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Research Article

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Effect of dietary protein source and *Saccharina latissima* on nutritional and safety characteristics of milk

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Abstract

BACKGROUND: Wheat distillers' grains (WDG) and seaweeds are recommended as alternative protein sources and enteric methane mitigators in dairy cow diets, respectively, but little is known about their impact on milk quality and safety. In the present study, 16 cows in four 4 × 4 Latin squares were fed isonitrogenous diets (50:50 forage:concentrate ratio), with rapeseed meal (RSM)-based or WDG-based concentrate (230 and 205 g kg^{-1} dry matter) and supplemented with or without *Saccharina latissima*.

RESULTS: Replacement of RSM with WDG enhanced milk nutritional profile by decreasing milk atherogenicity ($P = 0.002$) and thrombogenicity ($P = 0.019$) indices and the concentrations of the nutritionally undesirable saturated fatty acids – specifically, lauric ($P = 0.045$), myristic ($P = 0.022$) and palmitic ($P = 0.007$) acids. It also increased milk concentrations of the nutritionally beneficial vaccenic ($P < 0.001$), oleic ($P = 0.030$), linoleic ($P < 0.001$), rumenic ($P < 0.001$) and α-linolenic ($P = 0.012$) acids, and total monounsaturated ($P = 0.044$), polyunsaturated ($P < 0.001$) and n-6 ($P < 0.001$) fatty acids. Feeding *Saccharina latissima* at 35.7 g per cow per day did not affect the nutritionally relevant milk fatty acids or pose any risk on milk safety, as bromoform concentrations in milk were negligible and unaffected by the dietary treatments. However, it slightly reduced milk concentrations of pantothenate.

CONCLUSION: Feeding WDG to dairy cows improved milk fatty acid profiles, by increasing the concentrations of nutritionally beneficial fatty acids and reducing the concentration of nutritionally undesirable saturated fatty acids, while feeding seaweed slightly reduced pantothenate concentrations. However, when considering the current average milk intakes in the population, the milk compositional differences between treatments in this study appear relatively small to have an effect on human health.

Supporting information may be found in the online version of this article.

Keywords: bromoform; B vitamins; fatty acids; seaweed; rapeseed meal; wheat distillers' grains

INTRODUCTION

Meeting the rising needs for high-quality affordable feed protein to support the increased demand for production of animal-derived foods globally presents a significant challenge for global livestock production.[1] Soybean meal and rapeseed meal (RSM) are commonly used (at inclusion rates of approximately 150–200 g kg^{-1}, dry matter (DM) basis) as the concentrated protein source in dairy cow rations but soybean meal is primarily imported, while recent legislation on reduction of the use of pesticides in the UK and EU, as well as climate change, may pose significant threats to rapeseed yields in the UK and EU (19.5 Mt; the leading producer in 2022/2023).[2-4] Some rapeseed varieties are also high in glucosinolates that have shown adverse effects on ruminal fermentation and nutrient digestibility in a study with

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steers,[5] and posing a risk to food safety if extensively used in dairy rations.[6] In addition, the use of rapeseed could reduce iodine concentration in milk, thus restricting the supply of iodine to the population from its most important sources, which are milk and dairy products.[7,8] Wheat distillers' grains (WDG) are by-products of the ethanol industry that are commonly used as a feed ingredient in dairy cow diets and are an alternative protein source that can replace RSM.[3] It was previously shown that WDG with solubles produced in Europe can be used at high inclusion levels to up to 225 g kg^{-1} of diet DM without affecting DM intake and milk yield.[3]

In addition, there is a strong need to reduce enteric methane emissions from ruminant animals to meet future challenges around sustainable livestock production and climate change.[9] One of several dairy management interventions that have been recommended with the capacity to reduce enteric methane emissions is the supplementation of dairy cow diets with certain marine macroalgae (commonly known as seaweed) species.[10] Certain seaweeds contain compounds such as bromoform that have been shown to reduce methane emissions in ruminants; for instance, the seaweeds *Asparagopsis taxiformis* and *Asparagopsis armata*, included at low levels in the feed of cattle and sheep, inhibit methanogenesis by up to 98%, with evidence of improvements in feed utilization efficiency.[11,12] The bromoform content in seaweed can vary significantly, with the brown seaweed species *Ascophyllum nodosum* and *Fucus vesiculosus* showing a much lower bromoform content than *Asparagopsis* sp.[13] Seaweeds have also attracted increased attention due to their high concentrations of specific macrominerals and trace elements such as iodine, iron and zinc, which are important for ruminant health and may also enrich the nutritional profile of milk.[7,14,15]

Milk fat contains saturated fatty acids (SFA) (current recommendations are to reduce these in human diets, particularly C12:0, C14:0 and C16:0), and milk and dairy products are one of the main dietary sources.[16] However, it also contains beneficial fatty acids (FA), including monounsaturated FA (MUFA) such as t11 C18:1 (VA, vaccenic acid) and c9 C18:1 (OA, oleic acid); and polyunsaturated FA (PUFA) including omega-3 PUFA (n-3), such as c9c12c15 C18:3 (ALA, α-linolenic acid), c5c8c11c14c17 C20:5 (EPA, eicosapentaenoic acid), c7c10c13c16c19 C22:5 (DPA, docosapentaenoic acid), and c4c7c10c13c16c19 C22:6 (DHA, docosahexaenoic acid), omega-6 PUFA (n-6) such as c9c12 C18:2 (LA, linoleic acid), as well as c9t11 C18:2 (rumenic acid (RA), which is a conjugated linoleic acid (CLA)). Animal diet is the most influential driver of milk FA profile.[17] Pasture intake, dietary forage content and different forage types can be major drivers for improving milk FA composition, including profiles with less SFA and more PUFA and CLA when cows were fed fresh green forage.[18] In addition, use of rumen-protected linseed fat or oils rich in PUFA also improves milk FA profiles.[19-21] Previous work has shown that the inclusion of WDG in beef cattle feed can modify the FA composition of meat by increasing total PUFA, LA and CLA, when replacing barley grain with 300 g kg^{-1} WDG in diets.[3,22] In other studies, algae (*Schizochytrium* sp.) supplementation (43.0 g kg^{-1} of DM intake), provided via a rumen fistula, increased concentrations of t10 C18:1, VA, RA, t9c11 CLA and DHA, potentially by altering rumen biohydrogenation of LA and ALA.[23] Similarly, B vitamin concentrations in milk can be affected by cows' diet and their origins can be directly determined from feed and from *de novo* synthesis in the liver or rumen microbiota.[24] Factors that have been previously reviewed[24] to affect B vitamin concentrations in milk include vitamin supplementation (folic acid and cobalamin supplementation increased their concentrations in milk) and feeding system, with maize-based diets showing increased milk B12 concentrations, but with pasture-based diets showing increased milk B2 and B9 concentrations.[25,26]

However, despite the increased importance of including WDG and seaweed in dairy cows' diets, either as alternative protein source to rapeseed or mineral/methane mitigation supplement respectively,[3,7] little is known about the effect of these feeding practices on FA profile and B vitamin and bromoform concentrations in milk. In addition, previous work highlighted the importance of assessing the potential bromoform contamination risk of milk from cows fed seaweeds,[12] but this has only been explored for the addition of a high-level bromoform-containing red seaweed (*Asparagopsis armata* at 10 g kg^{-1} organic matter (OM) basis, 1.32 mg g^{-1} DM of bromoform[27] or *Asparagopsis taxiformis* at 5 g kg^{-1} OM basis), which showed no significant changes in milk bromoform concentration,[27,28] although the latter study found an 8.5-fold increase (from 5.1 to 43.2 mg L^{-1}) in the bromine content of milk when *Asparagopsis taxiformis* was fed.[28] Despite the growing interest in the use of seaweed and alternative proteins as feed, there remains a significant gap in the literature regarding their impact on the nutritional quality and safety characteristics of milk. The present study aims to address this novel area of research by investigating the effect of substituting RSM with WDG as a protein source in dairy rations, including *Saccharina latissima* as a supplement (previously shown to increase iodine concentrations),[7] as well as the potential interaction between these two feeding practices on the FA profiles and B vitamin concentrations of milk. In addition, the present study explored the extent of the transfer of bromoform from feed to milk, to ensure that feeding *S. latissima* at the given amounts would not pose any threats to food safety.

MATERIALS AND METHODS

Animals, diets, and experimental design

All experimental procedures were performed under licence by the UK Home Office under the Animals (Scientific Procedures) Act, 1996. The experimental design and animal diets have been previously described.[7] In brief, 16 multiparous Holstein cows were blocked by parity (mean ± SD = 4 ± 2, range = 2–7), days in milk (167 ± 53, 106–259 days), liveweight (678 ± 69, 590–821 kg) and milk yield (32.8 ± 4.4, 25.0–41.3 kg d^{-1}) into four 4 × 4 Latin square change-over designs, each for 4-week experimental periods. Each experimental period consisted of a 7-day washout period and a 21-day feeding period. The cows were first fed the WDG diet without seaweed supplementation (C-WDG) during the washout period before being randomly assigned to one of the experimental diets for the next 21-day feeding period. The four dietary treatments were: (i) a basal diet based on WDG as the primary protein source without seaweed supplementation (C-WDG); (ii) a basal diet based on WDG as the primary protein source with 35.7 g per cow per day (DM basis) of dried seaweed supplementation (*S. latissima*) (S-WDG); (iii) a basal diet based on RSM as the primary protein source without seaweed supplementation (C-RSM); and (iv) a basal diet based on RSM as the primary protein source with 35.7 g per cow per day (DM basis) of dried seaweed supplementation (*S. latissima*) (S-RSM). The seaweed was fed at this amount to avoid exceeding the iodine supplementation allowance for dairy cows, which was explained in our previous study.[6] The ingredient and chemical composition of the four

diets were previously described.[7] The FA profile and bromoform concentrations of the experimental diets are shown in Table 1 and those of the individual feed ingredients in Supporting Information, Table S1.

Sample collection and analysis

Individual feed intake and milk yield were recorded daily through each 21-day feeding period, with the last 6 days of each period used for measurement of feed intake and milk yield. Milk yield

Table 1. Fatty acid composition and bromoform concentrations of the experimental diets

	Seaweed		Protein source		Seaweed × concentrate			
Parameters	C	S	WDG	RSM	C-WDG	C-RSM	S-WDG	S-RSM
Fatty acid profile (g kg^{-1} diet dry matter)								
C4:0	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
C6:0	0.14	0.14	0.14	0.13	0.14	0.13	0.14	0.13
C7:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C8:0	0.08	0.08	0.04	0.13	0.04	0.13	0.04	0.13
C9:0	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04
C10:0	0.06	0.06	0.02	0.10	0.02	0.10	0.02	0.10
C10:1 c9	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C12:0	0.74	0.73	0.11	1.36	0.11	1.36	0.11	1.35
C12:1 c9	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C13:0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C13:0 iso	0.29	0.29	0.28	0.30	0.28	0.30	0.28	0.30
C14:0	0.29	0.29	0.06	0.51	0.06	0.51	0.06	0.51
C14:0 iso	0.27	0.27	0.27	0.27	0.27	0.27	0.27	0.27
C15:0	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
C15:0 anteiso	0.03	0.03	0.03	0.04	0.03	0.04	0.03	0.04
C16:0	4.10	4.13	4.48	3.75	4.46	3.75	4.50	3.75
C16:1 c9 + C17:0 anteiso	0.07	0.07	0.06	0.08	0.06	0.08	0.06	0.08
C16:1 t11/13	0.37	0.37	0.36	0.38	0.36	0.38	0.36	0.38
C17:0	0.04	0.04	0.03	0.05	0.03	0.05	0.04	0.05
C17:1 c9	0.03	0.03	0.00	0.06	0.00	0.06	0.00	0.06
C18:0	0.19	0.20	0.22	0.17	0.22	0.17	0.22	0.17
C18:0 iso	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C18:1 c9 (OA)	2.08	2.09	1.96	2.21	1.94	2.22	1.99	2.20
C18:1 c11	0.30	0.30	0.20	0.40	0.20	0.40	0.20	0.40
C18:1 c13	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C18:1 c16	0.01	0.01	0.00	0.01	0.00	0.01	0.00	0.01
C18:1 t11	0.02	0.01	0.01	0.02	0.01	0.03	0.01	0.02
C18:2 c9c12 (LA)	4.59	4.63	5.47	3.75	5.43	3.76	5.51	3.75
C18:2 c9t14	0.02	0.02	0.01	0.02	0.01	0.02	0.01	0.02
C18:2 c10t14	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
C18:2 t11c15	0.04	0.04	0.03	0.04	0.03	0.04	0.03	0.04
C18:2 t9t12	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
C18:2 t11t15	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C18:3 c6c9c12	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.08
C18:3 c9c12c15 (ALA)	6.69	6.68	6.72	6.65	6.76	6.63	6.69	6.67
C19:0 + C18:1 c15	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C20:0	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
C20:2 c11c14	0.02	0.02	0.02	0.03	0.02	0.03	0.02	0.03
C20:5 c5c8c11c14c17	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
C22:2 c13c16	0.05	0.05	0.04	0.06	0.04	0.06	0.04	0.06
C22:3 c13c16c19	0.09	0.09	0.09	0.09	0.09	0.09	0.10	0.09
C22:6 c4c7c10c13c16c19	0.02	0.02	0.02	0.01	0.02	0.01	0.02	0.01
C24:0	0.13	0.13	0.13	0.13	0.13	0.13	0.13	0.13
Total fatty acids	21.1	21.2	21.2	21.2	21.1	21.2	21.2	21.1
Bromoform (μg kg^{-1})	48.6	50.4	51.6	47.4	50.7	46.5	52.6	48.3

The four dietary treatments were: (i) a basal diet based on WDG as the primary protein source without seaweed supplementation (C-WDG); (ii) a basal diet based on WDG as the primary protein source with 35.7 g per cow per day on DM basis of dried seaweed supplementation (*Saccharina latissima*) (S-WDG); (iii) a basal diet based on rapeseed meal (RSM) as the primary protein source without seaweed supplementation (C-RSM); and (iv) a basal diet based on RSM as the primary protein source with 35.7 g per cow per day on DM basis of dried seaweed supplementation (*S. latissima*) (S-RSM).

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was recorded from twice daily milking at 05:00 h and 15:00 h. Milk samples, proportional to the yield at each milking, were collected over four consecutive milkings during the measurement week (3rd week in the measurement period; days 16–17) and stored at 4 °C. After collecting the final milk sample, all samples were heated in a water bath at 38 °C and combined (providing one sample per cow per period). The milk samples were then frozen at −20 °C for FA and bromoform analysis in two separate containers. Similarly, one composite sample per feed (forage, concentrate, seaweed) per experimental group per period was collected, oven dried and milled for FA and bromoform analysis.

Milk FA analysis

The procedures for esterification and methylation of milk FA, as well as peak identification, integration and quantification, were carried out based on previous studies.[29] The FA composition in fresh grass, grass silage, concentrates and seaweed were analysed according to previously published methods,[30] but using the same equipment and chromatographic conditions as the milk samples.[29] All FA analyses were performed in the laboratories of the School of Agriculture, Policy and Development of the University of Reading. The transfer rate of LA and ALA was determined based on their intake from dietary sources and output in milk, as previously described.[31] In addition, the atherogenicity index (AI) = (C12:0 + (4 × C14:0) + C16:0)/(MUFA + PUFA), and thrombogenicity index (TI) = (C14:0 + C16:0 + C18:0)/(0.5 × MUFA) + (0.5 × n-6) + (3 × n-3) + (n-3:n-6) were calculated following a previous study.[32]

Milk B vitamin analysis

The analysis of B vitamins in milk was performed in the laboratories of the School of Agriculture, Policy and Development and the Chemical Analysis Facility at the University of Reading. The extraction and detection of metabolites were performed following a previous study.[33] In detail, in the metabolite extraction process, a 100 µL milk sample was added to a 1.5 mL Eppendorf tube, followed by 300 µL methanol for protein precipitation and vortexed for 2 min. The mixture was centrifuged at 19 098 × *g* for 10 min at 4 °C, and 200 µL of the supernatant was transferred to a black centrifuge tube and dried under a gentle nitrogen stream. The residue was reconstituted in 200 µL of 10 mmol L^{-1} NH_4-formate aqueous/acetonitrile (99/1, v/v) and kept on ice. Subsequently, 400 µL methyl *tert*-butyl ether was added and vortexed for 30 s; after centrifugation, the upper phase was discarded and the lower phase was transferred to a fresh 2 mL liquid chromatography–mass spectrometry (LC-MS) glass amber vial with a 250 µL glass insert. For calibration standard preparation, each external standard (thiamine, riboflavin, pantothenate, pyridoxal, pyridoxine, biotin, folic acid, cyanocobalamin) was weighed into a 100 mL beaker, followed by the addition of 50–80 mL pure water (high-performance liquid chromatography grade). Once dissolved, the solution was transferred to a 100 mL volumetric bottle and used for the development of the standard linear calibration curves for the subsequent analysis, including 12 dilution gradients. Whole milk powder (ERM-BD600) was used for the certificate of analysis: VB1 = 4.5 mg kg^{-1}; VB2 = 16.7 mg kg^{-1}; VB12 = 0.32 mg kg^{-1}.

Liquid chromatographic–tandem mass spectrometric analysis was conducted using a triple-quadrupole mass spectrometer (LCMS-8050, Shimadzu, Kyoto, Japan), with the analysis carried out on a reversed-phase ACQUITY UPLC HSS T3 column (2.1 × 100 mm, 1.8 µm; Waters Corp., Milford, MA, USA) at 40 °C. The samples were maintained at 4 °C until injection, with 1.0 µL of sample injected. The mobile phases were 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B), with a flow rate of 0.3 mL min^{-1}. The gradient was set to change from 55% B to 15% B at 0–1 min; 15–60% B at 1–2.0 min; held 60% B at 2–2.5 min; 60–5% B at 2.5–2.6 min; held 5% B at 2.6–7 min. The needle was washed with a solution of water–acetonitrile–methanol–2-propanol (1:1:1:1). The mass spectrometer operated in positive ion mode electrospray ionization (ESI) and vitamins were detected in multiple reaction monitoring (MRM) with unit resolution at both Q1 and Q3. The heat block, desolvation temperature and interface temperatures were set to 400, 526 and 300 °C, respectively. Nitrogen gas served as the nebulizing agent, flowing at a rate of 3 L min^{-1} and as the drying gas at 10 L min^{-1}, while air was employed as the heating gas, also at a flow rate of 10 L min^{-1}. The MRM transitions used are presented in Supporting Information, Table S2, and voltages were optimised via an automated procedure using repeated no-column injections. Whole milk powder and the highest concentration of the mixed standard were used as quality controls at a frequency of every 16 samples within a run. All samples were injected in triplicate. Data analysis was performed using LabSolutions Insight (Shimadzu).

Bromoform analysis

The analysis of bromoform in milk started by transferring milk (3.00 ± 0.05 mL) to a 20 mL headspace vial (Supelco, Munich, Germany), which was sealed with a screw-top lid. The samples were incubated at 70 °C for 20 min using a CTC 120 autosampler (Agilent, Santa Clara, CA, USA). The volatiles in the headspace were extracted for 20 min at 70 °C using a Carboxen/DVB/PDMS SPME fibre (Supelco, Munich, Germany). The fibre was desorbed at 250 °C for 20 min in the injection port of a 7890A GC coupled to a 5975C Inert MS detector (both Agilent), fitted with a ZB-5Msi column (30 m, 0.25 mm, 1 µm) (Phenomenex, Torrance, CA, USA). The oven temperature was increased from 40 to 200 °C using a 4 °C min^{-1} ramp, increased to a maximum temperature of 300 °C at 20 °C min^{-1} and held for 16 min. The carrier gas was helium at a constant flow rate of 0.9 mL min^{-1}. Mass spectra were recorded in single ion monitoring (SIM)/scan mode at an ionization voltage of 70 eV and source temperature of 250 °C. In scan mode the *m/z* range was 20–450. In SIM mode *m/z* 171, 173, 175, 252 and 254 were monitored, each with a dwell time of 100 ms. An authentic standard of bromoform confirmed that the peak observed was indeed bromoform, with a linear retention index of 889 calculated from injection of an alkane standard C5-C25 (10 mg L^{-1} in diethyl ether). Quantification was via external calibration carried out using bromoform (Lancaster Synthesis, Lancaster, UK; >97% purity and stabilized with ethanol ∼1%). Bromoform solutions (2.90, 1.45, 0.29 and 0.015 µg L^{-1}) were made up in whole milk purchased from Milk and More (Camberley, UK) in glass bottles ($y = 1178x - 301.4$, $R^2 = 0.9997$). The intercept on the *x*-axis (0.26 µg L^{-1}) represents the concentration of bromoform in the purchased whole milk. For the bromoform analysis of feed samples, distilled water (18.2 MΩ, 3 mL) was added to feed (1.00 ± 0.01 g) in a 20 mL headspace vial and vortexed for 30 s. The extraction and analysis procedures were the same as for the milk. Calibration curves (29, 14.5, 2.90, 1.45, 0.29 and 0.015 µg kg^{-1}) were prepared in 1 g fresh grass feed in 3 mL distilled water (18.2 MΩ, 3 mL), ($y = 1710x - 229.3$, $R^2 = 0.9992$). The intercept on the *x*-axis (0.13 µg kg^{-1}) represents the concentration of bromoform in the fresh grass feed.

Statistical analysis

Statistical analysis was conducted using Minitab 21. A linear mixed model was used to analyse the experimental data, having seaweed supplementation (with/without), the main protein source in the concentrate (WDG/RSM) and their interaction as fixed factors, and cow ID and experimental period as the random factors. Normal distribution of the residuals was visually assessed, and data showed no deviation from normality, resulting in data being analysed untransformed. Where the effect of the fixed factors, or their interaction, was statistically significant ($P < 0.05$), significant differences between treatment means were calculated using Tukey's honest significant difference test ($P < 0.05$).

RESULTS

FA profiles and bromoform content of the feeds

The FA concentrations of all experimental diets are shown in Table 1, while the FA concentrations of individual feeds are listed in Supporting Information, Table S1. On a DM basis, the fresh grass and grass silage had more ALA compared to the seaweed and two concentrates (8.74 and 9.18 *vs*. 0.53 and 1.08 and 0.76 g kg^{-1}, respectively) while the two concentrates had higher LA concentrations, with WDG also having higher LA concentrations than RSM (11.95 *vs*. 6.21 g kg^{-1}). The average value of ALA was similar under the different experimental treatments. The bromoform content of the feeds, on a DM basis, averaged across the four periods was 53 μg kg^{-1} for grass silage, 45 μg kg^{-1} for fresh grass, 6.5 μg kg^{-1} for RSM concentrate, 23 μg kg^{-1} for WDG concentrate and 962 μg kg^{-1} for *Saccharina latissima*. The bromoform content of the experimental diets, on a DM basis, was only marginally different, with seaweed diets containing +1.8 μg kg^{-1} DM (+3.7%) more bromoform than the control diets, while WDG diets had +4.2 μg kg^{-1} DM more bromoform than the RSM diets.

FA profiles in milk

Milk FA composition and bromoform concentrations are presented in Table 2. Seaweed supplementation did not affect nutritionally relevant milk FA ($P \geq 0.05$), and significant differences were only found for c5c8c11c14 C20:4 (−5.8%) and c15 C24:1 (−10.0%) which decreased when seaweed was fed ($P < 0.05$; Supporting Information, Table S3). Protein source significantly affected the concentrations of several individual FA and FA groups (Table 2). When compared with milk from cows fed WDG, milk from cows fed RSM had higher ($P < 0.05$) concentrations of C12:0 (+4.7%), C14:0 (+3.3%), C16:0 (+3.8%) and total SFA (+1.7%), as well as higher ($P < 0.05$) n-3:n-6, AI and TI. In contrast, WDG milk had higher ($P < 0.05$) concentrations of C18:0 (+8.4%), VA (+16.9%), OA (+5.2%), LA (+30.7%), RA (+12.9%), ALA (+6.5%), total MUFA (+3.6%), *trans* MUFA (+7.4%), total PUFA (+13.3%), *cis* PUFA (+18.8%), n-6 (+7.1%), *cis* n-3 PUFA (+4.9%), and *cis* n-6 PUFA (+25.3%), total CLA (+10.5%), and total *trans* FA (+4.7%), as well as higher ($P < 0.001$) n-6:n-3. There were no significant interactions between seaweed supplementation and the protein source ($P \geq 0.05$).

B vitamin concentrations in milk

The effects of seaweed supplementation and different protein sources (WDG and RSM) on the concentration of B vitamins in milk are presented in Table 3. The analysis revealed that seaweed supplementation did not significantly affect the concentrations of most of the B vitamins. Notably, a significant decrease was observed in pantothenate level when seaweed was fed, with a reduction from 7440 to 6774 μg L^{-1} ($P < 0.05$). In terms of the protein source, the results indicated no significant changes for all detected vitamins ($P > 0.05$). However, pyridoxine concentration in the RSM group had a tendency to be higher than that in the WDG group ($P = 0.065$). When examining the interaction between seaweed supplementation and protein source, the data suggest no statistically significant changes in all the detected B vitamins, but pyridoxine showed a tendency to be significantly different ($P = 0.099$).

Bromoform concentrations in milk

The bromoform concentrations in milk were negligible across all treatments and individual samples and were not significantly affected by any of the dietary treatments or their interactions ($P \geq 0.05$; Table 2).

Transfer rates of FA and bromoform from feed to milk

The intake, output in milk, and transfer rates of LA, ALA and bromoform from feed to milk are presented in Table 4. There were no significant differences in the intake and output of LA, ALA and bromoform. Transfer rates for LA were higher ($P < 0.001$) when cows were fed RSM compared to WDG as protein source (+2.3 g output in milk per 100 g of LA intake), but transfer rates for ALA and bromoform were not affected by the dietary treatments or their interaction.

DISCUSSION

Implications of replacing RSM with WDG in cows' diets on FA, B vitamins and bromoform concentrations

Milk SFA concentrations are considered an important parameter of milk and dairy products, because some SFA (C12:0, C14:0, C16:0) are associated with negative effects on human health, including increased risk of coronary heart disease, while milk and dairy products are one of the main sources of SFA in human diets.[34,35] In ruminants, the milk FA are derived almost equally from three sources: the *de novo* FA synthesis (which are SFA C4:0-C14:0, and approximately 50% of C16:0[36]), the uptake of circulating FA from feed, and the new FA formed as a result of the FA biohydrogenation in the rumen by the rumen microbiome.[37] The present study found reduced SFA in milk by feeding WDG, which is similar to a previous study containing increasing amounts of WDG in the concentrate mix (60 and 120 g kg^{-1} on DM basis) of lactating ewes.[38] However, several studies found no significant differences in the SFA concentrations in milk from 60 up to 200 g kg^{-1} DM inclusion levels of WDG.[39,40] The reduction of SFA was reported to be due to either a higher secretion of long-chain FA from the blood and/or a lower *de novo* synthesis of FA in the mammary gland.[38] WDG diets contained higher levels of long-chain FA in this study, which might result in decreased milk SFA.

LA and ALA are not produced *de novo* in mammals, due to a lack of appropriate enzymes, and hence are essential in human diets.[41] In addition, RA is considered nutritionally beneficial due to its anticarcinogenic and other health-promoting properties.[42] Other nutritionally relevant FA include VA, which has been associated with reduced tumour growth in animal studies and the reduced risk of coronary heart disease in epidemiological studies,[43] and is also a precursor for the synthesis of RA in the mammary cells[44]; as well as n-3 FA, which are associated with healthy aging throughout life such as foetal development, cardiovascular

Table 2. Effect of seaweed inclusion in dairy cows' diets, the type of protein source and their interaction[a] on milk fatty acid (FA) profile[b] and bromoform concentrations

	Seaweed				Protein source				Seaweed × Protein source					
Parameters	C $n = 32$	S $n = 32$	SEM	*P*-value[c]	WDG $n = 32$	RSM $n = 32$	SEM	*P*-value[c]	C-WDG $n = 16$	C-RSM $n = 16$	S-WDG $n = 16$	S-RSM $n = 16$	SEM	*P*-value[c]
Individual FA (g kg^{-1} total FA)														
C4:0	25.0	25.0	0.68	0.838	25.1	24.9	0.68	0.465	25.1	24.8	25.1	25.0	0.71	0.662
C6:0	18.6	18.8	0.33	0.358	18.7	18.6	0.33	0.616	18.6	18.6	18.9	18.7	0.36	0.795
C8:0	11.4	11.6	0.21	0.425	11.6	11.4	0.21	0.317	11.5	11.3	11.7	11.5	0.24	0.898
C10:0	28.0	28.4	0.75	0.522	28.3	28.1	0.75	0.705	28.1	27.9	28.5	28.3	0.86	0.993
C12:0	36.3	37.1	0.99	0.388	35.9	37.5	0.99	**0.045**	35.4	37.2	36.3	37.9	1.15	0.907
C14:0	118	120	2.5	0.315	117	121	2.5	**0.022**	116	120	118	121	2.7	0.743
C16:0	367	371	7.9	0.494	362	376	7.9	**0.007**	358	377	366	375	8.6	0.262
C18:0	80.9	78.4	2.48	0.242	82.8	76.4	2.48	**0.005**	85.0	76.8	80.6	76.1	2.91	0.397
OA (C18:1 c9)	161	158	5.0	0.414	163	155	5.0	**0.030**	167	155	160	155	5.6	0.363
VA (C18:1 t11)	8.00	8.15	0.533	0.647	8.71	7.45	0.533	**<0.001**	8.79	7.21	8.63	7.68	0.583	0.353
LA (C18:2 c9c12)	12.6	12.2	0.49	0.240	14.1	10.8	0.49	**<0.001**	14.3	11.0	13.9	10.6	0.55	0.883
RA (CLA9, C18:2 c9t11)	4.62	4.63	0.403	0.927	4.91	4.35	0.403	**<0.001**	4.93	4.32	4.89	4.38	0.408	0.585
ALA (C18:3 c9c12c15)	4.31	4.32	0.302	0.874	4.45	4.18	0.302	**0.012**	4.42	4.19	4.48	4.17	0.310	0.718
EPA (C20:5 c5c8c11c14c17)	0.49	0.47	0.029	0.455	0.48	0.49	0.029	0.529	0.48	0.51	0.47	0.48	0.034	0.701
DPA (C22:5 c7c10c13c16c19)	0.72	0.70	0.048	0.247	0.72	0.70	0.048	0.461	0.74	0.71	0.70	0.70	0.050	0.352
DHA (C22:6 c4c7c10c13c16c19)	0.04	0.04	0.005	0.191	0.04	0.04	0.005	***0.060***	0.04	0.04	0.04	0.04	0.005	0.901
FA groups (g kg^{-1} total FA)														
SFA	733	737	6.3	0.412	729	741	6.3	**0.006**	725	741	732	741	7.0	0.381
MUFA	232	229	5.5	0.456	235	227	5.5	**0.044**	238	227	232	227	6.1	0.365
cis MUFA	208	205	5.2	0.477	210	203	5.2	***0.078***	213	203	207	204	5.8	0.321
trans MUFA	24.8	24.4	1.24	0.506	25.4	23.7	1.24	**0.006**	25.7	23.9	25.2	23.5	1.32	0.953
PUFA	34.5	33.9	0.95	0.262	36.3	32.0	0.95	**<0.001**	36.7	32.2	35.9	31.8	1.03	0.694
cis PUFA	21.2	20.6	0.68	0.217	22.7	19.1	0.68	**<0.001**	23.1	19.3	22.4	18.9	0.74	0.807
trans PUFA	0.31	0.31	0.032	0.986	0.30	0.32	0.032	0.187	0.31	0.31	0.29	0.33	0.034	0.277
cis, *trans* + *trans*, *cis* PUFA	13.0	12.9	0.58	0.713	13.2	12.6	0.58	**0.017**	13.3	12.6	13.1	12.6	0.60	0.704
n-3	8.58	8.51	0.373	0.678	8.60	8.49	0.373	0.535	8.62	8.55	8.59	8.42	0.396	0.776
n-6	16.1	15.6	0.60	0.177	17.6	14.1	0.60	**<0.001**	17.9	14.3	17.3	14.0	0.66	0.766
cis n-3 PUFA	5.75	5.72	0.274	0.838	5.87	5.60	0.274	**0.039**	5.88	5.62	5.87	5.58	0.289	0.902
cis n-6 PUFA	15.4	14.9	0.58	0.166	16.9	13.5	0.58	**<0.001**	17.2	13.7	16.6	13.3	0.63	0.738
n-3:n-6 ratio	0.55	0.56	0.033	0.389	0.50	0.61	0.033	**<0.001**	0.49	0.60	0.51	0.61	0.034	0.768
n-6:n-3 ratio	1.89	1.86	0.102	0.507	2.06	1.69	0.102	**<0.001**	2.08	1.69	2.03	1.68	0.108	0.636
EPA + DHA	0.54	0.51	0.032	0.388	0.52	0.53	0.032	0.654	0.52	0.55	0.51	0.51	0.037	0.711
EPA + DPA + DHA	1.26	1.21	0.077	0.215	1.24	1.23	0.077	0.942	1.26	1.25	1.21	1.21	0.081	0.815
Total CLA	6.11	6.12	0.443	0.968	6.42	5.81	0.443	**<0.001**	6.46	5.76	6.38	5.86	0.451	0.438
trans FA	30.5	29.9	0.66	0.259	30.9	29.5	0.66	**0.013**	31.5	29.5	30.3	29.4	0.77	0.341
trans FA (excl. VA)	22.5	21.7	0.84	0.103	22.2	22.0	0.84	0.687	22.7	22.3	21.7	21.7	0.91	0.638

Table 2. Continued

Parameters	Seaweed				Protein source				Seaweed × Protein source					
	C $n = 32$	S $n = 32$	SEM	*P*-value[c]	WDG $n = 32$	RSM $n = 32$	SEM	*P*-value[c]	C-WDG $n = 16$	C-RSM $n = 16$	S-WDG $n = 16$	S-RSM $n = 16$	SEM	*P*-value[c]
Human health-related indices														
AI	3.33	3.41	0.104	0.301	3.24	3.50	0.104	**0.002**	3.17	3.49	3.31	3.51	0.118	0.470
TI	3.69	3.75	0.113	0.417	3.62	3.81	0.113	**0.019**	3.57	3.80	3.68	3.82	0.125	0.531
Δ^9-Desaturase activity indicators														
Δ^9-Desaturase activity index	0.23	0.23	0.007	0.404	0.24	0.23	0.007	**0.029**	0.24	0.23	0.23	0.23	0.008	0.344
C14:1:C14:0	0.02	0.02	0.001	0.323	0.02	0.02	0.001	0.198	0.02	0.02	0.02	0.02	0.001	0.172
C16:1:C16:0	0.03	0.03	0.002	0.166	0.03	0.03	0.002	0.343	0.03	0.03	0.03	0.03	0.002	0.546
OA:C18:0	2.00	2.04	0.080	0.350	1.98	2.06	0.080	***0.065***	1.96	2.04	2.01	2.07	0.084	0.822
RA:VA	0.21	0.19	0.017	0.433	0.19	0.21	0.017	0.343	0.19	0.23	0.19	0.19	0.023	0.338
Bromoform, μg L^{-1}	1.37	1.27	0.200	0.743	1.36	1.27	0.200	0.749	1.29	1.44	1.44	1.10	0.283	0.395

[a] The four dietary treatments were: (i) a basal diet based on WDG as the primary protein source without seaweed supplementation (C-WDG); (ii) a basal diet based on WDG as the primary protein source with 35.7 g per cow per day on DM basis of dried seaweed supplementation (*Saccharina latissima*) (S-WDG); (iii) a basal diet based on rapeseed meal (RSM) as the primary protein source without seaweed supplementation (C-RSM); and (iv) a basal diet based on RSM as the primary protein source with 35.7 g per cow per day on DM basis of dried seaweed supplementation (*S. latissima*) (S-RSM).
[b] Summaries of fatty acids, as reported in the Parameters column, are: SFA: C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0. MUFA:C10:1 c9, C11:1 c10, C12:1 c9, C13:1, C14:1 t9, C15:1 c9, C16:1 t6-8, C16:1 t9, C16:1 t11 + t12 + t13, C16:1 (co-elutes with C17:0 anteiso), C16:1 c11,C16:1 c13, C17:1 t10, C17:1 c9, C18:1 t4, C18:1 t5, C18:1 t6-8, C18:1 t9, C18:1 t10, C18:1 t11 (VA), C18:1 c6 + t12, C18:1 c9 (OA), C18:1 t15, C18:1 c11, C18:1 c12, C18:1 c13, C18:1 c14 (co-elutes with C18:1 t6), C18:1 c15 (co-elutes with C19:0), C18:1 c16, C19:1, C20:1 c8, C20:1 c11, C22:1 c13, C24:1 c15. PUFA: C18:2 t11t15, C18:2 t9t12, C18:2 c9t13, C18:2 c10t14, C18:2 c9t14, C18:2 c9t12, C18:2 t11c15, C18:2 t9c12, C18:2 c9c12 (LA), C18:2 t12c15, C18:3 c6c9c12, C18:3 c9c12c15 (ALA), 18:2 c9t11 conjugated (RA) (co-elutes with C18:2 t7c9 + t8c10 + t6c8), other conjugated FA of unknown isomerisms of C18:2, C20:2 c11c14, C20:3 c8c11c14, C20:3 c11c14c17, C20:4 c5c8c11c14, C22:2 c13c16, C20:5 c5c8c11c14c17 (EPA), C22:3 c13c16c19, C22:4 c7c10c16c19, C22:5 c7c10c13c16c19 (DPA), C22:6 c4c7c10c13c16c19 (DHA). *cis* MUFA: C10:1 c9, C11:1 c10, C12:1 c9, C13:1 c9, C14:1 c9, C16:2 c9 (co-elutes with C17:0 anteiso), C16:1 c11, C16:1 c13, C17:1 c9, C18:1 c6 (co-elutes with C18:1 t16), C18:1 c9 (OA), C18:1 c11, C18:1 c12, C18:1 c13, C18:1 c14 (co-elutes with C18:1 t6), C18:1 c15 (co-elutes with C19:0), C18:1 c16, c19:1 c9, C20:1 c8, C20:1 c11, C22:1 c13, C24:1 c15. *trans* MUFA:C14:1 t9, C16:1 t6 + t7 + t8, C16:1 t9, C16:1 t11 + t12 + t13, C17:1 t10, C18:1 t4, C18:1 t5, C18:1 t6 + t7 + t8, C18:1 t9, C18:1 t10, C18:1 t11 (VA), C18:1 t12 (co-elutes with C18:1 c6), C18:1 t15, C18:1 t16 (co-elutes with C18:1 c14). *cis* PUFA:C18:2 c9c12 (LA), C18:3 c6c9c12, C18:3 c9c12c15 (ALA), 20:2 c11c14, C20:3 c8c11c14, C20:3 c11c14c17, C20:4 c5c8c11c14, C22:3 c13c16, C20:5 c5c8c11c14c17 (EPA), C22:3 c13c16c19, C22:4 c7c10c13c16, C22:5 c7c10c13c16c19 (DPA), C22:6 c4c7c10c13c16c19 (DHA). *trans* PUFA: C18:2 t11t15, C18:2 t9t12. *cis*, *trans* + *trans*, *cis* PUFA: C18:2 c9t13, C18:2 c10t14, C18:2 c9t14, C18:2 c9t12, C18:2 t11c15, C18:2 t9c12, C18:2 t12c15, C18:2 c9t11 (RA) (co-elutes with C18:2 t7c9 + t8c10 + t6c8), other conjugated FA of unknown isomerism (CLA other a–h). omega-3 PUFA (n-3): C18:2 t11t15, C18:2 t11c15, C18:2 t12c15, C18:3 c9c12c15, C20:3 c11c14c17, C20:5 c5c8c11c14c17 (EPA), C22:3 c13c16c19, C22:5 c7c10c13c16c19 (DPA), C22:6 c4c7c10c13c16c19. omega-6 PUFA(n-6): C18:2 t9t12, C18:2 c9t12, C18:2 t9c12, C18:2 c9c12 (LA), C18:3 c6c9c12, C20:2 c11c14, C20:3 c8c11c14, C20:4 c5c8c11c14, C22:2 c13c16, C22:4 c7c10c13c16. *cis* n-3 PUFA: C18:3 c9c12c15, C20:3 c11 c14 C17, C20:5 c5c8c11c14c17, C22:3 c13C16C19, C22:5 c7c10c13C16C19, C22:6 c4c7c10c13C16C19. *cis* n-6 PUFA: C18:2 c9c12, C18:3 c6c9c12, C20:2 c11c14, C20:4 c5c8c11c14, C22:2 c13c16. *trans* FA: C14:1 t9, C16:1 t6 + t7 + t8, C16:1 t9, C16:1 t11 + t12 + t13, C17:1 t10, C18:1 t4, C18:1 t5, C18:1 t6 + t7 + t8, C18:1 t9, C18:1 t10, C18:1 t11 (VA), C18:1 t12 (co-elutes with C18:1 c6), C18:1 t15, C18:1 t16 (co-elutes with C18:1 c14), C18:2 t11t15, C18:2 t9t12. Atherogenicity index = (C12:0 + (4 × C14:0) + C16:0)/(MUFA + PUFA), as described in Średnicka-Tober *et al.*[32] Thrombogenicity index = (C14:0 + C16:0 + C18:0)/(0.5 × MUFA) + (0.5 × n-6) + (3 × n-3) + (n-3:n-6) as described in Średnicka-Tober *et al.*[32] Δ^9-desaturase activity index = (c9 C14:1 + c9 C16:1 + OA + RA)/(c9 C14:1 + c9 C16:1 + OA + RA + C14:0 + C16:0 + C18:0 + VA) as described in Kay *et al.*[62]
[c] Significances were declared at $P < 0.05$ (bold regular fonts), and trends were indicated at $0.05 \leq P < 0.10$ (bold italics fonts).

Table 3. Effect of seaweed inclusion in dairy cows' diets, the type of protein source and their interaction[a] on milk concentrations of B vitamins

	Seaweed				Concentrate				Seaweed × concentrate					
Parameters, μg L^{-1}	C $n = 32$	S $n = 32$	SEM	*P*-value[b]	WDG $n = 32$	RSM $n = 32$	SEM	*P*-value[b]	C-WDG $n = 16$	C-RSM $n = 16$	S-WDG $n = 16$	S-RSM $n = 16$	SEM	*P*-value[b]
B vitamins (μg L^{-1})														
Thiamine (B1)	236	228	8.7	0.460	236	228	8.7	0.506	239	234	233	222	12.1	0.778
Riboflavin (B2)	1918	1832	80.1	0.448	1892	1857	80.1	0.760	2012	1824	1772	1891	113.2	0.180
Pantothenate (B5)	7440	6774	219.6	**0.034**	7293	6921	219.6	0.230	7765	7114	6820	6727	308.5	0.367
Pyridoxal (B6)	3266	3743	251.3	0.145	3721	3289	251.3	0.187	3539	2993	3902	3585	339.4	0.723
Pyridoxine (B6)	2.20	2.05	0.318	0.716	1.75	2.50	0.318	***0.065***	2.16	2.24	1.34	2.76	0.425	***0.099***
Biotin (B7)	28.85	29.50	2.487	0.851	29.02	29.33	2.487	0.929	28.40	29.30	29.64	29.37	3.497	0.867
Folic acid (B9)	4.34	5.60	1.162	0.150	5.31	4.63	1.162	0.432	4.30	4.38	6.33	4.88	1.314	0.380
Cyanocobalamin (B12)	9.40	10.50	0.905	0.384	10.47	9.44	0.905	0.419	9.56	9.23	11.37	9.64	1.270	0.583

[a] The four dietary treatments were: (i) a basal diet based on WDG as the primary protein source without seaweed supplementation (C-WDG); (ii) a basal diet based on WDG as the primary protein source with 35.7 g per cow per day on DM basis of dried seaweed supplementation (*Saccharina latissima*) (S-WDG); (iii) a basal diet based on rapeseed meal (RSM) as the primary protein source without seaweed supplementation (C-RSM); and (iv) a basal diet based on RSM as the primary protein source with 35.7 g per cow per day on DM basis of dried seaweed supplementation (*S. latissima*) (S-RSM).
[b] Significances were declared at $P < 0.05$ (bold regular fonts), and trends were indicated at $0.05 \leq P < 0.10$ (bold italics fonts).

Table 4. Effect of seaweed inclusion in dairy cows' diets, the type of protein source and their interaction[a] on the transfer rate of linoleic acid, α-linolenic acid and bromoform from feed to milk

	Seaweed				Concentrate				Seaweed × concentrate					
Parameters	C $n = 32$	S $n = 32$	SEM	*P*-value[b]	WDG $n = 32$	RSM $n = 32$	SEM	*P*-value[b]	C-WDG $n = 16$	C-RSM n = 16	S-WDG $n = 16$	S-RSM $n = 16$	SEM	*P*-value[b]
Linoleic acid														
Intake (g d^{-1})	83.5	83.2	3.43	0.722	98.4	68.4	3.43	**<0.001**	99.2	67.8	97.5	68.9	3.48	0.100
Output (g d^{-1})	11.4	10.9	0.78	0.192	12.3	10.0	0.78	**<0.001**	12.6	10.2	11.9	9.8	0.83	0.711
Transfer rate (% intake)	14.0	13.3	1.12	0.157	12.5	14.8	1.12	**<0.001**	12.9	15.1	12.2	14.4	1.17	0.950
α-Linolenic acid														
Intake (g d^{-1})	124	122	10.4	0.535	123	123	10.4	0.988	126	121	120	125	10.6	***0.060***
Output (g d^{-1})	3.93	3.89	0.456	0.821	3.94	3.87	0.456	0.606	3.95	3.90	3.94	3.85	0.466	0.923
Transfer rate (% intake)	3.26	3.18	0.346	0.461	3.25	3.19	0.346	0.605	3.27	3.25	3.22	3.13	0.356	0.740
Bromoform														
Intake (μg d^{-1})	819	822	96.0	0.981	848	793	96.0	0.686	857	781	839	805	135.8	0.881
Output (μg d^{-1})	26.9	28.2	4.71	0.850	27.7	27.4	4.71	0.960	24.0	29.9	31.5	24.9	6.67	0.357
Transfer rate (% intake)	4.56	4.44	0.780	0.919	4.22	4.78	0.780	0.611	3.51	5.60	4.93	3.96	1.104	0.171

[a] The four dietary treatments were: (i) a basal diet based on WDG as the primary protein source without seaweed supplementation (C-WDG); (ii) a basal diet based on WDG as the primary protein source with 35.7 g per cow per day on DM basis of dried seaweed supplementation (*Saccharina latissima*) (S-WDG); (iii) a basal diet based on rapeseed meal (RSM) as the primary protein source without seaweed supplementation (C-RSM); and (iv) a basal diet based on RSM as the primary protein source with 35.7 g per cow per day on DM basis of dried seaweed supplementation (*S. latissima*) (S-RSM).
[b] Significances were declared at $P < 0.05$ (bold regular fonts), and trends were indicated at $0.05 \leq P < 0.10$ (bold italics fonts).

function and Alzheimer's disease.[45] The present study found that feeding cows with WDG resulted in milk with higher content of PUFA, especially the nutritionally essential LA and ALA, when compared with milk from cows fed RSM, as well as increased values of VA (synthesized by the rumen microbial biohydrogenation of dietary PUFA[34,46]) and RA (synthesized in the mammary gland under the effect of Δ^9-desaturase using VA as precursor).[46] The increased milk concentrations of key FA such as LA, ALA and RA were also found in previous studies when feeding cows WDG, instead of barley silage or corn grain and soybean meal or canola meal.[38-40]

In the present study, dietary LA intake was higher when WDG was fed (which may explain the increase in milk LA concentrations), while the transfer rates from feed to milk were lower, thus indicating extensive biohydrogenation, which may have increased the synthesis of VA in the rumen and RA in the mammary gland[34,46] and explain their higher concentrations in milk. Despite the higher feed-to-milk transfer rate of LA in cows fed RSM, milk from RSM cows had lower concentrations in LA, indicating that the higher intakes of LA when cows fed WDG (+44%; +30 g d^{-1}) were enough to increase milk LA concentrations but also provide adequate substrate for the higher synthesis of VA and RA (which originate from dietary LA and ALA[34,46]). In general, dietary supplementation with oils rich in LA and ALA is considered an effective way to enhance milk RA concentrations in dairy cows.[19,21] Given that part of the observed effects on milk FA may be microbial driven,[47] a longer-term study with animals on a continuous study design, remaining on the same diets for longer periods (e.g., 84 days or more), would be beneficial to assess the persistence of these effects over time.

Implications for including seaweed in dairy cows' diets on FA, B vitamins and bromoform concentrations

Including different seaweeds in animal diets has been found to (i) reduce CH_4 production (g d^{-1}) and CH_4 yield (g kg^{-1} DM intake) by 26.4% and 20.3%, respectively, when the bromoform-rich red seaweed *Asparagopsis armata* was fed at 5 g kg^{-1} diet DM; and by 60% and 54%, respectively, when the bromoform-rich red seaweed *Asparagopsis taxiformis* was fed at 5 g kg^{-1} diet OM,[28] without affecting milk production and fat/protein contents,[27,28] (ii) reduce rumen ammonia, shift hydrogen disposal towards propionic acid and increase volatile FA synthesis by the rumen microbiota in dairy cows fed the brown seaweed Kelp,[48] (iii) reduce oxidative stress and improve stress marker profile in cattle fed the brown seaweed *Ascophyllum nodosum*,[49,50] and (iv) lower milk somatic cell counts (indicator of mastitis) in dairy cows fed *Ascophyllum nodosum*.[51] However, the present study, using 35.7 g d^{-1} of a different seaweed (*S. latissima*), did not show an effect on milk FA profiles, which were only marginally affected. Different findings between the studies may be affected by animal and seaweed species, basal diet, seaweed inclusion rates, the chemical composition of the seaweed and the composition of the bioactive components, which may all affect dietary supply of FA and their rumen biohydrogenation.[52-54]

Although dietary intake of vitamins has less of an effect on milk B vitamin concentrations, changes in their microbial synthesis, bioavailability, and possibly co-factor activities in biochemical pathways, all affected by cows' diet, are expected to have an indirect effect.[24] Seaweed supplementation had a limited effect on most B vitamin concentrations, except for pantothenate (vitamin B5), which was reduced by feeding seaweed. Previous studies have reported that κ-carrageenan and fucoidan from seaweed have the ability to inhibit pantothenate synthetase (an enzyme that catalyses the synthesis of B5).[55] The reduction observed might suggest an alteration in metabolic pathways in dairy cows[56] due to seaweed constituents. Similar to the case of milk FA profile, part of the variation in milk B vitamin content may originate from microbial processes;[24] hence longer-term studies with animals remaining on the experimental diets for longer periods could be useful to quantify the effect of SWD supplementation on milk pantothenate concentrations over time.

Bromoform is considered a toxic compound to humans and previously showed potential kidney and liver toxicity in experiments performed in rats and mice.[57] The US Environmental Protection Agency recommends that drinking water should contain no more than 700 μg L^{-1} of bromoform.[58] Regarding the potential risk of increased milk bromoform concentrations when the brown seaweed *S. latissima* is included in dairy cows' diet, the present study showed that supplementing dairy cow diets with 35.7 g d^{-1} of *S. latissima* is not a risk because bromoform concentrations were not affected by the dairy cows' diets. In addition, the transfer rates of bromoform from feed to milk are extremely small; for example, for every 100 μg ingested by the dairy cow via feed, less than 0.03 μg was transferred to milk. The mean concentrations of bromoform in milk in all treatments in the present study were only traces (1.32 μg L^{-1} milk), representing a marginal 0.26% of the maximum recommended concentration for water by the US Environmental Protection Agency.[58] Previous studies have also shown that bromoform does not accumulate in animal tissue but is excreted in urine, although they also mentioned milk as a potential pathway for excretion but without providing the transfer rates.[57] Other studies have claimed that low inclusion of the seaweed *Asparagopsis* may limit tissue absorption of bromoform due to the metabolic dehalogenation of bromoform in rumen.[12] The present study provides evidence that milk is not a source of bromoform in human diets and there are no bromoform-related safety concerns when feeding cows with *S. latissima* at 35.7 g d^{-1} on a DM basis.

Nutritional implications for consumers

Overall, the results suggest that feeding WDG to dairy cows, instead of RSM, can improve milk FA profiles by reducing nutritionally undesirable SFA and increasing certain nutritionally desirable unsaturated FA, which are associated with benefits to human health, such as PUFA, LA, RA and ALA. The reduction in AI and TI may also be considered a desirable effect from a nutritional point of view. The latest UK National Diet and Nutrition survey[16] reports the following dairy fat intakes across the different demographics: children 1.5–3.0 years of age, 13.4 g d^{-1}; for children 4–10 years of age, 9.8 g d^{-1}; adolescents 11–18 years of age, 8.3 g d^{-1}; adults 19–64 years of age, 8.8 g d^{-1}; adults 65–74 years of age, 9.8 g d^{-1}; adults 75+ years of age, 10.6 g d^{-1}. Based on current nutritional recommendations and dietary reference values (DRV; <10% total energy intake) for SFA intakes,[59] consuming WDG milk instead of RSM milk would reduce SFA intake from dairy fats (relative to DRV) from 79.0% to 77.7% for children 1.5–3.0 years of age, from 42.3% to 41.6% for children 4–10 years of age, from 31.1% to 30.6% for adolescents 11–18 years of age, from 30.0% to 29.5% for adults 19–64 years of age, from 36.5% to 35.9% for adults 65–74 years of age and from 41.5% to 40.8% for adults 75+ years of age. Despite the significant effect of WDG on milk SFA concentration, the relative impact that these would have in consumers' diets when consuming dairy fat produced by cows fed WDG instead of RSM appears relatively small, and it is difficult

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to imply that these would affect consumers' health. Similarly, consuming WDG milk instead of RSM milk would increase LA and ALA intake from dairy fats (relative to DRV) from 11.5% and 22.3% to 15.0% and 23.7% for children 1.5–3.0 years of age, from 6.2% and 11.9% to 8.0% and 12.7% for children 4–10 years of age, from 4.5% and 8.8% to 5.9% and 9.4% for adolescents 11–18 years of age, from 4.4% and 8.5% to 5.7% and 9.0% for adults 19–64 years of age, from 5.3% and 10.3% to 6.9% and 10.9% for adults 65–74 years of age, and from 6.1% and 11.7% to 7.9% and 12.5% for adults 75+ years of age, for LA and ALA, respectively. These differences, similarly to those in milk SFA, appear relatively small (in the case of ALA) or refer to an essential nutrient that is not in short supply in human diets (in the case of LA[60]), and it is difficult to imply that these would affect consumers' health.

The only difference observed in the concentrations of B vitamin concentration between experimental treatments was the 9% reduction in milk pantothenate concentrations. The European Food Safety Authority's Adequate Intake (AIn) level is set at 3 mg d^{-1} for infants and children, 5 mg d^{-1} for adolescents, adults, and pregnant women, and 7 mg d^{-1} for lactating women.[61] At the measured concentrations of pantothenate in the milk in this study, 200 mL of control or seaweed milk would provide 1.49 mg or 1.35 mg of pantothenate respectively. In children, this would represent a reduction in contribution towards pantothenate AIn requirements from 50% AIn to 45% AIn; while this reduction would be from 30% to 26% AIn for adolescents and adults and 21% to 19% for pregnant women. Given that this reduction is rather small, and that the European population shows no signs of pantothenate deficiency, these levels of reduction are unlikely to be associated with negative effects on human nutrition and health.

CONCLUSIONS

Feeding WDG to dairy cows improved the milk FA profiles by increasing the concentrations of the nutritionally beneficial PUFA, RA, LA and ALA and reducing the concentration of the nutritionally undesirable SFA. However, when accounting for the current average milk intakes in the UK population, these compositional differences appear relatively small to affect human health. Feeding *S. latissima* at 35.7 g per cow per day does not affect milk FA profiles and does not pose any risks around bromoform contamination of milk; while the small reductions in pantothenate concentrations in milk are unlikely to be associated with impacts on human nutrition and health.

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CONFLICT OF INTEREST

The authors declare no competing financial interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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