Efficacy of incorporating spent seaweeds in aqua feeds as a nutritional source

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Abstract:
Spent biomass of different seaweeds such as *Sargassum wightii*, *Turbinaria conoides*, *Padina tetrastomata* and *Ulva lactuca* were evaluated for the nutritional benefits in aqua feeds. Basic nutritional value, of seaweeds contains a number of pigments, defensive and storage compounds, and secondary metabolites that could have beneficial effects in farmed fish. Only a small fraction of algal species have so far been investigated as potential components in finfish diets and a number of knowledge gaps that current research has yet to be addressed. The effects of seaweed supplementation most relevant in aquaculture include stimulation of growth performance, enhancement of feed utilization efficiency, improvement of nutrient assimilation, and improvement of fatty acid profile (increase in long chain n-3 polyunsaturated fatty acids) in muscle. Spent seaweeds shows enriching nutritive values such as protein, vitamin, mineral, essential amino acids, essential fatty acids and also improves the digestibility of spent seaweed based feeds. The anti-nutritional factors in spent seaweed were also recorded as low in *Sargassum wightii* phenolics (1.60±0.05) tannins (3.20±0.43), in *Turbinaria conoides*, saponins (0.83±0.15), in *Ulva lactuca* Oxalates (0.13±0.02) Phytate (16.54±0.23). Hence, the spent residues from industries, and seaweed spent biomass generated in the laboratory after pigment extraction were considered to be a good source of biomass for aqua feed production.

Keywords: Spent seaweeds, Anti-nutritional, Biochemicals.

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Introduction

Marine ecosystem provides the most extensive resources like food, medicines, fuel and livelihoods. Among them seaweeds are crucial, economically valuable, macroscopic, multicellular algal resources (Cao *et al*., 2021). They are rich in carbohydrates, growth promoting phytohormones (IAA and IBA), trace elements, bioactive compounds, amino acids, vitamins, micro and macro nutrients (Wei *et al*., 2013; Matsumura *et al*., 2014; Elansary *et al*., 2016). Seaweed based industrial discharge contains organics which remains unexplored. Proper utilization of these organic waste into valuable products which enhances recycling, ecofriendly waste management.

Nutritional status of seaweeds

Seaweeds are good source of proteins, vitamins and minerals (Burtin, 2003). Seaweeds with good protein level are receiving considerable attention as novel feeds with potential nutritional benefits (Buschman *et al.*, 2001). Simultaneously other studies have reported that seaweeds contain more than 60 trace elements in much higher concentration than in terrestrial plants including the essential primary and secondary micro and macro nutrients, which is required for plant cell division, growth and development and making it as excellent fertilizer (Chennubhotla *et al*., 1991; Zopade, 2001; Jayasree *et al*., 2012; Mohanty *et al*., 2013). In this regard several other studies on the biochemical and nutritional composition of various seaweeds collected from different parts of the world have been conducted with a view to utilize their nutritional value (Dhargalkar *et al*. 1980; Norziah and Ching, 2000; Manivannan *et al*. 2008; Jayshree *et al*. 2012; Kiuomars *et al*. 2012; Cosman *et al*. 2013; Parthiban *et al*. 2013; Rajababu *et al*. 2017; Noorjahan *et al*., 2019). In recent years, great interest has been taken to develop commercial seaweed farming to harmless seaweeds as food, feed and fuel (Chennubholta *et al*. 1991; Vinoj and Kaladharan, 2007; Bindu and Levine, 2010; Bjorn *et al*. 2012).

Nutrition plays an important role to achieve an efficient aquaculture production as it brings great influences not only to the production costs but also to the fish health, growth and production of waste (Bhosale *et al.*, 2010). To produce nutritious and cost-effective diets, the specific nutritional requirement of a particular fish should be known before the formulation of any fish feed. However, in intensive aquaculture systems, commercial diets are quite expensive due to the inclusion of high priced fishmeal and fish oil, which are recognized as the best source of protein and lipid for most fish species (Davidson *et al.,* 2016). Fishmeal and fish oil are one of the most important ingredients for aqua feeds, however, both are a limited resource. Decreased availability and the increased price of the fish meal and fish oil have stimulated the search for sustainable alternatives for aquaculture feeds to replace fishmeal with readily available inexpensive plant sources.

Spent seaweeds

As it is well recorded that marine seaweed resources have been used as food, feed, and fertilizer and also as a unique source of traditional medicines in many countries (Zahid, 1999; Lakshmana *et al*., 2013; Evans and Critchley, 2014; Massoumeh *et al*., 2014; Maria and Combet, 2015).The huge amount of spent biomass generated from seaweed industry worldwide and effective utilization of those spent is really a challenging task. In India, some seaweed industries are converting the spent seaweed biomass to agricultural manures through composting and energy generation is another milestone from waste. The major thriving need of aquaculture industry is protein feed which is mainly depending on fish meal. In order to replace fish meal protein from spent seaweed provoke an innovative method to convert waste into a more valuable product.

Finding novel sustainable protein sources has become a major drive in the aquaculture sector in order to reduce the dependency on fish meal as the main protein component in aqua feeds. In order to further improve the use of spent seaweed incorporated diets, this study was conducted with the aim of evaluating the nutritional compounds of *Sargassum sp. Ulva sp. Turbinaria sp. Padina sp* with the potentiality of producing nutritious Aqua feed.

The aim of the present study to screen the spent seaweeds for proximate, mineral and anti-nutrients and to determine the growth response and feed utilization of Tilapia (*Oreochromis niloticus*) fed with graded dietary levels of spent seaweed incorporated meal.

Materials and methods

Spent seaweed

The seaweeds A. *Sargassum wightii*. B, *Turbinaria conoides*. C. *Padina tetrastomata* D. *Ulva lactuca* collected from the Rameshwarem coast (9.3069ºN, 79.3288ºE) were shaded dried, pulverized and sieved through a 0.5 mm mesh. Seaweed powder and water in the ratio (1: 20) were taken and autoclaved at 120°C for 15 min later cooled and filtered. The residue is taken and oven dried (45ºC) and stored in air tight container as spent seaweed powder.

Determination of nitrogen free extract (NFE)

The nitrogen free extract (NFE) was calculated by using the following formula,

This was determined by difference:

% Carbohydrate = [100 – (% Crude protein + % Lipid + % Crude fiber + % Ash + % Moisture)].

Anti-nutritional analysis of spent seaweeds

Samples of Spent seaweeds were taken for the determination of anti-nutritional Factors. The anti-nutritional compounds that were determined included such as phytate (Reddy *et al.*, 1982), Tannins (AOAC, 1980), Saponins (Hudson, 1979), Total oxalates Abeza *et al.*

(1968) and Total polyphenol (Slinkard and Singleton, 1977).

Proximate composition of formulated feed

Proximate analysis is usually the first step in the chemical evolution of a feed ingredient, where the materials is subjected to a series of relatively simple chemical tests so as to determine the content of moisture (AOAC, 2000), crude protein (Lowry *et al*., 1951), crude fiber (AOAC, 2000), lipid (Bragdon, 1951), Ash (Lovell, 1981) and digestible carbohydrate (Seifter *et al.*, 1950).

Experimental animal

Oreochromis niloticus (Tilapia) used in the experiment were obtained from in a standardized hatchery, fish farm, Four hundred and eighty (500) *Oreochromis niloticus* juveniles were transported in two (50 litres) jerrycans between 7.00am and 8.00am, to an outdoor concrete tank (100cm x 150cm x 120cm) at the Hatchery, CAS in Marine Biology, Annamalai university for two weeks to acclimatize prior to feeding trials. Fish were fed during the acclimatization period with 42% crude protein commercial feed (control diet) at 5% body weight twice daily. During the acclimatization period, the water temperature, pH and dissolved oxygen (D.O) were monitored. Water in the outdoor concrete tank was replaced at two days interval with dechlorinated water, stored for 24 to 48 hours prior to utilization.

Growth performance and feed utilization parameters

At the beginning of the experiment, the total wet weight of the fish in each group was determined by weighing in an electronic balance. All fish in each group were weighed at the end of the experiment.

Mean body weight gain

This was calculated as the difference between the initial and final body weights for fish

$W2 – W1$

Where $W2$ = Final body weight

$W1$ = Initial body weight

Mean increase in standard length (CM)

$L2 – L1$

Where $L2$ = Finial standard length

$L1$ = Initial standard length

Percentage live weight gain (PLWG) - (Wannigama *et al*., 1985)

The PLWG was computed as the difference between the initial and final fish weight divided by the initial weight expressed as percentage

$W2 – W1 / W1 \times 100$

Specific growth rate (SGR) in percentage body weight per day

$SGR = 100 (LnW2 – LnW1) / t$

Where $W2$ = Final Weight

$W1$ = Initial weight

t = Period of experiment in days

Ln = base of natural logarithm

Feed utilization (Total unit of feed consumed)
The feed conversion ratio (FCR) (Arunletaree and Moolthongnoi, 2008)
FCR = Amount of Feed Fed/ Weight gain (g)

Protein efficiency ratio (PER) (Olvera-Novoa, 2002)
PER= Weight gain/Protein fed
Where protein Fed= % protein in the diet /100 x total diet consumed

Net protein utilization (NPU) (Dabrowski and Kozak, 1979)
Net protein utilization (NPU)= Fish protein gain / Protein Fed x 100
Where,
Protein gain= Final body Protein – Initial body protein
Protein consumed= Total Dietary protein fed

Analysis of variance (ANOVA)
Variance between different means of a single variable was tested following one way calculation of variance described by Zar (1984).

Results

The proximate composition of spent seaweed were displayed in Table 1 and Figure 1. The maximum carbohydrate content was observed in *Ulva lactuca* (SUL) 45.3%>*Sargassum wightii* (SSW) 42.01%>*Turbinaria conoides* (STC) 36.2 % and *Padina. tetrastromatica* (SPT) 21.08 %. The crude fiber (20.09%) and moisture (15.2%) was maximum in *Ulva lactuca* (SUL). Total protein (18.1%), Lipid (2.2) and ash (31.3%) content was found to be maximum in *Turbinaria conoides* (STC).

Table 1: Proximate composition of spent seaweeds.

Proximate composition	SUL	SSW	SPT	STC
Moisture	15.2	10.4	11.3	8.1
Carbohydrate %	45.3	42.01	21.08	36.2
Total ash %	7.2	24	23.1	31.3
Crude fiber %	20.09	12.2	5.4	3.3
Total lipid %	1.18	1.22	0.42	2.2
Total protein %	17.3	18.1	16.3	18.1

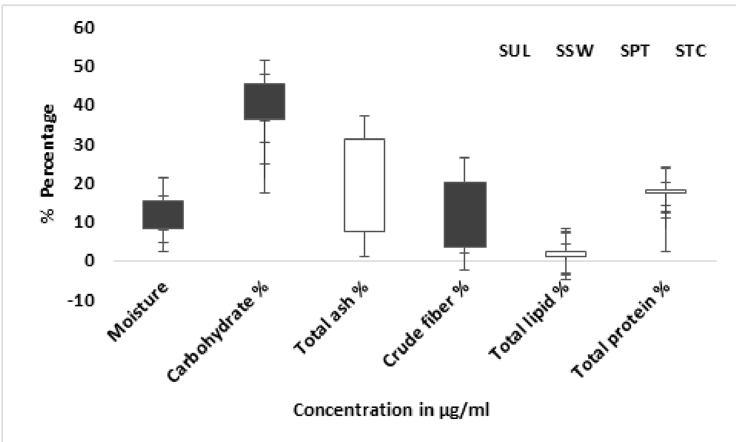

Figure 1: Proximate composition of spent seaweeds.

The anti-nutritional factors of the spent seaweed was determined and summarized in Table 2 and Figure 2. Total oxalates in all the species were in the range 0.13 to 0.24 mg g-1. Phenolics (1.97 mg g-1) and Saponins (2.03 mg g-1) content was found to be low in *Turbinaria conoides*. In *Sargassum wightii* Tannin content (2.32 mg g-1) was recorded as low on comparing with other species.

Table 2: Anti-nutritional contents of spent seaweeds.

Anti-nutritional factors(mg g^{-1})	SUL	SSW	STC	SPT
Total phenolic	6.46±0.65	3.72±0.04	1.97 ± 0.03	10.01±0.11
Total Tannins	6.34±0.22	3.21±0.01	10.83 ± 0.22	8.23±0.37
Total Saponin	3.07±0.49	2.32±0.04	2.03 ± 0.01	7.42±0.43
Total oxalates	0.13±0.02	0.16±0.01	0.24 ± 0.03	0.14±0.01
Total Phytate	17.21±0.2	20.02±0.2	17.23 ± 0.38	19.12±0.21

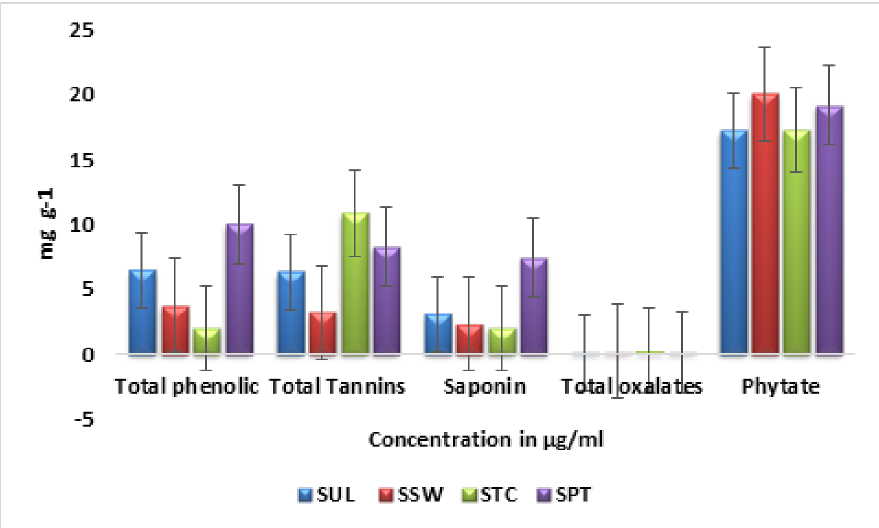

Figure 2: Anti-nutritional contents of spent seaweeds.

Minerals such as Ca, Cu, Fe, P, K, Na and Zn are essential for the digestibility, carcass development and for muscle growth. Spent seaweeds are rich in mineral content among the species *Ulva lactuca* was recorded high in Ca (209.0±0.1mg/100g) and Na (27.12±0.5 mg/100g), *Padina tetrastromatica* rich in Cu (0.24 ± 0.03mg/100g), *Sargassum wightii was* rich in Zn (9.37±0.27 mg/100g) and *Turbinaria conoides* rich in maximum number of minerals Fe (93.37±0.5 mg/100g), P (1.13±0.12 mg/100g) and K (114.2±0.27mg/100g) summarized in Table 3.

Table 4 shows the formulated seaweed feed. The seaweed meal incorporated in the feed at 10%, 20%, and 30% of the protein by replacing Fish meal which is already in practice. The fish were fed with three times in the rate of 4% of the body weight of the experimental animal taken for the study period.

Table 3: Mineral composition of Spent Seaweeds.

Anti-nutritional factors(mg g^{-1})	SUL	SSW	STC	SPT
Ca	209.0±0.1	137 ± 1.07	117.71±0.34	141.13±1.02
Cu	0.01±0.01	0.24 ± 0.03	0.02±0.01	0.02±0.01
Fe	14.63±1.01	29.36 ± 0.01	0.01±0.01	93.37±0.5
P	0.07±0.1	0.37±0.01	0.46±0.01	1.13±0.12
K	25.82±0.4	40.23 ± 0.22	56.57±0.03	114.2±0.27
Na	27.12±0.5	14.58 ± 0.01	15.61±0.26	19.73±0.13
Zn	0.8±0.2	0.24 ± 0.03	9.37±0.27	0.47±0.01

Table 4: Ingredient composition of spent seaweed meal incorporated feeds.

Ingredients (g)	Control (C)	Spent (SUL, STC, SPT, SSW)		
		10%	20%	30%
Groundnut cake	40	40	35	25
Fish meal	10	-	-	-
Wheat flour	10	10	10	10
Yellow corn	10	10	10	10
Wheat bran	20	20	15	15
Spent Seaweed	-	10	20	30
Vegetable oil	5	5	5	5
Vitamin & Mineral premix	2.5	2.5	2.5	2.5
Di calcium phosphate	2.5	2.5	2.5	2.5

The proximate composition of the formulated feed was determined and were displayed in Table 5. Among the species Protein (36.96%), Lipid (7.45%), Moisture (9.6%) and Ash (11.2%) was maximum in *Turbinaria conoides*, Whereas Carbohydrate content was rich in *Ulva lactuca* (34.89%).

Table 5: Proximate composition of Formulated feed.

Proximate composition	Commercial feed	SUL	SSW	SPT	STC
Moisture %	8.5±0.12	9.5±0.11	9.4±0.09	9.5±0.01	9.6±0.13
Carbohydrate %	31.69±2.1	34.89±1.9	33.78±1.01	32.69±2.9	33.89±1.29
Total ash %	9.0±0.11	10.2±0.12	10.62±0.42	10.12±1.12	11.2±0.12
Total lipid %	7.0±0.80	6.1±0.99	6.11±1.99	6.41±1.19	7.45±1.99
Total protein %	34.71±1.4	36.94±1.2	35.84±1.21	36.74±0.2	38.96±1.12

The growth performance and feed utilization of spent seaweeds (SUL, STC, SSW, and SPT) incorporated diets at various concentration 10%, 20%, & 30% on Tilapia, *Oreochromis niloticus* were monitored. The final weight, total weight gain (g) and weight gain (%) were significantly (p<0.05) increased by increasing the seaweed level in the diets. Fish fed spent seaweeds have exhibited the highest value (247g) was obtained in fish group fed with spent seaweed SUL=STC>SPT>SSW feed presented in Table. 6a, 6b, 6c and 6d.

Discussion

The nutrient rich seaweed hydrolysates are utilized as growth medium for culturing the microalgae in aquaculture to provide live feedstock for fish, molluscs and crustacean (Alvarado *et al*., 2008). The decrease of fish growth and feed utilization when diet supplemented with seaweed at levels higher than 5% could be explained by the presence of anti- nutritional factors such as saponins, tannins and phytate which occurred in several plants (Francis *et al*., 2001). The plants contain high amounts of indigestible fiber, carbohydrates, and some anti-nutritional factors, and are often insufficient in certain essential amino acids methionine and lysine, compared to animal protein, which causes adverse effects on feed absorption, digestion, and utilization and could have lowered the growth performance when included at a high-level (Krogdahl *et al*., 2014). There is no interference in the growth was observed in the experimental animals relating with anti-nutritional factors in spent seaweeds. From this it is clearly understood that spent seaweeds have permissible amount of nutritional factors. The tolerable limit for fish is not yet established. However, it has been indicated that fish are sensitive to tannins and that caution should be exercised in incorporating seeds and agro- industrial by-products containing high levels of tannins in fish feed (Francis *et al.*, 2001).

From the result it was evident from the previous study reported by Kiuomars *et al*. (2012) evaluated mineral composition of green, brown and red seaweeds from the Persian Gulf of Iran and it was reported that seaweeds contained higher amount of K, Mg, Fe, Mn, Cu, Zn and Co compared to terrestrial vegetables. Murugaiyan and Narasimman (2013) reported that in red seaweed *Amphiroa fragilissima* from Gulf of Mannar region, Southeast coast of India, the concentration of elements (in ppm) found to be as Al (97.68±7.93), B (9.17±0.61), Cd (0.38±0.63), Co (1.29±0.71), Cr (7.36±0.96), Cu (3.42±0.73), Fe (100.6±7.89), Mg (529.1±45.96), Mn (29.67±2.39), Ni (1.89±0.53), Pb (4.69±0.86), Zn (3.75±0.89).

Fish fed with gradient concentration of different seaweed species supplemented diets which showed increase in growth performance of the experimental animal during the experimental period in terms of the final weight, total weight gain (g) and weight gain (%) were statistically significant (p<0.05, Tables 6a, 6b, 6c & 6d). These results differed with (Alves *et al*., 2011) who found reduction, in weight gain of Nile tilapia (*O. niloticus*) fingerlings fed brown seaweed (*A. nodosum*) at level of 3%. This may be due to the raw seaweed contains anti-nutritional factors which may interfere with the performances. Also, it has been observed a decrease in juvenile grey mullet (*Chelon labrosus*) fed red seaweed (*Porphyra purpurea*) at 0, 16.5, and 33% (Davies *et al*., 1997). Moreover, (Linares *et al*., 2014; Queiroz *et al*., 2014) found no effects on gilthead seabream (*Sparus aurata*) performance and Senegalese sole (*Solea senegalensis*), respectively when fish diets were incorporated with seaweed. On contrast, higher growth performance was observed in Red tilapia (*Oreochromis sp*.) fed up to 15% level of seaweed (El-Tawil, 2010) and Spent seaweeds can be incorporated in feed upto 30% incorporation level with spent seaweed species such as *Ulva lactuca, Sargassum wightii, Padina tetrastromatica* and *Turbinaria conoides* (Akhilamole *et al*., 2019).

Table 6a: Growth related parameters of Tilapia *Oreochromis niloticus* fed with *Ulva lactuca* incorporated diets at different concentration.

Parameters	Control (C)	SUL 10%	SUL 20%	SUL 30%
IW(g)	50±0.1	50±0.16	50±0.16	50±0.16
FW(g)	178±0.03	193±0.02	198±0.02	297±0.02
WG(g)	128.32±0.1	143.01±0.015	148.01±0.015	247.01±0.015
WG%	78±0.1	93±0.2	98±0.21	197±0.21
DGR (g/day)	1.13±0.1	0.045±0.0021	1.14±0.0021	1.175±0.0024
SGR (% /day)	0.81±0.03	2.15±0. 36	2.55±0. 34	2.55±0. 34
FCR	1.82±0.01	1.8 ±0.1	1.89 ±0.1	1.98 ±0.1
FER	1.2±0.1	1.62±0.1	1.72±0.01	1.92±0.01
PER	1.32±0.02	5.03±0.3	5.43±0.3	5.93±0.3

Means with different letters are significantly different at (p<0.05)

Table 6b: Growth related parameters of Tilapia *Oreochromis niloticus* fed with *Sargassum wightii* incorporated diets at different concentration.

Parameters	Control (C)	SSW 10%	SSW 20%	SSW 30%
IW(g)	50±0.1	50±0.16	50±0.22	50±0.16
FW(g)	17 8±0.03	190±0.02	197±0.02	230±0.12
WG(g)	128.32±0.1	140±0.015	147±0.012	180±0.025
WG%	78±0.1	90.01±0.265	97±0.12	130.2±0.26
DGR (g/day)	1.13±0.1	0.041±0.0021	0.095±0.0021	0.065±0.0021
SGR (% /day)	0.81±0.03	1.58±0.036	1.75±0.036	1.95±0.036
FCR	1.82±0.01	1.7 ±0.1	1.98 ±0.1	1.9 ±0.1
FER	1.2±0.1	0.97±0.1	0.87±0.1	0.97±0.1
PER	1.32±0.02	3.03±0.3	4.13±0.3	4.13±0.3

Means with different letters are significantly different at (p<0.05).

Table 6c: Growth related parameters of Tilapia *Oreochromis niloticus* fed with *Padina tetrastromatica* incorporated diets at different concentration.

Parameters	Control (C)	SPT 10%	SPT 20%	SPT 30%
IW(g)	50±0.1	50±0.16	50±0.22	50±0.16
FW(g)	17 8±0.03	195±0.02	199±0.02	280±0.12
WG(g)	128.32±0.1	145±0.015	149±0.012	230±0.025
WG%	78±0.1	95±0.25	99±0.12	180.2±0.26
DGR (g/day)	1.13±0.1	0.061±0. 021	1.085±0.021	1.95±0. 21
SGR (% /day)	0.81±0.03	1.88±0. 36	1.75±0.036	1.95±0.036
FCR	1.82±0.01	1.7 ±0.1	1.98 ±0.1	1.9 ±0.1
FER	1.2±0.1	0.97±0.1	0.87±0.1	0.97±0.1
PER	1.32±0.02	4.03±0.3	4.73±0.3	5.13±0.3

Means with different letters are significantly different at (p<0.05).

Table 6d: Growth related parameters of Tilapia *Oreochromis niloticus* fed with *Turbinaria conoides* incorporated diets at different concentration.

Parameters	Control (C)	STC 10%	STC 20%	STC 30%
IW(g)	50±0.1	50±0.16	50±0.22	50±0.16
FW(g)	178±0.03	193±0.02	199±0.02	297±0.02
WG(g)	128.32±0.1	143.01±0.015	149±0.012	247.01±0.015
WG%	78±0.1	93±0.2	99±0.12	197±0.21
DGR (g/day)	1.13±0.1	0.045±0.0021	1.085±0.021	1.175±0.0024
SGR (% /day)	0.81±0.03	2.15±0. 36	1.75±0.036	2.55±0. 34
FCR	1.82±0.01	1.8 ±0.1	1.98 ±0.1	1.98 ±0.1
FER	1.2±0.1	1.62±0.1	0.87±0.1	1.92±0.01
PER	1.32±0.02	5.03±0.3	4.73±0.3	5.93±0.3

Means with different letters are significantly different at (p<0.05).

The utilization of several seaweed meals was evaluated in snakehead fry and found that among the seaweeds tested, *Ulva* sp provided the highest relative growth performance (Immanuel *et al*., 2004).

This high protein content of the species is one of the important constituents for projecting the species as supplemental food and can also be beneficial as aquaculture, including farm animals, animal feed, and pets. Protein has crucial functions in all the biological process and an estimated 30% of global algal production is used for animal feed due to its excellent nutritional profile (Becker, 2004). It has been reported that macroalgae can be incorporated as protein sources into the diets of poultry, pigs, cattle, sheep, and rabbits (Akhilamole *et al*., 2019).

Conclusion

The present study is a humble approach for standardization of the fed from spent seaweed associate in the aquaculture sector, which results not only in the economic upliftment of the coastal culture through sustainable fish culture, but also may open up an avenue of alternative livelihood, through development of small scale endemic seaweed based fish feed industry. Research findings reveal that feed incorporated with seaweed meal has resulted in improved performance of tilapia, better feed efficiency, better pellet stability and improved animal product quality.

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