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# Ulva Species Usage in Aquaculture: Current Status and Future Prospective

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**ABSTRACT**

Concerns about the economy, the environment, and production have sparked a search for long-acting immune-stimulants and growth promoters for aquatic species. *Ulva spp*. as green macro-algae is currently being studied as a potential alternative source for a variety of antibiotics and its wide range of availability and chemical composition creates both opportunities and challenges. Various studies have recently been undertaken on the utilization of *Ulva spp*. as a protein, lipids, carbohydrates, and vitamins source; besides, the existence of a wide range of bioactive compounds which can be used as antihypertensive, antioxidative, antitumor, antimicrobial, and anticoagulant components in functional foods or pharmaceuticals and nutraceuticals due to their health benefits and therapeutic potentials. Moreover, different researchers have used *Ulva spp*. in different forms, such as fresh, extract, and powder, to examine its effects on different aquatic organisms like fish, crustaceans, and molluscs. As a result, this review article aims to highlight the properties, impacts, and application methods of various *Ulva spp*.

**Keywords:** *Ulva spp*.; Aquatic Organisms; Feed; Effects

## Introduction

Seaweeds have been used for a variety of applications, including human-eating [1], paper manufacturing, cosmetics, animal feeding, wastewater treatment, medical research, and fertilizers [2-4]. Furthermore, green seaweed provide a sustainable biomass feedstock for the biotech industries and food, including bioremediation, future biofuel generation, and integrated aquaculture systems, from an economic standpoint [5,6].

Ulva is a chlorophyte, an informal collection of three traditional groups (*Ulvophyceae, Trebouxiophyceae*, and *Chlorophyceae*) [7-9]. The *Trebouxiophyceae* and *Chlorophyceae* diversified significantly in terrestrial and freshwater settings, while the *Ulvophyceae* dominated marine environments [10]. Furthermore, the morphological and cytological diversity of *Ulvophyceae* is amazing [11]. Sheet, unicells-like thalli, filaments, and giant-celled coenocytic or siphonal macro-algae [12] branch and fuse to form morphologies with root-, leaf-, and stem-like structures similar in size to large shrubs on land [13-15].

Due to eutrophication-driven tides in shallow areas, Ulva is becoming more essential in coastal ecosystem management [16,17]; besides, it has become available to do much research in the field of aquaculture; for example, *U. fasciata* [18], *U. lactuca* [19], *U. rigida* [20], and *U. clathrata* [21].

Although some studies have reported that different *Ulva spp*. have positive effects on different parameters such as growth and immunity, other studies have negative effects, and others have proven that there is no effect. These data varied according to fish spp., *Ulva spp*., concentrations used, and method of use. However, most of them dealt with Ulva as powder and few studies reported on Ulva as fresh and extract; besides, few studies documented its effects on growth, immune, and antioxidant-related gene expressions [18]. In general, the economic, academic, environmental, and biological significance of macro-algae, especially of *Ulva spp*. is not widely valued and discussed. As a result, this review targets to integrate the literature and delivers an essential perception of the use of different *Ulva spp*. in aquatic organisms' feed.

This review study achieves three (3) main purposes:

a) To describe the characteristics and chemical composition of different *Ulva spp*.

b) To describe the potential methods of different *Ulva spp*. used in different aquatic organisms' feeds.

c) To explore the impacts of dietary supplementation of different *Ulva spp*. on immunological and growth parameters.

## General Structure and Characteristics of *Ulva spp.*

*Ulva spp*. is available in a diverse group of around 125 species that are currently accepted taxonomically over the world [22] with a common structure as shown in Figure 1.

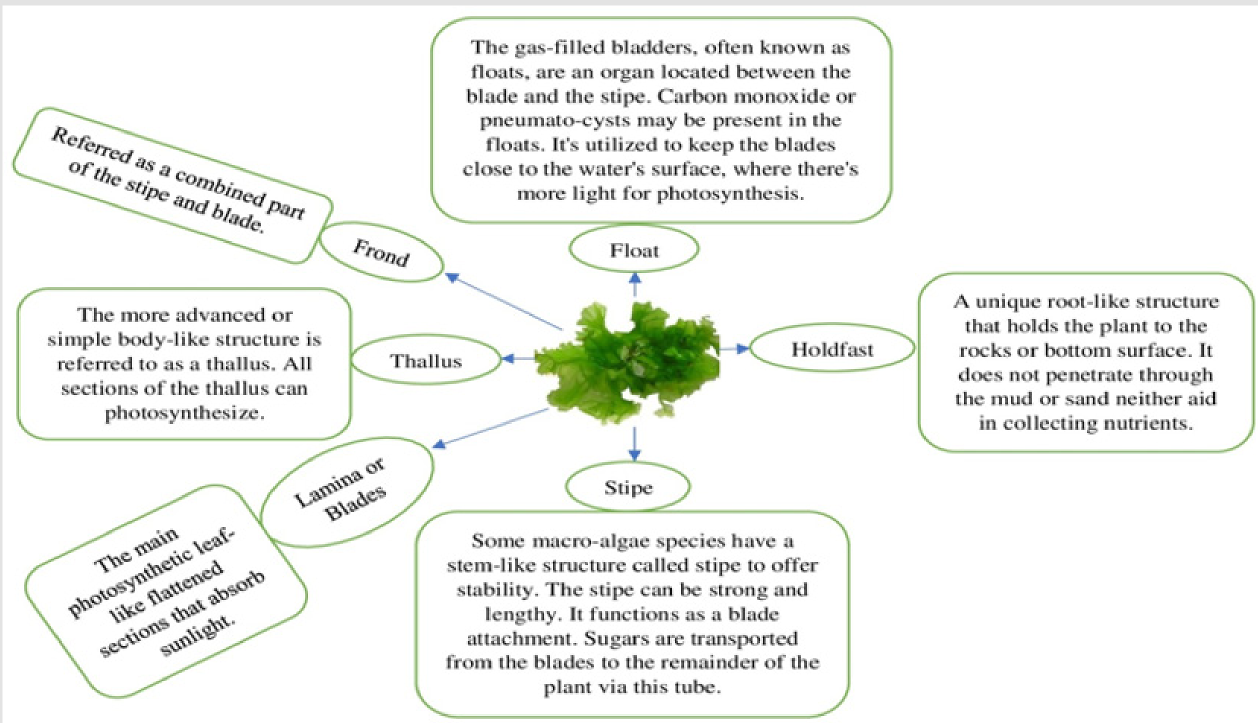


**Figure 1:** Typical structure of Ulva spp. with different parts.

They do not have real roots, leaves, and stems; moreover, because of its enlarged leaf-like structures that resemble garden lettuce, it's also known as sea lettuce. Ulva's morphology shows distinct seasonal fluctuations; for example, small plants are sensitive to the touch and dark green in colour, but older thalli turn light green and their surface becomes slimy [22]. The specific characteristics of different *Ulva spp*. are shown in Table 1 [23-37]. They have simple reproductive structures with no vascular tissue and form multilayered and stable vegetation that captures available photons from sunlight.

**Table 1:** The specific characteristics of most common *Ulva spp.*

| | Description | Habitat and distribution | References |
|---|---|---|---|
| *U. fasciata* | **a.** Thalli are thin, sheet-like plants with wide blades that are 10-15 cm wide at the base and taper upward to less than 2.5 cm wide at the tip. **b.** Thalli can be up to 1 meter long. **c.** When reproductive, bright grass green to dark green with gold at the borders. | **a.** Commonly found on intertidal rocks, tidepools, and reef flats. **b.** Frequently plentiful in places of high-nutrient freshwater runoff, such as near stream mouths and run-off pipes. | [23] |

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| | | | |
|---|---|---|---|
| *U. lactuca* | **a.** The margin of *U. lactuca* thin flat green algae growing from a discoid holdfast is ruffled and often broken.<br>**b.** It can grow up to 18 centimetres in length and 30 centimetres in width, however, it is usually considerably smaller with color ranges from light to dark green. | **a.** Europe, Central America, North America (west and east coasts), the Caribbean Islands, Africa, South America, the Indian Ocean Islands, China, South-west Asia, the Pacific Islands, New Zealand, and Australia. | [24-26] |
| *U. clathrata* | **a.** The plant is light green in colour.<br>**b.** Height: 20–80 millimetres (0.79–3.15 in). | **a.** It is common in Asia and Africa, including Kenya, Mauritius, South Africa, Tanzania, Japan, Portugal, and Tunisia.<br>**b.** It is also found in the Americas, including Alaska, Argentina, Brazil, Cuba, Grenada, Hispaniola, and Venezuela (including the mediterranean Sea). | [27] |
| *U. reticulata* | **a.** Thallus tough, ribbon-like, hard in texture, reticulate, netlike (membrane with several big and small holes).<br>**b.** Deeply and irregularly lobed, light to dark green, to 80 cm across, with minute serrations along the thallus margins and edges around the pores. | **a.** Found in the Western Atlantic, Indian, and Pacific Oceans; as well as, Antarctica, and subantarctic islands.<br>**b.** The continent of Asia (China, Korea, Japan, and Taiwan) | [28] |
| *U. rigida* | **a.** The thallus is sheet-like, inflexible, coarse, solitary, or aggregated into tufts, often deeply lobed, widely orbicular, and with a short firm stipe.<br>**b.** Dark green, up to 1 m in diameter.<br>**c.** Flat or ruffled blades, often perforated, smooth borders, undulating with microscopic teeth. | **a.** Widely widespread from the Arctic to the Antarctic and subantarctic islands, with a strong presence in Spain. | [29] |
| *U. pertusa* | **a.** Thallus produces a distromatic blade that is 5–20 cm long, irregularly orbicular, lobed, thick, and tough when tiny but oval and more delicate towards the borders when large. | **a.** The Indo-Pacific native U. pertusa can be found from the Sakhalin-Kuril Islands in the north to New Zealand in the south.<br>**b.** It can be found as an exotic in Europe's atlantic and Mediterranean coasts (the Netherlands, France, and Spain); as well as, North America's pacific shores (Mexico) | [30-32] |
| *U. conglobata* | **a.** It's 10 centimetres long, with rounded edges that are 9–16 micrometres in long and 7–12 micrometres in wide, and its base is made up of two 50 centimetres in long lines of cells. | **a.** Found on Korea's Jeju Island, China's Qingdao Province, and Japan's Yokohama. | [33,34] |
| *U. prolifera* | **b.** Thallus: bright green or dark green, densely branching or simple, tubular, flattened, deformed, with very thin, twisted stem gradually spreading toward apex.<br>**c.** Blades are 10–50 cm long (sometimes 2–4 m) and 0.1–1.5 cm broad. | **b.** Found in Europe, the White Sea, the Atlantic Ocean, the Indian Ocean, and the Pacific Ocean.<br>**c.** The continent of Asia (Korea, Japan, China, Taiwan, and Russia: Bering and Okhotsk seas, the Sea of Japan). | [35] |

| | | | |
|---|---|---|---|
| *U. ohnoi* | **a.** light-green in colour<br>**b.** Its tiny serrations are 30–55 micrometres in thick in the upper and middle regions and 80–90 micrometres in thick on the bottom, its thallus is 20–30 centimetres in high and expanded, and its thallus is 20–30 centimetres in high and expanded. | **a.** Endemic to western and southern of Japan | [36] |
| *U. compressa* | **a.** Thallus light green to dark green, 1–5 cm high, gregarious, forming thick tufts or turfs, tubular, and compressed | **a.** Widespread world wide including in the North and South Atlantic and in the Pacific Ocean and can be found in the intertidal zone in sheltered to open coastal sites, in shallow water, tide pools, and also on rock pools and sand. | [37] |

**Table 2:** Proximal composition of different Ulva spp. reported in different studies.

| ***Ulva spp.*** | **Inclusion level %** | **Dry matter %** | **Moisture %** | **Protein content %** | **Lipid content %** | **Ash %** | **Fiber %** | **NFE %** | **energy** | **References** |
|---|---|---|---|---|---|---|---|---|---|---|
| *U. lactuca* | | | | 19.3 ± 0.2 % | 3.4 ± 0.1 % | 44.1 ± 0.1% | 25.81 ± 0.2% | | | [38] |
| *U. clathrata* | | | | 20.1 ± 0.1 % | 2.2 ± 0.1 % | 27.5 ± 0.2 % | 40.6 ± 3.0 % | | | [38] |
| *U. clathrata* | 20g/kg feed | | 930.1g/kg dw | 990 g/kg dw | 960.3g/kg dw | 942 g/kg dw | 962g/kg dw | | | [39] |
| *U. lactuca* | | | 11.2 ± 0.3 % | 32.2 ± 2.8 % | 1.9 ± 0.2 % | 24.4 ± 2.1 % | 15.0 ± 1.3 % | | | [42] |
| *U. lactuca* | 25, 50, 75, and 100 % | | | 14% | 1.50% | 24.30% | 5.20% | 13.60% | | [19] |
| *U. lactuca* | 10%, 20%, and 30% | | 10.40% | 10.40% | 6% | 24.20% | | | | [41] |
| *U. lactuca* | 10% | 96.30% | | 17.40% | 2.50% | 32.80% | 5.40% | 41.70% | | [42] |
| *Ulva sp.* | 10%, 20%, and 30% | | 6.90% | 13.20% | 2.50% | 4.80% | | | | [43] |
| *U. fasciata* | 0, 50 and 100 % | 23.90% | 76.10% | 27% | 0.50% | 20% | 9.80% | 42.50% | 3.70% | [44] |
| *Ulva spp.* | 2.5 and 7.5 % | 85.70% | | 23.20% | 1.50% | 34.80% | | | 12.10% | [45] |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *U. rigida* | 5% and replaced soybean meal 10% | | 11.90% | 29.50% | 1.40% | | | | | [46] |
| *U. rigida* | 0%, 10%, 20%, and 30% of Soybean meal | 82% | | 16.40% | 2% | 4.50% | 12.30% | 46.80% | | [47] |
| *Ulva spp.* | 5% | | 3.60% | 8.90% | 1.40% | 32.80% | 5.70% | 51.10% | | [48] |
| *U. rigida* | 0% and 5% replaced corn starch | | | 9.90% | 0.10% | 26.60% | | | | [49] |
| *U. rigida* | 5% and 10% | | | 16% | 0.50% | 24.00% | | 59.50% | | [50] |
| *U. lactuca* | 0, 5, 10, and 15 % | | 3.60% | 17.40% | 2.50% | 32.80% | 5.70% | 41.40% | | [51] |
| *U. lactuca* | 0, 2.5 and 5% | 80.90% | | 20.30% | 3.20% | 17.90% | 9.87% | 48.30% | | [52] |
| *U. clathrata* | >10% | | 90% | 2.20% | 0.20% | 4.50% | 0.60% | 3.50% | | [53] |
| *U. clathrata* | 33g $kg^{-1}$ | | 14.2gkg | 23.4gkg | 1 g/kg | 16 g/kg | 4.6gkg | 40.8gkg | 12 kJ $g^{-1}$ energy | [54] |
| Low N-*U. lactuca* | 20% | 13.40% | | 1.60% | 0.10% | 4.40% | | 3.60% | 1.60% | [55] |
| high N-*U. lactuca* | 20% | 16.80% | | 7.30% | 0.10% | 2.90% | | 4% | 2.60% | [55] |
| *U. fasciata* | 10% | | 7.10% | 8.70% | 1.90% | 17.70% | 3% | 61.40% | | [56] |

**Ulva chemical composition:**

As shown in Table 2 [38-56]. Ulva has an intriguing chemical composition that enables them to be used in the production of functional or health-promoting foods appealing. Crude protein, ash, crude fat, and fiber contents in Ulva meals range from 1.6 to 32% percent, 2.9 to 44 percent, 0.1 to 6 percent, and 0.6 to 40%, respectively.

The chemical composition of Ulva varies based on species, geographical distribution, physiological status, and other factors, such as the primary environmental determinants like salinity, water temperature, nutrients, light, and minerals availability [57]. In general, macro-algae is rich in vitamins, minerals, and non-starch polysaccharides [58].

Because of their mineral richness or the useful qualities of their polysaccharides, seaweed is typically employed in human or animal food. However, the importance of seaweed proteins for nutrition is rarely emphasized [59]. Species and seasons have an impact on the protein content of seaweed. Ulva's amino acid profile has been the subject of in-depth research. Aspartic and glutamic acids make up a sizable portion of the amino acid composition in many organisms. Between 26 to 32 percent of the total amino acids are found in green seaweed [60]. Red and brown seaweeds are additionally higher potential sources of DHA and EPA than green seaweeds due to changes in colour and fatty acid contents [60].

**Cultivation of *Ulva spp.*:**

Ulva cultivation and harvesting are quite simple and, due to its size compared to micro-algae, a substantial amount of biomass can be collected [61]. Three basic sources of macro-algal biomass for downstream processing are used:

a) harvesting seaweed directly from the sea,

b) collecting dead macro-algae from the coast, and

c) cultivating selected seaweed species [62]. Direct cultivation of Ulva is possible in open water. Onshore, offshore, and integrated seaweed cultivation are some of the different techniques for growing seaweed [62].

The macro-algal growth life cycle depends on different factors, such as nutrients availability and photosynthesis; besides, various environmental parameters such as water salinity, temperature, current, and depth [63].

**Harvesting of Ulva:**

There is a lengthy history of macro-algae harvesting in many coastal regions [64]. The two types techniques of harvesting are:

a) Mechanical (moving boat, dredge, and mesh conveyor)

b) Manual (hand)

Harvesting takes place three times a year on average in natural water bodies. However, intense mechanical harvesting has negative repercussions for marine ecosystems and significantly reduces macro-algae development [65]. Moreover, harvesting macro-algae blooms has not proven to be economically viable in the world [66]. However, in some other areas, the elimination of proliferating macro-algae development as a means of eliminating surplus nutrients from the environment is advantageous.

**Integrating Ulva with Mariculture:**

Mariculture refers to the practice of raising aquatic organisms in regulated or semi-controlled environments [67]. *Ulva spp*., a type of seaweed, has a variety of applications and is gaining popularity as a new experimental system for biological research, as well as a component of integrated aquaculture systems [68].

## Different Methods of *Ulva spp*. Application

As illustrated in Figure 2, Ulva can be added through different methods.

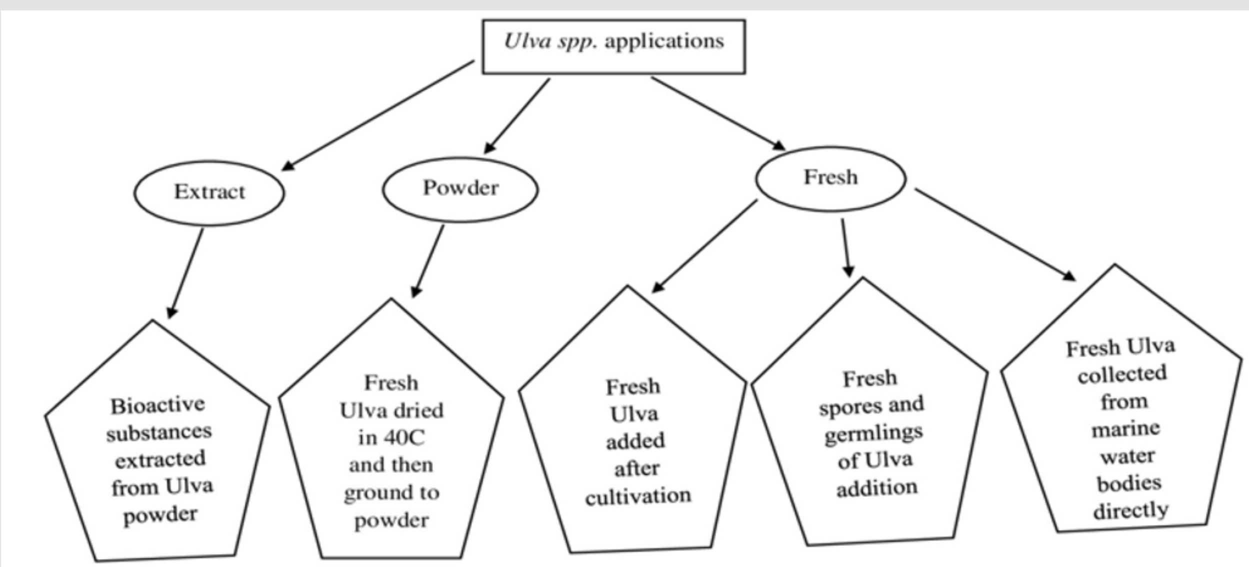


**Figure 2:** Different methods of Ulva spp. Application.

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**Table 3:** Studies on the use of Ulva as a fresh in crustaceans feed.

| *Ulva spp.* | Kind of Addition | *Crustacean spp.* | Initial Weight/ Stock Denisty | References |
|---|---|---|---|---|
| *U. clathrata* | fresh | Pacific white shrimp (*Litopenaeus vannamei*) | 25 adults.$m^{-2}$ | [21] |
| *U. lactuca* | fresh | Pacific white shrimp (*Litopenaeus vannamei*) | 4.54±0.09g | [69] |
| *U. clathrata* | fresh | Pacific white shrimp (*Litopenaeus vannamei*) | 3±0.01g | [70] |
| *U. lactuca* | fresh | Pacific white shrimp (*Litopenaeus vannamei*) | 1.17 ± 0.12 g | [40] |
| *U. clathrata* | fresh | Brown shrimp (Farfantepenaeus californiensis) | 60 ± 10 mg (pl 30) | [71] |
| *U. lactuca* | fresh | Pacific white shrimp (*Litopenaeus vannamei*) | 3.7±0.2 g | [19] |
| *U. lactuca* | fresh | white shrimp (*Litopenaeus vannamei*) | 0.59 ± 0.09 g | [72] |
| *U. clathrata* | fresh | Pacific white shrimp (*Litopenaeus vannamei*) | 188 ± 28 mg | [73] |
| *U. clathrata* | fresh | brown shrimp, (*Farfantepenaeus californiensis*) | 0.12 g | [74] |
| *U. clathrata* | fresh | Pacific white shrimp (*Litopenaeus vannamei*) | 3.5 g | [2] |

**Table 4:** Studies on the use of Ulva as a fresh in molluscs feed.

| *Ulva spp.* | Kind of Addition | *Molluscs spp.* | Initial Length/ Stock Denisty | References |
|---|---|---|---|---|
| *Ulva sp.* | fresh Spores and germlings | Juvenile greenlig abalone (*Haliotis Laevigata*) | 5.9±0.6 mm in initial shell length/2,000 juveniles per $m^2$ | [75] |
| *U. lactuca* | fresh | juvenile European Abalon (*Haliotis tuberculata*) | 0.8-2.1 g | [55] |
| *U. rigida* | fresh | Juvenile abalone (*Halitis discus hannai*) | | [76] |
| *Ulva sp.* | fresh | South African abalone (*Haliotis midae*) | | [77] |
| *U. lactuca* | fresh | Abalone (*Haliotis iris*) | wet weight (0.01 g) | [78] |
| *Ulva spp.* | fresh | *Blacklip abalone larvae (Haliotis rubra)* | Larvae were released at two densities (50,000 and 100,000 larvae per 1000 l tank | [79] |
| *U. rigida* | fresh | Gray juveniles abalone (Haliotis roei) | 32 mm in shell length | [20] |
| *Ulva spp.* | Fresh Spores and germlings | Greenlip abalone juvenile (*Haliotis laevigata*) | ≥3.5 mm shell length | [80] |

**Ulva Addition as Fresh:**

As illustrated in Table 3 [69-74] and Table 4 [75-80], different Ulva species can be added fresh to the diets of crustaceans and molluscs, but they are never used in fish feed. There are various techniques to add fresh Ulva.

A. Fresh Ulva Addition from Marine Water Bodies Directly:

Ulva is gathered from various aquatic water bodies and cleaned with sterilized marine water to get rid of epiphytes, after that set in laboratory conditions, in 5-L marine water containers, at 25°C, with a photoperiod of 12 h:12 h light: dark, with fluorescent light tubes of 75 W; besides, using Provasoli medium at a constant concentration of 0.5 ppm of nitrogen in water for two weeks before the feeding trial [72].

B. Fresh Ulva addition after Culturing:

Ulva is enriched with nitrogen and given 5-6 volume exchanges per day of filtered water pumped from the sea at a depth of 20 meters (41 ppt, 19.5-25.3°C). Ulva thalli that have been separated from the sea are grown in 1m2, 600-L tanks that are vigorously agitated [81,82]. A concentrated solution of inorganic nutrients containing disodium phosphate and ammonium sulphate is added to the media (at fluxes determined by the experimental treatment). Every morning, the cultures receive the solution over a 4-hour period, which is regarded to be sufficient for *Ulva spp.* to fulfil its daily ammonia-N needs [83].

Ammonia-N is added at concentrations ranging from 0.5 g ("low-N" Ulva culture) to 10 g ("high-N" culture). These levels are chosen based on Neori, et al. [82], which stated that various N-fluxes would provide Ulva with noticeably altered tissue-nitrogen levels while maintaining sufficient production to permit harvesting for feeding. Several Ulva cultures, both low-N and high-N, are developed. Once a week, the algae are replenished at their original density after being centrifuged (at 500 rpm for 3 min) to remove surface water and weighed to estimate biomass production.

 

C. Fresh Spores and Germlings of Ulva Addition

To stimulate gametogenesis, *Ulva spp*. thalli are gathered from submerged limestone rocks and treated with a cold (4°C) treatment. the Ulva thalli are placed between wet newspaper and then refrigerated. After 7 days of cold treatment, each of the five 400 L tanks is loaded with a modified culture medium with 10 kg of the blotted wet weight of Ulva thalli [84], that lacked sodium meta silicate, PII metals, and vitamin stock solutions. Each tank carried three horizontally stacked baskets of 12, 30 x 60-centimeter PVC plates. Only light aeration is used in the tanks to decrease water movement and allow for optimum spore adhesion. Ulva thalli are withdrawn from the 5 tanks after six days, and the germlings seeded PVC plates are distributed into three 400 L tanks with three containers of 20 plates, all of which are positioned vertically. The modified medium is then used to grow the germlings for 5 weeks, with media replacements occurring every two weeks [80].

**Table 5:** Studies on the use of Ulva as a powder in fish feed.

| *Ulva spp.* | Kind of Addition | Fish spp. | Initial Weight | References |
|---|---|---|---|---|
| *U. fasciata* | powder | California yellowtail *(Seriola dorsalis)* | 7.93 ± 0.24 g | [85] |
| *U. pertusa* | powder | Black sea bream (Acanthopagrus schlegeli) | 24 g | [86] |
| *U. rigida and U. lactuca* | powder | Nile tilapia (*Oreochromis niloticus*) | 13 g | [87] |
| *Ulva sp.* | powder | Nile tilapia (*Oreochromis niloticus*) | | [88] |
| *Ulva* sp. | powder | Nile tilapia (*Oreochromis niloticus*) | 12.1 g | [89] |
| *U. lactuca* | powder | Gilthead seabream (*Sparus aurata*) | 20 gilthead sea bream of 10.5 ± 0.45 g and 20 gilthead sea bream of 7.1 ± 2.1 g in the LRU and HRU experiment respectively | [90] |
| *U rigida* | powder | European sea bass juveniles (*Dicentrarchus labrax*) | 4.7 g | [46] |
| *U. rigida* | powder | Nile tilapia (*Oreochromis niloticus*) | 21.37± 0.193 g | [47] |
| *Ulva spp.* | powder | Gray mullet (*Liza spp.*) | 0.094g | [91] |
| *Ulva spp.* | powder | Snakehead fry (*Channa striatus*) | 0.24 g | [48] |
| *U. lactuca* | powder | African catfish (*Clarias gariepinus*) | 9.59 ± 0.43 g | [41] |
| *U. rigida* | powder | Common Carp (*Cyprinus carpio*) | 3.1 g | [92] |
| *U. rigida* | powder | Rainbow trout (*Oncorhynchus mykis*) | 7 g | [50] |
| *Ulva spp.* | powder | European seabass (*Dicentrarchus labrax*) | 25.5±4.1 g | [93] |
| *U. pertusa* | powder | Red Sea Bream (Pagrus major) | 2.1 g | [94] |
| *U. rigida* | powder | Nile Tilapia (*Oreochromis niloticus*) | 4.5 g | [95] |
| *U. rigida* | powder | Nile tilapia (*Oreochromis niloticus*) | 10 g | [48] |
| *Ulva spp.* | powder | Japanese Flounder (Paralihthys olivaceus) | 5 g | [96] |
| *U. lactuca* | powder | European seabass fry (*Dicentrarchus labrax L.*) | 0.23±0.02 g | [97] |

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|---|---|---|---|---|
| *U. lactuca* | powder | Gilthead seabream (*Sparus aurata*) | 0.1+0.05 g | [97] |
| *U. lactuca* | powder | striped mullet (*Mugil cephalus*) | 6.4±0.5g | [98] |
| *Ulva sp.* | powder | Red Tilapia (*Oreochromis Sp.*) | 1.15 g | [99] |
| *Ulva sp.* | powder | *Labeo rohita* fry | 0.62 ± 0.04 g | [100] |
| *U. lactuca* | powder | Rainbow Trout (*Oncorhynchus mykiss*) | 32.96±0.29 g | [116] |
| *U. lactuca* | powder | Nile tilapia fingerlings (*Oreochromis niloticus*) | 18.47 ± 1.25 g | [52] |
| *U. fasciata* | powder | Rabbitfish fry (*Siganus rivulatus*) | 0.18 g | [44] |
| *Ulva sp* | powder | Snakehead fry (*Channa striatus*) | 0.24 g | [48] |
| *U. pertusa* | powder | Black sea bream (*Acanthopagrus Schlegeli*) | | [100] |
| *U. conglobata* | powder | Nibbler fish (*Girella punctata*) | | [101] |
| *U. fasciata* | powder | Indian major carp (*Labeo rohita*) | 1.99 ± 0.10 to 2.05 ± 0.13 g | [57] |
| *U. pertusa* | powder | Black sea bream (*Sparus macrocephalus*) | 24g | [102] |

**Table 6:** Studies on the use of Ulva as a powder in crustaceans feed.

| *Ulva spp.* | Kind of Addition | *cructaceans spp.* | Initial Weight | References |
|---|---|---|---|---|
| *U. clathrata* | powder | Pacific white shrimp (*Litopenaeus vanname*i) | 600 females (53 ± 8 g) and 600 males (36 ± 7 g) | [39] |
| *U. clathrata* | powder | Pacific white shrimp (*Litopenaeus vannamei)* | 1.6 g | [54] |
| U. lactuca | powder | Black tiger shrimp juvenile (*Penaeus monodon*) | | [103] |
| *U. lactuca* | powder | Pacific white shrimp *(Litopenaeus vannamei)* | 0.02 g | [104] |
| *U. lactuca and U. clathrata* | powder | Pacific white shrimp (*Litopenaeus vannamei*) | *U. lactuca* 0.3g *and U. clathrate 1.59 g* | [38] |
| *U. lactuca* | powder | Pacific white shrimp(*Litopenaeus vannamei*) | 0.59 ± 0.09 g | [72] |
| *U. clathrata* | powder | Pacific white shrimp (*Litopenaeus vannamei*) | adult | [21] |

**Ulva Addition as Powder**

As illustrated in Table 5 [85-102] & Table 6[103,104], different *Ulva spp*. can be added as a powder in crustaceans and fish feed but not to momollusc'seed.

A. Preparation of Ulva as Powder

*Ulva spp*., a green macro-alga, is collected fresh from the near-shore waters of diverse coastal water bodies or gathered after cultivation. Ulva samples are completely rinsed with salt water, dried in a 40°C oven for 48 hours, ground to powder for proximate analysis, and stored dry until utilized in the diet formulation [49].

B. Ulva Addition as Extract

As illustrated in Table 7 [105-107] & Table 8 [108-112], different *Ulva spp*. can be added as extract either through injection or on feed for fish and crustaceans but not molluscs.

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**Table 7:** Studies on the use of Ulva as an extract in fish feed.

| *Ulva spp.* | Fish | Extraction Method | Solvent | Analytical Methods | Main Bioactive Compounds | References |
|---|---|---|---|---|---|---|
| *U. fasciata* | Nile tilapia (*Oreochromis niloticus*) | Maceration | Methanol | Gas chromatography mass spectrophotometer (GC-MS) | hexadecanoic acid | [18] |
| *U. rigida* | Grey mullet (Mugil cephalus) | Maceration | water | | polysaccharides | [105] |
| *U. rigida* | Turbot (Psetta maxima L.) | Maceration | | | Uronic acids, rhamnose, and xylose with smaller amounts of other sugars. | [106] |
| *U. clathrata* | Nile tilapia (Oreochromis niloticus) | Maceration | | Fourier transform infrared spectrometry (FTIR) | The sulfated α-rhamnose and the methyl protons of the α-L-rhamnosyl residues. | [107] |

**Table 8:** Studies on the use of Ulva as an extract in crustaceans feed.

| *Ulva spp.* | Crustaceans | Extraction Method | Solvent | Analytical Methods | Main Bioactive Compounds | References |
|---|---|---|---|---|---|---|
| *U. prolifera* | Pacific white shrimp (*Litopenaeus vannamei*) | Maceration | cold water extract (PC), hot water extract (PH) | | polysaccharides | [108] |
| *U. clathrata* | Pacific white shrimp (*Litopenaeus vannamei*) | Maceration | chloroform: methanol (2:1 v/v) | CG–FID | palmitic acid (35.5%) | [109] |
| *U. rigida* | Pacific white Shrimp (*Litopenaeus vannamei*) | Maceration | water | | polysaccharides extracts | [110] |
| *U. fasciata* | Shrimp (*Penaeus monodon*) | Soxhlet extractor | methanol | | | [111,112] |

Bioactive substances classification into various classes is currently unclear and is dependent on the classification's aim. Because of the ease of characterizing biosynthetic pathways, biosynthetic classifications will not be able to match the scope of pharmacological categorization. According to Croteau, et al. [113], plant bioactive chemicals are divided into three categories:

a. terpenes and terpenoids (about 25,000 kinds),

b. alkaloids (approximately 12,000 sorts), and

c. phenolic compounds (approximately 8000 types). Furthermore, many bioactive compounds are divided into various families, each with unique structural characteristics based on how they are generated in nature (biosynthesis).

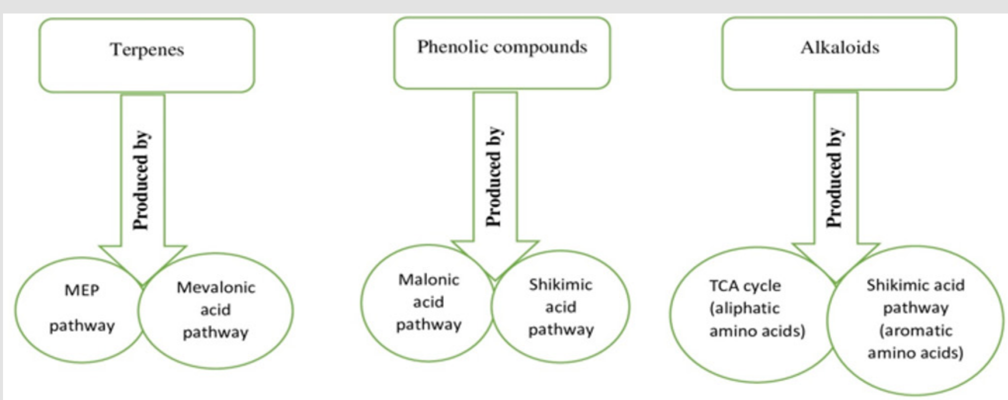


**Figure 3:** A simplified view for production of three major groups of plant bioactive compounds.

As shown in Figure 3, secondary metabolites, often known as bioactive compounds, are produced in four ways: The four pathways include the mevalonic acid pathway, the non-mevalonate (MEP) pathway, the shikimic acid pathway, and the malonic acid pathway [114]. Either aliphatic amino acids or aromatic amino acids (from the shikimic acid pathway) can create alkaloids. Malonic and shikimic acids can create phenolic chemicals. Terpenes can be made by two different processes: mevalonic acid and MEP.

Furthermore, as illustrated in Figure 4, Ulva bioactive substances can be extracted through many conventional and non-conventional extraction techniques.

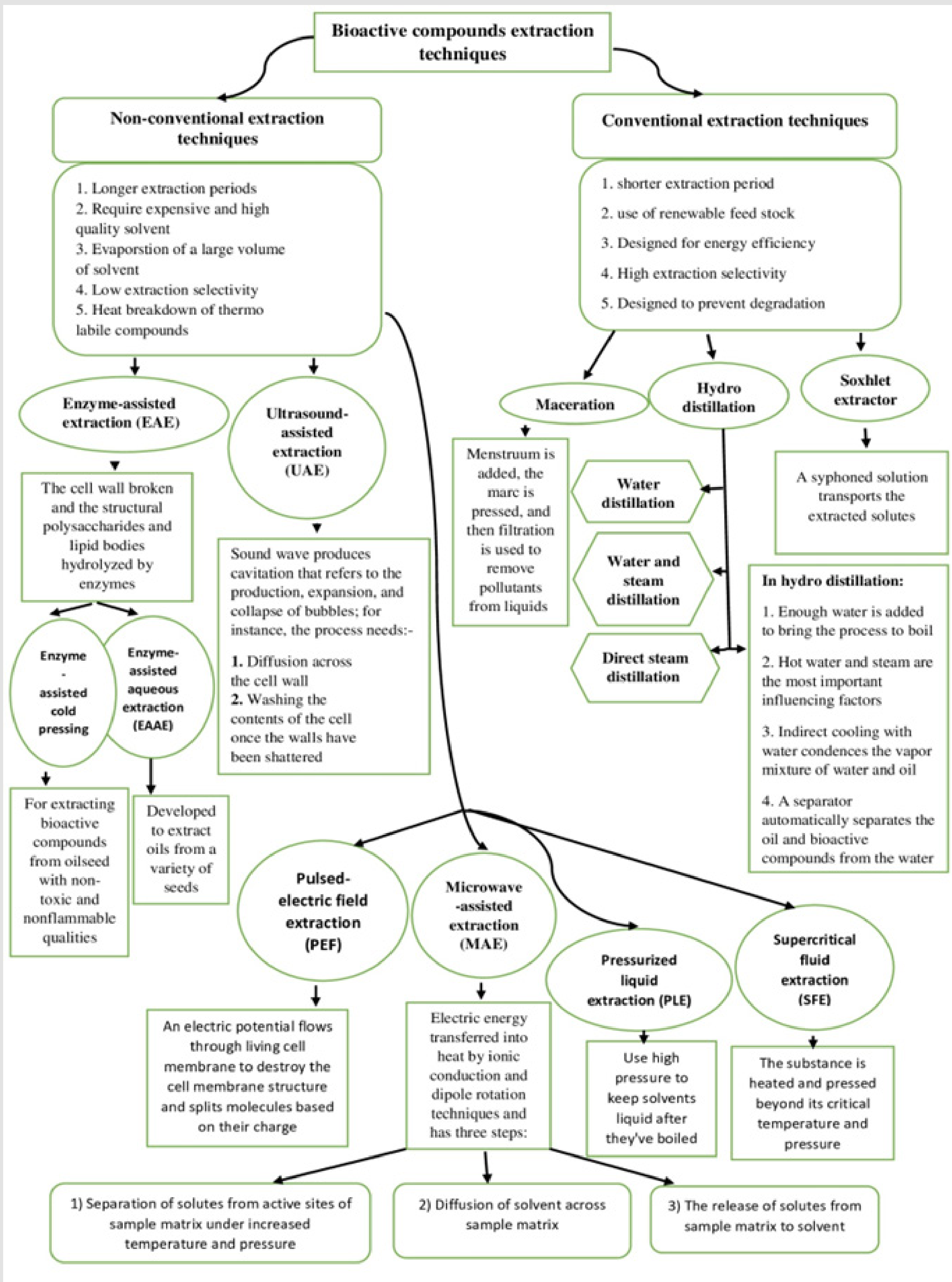


**Figure 4:** Different extraction techniques of bioactive substances.

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**Table 9:** Effects of different Ulva types dietary inclusion as powder, extract, and fresh on growth performance of fish, crustaceans, and molluscs.

| *Ulva spp.* | *Fish/crustacean/Mollusc spp.* | Initial Body Weight/ Length | Type of Addition/ Solution Type | Dose | Duration | Effect on Growth Performance | References |
|---|---|---|---|---|---|---|---|
| *U. clathrat*a | Nile tilapia (*Oreochromis niloticus*) | 25 ± 3 g | Extract | Ulvan (0.1, 0.5, and 1 % $kg^{-1}$ feed). | 60 days | No effect on growth | [107] |
| *U. rig*ida | Grey mullet (*Mugil cephalus*) | 15 ± 0.1 g | Extract/ water | 5, 10, and 15 mg $kg^{-1}$ | 8 weeks | 10 mg $kg^{-1}$ ↑ growth | [115] |
| *U. fasciata* | Nile tilapia (*Oreochromis niloticus*) | 1.32±0.12g | Methyl extract | 50, 100, and 150 $mgkg^{-1}$ | 90 days | No effects | [116] |
| *U. pertusa* | Shrimp (*Penaeus Japonicus*) | | extract | | 20 days | ↑ Growth rate. | [117] |
| *U. prolifera* | Pacific white shrimp (*Litopenaeus vannamei*) | 4.08±0.03 g | extract | 0.78, 1.33, and 31.57 g | 21 days | ↑ Growth | [109] |
| *U. rigida* | Pacific white shrimp (*Litopenaeus vanname*) | 1.0 g | extract | 0.5, 1 and 1.5 g/kg of WPU | 8 weeks | 1.5 g/kg ↑ Growth | [111] |
| *U. clathrata* | Pacific white shrimp (*Litopenaeus vanname*) | 1.6 g | powder | 33g $kg^{-1}$ | 28 day | ↑ Final body weight | [54] |
| *U. lactuca* | Pacific white shrimp (*Litopenaeus vanname*) | 0.59 ± 0.09 g | powder | 0, 1, 2, and 3% *U. lactuca* meal | 28 days | 3 UL diet ↑ Growth | [72] |
| U. lactuca | Black tiger shrimp juvenile (*Penaeus monodon*) | | powder | 15% and 30% replacement of soybean meal | 90 days | 30% ↑ Growth | [104] |
| *U. lactuca* | Pacific white shrimp (*Litopenaeus vannamei*) | 0.02 g | Powder | 4% (w/w) to replace wheat flour | 2weeks | ↑ Growth | [105] |
| *U. fasciata* | Rabbitfish fry (*Siganus rivulatus*) | 0.18 g | fresh | 50 and 100% | 70 days | 50% ↑ Growth rate | [43] |
| *U. lactuca* | Pacific white shrimp (*Litopenaeus vannamei*) | 3.7±0.2 g | fresh | 25, 50, 75, and 100 % | 28 days | 50 % ↑ Growth | [19] |
| *U. clathrata* | Brown shrimp (*Farfantepenaeus californiensis*) | 60 ± 10 mg (pl 30) | fresh | 60% of the pond surface covered with *Ulva* during the entire culture cycle of the shrimp | 18weeks | ↑ SGR | [74] |
| *U. clathrata* | Pacific white shrimp (*Litopenaeus vannamei*) | 188 ± 28 mg | fresh | 25%,50%, 75%, and 100% | 28 days | 25% ↑ Growth rate | [73] |
| *U. clathrata* | Brown shrimp (*Farfantepenaeus californiensis*) | 0.12 g | fresh | 30 g wet weight per tank | 8-weeks | ↑ Survival and growth rate | [74] |

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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *U. clathrata* | Pacific white shrimp (*Litopenaeus vannamei*) | 3.5 g | fresh | 10%, 45%, and 100% | 45 days | 10 and 45% ↑ Growth rate | [2] |
| *Ulva sp.* | Juvenile greenlip abalone (*Haliotis Laevigata*) | 5.9±0.6 mm in initial shell length 2,000 juveniles per m2 | Spores and germlings | 2% of total biomass weight | 17-week | ↑ Growth rate | [75] |
| *U. lactuca* | Juvenile European Abalone (*Haliotis tuberculate*) | 0.8-2.1 g | fresh | 20% | 16-week | ↑ Growth rate | [55] |
| *U. rigida* | Japanese abalone (*Haliotis discus hannai*) | | fresh | | | ↑ SGR | [76] |
| *Ulva sp.* | South African abalone (*Haliotis midae*) | | fresh | | 6-month | ↑ Growth rates. | [77] |
| *U. rigida* | Gray juveniles abalone (Haliotis roei) | 32 mm in shell length | fresh | | | ↑ Growth rate | [20] |
| *Ulva sp.* | Juvenile abalone (*Haliotis laevigata*) | ≥3.5 mm shell length | Spores and germlings | At first month consumption of germlings peaked at 500 germling blades.abalone$^{-1}$.day$^{-1}$ but by week 6 the consumption had decreased to 100 germling blades.abalone$^{-1}$.day$^{-1}$ | 14-week | ↓ Growth rates | [80] |
| *U. fasciata* | California yellowtail (Seriola dorsalis) | 7.93 ± 0.24 g | powder | 5, 10, and 20 g kg-1 | 48 days | No effects | [85] |
| A 50/50 % mixture of *Ulva* spp. (*U. rigida* and *U. lactuca*) | Nile tilapia (*Oreochromis niloticus*) | 13 g | powder | replaced fishmeal 10 % (U10), 15 % (U15), and 20 % (U20) of *Ulva spp.* | 63 days | No effects | [87] |
| *Ulva sp.* | Nile tilapia (*Oreochromis niloticus*) | | powder | | | ↑ Growth rate | [88] |
| *Ulva* sp. | Nile tilapia (*Oreochromis niloticus*) | 12.1 g, | powder | *Ulva* sp. from IMTA replaced fishmeal up to 10% | 84-day | No effect | [89] |
| *Ulva sp.* | Grey mullet (Liza ramada) | 0.094g | powder | | | ↑ SGR | [91] |
| *U. rigida* | Common Carp (Cyprinus carpio) | 3.1 g | powder | 0, 5, 10, 15 and 20% replaced wheat meal starch | 112-day | **1.** 5% ↑ Growth **2.** 20% ↓ Growth | [92] |
| *Ulva spp.* | European seabass (*Dicentrarchus labrax*) | 25.5±4.1 g | powder | 2.5 and 7.5 % | 49 days | No effect | [93] |
| *U. rigida* | European sea bass juveniles (*Dicentrarchus labrax*) | 4.7 g | powder | 5% and 10% replaced soybean meal | 10 weeks | 10% ↑ Growth performance | [46] |
| *U. lactuca* | African catfish (Clarias gariepinus) | 9.59 ± 0.43 g | powder | 10%, 20% and 30% | 10 weeks | 20% and 30% ↓ Growth | [41] |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| *Ulva spp.* | Snakehead fry (Channa striatus) | 0.24 g | powder | 5% | 8 weeks | ↑ Growth rate | [48] |
| *U. pertusa* | Red Sea Bream (Pagrus major) | 2.1 g | powder | 5% replaced fish meal | 41 days | ↑ Growth rate | [49] |
| *U. rigida* | Rainbow trout (Oncorhyn-chus mykis) | 7 g | powder | 5% and 10% | 12 weeks | 5% ↑ Growth rate | [50] |
| *U. rigida* | Nile Tilapia (*Oreochromis niloticus*) | 4.5 g | powder | 5%, 10%, or 15% replaced fish meal | 12-week feeding trial | 5% ↑Growth | [95] |
| *U. rigida* | Nile tilapia (*Oreochromis niloticus*) | 10 g | powder | 5% replaced corn starch | 16 weeks | ↑ SGR | [49] |
| *Ulva spp.* | Japanese Flounder (*Parali-hthys olivaceus*) | 5 grams | powder | 2 %, 4 %, and 8 % | 120 days | 1. 2% ↑ SGR 2. More than 4% ↓ Growth. | [96] |
| *U. lactuca* | European seabass fry (*Di-centrarchus labrax L.*) | 0.23±0.02 g | powder | 5, 10, and 15 % | 8 weeks | 5% ↑ Growth | [51] |
| *U. lactuca* | Giltheah seabream (*Sparus aurata*) | 0.1+0.05 g | powder | 0, 5, 10 & 15 % | 8week | 5% ↑ Growth | [97] |
| *U. lactuca* | Striped mullet (*Mugil cephalus*) | 6.4±0.5g | powder | 10, 15, 20 and 25% | 15 weeks | 20% ↑ Growth | [98] |
| *U. rigid*a | Nile tilapia (*Oreochromis niloticus*) | | powder | 5% | | ↑ Growth | [18] |
| *Ulva sp.* | Red Tilapia (Oreochromis Sp.) | 1.15 g | powder | 5, 10, 15, 20 and 25% replaced wheat flour and wheat bran up to 25% (fish meal was increased) | 9 weeks | 1. 5% and 10% ↑ Growth 2. 15% and 20% had no effect | [99] |
| *Ulva sp.* | Indian major Carp (Labeo rohita) | 0.62 ± 0.04 g | powder | 10%, 20%, and 30% | 45days | 10% ↑ SGR | [43] |
| *U. lactuca* | Rainbow Trout (Oncorhyn-chus mykiss) | 0.62 ± 0.04 g | powder | 10% replaced wheat meal | 60 days | 10% ↓ Growth | [118] |
| *U. lactuca* | Nile tilapia fingerlings (*Oreochromis niloticus*) | 18.47 ± 1.25 gm | powder | 2.5 and 5% | 12 weeks | ↑ Growth rates | [52] |
| *U. fasciata* | Rabbitfish fry (Siganus rivulatus) | 0.18 g | powder | 50 and 100 % | 70 days | ↑ Growth rate | [44] |
| *U. conglo-bata* | Nibbler (Girella punctata) | | powder | 5.00% | | ↑ Growth rate | [101] |
| *U. pertusa* | Black sea bream (*Acantho-pagrus schlegeli*) | | powder | 2.5,5.0,10.0, and 15.0% replaced fish meal | | 15 % ↓ Growth | [102] |
| *Ulva sp.* | Snakehead fry (Channa striatus) | 0.24 g | powder | 5% | 8 weeks | ↑ Growth rate and | [48] |
| *U. fasciata* | Indian major Carp (Labeo rohita) | 1.99 ± 0.10 to 2.05 ± 0.13 g | powder | 10% | 120 days | ↑ Growth | [56] |
| *U. pertusa* | Black sea bream (Acantho-pagrus schlegeli) | 24g | powder | 10% | 143 days | No effect | [101] |

## Effects of different *Ulva spp*. addition on different performance parameters

**Growth Performance:**

As shown in Table 9 [115-118], adding Ulva meal as a powder generally boosted growth at low levels but had the lowest growth performance at high levels, depending on the specific Ulva species, fish species, and addition method. Diler, et al. [92] discovered that fish fed a diet supplemented with 0, 5, 10, 15, and 20 percent *U. rigida* that replaced wheat meal starch showed the best growth by 5 percent meal inclusion, while fish fed a diet supplemented with 20 percent Ulva meal showed the worst growth performance. The growth of European seabass fry (*Dicentrarchus labrax L.*) was also positively impacted by the addition of 5% *U. lactuca* powder to the meal [51]. When compared to a control diet, the growth rate of Nile tilapia fingerlings (*Oreochromis niloticus*) (18.47±1.25 gm) fed meals containing 2.5 and 5% of *U. lactuca* powder dramatically increased by 53 and 68% respectively [52]. Additionally, Abdel Warith, et al. [41] reported that African catfish (*Clarias gariepinus*) fed diets containing 20 or 30 percent *U. lactuca* meal had the worst growth performance and feed utilization compared to control diets. These adverse effects were attributed to the experimental diet's low protein content, high ash content, and high level of soluble fiber [41]. In contrast, Abdel-Aziz and Ragab discovered that increasing the content of *U. fasciata* powder in the feeding of Rabbit fish fry (*Siganus rivulatus*) by 50% or 100% increased growth rate [48]. Nile tilapia (*Oreochromis niloticus*) growth was accelerated by up to 10% Ulva sp. in the fish feed [88]. *Ulva spp*. supplementation increased final body weight, weight gain, and specific growth rate in grey mullet (*Liza ramada*) considerably with an increase in *Ulva spp*. level up to 28% in the fish diet [91]. On the other hand, Legarda, et al. [85] found that increasing the amount of *U. fasciata* powder (5, 10, and 20 g kg-1) had no impact on the somatic characteristics and growth performance of (*Seriola dorsalis*). The growth parameters of European seabass (*Dicentrarchus labrax*) fed a diet supplemented with 2.5 and 7.5 percent Ulva meal were unaffected [93]. The discrepancies in results between studies may be due to different *Ulva spp*. and seasonal variables, as well as variations in the make-up of control diets.

As for the relationship of using Ulva extracts on growth performance parameters, when Nile tilapia (*Oreochromis niloticus*) were fed various quantities of *U. clathrate* [108] and *U. fasciata* [116]as extract, no significant effects were observed. But, Akbary and Aminikhoei [115] discovered that feeding grey mullet (*Mugil cephalus)* on 10 mg/kg of *U. rigida* extract resulted in a faster growth rate. Additionally, Yamasaki, et al. [117]; Ge et al. [109]; Akbary and Aminikhoei [115] reported that shrimp development improved because of the use of Ulva extract.

Regarding the impact of applying fresh Ulva on growth efficiency, the results showed that a 50 percent fresh *U. fasciata* replacement in rabbit fish fry (*Siganus rivulatus*) (0.18 g) diet resulted in the highest final weight, total weight gain, average daily gain, growth rate, and specific growth rate when compared to a 100 percent replacement [44]. The growth performance of pacific white shrimp (*Litopenaeus vannamei*) can be significantly improved by substituting up to 50% fresh *U. lactuca* for commercial feed [19]. Additionally, fresh *U. clathrate* improved the growth rate of pacific white shrimp (*Litopenaeus vannamei*) and brown shrimp (*Farfantepenaeus californiensis*) according to studies by Peña-Rodríguez, et al. [71]; Gamboa-Delgado, et al. [73]; Cruz-Suárez, et al. [2].

In terms of how employing Ulva spores and germlings affects growth performance, the inclusion of fresh Ulva lens and *Ulva spp*. germlings improved the growth performance of molluscs, according to Daume et al. [75]; Shpigel, et al. [55]; Corazani and Illanes [76]; Boarder [20]. On the other hand, the growth rate of abalone (*Haliotis midae* and *Haliotis laevigata*) was found to be lowered by fresh *Ulva spp*.' spores [77] and fresh *Ulva spp*.' Germlings [80], respectively. It is unclear whether the Ulva species' active ingredient is responsible for improving growth; instead, the advantage has been attributed to the plants' mineral and vitamin content, lipid mobilization, and improved absorption and digestion efficiency ratios.

**Table 10:** Effects of different Ulva types dietary inclusion on feed intake.

| *Ulva spp.* | **Fish/crustacean/** *Mollusc spp.* | **Initial Body weight/ Length** | **Type of Addition/ Solution Type** | **Dose** | **Duration** | **Effect on Feed Intake** | **References** |
|---|---|---|---|---|---|---|---|
| *U. lactuca* | Rainbow Trout (*Oncorhynchus mykiss*) | 32.96±0.29g | powder | 10% replaced wheat meal | 60 days | ↓ Feed intake | [118] |
| U. lactuca | Black tiger shrimp juvenile (*Penaeus monodon*) | | powder | 15% and 30% replacement of soybean meal | 90 days | No effect | [104] |

**Feed Intake:**

When Nile tilapia (*Oreachromis niloticus*)[18] and black tiger shrimp (*Penaeus monodon juvenile*) [104] were fed *U. fasciata* as extract and *U. lactuca* as powder respectively, found no influence on feed intake, as indicated in Table 10 [104,118]. In contrast, Yıldırım, et al. [118] reported that fish groups fed with the diet containing *U. lactuca* powder of Rainbow Trout (*Oncorhynchus mykiss*) (32.96 0.29g) had lower daily dry feed intake and total feed intake than those of the control group. Moreover, according to studies done on abalone and fish,

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dimethyl-beta-propionthein (DMTP) and dimethyl sulfonyl propionate (DMSP); besides, other compounds found in seaweed extracts, such as amino acids, phosphatidylcholine, digalactosyl-diacylglycerol, 6-sulfoquinovosyldiacylglycerol, and phosphatidylethanolamine, can act as attractants in pelleted diets [53, 119,120]. According to Cruz Suárez, et al. [53], these compounds act as attractants and enhance shrimp growth performance, feed efficiency, and feed intake.

**Table 11:** Effects of different Ulva types dietary inclusion on FCR.

| *Ulva spp.* | *Fish/crustacean/Mollusc spp.* | Initial Body Weight/ Length | Type of Addition/ Solution Type | Dose | Duration | Effect on FCR | References |
|---|---|---|---|---|---|---|---|
| *U. clathrata* | Brown shrimp (*Farfantepenaeus californiensis*) | 0.12 g | fresh | 30 g wet weight per tank | 8-week | ↓ FCR | [74] |
| *U. clathrata* | Pacific white shrimp (*Litopenaeus vannamei*) | 3.5 g | fresh | 10%, 45%, and 100% | 45-days | ↓ FCR | [2] |
| *U. lactuca* | Juvenile European Abalone (*Haliotis tuberculate*) | 0.8±2.1 g | fresh | 20% | 16-week | ↓ FCR | [55] |
| *U. lactuca* | Gilthead seabream (*Sparus aurata*) | 10.5 ± 0.45 g | powder | replaced 2.6% and 7.8% of the total feed biomass (and 6.8% and 23.5% of the fishmeal feed ingredient excluding fish oil) | 111-days | ↓ FCR | [90] |
| *U. fasciata* | Nile tilapia (*Oreochromis niloticus*) | 1.32±0.12g | Methyl extract | 50, 100, and 150 $mgkg^{-1}$ | 90 days | No effect | [116] |
| *U. rigida* | Nile tilapia (*Oreochromis niloticus*) | 21.37±0.193 g | powder | Soybean meal was replaced by 0%, 10%, 20% and 30% of UM | 7-days | ↓ FCR | [47] |
| *U. rigida* | Nile tilapia (*Oreochromis niloticus*) | 10 g | powder | 5% replaced corn starch | 16-week | ↓ FCR | [49] |
| *Ulva sp.* | Red Tilapia (*Oreochromis Sp.*) | 1.15 g | powder | 5, 10, 15, 20 and 25% replaced wheat flour and wheat bran up to 25% (fish meal was increased) | 9-weeks | 20% ↓ FCR | [99] |
| *Ulva sp.* | Indian major carp fry (Labeo rohita) | 0.62 ± 0.04 g | powder | 10% , 20%, and 30% | 45-days | ↓ FCR | [43] |
| *U. clathrata* | Pacific white shrimp (*Litopenaeus vannamei*) | 1.6 g | powder | 33g $kg^{-1}$ | 28-day | ↓ FCR | [54] |
| *U. lactuca* | Pacific white shrimp (*Litopenaeus vannamei*) | 0.59 ± 0.09 g | powder | 0, 1, 2, and 3% *U. lactuca* | 28-days | ↓ FCR | [72] |
| *U. lactuca* | Black tiger shrimp juvenile (*Penaeus monodon*) | | powder | 15% and 30% replacement of soybean meal | 90-days | No effect | [104] |

**Feed Conversion Ratio (FCR):**

As shown in Table 11, in a meal containing 20% fresh *U. clathrate*, FCR was decreased in young European Abalone (*Haliotis tuberculate*) [71]. Furthermore, in European abalone (*Haliotis tuberculate*) FCR was declined by *U. lactuca* [55]. Similarly, fresh *U. clathrata* decreased FCR in pacific white shrimp (*litopenaeus vannamei*) (3.5 g) [2]. According to Vyas [43], adding 10 percent Ulva meal powder to (*Labeo rohita*) fry (0.62 0.04 g) diet produced the lowest FCR. In addition, the impact of *Ulva spp*. as powder on FCR in crustaceans differed between studies. Cruz-Suárez, et al. [53] discovered that a concentration of 33 g kg-1 Ulva clathrate improved FCR in white shrimp (*Litopenaeus vannamei*) (1.6 g), but Elizondo-González et al. [72] found that the concentration of 3 percent *U. lactuca* improved FCR in pacific white shrimp (*Litopenaeus vannamei*) (0.59 0.09 g). In contrast, Serrano Jr, et al. [104] showed no effect on FCR in young black tiger shrimp after 90 days of feeding *U. lactuca* at a rate of 15% and 30% replacement of soybean meal (*Penaeus monodon*). Additionally, after a 90-day feeding trial using various quantities of *U. fasciata* extract, Nile tilapia (*Oreachromis niloticus*) fed the extract showed no influence on the FCR [18].

**Table 12:** Effects of different Ulva types dietary inclusion on PER.

| *Ulva spp.* | Fish/*crustacean/Mollusc spp.* | Initial Body Weight/Length | Type Of Addition/ Solution Type | Dose | Duration | Effect on PER | References |
|---|---|---|---|---|---|---|---|
| *U. rigida* | Grey mullet (Mugil cephalus) | 15 ± 0.1 g | water extract | 5, 10, and 15 mg $kg^{-1}$ | 8 weeks | ↑ PER | [104] |
| *U. clathrata* | Brown shrimp (Farfantepenaeus californiensis) | 0.12 g | fresh | 30 g wet weight per tank | 8-week | ↑ PER | [73] |
| *U. rigida* | Nile tilapia (*Oreochromis niloticus*) | 10g | powder | 5% replaced corn starch | 16 weeks | ↑ PER | [48] |
| *Ulva spp.* | Japanese Flounder (Paralihthys olivaceus) | 5 grams | powder | 2 %, 4 %, and 8 % | 120 days | 2% ↑ PER | [95] |
| *Ulva sp.* | Indian major carp fry (Labeo rohita) | 0.62 ± 0.04 g | powder | 10% , 20%, and 30% | 45-days | 10% ↑ PER | [115] |
| *U. lactuca* | Rainbow Trout (Oncorhynchus mykiss) | 32.96±0.29 g | powder | 10% replaced wheat meal | 60-days | ↓ PER | [116] |
| *U. pertusa* | Black sea bream (Sparus macrocephalus) | 24g | powder | 10% | 143-days | ↑ PER | [103] |
| *U. clathrata* | *Shrimp (Liptopeanaus vannamei)* | 1.6 g | powder | 33g $kg^{-1}$ | 28-days | ↑ PER | [53] |
| U. lactuca | Black tiger shrimp juvenile (*Penaeus monodon*) | | powder | 15% and 30% replacement of soybean meal | 90-days | No effect | [104] |

**Protein Efficiency Ratio (PER)**

PER in brown shrimp (*Farfantepenaeus californiensis*) (0.12 g) was shown to grow at a rate of 30 g wet weight per tank of fresh *U. clathrate* (Table 12) [74]. Furthermore, Vyas [43] found that integrating Ulva meal increased PER in (Labeo rohita) fry by the lowest concentration of 10 percent, and this result correlated with Nakagawa et al. [103]. Additionally, Japanese Flounder (*Paralihthys olivaceus*) (5 g) had the highest protein efficiency ratio when fed the lowest concentration of Ulva meal (2 percent) [96]. It has been reported that algae can boost the absorption and assimilation of dietary protein [121] or adjust lipid metabolism [103]. On the other hand, *U. lactuca* powder substituted for wheat meal showed reduced protein retention and protein efficiency ratio by 10% [118]. Furthermore, the PER of black tiger shrimp juveniles (*Penaeus monodon*) fed a *U. lactuca* diet was comparable throughout the experimental shrimp groups [104]. Similarly, no effect of *U. fasciata* methyl extract on PER in Nile tilapia (*Oreachromis niloticus*) (1.320.12 g) was found by Abo-Raya ,et al. [18].

**Immune and Ani-Oxidant Parameters**

As shown in Table 13 [122-124], we are not aware of any studies on the impact of fresh Ulva on the defense mechanisms and antioxidant activity of fish or crustaceans. On the other hand, few studies, including one by Peixoto, et al. [124], showed that European seabass (*Dicentrarchus labrix*) (25.5±4.1 g) fed Ulva powder at 2.5 and 7.5 percent had better innate immune and antioxidant responses. Additionally, Nile tilapia's immunological response was stimulated by 5% *U. rigida* powder [95]. Furthermore, most studies have shown that Ulva extract improves immunological and antioxidant activities in fish

[18,104,107,108,123,125,126]. Additionally, most research demonstrated that higher Ulva extract concentrations can have undesirable effects. 10 mg/kg of Ulva rigida water extract enhanced lysozyme, phagocytic, and respiratory burst activities in grey mullet (*Mugil cephalus*) than 5 and 15 mg/kg [115]. Like this, *U. fasciata* methyl extract enhanced lysozyme, phagocytic, and antioxidant activities in Nile tilapia (*Oreachromis niloticus*) (1.32±0.12 g) at a dose of 100 mg/kg when compared to 50 and 150 mg/kg [18]. Moreover, Ulva extract has also been shown to improve immunological and antioxidant activities in crustaceans [109,111,112,126] . The only study that we are aware of that used Ulva extract administered intraperitoneally in fish discovered that Senegalese sole's immune response was improved by 0.5 mg/fish *U. Ohnoi* extract [122]. According to all of this research, the presence of several bioactive substances such as 9-octadecenoic acid [127], hexadecanoic acid [128], phytol [129-131], 13-octadecenoic acid [132], arachidonic acid [133], and neophytadiene [130,131], could be connected to the greater lysozyme activity, phagocytic activity, and WBCs count.

**Table 13:** Effects of different Ulva types dietary inclusion on immune and ani-oxidative parameters.

| *Ulva spp.* | Fish/*Crustacean/ Mollusc Spp.* | Initial Body Weight/ Length | Type of Addition | Dose | Duration | Effect on immune and ani-oxidant parameters | References |
|---|---|---|---|---|---|---|---|
| *U. ohnoi* | Senegalese sole (*Solea senegalensi*) | 11.88 ± 2.08 gr | Extract intraperitoneal injection | (0.5 mg/fish) | | ↑ Immunity | [122] |
| *U. fasciata* | Shrimp (*Penaeus monodon*) | | extract | 1000 mg/kg | | ↑ Immunity | [112] |
| *U. prolifera* | *Shrimp (Litopenaeus vannamei)* | 4.08±0.03 g | extract | 0.78, 1.33, and 31.57 g | 21 days | ↑ Immunity | [109] |
| *U. rigida* | Shrimp (*Litopenaeus vannamei*) | 1.0 g | extract | 0.5, 1 and 1.5 g/kg of WPU | 8 weeks | 1.5 ↑ Immunity | [111] |
| *U. clathrata* | Nile tilapia (*Oreochromis niloticus*) | 25 ± 3 g | extract | ulvan (0.1, 0.5, and 1 % $kg^{-1}$ feed). | 60 days | ↑ Immunity | [108] |
| *U. fasciata* | Nile tilapia (*Oreochromis niloticus*) | 1.32±0.12g | methyl extract | 50, 100, and 150 $mgkg^{-1}$ | 90 days | 100 $mgkg^{-1}$ ↑ Immunity | [116] |
| *U. rigida* | Turbot (Psetta maxima L.) | 100 g | water extract | (0–50 µl $ml^{-1}$) | | ↑ Immunity | [123] |
| *U. rigida* | Turbot (Psetta maxima L.) | 100 g | extract | (0–100 µl $ml^{-1}$) | | ↑ Immunity | [107] |
| *U. rigida* | Grey mullet (*Mugil cephalus*) | 15 ± 0.1 g | water extract | 5, 10, and 15 mg $kg^{-1}$ | 8 weeks | 10 $mgkg^{-1}$ ↑ Immunity | [111] |
| *Ulva spp.* | European seabass (*Dicentrarchus labrax*) | 25.5±4.1 g | powder | 2.5 and 7.5 % | 49 days | ↑ Immunity | [121] |
| *U. rigida* | Nile tilapia (*Oreochromis niloticus*) | 4.5 g | powder | 5 to 10% | 12 weeks | 5% ↑ Immunity | [124] |

**Haemato-Biochemical Parameters:**

No investigations on fish and crustaceans by fresh Ulva demonstrated impacts on haemato-biochemical activities, as shown in Table 14, like immune and antioxidant responses. On the other hand, Seriola dorsalis given 20g/kg *U. fasciata* powder had a higher haematocrit (7.93±0.24 g) (Legarda et al., 2021)[51]. *U. fasciata* methyl extract improved haemato-biochemical parameters in Nile tilapia (Oreachromis niloticus) [18]. These results were suggested due to the presence of palmitic acid (17.25 %) and oleic acid (10.25 [18]. In contrast, research by Nakagawa [102] shown that U. pertusa at concentrations of 2.5, 5.0, 10.0, and 15.0 percent replaced fish meal, inhibited lipid buildup in intraperitoneal body fat in Black Sea bream (*Acanthopagrus schlegeli*). Additionally, Nile tilapia fingerlings (*Oreochromis niloticus*) fed *U. lactuca* meal powder with a concentration of 2.5 and 5 percent exhibited no significant influence on liver enzyme activity, serum total protein, globulin, and albumin [52].

**Table 14**: Effects of different Ulva types dietary inclusion on Haemato-biochemical parameters.

| *Ulva spp.* | *Fish/crustacean/ Mollusc spp.* | Initial body weight/ length | Type of addition | Dose | Duration | Effect on haemato-biochemical parameters | References |
|---|---|---|---|---|---|---|---|
| *U. fasciata* | *California yellowtail (Seriola dorsalis )* | 7.93 ± 0.24 g | powder | 5, 10, and 20 g kg$^{-1}$ | 48 days | 20 g kg$^{-1}$ PCV | [85] |
| *U. lactuca* | Nile tilapia fingerlings (*Oreochromis niloticus*) | 18.47 ± 1.25 gm | powder | 2.5 and 5% | 12 weeks | No effect | [52] |
| *U. fasciata* | Nile tilapia fingerlings (*Oreochromis niloticus*) | 1.32±0.12g | methyl extract | 50, 100, and 150 mgkg$^{-1}$ | 90 days | ↑ RBCs, PCV, albumin, and globulin | [116] |
| *U. pertusa* | Black sea bream (*Acanthopagrus schlegeli*) | | powder | 2.5, 5.0, 10.0 & 15.0% replaced fish meal | | ↓ Lipid accumulation | [102] |
| *U. pertusa* | Black sea bream (Acanthopagrus schlegeli) | 24g | powder | 10% | 143 days | ↑ Lipid contents | [103] |
| *U. lactuca* | Pacific white shrimp (*Litopenaeus vannamei*) | 0.59 ± 0.09 g | powder | 0, 1, 2, and 3% *U. lactuca* meal | 28 days | 3 % ↑ Lipid and carotent | [72] |
| U. lactuca | Black tiger shrimp juvenile (*Penaeus monodon*) | | powder | 15% and 30% of soybean meal | 90 days | No effect | [104] |

**Table 15:** Effects of different Ulva types of dietary inclusion on Antioxidant and immune-related genes expressions.

| *Ulva spp.* | Fish/*Crustacean/ Mollusc Spp.* | Initial Body Weight/ Length | Type of Addition | Dose | Duration | Effect on Anti-Oxidant and Immune-Related Genes Expressions | References |
|---|---|---|---|---|---|---|---|
| *U. ohno*i | Senegalese sole (*Solea senegalensis*) | 11.88 ± 2.08 g | extract | (0.5 mg/ fish) | | ↑ *TLRs* | [122] |
| *U. clathrata* | Pacific white shrimp (*Litopenaeus vannamei*) | 3±0.01g | fresh | 8 g, Daily | 20 days | ↑ Immune and lipid metabolism genes | [70] |
| *U. rigida* | Turbot (Psetta maxima L.) | 100g | extract | (0–100 µl ml$^{-1}$) | | ↑ *IL-1β* genes | [107] |
| *U. fasciata* | Nile tilapia (*Oreochromis niloticus*) | 1.32±0.12g | extract | 50, 100, and 150 mgkg$^{-1}$ | 90 days | 100 mgkg$^{-1}$ ↑ Catalase and SOD genes | [116] |

**Antioxidant and Immune-Related Genes Expressions:**

According to Table 15. In Pacific white shrimp (*Litopenaeus vannamei*), fresh *U. clathrate* (30.01g) was found to trigger immune and lipid metabolism genes [70]. Injecting *U. ohnoi* extract intraperitoneally led to the activation of immune-related genes like toll-like receptors (TLRs) in Senegalese sole (*Solea senegalensis*) [122]. Additionally, it was shown that *U. rigida* extract increased the expression of interleukin-1 (IL-1) in turbot peritoneal leucocytes [107]. To the best of our knowledge, no studies utilizing Ulva meal powder on fish and crustaceans have revealed the expression of immune and antioxidant-related genes.

**Growth-Related Genes Expressions:**

To our knowledge, Abo-Raya, et al. [18] is the only study that has examined the impact of Ulva species on growth-related genes in fish. They discovered that growth hormone (GH) and Insulin-like growth factor-1 (ILGF-1) expressions in Nile tilapia fed *U. fasciata* methyl extract made slight and non-significant increases, and they attributed these findings to the presence of bioactive compounds that inhibit growth; for example, clionasterol [Gamma sitosterol ($C29H50O$)] that has been mentioned to influence cholesterol synthesis in intestinal cell [18,134,135].

## Evaluation and future work

This review has discussed the potential of *Ulva spp*. as an alternative sustainable feed additive.

A. The outcomes of most Ulva research clearly show the following:

a) Ulva can be used in different forms (fresh, powder, and extract) and may have positive, negative, and non-effect on the different performance parameters.

b) Ulva contains numerous significant and useful bioactive compounds such as oleic acid, hexadecenoic acid, phytol, neophytadiene, arachidonic acid, and clionasterol which have significant effects on growth, haemato-biochemical, immune, and antioxidant parameters.

B. Ulva is a promising feed additive for fish, crustaceans, and molluscs; in addition, it can be a sustainable aquaculture resource in the future due to:

a) It's wide geographical composition, dispersion, and advantages over terrestrial plants; besides, it can generate a thriving and lucrative industry for coastal fishing communities.

b) When compared to the number of aquatic organisms that perish every day throughout the world and the negative effects of antibiotics on aquatic species and people, the extraction of Ulva bioactive substances is affordable.

C. On the other hand, in all the published studies involving Ulva, there are numerous research gaps:

a) Most research indicated that using Ulva at high doses had adverse effects; therefore, determining the appropriate concentration is vital. Therefore, regression statistical methods are more accurate than ANOVA since it is simple to determine the appropriate concentration to affect the various parameters.

b) Because all extracts contained a variety of bioactive substances, including both harmful and beneficial ones with varying concentrations, it is impossible to be certain of the reasons for the effects in any research. As a result, all researchers built the reasons for their results on the expectation.

c) Even though all research focused on showing how Ulva affected growth performance, nearly only one of them showed how Ulva affected the genes involved in growth. Additionally, no studies highlighted how Ulva as fresh affected haematological, biochemical, immunological, and oxidative parameters.

d) Most of the research used traditional methods of extraction, such as Maceration, for ease of extraction, these methods have severe drawbacks, such as low extraction selectivity and heat breakdown of thermo-labile compounds.

D. Therefore, future research should include the following:

a. Before examining the impacts of Ulva as powder, researchers must first study the effects of Ulva as an extract to identify any potentially dangerous or beneficial bioactive compounds.

b. Researchers should concentrate on using non-conventional extraction techniques to extract bioactive ingredients and be interested in learning how growth-related genes are affected.

c. The researchers must do additional studies to identify the precise bioactive ingredient that has the exact impact on the various studied parameters and not just to do expectations for how the extract responded. Therefore, using the liquid-liquid partition technique, the extract must be divided into three parts (hexane, chloroform, and ethyl acetate). Each part must then be tested again for various activities, and once it is known which part has the desired effect, fractionation and isolation of the precise effective bioactive compounds must be done using an open-column chromatography system [136].

## Conclusion

Ulva is a green macro-alga responsible for the disastrous green tides seen all over the world. These green blooms are a result of human activity. Ulva blooms mostly occur in shallow seas, and this alga's decomposition can release potentially harmful gases. Ulva has undergone in vivo testing for its pharmacological effects as an antioxidant, an anti-inflammatory, and a growth promoter. Furthermore, Ulva is a promising feed additive that can be utilized in the aquaculture industry. According to numerous studies, isolating and extracting its extracts needs a few steps, procedures, and strategies. Moreover, it can also be used fresh and in powder form. However, it's important to be cautious while choosing the right dosage. Future studies should prioritize their research on Ulva's biological activity and other health advantages since there is currently a dearth of information, particularly about its extract.

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## Ethical Approval

This article does not contain any experiments with humans or animals.

## Conflict of interest

The authors declare that they have NO conflict of interest that might be perceived to influence the discussion reported in this review.

## Authors' Contributions

All authors contributed equally to this work (writing the main manuscript text, preparing figures and tables, and reviewing the manuscript).

## Availability of Data and Materials

All data used has references that can be used to access it and no permissions are required.

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