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Addition of the red macroalgae Turkish Towel *Chondracanthus exasperates* and taurine improves the performance of alternative plant based feeds for juvenile sablefish *Anoplopoma fimbria*

Running title: Red macroalgae in sablefish feeds

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Abstract

Turkish Towel *Chondracanthus exasperates* and taurine were added to alternative plant based feeds for juvenile sablefish *Anoplopoma fimbria* to evaluate the effect of these ingredients, alone or combined, on fish growth, feed intake and efficiency, whole body nutrient composition, and liver histomorphology. Fish growth was significantly increased with the addition of taurine, and to a lesser extent, Turkish Towel to the experimental feeds. Feed efficiency and protein retention were significantly improved with the addition of taurine, but were unaffected by Turkish Towel addition. As expected, whole body taurine content was significantly affected by taurine addition. Increases in whole body lipid were observed with both taurine and Turkish Towel addition, with a significant interaction between the two factors. Liver histomorphology was generally normal; however histopathologic changes were observed in some fish at the end of the experiment. The occurrence of hepatocellular nuclear pleomorphism and clear cell foci was less among fish that had received the Turkish Towel feeds. The addition of taurine had no effect on the number of fish with a histopathologic change. Overall, results from this study reaffirm taurine supplementation is beneficial to sablefish receiving plant based feeds, and indicate Turkish Towel may be a promising feed ingredient for this species.

KEYWORDS Sablefish, alternative feeds, Turkish Towel, red macroalgae, taurine

1. INTRODUCTION

Aquaculture is the fastest growing food producing sector in the world today, and demands for feed ingredients, especially fish meal and fish oil, have increased dramatically in recent years (Hardy, Higgs, Lall & Tacon 2001; Barrows et al. 2008; FAO 2014). Alternative protein and oil sources are needed if further development of the aquaculture industry is to be sustained. Terrestrial plant proteins, such as those from soybean, pea, wheat, and corn, have been shown to adequately replace a portion of the fish meal used in aquaculture feeds for a number of species. The cost of plant proteins is typically lower than that of animal proteins and there is often immediate economic benefit associated with the replacement of fish meal in fish feeds with these ingredients. Complete replacement of fish meal and fish oil has yet to be achieved in commercial marine fish feeds due to nutrient deficiencies, species-specific sensitivities, palatability issues, and unknown factors.

There is emerging interest in the use of aquatic plants, such as macroalgae, in animal and fish feeds (Garcia-Vaquero & Hayes 2016; Wan, Davies, Soler-Vila, Fitzgerald & Johnson 2019). As reviewed initially by Mustafa & Nakagawa (1995) and later by Wan et al. (2019), macroalgae has successfully been incorporated into marine fish feeds as a minor ingredient without compromising growth or survival. Occasionally, increases in feed intake and growth have been reported (Mustafa, Wakamatsu, Umino & Nakagawa 1995; Ragaza et al. 2015; Vizcaino et al. 2016). In addition, macroalgae contains many essential nutrients from the marine environment that are limiting in terrestrial plants and can facilitate the replacement of fish proteins by plant proteins (Ragaza et al. 2015). Macroalgae also contains many unique compounds with poorly defined nutritional impacts. Lastly, there appears to be health benefits associated with the use of macroalgae in aquaculture feeds for some fish species (Xu et al. 2011; Lozano, Wacyk, Carrasco & Cortez-San Martin. 2016).

Global production of macroalgae now exceeds 30 million metric tons, annually (FAO 2018). The cultivation of macroalgae removes nitrogen and carbon from eutrophic marine ecosystems (Zhou et al. 2006; Wei et al. 2017) and can help remediate anthropogenic change in urbanized coastal waters (Yang et al. 2006; Yang et al. 2015; Kim, Kraemer & Yarish 2014). In contrast to the cultivation of terrestrial plants, which use large amounts of fresh water, macroalgae does not require fresh water for growth and is more economical and environmentally sustainable for some regions. By using macroalgae, the sustainability of aquaculture feeds can be increased and the environmental impacts associated with meeting the expanding global demand for seafood can be minimized.

Turkish Towel *Chondracanthus exasperates* is a red macroalgae native to the eastern Pacific Ocean and inland waters. Wild harvest of Turkish Towel is limited, but the species has been raised commercially for cosmetic products for over 15 years and products have been recently developed for the human foods market. Scientists at our research center have demonstrated the ability to intensively rear this macroalgae species, year-round, in land based tanks, and produce plants of consistent nutrient composition (Gadberry et al. 2018). From the results of this prior research, intensively reared Turkish Towel was selected for the present study for its moderate protein content and well-balanced amino acid profile for marine fish. In addition, it was found that Turkish Towel contains moderate levels of taurine, a nutrient that has been shown to improve growth, nutrient retention, and feed efficiency in sablefish fed plant based feeds (Johnson et al. 2015). As with most macroalgae, Turkish Towel also contains minerals and trace nutrients that are absent from plant proteins and likely beneficial to marine fish health.

Sablefish *Anoplopoma fimbria* is a cold water marine fish of the eastern north Pacific Ocean with high market value and is an emerging species for marine aquaculture. Juvenile growth is extremely good (Sogard & Spencer 2004; Forster, Campbell, Morton, Hicks & Rowshandeli 2017) and the species adapts well to tank and salmon style net-pen culture (Luckenbach, Fairgrieve & Hayman 2017; Reid et al. 2017). In addition, sablefish are non-discriminant eaters and have proven useful for alternative feeds research (Friesen, Balfry, Skura, Ikonomou & Higgs 2013a, 2013b; Johnson et al. 2015; Rhodes, Johnson & Myers 2016). In this study, Turkish Towel and taurine were added to plant based feeds for juvenile sablefish to evaluate the potential of these ingredients, alone or combined, to increase the performance of alternative feeds for a cold water marine fish species.

2. MATERIALS AND METHODS

2.1 Fish culture

Juvenile sablefish, approximately 0.5g, were obtained from NOAA's Manchester Research Station, Port Orchard, WA, USA in April 2016 and transitioned to an indoor, recirculating seawater system at the Northwest Fisheries Science Center (NWFSC), Seattle, WA, USA. Water temperature and salinity averaged 13°C and 29 g L^{-1}, respectively, throughout the study. Ammonia, nitrite, and nitrate levels averaged 0.04, 0.17, and 3.4 mg L^{-1}, respectively. Dissolved oxygen averaged 7.9 mg L^{-1} and pH averaged 8.0. Lighting was programmed to mirror the natural photoperiod of Seattle (47° 40' N). Fish were fed a salmon fry feed containing primarily marine ingredients (Bio-Fry, Skretting, Longview, WA, USA) until July 2016, when fish weighed approximately 30g. Fish were then sorted for uniform size and transitioned from the salmon fry feed to a plant based, conditioning feed. Average fish weight was 29.5g at the beginning of the conditioning period. The formulation of the conditioning feed was identical to that of feed 4 used in the subsequent growth trial (Table 1). The purpose of the conditioning period was to increase feed acceptance of experimental feeds during the growth trial and exclude any fish from the study that would not accept plant-based feeds. During conditioning, fish were occasionally sorted to remove small fish that appeared to be refusing the plant based feed. The conditioning period lasted for 4 weeks, after which, average fish weight was approximately 50g.

In August 2016, conditioned fish were sorted for uniform size and randomly distributed among 24 replicate 160L tanks for the growth trial. There were 16 fish in each tank. Six experimental feeds were randomly assigned to four tanks each. The experimental feeds had similar formulations, with the exception of the added taurine and Turkish Towel. Average fish weight at the beginning of the trial was 48.8 ± 0.5g. Sablefish were fed to apparent satiation at a single event, every other day, for 8 weeks and the amount of feed consumed was recorded. From previous research at our laboratory, we have observed juvenile sablefish to have an extended gut evacuation period and consistent feed intake and satisfactory growth can been achieved by feeding this species every other day. Fish were cared for humanely in accordance with guidelines established for laboratory aquatic animals by the National Research Council (National Research Council 2011). Fish growth was assessed at the end of the trial by bulk weighing final fish, by tank, after a 3 day fast and computing percent weight gain and the thermal growth coefficient (TGC) for each tank, from the following equations.

$$\text{weight gain (\%)} = \frac{final\ weight(g) - initial\ weight(g)}{initial\ weight(g)} \times 100\%$$

$$TGC = 1000 \, x \frac{(final\ weight(g)^{1/3} - initial\ weight(g)^{1/3})}{T(°C) \times t(days)}$$

2.2 Experimental feeds

Formulations and chemical compositions of the experimental feeds are shown in Table 1. The study was a 3 × 2 factorial design, resulting in six experimental feeds. The two factors added were taurine and Turkish Towel. No taurine was added to feeds 1-3, while 1% taurine was added to feeds 4-6. In addition, Turkish Towel was added at 5 and 10% in feeds 2 and 3, respectively, and feeds 5 and 6. Experimental feeds were practical plant based feeds produced at the Bozeman Fish Tech Center (Bozeman, MT, USA). The red macroalgae, Turkish Towel, was intensively reared in land based tanks at NOAA's Manchester Research Station, Port Orchard, WA, USA as described previously (Gadberry et al. 2018). Wet Turkish Towel was pressed and then dried in a heated ball mill at 95 °C as described previously (Nicklason, Xu, Johnson, Sommers & Armbruster 2016). Proximate composition and amino acid content of the Turkish Towel ingredient were summarized in Table 2.

The majority of feed protein originated from soy protein concentrate (Solae LLC, St. Louis, MO, USA) and corn protein concentrate (Cargill Incorporated, Minneapolis, MN, USA). Previous studies in our laboratory verified that taurine is absent from these plant ingredients. A small amount of taurine was supplied through the inclusion of fish meal as a minor feed ingredient and the taurine content of the unsupplemented feed (feed 1) was 1.7 g kg^{-1}, significantly below the recommended dietary requirement of 15 g kg^{-1} for optimal growth in this species (Johnson et al. 2015). Experimental feeds were formulated on an isonitrogenous basis. As taurine and Turkish Towel were added, soy and corn protein concentrates, and wheat flour were removed from the formulation to maintain similar nitrogen content among experimental feeds.

Turkish Towel addition had a measurable effect on the ash content of the experimental feeds, with percent ash increasing by about 10 g kg^{-1} for every 5% of Turkish Towel incorporated into a feed. As expected, taurine addition had a significant effect on the taurine content of the feeds with Turkish Towel addition having much less of an effect (Table 1). Feeds were assessed for feed efficiency (FE) and protein retention efficiency (PRE), which were computed by tank, from the following equations,

$$\mathrm{FE} = \frac{final\ weight(g) - initial\ weight(g)}{feed\ consumed(g)} \times\ 100\%$$

$$\mathrm{PRE} = \frac{final\ fish\ protein(g) - initial\ fish\ protein(g)}{protein\ consumed(g)} \times\ 100\%$$

2.3 Sampling

2.3.1. Body indices

At the end of the study, eight fish per tank were euthanized to determine body indices. Fish were euthanized in accordance with procedures developed by the American Veterinary Medical Association (AVMA 2007) prior to collection of tissue samples; namely, fish were anesthetized by immersion in a tricaine methanesulfonate (MS-222, Argent Laboratories, Redmond, WA, USA) bath followed by stunning with an irrecoverable blow to the head. Individual fish lengths were recorded, along with whole body weights and liver weights. Condition factor (CF) and hepatosomatic index (HSI) were determined using the equations below.

$$\mathrm{CF} = \frac{fish\ weight\ (g)}{(fish\ length\ (cm))^3} \times 100$$

$$\mathrm{HSI} = \frac{liver\ weight(g)}{fish\ weight(g)} \times\ 100$$

Tank averages were computed from individual body indices and used for statistical evaluations.

2.3.2. Chemical analysis

Fish were sacrificed for whole body proximate analyses and taurine analyses at the beginning and end of the growth trial. At the beginning of the trial, three whole body composite samples were prepared from five fish each. At the end of the trial, whole body composite samples were prepared from eight fish per tank. Fish were composited by tank and the composites were analyzed in triplicate to determine tank means. In addition, proximate composition and taurine analyses were performed on feed samples in duplicate and results averaged. Moisture content was determined gravimetrically by drying samples to a constant weight in a 105°C oven. Protein, lipid, and ash content were determined in accordance with AOAC Official Methods 968.06, 920.39, and 942.05, respectively (AOAC 2000). Taurine content of feed samples was determined by the University of Missouri Experimental Station Chemical Laboratory (Columbia, MO, USA) in accordance with AOAC Official Method 982.30.

2.3.3. Histology

Liver cross sections from 12 fish per treatment were initially fixed in Dietrich's fixative, rinsed with 70% ethanol after 48 hours, and saved in 70% ethanol for subsequent histological analysis. Fish were selected based on their weight being near the mean weight for the treatment. Fixed samples were sent to Fish Vet Group, Portland, ME, USA for embedding in paraffin wax, sectioning, and hematoxylin and eosin (H&E) staining. Slides were scanned digitally at 40x magnification on a Leica AT2 Scanner and tissues were examined blindly by a single pathologist (Aperio ImageScope). Sections were scored for incidence and intensity of pathological lesions previously observed in this species when reared on alternative feeds (Rhodes et al. 2016). Liver cross sections were quantitatively scored from 0 to 3 to indicate the severity of tissue alterations; 0 = no histologic changes observed; 1 = mild change; 2 = moderate change; 3 = diffuse change. For statistical analyses, liver cross sections scored 1 to 3 were grouped as fish possessing a histopathologic change, while liver cross sections scored as 0 represented fish absent of a histopathologic change.

2.4 Statistical analysis

Differences in fish growth, body indices, feed performance, and whole body chemical composition attributable to the addition of algae or taurine to an experimental feed were statistically evaluated using type III, two-way analysis of variance (ANOVA) with interaction. When the algae × taurine interaction term was not deemed significant, results were re-evaluated using type III, two-way ANOVA without interaction (main effect). In addition, differences in fish growth, body indices, feed performance, and whole body chemical composition means among the six experimental treatments were statistically evaluated using one-way ANOVA, followed by the Tukey HSD *post hoc* test. All differences were deemed significant when $p \leq 0.05$.

Differences in the occurrence of a histopathologic change in the liver attributable to a feed additive were statistically evaluated using Fisher's exact test. Differences were deemed significant when $p \leq 0.05$. All statistical evaluations were performed on R version 3.4.3 statistical software (The R Foundation for Statistical Computing, Vienna, Austria).

3. RESULTS

3.1 Growth and feed performance.

Fish grew well during the growth study with over 93% survival in all tanks. Cannibalism was observed in four tanks that were consequently excluded from the study. Fish percent weight gain increased with the addition of taurine ($p < 0.001$), and to a lesser extent, Turkish Towel ($p = 0.044$) to the experimental feeds with no interaction observed between these two factors (Table 3). As expected, fish TGC followed a similar trend to percent weight gain. Feed intake increased with the addition of taurine ($p < 0.001$) and increased slightly with Turkish Towel ($p = 0.066$). As observed previously at our laboratory, feed efficiency significantly improved with the addition of taurine to the experimental feeds ($p = 0.003$), and there was a slight improvement in protein retention ($p = 0.060$). There was no effect of Turkish Towel addition on feed efficiency or protein retention.

3.2 Body indices and whole body tissue nutrient composition

Fish length significantly increased with the addition of taurine to the experimental feeds ($p = 0.002$, Table 4). There was no effect, however, of either taurine or Turkish Towel addition on fish condition factor. Results were variable, but a significant effect of taurine addition was detected on HSI ($p = 0.004$), with lower HSI observed among fish that had received taurine supplemented feeds.

As expected, whole body taurine content was significantly affected by taurine addition to the experimental feeds ($p < 0.001$). Whole body taurine content slightly increased with the addition of Turkish Towel, but the effect was not significant ($p = 0.095$, Table 4). Increases in whole body lipid were observed with both taurine ($p = 0.008$) and Turkish Towel ($p = 0.012$) addition, with a significant interaction between the two factors ($p = 0.052$). In particular, whole body lipid was higher among fish that had received taurine supplemented feeds and increases in whole body lipid with Turkish Towel addition were greater among fish that had not received taurine supplemented feeds. Whole body protein was similar among treatments, with feed 1 fish having the lowest protein content, numerically. Whole body moisture content was inversely correlated with whole body lipid content and decreases in whole body moisture were observed with taurine ($p = 0.005$) and Turkish Towel ($p = 0.002$) addition, with a significant interaction observed between the two factors ($p = 0.028$).

3.3 Liver histology

Histological examination of fish livers revealed liver histomorphology was generally good among study fish, but some adverse cellular alterations were observed (Table 5). There was no effect of taurine addition on the occurrence of any histopathologic variable, but we did detect reductions in the occurrence of two cellular alterations attributable to Turkish Towel addition (Table 6). In particular, the occurrence of hepatocellular nuclear pleomorphism was reduced to almost a third ($p = 0.050$) and the occurrence of focal areas of vacuolation was reduced by more than half ($p = 0.030$, Figure 1) when Turkish Towel was added to an experimental feed. There was also a trend towards lower occurrence of hepatocellular karyomegaly among Turkish Towel fish, although it did not reach statistical significance. Low amounts of inflammatory cell infiltrates were observed in the majority of study fish livers, and neither taurine nor Turkish Towel addition affected the occurrence of these infiltrates. The presence of inflammatory infiltrates in the livers of apparently healthy cultured fish is not uncommon and typically attributed to the presence of chronic antigenic stimulation; however, the cause of these infiltrates in the present study is unknown. Lastly, there was no detection of necrosis or proliferative lesions in any of the study fish.

4. DISCUSSION

The addition of taurine and the red macroalgae Turkish Towel improved the performance of alternative, plant based feeds for juvenile sablefish. Improvements in fish growth, feed efficiency, and protein retention with taurine addition are similar to those observed previously in our laboratory with sablefish (Johnson et al. 2015), and mirror results from studies with other marine fish species (Lunger, McLean, Gaylord, Kuhn & Craig 2007; Chatzifotis, Polemitou, Divanach & Antonopoulou 2008; Matsunari et al. 2008; Rossi & Davis 2012). Some researchers have reported incremental increases in feed efficiency as taurine is initially added to a feed, followed by decreasing feed efficiencies at higher dietary taurine concentrations (Park, Takeuchi, Yokoyama & Seikai 2002; Jirsa, Davis, Salze, Rhodes & Drawbridge 2014; Kim et al. 2015). It was anticipated that the addition of taurine at a concentration of 10 g kg^{-1} to feeds 4, 5, and 6 in this study would improve feed efficiency. While many of the physiological processes responsible for these increases in feed efficiency have yet to be identified, it is generally believed that taurine modulates fish metabolism and improves the utilization of dietary nutrients (Salze & Davis 2015).

Taurine has antioxidant and cytoprotective characteristics and is suspected to improve health and immunity in fish (Salze & Davis 2015). Taurine is a small water soluble molecule and the leaching of synthetic taurine is an issue with formulated feeds. Leaching rates up to 0.8 mg g^{-1} min^{-1} taurine have been reported in alternative plant based feeds for juvenile cobia *Rachycentron canadum* (Watson, Barrows & Place 2015). As observed in some red macroalgae species, Turkish Towel is a good source of taurine. Supplementing feeds with Turkish Towel, rather than synthetic taurine could alleviate leaching difficulties as macroalgae has evolved to retain small water soluble nutrients in the marine environment. The inclusion of Turkish Towel in the experimental diets in this study resulted in an increase in taurine of 0.2 g kg^{-1} for every 5% Turkish Towel added. This resulted in a total taurine content of 2.1 g kg^{-1} in the 0% taurine, 10% Turkish Towel feed (feed 3). This is well below the recommended levels of 15 g kg^{-1} and 11 g kg^{-1} for optimum weight gain and feed efficiency of sablefish fed plant based feeds (Johnson et al. 2015). Regardless of any reduced leaching that may have occurred with the addition of Turkish Towel in feeds 2 and 3, significant improvements in percent weight gain and feed efficiency were observed when synthetic taurine was further added to these formulations (feeds 5 and 6), and there was no significant interaction detected between taurine and Turkish Towel addition for these parameters. As such, any benefit derived from endogenous taurine in Turkish Towel at a dietary inclusion level of 10% or less is suspected to be minor for juvenile sablefish.

In addition to being a feed for mollusks, macroalgae has been successfully incorporated into the diets of several marine fish species as a minor ingredient (Mustafa et al. 1995; Valente et al. 2006; Walker, Fournier, Neefus, Nardi & Berlinsky 2009; Ragaza et al. 2015; Vizcaino et al. 2016). Unlike many terrestrial plant proteins, macroalgae protein is typically well balanced for fish with only minor deficiencies in a few essential amino acids (Johnson, Kim, Armbruster & Yarish 2014; Garcia-Vaquero & Hayes 2016; Wan et al. 2019). Macroalgae protein is highly digestible by fish, although digestibility is dependent on both macroalgae species and fish species (Pereira, Valente, Sousa-Pinto & Rema 2012). Amino acid deficiencies can be balanced with the use of other feed ingredients. In a 12 week feeding trial, Walker et al. (2009) successfully replaced a portion of the fish meal in Atlantic cod *Gadus morhua* feeds with *Porphyra spp*. As observed with other macroalgae proteins, histidine is limiting in *Porphyra* protein, but is present in ample concentrations in blood meal. As macroalgae was added to the experimental diets, fish meal was reduced, and blood meal was concomitantly increased to yield experimental feeds with similar nitrogen and amino acid content. Feed intake was very similar among groups. Fish growth was also similar, although growth dropped slightly at 11% fish meal replacement. Feed efficiency increased with *Porphyra* addition, but not significantly.

The addition of Turkish Towel to experimental feeds 2, 3, 5, and 6 resulted in increases in fish growth and feed intake, with no significant improvements in feed efficiency. In previous feeding studies with marine fish, the effects of macroalgae addition on feed efficiency are mixed with both increases (Mustafa et al. 1995; Ragaza et al. 2015) and decreases (Davies, Brown & Camilleri 1997; Valente et al. 2006; Xu et al.

2011; Vizcaino et al. 2016) in feed efficiency reported. It has been proposed that differences in feed efficiencies may be related to the amount and type of phycocolloid present in the macroalgae as well as the biology of the fish (Wan et al. 2019). Phycocolloids include alginates, carrageenans, agars, and ulvans which are soluble, non-starch polysaccharides with gelling and emulsification properties. They can improve feed pellet stability, extend gastric passage times, and serve as prebiotics, but they can also chelate nutrients and act as physical barriers to nutrient absorption. In the current study, improvements in sablefish growth with macroalgae addition were associated with increases in feed intake rather than improvements in feed efficiency. Fish fed feeds containing both taurine and Turkish Towel (feed 5 and feed 6) displayed the greatest feed intake and fish growth.

Macroalgae can also facilitate the replacement of fish meal in marine fish feeds with lower cost terrestrial plant proteins as macroalgae contains many essential nutrients from the marine environment that are missing from these terrestrial ingredients (Garcia-Vaquero & Hayes 2016; Wan et al. 2019). In an 8 week feeding trial with Japanese flounder *Paralichthys olivaceus*, Ragaza et al. (2015) incrementally added the red macroalgae *Eucheuma denticulatum* to alternative diets containing 15% soy protein concentrate (SPC). Feed intake was similar among treatments, but fish growth and feed efficiency of fish receiving the SPC feed without macroalgae addition were significantly less than those receiving the fish meal control feed. The addition of macroalgae to the SPC diet improved fish growth and feed efficiency, with the highest improvements observed with 3% *E. denticulatum* addition.

The lower protein content of macroalgae can render direct substitution of macroalgae for fish meal problematic in fish feeds. Valente et al. (2006) investigated the effects of adding macroalgae to European sea bass *Dicentrarchus labrax* feeds. Three different macroalgae species were included in the diet, up to 10% inclusion. The study evaluated the red macroalgae, *Gracilaria bursa-pastoris* and the green macroalgae, *Ulva rigida*, which contained approximately 30% protein. The study also evaluated the red macroalgae, *Gracilaria cornea*, which contained 11% protein. As macroalgae was added to the diet, fish meal and fish protein hydrolysate were removed. As a result, the macroalgae feeds had lower protein content than the control feed. Fish growth and feed efficiency were inferior among fish receiving the *G. cornea* feeds. Fish receiving *G. bursa-pastoris* feeds, however, had comparable growth and feed efficiency to that of the control fish. Fish growth and feed efficiency of fish receiving *U. rigida* feeds were intermediate to fish receiving the two *Gracilaria* feeds.

In a similar study with gilthead sea bream *Sparus aurata*, Vizcaino et al. (2016) fed fish feeds containing up to 25% of either *G. cornea* or *U. rigida* for 10 weeks. As macroalgae was added, maltodextrin, cellulose, and fish meal were removed from the diets to yield feeds that were isonitrogenous and isocaloric. Despite the similarity in feed nutrient profile, fish growth varied with macroalgae species and inclusion level. Fish growth was inversely correlated with the inclusion of *G. cornea* in the diet, while fish growth was the highest among 25% *U. rigida* fish. Combined, results from Valente et al. (2006) and Vizcaino et al. (2016) reinforce the belief that fish response to macroalgae inclusion is often dependent on the macroalgae species, inclusion level, and fish species (Pereira et al. 2012).

In this study, increases in sablefish whole body lipids were observed with both taurine and Turkish Towel addition to the experimental feeds, suggesting an increase in fish nutritional status with these ingredients. Taurine is a principal component of bile acids in fish (Salze and Davis 2015). Taurine addition to marine fish diets increases the concentration of bile acids in the gall bladder (Kim et al. 2007; Kim et al. 2015) and improves lipid digestion (Nguyen et al. 2013; Satriyo, Galaviz, Salze & Lopez 2017; Richard, Colen & Aragao 2017). Increases in whole body lipids have been observed in marine fish when taurine is added to the feed (Qi et al. 2012), but such increases are not typical (Yun et al. 2012; Khaoian, Nquyen, Ogita, Fukada & Masumoto 2014; Wu, Han, Qin & Wang 2015; Satriyo et al. 2017). In a previous feeding study, sablefish whole body lipids were unaffected by taurine addition to plant based feeds (Johnson et al. 2015). Similarly, increases in whole body lipids with macroalgae addition to marine fish feeds has been observed in some studies (Yone, Furuichi & Urano 1986; Davies et al. 1997; Khan, Yoshimatsu, Kalla, Araki & Sakamoto 2008) but not all (Nakagawa, Umino & Tasaka 1997; Valente et al. 2006; Ragaza et al. 2015). Despite these inconsistences, sablefish flesh is valued for its high lipid content and any potential increase in whole body lipids that accompany the addition of either taurine or macroalgae to sablefish feeds would complement industry efforts to produce high quality fish.

It has been suggested that macroalgae protein concentrates may ultimately prove better substitutes for fish meal than whole macroalgae in alternative fish feeds. Protein is naturally concentrated in the spheroplasts of macroalgae and researchers have explored fractionating these spheroplasts from the plant and incorporating them into fish diets. Khan et al. (2008) fed black sea bream *Acanthopagrus schlegeli* feeds containing up to 5% *Porphyra spheroplasts* (PS) for 8 weeks. As PS was added to the feeds, fish meal and starch were removed. All fish exhibited similar feed intake and growth. Fish fed the 3% PS feed, however, had improved feed efficiency, protein retention and lipid retention. Similar to the current study, muscle lipids increased with PS addition and were higher in the 3% and 5% PS fish than the control and 1% PS fish.

An interesting observation in the present study was the reduction in the number of fish with detectable hepatocellular nuclear pleomorphism and focal areas of vacuolation when Turkish Towel was added to an experimental feed. Similar cellular alterations, interpreted to be dysplastic and degenerative changes, and other more advanced pathologies were previously observed in sablefish fed plant based feeds and were speculated to be associated with either a taurine or essential fatty acid deficiency (Rhodes et al. 2016). In extreme cases, taurine deficiency has been associated with green liver syndrome in marine fish due to excessive hemolysis and biliverdin accumulation in the liver (Watanabe et al. 1998, Goto et al. 2001, Takagi et al. 2006a, 2006b). In the present study, final fish livers had normal coloration and the addition of taurine to the experimental diets did not reduce the occurrence of any pathologies. Also, unlike the study conducted by Rhodes et al. (2016), all experimental diets in the present study were formulated to include 14% marine fish oil. As such, the occurrence of these liver changes are unlikely to be a result of a taurine or essential fatty acid deficiency, but may be indicative of other nutritional deficiencies or metabolic problems that are alleviated with the addition of Turkish Towel to the diet.

As reviewed by Garcia-Vaquero & Hayes (2016) and Wan et al. (2019), the consumption of macroalgae has been shown to improve human and animal health. Macroalgae contains antioxidants, immunostimulants, and other bioactive molecules that possess antimicrobial properties in fish (Reverter, Bontemps, Lecchini, Banaigs & Sasal 2014). Xu et al. (2011) found the substitution of starch with dried *Gracilaria lemaneiformis* in the diet of rabbitfish *Siganus canaliculatus* was associated in an increase in lysozyme and alternative complement activity (ACH50), suggestive of an enhanced innate immunity. Kim, Kim, Oh, Jung & Kang (2011) demonstrated the red macroalgae *Polysiphonia morrowii* contains bromophenols that have anti-virial activity against fish pathogenic infectious hematopoietic necrosis virus (IHNV) and infectious pancreatic necrosis virus (IPNV). Similarly, Lozano et al. (2016) demonstrated the addition of *Gracilaria chilensis* and *Pyropia columbina* to the diet of Atlantic salmon *Salmo salar* resulted in fish with sera that had increased antiviral activity against infectious salmon anemia (ISA). Lastly, in a comprehensive evaluation of 26 species of cultured macroalgae, Bansemir, Blume, Schröder & Lundequist (2006) measured the antibacterial activity of dichloromethane, methanol, and aqueous macroalgae extracts against 5 species of pathogenic bacteria common to fish culture systems. The dichloromethane extracts of several macroalgae species showed strong antibacterial activity. Interestingly, the highest level of inhibition was from extracts of red macroalgae species. Aqueous extracts showed no antibacterial activity. As such, these lipophilic antibacterial compounds are likely to accumulate in fish and may eventually reach therapeutic levels when red macroalgae is added to the diet. The red macroalgae Turkish Towel was not included in the former study, but may similarly prove to additionally possess antibacterial properties.

5. CONCLUSION

Sablefish growth significantly increased with the addition of taurine, and to a lesser extent, Turkish Towel to the experimental feeds. Increases in whole body lipid were observed with Turkish Towel and taurine addition and there was an interaction between these two factors. In particular, whole body lipid was higher among 1% taurine fish and increases in whole body lipid with Turkish Towel addition were greatest among 0% taurine fish. Feed efficiency and protein retention significantly improved with the addition of taurine, but were not affected by Turkish Towel addition. Examination of liver histomorphology of final fish indicates Turkish Towel addition may improve liver health. The addition of taurine had no effect on liver histomorphology. Overall, results from this study reaffirm taurine supplementation is beneficial to sablefish receiving plant based feeds, and indicate Turkish Towel may be a promising functional feed ingredient for cold water marine fish. Further research is needed to fully evaluate potential health benefits a Turkish Towel feed ingredient may offer juvenile sablefish receiving alternative feeds.

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DATA AVAILABILITY STATEMENT. Data generated in this study will be made available to interested readers upon request.

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TABLE 1 Formulations and chemical composition of experimental diets (g kg^{-1}) with varying amounts of the red macroalgae Turkish Towel (A) and taurine (T).

Ingredient	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6
	A 0, T 0	A 5, T 0	A 10, T 0	A 0, T 1	A 5, T 1	A 10, T 1
Soy protein concentrate	240	237	234	236	232	229
Corn protein concentrate	200	197	195	195	193	190
Wheat flour	250	206	161	249	205	162
Marine fish oil	140	140	140	140	140	140
Fish meal	100	100	100	100	100	100
Turkish Towel	**-**	**50**	**100**	**-**	**50**	**100**
Taurine	**-**	**-**	**-**	**10**	**10**	**10**
L-Methionine	1.6	1.6	1.6	1.6	1.6	1.6
L-Lysine	2.0	2.0	2.0	2.0	2.0	2.0
Vitamin pre-mix[†]	15.0	15.0	15.0	15.0	15.0	15.0
Stabilized Vitamin C[‡]	1.0	1.0	1.0	1.0	1.0	1.0
Mineral pre-mix[§]	1.0	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	20.0	20.0	20.0	20.0	20.0	20.0
Choline, 70%	5.0	5.0	5.0	5.0	5.0	5.0
Fish gelatin (cod)	20.0	20.0	20.0	20.0	20.0	20.0
Betaine	2.5	2.5	2.5	2.5	2.5	2.5

Chemical analysis (dry wt. basis)						
Protein	481	484	493	480	489	492
Lipid	147	153	152	150	147	151
Ash	40	53	63	42	52	65
Taurine	1.7	1.9	2.1	12.8	13.0	13.2

†ARS 702, contributed per kg diet: vitamin A 14475 IU, vitamin D 9600 IU, vitamin E 198 IU, vitamin K3 1.6 g, thiamin mononitrate 13.7 mg, riboflavin 14.4 mg, pyridoxine hydrochloride 20.6 mg, pantothenate DL-calcium 70 mg, cyancobalamin 0.05 mg, nicotinic acid 32.7 mg, biotin 0.50 mg, folic acid 3.75 mg, inositol 900 mg.

‡L-Ascorbyl-2-polyphosphate, 35% ascorbic acid activity.

§ARS 860, contributed per kg of diet: manganese 13 mg, iodine 5 mg, copper 9 mg, zinc 40 mg.

TABLE 2 Proximate and amino acid composition of intensively reared Turkish Towel at NOAA's Manchester Research Station, Port Orchard, WA, USA. Values are annual means, summarized from Gadberry et al. (2018).

Nutrient	g (kg dry Turkish Towel)$^{-1}$
Proximate composition	
Protein	236.3
Lipid	9.6
Ash	360.5
Essential amino acids	
Arginine	13.5
Histidine	4.0
Isoleucine	10.3
Leucine	16.1

Lysine	11.8
Methionine	3.9
Phenylalanine	10.2
Threonine	9.7
Tryptophan	2.0
Valine	12.5
Non-essential amino acids	
Alanine	14.4
Aspartic Acid	22.9
Cysteine	5.9
Glutamic Acid	27.1
Glycine	11.1
Proline	9.6
Serine	8.7
Tyrosine	6.5
Sulfonic acids	
Taurine	8.2
Total amino acids	200.0

TABLE 3 Growth of juvenile sablefish and feed performance of experimental plant based feeds with varying amounts of the red macroalgae Turkish Towel (A) and taurine (T). Values are mean ± SD. Within a row, mean values with different subscript letters are significantly different by Tukey HSD *post hoc* test at $p \leq 0.05$.

	Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6	*p*-value[§]		
	A 0, T 0	A 5, T 0	A 10, T 0	A 0, T 1	A 5, T 1	A 10, T 1	Algae	Taurine	Algae x Taurine
	n=4	*n=4*	*n=2*[†]	*n=4*	*n=3*[‡]	*n=3*[‡]			
Survival (%)	100 ± 0	98 ± 3	97 ± 4	100 ± 0	98 ± 4	98 ± 4	0.080	0.908	NS
Initial weight (g)[¶]	48.8 ± 0.5	48.4 ± 0.3	48.8 ± 0.2	48.9 ± 0.9	48.9 ± 0.6	48.6 ± 0.2	0.591	0.411	NS
Final weight (g)	157.9 ± 8.7 ^{a}	161.7 ± 6.4 ^{ab}	169.5 ± 12.1 ^{abc}	176.3 ± 7.3 ^{bc}	182.1 ± 1.9 ^{c}	182.5 ± 6.7 ^{c}	**0.044**	**<0.001**	NS
Weight gain (%)	223.7 ± 15.5 ^{a}	234.3 ± 13.6 ^{ab}	247.4 ± 23.5 ^{abc}	261.0 ± 21.1 ^{bc}	272.3 ± 1.1 ^{bc}	275.3 ± 14.3 ^{c}	**0.039**	**<0.001**	NS
TGC	2.37 ± 0.12 ^{a}	2.44 ± 0.10 ^{ab}	2.54 ± 0.17 ^{abc}	2.64 ± 0.13 ^{bc}	2.72 ± 0.01 ^{c}	2.73 ± 0.10 ^{c}	**0.038**	**<0.001**	NS
FI (g fish^{-1})	115.6 ± 7.3 ^{a}	119.5 ± 4.7 ^{ab}	124.9 ± 13 ^{ab}	128.9 ± 9.5 ^{ab}	132.7 ± 1.6 ^{ab}	135.2 ± 7.7 ^{b}	0.066	**<0.001**	NS
FE (%)	94.4 ± 2.9 ^{a}	95.2 ± 3.7 ^{ab}	98.0 ± 1.2 ^{ab}	100.3 ± 1.3 ^{b}	100.1 ± 0.2 ^{ab}	99.0 ± 0.9 ^{ab}	0.583	**<0.001**	NS

PRE (%)	27.6 ± 1.6	29.1 ± 2.7	29.8 ± 1.7	30.1 ± 1.4	30.6 ± 0.7	30.1 ± 1.7	0.279	0.060	NS

†Two tanks excluded from study due to cannibalism. ‡One tank excluded from study due to cannibalism. § Presented p-values are from Type III, 2-way ANOVA, with interaction, when a significant interaction was detected. When no significant interaction was detected (NS), presented p-values are from Type III, 2-way ANOVA, main effect. ¶Initial fish values are mean ± SD of 3 composite samples of 5 fish each.

TABLE 4 Body indices and nutrient composition of juvenile sablefish fed plant based feeds with varying amounts of the red macroalgae Turkish Towel (A) and taurine (T). Values are mean ± SD. Within a row, mean values with different subscript letters are significantly different by Tukey HSD *post hoc* test at $p \leq 0.05$.

								p-value¶		
		Feed 1	Feed 2	Feed 3	Feed 4	Feed 5	Feed 6			Algae
		A 0, T 0	A 5, T 0	A 10, T 0	A 0, T 1	A 5, T 1	A 10, T 1			x
	Initial fish†	*n=4*	*n=4*	*n=2*‡	*n=4*	*n=3*§	*n=3*§	Algae	Taurine	Taurine
Body Indices										
Length (cm)	-	24.9 ± 0.4 ^{a}	25.1 ± 0.3 ^{a}	25.3 ± 0.8 ^{ab}	25.7 ± 0.1 ^{ab}	26.2 ± 0.2 ^{b}	25.8 ± 0.3 ^{ab}	0.229	**0.002**	NS
CF (g/cm^3 x100)	-	1.01 ± 0.03	0.99 ± 0.01	0.98 ± 0.01	1.00 ± 0.05	0.99 ± 0.02	1.01 ± 0.01	0.379	0.804	NS
HSI	-	2.75 ± 0.12 ^{ab}	2.85 ± 0.20 ^{b}	2.38 ± 0.31 ^{a}	2.46 ± 0.13 ^{a}	2.41 ± 0.06 ^{a}	2.42 ± 0.10 ^{a}	0.167	**0.004**	NS

Whole Body Chemical Analysis (g/kg, wet weight)										
Protein	143 ± 1	141 ± 6	147 ± 8	148 ± 7	145 ± 4	148 ± 3	148 ± 2	0.129	0.473	NS
Lipid	80 ± 3	140 ± 3	144 ± 5	151 ± 8	147 ± 3	150 ± 4	147 ± 7	**0.012**	**0.008**	**0.052**
Ash	20 ± 1	19 ± 1	19 ± 1	20 ± 1	19 ± 1	19 ± 1	19 ± 1	0.118	0.906	NS
Moisture	751 ± 1	695 ± 5 ^{b}	686 ± 10 ^{ab}	676 ± 1 ^{a}	684 ± 3 ^{ab}	678 ± 5 ^{a}	682 ± 4 ^{ab}	**0.002**	**0.005**	**0.028**
Taurine	2.5 ± 0.1	0.8 ± 0.0 ^{a}	0.9 ± 0.1 ^{a}	1.0 ± 0.1 ^{a}	2.6 ± 0.1 ^{b}	2.5 ± 0.1 ^{b}	2.6 ± 0.0 ^{b}	0.095	**<0.001**	NS

[†]Initial fish values are mean ± SD of 3 composite samples of 5 fish each. [‡]Two tanks were excluded from study due to cannibalism. [§]One tank was excluded from study due to cannibalism. [¶]Presented *p*-values are from Type III, 2-way ANOVA, with interaction, when a significant interaction was detected. When no significant interaction was detected (NS), presented *p*-values are from Type III, 2-way ANOVA, main effect.

TABLE 5 Mean severity of observed sablefish liver histopathologic changes at the end of the 8 week feeding study (*n=12*). Liver cross sections were quantitatively scored from 0 to 3; 0 = no histologic changes observed; 1 = mild change; 2 = moderate change; 3 = diffuse change.

Histopathologic variable	Feed 1 A 0, T 0	Feed 2 A 5, T 0	Feed 3 A 10, T 0	Feed 4 A 0, T 1	Feed 5 A 5, T 1	Feed 6 A 10, T 1
Nuclear and cytoplasmic alterations						

Hepatocellular karyomegaly	0.25	0.17	0.17	0.33	0.08	0.08
Hepatocellular nuclear pleomorphism	0.33	0.08	0.17	0.25	0.08	0.08
Hepatocellular megalocytosis not due to liver/glycogen deposition	0	0.08	0.08	0.17	0.08	0
Focal hepatocellular vacuolation	0.25	0.17	0.08	0.50	0.08	0.25
Inflammatory infiltrates						
Mononuclear cell infiltrates	0.42	0.33	0.50	0.67	0.33	0.25
Eosinophilic granular cell infiltrates	1.2	1.0	0.9	1.1	0.9	1.0
Necrotic or proliferative lesions						
Hepatic fibrosis	0	0	0	0	0	0
Necrosis	0	0	0	0	0	0
Hepatocellular regeneration	0	0	0	0	0	0
Bile duct/ductile hyperplasia	0	0	0	0	0	0
Vacuolation of biliary epithelium	0	0	0	0	0	0
Oval cell proliferation	0	0	0	0	0	0

TABLE 6 Percentage of sablefish, by diet type, with histopathologic changes observed in the liver at the end of the 8 week feeding study. *P*-values for Fisher's exact test are shown. Number of fish examined for each feed is 12 fish.

Histopathologic variable	Feed type				*p*-value	
	Algae[†] *n=48*	No Algae[‡] *n=24*	Taurine[§] *n=36*	No Taurine[¶] *n=36*	Algae	Taurine
Nuclear and cytoplasmic alterations						
Hepatocellular karyomegaly	10	25	14	17	0.103	0.500
Hepatocellular nuclear pleomorphism	10	29	14	19	**0.050**	0.377
Hepatocellular megalocytosis not due to liver/glycogen deposition	6	8	8	6	0.544	0.500
Clear cell foci	15	38	28	17	**0.030**	0.200
Inflammatory infiltrates						
Mononuclear cell infiltrates	35	50	39	42	0.175	0.500
Eosinophilic granular cell infiltrates	88	96	89	92	0.250	0.500
Necrotic or proliferative lesions						
Hepatic fibrosis	0	0	0	0	-	-
Necrosis	0	0	0	0	-	-
Hepatocellular regeneration	0	0	0	0	-	-
Bile duct/ductile hyperplasia	0	0	0	0	-	-
Vacuolation of biliary epithelium	0	0	0	0	-	-

Oval cell proliferation	0	0	0	0	-	-

[†]Algae feeds include Feed 2, Feed 3, Feed 5, and Feed 6. [‡]No algae feeds include Feed 1 and Feed 4. [§]Taurine feeds include Feed 4, Feed 5, and Feed 6. [¶]No taurine feeds include Feed 1, Feed 2, and Feed 3.

Figure Legend

FIGURE 1 Histological slides of livers from juvenile sablefish fed experimental plant based feeds with (a) no taurine or Turkish Towel added (feed 1), and (b) 1% taurine and 10% Turkish Towel added (feed 6) at the end of the 8 week feeding study. A discrete area of extensive cytoplasmic vacuolation resulting in loss of normal cellular architecture is observed in the feed 1 fish liver. Normal hepatocytes with low amounts of clear to lightly eosinophilic intracytoplasmic material, interpreted to be lipid and glycogen, are observed in the feed 6 fish liver.