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Original Article

Supplementation of duckweed diet and citric acid on growth performance, feed utilization, digestibility and phosphorus utilization of TGGG hybrid grouper (*Epinephelus fuscoguttatus* x *Epinephelus lanceolatus*) juvenile

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Abstract

A feeding trial was conducted in juvenile TGGG hybrid grouper to investigate the growth performance, feed utilization, and digestibility after citric acid supplementation in a diet that was partially composed of duckweed. Five isoproteic and isolipidic diets (50% protein, 16% lipid levels) were formulated using *Lemna minor* and *Spirodela polyrrhiza* at 5% of fishmeal protein replacement level with or without 3% of citric acid supplementation. A diet without duckweed and citric acid was used as the control diet. Triplicate groups of fish (10.30±0.05 g) were randomly distributed in tanks with a flow through system at a stocking density of 20 fish per tank. The fish were fed twice daily with each experimental diet until apparent satiation for 10 weeks. As a result, the fish fed a diet with duckweed *S. polyrrhiza* and citric acid (DSC) achieved significantly higher growth, body weight gain (BWG) and specific growth rate (SGR) compared to the control group ($P<0.05$); however, it was not significantly different with other treatments ($P>0.05$). The growth, BWG and SGR in fish fed duckweed diets only (*L. minor* and *S. polyrrhiza*) were almost similar with the control without significant differences ($P>0.05$). Similarly in feed utilization, fish fed the DSC diet had a better feed conversion ratio, protein efficiency ratio, and net protein utilization without significant differences ($P>0.05$) compared to the control. The apparent digestibility coefficient for crude protein and crude lipid, and phosphorus absorption of DSC and the diet with duckweed *L. minor* and citric acid were comparable to the control without any significant differences ($P>0.05$). Survival was not affected by the experimental diets. This study showed that TGGG can utilize a diet partially composed of duckweed and better performance was observed with the aid of citric acid.

Keywords: hybrid grouper, citric acid, duckweed, growth, feed utilization

1. Introduction

The hybrid of tiger grouper (*Epinephelus fuscoguttatus*) x giant grouper (*Epinephelus lanceolatus*) (TGGG) was produced in Sabah, Malaysia in 2006 (Ch'ng & Senoo, 2008). TGGG has the potential of being food fish in the long run due to its fast growth, high survival, and high market potential (Ch'ng & Senoo, 2008; Senoo, 2006). In recent years, aquaculture sector has experienced a significant increase due to the need of high quality fish protein for human consumption (Hardy, 2010; Naylor *et al*., 2009). Fishmeal is an important ingredient in the production of aquafeeds due to its enriched nutrients profile such as minerals, fatty acids, essential amino acids, and vitamins (Zhou *et al*., 2004; National Research Council [NRC], 2011). However, fishmeal production is getting lower due to the stagnant landing of capture fisheries and demand of fishmeal for feed production is getting higher (Food & Agriculture Organization [FAO], 2014).

Plant protein alternatives are widely used in aquaculture feed as well as poultry and swine feed production

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(Hardy, 2010). However, local, cheap, and alternative protein sources that aim to reduce production costs without compromising fish quality are much more favourable. Duckweeds are small, fragile, free-floating aquatic plants that grow well in slow-moving and nutrient-rich freshwater or in a brackish aquatic environment (Leng *et al*., 1995). The biomass of duckweed also doubles in 2 to 3 days (Iqbal, 1999; Skillicorn *et al.*, 1993) under ideal conditions of nutrient availability, sunlight, pH (6.5-7.5), and temperature (20 °C to 30 °C). Generally, duckweed contains 6.8 to 45% crude protein (CP), 1.8 to 9.2% crude lipid (CL), 5.7 to 16.2% crude fiber, 12 to 27.6% ash, and the carbohydrate content is in the range of 14.1-43.6% on a dry matter basis (Landolt & Kandeler, 1987). The nutrient composition in each duckweed species varies depending on the condition of the water environment (Leng *et al*., 1995).

Duckweed has been included in the feed for various fish species including tilapia (*Oreochromis niloticus*) (Azim & Wahab, 2003; Fasakin *et al*., 1999; Tavares *et al*., 2008), rohu (*Labeo rohita*) (Das *et al*., 2007; Guru & Patra, 2007; Kaur *et al*., 2012), common carp (*Cyprinus carpio*) (Ansal *et al*., 2008; Yilmaz *et al*., 2004), and snakehead (*Channa striata*) (Raj *et al*., 2001). Various results were obtained from the studies conducted. The growth performance and feed utilization in tilapia reduced progressively at the inclusion level of more than 30% of duckweed (*Spirodela Polyrrhiza*) (Fasakin *et al*., 1999). The growth of rohu was significantly higher when 20% of dried duckweed *Lemna* sp. powder was incorporated as a protein supplement for mustard cake replacement in the diet (Kaur *et al*., 2012). On the other hand, carnivorous fish, such as snakehead, showed higher specific growth rates and weight gains when fed with a diet composed of 50% of duckweed (*Lemna Minor*) but reduced growth at levels higher than 50% (Raj *et al*., 2001). Although the inclusion and replacement levels of duckweed vary, the nutritional values of duckweed such as CP influence the formulation of feed for TGGG juveniles. A previous study also reported that replacement of 5% duckweed in common carp diet showed the best final weight without a significant difference compared with the group fed a 20% duckweed replacement diet (Yilmaz *et al*., 2004).

However, monogastric animals including fish have intestinal microflora that are incapable of digesting fibrous tissue due to the absence of endogenous enzymes that catalyze the hydrolysis of cellulose and other fibrous constituents of the diet (Stickney & Shumway, 1974). Generally, organic acids have been used as feed additives and act by altering gastrointestinal tract function, affect energy metabolism, and maximize nutrient availability (Lückstädt, 2008). In aquaculture, citric acid has been used in partial plant-based diets and fishmeal-based diets of some fish species to improve growth performance (Castillo *et al*., 2014; Hossain *et al*., 2007; Khajepour & Hosseini, 2012) and increase nutrient digestibility (Rabia *et al*., 2016) as well as mineral absorption (Baruah *et al*., 2005; Hossain *et al*., 2007; Sugiura *et al*., 1998). Therefore, this study was designed to investigate the growth performance of TGGG when fed diets composed of either duckweed *L. minor* or *S. polyrrhiza* with or without the supplementation of citric acid.

2. Materials and Methods

2.1 Diet preparation

The species of duckweeds tested were *L. minor* and *S. polyrrhiza. L. minor* contained 35.92% protein and 1.64% lipid while *S. polyrrhiza* had 24.83% protein and 1.79% lipid on a dry matter basis. The carbohydrate levels in *L. minor* and *S. polyrrhiza* were 15.15% and 15.87%, respectively. Due to the higher protein requirement of TGGG (Jiang *et al*., 2016), a lower level of duckweed at 5% of fishmeal protein replacement was tested in the present study. Fishmeal and fish oil were used as the main source of protein and lipid in the feed formulation (Table 1). The control diet (CON) was formulated without any addition of duckweed and citric acid. The other four diets were formulated with 5% protein replacement with duckweed with or without 3% of citric acid: (1) diet with duckweed *L. minor* (DL); (2) diet duckweed *L. minor* with citric acid (DLC); (3) diet with duckweed *S. polyrrhiza* (DS);

Table 1. Diet formulation for protein replacement of duckweed in dry matter (DM).

Ingredients (g/100g)	CON	DL	DS	DLC	DSC
Fishmeal[a]	66.3	62.9	62.9	62.9	62.9
Duckweed meal					
L. minor	-	7.0	-	7.0	-
S. polyrrhiza	-	-	10.1	-	10.1
Fish oil	10.3	10.6	10.4	10.6	10.4
Alpha starch[b]	15.4	11.5	8.6	8.5	5.6
Carboxymethylcellulose[c]	1.5	1.5	1.5	1.5	1.5
Vitamin Premix[d]	3.0	3.0	3.0	3.0	3.0
Mineral Premix[e]	2.0	2.0	2.0	2.0	2.0
Dicalcium phosphate	1.0	1.0	1.0	1.0	1.0
Citric acid[f]	-	-	-	3.0	3.0
Chromium oxide	0.5	0.5	0.5	0.5	0.5
Proximate composition (%)					
Moisture	7.0	8.3	8.1	7.7	8.1
Crude protein	50.3	51.8	50.7	50.1	50.4
Crude lipid	16.3	16.7	16.6	16.3	16.4
Crude fiber	0.6	1.7	2.2	1.7	2.2
Ash	13.2	14.5	15.1	14.2	15.2

[a] Danish fishmeal, Denmark
[b] Alpha starch, Zibo Zimao Co. Ltd.
[c] Carboxymethyl cellulose (CMC), Sigma
[d] Vitamin mixture (g/kg mixture): Ascorbic acid, 45.0; Inositol, 5.0; Choline chloride, 75.0; Niacin, 4.5; Riboflavin1.0; Pyridoxine HCL, 1.0; Thiamine HCL, 0.92; Dicalcium panothenate, 3.0; Retinyl acetate, 0.60; Vitamin D3, 0.083; Menadione, 1.67; Dialpha tocopherol acetate, 8.0; D-biotin, 0.02; Folic acid, 0.09; Vitamin B12, 0.001. All ingredients were diluted with cellulose to 1 kg.
[e] Mineral mixture (g/kg mixture): Calcium phosphate monobasic, 270.98; Calcium lactate, 327.0; Ferrous sulphate, 25.0; Magnesium sulphate, 132.0; Potassium chloride, 50.0; Potassium iodide, 0.15; Copper sulphate, 0.785; Manganese oxide, 0.8; Cobalt carbonate, 1.0; Zinc oxide, 3.0; Sodium salenite, 0.011; Calcium carbonate, 129.274.
[f] Citric acid, crystallized, 99.5% purity, Systerm
CON, control diet; DL, diet with duckweed *L. minor*; DS diet with duckweed *S. polyrrhiza*; DLC, diet with duckweed *L. minor* and citric acid; DSC, diet with duckweed *S. polyrrhiza* and citric acid (DSC).

and (4) diet with duckweed *S. polyrrhiza* with citric acid (DSC). The diet pellets (3 mm in diameter) were produced using a kitchen meat mincer, oven-dried at 40 ℃, and stored in a 5 ℃ chiller until use. Each experimental diet was subjected to proximate analysis after diet preparation. The moisture content in each diet ranged from 7.0 to 8.3% (P>0.05). The CP and CL contents were 50% and 16%, respectively (P>0.05). Crude fiber and ash content ranged from 0.60 to 2.20% and 13.2% to 15.2% (P>0.05), respectively.

2.2 Fish and experimental conditions

The juvenile TGGG were purchased from a local fish farm in Tawau, Sabah. Upon arrival, the fish were acclimatized to the experimental condition and fed a control diet for 1 week before starting the 10-week feeding trial. The fish were then randomly distributed into 150-liter conical tanks with 100 liters in volume of seawater at a stocking density of 20 fish per tank. Triplicate groups of fish were fed with each dietary treatment twice daily (07:00 and 14:00) until apparent satiation. The dissolved oxygen, pH, temperature, and salinity of the seawater were maintained at 4.19-5.31 mg/l, 7.07-7.64, 28.1-30.7 ℃, and 31.28-33.58 ppt, respectively.

2.3 Growth performance and body condition indices

Body weight and body length of fish were measured at the initial and final stages of the feeding trial. Body weight was also measured at 2-week intervals to determine the growth performance. Before every measurement, the fish were starved for 24 h. The fish were anaesthetized (Transmore, Nika Trading, Co.) to reduce handling stress. At the end of the feeding trial, fish (n=5) were sacrificed for an estimation of the viscerosomatic index (VSI), hepatosomatic index (HSI) and condition factor (CF) for each treatment. The whole body of the fish (n=4) and feces were kept frozen for the proximate composition analysis (AOAC, 1990). CP was determined by the Kjeldahl™ 2300 protein analyzer (FOSS Tecator, Sweden). CL was determined gravimetrically using the petroleum benzene-extraction method in a Soxtec™ 2043 Fat Extraction (FOSS Tecator, Sweden). Crude fiber was determined by weak acid and alkali digestion using a Fibertec System 1021 Cold Extractor and Hot Extractor (FOSS Tecator, Sweden). The ash content was determined as the residue remaining after incineration of samples at 550 ℃ in a muffle furnace for 5 h.

2.4 Fecal collection

Fecal collection for digestibility analysis was started after the feeding trial. The remaining fish in each treatment were pooled for fecal collection. The design of the tanks followed the description by Biswas *et al.* (2007) with slight modifications. After the last feeding, all tanks were properly cleaned after 1 h of feeding. The feces were collected in the fecal collection column at the bottom of each tank and rinsed with distilled water before storage at -20 ℃ in a freezer.

2.5 Apparent digestibility coefficient (ADC)

The apparent digestibility coefficient (ADC) of the diets was determined by the nitric acid-perchloric acid digestion method by Furukawa and Tsukahara (1966). Nutrient contents of the feed and feces, such as CP and CL, were analyzed according to AOAC (1990). The ADC of phosphorus in fish feces was determined using the Amidol method (Egsgaard, 1948) and measured in a spectrophotometer at 750 nm.

2.6 Statistical analysis

One-way analysis of variance (ANOVA) was used to evaluate the effects of different formulated feeds on the performance of hybrid grouper TGGG juveniles. Statistically different data were further compared using Tukey's test during the post-hoc test. All analyses of data were performed using the SPSS program for Windows. All data are presented as mean±SD. Data stated in percentage were transformed to arc-sine before data analysis.

3. Results

3.1 Growth performances and feed utilization

Fish fed DL and DS achieved higher body weight gain (BWG) and specific growth rate (SGR) compared to the CON without significant differences (P>0.05) (Table 2). Fish fed DSC and DLC achieved higher growth compared to the

Table 2. Growth performances of TGGG juveniles fed experimental diets.

Parameter	Dietary treatment				
	CON	DL	DS	DLC	DSC
Initial body weight (g)	10.30±0.02	10.32±0.07	10.32±0.05	10.31±0.05	10.30±0.04
Final body weight (g)	56.31±4.20^{a}	61.95±3.23^{ab}	58.50±1.90^{ab}	67.75±1.81^{b}	68.98±3.94^{b}
Initial body length (cm)	7.10±0.05	7.09±0.03	7.04±0.07	7.16±0.10	7.17±0.12
Final body length (cm)	12.12±0.25	12.63±0.17	12.63±0.10	12.90±0.25	12.77±1.03
Body weight gain (%)	446.47±40.06^{a}	500.59±33.37^{ab}	466.68±19.71^{ab}	557.12±19.00^{ab}	569.94±38.52^{b}
Specific growth rate (%/day)	2.83±0.12^{a}	2.99±0.09^{ab}	2.89±0.06^{ab}	3.14±0.05^{ab}	3.17±0.09^{b}
Survival (%)	96.97±5.77^{b}	100^{b}	96.67±2.89^{a}	93.33±2.89^{b}	80.00±5.00^{b}

Body weight gain (%) = (final weight (g) – initial weight (g)/initial weight (g) x 100)
Specific growth rate (%/d) = ln (final weight (g)) - ln (initial weight (g)/days x 100)
Survival (%) = final fish no./initial fish no. x 100 Values are mean±SD (n=3)
Different superscripted letters within the same row indicate significant differences (P<0.05)
CON, control diet; DL, diet with duckweed *L. minor*; DS diet with duckweed *S. polyrrhiza*; DLC, diet with duckweed *L. minor* and citric acid; DSC, diet with duckweed *S. polyrrhiza* and citric acid (DSC).

CON and the SGR of the DSC group was significantly higher compared to the CON (P<0.05). This indicated better growth of the fish fed the duckweed diet supplemented with citric acid than the CON. Survival of the fish was not affected by the experimental diets. Survival ranged from 80 to 100%.

All groups fed with duckweed diets had better feed performance compared to the CON. Fish fed with the duckweed diets (DL and DS) had better utilization than the CON (P>0.05) (Table 3). However, when fish were fed the duckweed diets supplemented with citric acid (DLC and DSC), the feed utilization was higher in the feed conversion ratio, protein efficiency ratio and net protein utilization (P>0.05). The daily feed intake was higher in the DS group compared to the CON but the CON was not significantly different compared with the DL, DLC, and DSC groups (P>0.05).

3.2 Body condition indices

There were no significant differences among treatments in the CF (P>0.05) (Table 4). The CON had a significantly higher HSI than fish fed the duckweed diets and duckweed diets supplemented with citric acid (P<0.05). The VSI of the CON was significantly higher than the DSC group (P<0.05) but comparable to other treatments (P>0.05).

3.3 Apparent digestibility coefficient (ADC) of the diets and phosphorus absorption

The ADCs of dry matter (DM) in the DL and DS groups were significantly lower than the CON, DLC, and DSC groups (P<0.05) (Table 5). Conversely, the ADCs of DM in the DLC and DSC groups were not significantly different to CON (P>0.05). The ADCs of CP in fish fed the DL and DS diets were significantly lower than the CON (P<0.05). However, the ADCs of CP in fish fed the DLC and DSC diets were similar to the CON (P>0.05). No significant differences were observed in the ADCs of CL among the treatments (P>0.05). The phosphorus absorption in fish fed the DL diet was significantly lower than the CON (P<0.05) although the DS group was comparable to the CON (P>0.05). The DLC and DSC groups showed significantly higher phosphorus absorption compared with the CON (P<0.05).

3.4 Whole body proximate composition

The body protein content in fish fed the DL, DS, and DLC diets was comparable with the CON (P>0.05) (Table 6). However, fish fed the DSC diet had a significantly higher body protein content than the CON, DL, and DS groups (P<0.05). The body lipid content in fish fed the DL, DS, and

Table 3. Feed utilization of TGGG juveniles after 10 weeks of feeding trial.

Feed utilization	Dietary treatment				
	CON	DL	DS	DLC	DSC
Daily feed intake (%)	1.80 ± 0.05^{a}	1.87 ± 0.03^{ab}	1.98 ± 0.05^{b}	1.84 ± 0.01^{a}	1.92 ± 0.12^{ab}
Feed conversion ratio	0.91 ± 0.07^{a}	0.92 ± 0.02^{a}	1.00 ± 0.05^{a}	0.90 ± 0.01^{a}	0.84 ± 0.11^{a}
Protein efficiency ratio (%)	2.55 ± 0.20^{a}	2.50 ± 0.11^{a}	2.24 ± 0.14^{a}	2.70 ± 0.27^{a}	2.75 ± 0.31^{a}
Net protein utilization (%)	31.72 ± 2.36^{a}	33.90 ± 2.27^{a}	31.30 ± 1.91^{a}	36.97 ± 0.15^{a}	35.02 ± 5.70^{a}

Daily feed intake (%) = Total feed (g) x 100/[(total final body weight (g) + total initial body weight (g)) x days/2]
Feed conversion ratio (FCR) = dry feed consumed (g)/wet weight gain (g)
Protein efficiency ratio (PER) = wet weight gain (g)/total protein intake (g)
Net protein utilization = (final body protein-initial body protein/total protein intake) x 100.
Values are mean±SD (n=3)
Different superscripted letters within the same row indicate significant differences (P<0.05)
CON, control diet; DL, diet with duckweed *L. minor*; DS diet with duckweed *S. polyrrhiza*; DLC, diet with duckweed *L. minor* and citric acid; DSC, diet with duckweed *S. polyrrhiza* and citric acid (DSC).

Table 4. Body indices (% wet weight) of TGGG juveniles after 10 weeks of feeding trial.

Body indices	Dietary treatment				
	CON	DL	DS	DLC	DSC
Condition factor	2.05±0.09	1.91±0.09	1.92±0.18	1.90±0.11	1.82±0.18
Hepatosomatic index	3.01 ± 0.55^{b}	1.85 ± 0.42^{a}	1.76 ± 0.69^{a}	1.41 ± 0.39^{a}	1.00 ± 0.20^{a}
Viscerosomatic index	13.43 ± 2.17^{b}	12.37 ± 1.17^{ab}	10.77 ± 1.76^{ab}	10.89 ± 0.85^{ab}	10.10 ± 0.35^{a}

Condition factor = fish weight (g)/(total length) ^{3} x100
Hepatosomatic index = liver weight (g)/fish weight (g) x 100
Viscerosomatic index = viscera weight (g)/fish weight (g) x 100
Values are mean±SD (n=3)
Different superscripted letters within the same row indicate significant differences (P<0.05)
CON, control diet; DL, diet with duckweed *L. minor*; DS diet with duckweed *S. polyrrhiza*; DLC, diet with duckweed *L. minor* and citric acid; DSC, diet with duckweed *S. polyrrhiza* and citric acid (DSC).

Table 5. Apparent digestibility coefficients of experimental diets.

Apparent Digestibility Coefficient	Dietary treatment				
	CON	DL	DS	DLC	DSC
Dry matter (%)	85.40±0.70^{b}	76.18±0.86^{a}	74.63±1.29^{a}	85.03±1.60^{b}	83.54±1.75^{b}
Crude protein (%)	95.94±0.27^{b}	93.57±0.21^{a}	93.25±0.13^{a}	95.99±0.30^{b}	95.46±0.30^{b}
Crude lipid (%)	98.35±0.87^{a}	97.70±0.20^{a}	97.40±0.97^{a}	97.82±0.35^{a}	96.95±0.50^{a}
Phosphorus absorption (%)	51.77±2.33^{b}	44.29±2.50^{a}	57.94±2.60^{bc}	63.20±2.13^{cd}	68.57±2.46^{d}

Apparent Digestibility Coefficient (%) = [100 – (C feed/C feces x N feces/N feed) x 100];
C, Chromium (III) oxide (Cr_2O_3); N, Nutrient (crude protein (CP) or crude lipid (CL)); DM, dry matter
Phosphorus absorption (%) = 100 – (100 x C feed (%)/C feces (%) x P feces (%)/P diet (%)); P, phosphorus
Values are mean±SD (n=3)
Different superscripted letters within the same row indicate significant differences (P<0.05)
CON, control diet; DL, diet with duckweed *L. minor*; DS diet with duckweed *S. polyrrhiza*; DLC, diet with duckweed *L. minor* and citric acid; DSC, diet with duckweed *S. polyrrhiza* and citric acid (DSC).

Table 6. Whole body proximate composition (% wet weight) of TGGG juveniles after a 10-week feeding trial.

Proximate composition	Dietary treatment				
	CON	DL	DS	DLC	DSC
Protein	16.88±0.14^{a}	16.57±0.09^{a}	16.63±0.13^{a}	16.91±0.19^{ab}	17.37±0.26^{b}
Lipid	8.08±0.10^{c}	7.70±0.33^{b}	6.51±0.13^{a}	7.54±0.08^{b}	7.82±0.04^{bc}
Moisture	70.05±1.26^{ab}	70.01±0.96^{ab}	69.14±0.38^{ab}	71.08±0.32^{b}	68.41±0.98^{a}
Ash	7.39±0.33^{a}	7.39±0.19^{a}	7.68±0.32^{a}	10.17±0.17^{b}	10.74±0.20^{b}

Values are mean±SD (n=3)
Different superscripted letters within the same row indicate significant differences (P<0.05)
CON, control diet; DL, diet with duckweed *L. minor*; DS diet with duckweed *S. polyrrhiza*; DLC, diet with duckweed *L. minor* and citric acid; DSC, diet with duckweed *S. polyrrhiza* and citric acid (DSC).

DLC diets was significantly lower than the CON (P<0.05). The lipid content in the DLC group was significantly lower than the CON (P<0.05) while the DSC group was not significantly different from the CON (P>0.05). The body moisture of fish fed the DLC and DSC diets was not significantly different to other treatments (P>0.05). The ash content was significantly higher in the DLC and DSC groups compared to the other treatments (P<0.05)

4. Discussion

The higher growth performance in the DSC and DLC groups was due to the citric acid supplementation in the diet. This is in agreement with other studies that used similar concentrations of citric acid in partial plant-based diets which showed a significant growth in beluga (Khajepour & Hosseini, 2012), rohu (Baruah *et al.,* 2007), and common carp (Khaje pour *et al*., 2012) demonstrated by increased weight gain and specific growth rate. Moreover, similar results of growth performance were also observed in red sea bream (*Pagrus major*) (Hossain *et al*., 2007; Sarker *et al*., 2007) with 1% citric acid and red drum (*Sciaenops ocellatus*) at 1.5% citric acid (Castillo *et al*., 2014). Other reports revealed that the suitable citric acid supplementation level was 0.2% for allogynogenetic crucian carp (Leng *et al*., 2006) and 0.3% for hybrid tilapia (Pan *et al*., 2004). Based on previous studies, carnivorous fish with a well developed stomach could tolerate citric acid at a higher level. However, omnivorous and herbivorous fish are limited to lower levels of citric acid, which prompts the distinction in the proper levels of dietary citric acid for different fish species (Li *et al*., 2015). Fresh-water carnivorous fish such as rainbow trout (*Oncorhynchus mykiss*) also showed similar growth with positive control at 1% citric acid (Pandey & Satoh, 2008). Supplementation with citric acid indicates that it might liberate adequate phosphorus (P) from fishmeal (tricalcium phosphate) and also duckweed to allow for fish growth (Hossain *et al*., 2007).

Duckweed diets with or without the citric acid supplementation did not influence the daily feeding intake of fish during the feeding trial. According to Gijzen and Khondker (1997), the unpalatability of duckweed is usually related to the genera *Lemna* and *Spirodela* as they may contain high amounts of calcium oxalate which might limit feed intake. However, the present study showed that the replacement of duckweed as protein at the level of 5% is still palatable for fish. In addition, duckweed intake is influenced by the quantity of nutrients in the plant and the bitter taste of some species of duckweed which is due to the level of tannins and toxins that might be present in the plant (Mwale & Gwaze, 2013). In a study using plant ingredients in feed such as cottonseed meal in tilapia diet, the feed intake was significantly reduced at higher replacement levels (Agbo *et al*., 2011).

The groups of fish fed duckweed diets had a comparable feed utilization to the control group but slightly higher with citric acid supplementation. This indicated that the

feed utilization can be improved through citric acid supplementation. At 3% inclusion level, the feed conversion ratio decreased in beluga (Khajepour & Hosseini, 2012), rohu (Baruah *et al.,* 2007), and common carp (Khajepour *et al*., 2012). The feed performance in red sea bream (Sarker *et al*., 2005) was also improved with 3% citric acid addition. The improved protein efficiency ratio in the present study was comparable to beluga (Khajepour & Hosseini, 2012) and rohu (Baruah *et al*., 2007) when citric acid was added into the diet at 3%. The increasing trend in net protein utilization showed that the supplementation of duckweed and citric acid in the diet can help to improve the feed utilization and also the growth of hybrid grouper.

The presence of a plant protein like duckweed influenced the HSI of TGGG in the present study. In a study of European seabass (*Dicentrachus labrax*) fed with a fish-based or plant-based diet, the fish fed a plant-based diet had reduced HSI compared to fish fed a fish-based diet (Geay *et al*., 2011). The significantly lower HSI in fish fed duckweed diets could be attributed to lower fat deposition in the liver that affected the size of the liver as reported in studies of tilapia when fed fermented plant-based diets (Velásquez *et al*., 2015). With citric acid supplementation, the HSI reduced further compared to the other groups. The possible reason may be the presence of anti-nutritional factors and other compounds in the duckweed. Previous studies using higher levels of duckweed in silver barb (*Barbodes gonionotus*) (Noor *et al*., 2000) showed the appearance of fat changes in the liver characterized by the presence of empty spaces in the hepatocyte. Another possible reason could be the concentration level of citric acid. No adverse effects were observed in HSI of the red drum when fed a diet with a lower concentration of citric acid at 0.75% and 1.5% (Castillo *et al*., 2014). Although citric acid has a positive impact on the growth and feed performance of fish in the present study, it should be a primary concern in the long-term application of citric acid as it may affect the histopathology of the fish. The lower VSI in citric acid-supplemented groups in the present study was supported in a study of beluga where muscle lipid content decreased as a result of citric acid addition at 3% concentration (Khajepour & Hosseini, 2012).

The lower ADC of CP in fish fed duckweed diets may be due to the presence of fiber in the duckweed in the formulated diet. Dietary plant ingredients can influence the gastrointestinal transit time of feed due to the presence of fibers and sugars which subsequently alter the digestibility of nutrients ingested by the fish (Eusebio *et al*., 2004; Storebakken *et al*., 1999; Zhou *et al*., 2004). However, the ADC of CP in fish groups fed the citric acid-supplemented diet was comparable to the control without significant difference. The apparent digestibility of protein and amino acids in growing pigs improved when organic acids were added into diets suggesting that it might stimulate mucosal morphology and hence influence pancreatic secretions (Partanen & Mroz, 1999).

Phosphorus is important for fish as it is the part of the skeleton, nucleic acids, ATP, and phospholipids which are present as hydroxyapatite and tricalcium phosphate in fishmeal (Sarker & Satoh, 2007) and phytate in plant protein. The improved absorption of phosphorus aided with the supplementation of citric acid in this study is similar to a report by Sugiura *et al.* (1998) where the apparent digestibility of many minerals including phosphorus in fishmeal increased with citric acid. In other studies, the supplementation of formic acid and citric acid in diets enhanced the apparent absorption of phosphorus in rainbow trout and red sea bream (Vielma & Lall, 1997; Sarker *et al*., 2005).

Higher body protein in the DSC diet indicated that citric acid can improve the digestion in fish and enhance fish growth. Supplementing the diet with citric acid also increased the whole body ash content in the fish which was similar to the studies in rainbow trout (Vielma & Lall, 1999). This suggested that mineral utilization of fishmeal and plant ingredients enhanced and therefore might have protected the inhibitory action in the experimental diet components (Khajepour & Hosseini, 2011).

5. Conclusions

The results of the present study demonstrate that duckweed can be used by the hybrid grouper juveniles without influencing their growth. Furthermore, the supplementation of citric acid enhanced the growth, feed utilization, digestibility, and phosphorus absorption in the fish.

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