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USE OF BIOFUEL BY-PRODUCT FROM THE GREEN ALGAE *Desmochloris* sp. AND DIATOM *Nanofrustulum* sp. MEAL IN DIETS FOR NILE TILAPIA *Oreochromis niloticus*

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Abstract:
Algal by-product meals from the Hawaiian biofuels industry were evaluated as ingredients in diets for juveniles of Nile tilapia (*Oreochromis niloticus)*. Four experimental diets were formulated to fulfill fish nutritional requirements. The diets were made with fish meal, soybean meal, whole diatom (*Nanofrustulum* sp.) meal, or defatted green algae (*Desmochloris* sp.) meal as the test ingredients. A feeding experiment with juvenile tilapia of 2.6 ±0.1 g initial weight was carried out in a freshwater recirculation system with each diet treatment tested in triplicate tanks. Fish were fed the experimental diets to apparent satiation twice a day for 12 weeks and fish weight was measured every three weeks. Water temperature was maintained at 22.7 ±0.8 °C, salinity at 0.1 ±0.0 ppt and dissolved oxygen at 5.6 ±0.5 mg/L. At the end of the experiment a significant effect ($P<0.05$) of diet treatments was found in fish growth and feed utilization, with specific growth rate, food conversion ratio, and retained nitrogen efficiency being highest for the green algae-based diet. Lipid content in the diets was lower than expected for the algae diets. Proximate composition analysis showed no significant difference ($P>0.05$) in the protein content of the fish bodies among the diets. Results indicate the suitability of the green algae by-product meal as an ingredient in Nile tilapia diets. Diatom meal also showed interesting potential for use as an ingredient in tilapia feeds.

Keywords: Nile tilapia, microalgae, diatom, green algae, fish feeds, Hawaii

Introduction

The Hawaiian Archipelago is one of the most isolated population centers in the world, located approximately 2,500 miles from the continental United States. Hawaii currently imports 85-90% of the food sold for human consumption (Leung and Loke, 2008), which clearly indicates sustainability and food security issues. Because no remnant processing facilities for animal products exist on the islands, there is little local protein available for use in animal feeds. The consequence of this is a high production cost for all animal production systems in the region since feeds need to be imported, including aquaculture feeds. Therefore, there is considerable interest in using locally available ingredients for fish feeds manufactured to support the growth of the aquaculture industry in Hawaii and the Pacific Basin region. Fish meal is widely used as a dietary protein source in most commercially farmed fish species, but also is the single most expensive major ingredient in aquaculture feeds (Tacon and Metian, 2008). Among the plant materials that have been tested to replace fishmeal in aquaculture feeds, soybean ingredients have performed satisfactorily for most species (Gatlin et al., 2007; Hardy, 2010). However, soybeans do not yield well as a crop under the prevalent climatic conditions in Hawaii.

The production of biofuels in Hawaii is important not only because of its renewable nature, but also because it is a potential local source of energy, which is relevant due to the fact that Hawaii is the most oil dependent state in the United States of America (Arent, 2009). A recent renewable energy model is to produce algae with high lipid contents which can be converted into biodiesel. This model also presents the opportunity to produce by-products. Thus, the algae-derived biofuels industry is a potential source of microalgae biomass for the animal feeds industry. Various strains of microalgae have been shown to possess appreciable protein content (Becker, 2007). Live microalgae are commonly used as food during the larval rearing of fish. However, few studies have been done with microalgae as a source of macronutrients in dry feeds for juvenile or adult fish. Positive effects on fish growth, protein assimilation, lipid metabolism and final product quality have been reported by the use of algae as a feed ingredient in fish diets (Mustafa and Nakagawa, 1995). Due to the high potential in Hawaii for the production of algal biomass, there is an opportunity to significantly reduce the dependence of imported ingredients for aquaculture feeds by using the by-products from the biofuel industry.

Tilapia aquaculture is widely practiced in many tropical and subtropical regions of the world. The Nile tilapia (*Oreochromis niloticus*) is the most distributed and commercially cultured species globally (El-Sayed, 2006). Nile tilapia is an omnivorous fish, with low stomach pH and a long digestive tract that allows for more effective digestion of plant material than most fish species (Rodrigues et al., 2012). The various enzymes that have been reported in tilapia fish including amylase, pepsin, trypsin, esterases and alkaline phosphatase increases their ability to utilize a wide variety of foods including aquatic larvae and insects as well as algae, weeds, and macrophytes (Tengjaroenkul et al., 2000). These attributes make the species attractive for aquaculture applications where less expensive plant derived protein can be used as the main protein source in feed. Therefore, the aim of this study was to evaluate the use of by-product green algae and diatom meals in diets for *O. niloticus*, and to compare their effect on fish growth and feed utilization to commercial fish meal and soybean-based diets.

Materials and Methods

Diets formulation and preparation

Four experimental diets were formulated to contain the maximum inclusion level of one of the following ingredients: fish meal (FM diet), soybean meal (SBM diet), whole diatom (*Nanofrustulum* sp.) meal (DIA diet), and defatted green algae (*Desmochloris* sp.) meal (ALG diet). Both algae meals were obtained from Cellana Inc., Kailua-Kona, HI, USA. All diets contained fish meal and soy protein concentrate in variable amounts depending on their formulation (Table 1). Diets were formulated to fulfill the nutritional requirements of early juveniles of Nile tilapia. Diet formulations were made with the software MIXIT WIN (V.6.14 Agricultural Software Consultants, San Diego, CA, USA). To prepare the diets, feed ingredients were thoroughly homogenized, mixed with water, and then ground in a 5.7 liter mixer (Professional 600 KitchenAid, St. Joseph, MI, USA). The resulting feed pellets were dried at 60°C for 24 hours in laboratory ovens (130D Thelco, Precision Scientific, VA, USA;

6542 Thermo Fisher Scientific, OH, USA). Feeds were then manually ground with a metal grain mill grinder and sieved to obtain particle size ranges of 0.8-1.2, 1.2-2.0 and 2.0-2.4 mm. The diets were stored in zip lock plastic bags and stored at 8°C in a refrigerator until further use.

Table 1. Formulation (g/kg dry weight) and proximate composition of the experimental diets.

	Diet			
Ingredient	**FM**	**SBM**	**DIA**	**ALG**
Fish meal[1]	584.33	50.29	244.51	143.13
Soybean meal[2]	50.00	746.58	-	-
Diatom meal[3]	-	-	401.25	-
Green algae meal[4]	-	-	-	577.62
Soy protein concentrate[5]	40.00	40.00	280.00	200.00
Dextrin[6]	191.10	-	-	-
Fish oil[7]	40.00	53.13	12.00	19.25
Canola oil[8]	44.57	60.00	12.24	10.00
Alginate[9]	20.00	20.00	20.00	20.00
Vitamin and mineral premix[10]	20.00	20.00	20.00	20.00
Di-calcium phosphate[11]	10.00	10.00	10.00	10.00
Proximate composition (% dw)				
Moisture	5.7	6.9	12.1	6.9
Protein	46.38	42.87	39.72	40.99
Lipid	13.35	12.05	8.61	5.09
Total saccharides	19.49	30.20	15.74	31.21
Ash	15.08	8.03	23.88	15.81

[1] Menhaden. Special Select, Omega Protein Inc., Houston, TX, USA. Crude protein 60% (dw).
[2] Solvent extracted. Hall Roberts' Son, Inc. Postville, IA, USA. Crude protein 46.0%.
[3] Whole cell. Cellana Kailuha-Kona, HI, USA. Crude protein 17.1%.
[4] Deffated. Cellana Kailuha-Kona, HI, USA. Crude protein 31.5%.
[5] Profine VF, Solae, St. Louis, MO, USA. Crude protein: 66.0%.
[6] Dextrin from corn D2131. Sigma-Aldrich Co., USA.
[7] Menhaden. Virginia Prime Gold, Omega Protein Inc., Houston, TX, USA.
[8] Cisco, The J.M. Smucker Co., OH, USA.
[9] Sodium alginate W201502, Sigma-Aldrich Co., USA.
[10] Rovimix, DSM Nutritional Products Mexico.
[11] Dihydrate. Squire, Neogen Corporation, KY, USA.

Experimental design

One feeding experiment was done with Nile tilapia (*O. niloticus*) juveniles produced at the PACRC freshwater fish hatchery in Panaewa, HI, USA. The experimental protocol applied in this research was approved by the Institutional Animal Care and Use Committee, University of Hawaii. The experiment was performed in a freshwater recirculation system which consisted of a biofilter coupled to a sump tank, with twelve rectangular glass aquaria of 72 L volume each. Each aquarium had a water inlet above the water surface and an outlet with a screen filter. Moderate aeration was provided to each aquarium with an air stone connected to a blower line. Water temperature, salinity and dissolved oxygen were monitored daily with a multi-meter (85D YSI, Yellow Springs, OH, USA) and were maintained at 22.7 ±0.8°C, 0.1 ±0.0 ppt, and 5.6 ±0.5 mg/L respectively. The water was maintained at ambient temperatures between 21 to 25°C. Photoperiod in the system was kept at 14L:10D.

Diet treatments were randomly assigned and each experimental diet was tested in triplicate aquaria. Ten tilapia juveniles were stocked in each aquarium with an initial individual weight for all treatments of 2.60 g ± 0.06 g. The fish were fed to apparent satiation twice daily at 09:00 and 16:00 h for 12 weeks. Every day the quantity of consumed food in each aquarium was recorded, and every three weeks the fish were group-weighed to determine growth. Fish samples for whole body proximate composition analysis were taken at the start and at the end of the experiment for each diet treatment. The fish samples were frozen at -10°C, cut into pieces with a knife and homogenized in a blender (SPB-600 Cuisinart, East Windsor, NJ, USA), then dried in an oven at 60°C for 24 h. Thereafter the samples were manually ground in a mortar and kept in zip lock bags at 8°C for further analysis.

Proximate composition analyses

Nutrient composition of experimental diets and fish bodies (three fish per tank) were confirmed through proximate composition analysis of triplicate samples. Total moisture or the inverse dry matter, was determined by the loss of mass from oven drying at 105°C until constant mass was achieved. Protein content was determined by the combustion method (AOAC Official Method 968.06, AOAC 2000). Briefly, a Costech Elemental Combustion System (ECS 4010, Valencia, CA, USA) was used to determine total nitrogen by yielding a percent nitrogen value for the sample. The nitrogen determined was converted to protein content in the sample using the protein conversion factor of 6.25. Ash was determined according to the AOAC Official Method 942.05 (AOAC 2000). Samples were dried in an oven prior to analysis to ensure that the samples were dry when analyzed. Samples were ashed at 500°C for 5.5 h and the percent ash was determined by gravimetrically measuring the difference in weight of the un-combusted and combusted samples. Lipid analysis was performed by use of an ANKOM XT10 (Macedon, NY, USA) extractor according to AOCS Official Procedure Am 5-04 (AOCS 2004). This method determines crude fat by a high temperature (90°C) extraction for 60 minutes using petroleum ether.

Amino acid analysis

All analyses were conducted by NP Analytical Labs (St. Louis, MO, USA) using standard methodologies. Specific procedures were performed to determine amino acid contents for the following groups of amino acids. Acid stable amino acids were determined by hydrolysis with hot HCl, separated on an ion-exchange column, and detected by reaction with ninhydrin. Cysteine and methionine (sulfur containing amino acids) content was determined by oxidation, and amino acid hydrolysis of the sample. The amino acids were then separated on an ion-exchange column, derivatized and quantitated upon comparison with standards that were taken through the same procedure. Tryptophan content was determined by alkaline hydrolysis of the sample, followed by HPLC using UV detection.

Fish growth and feed utilization

At the end of the experiment, for each diet treatment the specific growth rate (SGR) (% per day) of the fish was calculated as the weight gain divided by the time ((Ln final weight-Ln initial weight)/time in days * 100). The feed conversion ratio (FCR) was estimated as the feed intake divided by the fish weight gain (feed weight /weight gain) and the feed efficiency (FE) as the fish weight gain per unit of feed intake (weight gain/feed intake). The protein efficiency ratio (PER) was calculated as the fish weight gain divided by the consumed protein (weight gain/consumed protein). The retained nitrogen (RN) (g/fish) was calculated as the difference between the final and initial body nitrogen content. The retained nitrogen efficiency (RNE) (% nitro-

gen intake) was calculated as the retained body nitrogen multiplied by 100 and divided by the nitrogen intake (RN * 100/ N intake).

Statistical Analysis

All results were analyzed for normality and homogeneity of variance previous to a one-way ANOVA analysis to test for the effects of dietary treatments on fish growth, feed utilization and body proximate composition. If significant differences were found ($P<0.05$), the differences between treatments were analyzed by the Holm-Sidak method. Statistical analyses were done with SigmaStat 3.5 (Systat Software Inc., San Jose, CA, USA).

Results and Discussion

Experimental diets composition

Each of the experimental diets were formulated to contain the maximum level of one of the four test ingredients. Therefore, their proximate composition differed according to the nature of these ingredients (Table 1). At rounded values, all diets contained at least 40% protein, which fulfills the requirement of the species at this age (NRC, 2011). Lipid also varied among diets, with a lipid content lower than expected in the ALG diet, and the highest in the FM diet. Although a true lipid requirement is not specifically defined for each fish species and is influenced by diverse nutritional factors, it is generally assumed that freshwater fish as tilapia cannot tolerate high lipid levels in their diet. The lipid levels in the experimental diets in this study varied from moderate (13.4%) to low (5.1%). The ash content was high in the DIA diet (i.e. 23.9%), which is generally considered high for farmed species. The amino acid profile of the experimental diets is shown in Table 2. For all the essential amino acids, the experimental diets contained levels at or above the requirement for Nile tilapia (NRC 2011). In the case of amino acids known to be deficient in plant ingredients (i.e. methionine and lysine), their content in the diets were above requirement for the species (Furuya et al., 2001, 2012). The ash content in the DIA diet was similarly high as in other studies when diverse algae meals have been tested (Patterson and Gatlin, 2013; Vizcaíno et al., 2014).

Fish growth and feed utilization

The inclusion of algal meals in the diet had significant effects on fish growth and feed utilization. The highest final fish weight, weight gain, and specific growth rate were obtained with the ALG diet and they were significantly different ($P<0.05$) than that obtained with the other diet treatments (Table 3). The SBM diet produced the lowest fish growth, which was significantly different ($P<0.05$) than the other dietary treatments. During the first weeks of the experiment, fish growth was similar among treatments. However, it started to differentiate six weeks after the start of the experiment, and by week 12 the fish growth of the ALG diet compared to the other diet treatments was significantly higher (Figure 1). All experimental diets were well accepted by the fish and no rejection of any diet was observed. However, the feed intake was significantly different ($P<0.05$) among diet treatments, with the highest intake found in fish fed the ALG diet (Table 3). Similarly, the best FCR was obtained with the ALG diet (Table 3). The FE. RN and RNE were all significantly different ($P<0.05$) among feed treatments (Table 3). The highest protein utilization and nitrogen retention were found in fish fed the ALG diet. No significant differences ($P>0.05$) were found between the FM, SBM and DIA diet treatments in nutrient utilization. During the experiment no fish mortalities occurred, thus survival was 100% in all treatments.

Table 2. Essential and nonessential amino acid composition (g/100 g feed dry matter) of the experimental diets.

	Diet			
	FM	**SBM**	**DIA**	**ALG**
Essential amino acids				
Threonine	1.87	1.81	1.86	1.66
Valine	2.24	3.21	2.25	1.90
Methionine	1.26	0.69	0.91	0.74
Isoleucine	1.87	2.01	1.94	1.60
Leucine	3.15	3.33	3.22	2.76
Phenylalanine	1.88	2.29	2.08	1.88
Histidine	1.05	1.17	1.01	0.83
Lysine	3.30	2.67	2.80	2.07
Arginine	2.80	3.12	2.83	2.28
Tryptophan	0.40	0.52	0.45	0.48
Nonessential amino acids				
Aspartic acid	4.27	4.95	4.60	3.45
Serine	1.82	2.20	2.00	1.70
Glutamic acid	6.67	8.33	7.17	4.96
Proline	2.22	2.25	2.13	2.90
Glycine	3.31	2.04	2.53	2.03
Alanine	2.86	2.06	2.44	2.44
Tyrosine	1.35	1.54	1.41	1.13
Cysteine	0.42	0.61	0.56	0.47

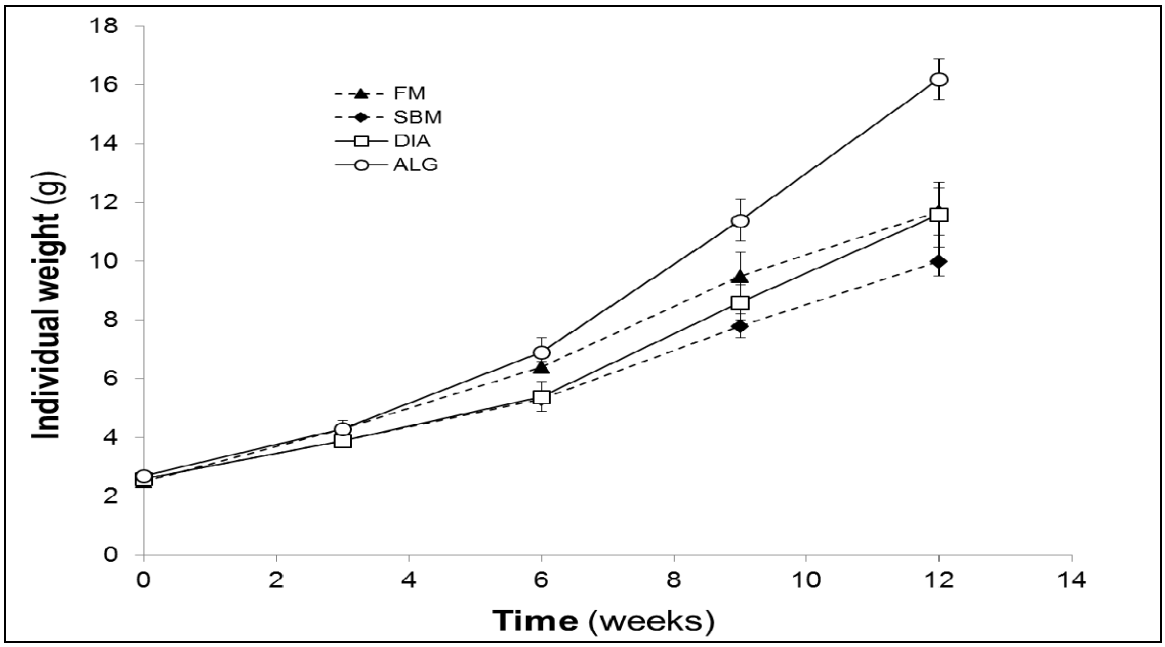

Figure 1. Mean individual weight of Nile tilapia juveniles fed experimental diets made with fish (FM), soybean (SBM), diatom (DIA) and green-algae (ALG) meals as the main protein source during a period of 12 weeks.

Table 3. Mean initial and final fish weight, weight gain, specific growth rate (SGR), feed intake, feed conversion ratio (FRC), feed efficiency (FE), protein efficiency ratio (PER), retained nitrogen (RN), retained nitrogen efficiency (RNE) and survival of Nile tilapia juveniles fed the experimental diets.

	Diet			
	FM	**SBM**	**DIA**	**ALG**
Initial weight (g)	2.53 ± 0.09	2.63 ± 0.03	2.56 ± 0.21	2.67 ± 0.12
Final weight (g)	11.73 ± 1.00^{b}	9.97 ± 0.57^{b}	11.60 ± 1.40^{b}	16.17 ± 0.85^{a}
Weight gain (%)	364.05 ± 41.35^{b}	278.36 ± 17.68^{c}	353.12 ± 38.30^{b}	504.77 ± 17.81^{a}
SGR (% day)	1.82 ± 0.11^{b}	1.58 ± 0.06^{c}	1.80 ± 0.10^{b}	2.14 ± 0.04^{a}
Feed intake (g/fish)	12.92 ± 0.36^{b}	12.10 ± 0.42^{b}	12.90 ± 0.54^{b}	15.57 ± 0.36^{a}
FCR	1.41 ± 0.12^{ab}	1.66 ± 0.10^{b}	1.44 ± 0.16^{ab}	1.16 ± 0.04^{a}
FE	0.71 ± 0.06^{b}	0.61 ± 0.03^{b}	0.70 ± 0.08^{b}	0.87 ± 0.03^{a}
PER	1.53 ± 0.13^{bc}	1.41 ± 0.08^{c}	1.76 ± 0.21^{ab}	2.11 ± 0.07^{a}
RN (g/fish)	0.85 ± 0.09^{b}	0.69 ± 0.05^{b}	0.82 ± 0.11^{b}	1.27 ± 0.07^{a}
RNE (% N intake)	25.97 ± 2.98^{b}	23.35 ± 1.87^{b}	29.85 ± 6.29^{b}	42.45 ± 3.27^{a}
Survival (%)*	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0

Values in the same row with the same superscript are not significantly different (P>0.05).
*No significant differences were found (P>0.05).

Fish proximate composition

At the end of the experiment, significant differences were found in the fish body proximate composition among diets treatments (Table 4). The moisture content was significantly higher (P<0.05) in fish fed the DIA and ALG diets than in the fish fed the FM and SBM diets. In contrast, the lipid content was highest (P<0.05) in fish fed the FM diet followed by the fish fed the SBM treatment. No significant differences (P>0.05) were found in protein and ash content for all diet treatments (Table 4).

Suitability of microalgae meals as protein source

Tilapias have herbivorous/omnivorous feeding habits which position them at the lower levels of the aquatic food chain. This is one of the main reasons plant ingredients are successfully and extensively used in feed formulations for cultured tilapia species. Most of the plant ingredients currently used as protein sources in fish feeds are of terrestrial origin. However, due to the increasing price, future availability, and concerns on the sustainability of terrestrial crops, more research has focused on aquatic organisms as alternative sources of nutrients in aquaculture feeds. Some studies have tested duckweeds (El-Sayed 1992, 1999) and seaweeds (Amor et al., 2005) as protein sources in tilapia diets. Other studies have instead used microalgae to replace fish meal as a protein source in fish diets including tilapia diets (El-Sayed, 1994; Olvera-Novoa et al., 1998; Tartiel et al., 2008;Walker and Berlinsky, 2011; Kiron et al., 2012). The biofuel industry converts the lipid from the microalgae to fuel, but currently there is no major use for by-products left over after the extraction process (by-product meal). Thus, the by-products from this industry are a potential and valuable source of nutrients that can be used in animal feeds. Due to the expanded production of microalgae-derived biofuels, a significant source of nutrients for fish feeds may be available from biofuel production (Brennan and Owende, 2010).

Table 4. Initial and final body composition (% wet weight) of Nile tilapia juveniles fed diets with fish meal, soybean meal, diatom meal and green algae meal as major sources of protein.

	Initial	Final			
		FM	SBM	DIA	ALG
Moisture	74.2 ± 0.0	73.4 ± 0.2[b]	73.4 ± 0.2[b]	74.2 ± 0.1[a]	74.1 ± 0.1[a]
Protein*	15.0 ± 0.0	15.3 ± 1.2	15.2 ± 0.6	14.4 ± 0.4	15.0 ± 0.6
Lipid	6.1 ± 0.0	7.7 ± 0.1[a]	6.8 ± 0.1[b]	6.5 ± 0.1[c]	5.5 ± 0.1[d]
Ash*	4.3 ± 0.2	3.9 ± 0.2	3.6 ± 0.3	4.0 ± 0.1	4.0 ± 0.2

Except for the initial, values in the same row with the same superscript are not significantly different (P>0.05).
*No significant differences were found (P>0.05).

Potential causes for the significant differences found in fish growth in the present study are the protein and lipid contents of the diets, which were higher in the FM and SBM diets compared to the algae-based diets. Although it may be expected that higher protein and lipid contents in the diet produce higher fish growth, these diets yielded lower growth than the ALG diet (Table 3). Although protein and lipid requirements for Nile tilapia were met in all of the experimental diets, higher protein and lipid contents in the FM and SBM diets did not produce better growth, possibly because of the high inclusion levels of fish and soybean meals in these diets. It has been reported that high levels of soybean meal similar to the level used in the present study (SBM diet) can be detrimental to fish growth (Alam et al., 2012). Other factors may have also contributed to the observed difference in fish growth. The essential amino acid requirements for Nile tilapia were fulfilled in all diets despite the differences in amino acid content among diets (Table 2), (NRC 2011). Thus, the difference in fish growth cannot be attributed to dietary essential amino acid deficiency as the highest growth rate was found in fish fed the ALG diet, which had lower methionine and lysine content compared to the FM diet. The combination of fish meal, soy protein concentrate, and algal meal contained in the DIA and ALG diets was sufficient enough to prevent any dietary deficiency in essential amino acids. From the total protein contained in the DIA diet (39.7%), only 6.9% was derived from the diatom *Nanofrustulum* meal, with the rest of the protein derived from fish meal and soy protein concentrate. As for the ALG diet, 18.2% or close to half of the total protein (41%) in the diet was derived from the green algae meal. The contribution of the green algae *Desmochloris* sp. protein in this study was higher than previously reported for Nile tilapia of similar weight fed with diets made with a mix of either microalgae *Chlorella* spp. or *Scenedesmus* spp. (Tartiel et al., 2008). The inclusion level of the diatom *Nanofrustulum* protein in our study was higher than the maximum level tested for common carp (3.8% of total protein) which also resulted in no significant difference in fish growth when compared with a fish meal/soybean meal-based diet (Kiron et al., 2012). However, the same study demonstrated that a level of 2.1% protein from the diatom meal proved detrimental for growth of Atlantic salmon. This is a clear indication that suitability of algal meals as protein source in fish diets and their level of dietary inclusion are dependent on the type of algae and the feeding habits of the target fish.

The level of the maximum microalgae meal inclusion in the ALG diet was similar to a study with *O. mossambicus* in which the fish meal in the diet was optimally replaced by the filamentous cyanobacteria *Spirulina maxima* up to a level of 40% (Olvera-Novoa et al., 1998). However, the protein content in *Spirulina* (i.e. 66.9%) was more than double the protein content in the *Desmochloris* meal (31.5%) used in the present study indicating a different grade of utilization of the algal ingredients based on their composition. The inclusion level of microalgae meals in fish diets may be limited due to their protein content and other factors such as specific nutrient requirements in different fish species, and ingredient digestibility. For example, despite the high protein content in *Spirulina* meal, it could only

replace up to 50% of fish meal in diets for silver sea bream and was suitable at even lower replacement levels for other species (El-Sayed 1994). With current processing practices of microalgae meals, it is still necessary to combine microalgae with other protein sources to partially or completely replace fish meal in diets for fish. The higher fish growth obtained with the green algae-based diet compared to the diatom-based diet may be due to the digestibility and attractiveness of the algal ingredients. The diatom meal was used in the diet as an intact cell which included the frustule or cell wall. The diatom cell wall is made of silica, and thus is difficult to digest or was possibly un-digestible for the fish. Consequently, the FE in fish fed the DIA diet was significantly lower than the one obtained in fish fed the ALG diet (Table 3). This can partially be discerned from the higher ash content in the DIA diet (Table 1) as typically, the higher the ash content in the diet the lower the feed efficiency in fish (Shearer et al., 1992). The breaking or removal of the silica cell wall of diatom material during processing may improve its value as a feed ingredient in fish feed. Nonetheless, high ash levels up to 25% in diets containing diatom meal did not produce detrimental effects on fish growth in tilapia (the present study) and common carp (Kiron et al., 2012), species that have herbivorous/omnivorous feeding habits. However, a different result is probable when these meals are tested in carnivorous fish. For example, feeding algae to rainbow trout, a carnivorous fish, is detrimental for growth (Barrows and Frost, 2014). Although feeding a diet with *Spirulina* meal as the sole protein source to common carp, an herbivorous fish, produced similar fish growth as a control diet made with fish meal (Nandeesha et al.,, 1998). Potential problems with aluminum accumulation in fish due to the algae mineral content were prevented by the dietary inclusion of di-calcium phosphate, which assists in the neutralization of aluminum in fish when fed diets containing high levels of algae meals (El-Sayed Hussein et al.,, 2014).

The feed intake was higher and the FCR was lower in tilapia fed the ALG diet compared to the values obtained with the FM diet; these values were also superior to those found in another study with Nile tilapia using fish meal, wheat bran, and sunflower cakes as protein sources (Maina et al., 2002). This may indicate one advantage (diet acceptability) of using microalgae as sources of protein over terrestrial plant proteins and fish meal by herbivorous/omnivorous fish species. Nitrogen retention in fish was not affected by algal meal inclusion in the diets compared to the fish meal-based control diet; it actually improved when the green algae *Desmochloris* was used as a protein source (Table 3). This could be the result of the type of protein present in the tested ingredients as the protein structure, size and functionality in fish meal may differ from those in the algal meals; these are factors that can affect protein utilization by fish. Omnivorous or herbivorous fish have different capacities for food digestion than carnivorous fish. Feeding tilapia with fish meal protein does not optimize the nitrogen retention as the species digestive system is adapted to utilize plant proteins. Using aquatic organisms (macrophytes, macroalgae, and microalgae) compared to angiosperms (cereals, legumes) as protein sources in fish feeds may follow the same rationale, as herbivorous and omnivorous fish have evolved ingesting and utilizing nutrients from aquatic sources.

Conclusions

As ingredients in fish feeds, microalgae meals derived from biofuel production represent an interesting alternative to fish meal and plant proteins of terrestrial origin, such as soybean products. In the present study, high inclusion levels of microalgae meal in the diets were achieved in comparison with previous research reported in tilapia. Nonetheless, the feasibility of using microalgae meals as protein and lipid sources in commercial fish feeds not only depends on their nutrient quality and utilization by fish, but also on their cost-effective production and sustained availability.

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