Digestibility and gastrointestinal transit of *Ulva fasciata* seaweed meal in tilapia (*Oreochromis niloticus*) juveniles: basis for the inclusion of a sustainable ingredient in aquafeeds

Digestibilidade e trânsito gastrointestinal do farelo de alga *Ulva fasciata* em juvenis de tilápia (*Oreochromis niloticus*): bases para a inclusão de um ingrediente sustentável na aquicultura

Digestibilidad y tránsito gastrointestinal de harina de alga *Ulva fasciata* en juveniles de tilapia (*Oreochromis niloticus*): bases para la inclusión de un ingrediente sostenible en la acuicultura

Received: 09/15/2020 | Reviewed: 09/24/2020 | Accept: 09/30/2020 | Published: 10/01/2020

Marcelo Duarte Pontes
ORCID: https://orcid.org/0000-0003-3344-1411
Fundação Instituto de Pesca do Estado do Rio de Janeiro, Brasil
E-mail: Marcelodpontes@gmail.com
Daniel Abreu Vasconcelos Campelo
ORCID: https://orcid.org/0000-0001-9204-3566
Universidade Federal do Pará, Brasil
E-mail: danielvc@ufpa.br
Rodrigo Takata
ORCID: https://orcid.org/0000-0003-3385-6895
Fundação Instituto de Pesca do Estado do Rio de Janeiro, Brasil
E-mail: takatarodrigo@gmail.com
Lidia Miyako Yoshii Oshiro
ORCID: https://orcid.org/0000-0002-9700-9866
Universidade Federal Rural do Rio de Janeiro, Brasil
E-mail: lidiaoshiro_ufrrj@yahoo.com.br
Beatriz Castelar
ORCID: https://orcid.org/0000-0002-0025-556X
Universidade de Turim, Itália
E-mail: beatrixcastelar@gmail.com

Abstract

The seaweed *Ulva fasciata* has many features favorable to integrated multi-trophic aquaculture (IMTA). It is efficient at biofiltering, shows high biomass production, and is rich in many nutrients useful in aquatic animal diets. We evaluated the digestibility of the seaweed meal of *U. fasciata* produced in IMTA and its effects on gastrointestinal transit time in tilapia (*Oreochromis niloticus*) juveniles. Juveniles (6.30 ± 1.80 g initial weight, and 5.5 ± 0.61 cm initial length) were cultivated in six tanks (50 individuals per tank) in a closed recirculating aquaculture system. The digestibility of *Ulva* meal was 57.92 ± 5.21% for dry material, 78.59 ± 1.91% for protein, and 69.87 ± 3.72% for energy. The inclusion of 10% seaweed meal did not alter the gastrointestinal transit time in tilapia juveniles as compared to controls. The earliest colored feces were observed four hours after first feeding in both treatments (feed diets with [10%] and without seaweed); all fecal material was colored after ten hours. The digestibility of seaweed meal was satisfactory for dry material, protein, and gross energy, and the inclusion of 10% of that meal did not change gastrointestinal transit time - indicating that the inclusion of 10% seaweed meal in tilapia diet is safe and without any nutritional use losses.

Keywords: Macroalgae; Complementary ingredient; Fish feed; Non-starch polysaccharides; Ulvan; Integrated multi-trophic aquaculture.

Resumo

A macroalga *Ulva fasciata* apresenta muitas características favoráveis à aquicultura multitrófica integrada (AMTI). É eficiente como biofiltro, apresenta alta produção de biomassa e é rica em muitos nutrientes úteis na dieta de animais aquáticos. Avaliamos a digestibilidade do farelo da macroalga *U. fasciata* produzida no AMTI e seus efeitos no tempo de trânsito gastrointestinal de juvenis de tilápia (*Oreochromis niloticus*). Juvenis (6,30 ± 1,80 g de peso inicial e 5,5 ± 0,61 cm de comprimento inicial) foram cultivados em seis tanques (50 indivíduos por tanque) em sistema de recirculação de água. A digestibilidade do farelo de *Ulva* foi 57,92 ± 5,21% para matéria seca, 78,59 ± 1,91% para proteína e 69,87 ± 3,72% para energia. A inclusão de 10% de farelo de ulva não alterou o tempo de trânsito gastrointestinal em juvenis de tilápia quando comparados aos controles. As primeiras fezes coloridas foram observadas quatro horas após a primeira alimentação em ambos os tratamentos (rações com [10%] e sem macroalga); todo o material fecal foi corado após dez horas. A digestibilidade do farelo de *Ulva* foi satisfatória para matéria seca, proteína e energia bruta, e a inclusão de 10% dessa refeição não alterou o tempo de trânsito gastrointestinal - indicando que a inclusão de 10% do farelo de *Ulva* na dieta da tilápia é segura e sem qualquer prejuízo nutricional.

Palavras-chave: Macroalgas; Ingrediente complementar; Ração para peixes; Polissacarídeos não amiláceos; Ulvana; Aquicultura multitrófica integrada.

Resumen

El alga *Ulva fasciata* tiene muchas características favorables a la acuicultura multitrófica integrada (AMTI). Es eficiente en el biofiltro, muestra una alta producción de biomasa y es rica en muchos nutrientes útiles en las dietas de los animales acuáticos. Se evaluó la digestibilidad de la harina de algas de *U. fasciata* producida en AMTI y sus efectos sobre el tiempo de tránsito gastrointestinal en juveniles de tilapia (*Oreochromis niloticus*). Se cultivaron juveniles (6,30 ± 1,80 g de peso inicial y 5,5 ± 0,61 cm de longitud inicial) en seis tanques (50 individuos por tanque) en un sistema de recirculación acuícola cerrado. La digestibilidad de la harina de *Ulva* fue 57,92 ± 5,21% para material seco, 78,59 ± 1,91% para proteína y 69,87 ± 3,72% para energía. La inclusión de harina de algas al 10% no alteró el tiempo de tránsito gastrointestinal en los juveniles de tilapia en comparación con los controles. Las heces de color más temprano se observaron cuatro horas después de la primera alimentación en ambos tratamientos (dietas con [10%] y sin algas); toda la materia fecal se coloreó después de diez horas. La digestibilidad de la harina de algas fue satisfactoria para el material seco, las proteínas y la energía bruta, y la inclusión del 10% de esa dieta no cambió el tiempo de tránsito gastrointestinal, lo que indica que la inclusión de un 10% de harina de algas en la dieta de la tilapia es segura y sin pérdidas nutricionales.

Palabras clave: Macroalgas; Ingrediente complementario; Alimento para peces; Polisacáridos sin almidón; Ulvan; Acuicultura multitrófica integrada.

1. Introduction

Food supply sustainability is integrally related to the development of high-efficiency eco-friendly production systems. Integrated Multi-Trophic Aquaculture (IMTA) systems are based on the efficient utilization of materials as one organism uses the wastes of another in a recycling loop (Troell et al., 2014). Seaweeds are often used as biofilter in IMTA with marine fish and shrimp and can produce green biomass rich in protein and bioactive compounds of great interest for animal nutrition (Øverland et al., 2018).

The possibility of producing plant ingredients within the same fish production system reduce costs, diminish gaseous emissions related to transport, and simplify the overall production chain. As such, the use of seaweed biomass as an ingredient in fish diets would contribute to the economic viability of IMTA and diminish its footprint (Marinho et al., 2013).

The green seaweed *Ulva* spp. presents an ideal fit for IMTA due to its fast growth and high capacity for nitrogen uptake (Nielsen et al., 2012; Ben-Ari et al., 2014; Castelar et al., 2015). *Ulva* biomass is rich in minerals and carotenoids such as lutein, and other antioxidants (Msuya & Neori, 2008; Cruz-Suárez et al., 2010). Has high protein content, a good essential amino acid profile, being able to be considered a source of phenylalanine and tryptophan demonstrating potential as anxiolytic in fish farming (Calheiros et al., 2019). These seaweeds are also rich in non-starch polysaccharides (NSP). Polysaccharides represent from 38 to 54% of the dry matter of *Ulva* sp.; the insoluble fraction is mostly cellulosic, while the soluble fraction is composed principally of ulvan (Lahaye & Robic, 2007). Ulvan may have bioactive effects in fish and thus act in disease prevention. The inclusion of ulvan extracted from *Ulva clathrata* in the diets of tilapia has been found to increase phagocytic activity and white blood cell counts, demonstrating the potential activity of ulvan on at least two immune responses (Quezada-Rodríguez & Fajer-Ávila, 2017). Additionally, the NSP of *Ulva pertusa* promoted the growth of beneficial bacteria in the gastrointestinal tract of *Siganus canaliculatus*, where they acted as prebiotics (Zhang et al., 2017).

Improvements in production performance and greater resistance to stress in fish have been reported in studies using *Ulva* spp. as a food ingredient (Valente et al., 2015; ZHu et al., 2015). However, the inclusion of high levels of NSP in a diet could modify the viscosity of the digesta and the gastrointestinal transit time (GTT) - thus impairing the enzymatic action and the absorption of nutrients (Leenhouwers et al., 2006; Sinha et al., 2011). It is therefore important to establish safe limits of *Ulva* meal inclusion in fish diets so that the NSP contents of those macroalgae do not negatively affect fish digestion and nutrient absorption (which can be assayed by changes in GGT).

The tilapia, *Oreochromis niloticus*, is one of the most produced and most studied fish in the world. Omnivorous fish with low protein requirements, which can use carbohydrates efficiently as an energy source, which allows the extensive use of vegetable ingredients in their diets, as well as reduced nitrogen content in the diet.

According to Turchini et al. (2019), the formulation of a diet for aquaculture can be understood as the process of identifying different combinations of “complementary” raw materials that satisfy the intended species dietary requirements and tolerances. In addition to the chemical composition of the ingredients, a set of information such as digestibility, palatability, utilization, and functionality should be investigated in representative species and in a variety of sources of the ingredient in question. In this sense, the information on the digestibility of *Ulva* meal from cultivation, as well as its influence on gastrointestinal transit, is still scarce. As such, we evaluated the energetic content, dry matter and protein digestibility of *Ulva* meal, as well as the effects of the inclusion of 10% seaweed meal in the diets of tilapia juveniles on gastrointestinal transit.

2. Methodology

Three hundred tilapia juveniles (mean weight 6.30±1.80 g; mean length 5.5 ± 0.61 cm) were used [Ethical Committee for Animal Uses of the Fisheries Institute of Rio de Janeiro State (FIPERJ) - number 001/2017]. The fish were randomly distributed in six cylindrical polyethylene tanks (0.1 m^3 volume; 50 juveniles per tank) in a closed water recirculation system. Each tank had a continuous water supply stream and drainage system (0.1 m^3 h^{-1}) with constant, soft, superficial aeration (15 cm deep) to avoid bottom disturbance. The effluents were conducted to a filtering and sterilizing system composed of a sedimentation tank (0.05 m^3), a physical filter (1 mm mesh), and six biological filter tanks (0.05 m^3 each) containing expanded clay as the substrate for nitrifying bacteria. The water then flowed by gravity to a reservoir (0.2 m^3), from which it was pumped (5.80 m^3 h^{-1}) by a submersible pump (JATO 6000; CUBOS®) through a UV filter, and finally redistributed to the fish tanks. The juveniles were acclimated for 15 days before initiating the experiments.

Two experiments were conducted: 1) to evaluate the gastrointestinal transit time (GTT); 2) to quantify the digestibility of the seaweed meal. The basal diet used in the two experiments was formulated from Soybean, Corn, Fish, Poultry by-product meals, in addition to Fish oil, Butyl-Hydroxy-toluene (BHT; antioxidant) and premix Nutrifish-GUABI®. It presented a proximate composition of 389.12 g kg^{-1} of crude protein, 92.91 g kg^{-1} of crude lipid, 13.27 g kg^{-1} of crude fiber, 114.21 g kg^{-1} of ash and 18.73 J kg^{-1} of gross energy.

To prepare the seaweed meal, the biomass of *U. fasciata* was harvested from an experimental IMTA of seaweed and marine fishes (*Eugerres brasilianus* and *Rachycentron canadum*) at the Almirante Paulo Moreira Experimental Aquaculture Station at FIPERJ. The seaweed biomass was dried in an oven (TE-394/3; TECNAL®) at 50 °C for 24 h, and then ground in a rotary mill (TE-651/2; TECNAL®) equipped with a 0.5 mm mesh; 853.9 g^{-1} of seaweed meal was produced. All ingredients cited in Table 1 were weighed in a centesimal balance, manually mixed, ground in a rotary mill (0.5 mm mesh), moistened with water (50% by weight), homogenized, pelletized (1.0 – 2.0 mm), and subsequently dried in a forced air recirculation oven at 50 °C for 24 h.

Ulvan, one of the most abundant polysaccharides produced by *Ulva* sp. (Lahaye & Robic, 2007) was extracted using a hot alcohol method (following Castelar et al., 2014) from three samples (1.0 g each) of seaweed meal. The ulvan yield (UY) was calculated as *UY(%) = [(dry mass of ulvan/ dry mass of seaweed) X 100]*.

In addition to the seaweed meal, two inert markers were used in the experimental feeds, chromium oxide (Cr_2O_3) and titanium dioxide (TiO_2), which lend green and white colors, respectively, to fish feces. During both experiments, the fish were fed until apparent satiety three times a day (at 08:00; 12:00; 16:00 h). The diets, feed administration, feces collections, and tank cleaning schedules varied according to the characteristics of each experiment, as described below.

***Experiment 1 - Gastrointestinal transit time* (GTT)**

The feeds used for GTT evaluations are presented in Table 2. GTT was evaluated using a color index (CI) representing the visual changes in the colors of the fish feces caused by the inclusion of chromium oxide (Cr_2O_3) or titanium dioxide (TiO_2) (which result in green or white tones respectively) (Storebakken, 1985). The CI is expressed as a score (0, 20, 40, 60, 80 and 100%) assigned to the colors of the feces – where 0% represents completely white feces(without any influence of the diet provided at the beginning of the time count) and 100% represents completely green matter (all feces were from the diet provided after the start of the time count). The feces for CI attribution were collected by siphoning (using a 4 mm silicone tube) and then transferred to a glass beaker. Tilapia feces are cohesive, which allows their collection by suction without affecting their physical structure. Color evaluations were performed on a gray bench illuminated by white light (LED 5w-6500k), facilitating distinctions of the different tones and colors. When collections coincided with feeding times, tank cleaning was performed immediately after feeding to avoid mixing the collected feces and feed remains.

For those experiments, the fish were fed with a reference diet containing TiO2 for two days, without any seaweed meal (*Wref*, Table 1) to dye all feces in white (and thus increase color contrasts). After the feces were completely white (CI-0%), feeding was initiated with a reference green test feed containing Cr2O3, without a seaweed meal (*Gref*). The feces of each tank (n=6) were then collected separately, every two hours for 24 h, and the CI assessed. The next step involved feeding the fish with a white test diet (*Wtest*, Table 1), containing TiO2 and seaweed meal, for two days, to dye all of the feces white again, and then restart the feeding-cleaning-feces collection cycle with green test feed (*Gtest*, Table 1) containing Cr2O3 and seaweed meal.

Similarly, the feces of each tank were collected every two hours, during 24 h, and the CI determined. Total GTT was determined by the time required for 100% CI in all replicates.

Table 1. Basal diet inclusion levels (g kg^{-1}), markers and test ingredients (seaweed meal of *Ulva fasciata*) in the experimental diets of experiment 1 - Gastrointestinal transit time evaluation.

Diet	Basal diet	Markers		seaweed meal
		chromium oxide	titanium oxide	
Gref	990	10	-	-
Wref	990	-	10	-
Gtest	890	10	-	100
Wtest	890	-	10	100

Source: Authors (2020).

Experiment 2 – Digestibility of seaweed meal

Two diets were prepared in the second experiment: the first (*reference diet*) contained the basal diet (995 g kg^{-1}) plus Cr_2O_3 (5g kg^{-1}); the second (*test diet*) contained the basal diet (695 g kg^{-1}), Cr_2O_3 (5g kg^{-1}), and seaweed meal (300 g kg^{-1}).

The fish in all six tanks were first fed with the reference diet, with feces collections beginning two days later. The feces were siphoned from each tank separately (n=6), every 2 hours, for five days. The collected material was dried at 60 °C for 12 hours and subsequently frozen at -15 °C for posterior analysis (gross energy, crude protein, dry matter, and Cr_2O_3 content). The same procedure was used for the test diet.

Composition analyses were made of the reference diet, test diet, the respective feces produced, and the seaweed meal (n=3). Gross energy was determined by burning the sample in a calorimetric pump; crude protein content was determined using the Dumas method; dry matter content was determined by drying loss; and Cr_2O_3 content was determined using atomic absorption spectroscopy, following the methodology recommended by the Brazilian Association of Animal Feeding Compendium (CBAA, 2009).

The coefficients of apparent digestibility of protein, energy generation, and the dry matters of both diets (CADd) and of the seaweed meal (CADs) were calculated following Pezzato et al.

(2002) and Sugiura et al. (1998) where, CADd (%) =100 - [100 X (diet Cr_2O_3 content / feces Cr_2O_3 content) X (feces protein (or energy) content / diet protein (or energy) content)], and CADs (%) = [(nutrient content of the reference diet X CAD of the reference diet – 0.7 X nutrient content of the test diet X CDA of the test diet)/ (nutrient content of seaweed meal X 0.3)].

Temperature and dissolved oxygen were recorded daily during the experimental period using a digital dissolved oxygen meter (HI9146, HANNA®); pH was recorded using a portable digital pH meter (HI 98129, HANNA®); ammonium concentrations in the water were estimated using an aquarium colorimetric kit and a photometer (HI83203, HANNA®). The means (±standard deviation) of the abiotic variables monitored during the experimental period were: dissolved oxygen 4.54 mg L^{-1} (±1.29); pH 7.76 (±0.45); ammonia (N-NH_3) 0.58 mg L^{-1} (±0.31); water temperature 20.32 ºC (±2.71).

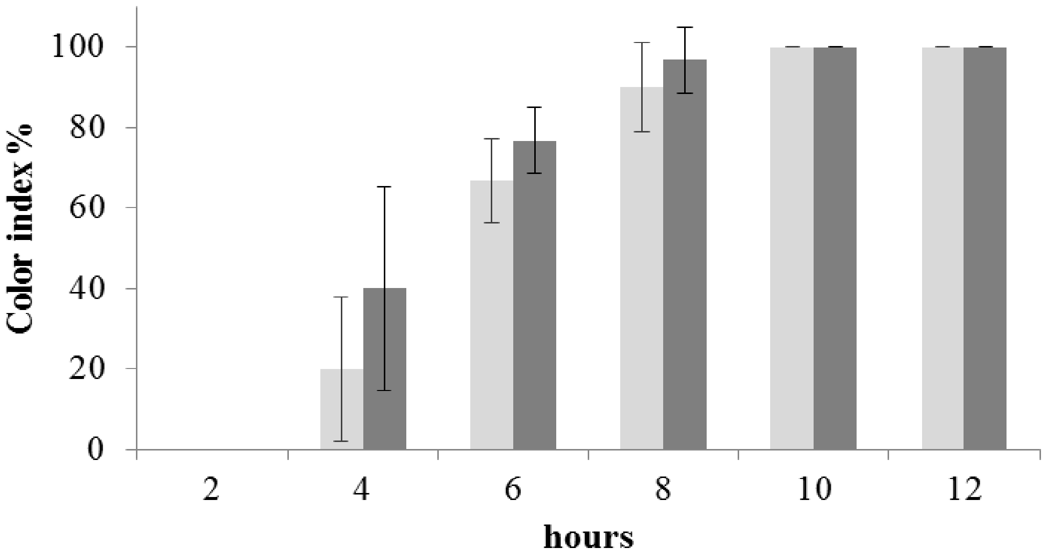
The normality (Shapiro-Wilk) and homogeneity of the variances (Cochran) of the IC data were tested according to Zar (1996). Analysis of variance (ANOVA) for repeated measures was used to verify differences between the ICs of diets with and without seaweed meal over time. The limit of tolerance for all tests was 95% ($p<0.05$). The statistical analyses were conducted using Statistica 6.0 StatSoft Inc. Software

3. Results

Seaweed meal obtained from *U. fasciata* showed a ulvan yield of 22.6 (±1.6) %.

Four hours after the beginning of feeding on diets with and without seaweed meal, the feces began to change color from white to green. The CI was 100% after 10 h in both cases for all treatments and replicates, suggesting that 10 h was the total GTT for both tested diets (Figure 1). Differences in ICs between diets were not observed at any time ($F = 1.569$; $p=0.271$).

Figure 1. Color index (CI %) of tilapia's feces (*Oreochromis niloticus*) fed with diets containing seaweed meal of *Ulva fasciata*. White bars represent feces of fish fed with a control diet (without seaweed meal) and dark gray bars represent feces of fish fed with a test diet (with seaweed meal).



Source: Authors (2020).

The seaweed meal contained 243.80 g kg^{-1} of crude protein and 12.73 KJ g^{-1} of gross energy, on a dry matter basis. Digestible protein was 191.60 g kg^{-1}, and digestible energy 8.89 KJ g^{-1}. The digestibility coefficients of both diets and the seaweed meal are presented in Table 2.

Table 2. Coefficients of apparent digestibility (%) of dry matter, protein and energy of both reference and test diets, and of seaweed meal of *Ulva fasciata* for tilapia (*Oreochromis niloticus*) juveniles.

	Coefficient of apparent digestibility (%)		
	Dry matter	Protein	Energy
Reference diet	71.29±0.67	87.19±0.57	78.95±0.53
Test diet	67.28±1.36	84.75±0.58	76.67±0.54
Seaweed meal	57.92 ± 5.21	78.59 ± 1.91	69.87 ± 3.72

Source: Authors (2020).

4. Discussion

Seaweed meal derived from *U. fasciata* contained more than 20% ulvan, an important component of NSP; it represented 2.26% of the dry matter in the diet used to determine gastrointestinal transit times. The inclusion of a rich NSP seaweed meal (10%) in the tilapia diet did not change its gastrointestinal transit time. That amount of seaweed meal may not alter the chime enough to affect the GTT or the activities of the digestive enzymes and diminish the expected growth rate of the species. The NSPs present in algae are viscous (Zhu et al., 2015) and, at high levels, can affect the viscosity of both the diet and feces, impairing nutrient digestion and production performance. Those effects were not observed at low inclusion levels, however, and our results, therefore, support the safe inclusion of up to 10% *Ulva* sp. for tilapia. Likewise, several other studies identified no production performance impacts at 10% inclusion levels (Güroy et al., 2007; Azaza et al., 2008; Ergün et al., 2009; El-tawil, 2010; Marinho et al., 2013).

The GTTs of fish depend on many factors, such as water temperature (Jobling et al., 1977; Carneiro et al., 1994), ingredient particle sizes (Hayashi et al., 1999; Gentelini et al., 2005) and nutritional composition – especially lipids (Meurer et al., 2002) and fibers (Meurer et al., 2003). Fiber enhancements in fish diets tend to diminish both the GTT and nutrient digestibly (Lanna et al., 2004; Rodrigues et al., 2010), although Meurer et al. (2003) observed that crude fiber enhancements of 8.5% in tilapia diets increased the GTT without fish productivity losses.

Fish, as well other monogastric animals, did not have gastrointestinal enzymes capable of degrading NSP (Sinha et al., 2011), although part of the energy contained in those carbohydrates could be obtained from the volatile fatty acids produced by fermentation by microorganisms. In addition to serving as energy sources, those fatty acids could promote immune system gains, ion absorption, the growth of beneficial microorganisms, and the inhibition of pathogens (Montagne et al., 2003; Amirkolaie et al., 2006). According to Haidar et al. (2016), the efficiency of volatile fatty acid use by tilapia depends on the polysaccharide source, feed practices, and processing.

The digestibility of the seaweed meal was similar to that of other plant ingredients. The coefficients of apparent digestibility (CAD) of the dry matter, protein, and energy of seaweed meal were within the ranges reported by Pezzato et al. (2002) for many vegetable meals of tilapia, ranging: from 23.44 to 71.04% for dry matter; from 67.83 to 94.86% for protein; and from 51.00 to 91.29% for energy. The CADs of dry matter and protein, however, were lower than those observed for corn, wheat, and soybeans fed to tilapia juveniles (Furuya et al., 2001).

Digestibility can be affected by anti-nutritional factors often observed in vegetable ingredients and, at high concentrations, they can be toxic and impact diet performance (Francis et al., 2001). Azaza et al. (2008) attributed low observed performances of tilapia juveniles to high contents of saponins, tannins, and phytic acid in diets containing more than 30% *U. rigida*. In comparison with other seaweeds, however, *Ulva* sp. showed one of the highest protein CAD values for tilapia, although Pereira et al. (2012) reported values of 63.4% and 57.1% for protein and energy respectively – values lower than those observed in the present study.

Variations of CAD values in *Ulva* sp. could be related to specific chemical (genotypic) heterogeneity or too high chemical plasticity in response to environmental (phenotypic) conditions (Fleurence, 1999). In this sense, IMTA has the potential to produce algae strains rich in proteins and with low chemical heterogeneity, as environmental conditions can be more controlled in closed aquaculture systems than in the open sea. However, optimal parameters have yet to be determined in cultivation, such as stocking density, nutrient availability, water flow and light intensity, as these parameters can determine algae growth and chemical composition (Diamahesa et al., 2017; Oca et al., 2019; Shpigel et al., 2019).

In addition to genotypic (specific) and phenotypic (environmental) expressions of chemical composition, differences in CAD values could be explained by diet processing methods. Extruding processes can alter the digestibility of nutrients and diminish protein digestibility through the denaturation that occurs during the high temperatures usually required (Cheng & Hardy, 2003). Fermentation can also improve seaweed digestibility, and Felix & Brindo (2014) reported that the fermentation of *Ulva lactuca* meal increased the digestibility of its dry matter, lipids, and proteins in relation to raw meal (used as feed for the giant freshwater prawn *Macrobrachium rosenbergii*). That fermentation process allowed the inclusion of up to 30% of *Ulva lactuca* meal with no detriment to productive performances.

The advance of the use of *Ulva* in animal nutrition demands improvements in utilization studies of nutrients present in the seaweed, nutraceutical effect of the present compounds and evaluation of productive performance. In addition, the deepening of the processing of the material, such as the extraction of the ulvan could increase the income of the activity, reduce a possible antinutritional factor and concentrate the other nutrients in this meal.

5. Conclusion

The seaweed meal of *U. fasciata* therefore proved to be a generally attractive complementary ingredient for tilapia diets, as the inclusion of 10% of that meal did not affect gastrointestinal transit times and its digestibility was found to be acceptable to that omnivorous fish.

Acknowledgments

This research was supported by *Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro* – FAPERJ [grant number FAPERJ/ E26/ 010.001971/ 2014]. The authors thank the company *d'Alga Aquicultura Urbana* for the IMTA grants and for providing facilities for the experiments.

References

Amirkolaie, A. K., Verreth, J. A., Schrama, J. W. (2006). Effect of gelatinization degree and inclusion level of dietary starch on the characteristics of digesta and faeces in Nile tilapia (*Oreochromis niloticus* (L.)). Aquaculture, 260, 194-205.

Azaza, M. S., Mensi, F., Ksouri, J., Dhraief, M. N., Brini, B., Abdelmouleh, A., Kraiem, M. M. (2008). Growth of Nile tilapia (*Oreochromis niloticus* L.) fed with diets containing graded levels of green algae *Ulva* meal (*Ulva rigida*) reared in geothermal waters of southern Tunisia. Journal of Applied Ichthyology, 24, 202-207.

Ben-Ari, T., Neori, A., Ben-Ezra, D., Shauli, L., Odintsov, V., Shpigel, M. (2014) Management of *Ulva lactuca* as a biofilter of mariculture effluents in IMTA system. Aquaculture, 434, 493–498.

Calheiros, A. C., Reis, R. P., Castelar, B., Cavalcanti, D. N., Teixeira, V. L. (2019). *Ulva* spp. as a natural source of phenylalanine and tryptophan to be used as anxiolytics in fish farming. Aquaculture, 509, 171-177.

Carneiro, D. J., Rantin, R. T., Dias, T. C. R., Malheiros, E. B. (1994). Interaction between temperature and dietary levels of protein and energy in pacu (*Piaractus mesopotamicus*). II. Effects on digestibility of protein and transit time through the gastrointestinal tract. Aquaculture, 124.

Castelar, B., Reis, R. P., & dos Santos Calheiros, A. C. (2014). *Ulva lactuca* and *U. flexuosa* (Chlorophyta, Ulvophyceae) cultivation in Brazilian tropical waters: recruitment, growth, and ulvan yield. Journal of applied phycology, 26(5), 1989-1999.

Castelar, B., Pontes, M. D., Costa, W. M., Fontes, L. C., Dias, G. E., Landuci, F. E. & Reis, R. P. (2015). Biofiltering efficiency and productive performance of macroalgae with potential for integrated multi-trophic aquaculture (IMTA). Boletim do Instituto de Pesca, 41, 763 – 770.

CBAA (2009). Compêndio brasileiro de alimentação animal. Sindirações (Ed). São Paulo, 390p.

Cheng, Z. J., Hardy, R. W. (2003). Effects of extrusion processing of feed ingredients on apparent digestibility coefficients of nutrients for rainbow trout (*Oncorhynchus mykiss*). Aquaculture nutrition, 9, 77-83.

Cruz-Suárez, L. E, León, A., Peña-Rodríguez, A., Rodríguez-Peña, G., Moll, B., Ricque-Marie, D. (2010). Shrimp/*Ulva* co-culture: a sustainable alternative to diminish the need for artificial feed and improve shrimp quality. Aquaculture 301, 64–68.

Diamahesa, W. A., Masumoto, T., Jusadi, D., Setiawati, M. (2017). Growth and protein content of *Ulva Prolifera* maintained at different flow rates in integrated aquaculture system. Jurnal Ilmu dan Teknologi Kelautan Tropis, 9(2), 429-441.

El-Tawil, N. E. (2010). Effects of green seaweeds (*Ulva* sp.) as feed supplements in red Tilapia (*Oreochromis* sp.) diet on growth performance, feed utilization and body composition. Journal of the Arabian Aquaculture Society, 5,179-193.

Ergün, S., Soyutürk, M., Güroy, B., Güroy, D., Merrifield, D. (2009). Influence of *Ulva* meal on growth, feed utilization, and body composition of juvenile Nile tilapia (*Oreochromis niloticus*) at two levels of dietary lipid. Aquaculture International, 17, 355-361.

Felix, N., Brindo, R. A. (2014). Evaluation of raw and fermented seaweed, *Ulva lactuca* as feed ingredient in giant freshwater prawn *Macrobrachium rosenbergii*. International Journal of Fisheries and Aquatic Studies, 1, 199-204.

Fleurence, J. (1999). Seaweed proteins: biochemical, nutritional aspects and potential uses, Trends in Food Science & Technology, 10, 25-28.

Francis, G., Makkar, H. P., Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture,199, 197-227.

Furuya, W. M., Pezzato, L. E., Miranda, E. C., Furuya, V. R. B., Barros, M. M. (2001). Coeficientes de digestibilidade aparente da energia e nutrientes de alguns ingredientes pela tilápia do Nilo, *Oreochromis niloticus* (L.)(linhagem tailandesa). Acta Scientiarum, 23, 465-469.

Gentelini, A. L., Boscolo, W. R., Feiden, A., Meurer, F., Hayashi, C. (2005). Graus de moagem dos ingredientes em rações peletizadas para alevinos de piavuçu (*Leporinus macrocephalus*). Acta Scientiarum Animal Sciences, 27, 93-97.

Güroy, B. K., Cirik, Ş., Güroy, D., Sanver, F., Tekinay, A. A. (2007). Effects of *Ulva rigida* and *Cystoseira barbata* meals as a feed additive on growth performance, feed utilization, and body composition of Nile tilapia, *Oreochromis niloticus*. Turkish Journal of Veterinary and Animal Sciences, 31, 91-97.

Haidar, M. N., Petie, M., Heinsbroek, L. T., Verreth, J. A., Schrama, J. W. (2016). The effect of type of carbohydrate (starch vs. nonstarch polysaccharides) on nutrients digestibility, energy retention and maintenance requirements in Nile tilapia. Aquaculture, 463, 241-247.

Hayashi, C., Boscolo, W. R., Soares, C. M., Boscolo, V. R., Galdioli, E. M. (1999). Uso de diferentes graus de moagem dos ingredientes em dietas para a tilápia-do-Nilo (*Oreochromis niloticus* L.) na fase de crescimento. Acta Scientiarum. Animal Sciences, 21, 733-737.

Jobling, M., Gwyther, D., Grove, D. J. (1977). Some effects of temperature, meal size and body weight on gastric evacuation time in the dab Limanda limanda (L). Journal of Fish Biology, 10, 291-298.

Lahaye, M., Robic, A. (2007). Structure and functional properties of ulvan, a polysaccharide from green seaweeds. Biomacromolecules, 8, 1765-1774

Lanna, E. A. T., Pezzato, L. E., Cecon, P. R., Furuya, W. M., Bomfim, M. A. D. (2004). Digestibilidade aparente e trânsito gastrintestinal em tilápia do Nilo (*Oreochromis niloticus*), em função da fibra bruta da dieta. Revista Brasileira de Zootecnia, 33, 2186-2192.

Leenhouwers, J. I., Adjei Boateng, D., Verreth, J. A. J., Schrama, J. W. (2006). Digesta viscosity, nutrient digestibility and organ weights in African catfish (*Clarias gariepinus*) fed diets supplemented with different levels of a soluble non starch polysaccharide. Aquaculture Nutrition, 12, 111-116.

Marinho, G., Nunes, C., Sousa-Pinto, I., Pereira, R., Rema, P., Valente, L. M. 2013. The IMTA-cultivated Chlorophyta *Ulva* spp. as a sustainable ingredient in Nile tilapia (*Oreochromis niloticus*) diets. Journal of applied phycology, 25, 1359-1367.

Meurer, F., Hayashi, C., Boscolo, W. R., Soares, C. M. (2002). Lipídeos na alimentação de alevinos revertidos de tilápia do Nilo (*Oreochromis niloticus* L.). Revista Brasileira de Zootecnia, 31, 566-573.

Meurer, F., Hayashi, C., Boscolo, W. R. (2003). Fibra bruta para alevinos de tilápia do Nilo (*Oreochromis niloticus,* L). Revista Brasileira de Zootecnia, 32, 256-261.

Montagne, L., Pluske, J. R., Hampson, D. J. (2003). A review of interactions between dietary fibre and the intestinal mucosa, and their consequences on digestive health in young non-ruminant animals. Animal feed science and technology, 108, 95-117.

Msuya, F. E., Neori, A. (2008). Effect of water aeration and nutrient load level on biomass yield, N uptake and protein content of the seaweed *Ulva lactuca* cultured in seawater tanks. Journal of Applied Phycology, 20, 1021-1031.

Nielsen, M. M., Bruhn, A., Rasmussen, M. B., Olesen, B., Larsen, M. M., Møller, H. B. (2012) Cultivation of *Ulva lactuca* with manure for simultaneous bioremediation and biomass production. Journal of Applied Phycology, 24, 449–458.

Oca, J., Cremades, J., Jiménez, P., Pintado, J., Masaló, I. (2019). Culture of the seaweed *Ulva ohnoi* integrated in a *Solea senegalensis* recirculating system: influence of light and biomass stocking density on macroalgae productivity. Journal of Applied Phycology, 3, 2461-2467.

Øverland, M., Mydland, L. T., & Skrede, A. (2019). Marine macroalgae as sources of protein and bioactive compounds in feed for monogastric animals. Journal of the Science of Food and Agriculture, 99(1), 13-24.

Pereira, R., Valente, L. M., Sousa-Pinto, I., Rema, P. (2012). Apparent nutrient digestibility of seaweeds by rainbow trout (*Oncorhynchus mykiss*) and Nile tilapia (*Oreochromis niloticus*). Algal Research, 1, 77-82.

Pezzato, L. E., Miranda, E. C. D., Barros, M. M., Pinto, L. G. Q., Furuya, W. M., Pezzato, A. C. (2002). Digestibilidade aparente de ingredientes pela tilápia do Nilo (*Oreochromis niloticus*). Revista Brasileira de Zootecnia, 31,1595-1604.

Quezada-Rodríguez, P. R., Fajer-Ávila, E. J. (2017). The dietary effect of ulvan from *Ulva clathrata* on hematological-immunological parameters and growth of tilapia (*Oreochromis niloticus*). Journal of Applied Phycology, 29, 423-431.

Rodrigues, L. A., Fabregat, T. E. H. P., Fernandes, J. B. K., Nascimento, T. M. T, Sakomura, N. K. (2010). Digestibilidade e tempo de trânsito gastrintestinal de dietas contendo níveis crescentes de fibra bruta para pacu. Acta Scientiarum. Animal Sciences, 32, 169-173.

Shpigel, M., Guttman, L., Ben-Ezra, D., Yu, J., Chen, S. (2019). Is *Ulva* sp. able to be an efficient biofilter for mariculture effluents? Journal of Applied Phycology, 31, 2449–2459.

Storebakken, T. (1985). Binders in fish feeds: I. Effect of alginate and guar gum on growth, digestibility, feed intake and passage through the gastrointestinal tract of rainbow trout. Aquaculture, 47, 11-26.

Sinha, A. K., Kumar, V., Makkar, H. P., De Boeck, G., Becker, K. (2011). Non-starch polysaccharides and their role in fish nutrition–A review. Food Chemistry, 127, 1409-1426.

Sugiura, S. H., Dong, F. M., Rathbone, C. K., Hardy, R. W. (1998). Apparent protein digestibility and mineral availabilities in various feed ingredients for salmonid feeds. Aquaculture, 159, 177-202.

Troell, M., Naylor, R. L., Metian, M., Beveridge, M., Tyedmers, P. H., Folke, C., Gren, Å. (2014). Does aquaculture add resilience to the global food system?. Proceedings of the National Academy of Sciences, 111, 13257-13263.

(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v9i10.8497

Turchini, G. M., Trushenski, J. T., Glencross, B. D. (2019). Thoughts for the future of aquaculture nutrition: realigning perspectives to reflect contemporary issues related to judicious use of marine resources in aquafeeds. North American Journal of Aquaculture 81(1), 13-39.

Valente, L. M., Araújo, M., Batista, S., Peixoto, M.J., Sousa-Pinto, I., Brotas, V., Rema, P. (2015). Carotenoid deposition, flesh quality and immunological response of Nile tilapia fed increasing levels of IMTA-cultivated *Ulva* spp. Journal of Applied Phycology, 28, 1-11.

Zar, J. H. (1996). Biostatistical Analysis. Prentice Hall. Upper Sadle River, NJ

Zhang, X., Wu, H., Li, Z., Li, Y., Wang, S., Zhu, D., Li, S. (2017). Effects of dietary supplementation of *Ulva pertusa* and non-starch polysaccharide enzymes on gut microbiota of *Siganus canaliculatus*. Chinese Journal of Oceanology and Limnology, 36, 438–449

Zhu, D., Wen, X., Xuan, X., Li, S., Li, Y. (2015). The green alga *Ulva lactuca* as a potential ingredient in diets for juvenile white spotted snapper *Lutjanus stellatus* Akazaki. Journal of Applied Phycology, 28, 703–711.

Percentage of contribution of each author in the manuscript

Marcelo Duarte Pontes – 40%

Daniel Abreu Vasconcelos Campelo – 10%

Rodrigo Takata – 10%

Lidia Miyako Yoshii Oshiro – 10%

Beatriz Castelar – 30%