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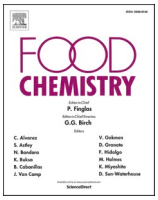

# Mineral concentrations in milk from cows fed seaweed (*Saccharina latissima*) under different basal protein supplementation

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ABSTRACT

Sixteen multiparous Holstein cows in four blocks of 4 × 4 Latin square over 4-week experimental periods were used to study the effects of seaweed (*Saccharina latissima*) supplement (with/without) and protein source (rapeseed meal (RSM)/wheat distiller's grain (WDG)) on milk mineral concentrations. Dietary treatments did not affect milk production and basic composition. Feeding seaweed slightly decreased milk Ca and Cu concentrations; whilst increased (by 3.3-fold) milk iodine (I) concentration, due to a higher dietary I supply. Substitution of WDG with RSM increased feed-to-milk transfer of Ca, Na, and Se and decreased that of Mg, P, Fe, and Mn; but only reduced milk Mn and I concentrations (the latter by 27 % as a potential result of increased glucosinolate intake). Seaweed supplement can improve milk I content when cows' I supply/availability is limited, but care should be taken to avoid excess milk I contents that may pose nutritional risks for young children.

## 1. Introduction

Cow milk is an important source of various minerals for human requirements (NDNS, 2020; Pennington, Wilson, Young, Johnson, & Vanderveen, 1987). Earlier studies reported that intake of 200 mL liquid milk per day provides about 25 % of Ca, 20 % of P, 8 % of K, 50 % of I, and 11 % of Mg relative to an adult's recommended nutrient intake (RNI) (Pennington et al., 1987). A recent study in the UK showed that approximately 37 % of I, 38 % of Ca, 24 % of P, 17 % of Zn, 13 % K, 11 % of Mg, and 7 % of Se in the diets of the adult population come from milk and dairy products; while these contributions are even higher in toddlers and children (NDNS, 2020). The mineral content of cow milk, however, is influenced by various factors, including cows' genetics, stage of lactation, milk solids composition, and intake and bioavailability of minerals in the diet (Flynn, 1992). Animals' diet is an effective pathway to improve the mineral profile of milk so that it delivers more minerals when consumed by humans, as positive correlations have been reported between concentrations of specific trace elements in milk, including I and Se, and their intakes by animals (Zwierzchowski & Ametaj, 2018). In contrast, the inclusion of feed ingredients containing anti-nutritive factors (i.e. phytates in legumes and cereals) or antagonists of minerals (i.e. glucosinolates in rapeseed) reduces the biological availability of specific minerals, and thus reduces the concentrations in milk (Samtiya, Aluko, & Dhewa, 2020). Reducing the use of such feed ingredients helps the improvement of milk's nutritional value with respect to mineral content.

Seaweeds are novel animal feed ingredients that have attracted increasing attention due to their high concentrations of specific macrominerals and trace elements (Makkar et al., 2016), providing them with an opportunity to be used as mineral supplements to livestock diets. Supplementation of specific brown seaweed species to dairy cows, represented by *Ascophyllum nodosum* and *Laminaria digitata*, has been reported to modify milk mineral concentrations (Newton et al., 2021), and a number of studies have shown that milk I concentration increased to

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more than 2-fold of the original level when cows were fed 100 to 200 g/d dried seaweed powder (Antaya, Ghelichkhan, Pereira, Soder, & Brito, 2019; Chaves Lopez et al., 2016; Newton et al., 2021). Given these results and the increasing demand for seaweeds as feed ingredients bring the necessity of screening more seaweed species as potential mineral supplements for dairy cows. *Saccharina latissima*, also known as sugar kelp, is a brown seaweed species considered a good source of I, Cu, Fe, Mn, and Se (Samarasinghe, van der Heide et al., 2021). Intake of *S. latissima* has been reported to influence the innate immune response of preweaning dairy calves by increasing the serum concentrations of acute-phase proteins (Samarasinghe, Sehested, Weisbjerg, Vestergaard, & Hernández-Castellano, 2021), while its effect on milk mineral concentrations has been seldomly reported. Considering the large between-species variation in chemical and mineral composition of brown seaweeds (Makkar et al., 2016), further investigation is needed to quantify the effect of feeding *S. latissima* on milk macrominerals and trace elements.

Rapeseed meal is the by-product of the extraction of oil from rapeseed, widely used as a protein source for dairy cows in temperate zones (Bell, 1984). Despite the high protein content, the inclusion of rapeseed meal in the diet is limited by the presence of anti-nutritive factors, namely erucic acid and glucosinolates (Bell, 1984). Since the 1960s, the cultivation of low-erucic, low-glucosinolate varieties has largely improved the nutritional value of rapeseed and its by-products as animal feed ingredients (Bell, 1984). However, even though the level of anti-nutritive factors has been reduced largely, the remaining presence of glucosinolates in rapeseed meal produced from the new varieties has still a negative impact on the nutritional value of milk (Emanuelson, Ahlin, & Wiktorsson, 1993; Papas, Ingalls, & Campbell, 1979; Trøan, Pihlava, Brandt-Kjelsen, Salbu, & Prestløkken, 2018). The most often reported adverse effect of rapeseed meal is the reduction of milk I content (Franke, Meyer, & Flachowsky, 2009; Franke, Meyer, Wagner, & Flachowsky, 2009; Trøan et al., 2018), as glucosinolates and their degradation compounds interfere with the normal functions of the thyroid and the transfer of I from feed to milk (Papas et al., 1979). Moreover, it has been reported that rapeseed meal has lower biological availability of other macrominerals and trace elements when compared with other commonly used protein sources (i.e. soybean meal, cottonseed meal, wheat) based on studies in broiler chicken (Liu et al., 2019; Nwokolo & Bragg, 1980). However, the effect of rapeseed meal intake on milk concentrations of other minerals, beyond I, has not been assessed in dairy cows.

The present study aimed to (i) investigate the effects of *Saccharina latissima* supplement and the inclusion of rapeseed meal or wheat distiller's grain as the main protein source in dairy cows' diet on milk macrominerals and trace elements concentrations (ii) quantify the feed-to-milk transfer efficiencies of macrominerals and trace elements in the same diets, and (iii) assess the impact of the consumption of milk produced with or without seaweed supplement and from different main protein sources on consumers' intake of macromineral and trace elements. It was hypothesized that (i) the seaweed supplement may increase concentrations of specific minerals (such as Ca, I, and Se) in milk; (ii) inclusion of rapeseed meal in the diet may decrease milk concentration and feed-to-milk transfer efficiency of I and possibly other minerals; (iii) consumers' I intake may be considerably increased by the consumption of milk produced with seaweed supplement, whilst it may be decreased by the consumption of milk produced from rapeseed meal.

## 2. Materials and methods

### 2.1. Animals, diets, and experimental design

All regulated experimental procedures used were authorized through licensing following ethical review by the UK Home Office under the Animals (Scientific Procedures) Act, 1996. Sixteen multiparous Holstein cows (average milk yield 35 kg/d) were allocated in four blocks of 4 × 4 Latin square change-over design with four 4-week experimental periods (details of the design are shown in the Supplementary Material, Table S1). The four diet treatments were (i) basal diet based on wheat distiller's grains (WDG) as the main protein source and without seaweed supplementation (C-WDG); (ii) basal diet based on WDG as the main protein source with dried seaweed supplementation (S-WDG; *S. latissima* 35.7 g/cow/d on DM basis); (iii) basal diet based on rapeseed meal (RSM) as the main protein source and without seaweed supplementation (C-RSM); (iv) basal diet based on RSM as the main protein source with dried seaweed supplementation (S-RSM; *S. latissima* 35.7 g/cow/d on DM basis). Each experimental period consisted of a 7-d washout period and a 21-d feeding period. During the washout period, C-WDG diet was provided before cows were allocated to their experimental diets for the next 21-d feeding period.

All experimental diets had a forage to concentrate ratio of approximately 75:25 on dry matter (DM) basis. The forage part of the diets was zero-grazed fresh-cut perennial ryegrass or grass silage (when grass growth was not sufficient) *ad libitum*. As a result of hot and dry weather that prevented adequate grass growth for zero-grazing, grass silage was offered to the animals as the only forage source during periods 1 and 2, while during periods 3 and 4 forage was made of 50:50 fresh weight ratio of ensiled: fresh-cut ryegrass. The two concentrate blends used in the study were formulated to be isonitrogenous (16 % crude protein, DM basis), while differences in the rest of the components of the concentrate blends, beyond the replacement of WDG with RSM, were kept to the minimum. The ingredient and chemical composition of concentrate blends are presented in Table 1. The chemical composition, including DM, crude protein, ether extract, total oil, acid detergent fibre, neutral-detergent fibre, starch, sugar as sucrose, and ash, were analysed in a commercial ISO 17025 accredited laboratory (Sciantec Analytical, Stockbridge Technology Centre, North Yorkshire, UK) using commercial wet chemistry methodologies. Mineral supplementation with or without seaweed (*S. latissima*, chemical composition also shown in Table 1) was mixed into concentrate blends in the form of dried powder. Concentrate feeds were distributed three times daily at 9.00, 16.00, and 20.00 in individual bowls and the consumption was supervised to ensure complete intake of the supplement.

In order to prevent I overfeeding, a risk previously associated with seaweed supplementation in dairy cow diets (when a mix of *Ascophyllum nodosum* and *Laminaria digitata* was fed at 0.5–1.5 % of concentrate DM; Newton et al., 2021), I content of seaweed was measured prior to feeding. According to book values for I content of the feeds used and a pre-trial measurement of *S. latissima* I content of 2005 mg/kg DM, the whole diets were designed so that I content was ca. 1 mg/kg DM (recommendation for diets not containing goitrogens; Flachowsky, Franke, Meyer, Leiterer, & Schone, 2014) in the diets without seaweed supplement (C-WDG and C-RSM) and ca. 4 mg/kg DM (below the EFSA limit for dairy diet iodine content of 5 mg/kg DM; Flachowsky et al., 2014) in the diets with seaweed supplement (S-WDG and S-RSM). The detailed ingredient and chemical composition of experimental diets are presented in Table 2.

### 2.2. Sample collection and analyses

Sampling and measurements took place for all cows individually during the last week (7-d measurement periods) of each 21-d feeding period. The cows were milked twice daily at 05.00 and 15.00 and milk yield was recorded. Body weight was measured on two consecutive days at the beginning and end of each 7-d measurement period, respectively. Dry matter intake (DMI) was assessed by recording the daily grass and/or grass silage intake via individual-access electronic gates and the amounts of offered and refused concentrate during the 7-day measurement weeks.

Representative samples of fresh grass, grass silage, and concentrates were collected during the measurement week of each period. Fresh grass and grass silage samples were collected daily over the measurement

**Table 1**
Ingredient and chemical composition of concentrate blends and seaweed.

| Concentrate blend | WDG | RSM | *Saccharina latissima* |
|---|---|---|---|
| **Diet components (g/kg DM)** | | | |
| Rapeseed meal (expeller) | – | 205 | – |
| Wheat distiller's grain | 230 | – | – |
| Rolled wheat | 90 | 80 | – |
| Rolled barley | 266 | 268 | – |
| Palm kernel (expeller) | – | 105 | – |
| Sugar beet pulp (un-molassed) | 120 | 140 | – |
| Soya hulls | 80 | 100 | – |
| Wheat blend (KW pellets) | 193 | 80 | – |
| Minerals (Dairy GP) | 21.9 | 21.9 | – |
| **Chemical composition (g/kg DM if not otherwise stated)** | | | |
| Dry matter (g/100 g) | 89.3 | 89.9 | 93.0 |
| Crude protein | 141 | 138 | 130 |
| Ether extract | 24 | 20 | 4 |
| Total oil | 34 | 28 | 16 |
| ADF | 128 | 169 | 199 |
| NDF | 223 | 284 | 111 |
| Starch | 230 | 230 | n.d. |
| Sugar as Sucrose | 36 | 33 | n.d. |
| Ash | 54 | 63 | 368 |
| NCGD | 808 | 799 | 610 |
| Ruminant ME (MJ/kg DM) | 12.4 | 12.1 | 9.0 |
| **Mineral concentrations (mg/kg DM if not otherwise stated)** | | | |
| Na (g/kg DM) | 5.20 | 3.53 | 25.3 |
| Mg (g/kg DM) | 3.59 | 3.72 | 7.87 |
| P (g/kg DM) | 5.71 | 6.04 | 2.36 |
| K (g/kg DM) | 8.75 | 9.19 | 112.4 |
| Ca (g/kg DM) | 14.9 | 11.6 | 14.1 |
| Cu | 48.7 | 40.7 | 0.78 |
| Co | 1.49 | 1.35 | 0.14 |
| Fe | 455.9 | 595.3 | 84.3 |
| I | 5.46 | 4.95 | 2435 |
| Mn | 98.6 | 112.3 | 6.05 |
| Mo | 1.50 | 1.23 | 0.37 |
| Se | 1.06 | 0.77 | 0.09 |
| Zn | 171.1 | 163.6 | 10.6 |
| Al | 298.0 | 561.0 | 35.9 |
| Sn | 0.07 | 0.09 | 0.07 |
| Ni | 1.55 | 2.07 | 0.46 |
| As | 0.17 | 0.22 | 69.0 |
| Cd | 0.12 | 0.08 | 2.11 |
| Cr | 3.47 | 3.61 | 0.29 |
| Hg | <0.01 | <0.01 | <0.01 |
| Pb | 0.30 | 0.40 | 0.07 |

WDG, concentrate blends with wheat dark distillers' grains as the main protein source; RSM, concentrate blends with rapeseed meal as the main protein source; ADF, acid-detergent fibre; NDF, neutral-detergent fibre; NCGD, neutral cellulase gammanase digestibility; ME, metabolisable energy; n.d., not determined.

weeks. Concentrates were sampled daily over 5 days during each measurement week. Composite samples of fresh grass, grass silage, and concentrates by period were analysed for DM, ash, neutral detergent fibre, acid detergent fibre, crude protein, ether extract, total oil, starch, and sugar as sucrose in a commercial laboratory (Sciantec, Bracknell, UK). In addition, fresh grass, grass silage, concentrates, and seaweed were analysed for macrominerals and trace element concentrations. The chemical composition of diets was calculated from the intake and chemical composition of the dietary components.

Milk samples, proportionate to the yield at each milking, were collected from four consecutive milkings during the measurement week and were stored at 4°C. After the collection of the last milk sample, all samples were pooled after being heated in a water bath of 38°C. 30 mL of the pooled samples were preserved with bronopol and sent for analyses of fat, protein, casein, lactose, urea, and somatic cell count (SCC) by fourier-transform infrared spectroscopy (FTIR) at a commercial laboratory (National Milk Laboratories, Wolverhampton, UK). Energy-corrected milk was calculated as 0.327 × milk yield + 12.95 × fat yield + 7.2 × protein yield. Fat-corrected milk was calculated as 0.4 × milk yield + 15 × fat yield. The remaining samples (without preservatives) were aliquoted to 7 mL bottle and stored at −20°C until the analyses of chemical elements.

Analyses of feed and milk chemical elements included the quantification of macrominerals (Ca, K, Mg, Na, P), essential trace elements (Cu, Fe, I, Mn, Mo, Se, Zn), non-essential trace elements (Al, Co, Sn, Ni) and heavy metals (As, Cd, Cr, Hg, Pb). Dried feed samples and frozen aliquots of milk samples were lyophilised at the University of Reading and sent on ice to Matis (accredited laboratory, Reykjavik, Iceland) for chemical element analyses. For all elements, except I, samples were digested using an Ultra-wave Acid Digestion System (Milestone Inc., Italy) and then proceeded to mineral analyses using inductively coupled plasma mass spectrometry (Agilent 7900, Agilent Technologies, Singapore) (NMKL 186, 2007). Certified reference material (CRM) DORM-4 (Fish protein certified reference material for trace metals, National Research Council Canada) and a relevant matrix matched CRM either skimmed milk powder ERM-BD150 (trace elements, European Reference Materials) or NMIJ 7405 b (Trace Elements and Arsenic Compounds in Seaweed (Hijiki), National Metrology Institute of Japan) were included with each analysis. Indium was applied as a continuous internal standard. The analysis of I was performed according to the methods described by Payling, Juniper, Drake, Rymer, and Givens (2015) and British Standards Institution Publication (BSI, 2017), where CRM skimmed milk powder, ERM-BD150, was included with each analysis. Based on the quantification of minerals in feed and milk, DMI, and milk yield, the transfer efficiencies of minerals from feed into milk (g yield/g intake) were calculated as follows: 100 × milk mineral concentration (mg/kg milk × milk yield (kg/d) / (diet mineral concentration (mg/kg DM) × DMI (kg/d)).

Glucosinolates in feed were extracted and quantified using previously published methods (Jasper, Wagstaff, & Bell, 2020) with the following modification: Fats and lipids were removed from the dried RSM samples prior to the analysis of glucosinolates, by mixing and vortexing samples (40 mg) with 1 mL of hexane three times (3 mL total). Results are presented in Table 3.

### 2.3. Statistical analysis

Statistical analyses were carried out in SAS (release 9.4, SAS Institute Inc., Cary, NC). Analyses of variance were performed with a mixed linear model using PROC MIXED, with seaweed supplementation (with/without), main protein source in the concentrate (WDG/RSM), and their interaction as fixed effects, with period as the random effect, and animal as the variable on which repeated measures were taken. The covariance structure was defined as a first-order autoregressive structure (AR (1)). The normality of variables was inspected with PROC UNIVARIATE, based on the distribution of residuals obtained from the statistical model described above. Milk mineral concentrations and SCC were log-transformed prior to the statistical analyses to achieve the normal distribution of the residuals, while all other variables were analysed using the untransformed values.

## 3. Results

### 3.1. Performance of animals, feed intake, milk production, and milk solids composition

Performance of animals, feed intake, milk yield, and milk composition are presented in Table 4. Seaweed supplementation and protein source did not affect animals' body weight and DMI. Milk production was not affected by the treatments, as reflected by the similar yields of milk, energy-corrected milk, fat-corrected milk, fat, protein, and lactose in all groups. Neither seaweed supplementation nor protein source influenced milk basic composition (contents of fat, protein, lactose, casein, urea, SCC, fat: protein ratio). In addition, all treatments resulted in similar efficiency values (feed conversion ratio, fat efficiency, and protein efficiency).

**Table 2**
Average ingredient and chemical compositions of diets in the four periods of the experiment.

| Parameter | Seaweed | | Concentrate | | Seaweed × concentrate | | | | StDev | Min | Max |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | S | WDG | RSM | C-WDG | S-WDG | C-RSM | S-RSM | | | |
| **Ingredient composition (g/kg DM)** | | | | | | | | | | | |
| Fresh grass | 116 | 116 | 117 | 116 | 117 | 116 | 116 | 116 | 119 | 0 | 275 |
| Grass silage | 589 | 586 | 586 | 589 | 590 | 582 | 587 | 591 | 124 | 410 | 753 |
| Rapeseed | 30.5 | 30.1 | 0 | 60.5 | 0 | 0 | 60.9 | 60.1 | 27.8 | 0 | 70.8 |
| Wheat distiller's grain | 33.7 | 34.7 | 68.4 | 0 | 67.4 | 69.4 | 0 | 0 | 30.8 | 0 | 80.4 |
| Rolled wheat | 25.1 | 25.3 | 26.8 | 23.6 | 26.4 | 27.2 | 23.8 | 23.5 | 34.8 | 17.8 | 31.5 |
| Rolled barley | 78.8 | 79.4 | 79.0 | 79.2 | 77.8 | 80.2 | 79.7 | 78.6 | 2.9 | 58.8 | 92.9 |
| Palm kernel | 15.6 | 15.4 | 0 | 31.0 | 0 | 0 | 31.2 | 30.8 | 7.4 | 0 | 36.2 |
| Sugar beet pulp | 38.4 | 38.6 | 35.7 | 41.3 | 35.2 | 36.2 | 41.6 | 41.1 | 15.8 | 26.6 | 48.3 |
| Soya hulls | 26.6 | 26.7 | 23.8 | 29.5 | 23.4 | 24.1 | 29.7 | 29.3 | 4.6 | 17.7 | 34.5 |
| Wheat blend | 40.1 | 40.8 | 57.3 | 23.6 | 56.4 | 58.1 | 23.8 | 23.5 | 3.8 | 17.8 | 67.3 |
| Minerals | 6.5 | 6.5 | 6.5 | 6.5 | 6.4 | 6.6 | 6.5 | 6.4 | 17.5 | 4.8 | 7.7 |
| *Saccharina latissima* | 0 | 2.01 | 1.02 | 0.97 | 0 | 2.04 | 0 | 1.97 | 1.02 | 0 | 2.35 |
| **Nutrient composition (g/kg DM if not otherwise stated)** | | | | | | | | | | | |
| DM (g/100 g) | 37.5 | 37.5 | 37.5 | 37.5 | 37.4 | 37.6 | 37.6 | 37.4 | 6.4 | 28.9 | 49.1 |
| CP | 155 | 156 | 156 | 155 | 156 | 157 | 155 | 155 | 17 | 129 | 182 |
| Ether Extract | 33.4 | 33.5 | 34.3 | 32.7 | 34.2 | 34.3 | 32.6 | 32.7 | 3.0 | 27.7 | 37.3 |
| Total Oil | 40.8 | 40.9 | 42.0 | 39.6 | 42.0 | 42.1 | 39.6 | 39.7 | 2.7 | 35.7 | 44.0 |
| ADF | 240 | 240 | 233 | 248 | 233 | 233 | 248 | 248 | 30 | 186 | 279 |
| NDF | 407 | 407 | 396 | 418 | 397 | 396 | 418 | 419 | 34 | 345 | 455 |
| Starch | 85 | 85.9 | 86.1 | 84.7 | 84.8 | 87.5 | 85.1 | 84.3 | 8.0 | 62.1 | 99.9 |
| Sugar as Sucrose | 67.3 | 67.4 | 68.3 | 66.5 | 68.3 | 68.3 | 66.4 | 66.6 | 38.1 | 19.0 | 113.4 |
| Ash | 85.9 | 85.9 | 84.3 | 87.5 | 84.4 | 84.2 | 87.4 | 87.5 | 9.6 | 70.2 | 99.3 |
| ME (MJ/kg DM) | 11.1 | 11.1 | 11.2 | 11.1 | 11.1 | 11.2 | 11.1 | 11.1 | 0.2 | 10.7 | 11.4 |
| **Macrominerals (g/kg DM)** | | | | | | | | | | | |
| Ca | 7.41 | 7.48 | 7.97 | 6.92 | 7.90 | 8.04 | 6.93 | 6.92 | 1.04 | 6.31 | 10.53 |
| K | 24.8 | 24.9 | 24.8 | 24.9 | 24.7 | 24.8 | 24.8 | 25.1 | 1.4 | 22.6 | 28.1 |
| Mg | 2.07 | 2.10 | 2.07 | 2.10 | 2.05 | 2.09 | 2.10 | 2.10 | 0.11 | 1.88 | 2.36 |
| Na | 2.89 | 2.95 | 3.17 | 2.66 | 3.13 | 3.21 | 2.64 | 2.69 | 0.38 | 2.28 | 3.63 |
| P | 3.86 | 3.87 | 3.81 | 3.91 | 3.80 | 3.83 | 3.91 | 3.91 | 0.18 | 3.43 | 4.21 |
| **Essential trace elements (mg/kg DM)** | | | | | | | | | | | |
| Cu | 18.8 | 18.9 | 20.2 | 17.6 | 20.0 | 20.3 | 17.7 | 17.5 | 2.9 | 14.5 | 26.9 |
| Fe | 293 | 293 | 273 | 313 | 272 | 275 | 314 | 312 | 32 | 237 | 349 |
| I | 2.25 | 7.14 | 4.83 | 4.56 | 2.32 | 7.33 | 2.17 | 6.95 | 2.56 | 1.68 | 8.43 |
| Mn | 78.3 | 78.2 | 76.3 | 80.2 | 76.1 | 76.4 | 80.4 | 80 | 15.5 | 56.1 | 104.9 |
| Mo | 2.00 | 2.00 | 2.04 | 1.96 | 2.04 | 2.04 | 1.96 | 1.96 | 0.32 | 1.63 | 2.63 |
| Se | 0.32 | 0.33 | 0.37 | 0.28 | 0.36 | 0.37 | 0.28 | 0.28 | 0.08 | 0.21 | 0.54 |
| Zn | 72.3 | 72.6 | 73.7 | 71.2 | 73.2 | 74.3 | 71.4 | 70.9 | 9.6 | 52.4 | 96.3 |
| **Non-essential trace elements (mg/kg DM)** | | | | | | | | | | | |
| Al | 212 | 212 | 174 | 251 | 173 | 175 | 251 | 250 | 47 | 143 | 309 |
| Co | 0.48 | 0.49 | 0.51 | 0.46 | 0.50 | 0.52 | 0.46 | 0.46 | 0.07 | 0.35 | 0.68 |
| Sn | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.06 | 0.02 | 0.04 | 0.10 |
| Ni | 1.10 | 1.10 | 1.02 | 1.17 | 1.02 | 1.03 | 1.18 | 1.17 | 0.13 | 0.82 | 1.29 |
| **Heavy metals (mg/kg DM)** | | | | | | | | | | | |
| As | 0.12 | 0.26 | 0.18 | 0.20 | 0.11 | 0.26 | 0.13 | 0.26 | 0.07 | 0.09 | 0.31 |
| Cd | 0.05 | 0.05 | 0.05 | 0.04 | 0.05 | 0.06 | 0.04 | 0.05 | 0.01 | 0.03 | 0.08 |
| Cr | 1.64 | 1.64 | 1.62 | 1.66 | 1.61 | 1.63 | 1.67 | 1.66 | 0.31 | 1.19 | 2.37 |
| Hg | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | <0.02 | n.a. | n.a. | n.a. |
| Pb | 0.28 | 0.28 | 0.26 | 0.29 | 0.26 | 0.26 | 0.29 | 0.29 | 0.04 | 0.22 | 0.34 |

The standard deviation, maximum, and minimum are across all treatments. C, control diets; S, seaweed-supplemented diets; WDG, dark distillers' grains diets; RSM, rapeseed meal diets; C-WDG, control with dark distillers' grains; S-WDG, seaweed-supplemented with dark distillers' grains; C-RSM, control with rapeseed meal; S-RSM, seaweed-supplemented with rapeseed meal; ME, metabolisable energy; n.a. not applicable.

**Table 3**
Concentrations of glucosinolates (GSL) in rapeseed meal.

| Glucosinolate (g/kg DM) | Period | | | | Mean | StDev | Min | Max |
|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | | | | |
| Pentyl GSL | 0.02 | 0.02 | 0.04 | 0.03 | 0.03 | 0.01 | 0.02 | 0.04 |
| Progoitrin | 1.24 | 1.25 | 1.29 | 1.17 | 1.24 | 0.04 | 1.17 | 1.29 |
| Gluconapoleiferin | 0 | 0 | 0.02 | 0 | 0.01 | 0.01 | 0 | 0.02 |
| Gluconapin | 0.19 | 0.15 | 0.04 | 0.18 | 0.14 | 0.06 | 0.04 | 0.19 |
| 4-hydroxyglucobrassicin | 0.01 | 0 | 0 | 0.02 | 0.01 | 0.01 | 0 | 0.02 |
| Total | 1.46 | 1.42 | 1.39 | 1.40 | 1.42 | 0.03 | 1.39 | 1.46 |

### 3.2. Milk mineral concentrations

Milk mineral concentrations and transfer efficiencies of minerals from diet to milk are presented in Table 5. The scatter plots of all measurements of mineral concentrations are presented in supplementary Figure S1. No significant effect of the interaction between seaweed supplement and protein source on milk mineral concentration was observed. However, the factorial design of seaweed supplement and

**Table 4**
Animal feed intake, productivity, milk basic composition and efficiency parameters.

| Parameter | Seaweed | | | | Concentrate | | | | Seaweed × concentrate | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C (n = 32) | S (n = 32) | SEM | *p*-value | WDG (n = 32) | RSM (n = 32) | SEM | *p*-value | C-WDG (n = 16) | S-WDG (n = 16) | C-RSM (n = 16) | S-RSM (n = 16) | SEM | *p*-value |
| **Animal parameters** | | | | | | | | | | | | | | |
| Body weight (kg) | 692 | 695 | 12.5 | 0.483 | 692 | 695 | 12.5 | 0.140 | 692 | 691 | 692 | 698 | 17.8 | 0.360 |
| DMI (kg/d) | 18.3 | 18.1 | 0.32 | 0.105 | 18.0 | 18.3 | 0.33 | 0.958 | 18.4 | 17.7 | 18.1 | 18.4 | 0.46 | 0.181 |
| Forage intake (kg DM/d) | 12.9 | 12.7 | 0.32 | 0.134 | 12.7 | 12.9 | 0.32 | 0.922 | 13.0 | 12.4 | 12.8 | 13.1 | 0.46 | 0.179 |
| Concentrate intake (kg DM/d) | 5.3 | 5.3 | 0.15 | 0.895 | 5.3 | 5.3 | 0.13 | 0.886 | 5.3 | 5.4 | 5.3 | 5.3 | 0.18 | 0.932 |
| **Productivity** | | | | | | | | | | | | | | |
| Milk yield (kg/d) | 22.1 | 21.9 | 0.94 | 0.932 | 21.6 | 22.4 | 0.92 | 0.244 | 21.8 | 21.3 | 22.3 | 22.5 | 1.33 | 0.870 |
| ECMY (kg/d) | 24.7 | 24.8 | 0.74 | 0.511 | 24.5 | 25.0 | 0.74 | 0.377 | 24.6 | 24.4 | 24.8 | 25.2 | 1.06 | 0.734 |
| FCMY (kg/d) | 25.2 | 25.3 | 0.78 | 0.464 | 25.0 | 25.6 | 0.78 | 0.319 | 25.1 | 24.9 | 25.4 | 25.8 | 1.12 | 0.846 |
| Fat yield (kg/d) | 0.95 | 0.96 | 0.032 | 0.579 | 0.94 | 0.97 | 0.032 | 0.413 | 0.94 | 0.94 | 0.97 | 0.98 | 0.046 | 0.956 |
| Protein yield (kg/d) | 0.72 | 0.73 | 0.021 | 0.762 | 0.72 | 0.73 | 0.021 | 0.660 | 0.73 | 0.71 | 0.72 | 0.74 | 0.029 | 0.463 |
| Lactose yield (kg/d) | 1.00 | 1.00 | 0.037 | 0.586 | 1.00 | 1.00 | 0.038 | 0.881 | 1.01 | 0.99 | 0.99 | 1.01 | 0.054 | 0.392 |
| **Milk basic composition** | | | | | | | | | | | | | | |
| Fat% | 4.19 | 4.15 | 0.118 | 0.904 | 4.15 | 4.19 | 0.119 | 0.539 | 4.15 | 4.16 | 4.23 | 4.15 | 0.168 | 0.720 |
| Protein% | 3.22 | 3.24 | 0.065 | 0.725 | 3.24 | 3.22 | 0.065 | 0.680 | 3.22 | 3.26 | 3.23 | 3.22 | 0.092 | 0.791 |
| Lactose% | 4.36 | 4.37 | 0.038 | 0.986 | 4.39 | 4.34 | 0.037 | 0.301 | 4.40 | 4.38 | 4.32 | 4.36 | 0.053 | 0.377 |
| Casein% | 2.56 | 2.57 | 0.043 | 0.825 | 2.57 | 2.57 | 0.043 | 0.749 | 2.56 | 2.57 | 2.57 | 2.57 | 0.062 | 0.891 |
| Urea (mg/l) | 227 | 225 | 12.5 | 0.646 | 230 | 222 | 12.5 | 0.278 | 239 | 220 | 214 | 231 | 17.8 | ***0.082*** |
| SCC (×1000/mL milk) | 659 | 1277 | 546.9 | 0.496 | 1586 | 350 | 462.6 | 0.689 | 964 | 2208 | 353 | 346 | 578.5 | 0.550 |
| Fat:protein | 1.32 | 1.32 | 0.024 | 0.822 | 1.31 | 1.33 | 0.023 | 0.501 | 1.30 | 1.31 | 1.34 | 1.32 | 0.033 | 0.454 |
| **Efficiency parameters** | | | | | | | | | | | | | | |
| Feed efficiency (kg milk/kg DMI) | 1.38 | 1.38 | 0.039 | 0.426 | 1.37 | 1.38 | 0.039 | 0.598 | 1.37 | 1.37 | 1.38 | 1.39 | 0.056 | 0.865 |
| Fat efficiency (kg fat/kg DMI) | 0.053 | 0.053 | 0.0018 | 0.564 | 0.053 | 0.054 | 0.0019 | 0.612 | 0.053 | 0.053 | 0.054 | 0.054 | 0.0026 | 0.798 |
| Protein efficiency (kg protein/kg DMI) | 0.040 | 0.040 | 0.0011 | 0.652 | 0.040 | 0.040 | 0.0011 | 0.836 | 0.040 | 0.040 | 0.040 | 0.041 | 0.0015 | 0.848 |

The *p*-value of SCC was obtained from the fitted linear model based on log2-transformed values. The *p*-values of other variables were obtained from the fitted linear model based on the observed values. SEM, averaged standard error of the mean. C, control diets; S, seaweed-supplemented diets; WDG, dark distillers' grains diets; RSM, rapeseed meal diets; C-WDG, control with dark distillers' grains; S-WDG, seaweed-supplemented with dark distillers' grains; C-RSM, control with rapeseed meal; S-RSM, seaweed-supplemented with rapeseed meal; DMI, dry matter intake; ECMY, energy-corrected milk yield; FCMY, fat-corrected milk yield; SCC, somatic cell count.

**Table 5**
Milk mineral concentrations and transfer efficiencies from diet to milk.

| Mineral | Seaweed | | | | Concentrate | | | | Seaweed × concentrate | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C (n = 32) | S (n = 32) | SEM | *p*-value | WDG (n = 32) | RSM (n = 32) | SEM | *p*-value | C-WDG (n = 16) | S-WDG (n = 16) | C-RSM (n = 16) | S-RSM (n = 16) | SEM | *p*-value |
| **Macrominerals concentrations (mg/kg milk)** | | | | | | | | | | | | | | |
| Ca | 1080 | 1031 | 32.3 | **0.036** | 1057 | 1055 | 32.6 | 0.557 | 1073 | 1040 | 1088 | 1023 | 46.3 | 0.843 |
| K | 1381 | 1357 | 29.8 | 0.389 | 1393 | 1347 | 29.7 | 0.247 | 1411 | 1375 | 1353 | 1340 | 42.4 | 0.696 |
| Mg | 98.0 | 95.2 | 2.30 | 0.192 | 98.1 | 95.0 | 2.28 | **0.050** | 98.8 | 97.5 | 97.1 | 92.9 | 3.24 | 0.894 |
| Na | 478 | 477 | 20.2 | 0.878 | 469 | 484 | 19.7 | 0.994 | 464 | 474 | 490 | 479 | 28.3 | 0.981 |
| P | 819 | 795 | 17.8 | 0.132 | 820 | 793 | 17.8 | **0.065** | 825 | 816 | 813 | 773 | 25.2 | 0.626 |
| **Essential trace elements concentrations (μg/kg milk)** | | | | | | | | | | | | | | |
| I | 208 | 695 | 58.7 | **<0.001** | 526 | 382 | 81.3 | **<0.001** | 267 | 816 | 158 | 615 | 79.7 | 0.445 |
| Cu | 48.1 | 41.9 | 2.68 | **0.029** | 46.8 | 43.3 | 2.75 | 0.299 | 52.2 | 41.1 | 44.1 | 42.6 | 3.69 | 0.451 |
| Fe | 241 | 233 | 12.9 | 0.835 | 248 | 227 | 12.9 | 0.374 | 250 | 246 | 233 | 220 | 17.8 | 0.904 |
| Mn | 30.2 | 29.8 | 1.99 | 0.863 | 31.8 | 28.2 | 1.94 | **0.012** | 31.2 | 32.3 | 29.1 | 27.3 | 2.76 | 0.550 |
| Mo | 58.6 | 59.6 | 2.40 | 0.634 | 60.3 | 57.9 | 2.39 | 0.355 | 60.5 | 60.2 | 56.8 | 59.0 | 3.42 | 0.317 |
| Se | 15.6 | 15.9 | 0.60 | 0.848 | 16.0 | 15.4 | 0.57 | 0.577 | 15.8 | 16.2 | 15.3 | 15.5 | 0.82 | 0.759 |
| Zn | 2847 | 2813 | 124.8 | 0.806 | 2870 | 2790 | 124.5 | 0.386 | 2891 | 2848 | 2803 | 2777 | 178.4 | 0.676 |
| **Macrominerals transfer efficiencies (g yield/100 g intake)** | | | | | | | | | | | | | | |
| Ca | 17.9 | 16.8 | 0.71 | ***0.070*** | 16.4 | 18.2 | 0.68 | **0.004** | 17.0 | 15.9 | 18.8 | 17.7 | 0.97 | 0.964 |
| K | 6.81 | 6.78 | 0.391 | 0.825 | 6.84 | 6.75 | 0.388 | 0.551 | 6.9 | 6.79 | 6.73 | 6.77 | 0.557 | 0.637 |
| Mg | 5.74 | 5.55 | 0.251 | 0.200 | 5.81 | 5.48 | 0.249 | **0.038** | 5.92 | 5.69 | 5.57 | 5.4 | 0.356 | 0.852 |
| Na | 20.9 | 19.7 | 0.88 | ***0.084*** | 18.7 | 21.9 | 0.82 | **<0.001** | 19.2 | 18.2 | 22.6 | 21.2 | 1.15 | 0.824 |
| P | 25.8 | 25.1 | 1.10 | 0.260 | 26.1 | 24.8 | 1.08 | **0.044** | 26.4 | 25.8 | 25.2 | 24.4 | 1.54 | 0.928 |
| **Essential trace elements transfer efficiencies (g yield/100 g intake)** | | | | | | | | | | | | | | |
| I | 13.2 | 12.6 | 1.95 | 0.757 | 14.3 | 11.4 | 1.94 | **<0.001** | 14.3 | 14.3 | 12.0 | 10.9 | 2.75 | 0.759 |
| Cu | 0.309 | 0.287 | 0.0197 | 0.331 | 0.292 | 0.304 | 0.0197 | 0.584 | 0.309 | 0.275 | 0.308 | 0.299 | 0.0274 | 0.567 |
| Fe | 0.100 | 0.095 | 0.0062 | 0.379 | 0.105 | 0.090 | 0.0062 | **0.008** | 0.109 | 0.102 | 0.091 | 0.088 | 0.0086 | 0.668 |
| Mn | 0.047 | 0.047 | 0.0035 | 0.923 | 0.053 | 0.041 | 0.0034 | **0.001** | 0.052 | 0.053 | 0.041 | 0.040 | 0.0047 | 0.800 |
| Mo | 3.63 | 3.66 | 0.208 | 0.854 | 3.64 | 3.65 | 0.204 | 0.957 | 3.71 | 3.56 | 3.54 | 3.75 | 0.291 | 0.229 |
| Se | 6.02 | 6.08 | 0.381 | 0.869 | 5.07 | 7.03 | 0.332 | **<0.001** | 5.16 | 4.99 | 6.89 | 7.18 | 0.461 | 0.522 |
| Zn | 4.83 | 4.8 | 0.295 | 0.868 | 4.83 | 4.8 | 0.294 | 0.876 | 4.82 | 4.84 | 4.84 | 4.75 | 0.420 | 0.802 |

The *p*-values were obtained from the fitted linear model based on log2-transformed values. SEM, averaged standard error of the mean. C, control diets; S, seaweed-supplemented diets; WDG, dark distillers' grains diets; RSM, rapeseed meal diets; C-WDG, control with dark distillers' grains; S-WDG, seaweed-supplemented with dark distillers' grains; C-RSM, control with rapeseed meal; S-RSM, seaweed-supplemented with rapeseed meal.

protein source effects produced an apparent numerical gradient in I concentration between groups (S-WDG > S-RSM > C-WDG > C-RSM), although the seaweed × concentrate interaction was not statistically significant. Milk I concentration was increased ($p < 0.001$) by seaweed supplement to 3.3-fold of the level in cows without the supplement (+485.4 μg/kg milk). Cows with seaweed supplement had lower milk Ca (-49.0 mg/kg milk; $p = 0.04$) and Cu (-6.2 μg/kg milk; $p = 0.03$) concentrations than those without the supplement. Concentrations of K, Mg, Na, P, Fe, Mn, Mo, Se, and Zn in milk were not affected by seaweed supplement.

Milk I concentration was reduced (-143.3 μg/kg milk; $p < 0.001$) when RSM was involved in the diet as the main protein source instead of WDG. Cows fed RSM had lower milk Mg (−3.1 mg/kg milk; $p = 0.04$) and Mn (−3.6 μg/kg milk; $p = 0.01$) concentrations and a tendency towards lower milk P concentration (−27.1 mg/kg milk; $p = 0.07$) than those fed WDG. Milk concentrations of Ca, K, Na, Cu, Fe, Mo, Se, and Zn were not affected by dietary protein source.

In addition, milk Co concentration was very low in all groups as the results of all individual measurements were below the limit of quantification (1.17 μg/kg milk). Similarly, all of the determined non-essential trace and heavy metal elements were present at very low levels in milk (Al, < 58.59 μg/kg milk; Cr, < 7.04 μg/kg milk; Ni, < 4.70 μg/kg milk; As, < 5.87 μg/kg milk; Cd, < 0.23 μg/kg milk; Sn, < 1.17 μg/kg milk; Hg, < 2.35 μg/kg milk; Pb, < 8.22 μg/kg milk). The proportions of the individual measurements that were below the limits of quantification were: Al (52 %), Cr (100 %), Ni (97 %), As (100 %), Cd (100 %), Sn (100 %), Hg (100 %), Pb (100 %).

### 3.3. Transfer efficiencies of minerals from feed to milk

No significant effect of the interaction between seaweed supplement and protein source on mineral transfer efficiency was observed. The effect of seaweed supplement on mineral transfer efficiency from feed to milk was minor, as reflected only by a tendency towards decreased ($p = 0.070$) efficiency of Ca transfer (-1.1 g yield/100 g intake). In contrast, the type of protein source showed significant effects on the transfer efficiencies of a number of minerals. Cows fed RSM had higher transfer efficiencies of (all expressed as g yield/100 g intake) Ca (+1.8, $p = 0.004$), Na (+3.2, $p < 0.001$), Se (+1.96, $p < 0.001$) but lower transfer efficiencies of Mg (-0.33, $p = 0.04$), P (-1.3, $p = 0.04$), I (-2.9, $p = <0.001$), Fe (-0.015, $p = 0.008$), and Mn (-0.012, $p = 0.001$).

## 4. Discussion

### 4.1. Effects of seaweed supplementation on animal productivity and efficiency

The supplement of *S. latissima* did not affect animals' performance, milk production, milk basic composition, and production efficiencies. The results are in line with other studies where brown seaweeds (including *A. nodosum* and *Undaria pinnatifida*) were included in the diet of dairy cows at a comparable level of 113–360 g DM/d (Antaya et al., 2019; Hong, Kim, Jin, Lee, Choi, & Lee, 2015). The potential explanation for the lack of seaweed effect on animal productivity, efficiency and milk basic composition could be the relatively low level of inclusion (0.22 g/100 g DMI), which might be insufficient to cause any effects on animal performance.

### 4.2. Effects of protein source on animal productivity and efficiency

The parameters related to performance and milk production were also not affected by the type of main protein source. Replacing other protein sources (distillers' grains from wheat or maize) with rapeseed meal had also no significant effect on milk production and basic composition in previous studies (Franke, Meyer, & Flachowsky, 2009; Mulrooney, Schingoethe, Kalscheur, & Hippen, 2009). However, the

lack of protein source effect on milk protein content in the present study is contradictory to Franke, Meyer, and Flachowsky (2009) where milk protein content was increased from 3.11 g/100 g to 3.29 g/100 g when dietary wheat distiller's grain was replaced by rapeseed meal. In addition, the substitution of soybean meal, cottonseed meal, and tallow with rapeseed meal had increased milk protein contents by 0.13 g/100 g in previous studies (Emanuelson et al., 1993; Martineau, Ouellet, & Lapierre, 2013). It is possible that the relatively low levels of rapeseed supplementation in the present study (230 g/kg concentrate), when compared with other studies (330 g/kg concentrate in Franke, Meyer, and Flachowsky (2009); 520 g/kg concentrate in Emanuelson et al. (1993)) were not sufficient to cause an effect on milk protein concentrations.

### 4.3. Effects of seaweed supplement on milk mineral concentrations and transfer efficiencies

Seaweed is generally considered a good source of Ca (Circuncisão, Catarino, Cardoso, & Silva, 2018) but milk Ca concentration was statistically lower in the seaweed-supplemented cows than in the controls in the present study although the difference (49.0 mg/kg) was numerically minor. The result is in contrast with the findings of Newton et al. (2021) who found similar milk Ca concentrations after supplementation of brown seaweeds in cows' diets. In the present study, the decreased milk Ca concentration from seaweed-fed cows may have resulted from the reduced feed-to-milk Ca transfer, as suggested by the tendency towards lower Ca transfer efficiency in seaweed-supplemented cows and the fact that dietary Ca intakes were similar across the two experimental groups. Approximately 70 % of Ca binds to casein micelles in milk and its concentration is relatively constant and determined mainly by cows' genetics (Flynn, 1992). A possible explanation for the reduced Ca transfer efficiency in seaweed-supplemented cows could be that the intake of seaweed decreased the biological availability of dietary Ca, although this was not assessed in the present study.

Milk Cu concentration was lower in seaweed-supplemented groups when compared with non-supplemented groups despite the similar dietary Cu contents and Cu intakes across the experimental groups. The decreased milk Cu concentration by seaweed supplementation is in line with a previous study where cows were fed a mixture of two brown seaweed species (*A. nodosum* and *L. digitata*; Newton et al., 2021). Moreover, the same study reported a decreased Cu transfer efficiency from feed to milk under seaweed supplementation and attributed this potentially to a lower Cu availability in seaweed-supplemented diets, although availability had not been measured (Newton et al., 2021). In the present study, the seaweed supplement did not affect Cu transfer efficiency. However, there was a numerical difference in Cu transfer efficiency between groups fed WDG as the main protein source (0.31 g yield/100 g in C-WDG and 0.27 g yield/100 g in S-WDG) but the same was not observed in the groups fed RSM, although the effect of seaweed × protein source interaction was not statistically significant. Therefore, the difference in milk Cu concentration was probably driven primarily by the differences in Cu transfer efficiency between S-WDG and C-WDG. It is however unclear why the same numerical differences were not seen between control and seaweed-fed groups when RSM was involved as the main protein source.

The seaweed supplement greatly elevated milk I content, thus aligning with the results from previous studies feeding different brown seaweed species, including *Thallus laminariae*, *A. nodosum*, and mixtures between *A. nodosum, L. digitata* and *U. pinnatifida* (Antaya et al., 2019; Chaves Lopez et al., 2016; Newton et al., 2021; Sorge, Henriksen, Bastan, Cremers, Olsen, & Crooker, 2016). Although the chemical composition of different brown seaweed species is variable, almost all species are good sources of I (Circuncisão et al., 2018). In the present study, the higher milk I concentrations in the seaweed-supplemented groups can be attributed to the greater I intake, as seaweed-fed cows ingested 2.2-fold more I (7.14 mg/kg DM) than their counterparts fed the non-supplemented diets (2.25 mg/kg DM); and it is worthy to note that the I contents in the seaweed-supplement diets exceeded the EFSA upper limit for dairy diet (5 mg/kg DM). This is in line with previous work that reported I intake to be the main driver for milk I concentration (Flachowsky et al., 2014). This is further reinforced by the similar I transfer efficiencies between the seaweed-fed and control groups, which is also in line with the results of Newton et al. (2021). The overfeeding of I in case of seaweed-fed cows was because (i) the I content of basal diet ingredients in the nutritional tables used when designing the diet, were lower compared with those eventually measured during the trial, and (ii) the I content measured in the seaweed pre-trial, used to design the diet, was lower than that measured during the trial. The latter which may also indicate considerable variation in I content of seaweed in samples between, but also within, batches and/or in the analytical techniques used for I quantification in feeds. This may also have implications and pose challenges, when aiming to maintain an I content below the EFSA's upper limit (5 mg/kg DM) in seaweed-based diets at commercial environment.

### 4.4. Effects of protein source on milk mineral concentrations and transfer efficiencies

Milk Ca and Na concentrations were similar in cows fed WDG and those fed RSM. However, both of their transfer efficiencies were lower in WDG-fed cows when compared with RSM-fed cows. As mentioned in the previous section, milk Ca concentration is in general constant and is only marginally affected by dietary Ca intake (Flynn, 1992). Moreover, milk Na concentration has been suggested not to be influenced by dietary Na intake within the normal range (Flynn, 1992). In the present study, feed analysis showed that Ca and Na contents of RSM diets were lower than those of WDG diets, which resulted in the lower transfer efficiencies of these minerals in WDG groups, given that their transport into milk may not be closely related to the intakes.

Milk Mg, P, and Mn concentrations were lower or tended to be lower in cows fed RSM than those fed WDG. Considering the highly similar intakes of these minerals in all groups, the lower concentrations in RSM-fed cows could be explained by their decreased transfer efficiencies. Previous work in other species suggested that rapeseed meal may have lower availability of various minerals when compared with other commonly used protein sources in feed (Liu et al., 2019; Nwokolo & Bragg, 1980). For example, Nwokolo and Bragg (1980) analysed the biological availability of minerals in rapeseed meal fed to broiler chicken and reported lower availability of Mg and P in rapeseed meal when compared with soybean meal (61.5 % and 75.3 % vs 77.8 % and 89.3 % for Mg and P). Liu et al. (2019) reported that rapeseed meal had lower availability of P when compared with wheat, wheat bran, cottonseed meal, corn distiller's dried grains with soluble, and corn gluten meal and lower availability of Mn when compared with wheat based on the evaluation in broiler chicken.

Milk Fe concentrations were similar in cows fed WDG and those fed RSM, although a lower feed-to milk transfer efficiency of Fe was observed in RSM-fed cows. In cow milk, Fe is associated with fat globule membrane, caseins, whey proteins, and low-molecular-weight fraction and its concentration is marginally influenced by dietary Fe intake (Flynn, 1992). Therefore, the lower Fe transfer efficiency in cows fed RSM could be partly explained by the higher dietary Fe intake of these cows (according to Fe concentrations in RSM and WDG diets) and the unchanged milk Fe concentrations. In addition, the decreased feed-to-milk Fe transfer by RSM may have resulted from the lower biological availability of Fe in rapeseed meal than in wheat, as suggested by the evaluation in broiler chicken (Liu et al., 2019).

Substitution of WDG with RSM considerably lowered milk I concentration. Given that I concentrations were similar in diets containing WDG or RSM (5.01 mg/kg DM and 4.74 mg/kg DM, respectively), the lower milk I concentration in RSM-fed cows was attributed to the decreased feed-to-milk transfer efficiency, as this was also observed to

**Table 6**
Estimated iodine intake of different age groups in the UK population from milk produced from different diets.

| Age group | Milk intake[1] (mL/d) | RNI of[2] iodine (μg/d) | Daily iodine intake from milk intake (%RNI) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | C | S | WDG | RSM | C-WDG | S-WDG | C-RSM | S-RSM | S over C | WDG over RSM |
| Children 1.5–3 years | 247 | 70 | 73.4 | 245.1 | 185.4 | 135.0 | 94.1 | 288.0 | 55.9 | 217.0 | 171.7 | 50.4 |
| Boys 4–10 years | 181 | 105 | 35.9 | 119.7 | 90.7 | 65.9 | 46.0 | 140.7 | 27.2 | 106.0 | 83.8 | 24.8 |
| Girls 4–10 years | 160 | 105 | 31.7 | 105.9 | 80.1 | 58.3 | 40.7 | 124.4 | 24.1 | 93.7 | 74.2 | 21.8 |
| Children 4–10 years | 170 | 105 | 33.7 | 112.5 | 85.1 | 61.9 | 43.2 | 132.1 | 25.6 | 99.6 | 78.8 | 23.2 |
| Boys 11–18 years | 129 | 135 | 19.9 | 66.4 | 50.2 | 36.5 | 25.5 | 78.0 | 15.1 | 58.7 | 46.5 | 13.7 |
| Girls 11–18 years | 113 | 135 | 17.4 | 58.1 | 44.0 | 32.0 | 22.4 | 68.3 | 13.3 | 51.5 | 40.7 | 12.0 |
| Adolescents 11–18 years | 121 | 135 | 18.7 | 62.3 | 47.1 | 34.3 | 23.9 | 73.1 | 14.1 | 55.1 | 43.6 | 12.8 |
| Men 19–64 years | 136 | 140 | 20.2 | 67.5 | 51.1 | 37.1 | 25.9 | 79.3 | 15.4 | 59.7 | 47.3 | 14.0 |
| Women 19–64 years | 106 | 140 | 15.8 | 52.6 | 39.8 | 28.9 | 20.2 | 61.8 | 12.0 | 46.6 | 36.8 | 10.9 |
| Adults 19–64 years | 121 | 140 | 18.0 | 60.1 | 45.4 | 33.1 | 23.1 | 70.5 | 13.6 | 53.1 | 42.1 | 12.3 |
| Men 65+ years | 186 | 140 | 27.6 | 92.3 | 69.9 | 50.8 | 35.5 | 108.4 | 21.0 | 81.7 | 64.7 | 19.1 |
| Women 65+ years | 150 | 140 | 22.3 | 74.4 | 56.4 | 41.0 | 28.6 | 87.4 | 16.9 | 65.9 | 52.1 | 15.4 |
| Adults 65+ years | 166 | 140 | 24.6 | 82.4 | 62.4 | 45.4 | 31.6 | 96.8 | 18.7 | 72.9 | 57.8 | 17.0 |
| Men 65–74 years | 154 | 140 | 22.9 | 76.4 | 57.9 | 42.1 | 29.4 | 89.8 | 17.4 | 67.6 | 53.5 | 15.8 |
| Women 65–74 years | 122 | 140 | 18.1 | 60.6 | 45.8 | 33.4 | 23.3 | 71.1 | 13.8 | 53.6 | 42.5 | 12.4 |
| Adults 65–74 years | 137 | 140 | 20.4 | 68.0 | 51.4 | 37.4 | 26.1 | 79.9 | 15.5 | 60.2 | 47.6 | 14.0 |
| Men 75+ years | 214 | 140 | 31.8 | 106.2 | 80.4 | 58.4 | 40.8 | 124.7 | 24.1 | 94.0 | 74.4 | 22.0 |
| Women 75 + years | 177 | 140 | 26.3 | 87.9 | 66.4 | 48.4 | 33.8 | 103.1 | 20.0 | 77.8 | 61.6 | 18.0 |
| Adults 75 + years | 193 | 140 | 28.7 | 95.8 | 72.5 | 52.7 | 36.8 | 112.5 | 21.8 | 84.8 | 67.1 | 19.8 |
| Pregnant/lactating women[3] | 106 | 200 | 11.1 | 36.8 | 27.9 | 20.2 | 14.1 | 43.3 | 8.4 | 32.6 | 25.8 | 7.6 |

C, control diets; S, seaweed-supplemented diets; WDG, dark distillers' grains diets; RSM, rapeseed meal diets; C-WDG, control with dark distillers' grains; S-WDG, seaweed-supplemented with dark distillers' grains; C-RSM, control with rapeseed meal; S-RSM, seaweed-supplemented with rapeseed meal; S over C, daily iodine intakes from milk produced from the seaweed-supplemented over that from control diet; WDG over RSM, daily iodine intakes from milk produced from WDG over that from RSM.

[1] Estimated based on energy intakes and % of energy intake from milk for the different demographics in UK's National Diet and Nutrition Survey (NDNS, 2020) and the average fat content of different milks according to the McCance and Widdowson's 'composition of foods integrated dataset' on the nutrient content of the UK food supply (CoFID, 2021).

[2] Reference iodine intakes from the UK's Scientific Advisory Committee on Nutrition (SACN, 2014).

[3] Assuming a similar milk intake with Women 19–64 years, and using the reference iodine intake for pregnant and lactating women from the European Food Safety Authority Panel on Dietetic Products & Allergies (EFSA, 2019).

be lower in cows fed RSM than those fed WDG. Previously, the reduction of milk I content and the feed-to-milk transfer efficiency of I have been prevalently reported when dairy cows consumed rapeseed or by-products made from rapeseed as the main protein source (Franke, Meyer, Wagner, & Flachowsky, 2009; Schöne, Spörl, & Leiterer, 2017; Trøan et al., 2018). The reduced I transfer efficiency was induced by glucosinolates and their degradation compounds, present at relatively higher levels in rapeseed than in other widely used feed protein sources (EFSA, 2008), act as antagonists that reduce I uptake by the thyroid and the transfer of I into milk (Papas et al., 1979). In the present study, the most abundant glucosinolate detected in RSM was progoitrin, present at an average concentration of 1.24 mg/g DM, comparable to that in the report of EFSA (2008) about glucosinolates content in rapeseed meal made from the double-low varieties.

Cows fed RSM had a higher feed-to-milk transfer efficiency of Se than their counterparts fed WDG, while milk Se concentration was not affected by the type of protein source. The concentration of Se was lower in RSM-containing diets than in WDG-containing diets, and the similar milk concentrations resulted in a higher Se transfer efficiency. Findings from a previous work suggested a potentially lower availability of Se in rapeseed meal than in soybean meal based on the observation of decreases in milk Se concentration and intake/output ratio of Se when rapeseed meal was replaced by soybean meal in the diet of dairy cows (Sustala, Trinacty, Illek, Kudrna, & Sustova, 2003).

### 4.5. Potential impacts of seaweed supplementation and concentrate protein source on UK consumers' mineral intakes

In the UK, 37 % of I intake in the adult population comes from milk and dairy products, while these contributions are increased to 40–64 % for children and adolescents (NDNS, 2020). However, studies have shown that milk I concentrations may be substantially lower under organic milk production management (Stevenson, Drake, & Givens, 2018; Zwierzchowski & Ametaj, 2018), although not consistent across all studies (Qin et al., 2021), or during summer (Qin et al., 2021; Stergiadis et al., 2021). In addition, a UK-based study suggested that sub-optimal I intake was observed in 23 % of female adolescents 11–18 years of age and 10 % of women 19 + of age (Miller, Spiro, & Stanner, 2016). Therefore, actions to improve milk I content when production includes high levels of pasture intake (which is negatively correlated to milk I concentrations; Qin et al., 2021; Stergiadis et al., 2021) would maintain a constant supply of I to the population and reduce the risk of further deficiencies. Current milk intakes in the present study were estimated from energy intakes and percentages of energy intake from milk for the different demographics in the UK's National Diet and Nutrition Survey (NDNS, 2020) and the average fat content of milk according to the McCance and Widdowson's composition of foods integrated dataset on the nutrient content of the UK food supply (CoFID, 2021). Based on the results of the present study, current milk intakes in the UK, and I's RNI (EFSA, 2019; SACN, 2014), consumption of milk produced from cows fed seaweed, relative to cows from the control group, would provide 52–120 μg more I per day in different consumer demographics. This would increase the contribution to RNIs for I from 73 % to 245 % for children 1.5–3.0 years of age, from 33 % to 113 % for children 4–10 years of age, from 18 % to 62 % for adolescents 11–18 years of age, from 18 % to 60 % for adults 19–64 years of age, from 25 % to 82 % for adults over 65 years of age and from 9 % to 30 % for pregnant and lactating women (the status of pregnant and lactating women was assessed according to EFSA (2019) RNI, the status of other age groups was assessed according to SACN (2014) RNI; Table 6). In addition, excluding rapeseed meal from dairy diets would increase contribution to I's RNIs from 135 % to 185 % for children 1.5–3.0 years of age, from 62 % to 85 % for children of 4–10 years of age, from 34 % to 47 % for adolescents 11–18 years of age, from 33 % to 45 % for adults 19–64 years of age, from 45 % to 62 % for adults over 65 years of age and from 16 % to 22 % for pregnant and lactating women.

In order to prevent hypothyroidism, hyperthyroidism and thyrotoxicosis, the upper limit (UL) of I intake for children and adolescents is set by the European Food Safety Authority, as 200 μg/day for children 1–3 years old, 250–300 for children 4–10 years old, 450–500 μg/day for adolescents, and 600 μg/day for adults (EFSA, 2019). The milk with the highest I content in this study (S-WDG, 816 μg/kg milk) would require consumption of 713 mL before reaching the UL of I intake in adults and approximately 565 mL before reaching the UL for adolescents; although given that seaweed-fed cows received marginally higher I intake than the regulation and milk I content may be lower if cows are fed less seaweed following the regulation. In any case, the milk produced by seaweed supplement does not pose a potential health risk as these age groups consume far lower amounts than the risky amounts. However, reaching the UL of I intake through the consumption of S-WDG milk would require 238 mL for children 1–3 years old and 327 mL for children 4–10 years old. Even the consumption of S-RSM milk, which contained less I as rapeseed meal was the main protein source, would require approximately only 315 mL for children 1–3 years old and 434 mL for children 4–10 years old to reach their UL of I intake. Consuming milk with such high I content may raise concerns in case of young children, and in particular, for children 1.5–3.0 years of age that already have a higher recorded milk intake than those above (247 mL/d; NDNS, 2020) and the generic recommendation for milk consumption from UK's National Health Service is 350 mL.

Based on the results of the present study, current milk intakes (NDNS, 2020), and I's RNI in the UK (SACN, 2014), notably consumption of milk from cows fed rapeseed and without seaweed supplementation, sharply reduced the contribution of milk to I's RNI to 25–56 % for children, 14 % for adolescents, 12–24 % for adults, and just 8 % for pregnant/lactating women. Given the drastic reduction of milk I concentration when rapeseed meal is fed, which is in line with several previous studies (Franke, Meyer, Wagner, & Flachowsky, 2009; Schöne et al., 2017; Trøan et al., 2018), it can be recommended that additional I is supplemented in cow diets that contain rapeseed to prevent the risk of further I deficiency in populations that rely heavily on milk and dairy products for I supply.

This study provided evidence that feeding seaweed to dairy cows considerably increases milk I concentrations and this will have a subsequent effect on the contribution of milk to the RNI of I in the different consumer demographics. Despite this being a highly potent practice to maintain milk I concentrations in cases that farming practices may cause a reduction, such as organic farming, rapeseed feeding or high pasture intake during the summer/grazing season (Franke, Meyer, Wagner, & Flachowsky, 2009; Qin et al., 2021; Schöne et al., 2017; Stergiadis et al., 2021; Stevenson et al., 2018; Trøan et al., 2018), extreme care should be taken when feeding seaweed to dairy cows, because the increase of milk I concentrations can be drastic, which poses a nutritional risk for young children, particularly 1–3 years of age. It should however be noted that the nutritional calculations in the present study assume that consumers would have access only to milk that has been produced exclusively by seaweed-fed cows, something which is not the case at present at retail level. In practice, milk from seaweed-fed cows may be mixed in the dairy plant with milk from farms that do not feed seaweed (e.g. feeding diets similar to the C-WDG in the present experiment). The mixing with milk of normal composition would dilute the composition of the milk from seaweed-fed cows and would reduce the risk of overconsumption of I. Similarly, mixing milk from rapeseed-fed cows with milk of normal composition in the dairy plant would dilute the negative impact of rapeseed on milk I concentrations, although rapeseed is much more widely used animal feed than seaweed at the moment.

Based on the results of the present study, current milk intakes (NDNS, 2020) and Ca's RNI (SACN, 2014) in the UK, consumption of milk produced from cows fed seaweed would provide 5.2–12.1 mg less Ca per day in different consumer demographics when compared with milk from non-supplemented cows. This would reduce the contribution to RNIs for Ca from 76.2 % to 72.8 % for children 1.5–3.0 years of age,

from 36.7 % to 35.1 % for children 4–10 years of age, from 14.5 % to 13.9 % for adolescents 11–18 years of age, from 18.7 % to 17.8 % for adults 19–64 years of age, from 25.6 % to 24.4 % for adults over 65 years of age and from 11.5 % to 10.9 % for pregnant and lactating women. These changes are rather small and thus cannot be concluded that they will be associated with any impact on consumers' health. The RNIs of Cu and Mn range from 400 to 1,500 μg/d and 0.5–3.0 mg/d, respectively (EFSA, 2019), depending on age and sex, and any differences in milk Cu and Mn concentrations as a result of feeding seaweed or rapeseed, respectively, would cause a marginal impact on the contribution towards RNI and cannot be associated with potential effects on human health. As in previous studies (Newton et al., 2021; Rey-Crespo, López-Alonso, & Miranda, 2014), the present work found only traces of heavy metals (below LOQ) and therefore milk is not a significant source of heavy metals in human diets.

## 5. Conclusions

Dietary supplementation of the seaweed *Saccharina latissima* and the substitution of wheat distillers' grains (WDG) with rapeseed meal (RSM) in cows' diet did not affect production, efficiency and milk basic composition. The seaweed supplement decreased milk Ca and Cu concentrations without affecting their transfer efficiencies, thus it is unclear whether this reduction relates to the lower availability of these minerals when seaweed is fed. Substitution of WDG with RSM decreased (or tended to decrease) milk Mg, P, and Mn concentrations and their transfer efficiencies from feed to milk. It also increased the feed-to-milk efficiencies of Ca, Na, and Se and decreased that of Fe without changing their concentrations in milk. The decreased transfer efficiencies of Mg, P, Fe, and Mn in RSM-fed cows were potentially associated with the lower biological availability of these minerals in RSM than in WDG; while the non-significant effect on milk Ca and Na concentrations, despite the higher transfer efficiencies, potentially relates to a homeostatic mechanism that reduces the effect of diet. Milk I concentration was increased markedly to 3.3-fold when seaweed was fed and reduced by 27 % when WDG was replaced by RSM. When cows were fed seaweed, a higher I intake was observed while the feed-to milk transfer efficiency of I was not affected. In contrast, the decrease of milk I concentration by RSM can be explained by the reduced transfer efficiency of I, potentially due to the glucosinolates content. The non-significant interaction between seaweed supplementation and main protein source suggested that the effect of seaweed supplementation on milk mineral composition and mineral transfer efficiencies is consistent, no matter rapeseed or wheat distillers' grains were provided as the main dietary protein source. Seaweed supplementation can benefit the I intake of certain populations or demographics when aiming to reduce I deficiency rates; although care should be taken when milk from seaweed-fed cows is offered to children 1.5–3.0 years, as they might exceed their I upper tolerable limit based on their current recorded milk intakes in the UK. Consumption of milk from cows with rapeseed meal as the main protein source would increase the risk of I deficiency in the population, particularly more in demographics with higher I requirements or lower milk intakes (e.g. adolescent, pregnant or lactating women).

## CRediT authorship contribution statement

**Nanbing Qin:** Methodology, Validation, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Ásta H. Pétursdóttir:** Methodology, Investigation, Resources, Data curation, Supervision, Writing – review & editing. **David J. Humphries:** Methodology, Resources, Validation, Investigation, Data curation, Writing – review & editing, Supervision. **Natasa Desnica:** Methodology, Validation, Investigation, Data curation, Writing – review & editing. **Eric E. Newton:** Methodology, Validation, Investigation, Data curation, Writing – review & editing, Data curation. **Aila Vanhatalo:** Methodology, Investigation, Resources, Supervision, Writing – review & editing. **Anni Halmemies-Beauchet-Filleau:** Methodology, Validation, Investigation, Data curation, Writing – review & editing. **Luke Bell:** Methodology, Validation, Investigation, Data curation, Writing – review & editing. **D. Ian Givens:** Methodology, Writing – original draft, Writing – review & editing. **Darren T. Juniper:** Methodology, Writing – original draft, Writing – review & editing. **Helga Gunnlaugsdóttir:** Investigation, Data curation, Resources, Writing – review & editing. **Sokratis Stergiadis:** Conceptualization, Methodology, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Data availability

Data will be made available upon request to the corresponding author.

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## Appendix A. Supplementary data

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