	2.98	60.9–68.3	61.0	4.10	52.4–64.5	57.1	2.97	53.4–61.2
Phosphorus	57.7	2.56	52.7–61.3	76.3	3.03	71.6–81.0	71.0	4.29	62.2–75.7	66.0	3.67	60.0–71.6
Potassium	416	33.3	363–458	456	42.8	406–530	440	63.2	315–520	430	58.0	336–502
Sodium	30.6	0.24	30.3–31.0	31.3	0.21	31.1–31.7	35.9	0.43	35.3–36.5	40.3	0.75	39.5–41.5
Sulfur	60.7	4.09	55.7–69.8	77.5	6.63	69.6–89.1	73.1	7.99	57.5–86.6	68.9	8.64	59.9–82.8
Trace element intake from experimental diet (mg/d)												
Cobalt	7.2	0.15	7.0–7.4	7.5	0.22	7.2–7.7	7.6	0.21	7.3–7.8	7.7	0.18	7.4–7.9
Copper	235	16.5	211–261	237	9.6	218–246	234	10.5	215–250	230	10.0	217–245
Iron	7,252	331.1	6,793–7,803	7,643	551.3	6,788–8,596	7,897	679.5	6,500–8,914	8,215	590.4	7,318–9,103
Iodine	24.1	0.15	23.7–24.2	24.2	0.27	23.9–24.6	25.2	0.36	24.5–25.6	26.1	0.21	25.9–26.5
Manganese	852	34.2	802–886	981	54.7	906–1,057	935	73.6	784–1,005	892	57.5	805–966
Molybdenum	9.9	1.43	7.4–11.4	11.1	1.25	9.4–12.7	10.4	1.97	6.7–12.3	9.7	1.39	8.1–12.1
Zinc	1,169	31.4	1,123–1,220	1,258	53.5	1,182–1,349	1,205	64.3	1,074–1,301	1,157	57.0	1,064–1,241

[1]CON= control, no microalgae; RSS = 2,550 g/d of rapeseed supplement; RSAL = 1,280 g/d of rapeseed supplement + 570 g/d of *Spirulina platensis* supplement; ALG = 1,130 g/d of *S. platensis* supplement.

[2]Expressed in µg/kg of DM.

Table 4. Mineral composition of milk, including means, SE, and ANOVA P-values for the effect of the dietary treatment[1]

Mineral	Diet: CON (n = 8)	RSS (n = 8)	RSAL (n = 8)	ALG (n = 8)	SE	ANOVA P-value[2]: Diet	Period
Macromineral (mg/kg)							
Calcium	1,229	1,139	1,178	1,173	43.9	0.287	0.001
Magnesium	123.7	115.3	118.3	119.7	4.68	0.342	<0.001
Phosphorus	957.5	919.7	936.1	935.3	28.85	0.713	<0.001
Potassium	1,510	1,468	1,471	1,508	36.2	0.758	<0.001
Sodium	597.1	531.4	601.3	576.3	37.46	0.383	0.009
Sulfur	667.3	650.1	669.2	667.6	48.87	0.991	0.136
Trace element (μg/kg)							
Copper	119.0	261.4	186.8	209.8	98.02	0.780	0.526
Iron	852.3	942.3	756.7	1,213.7	163.53	0.257	0.303
Iodine	140.3[a]	37.6[c]	70.7[b]	140.2[a]	22.06	<0.001	0.101
Manganese	70.3	51.4	50.4	58.0	9.19	0.413	0.067
Zinc	4,275	3,976	4,197	4,286	219.4	0.452	0.002
Macromineral transfer efficiency (g in milk/100 g ingested)							
Calcium	20.3[a]	17.5[b]	18.7[ab]	19.4[ab]	1.04	0.020	<0.001
Magnesium	6.0[a]	5.0[c]	5.3[bc]	5.7[ab]	0.20	0.003	<0.001
Phosphorus	44.4[a]	33.9[c]	36.3[bc]	38.7[b]	1.62	<0.001	<0.001
Potassium	9.8	9.0	9.1	9.7	0.38	0.137	<0.001
Sodium	51.7[a]	47.1[ab]	45.1[ab]	38.4[b]	2.70	0.018	0.002
Sulfur	29.6	23.3	24.7	26.4	2.01	0.143	0.071
Trace element transfer efficiency (g in milk/100 g ingested)							
Copper	1.3	3.2	2.2	2.5	1.16	0.700	0.466
Iron	0.32	0.34	0.27	0.40	0.055	0.414	0.153
Iodine	15.5[a]	4.3[b]	7.2[b]	14.2[a]	2.17	<0.001	0.032
Manganese	0.22	0.15	0.15	0.18	0.027	0.194	0.019
Zinc	9.8	8.9	9.6	10.1	0.51	0.227	<0.001

[a–c]Means for diet treatment within a row with different letters are significantly different, according to Fisher's least significant difference test ($P < 0.05$).

[1]CON= control, no microalgae; RSS = 2,550 g/d rapeseed supplement; RSAL = 1,280 g/d rapeseed supplement + 570 g/d *Spirulina platensis* supplement; ALG = 1,130 g/d *S. platensis* supplement.

[2]Significances were declared at $P < 0.05$.

feces that contained 36, 18, 21, 24, and 17% more Ca, Mg, S, Mn, and Zn, respectively, than CON. Fecal P concentrations were higher for RSS and RSAL than for CON and ALG, with average RSS and RSAL feces containing 24% more P than average CON and ALG. Cows fed RSAL and ALG excreted feces with more Co, Cu, and I than CON and RSS cows. The RSAL and ALG groups had feces that contained 50, 21, and 47% more Co, Cu, and I, respectively, than average CON and RSS feces. Fecal concentrations of Mo were highest in RSS and lowest in CON, with intermediate values in RSAL and ALG. Cows fed ALG had feces that contained 6, 24, and 33% more Fe than RSAL, RSS, and CON, respectively, and we detected an increasing Fe content as CON < RSS < RSAL < ALG, with all differences significant. We found a significant effect of dietary treatment on the apparent digestibility of Ca ($P < 0.001$), Mg ($P < 0.001$), Co ($P < 0.001$), Cu ($P = 0.001$), Fe ($P < 0.001$), I ($P = 0.001$), Mn ($P = 0.010$), and Zn ($P = 0.001$) in feces (Table 5). Apparent digestibility of all these minerals (except Mg) was higher in treatments fed microalgae (RSAL, ALG) compared with those that were not (CON, RSS). Magnesium apparent digestibility was highest for RSAL, lowest for CON, and had intermediate values for RSS and ALG; however, the difference between ALG with RSS and RSAL was not statistically significant.

Plasma Mineral Concentrations

The effect of dietary treatment did not influence the concentrations of any of the assessed minerals in blood plasma (Table 6).

DISCUSSION

Effect of Protein Supplementation on Milk Mineral Concentrations and Mineral Transfer Efficiencies from Feed to Milk

Milk I concentrations were significantly lowered when fed rapeseed (RSS, RSAL) as opposed to diets that did not contain rapeseed (CON, ALG). Given that we found no difference between CON and ALG milks, it

Table 5. Mineral composition of feces, including means, SE, and ANOVA P-values for the effect of the dietary treatment[1]

Mineral	Diet				SE	ANOVA P-value[2]	
	CON (n = 8)	RSS (n = 8)	RSAL (n = 8)	ALG (n = 8)		Diet	Period
Macromineral (g/kg DM)							
Calcium	9.91[c]	11.64[b]	13.51[a]	13.10[ab]	0.539	<0.001	0.006
Magnesium	3.73[c]	4.95[ab]	5.23[a]	4.73[b]	0.080	<0.001	0.005
Phosphorus	4.61[b]	6.42[a]	5.81[a]	5.24[b]	0.221	<0.001	0.499
Potassium	9.47	9.61	8.04	8.12	0.792	0.275	0.837
Sodium	1.91	1.55	2.18	2.16	0.377	0.342	0.412
Sulfur	3.36[b]	3.86[ab]	4.05[a]	3.89[ab]	0.136	0.027	0.020
Trace element (μg/kg DM unless otherwise indicated)							
Cobalt	470.4[b]	489.6[b]	738.9[a]	709.0[a]	39.72	<0.001	0.010
Copper[3]	22.3[b]	22.5[b]	27.1[a]	27.2[a]	0.94	<0.001	0.039
Iron[3]	665.0[d]	710.4[c]	831.1[b]	882.5[a]	15.65	<0.001	0.033
Iodine	413.2[b]	402.4[b]	592.5[a]	612.6[a]	29.21	<0.001	0.045
Manganese[3]	81.8[c]	96.1[b]	101.8[a]	96.7[b]	2.50	<0.001	0.130
Molybdenum	1,312[b]	1,566[a]	1,470[ab]	1,487[ab]	63.6	0.008	<0.001
Zinc[3]	97.2[c]	105.5[b]	115.0[a]	108.7[b]	2.61	<0.001	<0.001
Macromineral apparent digestibility [100 − (g in feces/100 g ingested)]							
Calcium	49.4[a]	48.2[a]	37.2[b]	37.0[b]	1.97	<0.001	0.007
Magnesium	43.7[a]	37.9[ab]	30.3[c]	34.0[bc]	1.93	<0.001	0.012
Phosphorus	33.7	32.1	34.0	37.7	2.64	0.335	0.827
Potassium	80.9	82.9	85.1	84.9	1.42	0.167	0.904
Sodium	48.2	60.9	50.4	58.0	8.94	0.437	0.422
Sulfur	53.9	59.2	54.6	54.7	1.99	0.247	0.001
Trace element apparent digestibility [100 − (g in feces/100 g ingested)]							
Cobalt	46.5[a]	46.8[a]	21.4[b]	26.1[b]	3.85	<0.001	0.006
Copper	21.3[a]	22.5[a]	5.6[b]	5.2[b]	3.27	0.001	0.002
Iron	23.9[a]	24.0[a]	14.3[b]	14.3[b]	1.46	<0.001	<0.001
Iodine	85.8[a]	86.4[a]	80.8[b]	81.2[b]	1.02	0.001	0.018
Manganese	20.6[a]	20.1[a]	11.6[b]	13.7[ab]	2.04	0.010	0.177
Zinc	31.1[a]	31.6[a]	22.6[b]	25.1[b]	1.51	0.001	<0.001

[a–c]Means for diet treatment within a row with different letters are significantly different, according to Fisher's least significant difference test ($P < 0.05$).

[1]CON= control, no microalgae; RSS = 2,550 g/d rapeseed supplement; RSAL = 1,280 g/d rapeseed supplement + 570 g/d *Spirulina platensis* supplement; ALG = 1,130 g/d *S. platensis* supplement.

[2]Significances were declared at $P < 0.05$.

[3]Expressed in mg/kg of DM.

appears that the addition of microalgae did not affect I concentration. Rather, the main driver of I concentration in milk was the amount of rapeseed in the cows' diet. This appears to be a dose-dependent response, as we detected 37.6 μg/kg of I in RSS milk (from cows fed 2.6 kg of DM per day of rapeseed), and 70.7 μg/kg of I in RSAL milk (from cows fed 1.3 kg of DM per day of rapeseed). This decrease in milk I concentration with increasing rapeseed inclusion may be due to the glucosinolates found in rapeseed. Glucosinolates interfere with I metabolism in cows, causing I to be diverted to the kidneys rather than the mammary gland (Křížová et al., 2016). This is further reinforced by previous work (Franke et al., 2009) that reported a decrease in milk I concentration by half when rapeseed meal replaced distillers dried grains with solubles in cows' diets. The potential diversion of I toward the kidneys (rather than the mammary gland) is further supported by I transfer efficiency from feed to milk in the present study; for instance, 14.2 and 15.5% of dietary I appeared in milk for ALG and CON treatments, respectively, whereas we detected a numerical dose-dependent response with only 4.3 and 7.2% of dietary I appearing in milk for RSS and RSAL treatments, respectively. Although milk I concentrations can vary widely based on dairy management practices, breed, teat dipping solutions, and diet composition (Flachowsky et al., 2014), the concentrations of 70.7 μg/kg, and furthermore 37.6 μg/kg (from cows fed rapeseed), are below the previously documented average I concentration of 150 μg/L in Finnish cow milk (Nyström et al., 2016). However, milk from the cows that did not consume rapeseed had similar values to those previously reported (Flachowsky et al., 2014; Nyström et al., 2016; Qin et al., 2021). Generally, it is recommended that when rapeseed is fed to dairy cows, additional I supplementation is to be

Table 6. Mineral composition of blood plasma, including the means, SE, and ANOVA P-values for the effect of the dietary treatment[1]

Mineral	Diet				SE	ANOVA P-value[2]	
	CON (n = 8)	RSS (n = 8)	RSAL (n = 8)	ALG (n = 8)		Diet	Period
Macromineral (mg/L)							
Calcium	114.1	122.5	117.6	117.2	3.84	0.486	0.300
Magnesium	25.5	27.4	25.9	25.6	0.90	0.270	0.264
Phosphorus	195.6	214.3	203.2	197.4	11.20	0.499	0.434
Potassium	215.4	240.5	218.5	219.2	8.34	0.127	0.421
Sodium	3,940	4,271	3,999	3,993	147.7	0.388	0.299
Sulfur	1,390	1,538	1,435	1,438	56.4	0.338	0.222
Trace element (μg/L)							
Copper	903.4	961.6	920.9	962.5	70.54	0.711	0.630
Iron	2,038	2,010	1,891	1,949	163.6	0.883	0.459
Iodine	78.4	92.8	113.1	95.3	9.12	0.087	0.025
Manganese	2.74	2.30	3.40	2.75	0.441	0.387	0.117
Selenium	80.5	90.1	86.9	87.4	4.06	0.380	0.418
Zinc	834.0	871.7	876.9	857.0	49.41	0.812	0.634

[1]CON= control, no microalgae; RSS = 2,550 g/d rapeseed supplement; RSAL = 1,280 g/d rapeseed supplement + 570 g/d *Spirulina platensis* supplement; ALG = 1,130 g/d *S. platensis* supplement.

[2]Significance was declared at $P < 0.05$.

provided, to reduce the risk of deteriorating milk nutritional quality by lowering I concentration (Flachowsky et al., 2014). In the present study, administration of I via the mineral-vitamin supplement was 70 mg/kg of DM (Table 1), and the whole diet of all experimental treatments contained approximately 1.0 to 1.1 mg of I per kg of DM (Table 3). Given that the European Food Safety Authority has maintained an upper level of I inclusion in dairy cows' diets of 5 mg of I per kilogram of feed DM (EFSA, 2013), the I supply in the present study was approximately 20% of the maximum recommendation, thus further contributing to the low I concentration of milk of RSS and RSAL in addition to the effect of rapeseed.

Other than I, we found no other statistically significant effects of diet on milk mineral concentrations, and, to the best of our knowledge, no other published studies have investigated the effect of feeding *S. platensis* to dairy cattle on milk mineral concentrations. In addition, this is the first study to present the effect of feeding rapeseed to dairy cattle on the wider milk mineral profile. Previous work has assessed the effect of feeding fava bean and rapeseed meal on the concentrations of Ca, P, and Mg in skim milk, and found these were not affected by their inclusion in the diet (Poulsen et al., 2021), which aligns with the effect of rapeseed and microalgae in the present study.

Effect of Protein Supplementation on Fecal Mineral Concentrations and Apparent Digestibility

We detected a significant effect of dietary microalgae on fecal I concentration, as RSAL and ALG treatments had increased fecal I compared with the CON and RSS treatments. This, however, did not appear to be a dose-dependent response, as there was only a 3.4% difference in fecal I concentration between the 2 treatments fed microalgae (RSAL and ALG), whereas the algae inclusion in the diet was 98% higher in the ALG than RSAL treatment. However, the extent of the difference on excretion, from 413.2 μg/kg of DM to 612.6 μg/kg of DM fecal I, may not be explained from the 2,000 μg/d difference in I intake between CON and ALG, and may also relate to a reduced apparent digestibility of I (81% in CON, 86% in ALG). A possible explanation that further adds to the reduced I apparent digestibility when algae is fed is that the I accumulation takes place in a form that is not as readily bioavailable in the gut and, therefore, a larger proportion of it ends up undigested in feces (Iwamoto and Shiraiwa, 2012; Han et al., 2016). Fecal I output in cattle could be higher with high I intakes and when I is already sufficient in the diet (Hemken, 1970; Franke, 2009).

Interestingly, the same pattern has been observed for several macrominerals (Ca, Mg) and trace elements (Co, Cu, Fe, Mn, Zn), where apparent digestibility was lower when cows were fed microalgae. Microalgal interactions with dietary minerals have been previously suggested for *S. platensis* in cows' diets, which resulted in reduced plasma Fe concentration due to a possible chelation with phycocyanin, a pigment-protein complex found in *Spirulina* (Bermejo et al., 2008; Suliburska et al., 2016). However, this reduction in plasma Fe concentration was not found, and increased excretion in macrominerals in the current trial did not influence the transfer into milk and subsequently milk nutritional quality.

Effect of Protein Supplementation on Mineral Concentrations of Bovine Plasma

The present study indicated that feed protein source (rapeseed, rapeseed and microalgae mix, and microalgae) had no significant effect on cow blood mineral concentrations. Despite the significant effect on milk I concentrations, we found only a tendency ($P = 0.087$) for reduced plasma I concentrations (average 78.4 to 113.1 μg/L) when rapeseed was fed. Research on the effect of diet on total plasma I concentration is scarce, but it has been reported that total plasma I can be affected by several factors such as blood composition, BW, breed, analysis method, and timing of parturition (Tong et al., 1986; Dillon et al., 2003). Previous studies (Sorge et al., 2016) reported a range of 305 to 311 μg/L in cow serum I concentration when TMR was fed, values which are substantially higher than those observed in the present study. This may be due to their use of a TMR of various kinds of hay, corn, and cottonseed (*Gossypium hirsutum*), which resulted in a dietary I concentration of 2.17 g/kg of DM, which is double the average I concentration (1.09 g/kg of DM) of all experimental diets in the present study. In the same previous study (Sorge et al., 2016), the mean dietary I intake was 62.5 mg/d for the cows in the control group, whereas cows in the present study ingested, on average, 24.8 mg/d across all experimental treatments. Overall, it could be concluded that rapeseed, microalgae supplementation, or both in dairy cow diets does not affect blood plasma mineral concentrations, including that of I.

Nutritional Implications of Milk from Microalgae-Fed Cows for Consumers

According to the Finnish Institute for Health and Welfare National FinDiet 2017 Survey (Kaartinen et al., 2020) the intakes of all types of liquid milk by men in the Finnish population were 319, 233, and 193 g/d in the age groups of 18 to 44, 45 to 64, and 65 to 74 years, respectively; additionally, intake by women was 183, 165, and 145 g/d in the age groups of 18 to 44, 45 to 64, and 65 to 74 years, respectively. The 2012 Nordic Nutritional Recommendations present a recommended nutritional intake (**RNI**) for I as 150, 175, and 200 μg/d for adults (18+ years of age), pregnant women, and nursing women, respectively (NNR, 2014). Therefore, based on the recorded liquid milk intakes from the FinDiet 2017 Survey and the milk I concentrations in the present study, CON and ALG milk would contribute (expressed as % RNI) (1) 30, 22, and 18% in men of age groups 18 to 44, 45 to 64, and 65 to 74 years, respectively, (2) 17, 15, and 14% in women of age groups 18 to 44, 45 to 64, and 65 to 74 years, respectively, (3) 15% in pregnant women, and (4) 13% in nursing women. In addition, even for the demographic with the highest milk intake, contribution of the experimental milk to the I upper tolerable limit (600 μg/d; NNR, 2014) if consumed would be marginal; someone would need to ingest substantially large amounts of CON or ALG milk (approximately 4.17 L per day) to reach the upper tolerable limit, and hence there are no public health safety concerns around excessive I consumption via milk from cows consuming microalgae.

Previous work in Finland (Nyström et al., 2016) reported that milk is a major source of I in the Finnish population; the adult population of Finland has also been classified as mildly I deficient. Previous work in Finland has suggested that goiter issues were frequent due to I deficiency 50 years ago, and iodized table salt and cattle feed (for increased milk I) has been used to attempt to remedy this issue (Elorinne et al., 2016). However, CON and ALG milk, produced in the present study, would provide less than 45 μg per day and contribute less than 30% of the recommended I intake (under the current milk intake levels). If such levels also appear within retail products, strategies to enhance milk I concentrations could be recommended, and the intake of I-rich foods could be increased (such as more milk and dairy, certain fish and seafood, or I-fortified foods; Bouga et al., 2018), with special considerations for pregnant and nursing women, as this could reduce the occurrence of I deficiency in the Finnish population.

As previously discussed, the feeding of rapeseed to cows decreases milk concentrations of I, as we found a significant difference between milk from cows fed rapeseed (RSAL, RSS) or diets containing no rapeseed (CON, ALG). By performing similar calculations (as above) regarding the contribution of RSAL and RSS milks to the RNI for I in the Finnish population, this would be (expressed as % RNI) (1) 9 to 15% along with 5 to 8% in men ages 18 to 44, 45 to 64, and 65 to 74, respectively, (2) 7 to 9% along with 4 to 5% in women ages 18 to 44, 45 to 64, and 65 to 74, respectively, (3) 7 and 4% in pregnant women, respectively, and (4) 6 and 3% in nursing mothers, respectively. Therefore, given that these values are substantially lower compared with milk from cows not consuming rapeseed, and that milk I concentration is strongly correlated with dietary I intake (Flachowsky et al., 2014), supplementing additional I in cow diets that contain rapeseed can be recommended as a potential way to mitigate the negative effect of rapeseed on milk I concentrations and the undesirable implications for consumer I intake.

CONCLUSIONS

Microalgal (*Spirulina platensis*) inclusion in dairy cow diets did not affect milk and plasma concentrations of macrominerals and trace elements. Rapeseed inclusion in cow diets did not affect blood plasma mineral concentrations but reduced milk I concentrations, an effect which may be associated with glucosinolates, which are known to reduce dietary I availability to the mammary gland. Based on Finnish population milk intakes and nutritional guidelines, the contribution of milk for I supply of the different population demographics could be reduced by approximately 25 to 50% when rapeseed partially or wholly substitutes other protein feeds. This may increase the need for higher I supply from either consuming more milk and dairy, or increasing the intake of other diet sources (fish, shellfish, I-fortified foods), especially in consumers with higher I requirements (pregnant and nursing women). This study showed that feeding microalgae to dairy cows maintained mineral concentrations in cows' blood and the milk produced. However, supplemental dietary I is recommended when rapeseed is fed to dairy cows, to prevent subsequent production of milk with lower I concentrations.

ACKNOWLEDGMENTS

This work was funded by the European Regional Development Fund and Raisioagro Ltd. (Raisio, Finland) and conducted in cooperation with Cursor Ltd. (Kotka, Finland). The authors acknowledge technical assistance by Juha Suomi and thank the farm staff at the University of Helsinki (Helsinki, Finland) for animal management and data collection. Special thanks to all laboratory personnel for feed, milk, feces, and blood plasma analyses at the University of Reading (Reading, UK), Harper Adams University (Newport, UK), and the University of Helsinki. Author contributions were as follows: Eric E. Newton: conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing—original draft, writing—review and editing, visualization; Marjukka Lamminen: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing—review and editing, project administration; Partha Ray: writing—review and editing, methodology, supervision; Alexander M. Mackenzie: methodology, validation, formal analysis, investigation, resources, data curation, writing—review and editing; Christopher K. Reynolds: writing—review and editing, supervision; Michael R. F. Lee: resources, writing—review and editing, project administration; Anni Halmemies-Beauchet-Filleau: resources, methodology, writing—review and editing, project administration; Aila Vanhatalo: conceptualization, methodology, resources, writing—original draft, writing—review and editing, supervision, project administration, funding acquisition; Sokratis Stergiadis: conceptualization, methodology, resources, data curation, writing—original draft, writing—review and editing, supervision, project administration. All authors reviewed and approved the manuscript. The dataset supporting the conclusions of this article is available on request from the corresponding authors. The authors have not stated any conflicts of interest.

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ORCIDS

Eric E. Newton https://orcid.org/0000-0002-5784-658X
Marjukka Lamminen https://orcid.org/0000-0002-2300-8887
Partha Ray https://orcid.org/0000-0001-8375-8279
Alexander M. Mackenzie https://orcid.org/0000-0002-1308-8529
Christopher K. Reynolds https://orcid.org/0000-0002-4152-1190
Michael R. F. Lee https://orcid.org/0000-0001-7451-5611
Anni Halmemies-Beauchet-Filleau https://orcid.org/0000-0001-6901-1400
Aila Vanhatalo https://orcid.org/0000-0003-0288-8237
Sokratis Stergiadis https://orcid.org/0000-0002-7293-182X