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Meta-Analysis of Dietary Supplementation with Seaweed in Dairy Cows: Milk Yield and Composition, Nutrient Digestibility, Rumen Fermentation, and Enteric Methane Emissions

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Abstract: This study used a meta-analytic approach to evaluate the effects of dietary supplementation with seaweed on milk yield, milk composition, nutrient digestibility, ruminal fermentation, and enteric methane (CH_4) emissions of dairy cows. Data used in statistical analyses were obtained from 23 peer-reviewed scientific articles. Effect size was assessed using weighted mean differences (WMD) between seaweed-supplemented and control treatments. Dietary supplementation with seaweed decreased ($p < 0.05$) dry matter intake, milk protein content, milk urea nitrogen, and somatic cell count. In contrast, milk fat content, milk lactose content, and milk iodine increased ($p < 0.05$) in response to dietary supplementation with seaweed. Dietary supplementation with seaweed did not affect ($p > 0.05$) nutrient digestibility, total volatile fatty acids, acetate, and propionate. Dietary supplementation with seaweeds increased ($p < 0.05$) ruminal pH and ruminal concentration of butyrate and valerate. In contrast, lower ($p < 0.05$) ruminal ammonia nitrogen concentration, acetate/propionate ratio, daily CH_4 emission, CH_4 yield, and CH_4 intensity were observed in response to dietary supplementation with seaweeds. In conclusion, dietary supplementation with seaweed modifies milk composition, improves ruminal fermentation, and decreases enteric methane emissions without negatively affecting milk yield or feed efficiency.

Keywords: macroalgae; *Asparagopsis taxiformis*; *Ascophyllum nodosum*; milk quality; meta-regression

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1. Introduction

Dairy cow production systems are important, as cow's milk is the most widely produced and consumed milk globally due to its high content of high-quality protein, bioavailable amino acids, vitamins, and minerals essential for humans [1]. However, enteric methane (CH_4) is produced in the rumen of dairy cows due to microbial fermentation of ingested feed [2]. Bačėninaitė et al. [3] indicate that CH_4 emitted by dairy cows contributes approximately 35–55% of total greenhouse gas (GHG) emissions from dairy farms worldwide. Consequently, in recent years, the interest of governments and researchers in finding solutions to reduce CH_4 emissions has increased dramatically [4]. To date, a large number of dietary additives have been evaluated for mitigating enteric CH4 in dairy cows, such as essential oils, condensed tannins, saponins, flavonoids, ionophores, 3-nitrooxypropanol, and seaweed [3–5]. Among these additives, seaweed is one of the most effective in mitigating CH_4 emissions in dairy cows without negatively affecting milk yield or feed efficiency [6,7].

Seaweeds (also known as macroalgae) are photosynthetic multicellular eukaryotic organisms that can have different sizes and morphologies [8]. According to some authors [6,9], seaweeds grow on the ocean bottom and are generally classified into three types: red seaweeds (Rhodophyta), brown seaweeds (Ochrophyta), and green seaweeds (Chlorophyta). Although their chemical profile may vary by seaweed type, seaweeds generally contain a wide variety of bioactive compounds, such as polysaccharides, phlorotannins, and halogenated alkanes (mainly bromoform) [6]. According to Glasson et al. [10], halogenated alkanes from seaweed can inhibit CH_4 emission in ruminants by blocking the activity of coenzyme M methyltransferase and the enzyme methyl-coenzyme M reductase (MCR), which are required in ruminal CH_4 formation [7]. On the other hand, seaweed polysaccharides have antimicrobial, antioxidant, antiviral, and anti-inflammatory properties in livestock [6]. Furthermore, seaweed phlorotannins improve ruminal protein metabolism and have antioxidant, anthelmintic, and anti-methanogenic activity in ruminants [7].

Specifically in dairy cows, a growing number of experiments have evaluated the effects of dietary seaweed supplementation on milk yield [11,12], milk composition [2,13], ruminal fermentation and nutrient digestibility [14,15], and enteric CH_4 emissions [16,17]. However, no fully reliable conclusions have been obtained regarding the effect of seaweed treatment on dairy cows because the results reported across studies have been contradictory. For example, some studies reported an increase in milk yield [13], improved milk composition [18], and a decrease in enteric CH_4 emissions [14] from dairy cows supplemented with seaweed. In contrast, other authors [2,17,19] show that seaweed negatively affects milk yield and milk composition of dairy cows. Likewise, other studies [11,15] reported that seaweed did not affect milk yield, milk composition, ruminal fermentation, nutrient digestibility, and CH_4 methane emissions of dairy cows.

Previously reported results from two meta-analyses [20,21] suggest that seaweeds can mitigate enteric CH_4 emissions in ruminants without negatively affecting productive performance. However, these two meta-analyses [20,21] only included a small number of studies (between six and 11) of dairy cows in their database. Furthermore, the meta-analysis by Lean et al. [21] only evaluated five response variables related to dairy cows. Likewise, in the meta-analysis by Sofyan et al. [20], the results for milk yield, milk composition, rumen parameters, and enteric CH_4 emissions were obtained by combining data from small ruminants, dairy cows, and beef cattle (in some response variables such as CH_4). According to Ioannidis [22], using databases with few studies or combining data from different animal species (e.g., small ruminants, dairy cows, and beef cattle) decreases the likelihood of obtaining robust and scientifically reliable results. On the other hand, Kebreab et al. [23] mention that periodically updating meta-analyses on a specific topic using larger databases can help obtain conclusive findings. In response to the increasing number of scientific articles published in recent years on the use of seaweed as a dietary additive for dairy cows, the current study aimed to evaluate the effects of dietary supplementation with seaweed on milk yield, milk composition, nutrient digestibility, ruminal fermentation, and enteric methane emissions of dairy cows through meta-analytical statistical procedures. The hypothesis of the present meta-analysis states that the inclusion of seaweed in diets for dairy cows will positively impact milk yield, milk composition, nutrient digestibility, and ruminal fermentation and will decrease methane emissions.

2. Materials and Methods

2.1. Literature Search

The research question was formulated using the PICO strategy proposed by Nishikawa-Pacher [24], in which P is the population, I is the intervention, C is the comparison, and O is the outcome. In the current study, the population was dairy cows, the intervention was the inclusion of seaweed in the diet, the comparison was between diets supplemented with seaweed and diets without seaweed, and the outcomes were the means of treatments obtained in milk yield and composition, nutrient digestibility, ruminal fermentation, and enteric CH_4 emissions. Subsequently, the scientific documents that evaluated the effects of dietary supplementation with seaweed in dairy cows were identified, selected, chosen, and included in the database following the guidelines of the PRISMA protocol [25], as shown in Figure 1. The identification of literature was carried out through systematic searches using the search engines Scopus, ScienceDirect, PubMed, and Web of Science. The keywords used in the searches were the following: (1) seaweed, (2) macroalgae, (3) dairy cows, (4) dairy cattle, (5) milk yield, (6) milk production, (7) milk composition, (8) milk quality, (9) nutrient digestibility, (10) ruminal fermentation, and (11) methane emission. To obtain updated information, literature searches were restricted to studies published in the recent decade (January 2014 to May 2024).

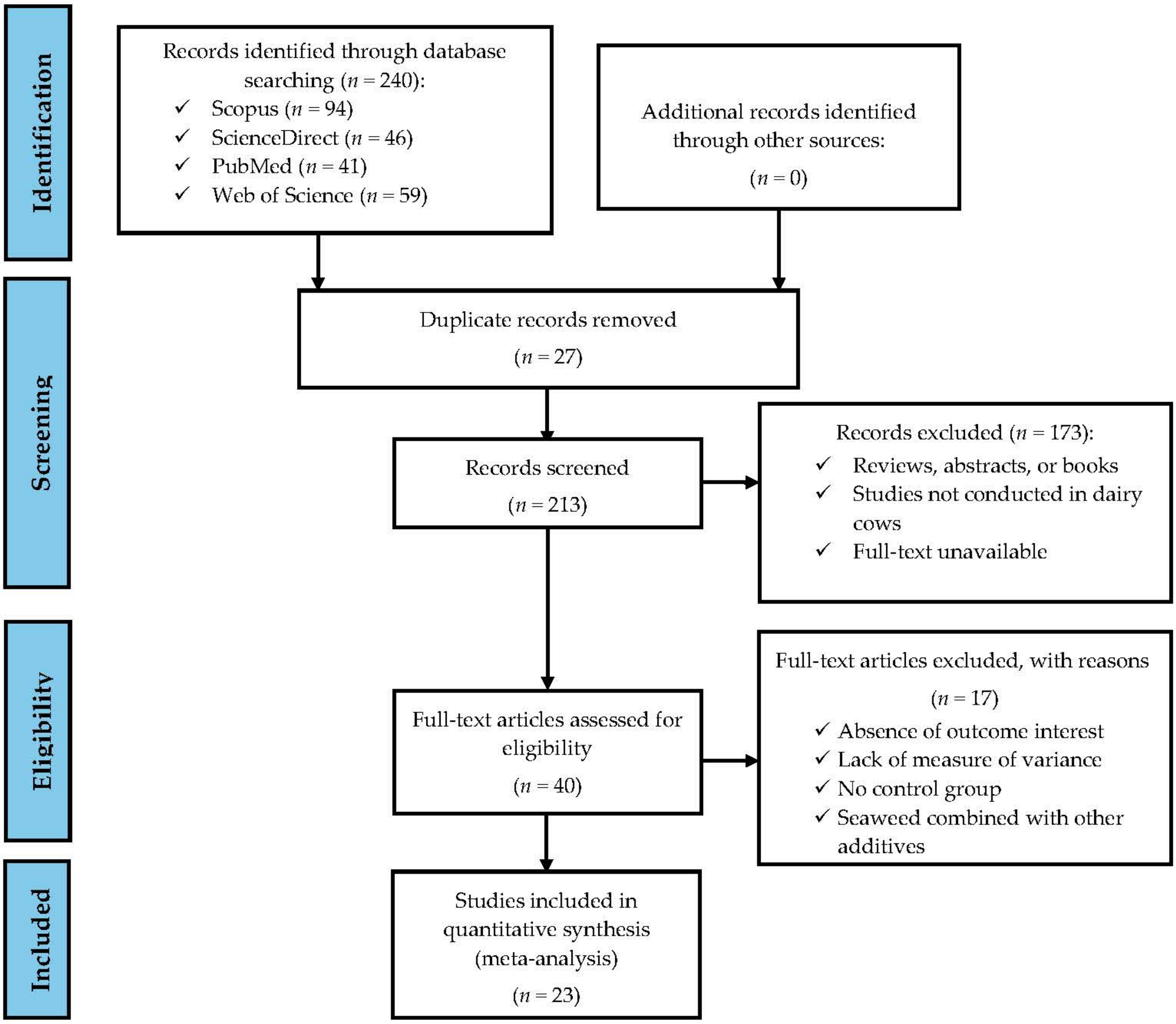

Figure 1. A PRISMA flow diagram detailing the literature search strategy and study selection for the meta-analysis.

2.2. Inclusion and Exclusion Criteria

Through the searches, 240 scientific documents were identified, which, after eliminating duplicate documents, were reduced to 213. After this, documents that had any of the following traits were eliminated: (1) conference proceedings, books, theses, review articles, and simulations; (2) studies that did not use dairy cows as experimental units; and (3) studies that did not use seaweed or combined seaweed with antibiotics, organic acids, or other products. The remaining documents were evaluated and only those that met all of the following inclusion criteria were used to form the meta-analysis database [26,27]: (1) scientific articles written in the English language and peer-reviewed; (2) studies that used dairy cows as experimental units and randomized experiments; (3) studies that compared the effect of dietary supplementation with seaweed against a control treatment (diets without the addition of seaweed) in dairy cows fed with the same basal diet; (4) studies reporting data on parameters related to milk yield and composition, nutrient digestibility, ruminal fermentation, and enteric CH_4 emissions; (5) studies that indicated the duration of the seaweed supplementation period and the doses of seaweed added to diets; and (6) studies that included data on standard deviation (SD), standard error (SEM), number of replicates (*n*), and means of treatments supplemented with seaweed and control (diets without the addition of seaweed).

2.3. Data Extraction

Table 1 shows the 23 scientific articles used to build the meta-analysis database. The following characteristics of each selected scientific article were extracted into an Excel spreadsheet: (1) name of the author, (2) year of publication, (3) breed of dairy cows, (4) experimental design (continuous or rotative), (5) days in milk from dairy cows, (6) duration (days) of the period of dietary supplementation with seaweed, (7) dose (g/kg DM) of seaweed added to the diets, (8) seaweed species (i.e., *Asparagopsis taxiformis*, *Ascophyllum nodosum*, among others), (9) type of seaweed (red or brown), and (10) level of forage (g/kg DM) included in diets. The variables extracted from the scientific articles were grouped as follows: (1) dry matter intake (DMI), milk yield (MY); energy-corrected milk yield (ECMY); feed efficiency (FE), milk fat yield (MFY), milk protein yield (MPY), milk lactose yield (MLY), milk fat content (MFC), milk protein content (MPC), milk lactose content (MLC), total solids (TC), milk urea nitrogen (MUN), somatic cell count (SCC), iodine and bromoform in milk; (2) dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD), neutral detergent fiber digestibility (NDFD), acid detergent fiber digestibility (ADFD), ruminal pH, ammonia nitrogen (NH_3-N), total volatile fatty acids (TVFA), acetate, propionate, butyrate, valerate, acetate/propionate ratio, daily CH_4 emission (g/d), CH_4 yield (g/kg DMI), CH_4 intensity (g/kg ECMY). The SEM, number of replicates, and treatment means were obtained for each response variable. The WebPlotDigitizer software version 4.4 was used to extract the data reported in graphs, following the procedures described by Drevon et al. [28].

Table 1. Description of the studies included in the meta-analysis database.

Reference	Breed	ED	DIM, d	SP, d	SW Dose (g/kg DM)	SW Specie	SW Type	Forage, g/kg DM
Antaya et al. [11]	Jersey	Rotative	40	21	3.2, 6.3, 9.7	*Ascophyllum nodosum*	Brown	642
Antaya et al. [29]	Jersey	Continuous	142	28	5.8, 6.6, 6.8	*Ascophyllum nodosum*	Brown	700
Bendary et al. [30]	Holstein	Continuous	7	150	2.9	*Ascophyllum nodosum*	Brown	600
Bošnjaković et al. [18]	Holstein	Rotative	174	21	20, 40	SL, SM, AN	Brown	611
Eikanger et al. [2]	Norwegian	Continuous	95	39	1.25, 2.50	*Asparagopsis taxiformis*	Red	650
Hong et al. [12]	Holstein	Continuous	30	360	20, 40	Blend-1	Brown	650
Katwal et al. [31]	Crossbreed	Rotative	NR	45	80.00	*Sargassum johnsonii*	Brown	600
Kidane et al. [32]	Norwegian	Rotative	164	28	8.9	*Ascophyllum nodosum*	Brown	840
Krizsan et al. [14]	Nordic Red	Rotative	122	21	5.0	*Asparagopsis taxiformis*	Red	600
López et al. [33]	Holstein	Continuous	302	38	4.8	*Ascophyllum nodosum*	Brown	770
Muizelaar et al. [16]	Holstein	Rotative	91	63	6.6, 6.5, 6.7	CHC, SL, Blend-2	Red, Brown	750
Newton et al. [13]	Iceland	Continuous	168	49	0.9, 3.5	Blend-3	Brown	550
Newton et al. [34]	Holstein	Continuous	168	63	13.4	*Ascophyllum nodosum*	Brown	710
Nyloy et al. [35]	Norwegian	Continuous	95	52	1.25, 2.5	*Asparagopsis taxiformis*	Red	650
Qin et al. [36]	Holstein	Rotative	NR	28	2.1	SL	Brown	750
Reyes et al. [37]	Holstein and Jersey	Continuous	150	84	60	CHC	Red	670
Roque et al. [19]	Holstein	Rotative	201	21	5.0, 10.0	*Asparagopsis armata*	Red	NR
Rey-Crespo et al. [38]	Holstein	Continuous	154	70	5.3	Blend-4	Blend	765
Sharma y Datt [39]	Crossbreed	Continuous	NR	150	15, 30	*Laminaria digitata*	Brown	NR

Table 1. *Cont.*

Reference	Breed	ED	DIM, d	SP, d	SW Dose (g/kg DM)	SW Specie	SW Type	Forage, g/kg DM
Silva et al. [40]	Jersey	Rotative	102	28	2.7, 5.4, 8.1	*Ascophyllum nodosum*	Brown	663
Singh et al. [41]	Sahiwal	Continuous	56	56	20	*Sargassum wightii*	Brown	750
Stefenoni et al. [17]	Holstein	Rotative	95	28	2.5, 5.0	*Asparagopsis taxiformis*	Red	603
Thorsteinsson et al. [15]	Holstein	Continuous	174	21	0.23, 0.46	*Ascophyllum nodosum*	Brown	534

ED: experimental design; DIM: days in milk; d: days; SP: supplementation period; SW: seaweed; NR: not reported; CHC: *Chondrus crispus*; SL: *Saccharina latissima*; SM: *Sargassum muticum*; AN: *Ascophyllum nodosum*; Blend-1: unreported species; Blend-2: SL and *Fucus serratus*; Blend-3: *Ascophyllum nodosum* and *Laminaria digitata*; Blend-4: SM, *Ulva rígida*, and *Sacchorhiza polyschides*.

2.4. Calculations, Statistical Analysis, Heterogeneity, and Publication Bias

The "meta" [42] and "metafor" [43] packages available in the R statistical software (version 4.1.2) were used to analyze all data in the current study. The effect size (ES) of seaweed supplementation in dairy cow diets was assessed by examining the weighted mean differences (WMD) between the experimental (diets with seaweed) and control (diets without seaweed) treatments. According to Takeshima et al. [44], WMD have greater statistical power and are easier to interpret than other ES measures, which makes their use advisable. The methods and procedures previously proposed by Der-Simonian and Laird [45] for random effects models were used to weight the treatment means by inverse variance.

Heterogeneity between studies was rigorously assessed using I^2 and Cochran's Q statistics, and significant heterogeneity was declared when Q had $p \leq 0.05$ and $I^2 > 50\%$ [46,47]. Furthermore, publication bias was assessed with Rosenberg's fail-safe number (NFS) using a significance level of $p \leq 0.05$ [48]. Although publication bias was detected in one response variable, the result was considered valid and robust when NFS > [5 (n) + 10], where n is the number of comparisons included in the analysis [49].

2.5. Meta-Regression and Subgroup Analysis

Univariate meta-regression analyses were used to test the effects of experimental design, days in milk, level of forage in the diet, seaweed dose, supplementation period, seaweed type, and seaweed species on the observed heterogeneity in response variables. Meta-regression analyses were performed using Der-Simonian and Laird's [45] method of moments only on response variables that had the following traits: (1) being reported in at least ten different scientific articles [26,27,46]; (2) have Cochran's Q statistic with $p \leq 0.05$ and I^2 statistic > 50% [46,47]; and (3) have $p > 0.05$ in the Rosenberg's fail-safe number [48]. The covariates evaluated were divided as follows: (1) experimental design (continuous and rotative); (2) days in milk (≤100 and >100 days); (3) level of forage in the diet (≤600 and >600 g/kg DM); (4) doses of seaweed added to diets 0.23–5.0, 5.1–10.0, and >10.0 g/kg DM; (5) dietary supplementation period with seaweed ≤ 30 and >30 days; (6) type of seaweed (red seaweed and brown seaweed); and (7) species of seaweed (i.e., *Asparagopsis taxiformis*, *Ascophyllum nodosum*, among others). The significant covariates ($p \leq 0.05$) were evaluated through subgroup analysis, including only subgroups with at least three comparisons, as recommended by other authors [27,50].

3. Results

3.1. Milk Yield and Composition

Table 2 shows that dietary supplementation with seaweeds decreased ($p < 0.05$) DMI, MPC, MUN, and SCC. In contrast, MFC, MLC, and milk iodine increased ($p < 0.05$) in response to dietary seaweeds supplementation. On the other hand, MY, ECMY, FE, MFY, MPY, MLY, TS, and bromoform content in milk were not affected ($p > 0.05$) by dietary supplementation with seaweeds. Table 2 shows that there was significant heterogeneity ($Q \leq 0.05$ and $I^2 > 50\%$) in DMI, MY, ECMY, and iodine.

Table 2. Milk yield and milk composition in dairy cows supplemented with seaweed.

Outcomes	N (NC)				Heterogeneity	
		Control Means (SD)	WMD (95% CI)	*p*-Value	*p*-Value [1]	I^2 (%)
DMI, kg/d	21 (41)	19.85 (4.93)	−0.218 (−0.488; 0.052)	0.113	<0.001	67.99
MY, kg/d	18 (35)	26.17 (7.43)	−0.100 (−0.718; 0.519)	0.752	<0.001	78.85
ECMY, kg/d	11 (21)	25.59 (8.55)	−0.525 (−1.823; 0.774)	0.428	<0.001	91.92
FE, DMI/MY	9 (17)	1.164 (0.351)	−0.010 (−0.028; 0.009)	0.296	0.309	12.39
MFY, kg/d	8 (18)	1.06 (0.33)	−0.004 (−0.037; 0.030)	0.829	0.769	0.00
MPY, kg/d	8 (18)	0.87 (0.31)	0.008 (−0.013; 0.029)	0.446	0.978	0.00
MLY, kg/d	7 (17)	1.17 (0.46)	0.016 (−0.018; 0.049)	0.356	0.998	0.00
MFC, g/kg	17 (33)	4.13 (0.55)	0.070 (0.046; 0.095)	<0.001	0.506	0.00
MPC, g/kg	17 (33)	3.34 (0.30)	−0.039 (−0.068; −0.009)	0.010	0.071	40.93
MLC, g/kg	15 (32)	4.67 (0.15)	0.015 (0.001; 0.029)	0.040	0.506	0.00
TS, g/100 g	6 (13)	12.61 (1.38)	0.122 (−0.038; 0.282)	0.135	0.989	0.00
MUN, mg/dL	10 (19)	12.27 (2.58)	−0.478 (−0.755; −0.201)	<0.001	0.060	37.63
SCC, $\times 10^3$ cell/mL	9 (17)	4.98 (2.55)	−0.275 (−0.424; −0.125)	<0.001	0.073	48.34
Iodine, mg/dL	9 (15)	0.32 (0.19)	0.805 (0.574; 1.036)	<0.001	<0.001	96.87
Bromoform, µL	3 (4)	0.20 (0.11)	0.047 (−0.008; 0.102)	0.096	0.917	0.00

N: number of studies; NC: number of comparisons between seaweed treatment and control treatment; SD: standard deviation; WMD: weighted mean differences between control and treatments with seaweed; CI: confidence interval of WMD; [1] *p*-Value to Cochran's Q statistic; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; DMI: dry matter intake; MY: milk yield; ECMY: energy-corrected milk yield; FE: feed efficiency; MFY: milk fat yield; MPY: milk protein yield; MLY: milk lactose yield; MFC: milk fat content; MPC: milk protein content; MLC: milk lactose content; TS: total solids; MUN: milk urea nitrogen; SCC: somatic cell count.

3.2. Nutrient Digestibility, Ruminal Fermentation, and Enteric Methane Emissions

Dietary supplementation with seaweeds did not affect ($p > 0.05$) DMD, OMD, CPD, NDFD, ADFD, TVFA, acetate, and propionate (Table 3). Dietary supplementation with seaweeds increased ($p < 0.05$) ruminal pH and ruminal concentration of butyrate and valerate. In contrast, lower ($p < 0.05$) ruminal NH_3-N concentration, acetate/propionate ratio, daily CH_4 emission, CH_4 yield, and CH_4 intensity were observed in response to dietary supplementation with seaweeds. Likewise, Table 3 shows that significant heterogeneity ($Q \leq 0.05$ and $I^2 > 50\%$) was observed in NH_3-N, TVFA, acetate, propionate, butyrate, acetate/propionate ratio, daily CH_4 emission, CH_4 yield, and CH_4 intensity. In the current study, meta-regression analyses were only performed on DMI, MY, ECMY, and daily CH_4 emissions. This is justified because the other response variables that had significant heterogeneity were reported in fewer than ten studies, and under these conditions, the power of the test is low [46].

Table 3. Nutrient digestibility, ruminal fermentation, and enteric methane emission in dairy cows supplemented with seaweed.

Outcomes	N (NC)				Heterogeneity	
		Control Means (SD)	WMD (95% CI)	*p*-Value	*p*-Value [1]	I^2 (%)
DMD, g/100 g	5 (11)	69.95 (3.56)	−0.241 (−0.639; 0.156)	0.234	0.724	0.00
OMD, g/100 g	7 (15)	72.63 (2.91)	−0.113 (−0.498; 0.272)	0.565	0.660	0.00
CPD, g/100 g	7 (15)	66.67 (5.37)	−0.230 (−1.199; 0.740)	0.642	0.065	43.97
NDFD, g/100 g	6 (15)	62.42 (5.75)	0.308 (−0.366; 0.981)	0.371	0.327	11.27
ADFD, g/100 g	3 (9)	62.02 (5.93)	0.248 (−1.113; 1.609)	0.721	0.236	23.29
Ruminal pH	4 (9)	6.21 (0.37)	0.074 (0.014; 0.134)	0.016	0.242	22.61
NH_3-N, mg/dL	4 (6)	11.88 (4.98)	−1.643 (−3.270; −0.016)	0.048	<0.001	83.53
TVFA, Mm	6 (12)	122.73 (16.94)	−4.427 (−11.877; 3.023)	0.244	<0.001	90.85
Acetate, mol/100 mol	5 (10)	61.69 (5.02)	−1.204 (−2.412; 0.003)	0.061	<0.001	68.73
Propionate, mol/100 mol	5 (10)	21.00 (2.57)	0.978 (−0.219; 2.175)	0.109	<0.001	71.80
Butyrate, mol/100 mol	5 (10)	13.51 (2.22)	1.073 (0.385; 1.761)	0.002	<0.001	85.87
Valerate, mol/100 mol	5 (10)	1.59 (0.41)	0.141 (0.025; 0.257)	0.017	0.063	47.05

Table 3. *Cont.*

Outcomes	N (NC)				Heterogeneity	
		Control Means (SD)	WMD (95% CI)	*p*-Value	*p*-Value [1]	I^2 (%)
Acetate/propionate	4 (8)	3.25 (0.70)	−0.321 (−0.550; −0.093)	0.006	<0.001	82.42
CH_4 production, g/d	10 (22)	411.00 (87.40)	−29.422 (−41.565; −17.280)	<0.001	<0.001	89.18
CH_4 yield, g/kg DMI	9 (20)	18.06 (3.61)	−1.578 (−2.204; −0.951)	<0.001	<0.001	81.01
CH_4 intensity, g/kg ECMY	9 (19)	15.82 (5.26)	−1.710 (−2.344; −1.076)	<0.001	<0.001	66.10

N: number of studies; NC: number of comparisons between seaweed treatment and control treatment; SD: standard deviation; WMD: weighted mean differences between control and treatments with seaweed; CI: confidence interval of WMD; [1] *p*-Value to Cochran's Q statistic; I^2: proportion of total variation of size effect estimates that is due to heterogeneity; DMD: dry matter digestibility; OMD: organic matter digestibility; CPD: crude protein digestibility; NDFD: neutral detergent fiber digestibility; ADFD: acid detergent fiber digestibility; NH_3-N: ammonia nitrogen; TVFA: total volatile fatty acids; CH_4: methane; DMI: dry matter intake; ECMY: energy-corrected milk yield.

3.3. Publication Bias and Meta-Regression

Table 4 shows that the Rosenberg fail-safe number was only significant ($p < 0.05$) for MFC, MPC, MUN, SCC, iodine, daily CH_4 emission, and CH_4 yield, which suggests that there was publication bias in these response variables. However, the results obtained can be considered solid because NFS > [5 (n) + 10] was observed in all these variables [49].

Table 4. Analysis of publication bias.

Outcomes	Observed Significance	Target Significance	NFS Number	Number of Comparisons (n)	NFS > [5 (n) + 10]
DMI	0.2878	0.05	0	41	NA
MY	0.1915	0.05	0	35	NA
ECMY	0.2708	0.05	0	21	NA
FE	0.0692	0.05	0	17	NA
MFY	0.8589	0.05	0	18	NA
MPY	0.4246	0.05	0	18	NA
MLY	0.3332	0.05	0	17	NA
MFC	<0.001	0.05	283	33	175
MPC	<0.001	0.05	247	33	175
MLC	0.0775	0.05	0	32	NA
TS	0.6746	0.05	0	13	NA
MUN	0.0008	0.05	253	19	105
SCC	<0.001	0.05	324	17	95
Iodine	<0.001	0.05	1010	15	85
Bromoform	0.0962	0.05	0	4	NA
DMD	0.2671	0.05	0	11	NA
OMD	0.6511	0.05	0	15	NA
CPD	0.1890	0.05	0	15	NA
NDFD	0.4562	0.05	0	15	NA
ADFD	0.6531	0.05	0	9	NA
Ruminal pH	0.0648	0.05	0	9	NA
NH_3-N	0.0729	0.05	0	6	NA
TVFA	0.3391	0.05	0	12	NA
Acetate	0.1211	0.05	0	10	NA
Propionate	0.0634	0.05	0	10	NA
Butyrate	0.1941	0.05	0	10	NA
Valerate	0.0740	0.05	0	10	NA
Acetate/propionate	0.0647	0.05	0	8	NA
CH_4 production	<0.0001	0.05	201	22	120
CH_4 yield	<0.0001	0.05	187	20	110
CH_4 intensity	0.3276	0.05	0	19	NA

DMI: dry matter intake; MY: milk yield; ECMY: energy-corrected milk yield; FE: feed efficiency; MFY: milk fat yield; MPY: milk protein yield; MLY: milk lactose yield; MFC: milk fat content; MPC: milk protein content; MLC: milk lactose content; TS: total solids; MUN: milk urea nitrogen; SCC: somatic cell count; DMD: dry matter digestibility; OMD: organic matter digestibility; CPD: crude protein digestibility; NDFD: neutral detergent fiber digestibility; ADFD: acid detergent fiber digestibility; NH_3-N: ammonia nitrogen; TVFA: total volatile fatty acids; CH_4: methane; n: number of comparisons; NFS: fail-safe number; NA: Rosenberg's test does not apply since the observed significance level was greater than 0.05.

Table 5 shows that the covariate of experimental design, days in milk, level of forage in the diet, seaweed dose, and supplementation period did not have a significant relationship

($p > 0.05$) with any of the response variables evaluated. The seaweed type covariate explained ($p < 0.001$) 61.18, 66.68, 72.40, and 58.26% of the heterogeneity observed in DMI, MY, ECMY, and daily CH_4 emission, respectively. The seaweed species covariate explained ($p < 0.001$) 75.97, 66.10, 83.08, and 39.68% of the heterogeneity observed in DMI, MY, ECMY, and daily CH_4 emission, respectively.

Table 5. Meta-regression of the effects of dietary seaweed supplementation on growth performance of dairy cows.

Outcomes		**QM**	**Df**	***p*-Value**	**R^2 (%)**
Dry matter intake (DMI)	Experimental design	2.879	1	0.090	3.53
	Days in milk	1.470	1	0.480	0.62
	Forage level	0.738	1	0.607	0.00
	Seaweed dose	2.133	2	0.344	4.27
	Suplementation period	0.037	1	0.848	0.00
	Seaweed type	19.683	1	<0.001	61.18
	Seaweed specie	38.266	8	<0.001	75.97
Milk yield (MY)	Experimental design	0.847	1	0.357	0.00
	Days in milk	0.028	1	0.986	0.00
	Forage level	4.184	1	0.123	0.00
	Seaweed dose	1.551	2	0.460	2.41
	Suplementation period	0.080	1	0.778	0.00
	Seaweed type	28.541	1	<0.001	66.68
	Seaweed specie	44.459	10	<0.001	66.10
Energy corrected milk yield (ECMY)	Experimental design	0.041	1	0.840	0.00
	Days in milk	1.544	1	0.642	1.74
	Forage level	0.003	1	0.955	0.00
	Seaweed dose	3.212	2	0.201	1.07
	Suplementation period	1.631	1	0.202	3.18
	Seaweed type	25.074	1	<0.001	72.40
	Seaweed specie	44.150	5	<0.001	83.08
Methane (CH_4) production, g/d	Experimental design	2.327	1	0.127	2.79
	Days in milk	0.536	1	0.464	0.00
	Forage level	0.032	1	0.858	0.00
	Seaweed dose	2.836	2	0.242	3.17
	Suplementation period	0.269	1	0.604	0.00
	Seaweed type	12.184	1	<0.001	39.68
	Seaweed specie	27.526	7	<0.001	58.26

QM: coefficient of moderators; QM is considered significant at $p \leq 0.05$; df: degree of freedom; R^2: the amount of heterogeneity accounted for.

3.4. Subgroup Analysis

Figure 2a shows that DMI decreased (WMD = −1.507 kg/d; $p = 0.010$) when red seaweeds were used. However, when brown seaweeds were used, DMI was not affected (WMD = 0.059 kg/d; $p = 0.501$). Figure 2b shows that MY decreased when red seaweed was used (WMD = −1.542 kg/d; $p = 0.006$). In contrast, when brown seaweeds were used, MY increased (WMD = 0.595 kg/d; $p = 0.003$). Figure 2c shows that ECMY decreased (WMD = −2.780 kg/d; $p < 0.001$) when red seaweeds were used. However, when brown seaweeds were used, ECMY was not affected (WMD = 0.489 kg/d; $p = 0.125$). Daily CH_4 emissions decreased ($p < 0.01$) regardless of the type of seaweed used (Figure 2d). However, the effect was greater when studies used red seaweed (WMD = −104.020 g/d) than when they used brown seaweed (WMD = −13.755 g/d).

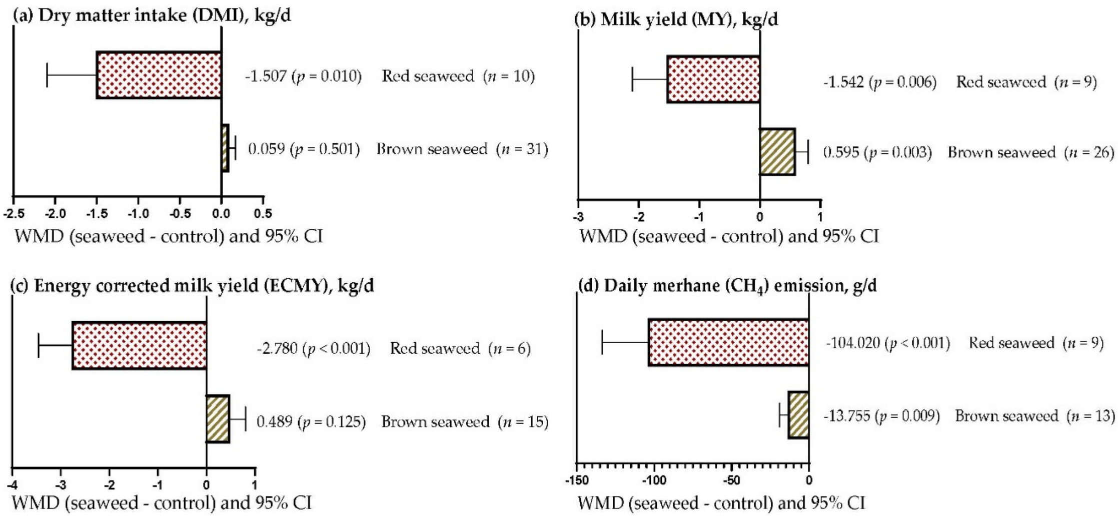

Figure 2. Subgroup analysis (subgroup = seaweed type) of the effect of including seaweed in dairy cows' diets, WMD = weighted mean differences between seaweed treatments and control.

Figure 3a shows that DMI was not affected (WMD = 0.198 kg/d; $p = 0.561$) when the seaweed used was *Saccharina latissima*. However, DMI decreased (WMD = −1.812 kg/d; $p = 0.003$) when seaweed *Asparagopsis taxiformis* was included in the diets. In contrast, DMI increased (WMD = 0.277 kg/d; $p = 0.049$) when seaweed *Ascophyllum nodosum* was added to the diets. Figure 3b shows that MY increased (WMD = 1.004 kg/d; $p = 0.038$) when the seaweed used was *Saccharina latissima*. However, MY decreased (WMD = −2.215 kg/d; $p < 0.001$) when seaweed *Asparagopsis taxiformis* was included in the diets. In contrast, MY increased (WMD = 0.823 kg/d; $p < 0.001$) when seaweed *Ascophyllum nodosum* was added to the diets. Figure 3c shows that ECMY was not affected (WMD = 0.054 kg/d; $p = 0.958$) when the seaweed used was *Saccharina latissima*. However, ECMY decreased (WMD = −3.402 kg/d; $p < 0.001$) when seaweed *Asparagopsis taxiformis* was included in the diets. In contrast, ECMY increased (WMD = 0.734 kg/d; $p = 0.050$) when seaweed *Ascophyllum nodosum* was added to the diets. Daily CH_4 emissions decreased ($p < 0.01$) regardless of the seaweed type used (Figure 3d). However, the effect was greater when studies used seaweed *Asparagopsis taxiformis* (WMD = −105.673 g/d) than when they used seaweed *Ascophyllum nodosum* (WMD = −24.451 g/d).

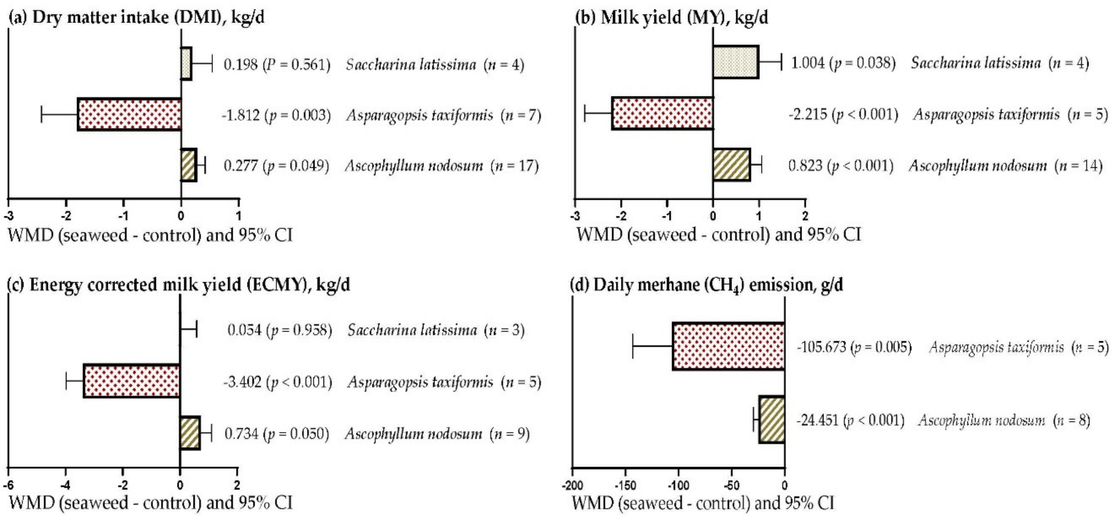

Figure 3. Subgroup analysis (subgroup = seaweed species) of the effect of seaweed supplementation in dairy cows' diets; WMD = weighted mean differences between seaweed treatments and control.

4. Discussion

4.1. Milk Yield and Composition

Dietary supplementation with seaweed did not affect DMI, suggesting that seaweed does not negatively affect dietary palatability or nutrient intake in dairy cows. However, subgroup analyses revealed that DMI decreased when red seaweed *Asparagopsis taxiformis* was used and increased when brown seaweed *Ascophyllum nodosum* was used. An in vitro study [51] reported that several brown seaweeds (*Undaria pinnatifida*, *Sargassum fusiforme*, and *A. nodosum*) can increase the relative abundance of bacteria *Fibrobacter succinogenes* (+19.4 to +58.4%), *Ruminococcus albus* (+7.4 to +11.1%), *Ruminococcus flavefaciens* (+4.8 to +14.9%), and rumen fungi (+86.8 to +100%) in ruminal fluid of beef cattle fed high forage diets (600 g/kg DM). Similar effects of brown seaweed consumption on dairy cows could explain the higher DMI observed since, according to Comtet-Marre et al. [52], a high abundance of *F. succinogenes*, *R. albus*, *R. flavefaciens*, and rumen fungi can decrease the residence time of feed particles in the rumen and lead to higher DMI. On the other hand, under in vitro conditions [53], *A. taxiformis* decreases (−41.1 to −64.2%) the relative abundance of the microbial genera *Fibrobacter* and *Ruminococcus* in the ruminal fluid of steers fed high forage diets (500 g/kg DM). Similar effects of *A. taxiformis* on dairy cows could reduce fiber digestibility and passage rate and result in low DMI.

MY, ECMY, FE, MFY, MPY, MLY, and TS were not affected by seaweed supplementation. However, subgroup analyses showed that MY and ECMY decreased when red seaweed *A. taxiformis* was used and increased when brown seaweed *A. nodosum* was added to the diets. These differences could be related to the effects of *A. taxiformis* and *A. nodosum* on DMI. Likewise, under in vitro conditions, *A. taxiformis* and other red seaweed decrease the relative abundance of bacteria related to the degradation of fiber, carbohydrates, and proteins [53]. A similar effect of *A. taxiformis* in dairy cows would decrease nutrient digestibility and rumen production of volatile fatty acids, which could decrease the metabolic availability of nutrients and energy and negatively affect MY. In contrast, several brown seaweeds, including *A. nodosum*, improve (+12.7 to +22.5%) serum levels of antioxidant enzymes (glutathione peroxidase and catalase) and total antioxidant capacity (TAC) in the blood serum of dairy ruminants [40,54]. These effects of the brown seaweed *A. taxiformis* partially explain the higher MY detected in the present study since, according to Dong et al. [55], the serum concentration of catalase, superoxide dismutase, and TAC have a positive correlation (r of 0.38 to 0.64) with MY in dairy cows.

Dietary supplementation with seaweed increased MFC and MLC but decreased MPC. Although an increase in MFC is not considered a nutritional benefit in humans, it plays an essential role in milk quality because it directly influences its visual attributes and flavor [56]. For example, high MFC is positively associated with milk creaminess and flavor [57]. On the other hand, an increase in MLC could negatively affect the quality of some dairy products. For example, Portnoy and Barbano [58] indicate that high MLC can induce the excessive formation of lactose and lactate crystals, negatively affecting ice cream's texture and consumers' acceptability of cheddar cheese. Likewise, Osorio et al. [56] mention that a low MPC decreases the quality and value of cow's milk and derived dairy products.

The higher MFC observed in the current study could be related to the effects of seaweed on lipid metabolism. For example, a recent study [18] shows that dietary supplementation with seaweed increases serum triglyceride (TG) concentration by up to 61.5% in dairy cows. Likewise, another study [11] reported that the dietary inclusion of seaweed decreases serum levels of non-esterified fatty acids (NEFA) between 12.0 and 19.5% in dairy cows. According to Andjelić et al. [59], in dairy cows, MFC has a significant positive (r = 0.304) and negative correlation (r = −0.324) with serum levels of TG and NEFA, respectively. On the other hand, recent studies [18,40] reported that seaweed increases (4.2 to 13.4%) the serum glucose concentration in dairy cows. This seaweed effect could explain the increased MLC because lactose synthesis in the mammary gland is carried out mainly from plasma glucose [60]. Furthermore, the lower MPC observed in the current study could be explained by the reduction in DMI of cows supplemented with seaweed. This hypothesis is justified because a low DMI decreases crude protein intake (CPI) and lactation net energy intake (NE_LI) and, according to Hristov et al. [61], DMI, CPI, and NE_LI are positively correlated (r of 0.57 to 0.62) with MPC in dairy cows. Likewise, a recent study [40] reported that seaweed supplementation decreases dairy cows' serum insulin levels by up to 22.5%. A low serum insulin concentration can decrease the absorption of amino acids in the mammary gland [56], which could result in low MPC.

Hossein-Zadeh [62] mentions that MUN is a normal component in ruminant milk, which varies from 20 to 75% of the non-protein nitrogen of milk, and its values can be used as indicators of the efficiency of utilization of protein ingested in dairy cows. In the present study, lower MUN was observed in response to dietary supplementation with seaweed, suggesting that seaweed improves the utilization efficiency of dietary protein. Recent studies [29,40] reported that dietary supplementation with seaweed decreases blood urea nitrogen (BUN) levels in dairy cows by up to 16.4%. This effect of seaweed could explain the lower MUN observed in the current meta-analysis since, according to a recent study [62], BUN has a high positive correlation with MUN in dairy cows. On the other hand, SCC values are a simple and helpful tool to periodically evaluate ruminants' udder health and milk quality [63]. Although somatic cells are a naturally present component in milk, an increase in SCC values is generally associated with a health problem in the udder and negatively affects MY and milk quality [64]. In the current study, dietary supplementation with seaweed decreased SCC, suggesting that seaweed could reduce SCC in milk from dairy cows. Lopez et al. [33] reported that seaweed supplementation decreases the abundance of *Staphylococcus* spp. bacteria (including *Staphylococcus aureus*) in the milk of dairy cows. This effect could explain the lower SCC observed in the current study since, according to Kaskous et al. [64], *S. aureus* has a positive correlation with SCC in ruminants.

Dietary supplementation with seaweed increased milk iodine content but did not affect milk bromoform content. These effects were expected since, in dairy cows supplemented with seaweed, the transfer efficiency of dietary iodine to milk is high (37.5 to 41.8%) [13,34], while the transfer efficiency of dietary bromoform to milk is low (4.44%) [65]. A high iodine content in milk could be positive for human health since, according to Han et al. [66], more than 2 billion people are at risk of consuming amounts of iodine below their requirements worldwide. According to Newton et al. [13], the high iodine content in milk could affect consumers' health in human populations without iodine deficiencies. However, the maximum dose of iodine allowed in milk for human consumption is 500 mg/L [6], which is higher than the range iodine content (1.0 to 29.6 mg/L) observed in the milk of dairy cows supplemented with seaweed in the current study. On the other hand, the lack of significant changes in bromoform content in milk suggests that the inclusion of seaweed in diets for dairy cows does not represent any apparent risk of bromoform contamination [10].

4.2. Nutrient Digestibility, Ruminal Fermentation and Enteric Methane Emissions

In the current study, dietary supplementation with seaweed did not affect DMD, OMD, CPD, NDFD, and ADFD. Similarly, Maheswari et al. [54] also did not detect significant changes in DMD, OMD, CPD, NDFD, and ADFD of lactating buffaloes supplemented with high doses (25 g/kg DM for 90 days) of combinations of red and brown seaweeds (*Kappaphycus alvarezii*, *Gracilaria salicornia*, *Turbinaria conoids*). Likewise, dietary supplementation with up to 300 g/kg DM of various seaweeds (*Ruppia* sp., *Ulva* sp., or *Chaetomorpha* sp.) does not affect DMD, OMD, CPD, NDFD, and ADFD in sheep fed high forage diets [67].

Ruminal pH increased, and ruminal NH_3-N concentration decreased in response to dietary supplementation with seaweed. These effects could be positive since a low rumen pH is associated with rumen acidosis [68], while a high NH_3-N concentration generally indicates a low absorption of ammonia in the rumen, excessive deamination of amino acids or slow utilization of ammonia by ruminal microorganisms [69]. Some authors [70,71] reported that seaweed increases (40.0 to 142.1%) the rumen relative abundance of *Selenomonas ruminantium* and *Megasphera elsdenii* bacteria. A higher concentration of these bacteria could increase the ruminal pH because they are lactate-consuming, and the ruminal pH decreases when lactic acid accumulates [68]. On the other hand, Rémond et al. [72] found that a high ruminal concentration of butyrate stimulates ruminal absorption of NH_3-N. Therefore, the lower ruminal NH_3-N concentration could be partially explained by the increased ruminal butyrate concentration of dairy cows supplemented with seaweed. Likewise, several studies [40,51,70] show that seaweed decreases (−23.1 to −54.0%) the population of rumen protozoa, which have a positive correlation (r = 0.609) with the rumen concentration of NH_3-N in ruminants [73].

In the current study, dietary supplementation with seaweed increased the rumen concentration of butyrate and valerate but decreased the acetate/propionate ratio. The higher ruminal concentration of butyrate and valerate could be closely related to the reduction in CH_4 emission of dairy cows supplemented with seaweed. This hypothesis is justified because when ruminal methanogenesis decreases, the ruminal concentration of butyrate and valerate increases since it acts as an alternative sink for H_2 [74]. According to Min et al. [9], the acetate/propionate ratio changes when the individual rumen concentration of acetate and propionate is modified. These two volatile fatty acids did not change significantly in the present study. However, dietary supplementation with seaweed numerically modified the rumen concentration of acetate (−1.9%) and propionate (+4.7%), which could be related to the observed lower acetate/propionate ratio.

A recent study [3] shows that enteric CH_4 emitted by dairy cows accounts for a high proportion (between 35 and 55%) of total GHG emissions from dairy farms worldwide. According to recent reviews [10,75], mitigation of enteric CH_4 emissions in dairy cows is essential to improve environmental sustainability in dairy farms. In the present meta-analysis, dietary supplementation with seaweed decreased daily CH_4 emission (−7.16%), CH_4 yield (−8.74%), and CH_4 intensity (−10.81%). The simultaneous reduction of these three types of enteric CH_4 shows that seaweed has a consistent anti-methanogenic effect and could improve environmental sustainability in dairy farms. Similar to our results, Roque et al. [76] reported that dietary supplementation with low doses (2.5 and 5.0 g/kg DM for 147 days) of seaweed in beef cattle fed high forage diets (600 g/kg DM) decreased the daily emission of CH_4 (−36.28 to −58.64), CH_4 yield (−32.58 to 52.03%), and CH_4 intensity (−36.93 to −54.46%). Likewise, Li et al. [77] observed lower daily CH_4 emission (−14.92 to −81.34%) and CH_4 yield (−15.33 to −80.66%) in sheep fed with 40% forage in the diet and supplemented with increasing doses (5.0 to 30.0 g/kg DM for 72 days) of seaweed. Wanapat et al. [75] mention that the anti-methanogenic effects of seaweeds are related to their bromoform and phlorotannin content. Bromoform is a halogenated alkane that competitively binds to coenzyme M methyltransferase, inhibiting methyl transfer in methanogenesis [10]. Likewise, bromoform blocks the enzyme methyl-coenzyme M reductase (MCR) [6], which plays an essential role because it catalyzes the last step in the formation of ruminal CH_4 [10]. On the other hand, phlorotannins are compounds analogous to condensed tannins [5], which can inhibit the growth of methanogenic archaea in the rumen [78].

Although daily CH_4 emission decreased regardless of seaweed type, subgroup analyses showed that daily CH_4 emission decreased almost four times more when red seaweed *A. taxiformis* was used than when brown seaweed *A. nodosum* was added to the diets. Hutchings et al. [79] reported that *A. taxiformis* has a high bromoform content (10–50 g/kg DM), while *A. nodosum* has a low concentration of phlorotannins (34.9 mg/kg DM) but does not have bromoform [5]. This variation in the concentration of anti-methanogenic compounds in seaweed could explain the differences in their effects on daily CH_4 emissions.

5. Conclusions

The overall results indicate that dietary supplementation with seaweed does not affect dry matter intake, milk yield, energy-corrected milk yield, or feed efficiency in dairy cows. However, the use of red seaweed, specifically *Asparagopsis taxiformis*, decreases dry matter intake, milk yield, and energy-corrected milk yield in dairy cows. Furthermore, brown seaweed *Ascophyllum nodosum* increases dry matter intake, milk yield, and energy-corrected milk yield in dairy cows. Likewise, dietary supplementation with seaweed increases milk's fat, lactose, and iodine content while decreasing milk protein content, milk urea nitrogen, and somatic cell count. On the other hand, the inclusion of seaweed in dairy cow diets does not affect nutrient digestibility but improves ruminal fermentation through an increase in pH, butyrate, and valerate and a reduction in ruminal ammonia nitrogen concentration and acetate/propionate ratio. Furthermore, dietary supplementation with seaweed can be used as a nutritional strategy to reduce enteric methane emissions in dairy cows. The best enteric methane mitigation effect is achieved using red seaweed *Asparagopsis taxiformis*.

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