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Evaluation of Heterotrophic Algae Meal as a Diet Ingredient for Channel Catfish, *Ictalurus punctatus*

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Abstract

Most microalgae evaluated in aquaculture diets have been produced autotrophically. In order to produce a cost-efficient biomass at greater magnitudes for biofuel feedstock, heterotrophic production may be warranted. However, the chemical/nutritional attributes of these microalgae could differ from those grown autotrophically. An 8-wk feeding trial was conducted to evaluate *Chlorella* spp. algae meal (AM) that had been cultured heterotrophically. The oil (lipid) was extracted to simulate biofuel production. Juvenile channel catfish, *Ictalurus punctatus* (5.7 ± 1.4 g; 8.9 ± 0.8 cm), were stocked at 10 fish/tank into fifteen 37.7-L aquaria in a closed recirculating system and fed one of the five experimental diets to apparent satiation twice daily. Diets contained either 0 (control, CTL), 10, 20, or 40% AM and an additional diet containing 40% AM was supplemented with 2% lysine (40% AM+LYS). After 8 wk, there were no statistically significant differences in terms of survival, dressout percentages, whole-body proximate composition, or fatty acid composition of the fillets among fish fed the diets containing varying levels of AM without added lysine. Feed consumption and weight gain for fish fed the 10, 40, and 40% AM+LYS diets were significantly greater than those fed the CTL diet. Feed conversion ratio was significantly lower for fish fed the 40% AM+LYS diet compared to those fed all other diets, which did not differ significantly from each other. These data indicate that channel catfish readily accept and can efficiently utilize heterotrophically produced AM at levels up to at least 40% of the total diet and that AM may enhance diet palatability.

The identification, development, and use of alternative ingredients remain a research priority, and consequently, aquaculture feeds of the future will likely contain a wider variety of ingredients (Glencross et al. 2007). By-products from the growing biofuel industry are being considered for inclusion in aquaculture feed. Large-scale production of microalgae as a feedstock for producing second generation biofuels has received considerable attention (Chisti 2007; Brennan and Owende 2010; Mata et al. 2010; Singh and Gu 2010). During the production of microalgae for biofuels, the algal biomass is harvested and the lipid is removed by mechanical and/or chemical means. The residual material, known as algae meal (AM), may hold potential as a feed ingredient in aquaculture diets.

While most microalgae are cultured under autotrophic conditions using sunlight, there is increasing interest in their commercial production under heterotrophic conditions in fermentation tanks (Gladue and Maxey 1994; Richmond 2004; Perez-Garcia et al. 2011). From a biofuel perspective, heterotrophic production of microalgae appears promising. Cost effectiveness, relative simplicity of operations, and consistently higher biomass densities are the main attractions compared to autotrophic production (Gladue and Maxey 1994; Brennan and Owende 2010; Perez-Garcia et al. 2011). However, the biological composition of the resulting microalgae can differ substantially between these two production methods. Differences in protein, lipid, carbohydrate composition (Xu et al. 2006), amino acid composition (Khairy et al. 2011), and fatty acid composition (Kim and Hur 2013) have been reported. These differences

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could also affect their nutritional value and therefore the usefulness of the by-products remaining after oil extraction. These by-products can serve as additional revenue sources, helping to support the economic viability of algal biofuels (FAO 2010; Singh and Gu 2010).

Heterotrophically produced *Chlorella* spp. appears to be good candidate for biofuel production (Xu et al. 2006; Mata et al. 2010; Kim and Hur 2013) and the resulting defatted AM may be a potential ingredient in aquaculture diets. Harel et al. (2002) found that the inclusion of heterotrophically produced defatted *Crypthecodinium* meal in diets for striped bass, *Morone saxatilis*, yielded growth similar to those fed a commercial feed. Kiron et al. (2012) found no significant differences in growth or feed efficiency of Atlantic salmon, *Salmo salar*, common carp, *Cyprinus carpio*, or whiteleg shrimp, *Litopenaeus vannamei*, fed diets containing defatted marine microalgae compared to a control (CTL) diet. Li et al. (2009) observed increased weight gain and feed efficiency, as well as increased levels of n-3 long-chain fatty acids in channel catfish, *Ictalurus punctatus*, fed diets supplemented with heterotrophically produced *Schizochytrium* that had not been lipid extracted. The objective of this study is to evaluate AM from *Chlorella* spp. that had been grown heterotrophically with the lipid chemically extracted (to simulate biofuel production) as a diet ingredient for channel catfish.

Material and Methods

Spray-dried *Chlorella* spp. microalgae were obtained from Alltech Algae (Winchester, KY, USA). The algae were cultured under heterotrophic conditions in a 260,000-L air-lift fermenter. The lipid portion was extracted prior to use in the experimental diets to simulate AM that would be a by-product of biofuel production. Lipid extraction was conducted at the Center for Renewable and Alternative Fuel Technologies (CRAFT) Laboratory at Eastern Kentucky University (Richmond, KY, USA). Cells were lysed by ultrasonication using a Model 505 Sonic Dismembrator (Fisher Scientific, Waltham, MA, USA). Then, the oil was solvent extracted with hexane. The defatted algae biomass was separated from the hexane/oil supernatant via centrifugation. Residual hexane was allowed to evaporate overnight from the defatted algae biomass, hereafter referred to as AM. This process was repeated multiple times to accumulate approximately 2 kg of AM for chemical analysis and diet formulation.

Five diets containing different levels of AM were formulated to be isonitrogenous (33% dry matter protein) and isocaloric (3.7 kcal/g). The CTL diet contained no AM and was based on a diet previously described by Li et al. (2011). Experimental diets contained either 10, 20, or 40% AM. An additional experimental diet contained 40% AM and 2% lysine supplementation (40% AM + LYS) (Table 1). Lysine is often the first limiting amino acid in practical aquaculture diets and this treatment is used to identify lysine limitation with high inclusion levels of alternative ingredients. Based on table values (NRC 2011), lysine was estimated to decrease as AM increased in experimental diets. However, when analyzed, lysine levels increased as AM increased (Table 2).

All other diet ingredients were obtained from a commercial feed manufacturer (Rangen, Inc., Houston, TX, USA). Dry ingredients were mixed in a Hobart mixer (Hobart, Inc., Troy, OH, USA) for 45 min. Water was added to the dry mix to obtain a 35% moisture level. The moist mixture was then blended for an additional 45 min. Additional water was added to the 40% AM and 40% AM + LYS diets (50 and 45% moisture level, respectively) to allow proper mixing as these formulations were less malleable. Diets were then passed through a mincer with a 2-mm die (Hobart, Inc.) to form "spaghetti-like" strands and fan dried at room temperature overnight. Once dried to $\leq$10% moisture, strands were broken by hand and stored frozen (−20 C) until fed. Three samples of each diet were submitted to a commercial laboratory (Eurofins Scientific, Inc., Des Moines, IA, USA) for proximate analysis. Two samples of each of the finished diets were analyzed for amino acids (Table 2) and fatty acids (Table 3) by the same commercial laboratory.

TABLE 1. *The percentage composition of five experimental diets formulated to be isonitrogenous and isocaloric diets with increasing levels of algae meal (AM) and the analyzed composition of the diets.*

	Five experimental diets				
Ingredient	Control	10% AM	20% AM	40% AM	40% AM + lysine
Fish meal (Menhaden)	5	5	5	5	5
Soybean meal	40	37.1	37.1	37.1	37.1
Cottonseed meal	7.5	7	5	0	0
AM	0	10	20	40	40
Corn meal	30.6	25	18	3	1
Wheat	10	10	10	10	10
Fish oil	1	1	0	0	0
Vegetable oil	1	0	0	0	0
Lysine	0	0	0	0	2
Other[1]	4.9	4.9	4.9	4.9	4.9
Analyzed composition (dry matter basis)[2]					
Protein	33.1	32.8	32.8	32.3	34.3
Fat	3.9	2.6	1.5	1.3	1.4
Fiber	2.2	2.3	2.0	1.8	1.8
Ash	6.6	6.5	6.6	6.2	6.3
NFE[3]	53.8	55.6	56.9	58.2	55.9
Energy (kcal/g)[4]	3.8	3.7	3.7	3.7	3.7
DE : P[5]	11.5	11.5	11.3	11.5	10.8

[1] Includes 0.3% choline, 0.5% trace mineral premix, 0.5% vitamin premix, 0.1% vitamin C, 1.5% di-calcium phosphate, and 2% carboxymethyl cellulose (pellet binder).

[2] Values expressed as means of three replications per diet.

[3] NFE = nitrogen-free extract.

[4] Gross energy values of 4.0, 4.0, and 9.0 kcal/g for carbohydrate, protein, and lipid respectively (Trosvik et al. 2012).

[5] Estimated digestible energy to gram of protein ratio.

TABLE 2. *Amino acid composition (% of dietary protein) of five experimental diets formulated to be isonitrogenous with increasing levels of algae meal (AM). Values are means of two replicate analyses per diet ± SD.*

		Five experimental diets				
Amino acid	Requirement[1]	Control	10% AM	20% AM	40% AM	40% AM + lysine
Arginine	4.3	6.1 ± 0.0	6.2 ± 0.0	6.2 ± 0.0	6.3 ± 0.0	6.1 ± 0.0
Histidine	1.5	2.2 ± 0.0	2.1 ± 0.0	2.0 ± 0.0	1.9 ± 0.0	1.8 ± 0.0
Isoleucine	2.6	3.4 ± 0.0	3.3 ± 0.0	3.4 ± 0.1	3.3 ± 0.0	3.2 ± 0.0
Leucine	3.5	6.6 ± 0.0	6.2 ± 0.0	6.3 ± 0.2	6.0 ± 0.0	5.8 ± 0.1
Lysine	5.1	5.0 ± 0.0	4.4 ± 0.0	4.7 ± 0.1	4.9 ± 0.1	9.2 ± 0.2
Methionine + Cystine	2.3	2.5 ± 0.0	2.6 ± 0.0	2.5 ± 0.0	2.4 ± 0.0	2.4 ± 0.0
Phenylalanine + Tyrosine	5.0	6.6 ± 0.1	6.5 ± 0.0	6.4 ± 0.0	5.9 ± 0.0	5.8 ± 0.0
Threonine	2.0	3.4 ± 0.0	3.3 ± 0.0	3.3 ± 0.0	3.3 ± 0.0	3.2 ± 0.0
Tryptophan	0.5	1.1 ± 0.0	1.1 ± 0.0	1.1 ± 0.0	1.2 ± 0.0	1.1 ± 0.0
Valine	3.0	3.9 ± 0.0	3.8 ± 0.0	3.9 ± 0.1	3.9 ± 0.0	3.7 ± 0.0

[1]For channel catfish (Robinson et al. 2001; NRC 2011).

Juvenile channel catfish were obtained from Pfeiffer fish hatchery (Frankfort, KY, USA), graded to similar size (5.7 ± 1.4 g; 8.9 ± 0.8 cm), and randomly stocked at 10 fish/aquarium into fifteen 37.7-L glass aquaria in a G-Hab Tank system (Aquatic Habitats, Inc., Apopka, FL, USA). All fish were fed the CTL diet for 1 wk as a conditioning diet while being acclimated to the system and then switched to the experimental diets. Three replicate aquaria were randomly assigned to each of the five diets and fish were fed to apparent satiation twice daily (0900 and 1700 hours) for 8 wk. The amount of diet fed to fish in each aquarium was recorded daily.

TABLE 3. *Fatty acid composition (% relative) of five experimental diets formulated with increasing levels of algae meal (AM). Values are means of two replicate analyses per diet ± SD.*

Fatty acid	Control	10% AM	20% AM	40% AM	40% AM + lysine
14:0	0.5 ± 0.0	0.8 ± 0.0	1.09 ± 0.09	1.1 ± 0.0	1.3 ± 0.0
16:0	12.9 ± 0.1	14.8 ± 0.2	18.6 ± 0.5	22.6 ± 0.1	22.4 ± 0.0
16:1 n-7	0.9 ± 0.1	1.5 ± 0.0	2.7 ± 0.0	4.3 ± 0.1	4.6 ± 0.0
18:0	3.8 ± 0.0	3.7 ± 0.1	3.3 ± 0.2	3.8 ± 0.1	3.7 ± 0.1
18:1 n-9	24.8 ± 0.1	25.6 ± 0.0	28.3 ± 0.2	32.0 ± 0.6	32.3 ± 0.2
18:2 n-6	46.9 ± 0.5	41.7 ± 0.0	32.9 ± 0.0	21.2 ± 0.0	18.9 ± 0.1
18:3 n-3	4.2 ± 0.0	3.8 ± 0.0	3.0 ± 0.8	4.6 ± 0.1	4.7 ± 0.0
20:4 n-6	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.2
20:5 n-3	1.1 ± 0.0	1.4 ± 0.0	1.9 ± 0.0	2.0 ± 0.1	2.2 ± 0.0
22:6 n-3	1.0 ± 0.1	1.5 ± 0.0	1.9 ± 0.1	1.7 ± 0.4	2.1 ± 0.2
Sum n-3	6.9 ± 0.8	7.5 ± 0.4	7.9 ± 0.4	9.1 ± 0.6	9.9 ± 0.02
Sum n-6	46.4 ± 0.4	41.5 ± 0.0	32.5 ± 0.1	21.2 ± 0.0	18.9 ± 0.15

Additionally, evaporated salt was added to the system and make up water holding tank to maintain salinity at 3000 mg/L (3 ppt) to prevent nitrite toxicity (Tucker and Robinson 1990).

Water temperature, dissolved oxygen, and pH were measured daily using a YSI Professional Plus meter (YSI Company, Yellow Springs, CO, USA). Total ammonia-N and nitrite-N were measured three times per week using a HACH DR/3900 spectrophotometer (HACH, Loveland, CO, USA). Alkalinity was monitored three times per week by titration (HACH). Salinity was monitored three times per week using the YSI meter mentioned above.

At the end of 8 wk, all fish in each tank were bulk weighed, then individually weighed, and measured (total length). Three fish were randomly selected from each aquarium and dissected for liver weight (hepatosomatic index, HSI) and fillet weight. Tissues from three fish were homogenized together in a blender, and stored frozen in plastic bags for analysis of whole-body proximate composition (protein, lipid, ash, and moisture) by the commercial laboratory. For fatty acid analyses, three additional fish were randomly selected from each aquarium, homogenized in a blender, quick frozen with liquid nitrogen, and stored frozen in 40-mL glass vials for analysis by the commercial laboratory.

Growth performance variables were calculated as follows: Average harvest weight (AHW) = [bulk weight at harvest (g)/harvest number]. Total weight gain (TWG) = [final bulk weight − initial bulk weight]. Percent weight gain (PWG) = [weight gain/initial bulk weight] × 100. Specific growth rate (SGR) = % body wt/d = $[(\ln W_f - \ln W_i)/t] \times 100$, where W_f = final bulk weight (g), W_i = initial bulk weight (g), and t = time in days. Feed conversion ratio (FCR) = [total diet fed (g)/weight gain (g)]. Protein efficiency ratio (PER) = [weight gain (g)/protein fed (g)]. HSI = [weight of liver (g)/weight of whole body (g)] × 100. Whole dress percentage (WD%) = [weight of whole dress (g)/weight of whole body (g)] × 100. Fillet percentage (FL%) = [weight of fillet (g)/weight of whole body (g)] × 100. Condition factor (K) = [individual harvest weight (g)/average length^3 (cm)] × 100. Treatment effects were compared by ANOVA ($P \leq 0.05$) by Statistix version 9.0 (Statistix Analytical Software 2000). If significant differences were found among treatments, means were separated using Student's LSD test (Steel and Torrie 1980).

Results

During the trial, the overall water quality values averaged (±SD) dissolved oxygen 6.7 ± 0.2 mg/L; temperature 28.4 ± 0.4 C; pH 7.6 ± 0.1; un-ionized ammonia-N 0.005 ± 0.004 mg/L; total ammonia-N 0.17 ± 0.10 mg/L; nitrite-N 0.942 ± 0.740 mg/L; alkalinity 84.9 ± 14.3 mg/L; salinity 2.9 ± 0.1 ppt. These values represent suitable conditions for channel catfish (Tucker and Robinson 1990).

TABLE 4. *Mean (±SD) of survival, average harvest weight (g) (AHW), total weight gain (g) (TWG), percent weight gain (PWG), total feed consumption (TFC), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), condition factor (K), hepatosomatic index (HSI), whole dress percentage (WD%), and fillet percentage (FL%) of juvenile channel catfish fed five experimental diets with increasing levels of algae meal (AM) inclusion. Significant differences ($P \leq 0.05$) are indicated by different letters within lines.*

Variable	Control	10% AM	20% AM	40% AM	40% AM + lysine
Survival	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}	100.0 ± 0.0^{a}
AHW (g)	43.4 ± 9.4^{c}	54.4 ± 4.2^{ab}	48.3 ± 5.8^{bc}	57.6 ± 2.0^{ab}	63.1 ± 4.3^{a}
TWG (g)	376.8 ± 94.9^{c}	486.6 ± 42.3^{ab}	423.8 ± 58.6^{bc}	516.8 ± 17.9^{ab}	573.3 ± 42.0^{a}
PWG	665.6 ± 179.8^{c}	843.8 ± 77.5^{abc}	718.1 ± 105.7^{bc}	880.3 ± 7.3^{ab}	997.8 ± 61.1^{a}
TFC	490.2 ± 100.3^{c}	617.1 ± 42.7^{ab}	547.7 ± 74.0^{bc}	653.9 ± 5.9^{ab}	680.8 ± 61.3^{a}
SGR	3.7 ± 0.4^{c}	4.1 ± 0.2^{abc}	3.8 ± 0.2^{bc}	4.2 ± 0.0^{ab}	4.4 ± 0.1^{a}
FCR	1.3 ± 0.7^{a}	1.3 ± 0.0^{a}	1.3 ± 0.0^{a}	1.3 ± 0.0^{a}	1.2 ± 0.0^{b}
PER	2.3 ± 0.1^{a}	2.4 ± 0.0^{a}	2.3 ± 0.0^{a}	2.4 ± 0.0^{a}	2.4 ± 0.0^{a}
K factor	1.0 ± 0.1^{a}	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}	1.0 ± 0.0^{a}
HSI	1.6 ± 0.2^{a}	1.9 ± 0.1^{a}	2.1 ± 0.1^{a}	1.9 ± 0.1^{a}	2.0 ± 0.1^{a}
WD%	62.7 ± 0.4^{a}	61.9 ± 0.5^{a}	62.6 ± 0.2^{a}	63.3 ± 0.8^{a}	62.9 ± 1.0^{a}
FL%	31.0 ± 0.3^{a}	32.2 ± 0.9^{a}	32.7 ± 0.3^{a}	34.2 ± 0.7^{a}	32.5 ± 0.6^{a}

At harvest, there were no significant differences ($P > 0.05$) among treatments in terms of survival ($100 \pm 0.0\%$), PER (2.71 ± 0.06), *K* factor (0.87 ± 0.02), and HSI (1.89 ± 0.16) (Table 4). AHW for fish fed the 10% AM, 40% AM, and 40% AM + LYS diets were significantly greater ($P \leq 0.05$) than those fed the CTL diet. AHW in fish fed the 20% AM diet was not significantly different ($P > 0.05$) from fish fed the CTL diet. Weight gain percentage and SGR were significantly greater ($P \leq 0.05$) for fish fed the 40% AM + LYS diets compared to those fed the CTL diet and the 20% AM diet, but not significantly different ($P > 0.05$) from fish fed the 10% AM and 40% AM diets. Feed consumption by fish fed the 10% AM, 40% AM, and 40% AM + LYS was significantly greater ($P \leq 0.05$) than those fed the CTL diet. Feed consumption in fish fed the 20% AM diet was not significantly different ($P > 0.05$) from fish fed the CTL diet. FCR was significantly lower (more efficient) ($P \leq 0.05$) for fish fed the 40% AM + LYS diet compared to those fed all other diets, which did not differ significantly ($P > 0.05$) from each other (Table 4).

Whole-body proximate composition did not differ statistically ($P > 0.05$) among fish fed any of the five experimental diets with regards to protein, fat, ash, and moisture (Table 5). Fatty acid composition (% relative) in fillets also did not differ statistically ($P > 0.05$) among fish fed any of the five experimental diets. Dressout percentages in terms of whole dress (WD%) and fillet (FL%) (as a percentage of total body weight) also did not differ significantly ($P > 0.05$) among fish fed the five experimental diets (Table 4).

Discussion

According to the results of this study, defatted heterotrophically produced AM (*Chlorella* spp.) is well accepted and efficiently utilized by channel catfish. With regards to the PER and FCR, these data are in agreement with Kiron et al. (2012) who reported no significant differences for either performance parameter when two defatted marine microalgal meals (*Nanofrustulum* and *Tetraselmis*) were fed to Atlantic salmon, common carp, and white leg shrimp. Palmegiano et al. (2005) observed an improvement in PER and FCR when *Spirulina* was included in diets for Siberian sturgeon, *Acipenser baeri*.

The amino acid composition of all experimental diets appeared to meet the requirement levels reported for channel catfish, except for lysine (NRC 2011) (Table 2). Lysine is usually considered to be the first limiting amino acid in channel catfish feeds, especially those using high levels of plant proteins (Robinson et al. 2001). It was anticipated that lysine may be deficient, especially within the diet with the highest inclusion

TABLE 5. *Fatty acid composition (% relative) of fillets and proximate analysis of whole body of channel catfish fed five experimental diets with increasing levels of algae meal (AM). Values are means (±SEM) of three replicates. An ANOVA indicated no significant differences ($P > 0.05$) among diets for the fatty acid composition and proximate analysis of whole body.*

Fatty acid	Control	10% AM	20% AM	40% AM	40% AM + lysine
14:0	1.1 ± 0.2	1.1 ± 0.0	1.3 ± 0.3	1.0 ± 0.1	1.2 ± 0.0
16:0	18.1 ± 1.8	18.3 ± 0.2	18.6 ± 0.4	18.6 ± 1.4	19.1 ± 0.9
16:1 n-7	3.9 ± 2.1	3.6 ± 0.1	4.5 ± 0.1	4.6 ± 1.5	5.8 ± 0.4
18:0	4.4 ± 1.0	4.5 ± 0.4	4.3 ± 0.2	4.0 ± 0.5	3.5 ± 0.2
18:1 n-9	47.6 ± 4.4	50.1 ± 0.6	48.2 ± 11.4	51.9 ± 4.5	56.4 ± 2.5
18:2 n-6	13.7 ± 6.4	12.3 ± 0.2	10.0 ± 4.3	9.6 ± 6.3	5.6 ± 0.1
18:3 n-3	1.6 ± 0.4	1.4 ± 0.0	1.1 ± 0.3	1.3 ± 0.4	1.0 ± 0.0
20:4 n-6	0.9 ± 0.2	0.8 ± 0.0	1.0 ± 0.6	0.8 ± 0.1	0.6 ± 0.0
20:5 n-3	0.5 ± 0.0	0.4 ± 0.0	0.9 ± 0.7	0.4 ± 0.0	0.4 ± 0.0
22:6 n-3	2.0 ± 0.1	1.7 ± 0.1	3.0 ± 2.4	1.7 ± 0.0	1.5 ± 0.3
Sum n-3	4.6 ± 0.4	3.9 ± 0.1	5.8 ± 4.0	4.1 ± 0.1	3.6 ± 0.5
Sum n-6	16.2 ± 7.6	14.3 ± 0.1	12.2 ± 5.3	11.5 ± 7.2	6.4 ± 0.4
Proximate analysis of whole body					
Protein	18.8 ± 0.2	18.6 ± 0.0	17.2 ± 1.5	18.5 ± 0.1	18.8 ± 0.1
Fat	3.1 ± 0.5	4.2 ± 0.3	3.5 ± 0.0	4.2 ± 0.1	4.5 ± 0.1
Ash	1.2 ± 0.0	1.2 ± 0.0	1.0 ± 0.2	1.2 ± 0.0	1.2 ± 0.0
Moisture	77.0 ± 0.5	75.8 ± 0.4	78.1 ± 1.4	75.9 ± 0.2	75.5 ± 0.2

rate of AM (40% AM). The fact that fish fed the diet with supplemental lysine had a significantly better FCR than fish fed unsupplemented diets suggests that the 40% AM diet is limited in lysine. As the other experimental diets had lysine concentrations similar or less than the 40% AM, they were also likely limited in lysine. Lim et al. (2009) reported that with lysine supplementation, dried distiller grains with solubles could be included up to 40% in the diet of juvenile channel catfish without affecting the growth performance. These results appear similar, but lysine supplementation may have also been beneficial at lower AM inclusion levels in this trial.

The inclusion of AM in the diets appeared to enhance growth performance in channel catfish. Fish fed the 10% AM, 40% AM, and 40% AM + LYS diets were significantly larger than those fed the CTL diet and gained 29, 37, and 52% more weight, respectively. This is in agreement with Kim et al. (2002) who reported that dietary supplementation with 2% *Chlorella* spp. powder significantly improved the growth of juvenile flounder. Similarly, Li et al. (2009) indicated that adding 1–1.5% of dried microalgae (*Schizochytrium* sp.) increased weight gain in channel catfish. At higher inclusion rates, *Spirulina* has been reported to significantly improve the growth performance of rohu, *Labeo rohita*, and Siberian sturgeon, *A. baeri* (Nandeesha et al. 2001; Palmegiano et al. 2005). *Chlorella* spp. is not commonly utilized at high inclusion rates due to the cell wall which is composed of indigestible cellulose. This cell wall can restrict access of digestive enzymes to nutritional components within the cell. Janczyk et al. (2005) reported that ultrasonication (as used in this study) improves protein digestibility and efficiency of *Chlorella vulgaris* by degradation of the cell wall. While this process is used to facilitate the extraction of oil, it may similarly improve the nutritional quality of the AM and allow for higher inclusion levels.

Compared to fish fed the CTL diet, fish fed the 10% AM, 40% AM, and 40% AM + LYS consumed 25, 33, and 38% more feed, respectively. Fish are known to eat to satisfy an energy requirement. However, the energy concentration in all of the diets was similar. Increased performance of fish fed diets with high concentrations of AM appears to be due to enhanced palatability. As FCR was not negatively affected by AM inclusion (and actually more efficient for fish fed the added lysine), increased consumption

resulted in greater growth. These results are in agreement with Hussein et al. (2013) who report that microalgae positively influenced feed consumption of Nile tilapia, *Oreochromis niloticus*. However, Walker and Berlinsky (2011) observed reduced feed consumption and consequently reduced growth of Atlantic cod, *Gadus morhua*, when fed diets that included AMs *Nannochloropsis* and *Isochrysis*.

In conclusion, these data indicate that channel catfish find defatted AM produced from heterotrophically grown *Chlorella* spp. to be highly palatable and can efficiently utilize it at levels up to at least 40% of the total diet. At 40% inclusion, lysine supplementation significantly improved feed conversion efficiencies, likely indicating that lysine was becoming limiting. This promising feed ingredient may become more widely available as biofuel production continues to develop.

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