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Assessment of *Sargassum* sp., *Spirulina* sp., and *Gracilaria* sp. as Poultry Feed Supplements: Feasibility and Environmental Implications

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Abstract: Eutrophication, coupled with ocean acidification and warming, results in an increased concentration of marine algae, severely impacting some regions. Several algae are a rich source of protein and minerals. Marine algae are rich in bioactive molecules with antioxidants, anti-inflammatory, anti-fungal, and antimicrobial properties. These properties make them attractive for usage in the pharmaceutical industry. This study evaluated *Sargassum* sp., *Spirulina* sp., and *Gracilaria* sp. for use as poultry feed. Chemical analyses show that crude protein (CP) in analyzed algae was 9.07–63.63%, with a fiber content of 0.15–17.20%, and a crude fat range of 0.152–2.11%, suggesting that algae can partially substitute imported protein sources used for poultry feed. A rapid impact assessment matrix (RIAM) was used to assess the environmental footprint of algae usage in poultry feed. The environmental assessment results show promising opportunities to help harvest the algae from the marine area. However, the feasibility of establishing outdoor algal ponds is not environmentally viable in the Middle East.

Keywords: eutrophication; marine algae; crude protein; RIAM

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1. Introduction

Ocean acidification and ocean warming are obliterating the physicochemical characteristics of the oceans [1]. The growth of phytoplankton is likely to improve due to the exceedance of carbon dioxide in aquatic systems [2–6]. Algae are known for their higher biomass production capacity per unit area due to their enhanced photosynthetic efficiency. Microalgae and the macroalgae are found in littoral zones and in open oceans. The recurrence of *Sargassum* across the Atlantic has posed a significant environmental and socio-economic challenge. Caribbean people attempted to turn this catastrophe into an opportunity [7]. Algae utilize carbon dioxide and other nutrients including nitrogen and phosphorous for their growth and propagation [8–16]. Pelagic Sargassum has been known to fix carbon and nitrogen [17]. They also absorb and adsorb various contaminants from the aquatic environment [18,19]. Studies have demonstrated the presence of bacterial and fungal communities with seaweed rich in bioactive compounds, including some with antimicrobial properties [20,21]. *Sargassum* is rich in beta carotene and vitamins and free of anti-nutrients [22]. Several workers have reported metal uptake, and fewer have looked at organic contaminants like polycyclic aromatic hydrocarbons (PAHs) [23]. While the use of microalgae for the production of biofuels has been extensively researched, few studies have considered algae as poultry feed [24–26]. Using locally available natural resources may contribute to food security and sustainability, especially during a global crisis such as the COVID-19 pandemic.

Macroalgae are critical for carbon dioxide uptake and nutrient cycling, playing a significant role in providing habitats for marine organisms [27] in the Persian/Arabian Gulf

(hereafter "Gulf") [28]. The occurrence of harmful algal blooms (HABs) can pose a concern to the safety of algae use in animal feed. Only about 2% of the more than 5000 species of algae are toxic. The occurrence of the toxic species is rare in the Gulf [29,30].

Macroalgae are abundant in the Gulf, and they provide habitat and food for a wide variety of mollusks, crustaceans, annelids, and even fish [31]. A significant quantity of macroalgae washes ashore in Kuwait and over the Gulf during the summer season. A strategic environmental impact assessment (SEIA) was carried out to assess the viability of two commonly grown microalgae (*Spirulina* sp. and *Gracilaria* sp.) and macroalgae (*Sargassum* sp.) as poultry feed, considering their nutritional level and abundance [32]. However, we are also mindful of their capacity to concentrate metals that might reach toxic levels. Studies have shown preferential uptake and assimilation of ^{210}Po and ^{210}Pb in several algal species [4]. The quantities of the macroalgae washed ashore and concentrated at certain places are huge, whereas microalgae extraction from the marine area is not a very practical option. Hence, we assessed the macroalgae *Sargassum* sp., imported from India and collected from Kuwait's territorial water. The microalgae *Spirulina* sp. and *Gracilaria* sp. were also assessed because they are widely cultivated and used as a food supplement.

2. Materials and Methods

The local *Sargassum* sp. was harvested from different locations in Kuwait, such as Al-Salam beach (Shuwaikh) and the Fintas, Funaitees, and Al-Khairan areas (Figure 1). The amount washed up on the shores was plentiful, and covered the entire shore area, in addition to the ones floating on the shallow waters. The algal masses were collected in dark bags to prevent exposure to direct light and oxidation of lipids. They were first washed with sea water and then with fresh water, after which excess water was drained on cement slabs, and then packed into black bags to be transported to the laboratories for drying and grinding. The commercial algae were purchased powdered and ready to use (Farm Ocean Technologies India PVT. Ltd., Nagercoil, Tamil Nadu, India).

In this study, 595 one-day-old male Cobb 500 broiler chicks were raised in cages. This study utilized cages to improve space utilization and cost reduction. The experimental diet was given to chickens from 1 d until they were till slaughtered at 35 d. The experimental design was identical to Al-Khalaifah et al. [33] (Supplementary Materials).

The samples were analyzed in triplicate in the Kuwait Institute for Scientific Research. Ten random samples from each algae type were used. The following analyses were conducted: moisture, ash, crude protein, and crude fat using standard techniques. Two grams of algae was taken to determine the moisture content using the thermogravimetry method [34,35]. The ash content was determined by taking 2 gm of algae using oxidation drying at 575 °C [36]. Crude protein was determined using the Macro-Kjeldahl method [37] that entails the conversion of nitrogen into ammonium sulphate by acid digestion. Crude fat was determined using the procedure detailed in Al-Khalaifah et al. [31] using 2 gm of algae sample and extraction by a chloroform: methanol (2:1) solution, following the standard procedure of Chen, et al. [38]. The fiber content was measured in the samples using a Fibertec system. A moisture- and fat-free sample was first digested with a weak acid and a weak base solution. The digested residue was then collected in a filter crucible, dried, and then ignited. The loss of weight on ignition is the crude fiber.

The lipid profile was analyzed in these algal samples using gas chromatography [39]. The technique has been well established since the 1950s, utilizing the fact that fatty acids can be separated more efficiently at lower temperatures. Gas chromatography requires fatty acids to be derivatized to become sufficiently volatile and eluted at reasonable temperatures without thermal decomposition. This usually involves the substitution of functional-group-containing hydrogen to form esters, thioesters, or amides for analysis. Methyl esters are commonly studied derivatives that are produced by methylation. In this method, the ester bonds in complex lipids are hydrolyzed to release free fatty acids transmethylated to form fatty acid methyl esters (FAME). The resulting profile of FAME is determined by gas chromatography [40,41]. The technique allows both the proportions of individual fatty

acids and their concentrations to be measured [42]. The injection temperature was set to 140 °C, and helium was used as the carrier gas with a pre-column split ratio of 50:1 and a head pressure of 37.34 psi. This setting was optimal for determining the necessary fatty acids in chicken meat samples. The GC was standardized using the 37 FAME standard mix. The presence of metals including As, Ba, Mo, V, Ag, Ni, Pb, Cd, Cr, Cu, Zn, and Hg was determined in the algae samples using an inductively coupled plasma (ICP) following the USEPA method 6010B [43].

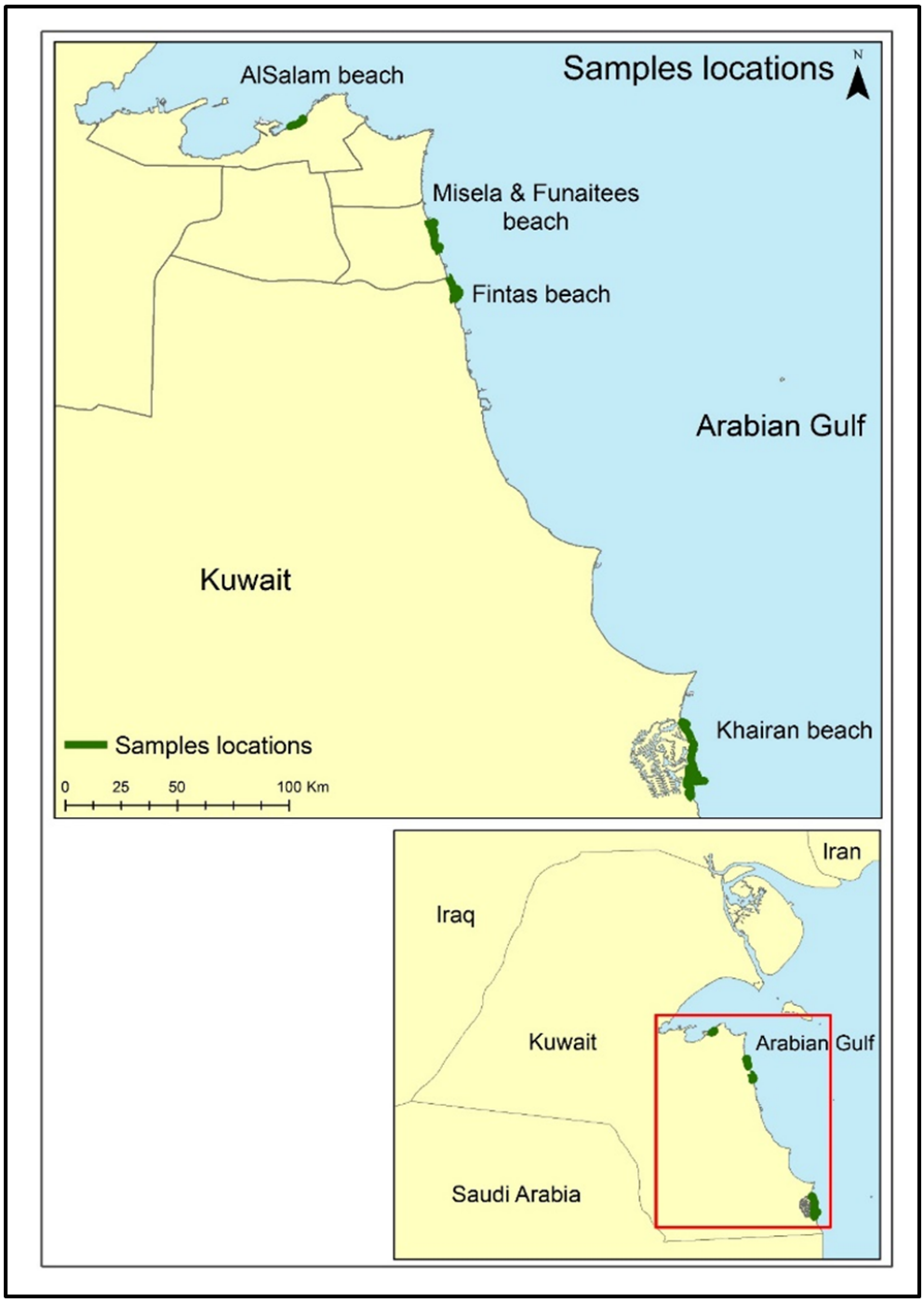

Figure 1. Collection locations on Kuwait's map.

The cellular immune response was measured in ten birds per treatment at 35 days of age using the phytohemagglutinin (PHA) test, represented by the subsequent swelling at the injection site (wattle) following the procedure stated in Al-Khalaifah [44,45]. The

humoral immune response was assessed in ten broiler chickens of five weeks of age from each treatment. Antibody titers were assessed using sheep red blood cells (RBC) following the procedure adopted by Schoeni and Doyle [46]. Furthermore, hindgut acidosis was measured by collecting the hindgut digesta into tubes, and the pH value was measured with a probe following Li et al. [47].

The environmental impact of the possibility of using algae as poultry feed was assessed using the rapid impact assessment matrix (RIAM) [48,49]. The RIAM method is holistic and provides a valuable tool for organizing and analyzing the coherent data sets for conducting an environmental impact assessment (EIA). This is a structured and straightforward method that provides flexibility in selecting parameters, and has been upgraded by coupling an environmental database for more realistic environmental scores [50]. In RIAM, the impacts of project activities are evaluated against the environmental components. For each component, a score (using the defined criteria) is determined, which measures the expected impact from the specific component.

3. Results

3.1. Chemical Characterization of the Algal Species

The analysis of the algal samples was conducted, and the proximate results of moisture, ash content, fat, proteins, and fiber are presented in Table 1. Results in Table 1 show that the moisture content of *Sargassum* sp. collected from Kuwait territorial waters varied between 18 ± 1%. The ash content was 13.05 ± 2.01%, while the crude protein, fat, and fiber content were 21.6 ± 0.02%, 0.152 ± 0.12, and 17.20 ± 0.50%, respectively. The algae from India were procured dry, so the moisture content was not determined for these samples. The ash content in *Sargassum* sp. from India was 24.39 ± 0.04, the fat content was 1.07 ± 0.10%, and the fiber was 13.90 ± 0.19%. The protein content in *Sargassum* samples was between 9.51 and 0.08% and the energy was 308.79 calories from 2 gm of dry sample. The chemical characterization highlights a significant difference in the two *Sargassum* samples obtained from Kuwait and India. This variance can be attributed to different species, as well as the environmental milieu. The *Sargassum* from Kuwait was collected from territorial water that is known to be higher in metal content, but the provenance of the *Sargassum* from India is not known.

Table 1. Proximate Analyses of the Various Algal Samples.

Sample	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Fiber (%)	Crude Protein (%)	Energy (Calories/g) Dry Weight
Sargassum sp. *	18.3 ± 0.3	24.39 ± 0.04	1.07 ± 0.10	13.90 ± 0.19	9.51 ± 0.08	154.4
Spirulina sp. *	5.3 ± 0.2	7.76 ± 0.05	2.11 ± 0.54	0.15 ± 0.17	63.63 ± 0.24	189.8
Gracilaria sp. *	19.05 ± 0.25	54.66 ± 0.10	1.01 ± 0.42	4.62 ± 0.20	9.07 ± 0.13	93.2
Sargassum sp. +	18.9 ± 0.4	13.05 ± 2.01	0.152 ± 0.12	17.20 ± 0.50	21.6 ± 0.02	157.2

* Sourced from India; + Sourced from Kuwait coastal waters.

The blue-green algae *Spirulina* was analyzed, and the ash content was low (7.76 ± 0.05%). The protein content was very high, with an average value of 63.63 ± 0.24%. The average crude fat and fiber contents were 2.11 ± 0.54% and 0.15 ± 0.17%, respectively.

The ash content of *Gracilaria* was very high at 54.66 ± 0.10%. The protein was lower compared to *Spirulina* and was only 9.07 ± 0.13%, and the fiber content was 4.62 ± 0.20%. The fat content of *Gracilaria* was 1.01 ± 0.42%. Based on these analytical results, it was evident that *Spirulina* sp. was the best candidate based on its protein content, low level of ash, and crude fiber. The protein content of *Sargassum* sp. and *Gracilaria* sp. was relatively low, while the ash content was higher than *Spirulina*. The prevalence of *Sargassum* is very high in marine areas across the globe, especially in the Caribbean. We believe it makes a very strong candidate for addition to the poultry feed.

3.2. Lipid Profile Analysis

The lipid profile analyses of the samples are presented in Tables 2 and 3. However, for comparison, the results are summarized considering the ratios of ∑n-6:∑n-3, which are vital to determine the feed quality. The ∑n-6:∑n-3 for *Sargassum* collected from Kuwait's coast varied between 0.61 and 0.63, whereas for the imported *Sargassum*, the ratio was 0.35. The ∑n-6:∑n-3 ratio for *Gracilaria* was 0.62, which was identical to the *Sargassum* collected from Kuwait. However, the ∑n-6:∑n-3 ratio for *Spirulina* sp. was 41.27, almost two orders of magnitude higher than the other analyzed algae.

Table 2. Fatty Acid Composition of Marine Algae (*Sargassum* sp.) Collected from Five Different Locations in Kuwait.

	Fatty Acids (wt%)				
	Algae 1	**Algae 2**	**Algae 3**	**Algae 4**	**Algae 5**
C4:0	0.22	0.24	0.39	0.32	0.40
C8:0	0.09	0.00	0.11	0.00	0.00
C12:0	0.18	0.27	0.00	0.00	0.19
C14:0	3.56	3.74	3.64	3.70	3.68
C15:0	0.64	0.65	0.63	0.62	0.65
C16:0	35.37	36.78	36.84	37.21	37.11
C16:1n7	6.90	7.65	7.07	7.28	7.01
C17:0	0.31	0.31	0.00	0.28	0.00
C17:1	0.29	0.32	0.00	0.49	0.32
C18:0	1.39	1.39	1.46	1.27	1.35
C18:1n9trans	0.26	0.00	0.00	0.00	0.00
C18:1n9cis	16.53	16.58	16.98	16.22	16.60
C18:2n6trans	0.35	0.39	0.46	0.39	0.41
C18:2n6cis	4.00	3.93	3.99	3.85	3.88
C20:0	0.95	0.98	1.00	0.91	0.94
C18:3n6	2.40	2.51	2.48	2.36	2.44
C18:3n3	0.97	0.96	1.07	1.09	1.03
C20:1n9	0.23	0.00	0.00	0.00	0.00
C21:0	1.65	1.82	1.82	1.65	1.75
C20:2	0.26	0.00	0.00	0.25	0.00
C22:0	0.77	0.69	0.64	0.68	0.64
C20:3n6	0.78	0.74	0.87	0.78	0.81
C20:3n3	10.63	10.67	10.56	10.31	10.37
C23:0	0.50	0.47	0.48	0.49	0.48
C22:2	0.00	0.11	0.16	0.00	0.00
C24:0	3.57	3.47	3.40	3.10	3.29
C20:5n3	0.55	0.50	0.63	0.58	0.59
C24:1n9	0.26	0.13	0.17	0.24	0.00
C22:1n9	1.89	1.75	2.02	2.00	1.98
C22:6n3	0.19	0.00	0.06	0.00	0.00
Total	95.65	97.03	96.93	96.06	95.92
∑SAT [1]	49.19	50.80	50.42	50.23	50.49
∑MONO [2]	26.34	26.43	26.25	26.23	25.91
∑PUFA [3]	17.73	17.30	17.79	17.24	17.09
∑n-6 [4]	7.53	7.57	7.80	7.38	7.53
∑n-3 [5]	12.34	12.14	12.31	11.97	12.00
∑n-6:∑n-3 [6]	0.61	0.62	0.63	0.61	0.62

[1] ∑SAT = Sum percentage of saturated fatty acids (C8:0, C10:0, C12:0, C14:0, C15:0, C16:0, C17:0 C18:0, C21:0, C22:0, C23:0, 24:0); [2] ∑MONO = Sum percentage of monounsaturated fatty acids (C14:1, C16:1n7, C17:1, C18:1n9t, C18:1n9cis, C20:1n9, C24:1n9, C22:1n9); [3] ∑PUFA = Sum percentage of polyunsaturated fatty acids (C18:2n6t, C18:2n6c, C18:3n-6, C18:3n3, C20:2, C20:3n6, C20:3n3, C22:2, C20:5n3, C22:6n3); [4] ∑n-6 = Sum percentage of n-6 polyunsaturated fatty acids (C18:2n-6t, C18:2n-6c, C18:3n-6, C20:3n-6, C22:4n6); [5] ∑n-3 = Sum percentage of n-3 polyunsaturated fatty acids (C18:3n-3, C20:3n-3, C20:5n-3, C22:6n-3); [6] ∑n-6:∑n-3 = ratio of ∑n-6 to ∑n-3, Algae 1–5: algae samples from Al-Salam, Al-Fintas, Al-Funaitees, Al-Khairan, Al-Misela, respectively.

Table 3. Fatty Acid Composition of the Commercial Algae Samples.

	Fatty Acids (wt%)		
	***Sargassum* sp.**	***Spirulina* sp.**	***Gracilaria* sp.**
C8:0	0.00	0.31	0.00
C10:0	0.16	12.56	2.20
C12:0	0.12	0.19	0.00
C14:0	4.59	0.69	1.77
C14:1	0.00	0.18	0.24
C15:0	0.00	0.12	0.27
C16:0	41.73	48.09	63.99
C16:1n7	5.34	3.13	1.71
C17:0	0.00	0.37	0.00
C17:1	0.80	0.42	0.00
C18:0	0.87	1.15	2.13
C18:1n9trans	0.00	0.26	0.00
C18:1n9cis	12.14	4.01	9.26
C18:2n6trans	0.00	0.12	0.00
C18:2n6cis	5.04	12.75	2.09
C18:3n6	0.51	13.25	0.30
C18:3n3	5.83	0.63	0.52
C20:1n9	0.56	0.00	0.00
C21:0	2.71	0.00	0.00
C20:2	0.00	0.09	0.23
C22:0	0.51	0.16	2.49
C20:3n6	0.50	0.00	0.67
C20:3n3	9.43	0.00	3.15
C23:0	0.48	0.00	0.00
C22:2	0.00	0.00	1.87
C24:0	1.65	0.00	0.00
C20:5n3	1.92	0.00	0.00
C24:1n9	0.00	0.00	1.37
C22:4n6	0.00	0.00	0.26
C22:6n3	0.00	0.00	1.24
Total	94.86	98.48	95.77
∑SAT [1]	52.81	63.64	72.85
∑MONO [2]	18.84	8.00	12.58
∑PUFA [3]	23.22	26.75	10.11
∑n-6 [4]	6.05	26.00	3.06
∑n-3 [5]	17.17	0.63	4.92
∑n-6:∑n-3 [6]	0.35	41.27	0.62

[1] ∑SAT = Sum percentage of saturated fatty acids (C4:0, C8:0, C12:0, C14:0, C15:0, C16:0, C17:0 C18:0, C20:0, C21:0, C22:0, C23:0, 24:0); [2] ∑MONO = Sum percentage of monounsaturated fatty acids (C16:1n7, C17:1, C18:1n9t, C18:1n9cis, C20:1n9, C24:1n9, C22:1n9); [3] ∑PUFA = Sum percentage of polyunsaturated fatty acids (C18:2n6t, C18:2n6c, C18:3n-6, C18:3n3, C20:2, C20:3n6, C20:3n3, C22:2, C20:5n3, C22:6n3); [4] ∑n-6 = Sum percentage of n-6 polyunsaturated fatty acids (C18:2n-6t, C18:2n-6c, C18:3n-6, C20:3n-6); [5] ∑n-3 = Sum percentage of n-3 polyunsaturated fatty acids (C18:3n-3, C20:3n-3, C20:5n-3, C22:6n-3); [6] ∑n-6:∑n-3 = ratio of ∑n-6 to ∑n-3.

3.3. Heavy Metal Concentration in Algae Samples

The trace metal concentrations in macroalgae and microalgae were determined. The average metal concentrations for arsenic (As), zinc (Zn), chromium (Cr), nickel (Ni), barium (Ba), molybdenum (Mo), copper (Cu), vanadium (V), lead (Pb), cadmium (Cd), silver (Ag), and mercury (Hg) are presented in Table 4. The concentrations in *Sargassum* collected from Kuwait's marine area were in the following order: As > Ni > Zn > Cr > Ba > Cu > Mo > V > Pb, whereas Cd, Ag, and Hg were below the detection limit of 0.01 μg g^{-1}. The As levels in *Spirulina* and *Gracilaria* were below the detection limit. However, a higher level of Zn in *Spirulina* can probably be related to its bioaccumulation potential.

Table 4. Trace Metals (in μg g^{-1}) in the Algae Samples.

Algae	As	Ba	Cd	Cr	Cu	Mo	Ni	Pb	V	Zn	Ag	Hg
Sargassum sp. (Kuwait)	0.59	0.23	<0.01	0.35	0.09	0.04	0.44	0.01	0.04	0.35	<0.01	<0.01
Sargassum sp.	0.55	0.12	0.02	0.05	0.03	<0.01	0.03	<0.01	0.02	0.12	<0.01	<0.01
Spirulina sp.	<0.01	0.06	<0.01	0.07	0.06	<0.01	0.07	<0.01	<0.01	0.43	<0.01	<0.01
Gracilaria sp.	<0.01	0.06	0.01	1.69	0.06	0.23	0.84	0.061	0.45	0.25	<0.01	<0.01

The higher levels of arsenic in seaweed are known [51,52]. The levels measured were within an acceptable limit based on the previous reporting, which stipulated 40 μg g^{-1} as the maximum permissible limits for arsenic in seaweed used for animal feed rations. Based on the trace metal results, all these algal samples can be considered suitable for use as a feed additive.

3.4. Performance of Broiler Chickens with Algal Feed Additives

Feed is considered the most significant financial input in Kuwait's poultry industry, accounting for about 70% of the operational cost [33,53]. Most of the corn, soybean, and minerals and vitamins used as feed are imported. Marine algae are considered an additive, which could also be used to partially replace the soybean for its protein content and reduce the cost of the feed. However, the algal protein content is insufficient for complete replacement. The replacement of *Sargassum* sp., *Spirulina* sp., and *Gracilaria* sp. at 2.5, 6, and 10% in the poultry feed was assessed for the performance and immune status of broiler chickens.

Results of the production performance parameters indicated that the algal inclusions had no adverse effect on production performance parameters such as feed efficiency, growth rate, feed consumption, and mortality. However, body weight was enhanced when broiler chickens were supplemented with *Gracilaria* sp. compared with the control group. The immune response functions were enhanced when birds were supplemented with the algal inclusions. A higher cellular response expressed as T-cells in the wattle area (p = 0.037) was observed in groups with algal inclusions. With *Sargassum* sp., the humoral response, represented by antibody titers, was also enhanced. The published results of the algal addition to broiler feed [33] show enhancement in the cellular and humoral immune status of broilers. At the same time, they promote healthy microflora in their guts, including the inhibition of *Salmonella* sp.

3.5. Environmental Impact Assessment of Using Algae as Poultry Feed

The RIAM provides an insight into the likely environmental implications of substituting algae as a protein source in poultry feed. The details of the RIAM scoring are provided elsewhere in a concept paper [48,54]. The scoring in RIAM falls under two distinct categories: A is the independent condition that can change the score, while B is criteria that add to the amplitude of change, its spatial extent, and its permanence; however, they cannot change the score independently. The environmental scores have been assigned considering how algae as a poultry feed is likely to affect the environmental baseline. The paper also sheds insight on the feed quality and the performance of poultry. The RIAM is presented in Table 5, and the visual assessment is in Figures 2 and 3.

Table 5. The Rapid Impact Assessment Matrix for Use of Algae as Additive to Poultry Feed.

Physical and Chemical Components (PC)							
	Components	ES	RB	A1	A2	B1	B2	B3
PC1	Removal of algal biomass	9	A	1	1	3	3	3
PC2	Uptake and accumulation of metals	18	B	1	2	3	3	3
PC3	Uptake and accumulation of radionuclides	18	B	1	2	3	3	3

Table 5. *Cont.*

Physical and Chemical Components (PC)													
	Components						ES	RB	A1	A2	B1	B2	B3
PC4	Accumulation of hydrocarbons						3	A	1	1	1	1	1
PC5	Growth of algal ponds for climate change mitigation						7	A	1	1	1	3	3
PC6	Carbon dioxide sequestration for biomass conversion						9	A	1	1	3	3	3
Biological and Ecological Components (BE)													
	Components						ES	RB	A1	A2	B1	B2	B3
BE1	Increase in algal biomass under changing climate						9	A	1	1	3	3	3
BE2	Performance of broiler chicken with substitution of 2.5% Sargassum in poultry feed						0	N	0	1	1	1	1
BE3	Performance of broiler chicken with substitution of 6% Sargassum in poultry feed						0	N	0	1	1	1	1
BE4	Performance of broiler chicken with 10% substitution of Sargassum in poultry feed						0	N	0	1	1	1	1
BE6	Performance of broiler chicken with substitution of 2.5% Spirulina in poultry feed						0	N	0	1	1	1	1
BE7	Performance of broiler chicken with 6% substitution of Spirulina in poultry feed						0	N	0	1	1	1	1
BE8	Performance of broiler chicken with substitution of 2.5% Gracilaria in poultry feed						0	N	0	1	1	1	1
BE9	Performance of broiler chicken with 6% substitution of Gracilaria in poultry feed						0	N	0	1	1	1	1
BE10	Performance of broiler chicken with 10% substitution of Gracilaria in poultry feed						0	N	0	1	1	1	1
BE11	Performance of broiler chicken with 10% substitution of Spirulina in poultry feed						0	N	0	1	1	1	1
Sociological and Cultural Components (SC)													
	Components						ES	RB	A1	A2	B1	B2	B3
SC1	Algal removal from coastline						32	C	2	2	3	3	2
SC2	Reduction in dependence of feed import						7	A	1	1	2	2	3
Economical and Operational Components (EO)													
	Components						ES	RB	A1	A2	B1	B2	B3
EO1	Cost of feed by using Sargassum from local marine area						7	A	1	1	3	2	2
EO2	Procured algae for addition to poultry feed						14	B	2	1	3	2	2
EO3	Feed efficiency of Gracilaria 2.5%						0	N	0	0	2	1	1
EO4	Feed efficiency of Gracilaria at 6%						0	N	0	1	2	1	1
EO5	Feed efficiency of Gracilaria at 10%						0	N	0	2	2	1	1
EO6	Feed efficiency of Sargassum 2.5%						0	N	0	0	2	1	1
EO7	Feed efficiency of Sargassum 6%						0	N	0	1	2	1	1
EO8	Feed efficiency of Sargassum 10%						0	N	0	2	2	1	1
EO9	Feed efficiency of Spirulina 5%						0	N	0	−1	2	1	1
EO10	Feed efficiency of Spirulina 7.5%						0	N	0	1	2	1	1
Summary of Scores													
Range	−108	−71	−35	−18	−9	0		1	10	19		36	72
	−72	−36	−19	−10	−1	0		9	18	35		71	108
Class	−E		−D	−C	−B	−A	N	A		B	C	D	E
PC	0		0	0	0	0	0	4		2	0	0	0
BE	0		0	0	0	0	9	1		0	0	0	0
SC	0		0	0	0	0	0	1		0	1	0	0
EO	0		0	0	0	0	8	1		1	0	0	0
Total	0		0	0	0	0	17	7		3	1	0	0

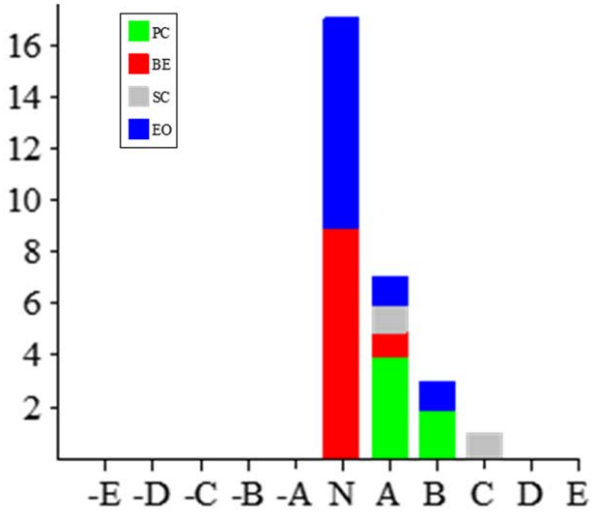

Figure 2. Summary of the RIAM assessment for considering algae as poultry feed additive.

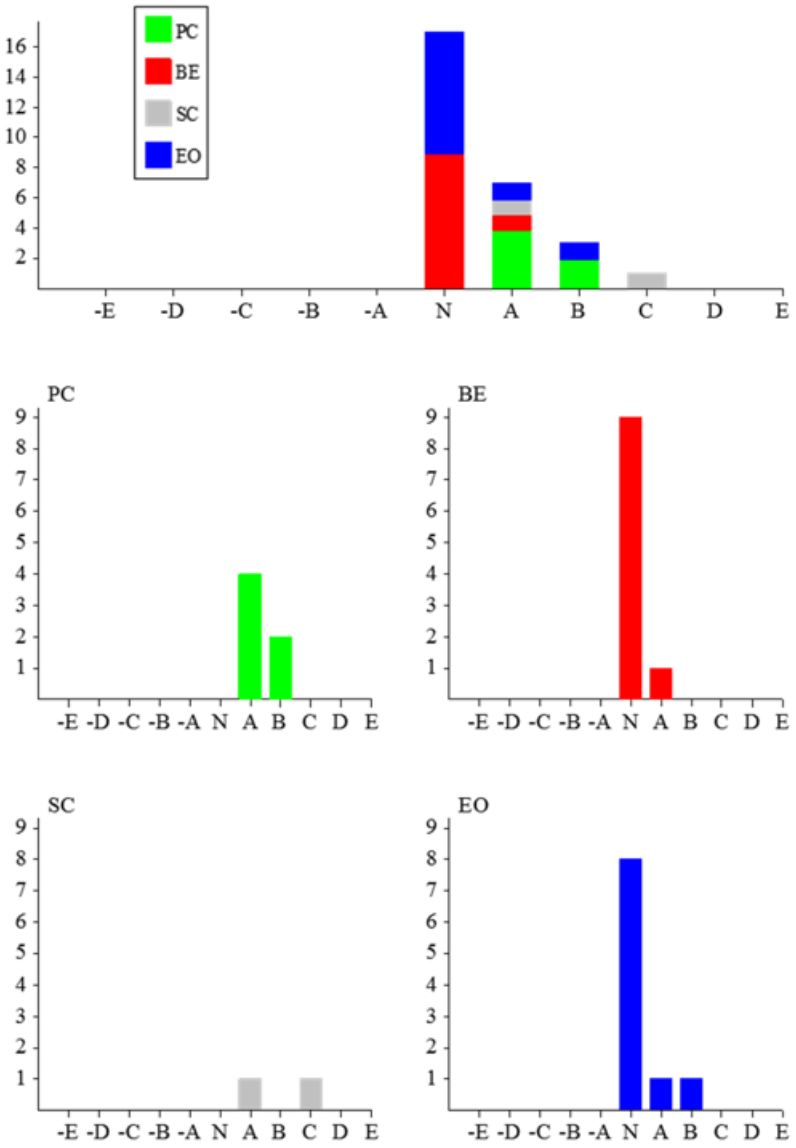

Figure 3. The RIAM scores for various EIA components including PC, BE, SC, and EO.

4. Discussion

The environmental implication of utilizing *Sargassum* sp., *Spirulina* sp., and *Gracilaria* sp. in poultry feed seems optimistic from the perspective of climate change mitigation. It has been found and hypothesized that with the increase in CO_2 concentration in seawater and eutrophication, marine phytoplankton growth will be positively affected [55–58]. Phytoplankton are essential components of marine ecosystems at the base of the food chain and are critical for CO_2 uptake. They are known to concentrate metals and transuranic elements from surrounding waters [59]. One of the overarching effects of ocean acidification on phytoplankton communities is the possible alternation in the chemical speciation of trace metals. Some of these metals, such as iron (Fe), copper (Cu), and zinc (Zn), are essential for the growth and development of phytoplankton. While others, like cadmium (Cd), mercury (Hg), lead (Pb), and polonium (Po), have no known biological functions and are highly toxic at even low concentrations [60,61].

The ability of macroalgae to accumulate pollutants from the environment and transfer them to higher trophic levels in the food chain has also attracted considerable attention [62–68]. A significant accumulation of ^{210}Po and ^{210}Pb by macroalgae was previously reported [19,68,69]. Thus, the radiation dose to the epifauna and ^{210}Po transport in the food chain is a topic of high scientific interest. In recent years, macroalgae have been used in the pharmaceutical industry and cosmetics, and it is also being considered as a food source for human beings and animals. The poisoning of red alga *Gracilaria coronopifolia* is also reported [70] and needs to be addressed if algae is considered for large-scale inclusion in poultry feed. Furthermore, there is a need to undertake comprehensive metal characterization of both the metals conventionally present in poultry feed and those that are toxic before algae substitution in poultry feed is considered on large scale.

Large quantities of macroalgae are washed ashore globally as part of the natural processes in the marine ecosystem. However, these may have implications for the biogeochemical cycle of elements and for human activities [71]. For example, the recent incidences of the Caribbean shores flooded with *Sargassum* sp. resulted in severe environmental and economic concerns [72]. The Gulf shores have witnessed marine macroalgae blooms from late February until mid-April each year, at least for the past decade [4]. During this time of the year, the shores of Kuwait turn green, and the decay of this stranded algal biomass develops slowly as fermentation progresses. The other pertinent issue is that several countries are looking forward to using these macroalgae as fertilizer additives for soil improvement. In addition, researchers are considering fortifying the poultry feed with macroalgae as a protein source.

One of the significant concerns emanated from radioactive lead and polonium that exist in algae. Even with the enriched ^{210}Po and ^{210}Pb concentrations in macroalgae varying between 1.533 and 2.947 Bq kg^{-1} wwt, and 0.170 and 1.057 Bq kg^{-1} wwt, respectively, they do not pose any concern to be used in feed. The highest concentration of ^{210}Po and ^{210}Pb among all the macroalgae analyzed was found in the brown algae *Sargassum boveanum* (class Phaeophyta). The ^{210}Po/^{210}Pb ratio in algae was always higher than the seawater (^{210}Po/^{210}Pb ratio ranging between 2.67 and 10.95), suggesting an enrichment in algae. The passive uptake via adsorption on the outer organic coating of macroalgae is likely to be the active process. Both ^{210}Po and ^{210}Pb concentrations were substantially higher in Phaeophytes than in Chlorophytes. The higher trace metal, including ^{210}Po concentration in Phaeophytes, is likely due to the presence of sulfated polysaccharides and alginates within the outer layer of the cell wall of these algae, which have a strong affinity for trace metals [73]. The lower ^{210}Po concentration in Chlorophytes is attributed to carboxyl groups in the polysaccharides, which are not as efficient in binding ^{210}Po [74]. The concentration factor of ^{210}Po observed is 5×10^3 to 1×10^4 relative to its concentration in ambient seawater. The trends are similar for other metals.

Current proximate analysis results suggest that the analyzed algae contained a considerable amount of fats and proteins to partially replace conventional soya and corn feed. The presence of significant omega-3 fatty acids in the algal samples is likely to benefit the

birds and can be a healthier option for human consumption. The trace metal analyses in the algae were within the levels observed elsewhere and were acceptable to be added to the poultry feed without posing any threat of contamination. Four heavy metals, including selenium, manganese, copper, and zinc are added to poultry feed as micro-nutrients. The concentration of these metals in commercial poultry feed in Bangladesh was 0.0347, 0.5788, 0.4399 and 0.0579 ppm [75]. The use of algae as feed is also likely to benefit the marine environment since a substantial amount of biomass can be harvested that quenches metals and radionuclides. In spite of this quenching, the level of metals within the analyzed algae is within the safe limits of usage as feed [76]; this will help maintain levels of dissolved metals in seawater. The utilization of the algae in poultry feed will also have a positive economic implication, as up to 10% of algae can be added to the feed, saving a considerable amount of soya and corn. The use of *Sargassum*, *Enteromorpha*, *Ulva*, and other varieties, which are commonly washed ashore in Kuwait, will be a multi-dimensional approach to the utilization of the algae washed ashore, thus maintaining the water quality and reducing dependence on conventional poultry feed sources.

5. Conclusions

The strategic environmental impact assessment provides a promising overview on the use of algae as poultry feed. The algae harvesting is likely to have a positive impact on the environmental baseline when utilized as a poultry feed. The harvesting of the algae will minimize the degradation of algae and the release of metals back into the marine environment. The use of microalgae can bring in the perspective of creating algal ponds; however, in this approach, the economic feasibility and environmental footprint are likely to be negative. Open ponds in Kuwait are not feasible due to the lack of surface water and extremely high evaporation rates in the summers. The use of algae as poultry feed is very likely to positively impact the poultry industry in Kuwait since these feed sources are cheaper and are comparable in performance to conventional feed. However, a more detailed investigation and chemical characterization should be taken up, including analyses of amino acids and metals like magnesium, iron, iodine, and selenium; consideration should also be given to assessing whether a higher percentage of substitution can be achieved. The palatability and capacity to extrude and palletize *Sargassum* should also be assessed in future assessments.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/su14148968/s1. The Supplementary Material is included that describes the experimental design and antibodies concentration as result of the experiment.

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Informed Consent Statement: Not applicable.

Data Availability Statement: All the data is available in the manuscript and the Supplementary Materials.

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