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Effects of protein source and seaweed supplementation on mineral intake, milk mineral concentrations, and iodine transfer efficiency from feed to milk in lactating dairy cows

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ABSTRACT

The objective of this experiment was to study the effects of protein source and seaweed supplementation on intake, milk concentration, and transfer efficiency of minerals from feed to cow milk. Twelve multiparous Nordic Red cows were used in a cyclic change-over study with a 2 × 3 factorial arrangement of treatments. The cows were divided into block 1 (DIM 151 ± 12.7 d, milk yield 29.7 ± 4.08 kg/d) and block 2 (DIM 115 ± 15.1 d, milk yield 39.5 ± 3.45 kg/d). The study consisted of three 17-d periods with an 18-d washout between periods. Cows were randomly assigned to treatments which included rapeseed cake (RSC) or pea (MFP) as protein feed without (CON) or with seaweed supplementation (*Ascophyllum nodosum* [AN; 57 g DM/d] or *Laminaria digitata* [LD; 7.8 g DM/d]). Cows were offered a concentrate mix of seaweed, sugar beet pulp, mineral supplement, and molasses, and ad libitum partial mixed ration consisting of grass silage, barley, and protein feed. Diets had I concentration (mg/kg DM) of 4.1 for RSC-CON, 6.8 for RSC-AN and RSC-LD, 1.9 for MFP-CON, and 4.7 for MFP-AN and MFP-LD. Despite decreased I intake, I transfer efficiency and concentration in milk were increased when rapeseed was substituted with pea. Seaweeds increased the intake, transfer efficiency, and milk concentration of I. Seaweeds increased the intake of As, but highly toxic trace elements (As, Cd, Cr, Hg, Pb) were not found in milk on any treatment. Milk I concentration was very low, especially in RSC-CON (38 μg/kg), possibly due to the unexpectedly high glucosinolate concentration (48 mmol/kg DM) of rapeseed. Milk I concentration on MFP-CON and I transfer efficiency on all pea diets were also lower than usual. In conclusion, pea diets resulted in more favorable milk I concentration for human consumers than rapeseed high in glucosinolates.

Key words: protein feed, macro algae, dairy cow, milk mineral composition, iodine

INTRODUCTION

Iodine nutrition of animals and humans is interwoven and linked to I geochemistry. Milk and dairy products are very important sources of I. In European countries, they contribute 12% to 53% of I intake (Bath et al., 2022). Pregnant women and infants are recognized as high-risk groups for I deficiency; in Finland, 66% to 70% of pregnant women and 29% of infants at the age of 3 mo experience I insufficiency (Miles et al., 2022). The only known and vital function of I in the body is as a constituent of thyroid hormones (Suttle, 2022). Depending on the severity of I deficiency and life-stage group, the consequences include impaired cognitive and neurological functions, physical development, increased hypothyroidism, goiter, and mortality (Bertinato, 2021). In livestock, I deficiency decreases fertility, growth, and milk yield (Suttle, 2022).

The I concentration of milk is highly variable (Koiranen and Stabel-Taucher, 1976; Niero et al., 2020), depending on factors such as geographical location, use and type of mineral supplements, presence of goitrogenic compounds in feeds, season, and use of disinfectant iodophors in cleaning (Miller et al., 1975; van der Reijden et al., 2018). The I concentration of organic milk is often lower than that of conventional milk (EFSA, 2013; van der Reijden et al., 2018), even though the I fortification of organic feeds is allowed in the European Union (**EU**) using potassium iodide (KI) and calcium iodate anhydrous

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(**CaI**; European Commission, 2021), which is similar to conventional production (European Commission, 2015). However, the interpretation of the production principles listed in the organic regulation may vary depending on the country, leading to differences in management of organic dairy production systems and use of I fortified feeds. Therefore, natural I-rich substances are an interesting alternative for organic milk production.

Seawater is an important reservoir of I, which is enriched in marine organisms, especially brown algae (seaweeds; Fuge, 2005). Brown seaweeds *Ascophyllum nodosum* and *Laminaria digitata* are species particularly high in I (Nitschke et al., 2018). Indeed, seaweeds have been identified as a potential mineral supplement for ruminants (Rey-Crespo et al., 2014). However, high concentrations of highly toxic trace elements are not uncommon in seaweeds (Qin et al., 2023), which may limit their use in animal diets.

Peas (*Lathyrus oleraceus*, syn. *Pisum sativum*) are appealing protein feed in organic production due to their ability to fix atmospheric N. However, protein source can also influence mineral nutrition; for example, Ca, P, and Zn are typically higher in rapeseed byproducts than in pea (Heuzé, 2017, 2020). Rapeseed byproducts are commonly used in dairy cow nutrition in Finland, but they contain goitrogenic compounds, such as glucosinolates (**GSL**), and their hydrolysis products, isothiocyanates, thiocyanates, and oxazolidine-2-thiones. The GSL can inhibit the metabolism of I either by impairing thyroid uptake into thyroxine (**T4**; cyanogenic goitrogens), or the transformation of T4 into the active form of thyroid hormone, triiodothyronine (**T3**; thiouracil-like goitrogens; Fenwick and Heaney, 1983; Suttle, 2022). The effects of cyanogenic goitrogens can be alleviated with sufficient dietary I supply, whereas that of thiouracil-like goitrogens cannot (Fenwick and Heaney, 1983). With plant breeding, the concentrations of GSL have been successfully decreased in rapeseed (Diederichsen and McVetty, 2011), however, even rapeseed with minimal concentrations of GSL has been reported to influence I transfer from feed to milk (Trøan et al., 2018).

The objective of this experiment was to study the effect of protein source (rapeseed cake vs. pea) and seaweed supplementation on mineral intake, transfer efficiency of minerals from diet to milk, and milk mineral composition of lactating dairy cows. We hypothesized that protein source and seaweed supplementation influence mineral intake and mineral profile of milk. The substitution of rapeseed cake with pea and the addition of seaweeds to the diets of lactating dairy cows was hypothesized to increase the I concentration of milk due to the presence of goitrogenic compounds in rapeseed and high concentration of I in seaweeds.

MATERIALS AND METHODS

Animals, Experimental Design, and Diets

Twelve multiparous Nordic Red cows were used in a cyclic change-over study with a 2 × 3 factorial arrangement of treatments and three 17-d periods, the last 4 d of each serving as the sampling period. Each period was followed by an 18-d washout, making the total experiment duration 87 d. The washout was implemented between experimental periods to ensure the complete elimination of any potential carryover effects of the dietary treatments on milk mineral concentrations. The duration of 18 d was selected because the equilibrium between I intake and secretion in milk has been reported to occur after 7 to 10 d of daily dosing (Miller et al., 1975), and the half-life of thiocyanate has been reported to vary from 1 d to over 2 wk (Bliss and O'Connell, 1984). The cows were divided into 2 blocks based on pre-experiment milk yield (block 1: DIM 151 ± 12.7 d, milk yield 29.7 ± 4.08 kg/d; block 2: DIM 115 ± 15.1 d, milk yield 39.5 ± 3.45 kg/d) and randomly assigned to 1 of 6 dietary treatments within the blocks. The treatments included cold-pressed rapeseed cake (**RSC**; Hauhon Myllärit, Hauho, Finland) or farm-grown milled feed peas (**MFP**) as the protein source, with or without seaweed supplementation (**CON**). The seaweed species studied were *Ascophyllum nodosum* (**AN** diet, 57 g DM/d; commercial seaweed producer in Iceland) and *Laminaria digitata* (**LD** diet, 7.8 g DM/d; commercial seaweed producer in Iceland). The diets were formulated to be isonitrogenous in terms of N supplied by the protein feed (rapeseed cake or pea). The I concentration of 0.9 mg/kg DM was targeted on diets without seaweed supplementation (RSC-CON and MFP-CON) based on I concentration of feedstuffs in Finnish feed tables (Luke, 2025) and information provided by the feed manufacturers. This concentration corresponds to the current Finnish I intake recommendation for dairy cow diets without goitrogenic feed compounds (Luke, 2025). On seaweed diets (RSC-AN, RSC-LD, MFP-AN, and MFP-LD), I concentration of 4 mg/kg DM was targeted, which is below the allowed maximum I concentration in complete feed for dairy cows in the EU (5 mg/kg with 12% moisture content of feed; European Commission, 2015). This limit corresponds to I concentration of 5.68 mg/kg DM.

Cows had ad libitum access to water, salt blocks, and partial mixed ration (**PMR**) consisting of grass silage, barley, and protein feed. Concentrates consisted of sugar beet pulp (Opti Leike, Lantmännen Feed, Turku, Finland), mineral-vitamin supplement (Seleeni-E-Melli TMR, Lantmännen Feed), molasses (Farmarin Seosmelassi, Hankkija Ltd., Hyvinkää, Finland), and seaweed. All experimental diets contained the same amount of mineral-vitamin supplement (239 g DM/d). No seaweed was given during the 18-d washout period, and if the dietary protein source changed between periods, the change was made in the beginning of washout period. The forage:concentrate ratio of diets was 65:35 on DM basis. Rapeseed cake originated from seeds grown in the Hämeenlinna region, Finland. Cows were housed in individual tiestalls and milked 2 times a day at 0600 and 1700 h. Iodine-free teat tipping solution was used during the experiment. The orts were weighed after morning milking.

Measurements, Sampling, and Chemical Analysis

Feed intake and milk yield of the cows were recorded daily throughout the experiment, but only the measurements on d 14 to 17 of each period were used for statistical analysis. The intake of PMR was recorded with scaled feeding troughs (Roughage Intake Control System, Insentec BV, Marknesse, the Netherlands). Representative samples of diet ingredients were collected on d 14 and 16 of each period. Silage samples were combined by period, and other feed ingredients were combined over all periods; both were stored frozen (−20°C) until analyses. Milk yield was measured with milk meters (WB Auto Sampler, Tru-Test Datamars, Auckland, New Zealand). Milk samples were collected into acid-washed (8% HNO_3, NORMATOM for trace metal analysis, VWR International Oy, Helsinki, Finland) plastic bottles over 4 consecutive milkings starting on d 15 at 0600 h and combined proportionally to milk yield by cow within period. Milk samples destined for mineral analysis were stored frozen (−20°C) until analyses. The concentrations of macrominerals (Ca, K, Mg, Na) and trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Hg, I, Mn, Mo, Ni, Pb, Se, Sn, Zn) were quantified from feeds and milk samples. For simplification, the following groupings were used: essential trace elements (Cu, Fe, I, Mn, Mo, Se, Zn), nonessential trace elements (Al, Co, Sn, Ni), and highly toxic trace elements (As, Cd, Cr, Hg, Pb). Milk samples were freeze-dried (Christ, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany), and silage samples oven-dried, except for milk I, that was analyzed in liquid samples. The minerals of concentrates were analyzed from air-dry samples. Analyses of I in feed were conducted at AGROLAB LUFA GmbH (Kiel, Germany) according to European Parliament (2017; DIN EN 17050:2017-11). All other mineral analyses were conducted at Matís, Reykjavík, Iceland. Following NMKL method 186 (Julshamn et al., 2007; modified in-house), samples for analysis of macrominerals (Ca, K, Mg, Na) and trace elements (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Mo, Ni, Pb, Se, Sn, Zn) were digested using an ultraWAVE Acid Digestion System (Milestone Inc., Italy). Briefly, 0.150 to 0.200 g of sample was weighed in quartz digestion vessels. Upon addition of 1 mL of concentrated HNO_3 and 1 mL of H_2O_2 (30%), digests were heated to 240°C for 30 min. Digests were diluted to 50 mL with Milli-Q water, and further diluted 50-fold in 2% (vol/vol) HNO_3 for macromineral analysis. Blank digests and certified reference materials (**CRM**), including skim milk powder (BCR-063R; Joint Research Center of European Commission, Belgium) and either brown seaweed hijiki (CRM 7305-a; National Metrology Institute of Japan) or fish proteins (CRM DORM-4; NRC of Canada), were included with each digestion batch for quality assurance and quality control. All samples were analyzed in duplicate using inductively coupled plasma MS (**ICP-MS**, Agilent 7900, Agilent Technologies, Singapore). External calibration curve (7–9 concentration levels) was used for quantification (ICP grade single element standards, 2% HNO_3, LabKings, the Netherlands). Analysis was repeated if the difference in concentration was >10% between duplicates. Run stability was monitored by applying 1 mg/L in 2% HNO_3 externally.

For analysis of I in milk, 100 µL of liquid milk was diluted with 9.9 mL of the standard diluent of 5 µg/L Rh (E3RH# PrimAg element reference solution, Romil Ltd., Cambridge, United Kingdom) in 2% tetramethylammonium hydroxide (**TMAOH**, Sigma-Aldrich). One gram of skim milk powder (ERM BD150, European Reference Materials, Geel, Belgium) containing 1.73 mg I/kg (±0.14 mg/kg) was used as CRM and dissolved in 10 mL of distilled water to yield a homogeneous solution approximating skimmed cow milk containing 173 ug/L of iodine. Samples and CRM were centrifuged (20°C, 30 min, 3,220 × *g*) in filter tubes with polyvinylidene fluoride membrane to remove any fat which could block the nebulizer. Before I analysis with ICP-MS, system was flushed with ultrapure water for 10 min followed by flushing with the alkaline solution (2% TMAOH [aq]) for 2 to 3 h. The quantification of I was made using 7 concentration levels of an external standard curve (0 to 10 µg/L, 1,000 mg/L iodide ion reference solution [Si04#, Romil Ltd.] in 2% TMAOH). Analyses of standard solutions and CRM were repeated multiple times during each run.

The GSL of rapeseed cake were extracted and quantified at the University of Reading (UK), as described in Jasper et al. (2020), using the modifications of Qin et al. (2023). Authentic standards (Phytoplan, Heidelberg, Germany) were used to quantify concentrations of all other GSL except gluconapoleiferin, which was semiquantified using gluconapin as a proxy. The purity of the standards was 99.07% for progoitrin, 98.66% for gluconapin, 96.19% for 4-hydroxyglucobrassicin, 99.22% for glucobrassicanapin, 99.68% for glucoerucin, 99.38% for glucobrassicin, 98.38% for gluconasturtiin, and 94.78% for

Table 1. Mineral composition of the experimental feeds[1]

Item (g/kg DM, unless noted)	Grass silage	Barley	Sugar beet pulp	Mineral-vitamin supplement	Molasses	Rapeseed cake	Pea	*Ascophyllum nodosum*	*Laminaria digitata*
Macrominerals, g/kg DM									
Ca	6.59	0.413	4.78	233	4.45	7.36	1.19	15.2	24.1
K	35.6	6.03	5.40	0.491	38.3	10.3	11.5	18.3	91.1
Mg	2.66	1.51	2.17	74.9	0.699	5.40	1.41	7.60	7.32
Na	1.49	0.061	0.738	84.1	5.65	0.033	0.078	30.6	40.2
P	3.03	4.33	3.17	0.157	1.38	11.7	4.86	1.01	3.07
Essential trace elements, mg/kg DM									
Cu	8.60	8.82	14.1	1,313	3.14	12.2	12.6	2.96	5.67
Fe	166	87.2	172	6,148	363	201	155	651	765
I	0.908	ND	1.23	119	0.331	31.1	ND	1,076	8,339
Mn	47.7	19.5	68.7	1,310	27.9	104	17.2	57.6	82.5
Mo	3.82	1.32	0.876	0.527	0.675	3.52	12.1	1.27	0.605
Se	0.044	0.048	0.402	47.5	0.177	0.231	0.050	0.145	0.292
Zn	43.8	65.3	73.5	1,817	33.4	117	71.5	9.34	23.5
Nonessential trace elements, mg/kg DM									
Al	87.1	20.3	119	2,940	46.1	17.6	82.9	380	345
Co	0.142	0.017	0.633	49.1	0.957	0.183	0.151	3.29	0.856
Ni	3.31	0.457	1.48	7.35	4.23	1.00	2.17	1.29	1.54
Sn	0.029	0.012	0.018	1.35	0.060	0.007	0.013	0.018	0.031
Highly toxic trace elements, mg/kg DM									
As	0.002[2]	ND	ND	2.39	ND	ND	ND	46.5	164
Cd	0.083	0.025	0.200	0.043	0.021	0.138	0.034	0.872	0.637
Cr	7.75	0.704	4.22	3.39	0.452	0.170	1.01	0.963	1.24
Hg	ND	ND	ND	ND	ND	ND	ND	0.040	0.025
Pb	0.223	ND	0.113	1.62	0.217	0.034	0.155	ND	0.087

[1] ND = not detected, concentration below detection limit.

[2] Concentration was below the detection limit in samples from periods 1 and 2 and therefore assumed to be 0 mg/kg DM in these samples; concentration 0.064 mg/kg DM in sample from period 3.

4-methoxyglucobrassicin. The purities of the compounds were considered when calculating the concentrations.

Calculations and Statistical Analysis

Daily DMI of individual cows was calculated as the difference between DM offered and DM residue. For converting milk I concentration from milligrams per liter to grams per kilogram, analyzed milk fat (on average, 42.5, 43.0, and 43.4 g/kg for RSC-CON, RSC-AN, and RSC-LD, respectively, and 44.2, 43.3, and 43.1 g/kg for MFP-CON, MFP-AN, and MFP-LD, respectively; our unpublished data) and DM concentration, and milk volume-to-weight conversion factors and equation of USDA (1965) were used. Mineral transfer efficiency from feed to milk (%) was calculated as follows:

$$100 \times \frac{\text{milk mineral concentration} \times \dfrac{\text{milk yield}}{1{,}000}}{\text{diet mineral concentration} \times \text{DMI}}.$$

The experimental data were subjected to ANOVA using the Mixed procedure of SAS 9.4 version (SAS Institute Inc., Cary, NC). Block, period, diet, and the interaction of block × diet were included as fixed effects, and animal within block as random effect in the statistical model. P-values ≤ 0.05 were considered as significant, and $0.05 < P \leq 0.10$ were accepted as a tendency. The sums of squares of the treatment effects were further separated into single df comparisons using orthogonal contrasts. The contrasts were as follows: 1 = the effect of protein source (RSC-CON + RSC-AN + RSC-LD vs. MFP-CON + MFP-AN + MFP-LD); 2 = the effect of addition of seaweeds to the diets (RSC-CON + MFP-CON vs. RSC-AN + RSC-LD + MFP-AN + MFP-LD); 3 = the effect of seaweed species (*A. nodosum* vs. *L. digitata*; RSC-AN + MFP-AN vs. RSC-LD + MFP-LD); 1 × 2 = the interaction of protein source and addition of seaweeds; and 1 × 3 = the interaction of protein source and seaweed species. The normality of the residuals was tested using the Univariate procedure of SAS with the Shapiro–Wilk test, and homoscedasticity was checked visually by inspecting a plot of residuals by predicted values. Logarithmic transformations were used to correct deviations from normality and homoscedasticity of residuals. If transformations were needed, LSM are reported from statistical analysis of untransformed

Table 2. Glucosinolate concentrations of rapeseed cake

Type	Glucosinolate common name	Chemical name	g/kg DM	mmol/kg DM	Proportion of total glucosinolate concentration; %
Aliphatic					
		Pentyl glucosinolate	0.691	1.56	3.36
	Progoitrin	2-Hydroxy-3-butenyl glucosinolate	2.07	4.84	10.1
	Gluconapoleiferin	2-Hydroxy-4-pentenyl glucosinolate	0.082	0.192	0.402
	Gluconapin	3-Butenyl glucosinolate	4.10	9.55	20.0
	Glucobrassicanapin	4-Pentenyl glucosinolate	0.068	0.153	0.320
	Glucoerucin	4-Methylthiobutyl glucosinolate	0.894	1.95	4.07
Indole					
	Glucobrassicin	3-Indolylmethyl glucosinolate	1.75	3.60	7.54
	4-hydroxyglucobrassicin	4-Hydroxy-3-indoylmethyl glucosinolate	7.17	14.3	29.9
	4-methoxyglucobrassicin	4-Methoxy-3-indolylmethyl glucosinolate	1.09	2.17	4.54
Aromatic					
	Gluconasturtiin	2-Phenylethyl glucosinolate	4.37	9.49	19.9
Total			22.3	47.8	100

values and LSM, SEM, and *P*-values from analysis of transformed data. Spearman rank correlation coefficients were calculated with the Corr procedure of SAS to estimate the correlations between milk mineral concentrations.

RESULTS

Composition of Feeds

The mineral composition of feeds is presented in Table 1. The concentration of Ca, Mg, I, Mn, and Se in pea was lower than in rapeseed cake and vice versa for Mo, Al, Ni, and Pb. The concentration of I in *L. digitata* was ~8 times higher than in *A. nodosum*. As I supply from seaweeds was designed to be equal, the proportion of seaweeds differed in the diets (0.3 g/kg DM vs. 2.5 g/kg DM for diets containing *L. digitata* vs. *A. nodosum*, respectively). The targeted I concentration of diets was 0.9 mg/kg DM for RSC-CON and MFP-CON and 4 mg/kg DM for seaweed-containing diets (RSC-AN, RSC-LD, MFP-AN, and MFP-LD). However, these concentrations were exceeded on all diets by the factor of 1.2 to 4.6 (Supplemental Table S1, see Notes). In RSC-CON, the dietary I concentration was 4.1 mg/kg DM and in MFP-CON 1.94 mg/kg DM. In seaweed diets supplemented with rapeseed cake (RSC-AN and RSC-LD), the dietary I concentration was on average 6.83 mg/kg DM and in pea-supplemented seaweed diets (MFP-AN and MFP-LD) 4.68 mg/kg DM. The glucosinolate concentration of rapeseed cake was 47.8 mmol/kg DM (Table 2). The major GSL in RSC were 4-hydroxyglucobrassicin (30% of total), gluconapin (20% of total), gluconasturtiin (20% of total), and progoitrin (10% of total; Table 2).

Intake of Feed, Nutrients, and Minerals and Digestibility of Nutrients

Diets had no effect ($P > 0.05$) on PMR or concentrate intake (Table 3). The substitution of rapeseed cake with pea resulted in lower ($P < 0.001$) intake of Ca, Mg, I, Mn, Se, Zn, and Cd (Table 3). In contrast, the intake of Mo, Al, Ni, and Pb was higher ($P < 0.001$) for pea than rapeseed cake diets. The addition of seaweeds to the diet increased intakes of Na ($P = 0.006$), I ($P < 0.001$), Se ($P = 0.048$), and As ($P < 0.001$), and tended to decrease that of Pb ($P = 0.051$). Intakes of Na ($P < 0.001$), Co ($P < 0.001$), As ($P < 0.001$), and Cd ($P = 0.020$) were higher with *A. nodosum* than with *L. digitata* diets, whereas the opposite was true for intake of I ($P = 0.002$).

Milk Mineral Composition and Transfer Efficiency from Feed to Milk

The substitution of rapeseed cake with pea resulted in decreased milk yield ($P = 0.011$) and increased concentration of DM ($P = 0.021$), Ca ($P = 0.011$), K ($P = 0.007$), and I ($P < 0.001$) in milk (Table 4). The transfer efficiency of I ($P < 0.001$), Ca ($P = 0.018$), and Mo ($P = 0.006$) from feed to milk was higher for pea than for rapeseed cake diets (Table 5). The addition of seaweeds to the diet increased the concentration of I ($P < 0.001$) in milk (Table 4), as well as the transfer efficiency of I ($P < 0.001$; Table 5). The increase of I concentration and transfer efficiency of I induced by seaweed addition were higher for pea than for rapeseed cake diets ($P < 0.001$ for 1 × 2 interaction). On rapeseed diets, *A. nodosum* resulted in higher Na transfer efficiency than *L. digitata*, but the opposite was observed for pea diets ($P = 0.043$ for 1 × 3 interaction).

Table 3. Effect of substituting mineral supplement with *Ascophyllum nodosum* or *Laminaria digitata* seaweeds on DM and mineral intake on rapeseed meal or pea-supplemented dairy cow diets

	Treatment[1]							Significance[2]				
Item	RSC-CON	RSC-AN	RSC-LD	MFP-CON	MFP-AN	MFP-LD	SEM	1	2	3	1 × 2	1 × 3
Intake (kg/d)												
Partial mixed ration DM[3]	21.3	21.4	21.0	20.8	21.1	21.1	0.60	0.356	0.729	0.578	0.512	0.501
Concentrate DM[4]	1.68	1.74	1.69	1.68	1.74	1.69						
Macromineral intake,[5] g/d												
Ca	175	175	173	164	165	165	3.0	<0.001	0.972	0.385	0.560	0.592
K	591	596	584	584	594	595	16.3	0.899	0.524	0.530	0.451	0.463
Mg	76.3	76.8	75.7	69.0	69.9	69.6	1.46	<0.001	0.614	0.382	0.528	0.587
Na	44.7	46.2	44.4	44.3	46.1	44.7	0.63	0.694	0.006	<0.001	0.288	0.447
Essential trace element intake,[5] mg/d												
Cu	523	523	519	522	523	523	5.4	0.682	0.801	0.513	0.457	0.484
Fe	4,987	4,984	4,898	4,882	4,928	4,903	88.5	0.154	0.863	0.186	0.297	0.452
I	92.7	154	159	43.5	104	109	1.39	<0.001	<0.001	0.002	0.788	0.537
Mn	1,383	1,372	1,358	1,225	1,227	1,226	24.6	<0.001	0.418	0.547	0.347	0.589
Mo	71.1	70.8	68.7	90.1	91.0	91.1	2.31	<0.001	0.824	0.374	0.257	0.315
Se	13.1	13.2	13.1	12.8	12.9	12.9	0.03	<0.001	0.048	0.661	0.442	0.611
Zn	1,689	1,683	1,665	1,592	1,601	1,603	30.5	<0.001	0.836	0.572	0.356	0.487
Nonessential trace element intake,[5] mg/d												
Al	2,313	2,296	2,252	2,415	2,431	2,417	43.6	<0.001	0.415	0.188	0.228	0.473
Co	15.2	15.3	15.1	15.2	15.4	15.2	0.072	0.343	0.151	<0.001	0.358	0.542
Ni	58.1	57.6	56.5	56.5	60.2	60.2	1.53	<0.001	0.642	0.451	0.229	0.384
Sn	0.871	0.863	0.853	0.866	0.868	0.869	0.0143	0.352	0.364	0.486	0.235	0.435
Highly toxic trace elements,[5] mg/d unless noted												
As	0.903	3.50	2.16	0.903	3.50	2.14	0.0181	0.777	<0.001	<0.001	0.834	0.660
Cd	1.87	1.90	1.83	1.68	1.73	1.68	0.042	<0.001	0.516	0.020	0.470	0.592
Cr	127	126	124	125	126	127	3.3	0.634	0.545	0.532	0.210	0.408
Hg, µg/d	ND[6]	2.3	0.2	ND	2.3	0.2						
Pb	4.01	3.90	3.85	4.23	4.17	4.18	0.104	<0.001	0.051	0.658	0.339	0.550

[1] MFP-AN = pea, *Ascophyllum nodosum*; MFP-CON = pea, control without seaweed; MFP-LD = pea, *Laminaria digitata;* RSC-AN = rapeseed cake, *Ascophyllum nodosum*; RSC-CON = rapeseed cake, control without seaweed; RSC-LD = rapeseed cake, *Laminaria digitata*. n = 6 on each treatment; n = 36 in the whole experiment (n represents number of observations used in the statistical analysis).

[2] The effect of (1) protein source (rapeseed meal vs. pea; RSC-CON + RSC-AN + RSC-LD vs. MFP-CON + MFP-AN + MFP-LD); (2) addition of seaweeds to the diets (RSC-CON + MFP-CON vs. RSC-AN + RSC-LD + MFP-AN + MFP-LD); and (3) seaweed species (*Ascophyllum nodosum* vs. *Laminaria digitata*; RSC-AN + MFP-AN vs. RSC-LD + MFP-LD). 1 × 2 = the interaction of protein source in the basal diet and addition of seaweeds; 1 × 3 = the interaction of protein source in the basal diet and seaweed species.

[3] Corrected DMI. Contained grass silage, barley, and protein supplement (rapeseed cake or pea).

[4] Contained molassed sugar beet pulp, molasses, and mineral-vitamin supplement. Diets RSC-AN, RSC-LD, MFP-AN, and MFP-LD also contained seaweed.

[5] Including nutrient intake from molasses.

[6] ND = not detected, concentration below detection limit.

Table 4. Effect of substituting mineral supplement with *Ascophyllum nodosum* or *Laminaria digitata* seaweeds on milk yield and milk mineral composition on rapeseed meal or pea-supplemented dairy cow diets

	Treatment[1]							Significance[2]				
Item	RSC-CON	RSC-AN	RSC-LD	MFP-CON	MFP-AN	MFP-LD	SEM	1	2	3	1 × 2	1 × 3
Milk yield, kg/d	29.4	30.4	29.3	28.1	28.5	28.0	1.45	0.011	0.531	0.192	0.748	0.638
Milk DM, g/kg	133	134	135	137	136	136	1.8	0.021	0.946	0.708	0.337	0.818
Macrominerals, mg/kg												
Ca	1,062	1,075	1,049	1,092	1,120	1,131	28.0	0.011	0.346	0.721	0.365	0.355
K	1,459	1,484	1,477	1,486	1,548	1,573	27.9	0.007	0.025	0.685	0.204	0.475
Mg	110	114	109	109	109	110	3.6	0.484	0.708	0.462	0.913	0.259
Na	456	488	443	453	457	472	26.0	0.913	0.566	0.465	0.972	0.161
Essential trace elements, µg/kg												
Cu	62.5	49.3	46.8	56.1	48.9	47.0	8.08	0.738	0.109	0.778	0.643	0.969
Fe[3]	346	302	342	323	297	279						
	(2.49)	(2.48)	(2.50)	(2.46)	(2.44)	(2.44)	0.058	0.327	0.817	0.817	0.882	0.803
I	38.3	158	166	106	475	419	21.1	<0.001	<0.001	0.204	<0.001	0.092
Mn	26.1	22.3	28.7	25.3	26.0	22.8	4.10	0.770	0.837	0.699	0.964	0.257
Mo[3]	95.2	72.7	79.8	82.1	84.0	80.6						
	(1.96)	(1.85)	(1.88)	(1.88)	(1.90)	(1.90)	0.049	0.914	0.359	0.751	0.239	0.744
Se	30.1	27.9	31.6	30.7	32.8	27.4	3.23	0.866	0.869	0.787	0.959	0.176
Zn	4,463	4,851	4,404	4,415	4,284	4,300	379.9	0.347	0.934	0.465	0.586	0.431
Nonessential trace elements	Al, Co, Ni, and Sn below detection limit[4]											
Highly toxic trace elements	As, Cd, Cr, Hg, and Pb below detection limit[4]											

[1] MFP-AN = pea, *Ascophyllum nodosum*; MFP-CON = pea, control without seaweed; MFP-LD = pea, *Laminaria digitata*; RSC-AN = rapeseed cake, *Ascophyllum nodosum*; RSC-CON = rapeseed cake, control without seaweed; RSC-LD = rapeseed cake, *Laminaria digitata*. n = 6 on each treatment; n = 36 in the whole experiment (n represents number of observations used in the statistical analysis).

[2] The effect of (1) protein source (rapeseed meal vs. pea; RSC-CON + RSC-AN + RSC-LD vs. MFP-CON + MFP-AN + MFP-LD); (2) addition of seaweeds to the diets (RSC-CON + MFP-CON vs. RSC-AN + RSC-LD + MFP-AN + MFP-LD); and (3) seaweed species (*Ascophyllum nodosum* vs. *Laminaria digitata*; RSC-AN + MFP-AN vs. RSC-LD + MFP-LD). 1 × 2 = the interaction of protein source in the basal diet and addition of seaweeds; 1 × 3 = the interaction of protein source in the basal diet and seaweed species.

[3] Logarithmic transformation of milk Fe and Mo concentration; logarithmic values are presented in parenthesis below the original values.

[4] Detection limit for Al <0.5 mg/kg DM (64% of samples below detection limit), Co <0.01 mg/kg DM (97% of samples), Ni <0.04 mg/kg DM (100% samples), Sn <0.01 mg/kg DM (94% of samples), As <0.05 mg/kg DM (100% of samples), Cd <0.002 mg/kg DM (100% of samples), Cr <0.06 mg/kg DM (97% of samples), Hg <0.02 mg/kg DM (100% of samples), and Pb <0.07 mg/kg DM (100% of samples).

Table 5. Effect of substituting mineral supplement with *Ascophyllum nodosum* or *Laminaria digitata* seaweeds on mineral transfer efficiency (excretion in milk:intake in feed; %) on rapeseed meal or pea-supplemented dairy cow diets

	Treatment[1]							Significance[2]				
Item	RSC-CON	RSC-AN	RSC-LD	MFP-CON	MFP-AN	MFP-LD	SEM	1	2	3	1 × 2	1 × 3
Macrominerals												
Ca	17.8	18.4	17.6	18.6	19.1	19.4	0.90	0.018	0.295	0.624	0.562	0.204
K	7.29	7.60	7.43	7.12	7.36	7.37	0.358	0.343	0.158	0.657	0.948	0.620
Mg	4.24	4.41	4.17	4.38	4.36	4.46	0.191	0.306	0.734	0.616	0.918	0.235
Na	30.5	30.8	28.4	28.3	27.1	30.2	1.31	0.207	0.776	0.787	0.565	0.043
Essential trace elements												
Cu	0.343	0.280	0.267	0.278	0.257	0.246	0.0454	0.274	0.162	0.753	0.539	0.991
Fe	0.203	0.179	0.206	0.170	0.159	0.166	0.0287	0.208	0.725	0.558	0.959	0.732
I	0.990	3.02	2.87	5.35	14.3	13.5	0.707	<0.001	<0.001	0.517	<0.001	0.659
Mn	0.056	0.048	0.060	0.055	0.056	0.053	0.0086	0.975	0.883	0.573	0.920	0.399
Mo[3]	4.04	3.11	3.37	2.38	2.53	2.57	0.407					
	(0.585)	(0.482)	(0.503)	(0.349)	(0.378)	(0.397)	0.0556	0.006	0.579	0.717	0.188	0.993
Se	6.67	6.26	6.95	6.26	7.00	6.30	0.747	0.858	0.788	0.995	0.715	0.322
Zn	7.85	8.61	7.73	7.52	7.23	7.44	0.805	0.176	0.888	0.549	0.621	0.324

[1] MFP-AN = pea, *Ascophyllum nodosum*; MFP-CON = pea, control without seaweed; MFP-LD = pea, *Laminaria digitata*; RSC-AN = rapeseed cake, *Ascophyllum nodosum*; RSC-CON = rapeseed cake, control without seaweed; RSC-LD = rapeseed cake, *Laminaria digitata*. n = 6 on each treatment; n = 36 in the whole experiment (n represents number of observations used in the statistical analysis).

[2] The effect of (1) protein source (rapeseed meal vs. pea; RSC-CON + RSC-AN + RSC-LD vs. MFP-CON + MFP-AN + MFP-LD); (2) addition of seaweeds to the diets (RSC-CON + MFP-CON vs. RSC-AN + RSC-LD + MFP-AN + MFP-LD); and (3) seaweed species (*Ascophyllum nodosum* vs. *Laminaria digitata*; RSC-AN + MFP-AN vs. RSC-LD + MFP-LD). 1 × 2 = the interaction of protein source in the basal diet and addition of seaweeds; 1 × 3 = the interaction of protein source in the basal diet and seaweed species.

[3] Logarithmic transformation of Mo transfer efficiency; logarithmic values are presented in parenthesis below the original values.

Milk I concentration was not correlated with any other mineral in milk ($P > 0.10$; Supplemental Table S2, see Notes). Minerals most frequently correlated were Ca, Cu, Fe, Mn, Mo, Se, and Zn; all also correlated ($P < 0.05$) or tended to correlate ($P < 0.10$) positively with each other, except for Ca-Fe, Cu-Fe, and Cu-Mn. Strongest correlations were found between Cu and Mo (r = 0.841, $P < 0.001$), Mn and Se (r = 0.833, $P < 0.001$), Mn and Mo (r = 0.783, $P < 0.001$), Cu and Se (r = 0.766, $P < 0.001$), Mo and Zen (r = 0.758, $P < 0.001$), Fe and Zn (r = 0.773, $P < 0.001$), Mn and Se (r = 0.723, $P < 0.001$), and Mn and Fe (r = 0.708, $P < 0.001$).

The low yielding block had higher milk I concentration ($P = 0.025$) than high yielding block (Supplemental Table S3, see Notes). There was also a tendency for lower I intake ($P = 0.087$) and higher I transfer efficiency ($P = 0.061$) for low than for high yielding block. Block × diet interaction was only found for iodine yield (mg/d; $P = 0.041$).

DISCUSSION

This study evaluated the use of 2 isonitrogenously given protein feeds (rapeseed cake or pea), differing in mineral composition and glucosinolate concentration, as well as supplementation of diets with I-rich seaweeds (*A. nodosum* and *L. digitata*) in lactating dairy cow diets. Differences between diets were observed in mineral intakes, milk mineral profile, and mineral transfer efficiency from feed to milk. A special emphasis is given to I, as the use of a GSL-containing protein source and I-rich seaweeds greatly altered the intake and excretion of this essential trace element into milk.

Feed Composition

The I concentration of grass silage in the current experiment (0.908 mg/kg DM) was relatively high compared with the respective average values of 0.22 in the UK and 0.32 in Germany, varying in the range of 0.105 to 0.949 mg/kg DM (Schöne et al., 2017; Newton et al., 2021). The geographical and temporal variation of I concentration in Finnish forages is poorly understood. During the 1970s, iodine concentrations of <1 to 4 mg/kg DM were reported in Finnish forages (Ettala and Kossila, 1985). The I flows of agroecosystems are still not fully comprehended (McKernan et al., 2020), and it is not clear how the I concentration of manure influences the concentration of manure-fertilized crops (Kao et al., 2020), and can I supplementation in animal diets accumulate I into the animal-manure-soil-crop axis. The I concentrations of *A. nodosum* (1,076 mg/kg DM) and *L. digitata* (8,339 mg/kg DM) agree with the literature, although variation is extensive: in *A. nodosum* from 466 to 1,365 mg/kg DM (Garcia-Vaquero et al., 2021; Samarasinghe et al., 2021; Silva et al., 2022), and in *L. digitata* from 2,400 to 12,000 mg/kg DM (He et al., 2002; Gall et al., 2004; Garcia-Vaquero et al., 2021).

The concentrations of I in rapeseed cake, grass silage, mineral-vitamin supplement, and seaweeds were higher than expected, therefore, the targeted dietary I concentrations were exceeded in all diets. Also, the I concentrations of rapeseed diets with seaweed supplementation (6.83 and 6.82 mg/kg DM for RSC-AN and RSC-LD, respectively) exceeded the allowed maximum I concentration for dairy cows in the EU (5.68 mg/kg DM; European Commission, 2015). The upper tolerated level of I for dairy cattle is not determined, however, it has been suggested that in fattening bulls (BW 223–550 kg), the upper tolerated level of I would be ~8.3 mg/kg DM (Meyer et al., 2008; EFSA, 2013).

Unexpectedly, rapeseed cake used in the current experiment had much higher total GSL concentration (total 47.8 mmol/kg DM, equivalent of 22.3 g/kg DM) and different GSL profile than typically reported for modern cultivars (Zhao et al., 1994; Qin et al., 2023). The used analytical methods and reference standards can influence GSL concentration (Wu et al., 2017). However, the difference of GSL concentration and profile to Qin et al. (2023) is likely authentic, as the GSL concentration of RSC was analyzed in the same laboratory using the same analytical methods and authentic reference standards as Qin et al. (2023). They observed an average total GSL concentration of 1.42 g/kg DM for rapeseed meal of UK origin. Zhao et al. (1994) reported total GSL concentrations of 9.93 to 23.7 mmol/kg for seeds of 00-variety rapeseed. Rapeseed cake used in the current experiment had much lower proportion of aliphatic to total GSL (38%) compared with Zhao et al. (1994; 77%–84%) and Qin et al. (2023; 99%–100%). The opposite was true for the proportion of indole GSL (42% in current experiment compared with 15%–31% in Zhao et al. 1994 and 0%–1% in Qin et al. 2023) and aromatic GSL (20% in current experiment compared with 0.3%–1.2% in Zhao et al. 1994 and 0% in Qin et al. 2023). Aliphatic GSL originate from AA Ala, Leu, Ile, Val, and Met (Ishida et al., 2014). Indole GSL are derived from Trp, aromatic GSL are derived from either Phe or Tyr (Ishida et al., 2014). Double-zero (00) varieties of rapeseed should contain <20 mmol/kg DM GSL in seed (EFSA, 2008), and canola <30 mmol/kg GSL in air-dry defatted meal (OECD, 2011). Finnish quality requirements set a maximum concentration of 18 mmol/kg GSL for sowing material of rapeseed; the condition is met when certified sowing seed and varieties commonly cultivated in Finland are used (VYR, 2022). In the older rapeseed varieties not bred for low GSL concentrations, the abundance varied from 60 to 100 mmol/kg (Wittkop et al., 2009). Despite breeding efforts for low-GSL rapeseed cultivars and quality criteria for marketable seeds, this study has demonstrated that rapeseed products high in GSL are still on the market, with implications on both animal and human nutrition. The extent of this problem is currently unknown.

Reasons for the high GSL concentration and different GSL profile of RSC in the current experiment are unknown because no detailed information was available about production conditions of the rapeseed. Glucosinolates are an important alleviation mechanism for various abiotic and biotic stressors in the *Brassica* family (Variyar et al., 2014). Thus, changes in glucosinolate quantity may have been caused by environmental factors, such as soil mineral composition, temperature, and pest/pathogen interactions (Yan and Chen, 2007; He et al., 2018). Different glucosinolate compounds are also produced differentially in response to various abiotic and biotic stressors. For example, mechanical wounding and insect damage have been observed to change the ratio of aliphatic and indole glucosinolates in oilseed rape (Koritsas et al., 1991). Also, N and S supply can influence the glucosinolate profile of rapeseed (Zhao et al., 1994). Although the types of GSL synthesized are strictly genetically controlled (Mithen, 2006), GSL content has been shown to be highly variable between environments, even when the plants tested are derived from highly inbred and uniform parents (He et al., 2018). Processing conditions of the seed and cake (Eklund et al., 2015) can also influence the GSL concentration of rapeseed.

The GSL profile can influence the sensory properties and physiological impacts (including goitrogenic effects) of the biomass (Fenwick and Heaney, 1983). The breakdown products of GSL, such as isothiocyanates, thiocyanates, and oxazolidine-2-thione (goitrin), have goitrogenic effects (Fenwick and Heaney, 1983). Isothiocyanates and thiocyanates are cyanogenic goitrogens that interfere with thyroid peroxidase-catalyzed I uptake by competitive inhibition of the sodium-iodide symporter (**NIS**) in the thyroid gland (Fenwick and Heaney, 1983; Laurberg et al., 2009; Suttle, 2022). This leads to decreased formation of T4 and excretion of I into milk (Fenwick and Heaney, 1983; Laurberg et al., 2009; Suttle, 2022). Oxazolidine-2-thione is a thiouracil-like goitrogen that is the breakdown product of progoitrin (Mithen, 2006) and interferes with the activation of T4 to T3 by inhibiting the activating enzyme, type 1 deiodinase (Suttle, 2022). These 2 goitrogen types differ in response to I supplementation: The effects of cyanogenic goitrogens can be alleviated with increased I intake, whereas the effects of thiouracil goitrogens cannot (Fenwick and Heaney, 1983). Based on the GSL profile of RSC that included progoitrin and a diverse profile of aliphatic, indole and aromatic GSL, both modes of goitrogenic activity were present in the current experiment.

Because seaweeds are considered as a feed material rather than a complementary feed in EU regulation (Tarja Root, Finnish Food Authority, Helsinki, Finland, personal communication, Nov. 4, 2023), the maximum allowed concentration for total As is 45.5 mg/kg DM (European Parliament and Council of the European Union, 2002). In the current experiment, this limit was exceeded in both seaweeds. However, the concentrations of other highly toxic trace elements remained below the maximum allowed levels.

Mineral Intake

The substitution of rapeseed cake with pea decreased the intake of many macrominerals and trace elements, including Ca, Mg, I, Se, and Zn. Nevertheless, the intake of all macrominerals and trace elements on all diets exceeded the requirements of lactating dairy cattle (Luke, 2025). This was also true for Mn on all diets (54–60 mg/kg DM), considering the DIM of cows (on average 151 d [block 1] and 115 d [block 2] in the beginning of the experiment). The recommended dietary concentration of Mn is 40 mg/kg DM for cows with DIM >90 d, and 80 mg/kg DM for early lactation cows (DIM <90 d; Luke, 2025). Thus, attention should be paid to sufficient Mn intake when feeding early lactation cows with similar diet types. While this challenge was common to all diets in the current experiment, it was more emphasized on pea diets, as Mn intake was decreased when rapeseed was substituted with pea. The adequate supply of Se is important to consider along with I, as Se is required in the metabolism of I (Suttle, 2022).

Milk Mineral Profile and Mineral Transfer Efficiency

The substitution of rapeseed cake with pea decreased milk yield. The concentrations of Ca, K, Mg, and Na in milk were within the normal range as summarized by Moreno-Rojas et al. (1994). Compared with Qin et al. (2023), the substitution of rapeseed with other protein feed caused fewer changes in transfer efficiencies of minerals and trace elements in the current study. Despite the decreased intake of Ca and unchanged intake and transfer efficiency of K, the concentrations of Ca and K in milk were increased when rapeseed cake was substituted with pea. Seaweed supplementation had no effect on intake or transfer efficiency of K, yet the concentrations of these minerals in milk were also increased on seaweed diets. The increased transfer efficiency of Ca might indicate higher availability of the mineral in pea and seaweed. Phytates can hinder the availability of many minerals, including P, Ca, Mg, Fe, and Zn (Schlemmer et al., 2009). Brown seaweeds also include phlorotannins that are analogous to condensed tannins in terrestrial plants

(Abbott et al., 2020). Tannins can bind especially to Fe but also to Cu, Mn, Al, Zn, and Co, potentially decreasing their availability (Naumann et al., 2017). However, the interactions between minerals or cow metabolism are also possible explanations for changes in transfer efficiency. Particularly, milk Ca, P, K, and S are influenced by metabolic status indicators, such as BCS, and plasma glucose, β-hydroxybutyrate, and urea (Toscano et al., 2023). In the case of Ca and I, the transfer efficiency from ingested feed to milk was increased in response to their decreased intake, and vice versa for Mo. The concentrations of Ca, Cu, Fe, Mn, Mo, Se, and Zn were correlated with ≥5 out of 12 analyzed minerals in milk. This is in stark contrast with milk I concentration, which, according to correlation analysis, was not influenced by any other analyzed mineral in milk. This agrees with the findings of Toscano et al. (2023).

The concentrations of highly toxic trace elements (As, Cd, Cr, Hg, Pb) were below the detection limit in milk, even though high As concentration was observed in both seaweeds. It has also been reported by Xiong et al. (2024) that feeding seaweed to dairy cows does not possess As-related safety concerns for milk. The allowed maximum concentration for As is not determined for milk (FAO/WHO, 1995; European Commission, 2023).

Despite lower dietary I concentration (5.92 g/kg DM vs. 3.77 g/kg DM for rapeseed and pea diets, respectively), pea resulted in higher milk I concentration than rapeseed diets (121 µg/kg vs. 333, equivalent to 119 µg/L vs. 322 µg/L). This was likely caused by the GSL in the rapeseed cake. Previously, rapeseed has reduced transfer efficiency of I from feed to milk even when cultivars low in GSL (1.107 mmol/kg DM) were used (Trøan et al., 2018). This led to a GSL intake as low as 0.96 mmol/d (Trøan et al., 2018). The GSL intake in the current experiment was 72.5, 72.4, and 76.2 mmol/d for RSC-C, RSC-AN, and RSC-LD, respectively.

Large variation in I concentration of Finnish milk has been reported both in the past (59–468 µg/L; Koiranen and Stabel-Taucher, 1976) and present (24–732 µg/kg; Honkanen et al., 2024). Large variation is often reported elsewhere, for example, in Italy, 24 to 499 µg/kg (Niero et al., 2020). The I concentration of raw milk is influenced by the amount and form of dietary I, milk yield, stage of lactation, season, goitrogens in feeds, and thyroid status of the cow, and teat-dipping practices (Miller et al., 1975; van der Reijden et al., 2018). Milk I concentration decreases with parity, is lowest in the beginning of lactation, and relatively stable ~61 to 240 DIM (Costa et al., 2021). In the current experiment, the higher yielding block tended to have lower I transfer efficiency (5.99%) and I concentration of milk (192 µg/kg) than lower yielding block (7.35% and 262 µg/kg); however, the effect was confounded with the phase of lactation, as DIM differed slightly between the blocks.

Mammary gland I uptake from blood circulation is facilitated by NIS (Tazebay et al., 2000; Aceves et al., 2005). Iodination occurs in the alveoli of the mammary gland, in lactocytes, where I is incorporated into casein by the action of the lactoperoxidase enzyme (Strum, 1978; Aceves et al., 2005; Opazo et al., 2021). Iodocasein is aggregated inside micelles, fused with the apical membrane, and released to milk by exocytosis (Opazo et al., 2021). The function of mammary NIS is otherwise similar to that in the thyroid gland, with the exception that the autoregulation of I uptake is lacking or much less developed in the mammary gland (Laurberg et al., 2009). Therefore, the variation in I intake or concentration of goitrogenic compounds is greatly associated with I concentration of milk, but less so with thyroid hormones in plasma, as observed by Trøan et al. (2018). The NIS in the mammary gland is inhibited by thiocyanates in a similar manner as in the thyroid gland (Brown-Grant, 1956).

It is well established that I concentration in milk decreases with increasing GSL intake (Trøan et al., 2015) and increases with increasing I intake (Franke et al., 2009; Flachowsky et al., 2014; Niero et al., 2023). This is also supported by the current experiment. In the GSL-containing RSC-CON diet with moderate dietary I concentration, the transfer efficiency was only ~1%, whereas the highest transfer efficiencies of ~14% were measured in MFP-AN and MFP-LD; that is, diets without GSL and higher dietary I concentration. However, low responses on pea diets were unexpected. Despite similar dietary I concentrations, MFP-CON resulted in an I transfer efficiency of 5.35%, compared with 14.3% for GSL and seaweed-free diets in Qin et al. (2023). Also Trøan et al. (2018) reported much higher I transfer efficiency (25%) for GSL and seaweed-free diets with dietary I concentration of 4.0 mg/kg DM. In Newton et al. (2023), a GSL and seaweed-free diet (I concentration of 0.5 mg/kg DM) resulted in milk I concentration of 166 µg/kg and I transfer efficiency of 45%. This is 60 µg/kg higher than on MFP-CON diet in the current experiment despite much smaller dietary I concentration.

The results of the current experiment (Figure 1) do not fit the previous regression equations developed for estimation of milk I concentration. Using the regression equations of Franke et al. (2009; developed for rapeseed meal diets), Flachowsky et al. (2014), and Niero et al. (2023), the estimated milk I concentration for RSC-CON, RSC-AN, and RSC-LD diets would exceed the measured I concentration by a factor of 4.5 to 12.9, 8.7 to 23.6, and 5.3 to 16.1, respectively. On MFP-CON, MFP-AN, and MFP-LD diets, the regression equations of Franke et al. (2009; developed for GSL-free diets), Flachowsky

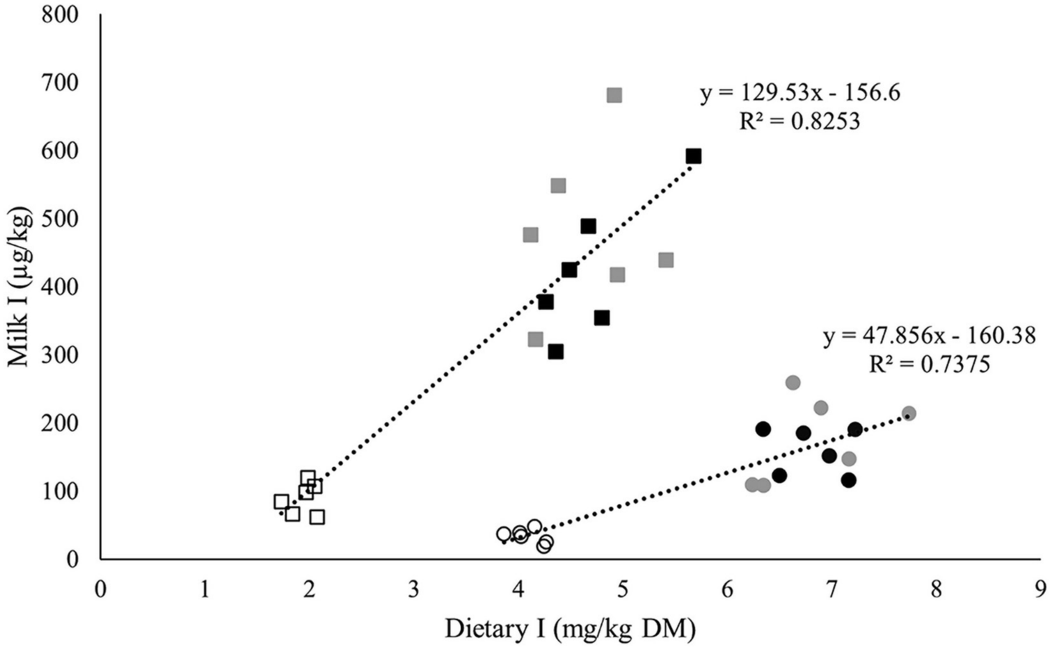

Figure 1. Iodine concentrations of milk (µg/kg) as a function of dietary iodine concentration (mg/kg DM). Open circle: rapeseed cake, control without seaweed (RSC-CON); circle filled with gray: rapeseed cake, *Ascophyllum nodosum* (RSC-AN); circle filled with black: rapeseed cake, *Laminaria digitata* (RSC-LD). Open square: pea, control without seaweed supplementation (MFP-CON); square filled with gray: pea, *Ascophyllum nodosum* (MFP-AN); square filled with black: pea, *Laminaria digitata* (MFP-LD).

et al. (2014), and Niero et al. (2023) overestimate milk I concentration by a factor of 2.8 to 5.6, 2.1 to 4.4, and 1.4 to 3.9, respectively. In all cases, the most overestimated milk I concentration was always on RSC-CON diet.

Due to a washout of 18 d between periods, it is unlikely that the low I transfer efficiencies on pea diets were caused by a carryover effect of GSL between diets. Washout resulted in a 31-d interval between diet eaten in a previous period and a sampling week of the following period. The half-life of thiocyanate has been mainly studied in humans in the context of tobacco smoking, and the results indicate that the half-life of thiocyanate in human plasma and saliva varies from 1 d to over 2 wk depending on exposure, the biological characteristics of the sample donor, and sampling (Bliss and O’Connell, 1984). In Weiss et al. (2015), the effect of rapeseed supplementation and change in dietary I concentration on milk I concentration was no longer observed 9 d after the treatments were ceased.

In the current experiment, milk samples were taken on d 14 to 17 of each period, which might have influenced the I concentration of milk to some extent. However, the optimal period for determining dietary effects on milk I is not easy to find. The equilibrium between I intake and secretion in milk has been reported to occur after 7 to 10 d of daily dosing, as summarized by Miller et al. (1975). However, in van der Reijden et al. (2019), the concentration of I in milk appeared to reach a near steady state after 21 d of I supplementation (0.5–2 mg/kg DM), whereas in Weiss et al. (2015), the steady state was reached already by 4 d. When cows were supplemented with 330 g/d of *A. nodosum*, milk I concentration continued to increase during the whole 8-wk measurement period (Newton et al., 2023). In Newton et al. (2021), the I concentration of milk peaked ~3 wk from the onset of seaweed supplementation (12.8 or 50.2 g DM/d) but decreased rapidly afterward and fluctuated slightly until the end of the 6-wk measurement period.

Soy isoflavonoids daidzein and genistein have goitrogenic effects on humans and other monogastrics (Divi et al., 1997) and dairy cows (Hemken et al., 1971) when dietary concentration of I has been low; symptoms disappeared when diets were supplemented with I (Block et al., 1961; de Souza dos Santos et al., 2011). These isoflavonoids are also present in peas. Timoracká and Vollmannová (2010) reported the daidzein concentration of green and yellow peas to be 1.7 to 2.7 and 0.38 to 0.78 mg/kg, respectively, and genistein concentration 0.41 to 0.71 and 0.12 to 0.16 mg/kg, respectively. The daidzein and genistein concentrations in raw, mature soybeans have averaged 1.3 and 1.9 mg/kg, respectively, although variation between samples is extensive (0.0–6.0 mg/kg; Bhagwat and Haytowitz, 2015). While the goitrogenic effects of soybeans are well described, the authors were unable to identify prior studies examining the goitrogenic activity of pea on ruminants.

The source of I is another possible explanation for differences in I transfer efficiency, as previous response studies are based on experiments using mainly inorganic I sources, for example, KI, CaI, ethylenediamine dihydroiodide, and pentacalcium orthoperiodate (Franke et al., 2009; Flachowsky et al., 2014; Niero et al., 2023). The comparisons between seaweeds and inorganic I supplements are lacking in dairy cow diets, but differences in I transfer efficiency have been observed between different inorganic forms of I (Miller et al., 1975). In humans, the bioavailability of I from seaweed is reported to be variable but lower than that of KI. Combet et al. (2014) reported the I bioavailability of *A. nodosum* to be 31% and that of KI 59% in vivo in humans. In Aquaron et al. (2002), I bioavailability of *Laminaria hyperborea* was 62% to 90% and that of KI 96% in vivo in humans. Therefore, it is plausible that the responses based on inorganic forms of I supplementation are not directly applicable to situations where seaweeds are used as an I supplement. However, this does not explain the difference in I transfer efficiency on seaweed-supplemented diets. For example, the regression equation developed by Niero et al. (2023) using data from Antaya et al. (2015), where *A. nodosum* was used as an I supplement, also overestimates the I concentration of milk in the current experiment by the factor of 1.7 to 18.6. Moreover, seaweed-supplemented diets with dietary I concentration 6.1 to 7.5 mg/kg DM have previously resulted in I transfer efficiency of 38% to 42% (Newton et al., 2021, 2023), compared with ~3% on RSC-AN and RSC-LD and 14% on MFP-AN and MFP-LD. Qin et al. (2023) reported I transfer efficiencies of 11% and 14% for seaweed-supplemented diets, including rapeseed meal or distillers grains as protein source, respectively; yet the dietary I concentration of ~7 mg/kg DM resulted in much higher milk I concentration (615–816 μg/kg) than in the current experiment (158–475 μg/kg). Further studies are needed to better understand the causes for low transfer efficiency of I in similar diets as in the current experiment.

Implications on Human Nutrition

Historically, along with many other European countries (Vitti et al., 2001), Finland has suffered from I deficiency, as indicated by endemic goiter in the population (Lamberg, 1986). The iodization strategy of Finland has relied heavily on the use of iodized animal feeds (Lamberg, 1986), I supply from dairy products and iodized salt, and unchanged consumption patterns (Nyström et al., 2016). This has proven to be a successful strategy, because the prevalence of I deficiency in Finland was dramatically decreased from the 1950s (I intake 50–70 μg/d; Lamberg, 1986). In 2017, I intake was estimated to be adequate or close to adequate in a Finnish population of adults (237 μg/d for men and 186 μg/d for women; Valsta et al., 2018). However, the consumption of milk and dairy products has already decreased by 18 kg/person per year since 2017 (Luke, 2024) when the survey of Valsta et al. (2018) was conducted. There is also pressure to decrease salt consumption from the current 7.5 g/d (Valsta et al., 2018) to 5.75 g/d (Blomhoff et al., 2023). These changes in dietary consumption patterns pose challenges to adequate I intake. The estimation of I supply of consumers is impeded by the huge variation in I concentration of milk, as was also demonstrated in this study, which is not always reflected in food composition databases (e.g., Finnish Institute for Health and Welfare, 2025) used in human nutrition modeling.

The regression equations of Franke et al. (2009) were used as a central argument in the EFSA (2013) recommendation for reducing the maximum dietary I concentrations for dairy cows from 5 to 2 mg/kg to avoid excess I intake in consumers. According to EFSA (2013) estimates, I concentration of 2 mg/kg in dairy cow diets would result in I concentration of 280 μg/L in pasteurized milk, which would be sufficient to meet the I requirements of adult consumers when I intake from other dietary sources is considered. Iodized salt intake of 9 g/d was assumed. The authorized maximum dietary I concentration for dairy cow diets in the EU is 5 mg/kg until June 2025 (European Commission, 2015), although the recommendation of 2 mg/kg is also mentioned in the regulation. Currently, the concentration of 5 mg/kg is followed in Finland (Tarja Root, Finnish Food Authority, Helsinki, Finland, personal communication, Aug. 2, 2023). Based on the results of the current experiment, decreasing the maximum dietary I concentrations from 5 to 2 mg/kg would likely lead to decreased I supply from dairy sources. In the current experiment, dietary I concentration of 1.94 mg/kg DM on MFP-CON resulted in I concentration of 106 μg/kg (103 μg/L) in raw milk; that is, it is clearly below the estimate of 280 μg/L by EFSA (2013). Because of the exceptionally high GSL concentration in rapeseed cake in the current experiment, the I concentration of milk on RSC-CON, RSC-AN, and RSC-LD diets is unlikely to represent a typical response to dietary I supplementation on Finnish dairy cow diets. Therefore, these diets are not used here as evidence to estimate the adequacy of dietary I concentration, even though rapeseed meal and cake are the most typical protein feeds used in Finnish dairy cow diets. However, the current experiment demonstrated that high levels of seaweed supplementation in diets including rapeseed high in GSL do not pose a threat for I overdose for human consumers, as these diets resulted in milk with relatively low I concentration. When comparing diets not supplemented with seaweeds, pea diets resulted in more favorable milk I concentration for human consumers than rapeseed with high GSL concentration.

CONCLUSIONS

All experimental diets met the mineral requirements of dairy cows. However, the dietary protein source and seaweed supplementation influenced mineral intake of dairy cows, which should be considered in diet planning. Ensuring adequate Mn intake is important when feeding early lactation cows with similar types of diets. Despite lower dietary I concentration, pea resulted in higher milk I concentration than rapeseed diets, potentially due to unexpectedly high GSL concentrations in rapeseed. Seaweed supplementation increased milk I concentration. All diets resulted in much lower I transfer efficiency from feed to milk compared with previous reports on rapeseed-supplemented, GSL-free, or seaweed-supplemented diets. The transfer efficiency of I was particularly low on rapeseed diets without seaweeds. Highly toxic trace elements (As, Cd, Cr, Hg, Pb) were not observed in milk. Further research is warranted on effects of substituting rapeseed with leguminous protein feeds on milk mineral concentration and especially I transfer efficiency.

NOTES

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Nonstandard abbreviations used: AN = *Ascophyllum nodosum* diet; CaI = calcium iodate anhydrous; CON = control diet without seaweed supplementation; CRM = certified reference materials; EU = European Union; GSL = glucosinolates; ICP-MS = inductively coupled plasma MS; LD = *Laminaria digitata* diet; MFP = milled feed pea diet; ND = not detected; NIS = sodium-iodide symporter; PMR = partial mixed ration; RSC = rapeseed cake diet; T3 = triiodothyronine; T4 = thyroxine; TMAOH = tetramethylammonium hydroxide.

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