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A defatted microalgae meal (*Haematococcus pluvialis*) as a partial protein source to replace fishmeal for feeding juvenile yellow perch *Perca flavescens*

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Abstract
The objective of this study was to investigate the potential of a co-product, defatted microalgae meal (*Haematococcus pluvialis*), as a feed ingredient for yellow perch (*Perca flavescens*). A mixture of the ingredient combining the algae meal and soy protein isolate (at a ratio of 1:1) was added to the control diet at levels of 10, 20, or 30% to replace 25, 50, or 75% of fishmeal in a control diet. Yellow perch (initial body weight, 13.1 ± 1.6 g; 30 fish/tank; $n = 3$ tanks) were fed the test diets for 8 weeks in an indoor system with flow-through water at 22 °C. The results showed that replacement of 25% fishmeal with the combined mixture had no adverse effect on the growth performance, proximate composition, and serum biochemical indexes compared with the control diet ($P > 0.05$). However, fish fed the diets with 50 or 75% fish meal replacement were shown to have significantly reduced growth compared to fish fed the control diet or the diet with 25% fish meal replaced ($P < 0.05$). Increased use of the combined ingredient to replace 50% fishmeal in the current formulations may have led to nutrient imbalance such as amino acids, or minerals in the test diets. Supplementation of limited nutrients into the defatted algae meal may potentially increase the potential of the byproduct used as a feed ingredient. This needs to be investigated in future study. Results of this study indicate that the defatted microalgae meal blended with soy protein isolate can be used to (10% of the diet) replace 25% of the fish meal in the test diet without compromising the performance of yellow perch under current testing conditions.

Keywords Alternative ingredient · Growth · Microalgae meal · Soy protein isolate · Yellow perch

Introduction

Fishmeal contains a high level of palatable, digestible, and essential nutrients, which makes it a highly desirable aquafeed component, especially for carnivorous finfish species (NRC 2011). However, with global fisheries approaching unsustainable limits, fishmeal production will be inadequate to support the cost-effective demand for aquafeeds (Tacon and Metian 2008; Shepherd and Jackson 2013). In response, the aquafeeds industry is shifting to crop-based feed ingredients (Hardy 2010; Olsen and Hasan 2012). Accordingly, the application of fishmeal and fish oil in fish feed has decreased significantly (Tacon and Metian 2008). The use of plant ingredients in aquafeeds alleviates the pressure on fish and fishmeal sources, but in response to increased application, crop commodity prices have also been increasing. The commodity prices of corn and soybean meal increased 100% from 2004 to 2014 (http://www.indexmundi.com/commodities), making these sources less attractive. Increased use in plant raw materials also puts aquaculture feed production in direct competition with the human food supply and increases the demand on natural resources such as arable land, water, energy, and fertilizer. Recently, Fry et al.(2016) estimated that between 31 and 35 km^3 of water were used to grow crops for commercial aquaculture feed in 2008, suggesting that the water footprint associated with aquaculture feed increases with the growing use of terrestrial crop-based ingredients.

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Therefore, there is a need to seek additional resource-efficient ingredients, which will enable the aquafeed industry to keep pace with the rapid expansion of worldwide aquaculture production, while preventing the losses due to decreased productivity, increased cost, or compromised product quality.

Microalgae have much higher biomass productivities with lower rates of water renewal than terrestrial crops and they may be cultivated in brackish water with fewer nutrient requirements (Amaro et al. 2011). Microalgae meals have been shown to be potentially used as a fishmeal replacement or feed additive in aquafeeds (Shah et al. 2018). They can be used to partially replace fishmeal in feeds for shrimp and fish including Pacific white shrimp *Litopenaeus vannamei* (Ju et al. 2012; Basri et al. 2015), gilthead sea bream *Sparus aurata* (Vizcaíno et al. 2016), Atlantic salmon *Salmon salar* (Kiron et al. 2016), and common carp, *Cyprinus carpio* (Kiron et al. 2012). Some microalgae meals have also been reported to play a role in enhancing pigmentation (Zaťková et al. 2011) and improving stress resistance (Dagar et al. 2010) in different culture species.

Haematococcus pluvialis is a microalgae species that has attracted the attention of aquatic feed researchers. As a natural source of astaxanthin, *Haematococcus* meal is still too expensive to be used as feed ingredient due to its high production cost (Lorenz and Cysewski 2000). However, the defatted meal of this microalgae, a co-product generated from astaxanthin production, has been demonstrated to be a potential protein source in Pacific white shrimp (Ju et al. 2012) and longfin yellowtail *Seriola rivoliana* (Kissinger et al. 2016). Application of this defatted meal in feeds may require validation owing to species-specific differences in nutrient requirements, feeding physiology and behavior. Therefore, it is critical to evaluate the potential of defatted meal as a feed ingredient in different culture species.

Yellow perch (*Perca flavescens*), a carnivorous cool water fish that are an ecologically and economically important species in the Great Lakes Region of the USA (Malison 2003; Hinshaw 2006). Yellow perch are sold to retailers and restaurants primarily as skin-on fillets with a retail value often exceeding US$33.2 kg^{-1} (Direct 2015). This fish has become a highly sought after seafood product in the Great Lakes region due to its firm flesh with low fat content, a long shelf life and minimal problems with off-flavor (Malison 2003). Existing commercial yellow perch feeds are based on formulations developed for rainbow trout. Thus, the diet is not cost-effective due to high protein sources from fishmeal and other land-based crops (Hinshaw 2006).

Haematococcus defatted meal contains a similar amino acid profile (amino acid % protein) compared to fishmeal, but it has a relatively low level of total protein (about 40% crude protein). Consequently, the low protein level of the defatted algae meal (DMM) may limit its application in a diet for carnivorous fish such as yellow perch. This limitation, however, may be compensated for when combined with another high protein ingredient, such as soy protein isolate (SPI), as a dietary protein source. Thus, the aim of this study was to investigate the effects of fishmeal replacement by defatted *Haematococcus* meal blended with SPI on growth performance body composition, and fish health based on serum biochemical indices of juvenile yellow perch.

Materials and methods

Test diet preparation

The defatted microalgae meal *Haematococcus pluvialis* was obtained from Cyanotech Corporation (Kona, Hawaii, USA). It is a co-product generated from production of astaxanthin from *H. pluvialis*. Nutrient compositions of this DMM, the soy protein isolate and fishmeal are presented in Table 1. Four test diets (Table 2) were iso-nitrogenous and iso-lipidic. The control diet composed of 40% fishmeal and the other three test diets included increasing levels (10, 20, and 30%) of the (1:1) mixture of DMM/SPI to replace 25, 50, or 75% of fishmeal in the control diet. The test diets were designated as D-0%: D-25%, D-50%, and D-75% based on the level of fishmeal replaced. All dry feed ingredients were pulverized to less than 400 μm particles, weighed accurately (~0.1 g), and mixed by using a Hobart mixer (K5-SS, Hobart Corporation, USA) to form a homogeneous mixture. The dry mixture was then blended with 50% boiled water (80 °C, *w*/*w* total dry mixture) and then oils to mix completely to form homogeneous moist dough, which was then extruded through a Hobart meat grinder. The resultant moist pellets were sealed by foil and then baked in an oven at 80 °C for 15 min to increase the gelatinization of carbohydrate. Subsequently, diets were dried at 21 °C for about 48 h with blowing air in a laboratory fume hood until the moisture content was less than 10%. The dry pellets were crumbled and sieved to generate suitable sizes (0.85~2.0 mm and 2~4 mm in diameter) of pellets used for the feeding trials. All test diets were packed and stored at 4 °C until use. The proximate composition and amino acids of the test diets are presented in Table 3.

Nutrient compositions of ingredients and test diets

The defatted microalgae meal contained 38.6% protein, 3.4% lipid, and 12.9% ash versus 63% protein, 9.3% lipid, and 19% ash in fishmeal (Table 1). The algae meal contained 9.6% fiber but fishmeal only had 0.7% crude fiber. The concentrations of indispensiable amino acids in the defatted meal are low compared to those in the fishmeal. The concentrations based on protein content (amino acid profiles) for most amino acids were similar to those from fishmeal except for arginine, histidine, lysine, methionine, and glutamate plus glutamine.

Table 1 Nutrient compositions (g per 100g diet) of defatted microalgae meal (DMM), soy protein isolate (SPI), and menhaden fishmeal (FM)

Nutrients	DMM	SPI	FM	DMM and SPI (1:1, g/g)
Crude protein	38.6	91	63	64.8
Crude lipid	3.4	0.8	9.3	2.1
Crude ash	12.9	4.1	19	8.5
Crude fiber	9.6	0.25	0.7	4.93
Moisture	2.75	6	7.2	4.38
Total carbohydrates	41.9	0.25	0.7	21.08
Calcium	0.63	0.15	5.19	0.39
Phosphorus	0.79	0.8	2.88	0.80
Dispensable amino acid	g per 100 g diet as fed (g per 100 g protein)			
Ala	3.73 (9.66)	3.60 (3.96)	4.27 (6.78)	3.67 (5.66)
Asp+Asn	3.81 (9.87)	10.20 (11.21)	6.11 (9.70)	7.01 (10.82)
Cys	0.54 (1.40)	1.10 (1.21)	0.64 (1.02)	0.82 (1.27)
Glu+Gln	3.73 (9.66)	17.50 (19.23)	8.68 (13.78)	10.62 (16.39)
Gly	2.89 (7.49)	3.60 (3.96)	4.36 (6.92)	3.25 (5.02)
Pro	2.08 (5.39)	4.90 (5.38)	3.12 (4.95)	3.49 (5.39)
Ser	2.00 (5.18)	4.50 (4.95)	2.47 (3.92)	3.25 (5.02)
Indispensable amino acids				
Arg	2.58 (6.68)	6.70 (7.36)	4.83 (7.67)	4.64 (7.16)
His	0.81 (2.10)	2.20 (2.42)	1.87 (2.97)	1.51 (2.33)
Ile	1.73 (4.48)	4.30 (4.73)	2.59 (4.11)	3.02 (4.66)
Leu	3.35 (8.68)	6.80 (7.47)	4.41 (7.00)	5.08 (7.84)
Lys	2.08 (5.39)	5.60 (6.15)	5.28 (8.38)	3.84 (5.93)
Met	0.77 (1.99)	1.10 (1.21)	2.01 (3.19)	0.94 (1.45)
Phe	2.04 (5.28)	4.70 (5.16)	2.82 (4.48)	3.37 (5.20)
Thr	2.39 (6.19)	3.20 (3.52)	2.89 (4.59)	2.80 (4.32)
Tyr	1.39 (3.60)	3.80 (4.18)	2.33 (3.70)	2.60 (4.01)
Val	2.66 (6.89)	4.20 (4.62)	3.15 (5.00)	3.43 (5.29)

Most of the amino acid concentrations were increased to those observed in fishmeal, except for methionine, arginine, histidine, and lysine when the DMM was equally blended with soy protein isolate (1:1). Very low levels of calcium and phosphorus were detected in the DMM and soy protein isolate compare to the fishmeal. With the increased levels of fishmeal replacement, the test diets tended to have decreased levels of ash, methionine, and lysine (Table 3).

Maintenance and feeding of fish

Yellow perch were produced from brood stock cultured at the Great Lakes Aquaculture Center (University of Wisconsin-Milwaukee, USA) and cultured in a 4 m^3 aquaria until they are used for the current study (Rosauer et al. 2011).

Two weeks before the feeding trial, 720 fish (60 fish per tank) were selected and distributed into 12 tanks (350 L) for acclimation. The indoor culture system was running with dechlorinated municipal flow-through water (about 5 L min^{-1}) at a temperature of 22 °C. During the acclimation period, fish were fed a mixture of four test diets, in equal proportion, to apparent satiation three times daily (9:00, 12:00, and 15:00). Yellow perch is vulnerable to stress including handling and diet weaning. With the current feed formulations varied with some major ingredients for protein sources, using the same mixture diet for conditioning would provide the fish the same initial status before they were started with a designated diet. The fish were easier to be weaned to their test diets.

Prior to the feeding trial, fish were fasted for 24 h and then pooled into a larger tank, and then 360 fish of similar size (average body weight 13.1 ± 1.6 g, $n = 30$) were selected and distributed into each tank with 30 fish per tank. Each test diet was randomly assigned to triplicate tanks. Fish were hand-fed three times daily (09:00, 12:00, and 15:00) at a daily feeding rate of 3% of body weight for 8 weeks. Fish were batch-weighed in water containing stress coat (1.5 mL per 10 L water; Fishcare North America, Inc., USA) every 2 weeks to obtain growth data, and feed rations were adjusted accordingly. The care, handling, and sampling of fish followed the animal care protocols approved by the Animal Care and Use Committee, University of Wisconsin-Milwaukee.

Table 2 Formulation of the experimental diets (% as fed)

Ingredients	D-0%[g]	D-25%[g]	D-50%[g]	D-75%[g]
Menhaden meal[a]	40	30	20	10
Wheat starch[b]	20	19	18	17
Corn protein concentrate[c]	19.75	19.75	19.75	19.75
Wheat gluten[d]	2	2	2	2
Menhaden oil[b]	5	6	7	8
Soybean oil[e]	3	3	3	3
Sodium alginate[d]	2	2	2	2
CMC-Na[d]	2	2	2	2
Mineral premix[b,e]	1.5	1.5	1.5	1.5
Vitamin premix[b,e]	3	3	3	3
Dicalcium phosphate[d]	0.9	0.9	0.9	0.9
Choline chloride[d]	0.1	0.1	0.1	0.1
Defatted *Haematococcus*[f]	0	5	10	15
Soy protein isolate[b]	0	5	10	15
Ascorbyl palmitate[d]	0.05	0.05	0.05	0.05
Cr_2O_3[d]	0.7	0.7	0.7	0.7
Total	100	100	100	100

[a] Omega Protein Corporation, Houston, TX, USA

[b] MP Biomedicals, LLC, Santa Ana, CA, USA

[c] Gavilon LLC, Omaha, NE, USA

[d] Sigma-Aldrich Co. LLC

[e] Aqua Solution, Inc.; the same formulation used by Moon and Gatlin (1991)

[f] Cyanotech Corporation, Kona, HI, USA

[g] The control diet was primarily composed of 40% fishmeal and the other three test diets were included increasing levels (10%, 20%, and 30%) of the (1:1) mixture of Deffated haematococcus: soy protein isolated to replace 25%, 50%, or 75% of fishmeal in the control diet. The test diets were designated as D-0%: D-25%, D-50%, and D-75% based on the level of fishmeal replaced

Table 3 Proximate composition and amino acids of the experimental diets (% as fed)

Index	D-0%	D-25%	D-50%	D-75%
Crude protein	41.7	40.8	40.8	40.9
Crude lipid	12.6	11.6	11.7	12.3
Crude ash	10.5	9.5	8.4	7.1
Moisture	7.7	6.7	6.8	8.1
Gross energy(kJ g^{-1})	17.5	17.3	17.9	17.8
Dispensable amino acid	g/100 g diet as fed			
Ala	2.9	2.77	2.73	2.64
Asp	3.1	3.14	3.26	3.37
Cys	0.43	0.44	0.46	0.48
Glu	7.14	7.22	7.44	7.61
Gly	2.23	2.03	1.87	1.71
Pro	2.47	2.48	2.47	2.54
Ser	1.89	1.87	1.96	2.06
Indispensable amino acids				
Arg	1.92	1.94	2.01	2.06
His	0.89	0.89	0.89	0.9
Iso	1.5	1.6	1.62	1.61
Leu	4.29	4.31	4.38	4.39
Lys	1.9	1.78	1.65	1.58
Met	1.03	0.94	0.88	0.79
Phe	2.02	2.09	2.12	2.15
Thr	1.54	1.5	1.52	1.53
Trp	0.32	0.34	0.35	0.39
Val	1.76	1.84	1.85	1.82

During the feeding trial, water quality and photoperiod were maintained to meet the optimal growth of this fish. Water temperature and dissolved oxygen were continuously monitored by automatic sensors. Other water quality parameters were monitored in the morning once a week. During the growth study, the water temperature was 22–24 °C; dissolved oxygen, > 6.0 mg L^{-1}; total ammonia nitrogen, < 0.08 mg L^{-1}; pH, 7.0–8.0. The photoperiod was maintained at 12 h:12 h light/dark.

Sample collection and analysis

At the end of the 8-week trial, all fish were fasted for 24 h before they were batch-weighed and counted to obtain final values for survival and tank biomass. Four individuals were euthanatized with overdose MS222 and collected for whole-body proximate composition (moisture, crude protein, lipid, ash) analyses, enabling evaluation of protein efficiency ratio, and protein and energy retention. Another three fish from each tank were also euthanatized for measurement of individual body weight and body length values to calculate the condition factor (CF). Subsequently, these three fish were dissected to measure their liver, viscera, and viscera fat. Value of heapatosomatic index (HSI), viscerosomatic index (VSI), and viscera fat index (VFI) were calculated accordingly.

Blood samples from four fish/tank were collected via caudal puncture of the hemal arch using a 1.5-mL non-heparinzed syringe. Blood samples were clotted in ice for 4 h and then centrifuged at 4000×*g* for 20 min at 4 °C. Serum was collected and stored at − 80 °C until used. Serum chemistry parameters were determined using the Abaxis VetScan VS2 Veterinary Chemistry Analyzer (USA). For each sample, 100 μL of serum was used to determine the following parameters using a disposable Comprehensive Diagnostic Rotor (part number #500-0038): albumin, alkaline phosphatase, alanine amino transferase, amylase, calcium, globulin, glucose, total bilirubin, inorganic phosphorus, and total protein.

Proximate analysis of experimental diets and fish samples were conducted following the methods by AOAC and methods described below. Fish moisture content was

measured by drying samples in a vacuum freeze dryer for 48 h to reduce moisture content to a level of < 95%, and then subsamples from the freeze-dried samples further dried in an oven at 105 °C for 24 h. Protein content was determined by measuring nitrogen (N×6.25) levels using an elemental combustion system (ECS 4010 Nitrogen/protein analyzer, Costech Analytical Technologies, USA). Lipid content was determined by ether extraction using a Soxhlet Unit (Soxtec 8000 Foss, Denmark). Ash content was determined using a muffle furnace at 550 °C for 12 h. Energy content was analyzed using an Automatic Adiabatic Bomb Calorimeter (SDACM 4000 Hunan Sundy Science and Technology Co., Ltd., China). Amino acid content was determined by AMINOLab of Evonik (Beijing, China).

Data calculation and statistical analysis

Data are presented as mean ± SD of three replicates. All data were subjected to one-way analysis of variance (ANOVA). When overall differences were significant ($P < 0.05$), Tukey's test was used to compare the mean values between the treatments. When the test of homogeneity of variances failed, the Games-Howell's test was used. Statistical analyses were performed using SPSS 19.0 for Windows (SPSS, USA).

Results

Growth performance

The growth performance and feed utilization of yellow perch fed the test diets for 8 weeks are shown in Table 4. Survival was 100% across all tanks and treatments. Yellow perch fed the D-0% and D-25% diets were shown to have similar mean final fish body weights and weight gain (WG), which were significantly higher than those of fish fed D-50% and D-75% ($P < 0.05$) diets. Feed conversion ratio (FCR) values did not vary significantly ($P \geq 0.05$) among all treatments except for fish fed the D-75% diet, which had significantly higher FCR than the fish fed the control D-0% diet. Fish fed the D-50% and D-75% diets exhibited significantly ($P < 0.05$) reduced HSI, VSI, and CF compared to those fed with the D-0% diet. VFI was similar among all fish fed the different test diets. Mean values for protein efficiency ratio (PER) and energy

Table 4 Growth performance of yellow perch fed test diets for 8 weeks

Index	D-0%	D-25%	D-50%	D-75%	*P* valve
IBW[a]	13.0 ± 0.6a	13.3 ± 0.4a	13.1 ± 0.3a	13.0 ± 0.1a	0.707
FBW[b]	37.7 ± 1.3b	36.9 ± 0.9b	31.6 ± 0.9a	31.3 ± 0.6a	0.000
WG[c]	191.5 ± 17.6b	177.3 ± 2.4b	142.3 ± 12.6a	141.2 ± 5.9a	0.001
FI[d]	31.0 ± 0.7a	31.4 ± 0.9a	28.3 ± 0.6b	27.8 ± 0.1b	0.002
FCR[e]	1.17 ± 0.08a	1.19 ± 0.01ab	1.39 ± 0.14ab	1.40 ± 0.05b	0.016
PER[f]	1.92 ± 0.14a	1.76 ± 0.06ab	1.58 ± 0.11b	1.62 ± 0.05b	0.010
PR[g]	36.4 ± 2.5a	31.0 ± 2.9a	30.3 ± 3.3a	30.2 ± 1.7a	0.054
ER[h]	42.3 ± 3.7a	38.2 ± 1.4ab	33.4 ± 2.6b	36.1 ± 1.8ab	0.014
CF[i]	1.15 ± 0.08a	1.09 ± 0.07ab	1.05 ± 0.08b	1.01 ± 0.05b	0.000
HSI[j]	2.47 ± 0.39a	2.00 ± 0.5ab	1.85 ± 0.26b	1.61 ± 0.59b	0.000
VSI[k]	15.7 ± 2.1ba	14.0 ± 1.3ab	13.3 ± 2.0b	13.5 ± 1.4b	0.011
VFI[l]	7.6 ± 1.4a	8.0 ± 1.0a	7.8 ± 1.2a	8.2 ± 1.0a	0.662

Data presented as mean ± SD, $n = 3$. Means in the same line sharing the same or none lowercase letters are not significantly different, as determined by Turkey's test ($P > 0.05$)

[a] IBW (g) = initial mean weight

[b] FBW (g) = (final total fish weight per tank, g)/final fish number per tank

[c] WG (percentage of weight gain, %) = (FBW − IBW)/IBW × 100

[d] FI (g) = feed intake per fish during 8 weeks

[e] FCR (feed conversion ratio) = (feed intake per tank, g)/(total final fish weight, g − total initial fish weight, g + dead fish, g)

[f] PER (protein efficiency ratio) = (fish weight gain, g)/(protein fed, g)

[g] PR (protein retention, %) = 100 × (final fish body protein, g − initial fish body protein, g)/(protein fed, g)

[h] ER (energy retention,%) = 100 × (final fish body energy, J − initial fish body energy, J)/(energy fed, J)

[i] CF (condition factor, g cm^{-3}) = (body weight, g)/(body length, cm)^3 × 100

[j] HSI (hepatosomatic index, %) = 100 × (liver weight, g)/(body weight, g)

[k] VSI (viscerosomatic index, %) = 100 × (viscera weight, g)/(body weight, g)

[l] VFI (viscera fat index, %) = 100 × (viscera fat, g)/(body weight, g)

Table 5 Proximate whole body composition of yellow perch fed test diets for 8 weeks

Index	D-0%	D-25%	D-50%	D-75%	*P* value
Moisture (%)	67.5 ± 1.0a	68.4 ± 0.2a	68.2 ± 0.6a	67.8 ± 0.4a	0.075
Crude protein (%)	16.5 ± 0.2a	15.5 ± 0.7a	16.1 ± 0.8a	15.8 ± 0.9a	0.402
Crude lipid (%)	11.0 ± 0.4a	10.5 ± 0.2a	10.3 ± 0.5a	11.3 ± 0.6a	0.092
Ash (%)	4.3 ± 0.1a	4.2 ± 0.1a	4.3 ± 0.0a	4.0 ± 0.1b	0.006

Data presented as mean ± SD, $n = 3$. Means in the same line sharing the same letter are not significantly different, as determined by Tukey's test ($P > 0.05$)

retention (ER) decreased in fish fed D-50% and D-75%, but there were no significant differences in protein retention (PE) among these diets.

Proximate composition of whole fish

Whole-body values for moisture, crude protein, and lipid did not vary significantly among dietary treatments (Table 5). However, yellow perch fed the D-75% diet showed the lowest ash content, which was significantly lower than the ash contents in fish fed the other three diets. Fish fed D-0%, D-25%, and D-50% had similar levels of ash contents.

Serum biochemical indices

Serum biochemical indices of yellow perch fed test diets for 8 weeks are presented in Table 6. Yellow perch fed the D-25% diet had a significantly higher serum phosphorus content than the fish fed the D-75% diet, and no significant difference was observed in the fish fed the other three treatments. Different dietary treatments had no significant effects on other serum biochemical indices including albumin alkaline phosphatase, alanine amino transferase, amylase, calcium, globulin, glucose, and total protein levels.

Discussion

Microalgae may provide nutrients such as protein, minerals, and fatty acids needed by aquatic species (Hemaiswarya et al. 2011; Shah et al. 2018). However, a high production cost and poor utilization of nutrients from some species of microalgae remain as drawbacks, and thus limit the extended applications of microalgae meal as a major ingredient (Sarker et al. 2016). Utilization of already existing co-products sources may be economically feasible for both aquatic feed industry and algae producers. This will need to be more systematically explored.

The DMM used in the current study contained a low level of crude protein (38% crude protein) as well as amino acids compared to the fishmeal (63% crude protein). Thus, it is not optimal to be used as a major protein source in feed for carnivore fish, such as yellow perch. The levels of crude protein, arginine, histidine, and lysine were improved when the DMM was combined with SPI. However, the overall levels of lysine and methionine in the DMM/SPI combinations were still not reaching the level in fishmeal. Apparently, this is one of the limitations for the combined mixture being used to replace fishmeal without impairing amino acid balance if those amino acids are not supplemented to a targeted feed.

Some factors related to dietary nutrients in the DMM may be responsible for reduced growth performance of yellow

Table 6 Fasting levels of serum biochemical indices from yellow perch fed test diets for 8 weeks

Index	D-0%	D-25%	D-50%	D-75%	*P* value
ALB	33.3 ± 3.5	31 ± 1.7	28.8 ± 0.6	29.8 ± 2.5	0.183
ALP	147.5 ± 10.0	166 ± 4.4	166 ± 4.0	142.7 ± 16.2	0.080
ALT	754.7 ± 9.4	754.2 ± 14.0	773.7 ± 31.8	762.7 ± 22.0	0.655
AMY	2397.3 ± 105.8	2288.3 ± 220.0	2143.8 ± 63.3	2235.3 ± 189.6	0.325
Ca	6.9 ± 0.1	7 ± 0.1	6.9 ± 0.1	7.0 ± 0.3	0.624
GLOB	20.7 ± 1.3	22.8 ± 0.6	22.8 ± 2.0	23.5 ± 1.5	0.163
GLU	16.2 ± 2.8	12.9 ± 0.9	12.5 ± 1.6	13.6 ± 2.7	0.228
Na	133.8 ± 2.4	135 ± 1.3	133.8 ± 0.8	136.2 ± 2.5	0.466
PHOS	3.7 ± 0.3ab	4.0 ± 0.3b	3.6 ± 0.2ab	3.3 ± 0.1a	0.046
TP	53.8 ± 4.2	54.2 ± 1.3	51.7 ± 2.6	53.2 ± 3.7	0.772

Data presented as mean ± SD, $n = 3$. Means in the same line sharing the same or none lowercase letter are not significantly different, as determined by Turkey's test ($P > 0.05$)

ALB albumin, g L^{-1}, *ALP* alkaline phosphatase, U L^{-1}, *ALT* alanine amino transferase, U L^{-1}, *AMY* amylase, U L^{-1}, *Ca* calcium, mEq L^{-1}, *GLOB* globulin, g L^{-1}, *GLU* glucose, mmol L^{-1}, *Na* sodium, mmol L^{-1}, *PHOS* inorganic phosphorus, mmol L^{-1}, *TP* total protein, g L^{-1}

perch fed with diets contain the mixed ingredient used to replace 50–75% fishmeal protein.

First, for all the dietary dispensable amino acids, only methionine and lysine decreased in their levels when fishmeal was replaced with the combined ingredients. The diet D-50% and D-75% contained only 0.79–0.88 g of methionine for 100 g diet, which was lower than the requirement of 1.0 g methionine per 100 g diet suggested for the growth of yellow perch (Twibell et al. 2000). The same study also suggested that dietary cyst(e)ine could spare up to 51% of the methionine requirement and thus the dietary total sulfur amino acid requirement (TSAA) was 0.85% based on weight gain. The test diets (D-50% and D-75%) contained only 0.79–0.88% methionine, but their total sulfur amino acids were 1.27–1.34%. Thus, the requirement estimated based on semi-purified feed formulation may be different from the requirement in a practical feed formulation. A higher level of requirement may be needed in the current feed formulation. A further study is needed to test this hypothesis. In a previous study, a higher level of the same DMM (12% in a diet) was used to replace 50% fishmeal protein and no adverse effect was observed on shrimp growth performance (Ju et al. 2012). Different from our current study, the DMM was used as a single fishmeal substitute in shrimp feed but it was combined with soy protein isolate to replace fishmeal in yellow perch feed. The soy protein isolate is also limited in methionine and thus might exacerbate the impacts on growth performance of yellow perch in the current study. This may be one of the reasons that a relatively low level of fishmeal replacement was accepted by yellow perch in the present study. Furthermore, dietary methionine requirement for Pacific white shrimp is about 0.67% (Lin et al. 2015), which is relatively lower than the requirement of yellow perch (1.0% in a diet). The methionine level in the shrimp test diets was higher than the requirement level of shrimp even though 50% fishmeal was replaced by the DMM (Ju et al. 2012). Thus, utilization of the defatted algae to replace fishmeal is different depending on the nutrient requirement of a targeted species and a basal feed formulation used to test the hypothesis. Also, a recent study on longfin yellowtail by Kissinger et al. (2016) reported that 80% fishmeal can be substituted by the blends of soy protein concentrate, squid meals, and defatted *Haematococcus* meal used. No compromising fish growth performance and feed utilization were observed, but the feed was supplemented with methionine, lysine, and taurine (Kissinger et al. 2016). Beside the methionine level, with the increasing level of fishmeal replacement lysine level in the test diets decreased from 1.90 to 1.58 g per 100 g diet, which is still in the range of lysine requirement (1.6–2.4 g per 100 g diet) determined for other species of freshwater fish (NRC, 2011). However, the available lysine level in D-50% and D-75% might not sufficient if digestibility was low in the diets. This is not known based on the current observations. Therefore, we hypothesized that yellow perch may be able to tolerate the DMM at a level higher than 5% in a diet with a supplementation of deficient nutrients such as methionine or lysine. This hypothesis is warrant for future study.

Second, the DMM/SPI combination had a lower level of calcium and phosphorous than the fishmeal. It is expected that these minerals were decreased in the diets when fishmeal was replaced by the DMM/SPI mixed ingredient, which is derived from plant sources. This partially explained the observation on decreased ash content in the fishmeal replacement diets and the lowest level of ash and phosphorus contents observed in whole fish fed the D-75%. Therefore, mineral deficiency may be another cause responsible for the reduced growth performance of yellow perch fed the diets with 50–75% fishmeal replaced.

Third, the DMM contained considerably higher levels of total carbohydrates (41.9%) compared to fish meal (0.7%). It is known that most of carbohydrates from microalgae meal are non-starch polysaccharides (NSPs), which can cause reduced gastric emptying and interfere nutrient digestion and absorption (Amirkolaie et al. 2005; Leenhouwers et al. 2006; Sinha et al. 2011; Haidar et al. 2016). Therefore, additional carbohydrate from the DMM that was used to make the DMM/SPI mixture may be another factor resulting in the depressed growth of yellow perch fed D-50% and D-75% diets. Further investigations, using DMM and SPI alone, in this species will enable us to answer specific questions about the effects of these ingredients alone, and in combination, on yellow perch performance.

On the other hand, the DMM and SPI do not significantly change the contents of protein, lipid, and moisture as well as the serum biochemical measurements except that the phosphorous was significantly decreased in the fish fed diet D-75%. This suggested that the overall fish health was not impacted at this stage when the fish were fed with the test diets under the current testing conditions. *Haematococcus* is cultured for astaxanthin production. Astaxanthin is primarily used as a pigmentation source in aquatic feed and is found to have different beneficial effects on growth, survival, tolerance to stress, and diseases of aquaculture species (Lim et al. 2017). Beneficial effects on pigmentation were previously documented on shrimp fed diets containing the same DMM used in the current study (Ju et al. 2012). In the current study, yellow perch fed all diets containing the DMM and SPI were also shown to have pelvic fin with significant orange coloration (data not shown). The enriched pigmentation is likely caused by the dietary astaxanthin from the DMM. Thus, the co-product of DMM maybe a promising feed additive in yellow perch feed to enhance the health and pigmentation of this fish. A long-term feeding trial is needed to verify beneficial functions of the DMM as a feed additive in feed for carnivores fish like yellow perch.

Conclusion

The results of this study demonstrate that the DMM/SPI blend (equal ratio) can be used to (10% of the diet) replace 25% of

menhaden fishmeal in practical diets with no any amino acid supplementation for yellow perch without posing any adverse effect on the growth performance, nutritional composition, and biochemical indexes related to fish health. Limitations of the combined ingredients in feed for yellow perch may be due to deficiency of certain essential nutrients or decreased digestibility of the ingredients, which can be addressed by nutrient supplementation or application of different feed processing methods. In the current study, we did not evaluate DMM (alone) as a single substitute protein source for yellow perch; further studies are to investigate the potential of the DMM supplemented with necessary amino acids. Furthermore, a long-term study with feed manufactured using similar processing methods applied in feed industry could enable comprehensive assessment on the potential of the DMM as a feed ingredient or feed additive for feeding of yellow perch.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest. The views contained in this document are those of the authors and should not be interpreted as necessarily representing the official policies, either expressed or implied, of the US Government. Mention of trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable. This manuscript is submitted for publication with the understanding that the United States Government is authorized to reproduce and distribute reprints for governmental purposes. The USDA is an Equal Opportunity Employer.

References

Amaro HM, Guedes AC, Malcata FX (2011) Advances and perspectives in using microalgae to produce biodiesel. Appl Energy 88:3402–3410

Amirkolaie AK, Leenhouwers JI, Verreth JAJ, Schrama JW (2005) Type of dietary fibre (soluble versus insoluble) influences digestion, faeces characteristics and faecal waste production in Nile tilapia (*Oreochromis niloticus* L.). Aquac Res 36:1157–1166

Basri NA, Shaleh SRM, Matanjun P, Noor NM, Shapawi R (2015) The potential of microalgae meal as an ingredient in the diets of early juvenile Pacific white shrimp, *Litopenaeus vannamei*. J Appl Phycol 27:857–863

Dagar A, Zilberg D, Cohen Z, Boussiba S, Khozin-Goldberg I (2010) Short-term dietary supplementation with the microalga *Parietochloris incisa* enhances stress resistance in guppies *Poecilia reticulata*. Aquac Res 41:267–277

Fry JP, Love DC, MacDonald GK, West PC, Engstrom PM, Nachman KE, Lawrence RS (2016) Environmental health impacts of feeding crops to farmed fish. Environ Internat 91:201–214

Haidar MN, Petie M, Heinsbroek LTN, Verreth JAJ, Schrama JW (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

Hardy RW (2010) Utilization of plant proteins in fish diets: effects of global demand and supplies of fishmeal. Aquac Res 41:770–776

Hemaiswarya S, Raja R, Ravi Kumar R, Ganesan V, Anbazhagan C (2011) Microalgae: a sustainable feed source for aquaculture. World J Microbiol Biotechnol 27:1737–1746

Hinshaw JM (2006) Species profile: yellow perch. Southern Regional Aquaculture Center Publication No. 7204. North Carolina State University

Ju ZY, Deng D, Dominy W (2012) A defatted microalgae (*Haematococcus pluvialis*) meal as a protein ingredient to partially replace fishmeal in diets of Pacific white shrimp (*Litopenaeus vannamei*, Boone, 1931). Aquaculture 354–355:50–55

Kiron V, Phromkunthong W, Huntley M, Archibald I, De Scheemaker G (2012) Marine microalgae from biorefinery as a potential feed protein source for Atlantic salmon, common carp and whiteleg shrimp. Aquac Nutr 18:521–531

Kiron V, Sørensen M, Huntley M, Vasanth GK, Gong Y, Dahle D, Palihawadana AM (2016) Defatted biomass of the microalga, *Desmodesmus* sp., can replace fishmeal in the feeds for Atlantic salmon. Front Mar Sci 3:67

Kissinger KR, García-Ortega A, Trushenski JT (2016) Partial fish meal replacement by soy protein concentrate, squid and algal meals in low fish-oil diets containing *Schizochytrium limacinum* for longfin yellowtail *Seriola rivoliana*. Aquaculture 452:37–44

Leenhouwers JI, Adjei-BoatengG D, Vereth JAJ, Schrama JW (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. Aquac Nutr 12:111–116

Lim KC, Yusoff FM, Shariff M, Kamarudin MS (2017) Astaxanthin as feed supplement in aquatic animals. Rev Aquac 10:738–773

Lin H, Chen Y, Niu J, Zhou C, Huang Z, Du Q (2015) Dietary methionine requirements of Pacific White Shrimp *Litopenaeus vannamei*, of three different sizes. Bamidgeh 67:1–10

Lorenz RT, Cysewski GR (2000) Commercial potential for *Haematococcus* microalgae as a natural source of astaxanthin. Trends Biotech 18:160–167

Malison JA (2003) A white paper on the status and needs of yellow perch aquaculture in the North Central Region. In: Special publication the North Central Regional Aquaculture Center. USA, Lansing, MI

Moon HY, Gatlin DM (1991) Total sulfur amino acid requirement of juvenile red drum, *Sciaenops ocellatus*. Aquaculture 95:97–106

NRC (2011) Replacement of marine resources of alternative ingredients and strategies on nutritional quality. In: Nutrient requirements of fish and shrimp. The National Acadmics Press, Washington D.C

Olsen RL, Hasan MR (2012) A limited supply of fishmeal: impact on future increases in global aquaculture production. Trends Food Sci Technol 27:120–128

Rosauer DR, Biga PR, Lindell SR, Binkowski FP, Shepherd BS, Palmquist DE, Simchick CA, Goetz FW (2011) Development of yellow perch (*Perca flavescens*) broodstocks: initial characterization of growth and quality traits following grow-out of different stocks. Aquaculture 317:58–66

Sarker PK, Kapuscinski AR, Lanois AJ, Livesey ED, Bernhard KP, Coley ML (2016) Towards sustainable aquafeeds: complete substitution of fish oil with marine microalga *Schizochytrium* sp. improves growth

and fatty acid deposition in juvenile Nile Tilapia (*Oreochromis niloticus*). PLoS One 11:e0156684

Shah MR, Lutzu GA, Alam A, Sarker P, Kabir Chowdhury MA, Parsaeimehr A, Liang Y, Daroch M (2018) Microalgae in aquafeeds for a sustainable aquaculture industry. J Appl Phycol 30:197–213

Shepherd CJ, Jackson AJ (2013) Global fishmeal and fish-oil supply: inputs, outputs and marketsa. J Fish Biol 83:1046–1066

Sinha AK, Kumar V, Makkar HPS, De Boeck G, Becker K (2011) Non-starch polysaccharides and their role in fish nutrition—a review. Food Chem 127:1409–1426

Tacon AGJ, Metian M (2008) Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. Aquaculture 285:146–158

Twibell RG, Wilson KA, Brown PB (2000) Dietary sulfur amino acid requirement of juvenile yellow perch fed the maximum cystine replacement value for methionine. J Nutr 130:612–616

Vizcaíno AJ, Saéz MI, López G, Arizcun M, Abellán E, Martínez TF, Cerón-García MC, Alarcón FJ (2016) *Tetraselmis suecia* and *Tisochrysis lutea* meal as dietary ingredients for gilthead sea bream (*Sparus aurata L.*) fry. J Appl Phycol 28:2843–2855

Zaťková I, Sergejevova M, Urban J, Vachta R, Štys D, Masojidek J (2011) Carotenoid-enriched microalgal biomass as feed supplement for freshwater ornamentals: albinic form of wels catfish (*Silurus glanis*). Aquac Nutr 17:278–286

Direct W (2015) http://www.walleyedirect.com/product/yellow-perch-11.html