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Dried *Enteromorpha intestinalis* could partially replace soybean meal in the diet of juvenile *Oreochromis niloticus*

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Abstract. This study aimed at evaluating raw dried *Enteromorpha intestinalis* to replace partially soybean meal in the diet of Nile tilapia *Oreochromis niloticus* fry. For 90 days fish fry were fed diets containing 0, 15 and 30 % replacement of soybean meal with the dried seaweed by weight, the replacement represented an equivalent to 0, 3.1 and 6.2 % soybean protein replacement. Results showed that survival for all treatments were statistically similar, quite high (93 – 96 %) and were independent of dietary treatment ($P>0.05$). Weight gain, specific growth rate and feed intake of *O. niloticus* fry fed on diet containing 15 % replacement were snot significantly different ($P>0.05$) with those fed with the control diet. Feed conversion ratios (FCR) of tilapia fed the experimental diets were excellent (1.0 - 1.1) and were not significantly different ($P\geq0.05$) from each other. It is concluded that 15 % replacement by weight of soybean meal with the raw dried *E. intestinalis* in the Nile tilapia fry or fingerling diet could be used to lessen the cost of the formulated feeds since it did not have any adverse effects on growth, nutrient utilization and body composition of the fish.
Key Words: Forage substituent, aquaculture, protein replacement, dietary treatment, fry.

Introduction. Production of soybean meal in tropical countries is very limited and in the Philippines, locally produced soybean meal is allotted almost solely for human consumption and those used for animal feeds are almost entirely imported. As a result, there is a problem of fluctuating prices of the ingredient partially due to changing foreign exchange rate and ultimately a problem of food security in terms of protein supply from fish. There is a need to look for other sources that could replace soybean meal as a feed ingredient in the aquaculture industry.

Enteromorpha sp. is a good candidate for partially replacing soybean meal for reasons that it multiplies rapidly effectively making it a nuisance macroalgae and are not highly consumed by grazers of epiphytes (Lougheed & Stevenson 2004). *Enteromorpha intestinalis* tends to dominate over other seaweeds due to its reproductive unicells which can withstand unfavorable conditions (Beach et al 1995). A few workers have evaluated this seaweed as feeds for aquaculture species. Inclusion of 10 % *Enteromorpha* in the diet of shrimps enhances growth, feed intake, and protein efficiency (Cruz-Suárez et al 2008). Inclusion of 5 to 15 % of *Enteromorpha prolifera* in juvenile large yellow croaker diet results in higher SGR than in those fed using the control (no inclusion) diet (Asino et al 2011).

Juvenile and adult stages of Nile tilapia basically feed on aquatic vegetation, phytoplankton, zooplankton, periphyton and detritus of plant origin, depending on size (El-Sayed 2006). Their larvae initially feed on phytoplankton and zooplankton, especially crustaceans (copepods), zooplankton, periphyton and detritus of plant origin, depending on size. Thus, this fish species is expected to accept diets containing macroalgae. As far as we are concerned, incorporating *Enteromorpha* in the diet of fry or juvenile Nile tilapia has not been evaluated. Thus, the aim of the present study was to evaluate *E. intestinalis*

as a partial replacement of soybean meal in the diet of fry to juvenile *Oreochromis niloticus* by determining its effects on growth, nutrient utilization and body composition.

Material and method

Preparation of dried E. intestinalis. *E. intestinalis* was collected from brackishwater ponds around Iloilo, namely Arevalo, Duangas and Leganes. Seaweeds were transported to the University multispecies Hatchery, Miagao, Iloilo and were manually washed with freshwater, shade-dried for 3 to 4 days, ground using a miller, and kept frozen (-20 ^{o}C) until use. The whole experiment was done from September 2012 to March 2013.

Diet preparation. Three practical diets were formulated to provide 37 to 40 % crude protein and 9 - 10 % crude fat containing varying amount of dried raw *E. intestinalis.* The dried raw seaweed replaced soybean meal by weight at 0 % (Control diet), 15 % (Diet 2) and 30 % (Diet 3) (Table 1). These weight replacements were equivalent to 0 %, 3.1 % and 6.2 % of soybean protein replacements, respectively.

Table 1

Feed formulation and proximate composition of experimental diets, namely a control and two diets with soybean meal replaced with raw dried *Enteromorpha intestinalis* at 15 % and 30% by weight

Ingredients	*Control diet (0 %)*	*Diet 1 (15 %)*	*Diet 2 (30 %)*
Sardines meal	310.0	310.0	310.0
Soybean meal	260.0	221.0	182.0
Seaweeds RDUP	0.0	39.0	78.0
Copra Meal	74.8	74.8	74.8
Rice Bran	120.9	120.9	120.9
Ipil- ipil leaf meal	101.0	101.0	101.0
Cod liver oil	30.0	30.0	30.0
Vegetable oil	20.0	20.0	20.0
Vitamin mix Biomax a	21.7	21.7	21.7
Mineral Mix b	21.6	21.6	21.6
Cornstarch	30.0	30.0	30.0
CMC	10.0	10.0	10.0
Total	1000.0	1000.0	1000.0
Proximate composition (dry weight basis) g kg^{-1}			
Dry matter	949.2	945.3	944.1
Crude Protein	400.2	388.1	374.9
Crude Fat	99.4	94.3	94.1
Crude Fiber	64.7	61.8	63.7
Ash	140.5	149.1	156.7
Nitrogen-free extract	295.2	306.8	310.8
Energy	3675.8	3628.0	3589.0
Total	1000.0	1000.0	1000.0

a Vitamin mix Biomax (mg kg-1 dry diet unless otherwise stated): Vitamin A 1 200 000 IU; Vitamin D3 200 000 IU; Vitamin E 20 000 IU; Vitamin B1 8000; Vitamin B2 8000; Vitamin B6 5000; Vitamin B12 1% 2000 mcg; Niacin 40 000; Calcium Pantothenate 20 000; Biotin 40; Folic acid 1 800; Ethoxyquin 500; Carrier q.s ad to make 1 kg.
b Mineral premix (mg kg-1 dry diet unless otherwise stated): Iron 40 000; Manganese 10 000; Zinc 40 000; Copper 400; Iodine 1 800; Cobalt 20; Selenium; Carrier q.s ad to make 1 kg.

The diets were formulated following that of Santiago et al (1982). The ingredients were ground and sieved at 400 µm prior to mixing; all dried ingredients were thoroughly mixed and liquid ingredients and vitamin/ mineral premix added last. Gelatinized cornstarch was added as a final step before pelletizing. The moistened mixture was pelleted (2 mm) in a meat grinder and oven-dried (60 ^{o}C) long enough to reduce

moisture to less than 10 %. Diets were crumbled into different pellet sizes (0.5 to 3.0 mm), sealed in plastic bags, and stored at -20 ^{o}C until use.

Experimental tilapia and set up. Four thousand Nile tilapia (*O. niloticus*) fry were procured from SEAFDEC-AQD, Tigbauan, Iloilo and acclimatized in the hatchery laboratory of UPV in a 1-ton fiber glass tank with continuous aeration for 10 days. During acclimatization, the fish were fed with the control diet. Two hundred seventy Nile tilapia fry were randomly stocked in eighteen 60 L tanks (15 fry tank^{-1}). The experimental diets were fed to triplicate groups of Nile tilapia fry (average body weight –ABW- of 0.03 ± 0.00 g) at a rate of 4 times daily. Every 10 days, fish were bulk-weighed during the entire feeding trial and feed amount was adjusted continually. The whole feeding trial lasted for 90 days.

The feeding trials were conducted in closed recirculating systems where filtered water from reservoir entered and left each tank at the rate of 1.3 L min^{-1}. About 70 % of the water volume in the system was replaced and filters cleaned every 2 days. Uneaten feeds and faeces were siphoned off every morning before the first feeding. Chlorinated tap water (100 ppm NaClO) was subjected to strong aeration 3 days before use. Water temperature and pH were measured twice a day (0800 and 1600 h) while dissolved oxygen (D.O.) was measured twice a week, and nitrite and total ammonia weekly using commercially available kits (AQUA- NITE^{TM} and AQUA-AM^{TM}, respectively).

Growth performance parameters. Growth and feed utilization efficiency were calculated using the following formulas:

$$\text{Weight gain, WG (g)} = W2 - W1$$

Where: W2 = Final weight (g)
W1 = Initial weight (g)

$$\text{Specific Growth Rate (SGR, \%/ day)} = [(\ln W2 - \ln W1) / (T2 - T1)] * 100$$

Where: W2 = Final weight (g)
W1 = Initial weight (g)
T2 = Final time (in days)
T1 = Initial time (in days)

$$\text{FCR} = \text{Feed intake (g)} / \text{Weight gain (g)}$$

$$\text{Nutrient Retention (\%)} = [(\%\ \text{final carcass nutrient} \times \text{final ABW (g)}) - (\%\ \text{initial carcass nutrient} \times \text{initial ABW (g)})] \times 100 / \text{total nutrient intake (g)}]$$

$$\text{Survival (\%)} = (\text{Survived fish} / \text{Initial fish stocked}) \times 100$$

Carcass analysis. Three thousand cohort fish were taken at the start of the experiment and kept frozen (-20 ^{o}C) until proximate analysis for initial carcass composition. At the end of the feeding trial, fish from each tank were pooled by treatment, weighed, dried and subjected to final carcass analysis. Moisture was measured using a thermo-balance (Mettler Toledo HB43 halogen moisture analyzer). Ash content was determined after incineration in a muffle furnace at 550 ^{o}C for 12 h (AOAC, 2002). Crude protein was measured after block digestion and steam distillation using Foss Tecator^{TM} digestion system and Foss Kjeltec^{TM} 8200 auto-distillation unit. Crude fat was extracted using Foss Soxtec^{TM} 2050 automatic system and fiber was determined using Foss Fibertec^{TM} 2010 system.

Statistical analysis. A Shapiro-Wilks *W* test was used to asses normality of data and Levene's test was performed to check homogeneity of variance. Data on growth performance (*i.e.* survival, weight gain, SGR, FCR, carcass composition, nutrient and

energy retention) were analyzed by one-way analysis of variance (ANOVA). Where significant difference detected, treatment means were compared using Tukey's HSD test at $P<0.05$. Values are expressed as means ± S.E.M. All statistical calculations were performed using SPSS for Windows (version 16).

Results

Water parameters quality. The following ranges of values for the water quality parameters were recorded: temperature 27.3 ± 0.1 ^{o}C; pH 9.0 ± 0.01; D.O. 10.1 ± 0.3 ppm; ammonia 0.02 ppm and nitrite 0.01 ppm. All measured parameters were within the optimal range known for tilapia.

Chemical composition of the raw dried E. intestinalis. Table 2 shows the proximate compositions of the dried *E. intestinalis* powder. Crude fiber and lipid contributed the least amount in analyzed samples and the major biochemical component in the dried seaweed was carbohydrate. The data were not replicated and thus, comparison was numerical.

Table 2

Proximate analysis of raw dried *Enteromorpha intestinalis* ingredient used in the experimental diets (dry weight basis)

Chemical composition	*(g kg^{-1})*
Dry matter	837.8
Crude protein	99.1
Crude lipid	1.43
Crude fiber	59.0
Ash	330.5
Nitrogen-free extract	510.0
Total	1000

Growth performance and nutrient utilization. WG, SGR, and feed intake of fish fed with the diet containing 15 % dried *E. intestinalis* powder exhibited no significant difference with those fed with the control diet; fish fed diets with 30 % replacement by seaweed was significantly lower ($P\leq0.05$) than the control and 15 % seaweed-replaced diets (Table 3).

Table 3

Growth performance, feed and nutrient utilization of Nile tilapia, *Oreochromis niloticus* fed with the control and soybean replacement with raw dried *Enteromorpha intestinalis* diets for 90 days

Parameter	*Control (0 %)*	*Diet 1 (15 %)*	*Diet 2 (30 %)*
Weight gain (g)	7.00 ± 0.55^{a}	8.28 ± 0.98^{a}	4.57 ± 0.13^{b}
SGR (%/day)	6.79 ± 0.10^{a}	6.84 ± 0.17 ^{a}	6.29 ± 0.04^{b}
Feed intake (g fish-1)	6.93 ± 0.48^{a}	8.78 ± 0.55 a	5.01 ± 0.18^{b}
FCR	1.00 ± 0.02	1.12 ± 0.06	1.09 ± 0.03
Survival rate (%)	95.6 ± 2.2	95.6 ± 2.2	93.3 ± 2.2
Protein retention (%)	29.59 ± 0.48^{a}	28.41 ± 1.63^{a}	26.71 ± 0.64^{b}
Lipid retention (%)	38.65 ± 0.63^{a}	29.33 ± 1.68^{b}	27.36 ± 0.66^{b}

Values are means ± SEM. Mean with common superscripts in the same column are not significantly different ($P<0.05$).

FCR and survival did not differ significantly among dietary treatments ($P\geq0.05$). Tilapia fed with the control diet exhibited higher protein and lipid retention than did fish fed diets

with soybean meal replaced by *E. intestinalis*. However, those fish fed diets containing seaweed exhibited numerically higher body crude protein than did fish fed the control diet (Table 4). Protein retention of fish fed the control and 15 % seaweed-replaced diet were not significantly different from each other ($P \geq 0.05$) while the 30 % seaweed-replaced diet was significantly lower. In contrast, lipid retention in fish fed the two seaweed-replaced diets were significantly lower ($P \leq 0.05$) than in those fed the control diet.

Table 4

Final carcass composition of Nile tilapia, *Oreochromis niloticus* fed the control and raw dried *Enteromorpha intestinalis* powder replacing soybean meal at 15 % and 30 % by weight

Components	*Final carcass (g kg-1)*		
	Control Diet (0 %)	*Diet 1 (15 %)*	*Diet 2 (30 %)*
Dry matter	954.0	945.1	944.3
Crude protein	630.4	643.4	643.7
Crude lipid	204.2	161.3	165.3
Crude fiber	2.31	2.01	3.18
Ash	163.1	177.3	161.6

Discussion. The present study evaluated the potential of raw dried *Ulva intestinalis* as a feed ingredient in the diet of fry and juvenile Nile tilapia replacing the imported soybean meal. The proximate composition of raw *E. intestinalis* showed it contained low amounts of protein (9.9 %) and as such, it could not be considered as a major protein source to replace a considerable amount of soybean meal containing on average 40 – 60 % crude protein content. At best, it could only replace 15 % by weight of the soybean meal or 3.1 % of soybean protein content without adverse effects on the growth performance such as weight gain, specific growth rate and feed intake and on survival. This was an indication that replacing 15 % of soybean meal by the raw dried seaweed could have a significant economic advantage on the part of a fishfarmer especially if the reckoning is on a per hectare basis.

Increasing the level of replacement of soybean meal by dried *E. intestinalis* powder in the present study beyond 15 % resulted in significantly poorer ($P \leq 0.05$) weight gain, specific growth rate and feed intake but resulted in statistically similar ($P \geq 0.05$) FCR and survival rate. This observation was not dissimilar with that of Hasan & Chakrabarti (2009) who find that there is a progressive decrease in fish performance when dietary incorporation of algal meal rose above 15 – 20 percent. However, soybean meal when replaced in Nile tilapia diets with *Ulva rigida* up to 20 % shows no negative effects on growth performance and feed utilization efficiency (Azaza et al 2008). Improvement on weight gain, low FCR and high survival rate are observed after including *Pterocladia capillacea* at 10 % and *U. lactuca* at 5 % in Gilthead seabream diets (Wassef et al 2005). Similarly, better weight gain, SGR and lower FCR are observed when 5 % *Ulva* meal was added in Nile tilapia diet (Ergun et al 2009). Incorporation of *U. lactuca* and *E. linza* at 10 %, however, resulted in poorer growth and feed utilization for rainbow trout than did the control diet (Yildrim et al 2009). The variability among the results could be due to differences in the feeding habits, age and the species of both algae and fish.

It has been known that protein from seaweeds is negatively affected by the presence of large amount of cell wall polysaccharides and phenol contents (Fleurence 1999; Higgs et al 1995; Ragan & Glombitza 1986). *Enteromorpha sp.* (*E. compressa, E. linza, and E. tubulosa*) have been demonstrated to contain a good amount of phenolic compounds (Ganesan et al 2011). The same authors find that protein digestibility has a strong negative linear correlation with the total phenolic content of the seaweed. The presence of oxidized phenolic compounds could have reacted with amino acids and proteins in the present study effectively inhibiting the activity of proteolytic enzymes. The ability of phenolic compounds to form insoluble complexes with protein interferes with

the utilization of dietary protein, thus lowering its nutritional value (Wong & Cheung 2001). In the present study, higher inclusion of the dried *E. intestinalis* could have resulted in a considerable increase in phenolic compounds causing poor digestibility of dietary protein.

Another possible reason for depression of growth at 30 % replacement level could be due to high carbohydrate and ash contents in the raw dried seaweed. Aquatic plants including algae contain 40 % or more of carbohydrate, of which only a small amount of mono- and di-saccharides are present (Hasan & Chakrabarti 2009). The dominance of complex and structural carbohydrates in algae contribute to low digestibility of plant materials. High ash content in diets could decrease protein digestibility and thus adversely affect fish performance (Koprucu & Ozdemir 2005).

The similar high survival rates of fish in all three dietary treatments in the present study indicated that the dried raw seaweed, even at the highest replacement level of 30 % were not lethal to Nile tilapia even after 90 days of culture. This agrees with the work of Aguilera-Morales et al (2005) who observe that anti-nutritional factors in the *Enteromorpha sp.* (*e.g.* tannins, alkaloids, cynogenic glucids and saponin) are scarce to none. Thus, it can be incorporated in diets of aquatic animals.

Conclusion. The present study suggested that the raw dried *E. intestinalis* powder could replace 15 % (w/w) soybean in the diet of Nile tilapia (replacing 3.05 % of soybean meal protein) exhibits no adverse effect on weight gain, specific growth rate, feed intake, food conversion ratio, protein retention and survival rate. This diet produced less fatty fish than did diet with no seaweed replacement.

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