International Journal of Agronomy and Agricultural Research (IJAAR)
ISSN: 2223-7054 (Print) 2225-3610 (Online)
http://www.innspub.net
Vol. 5, No. 6, p. 78-85, 2014

RESEARCH PAPER OPEN ACCESS

Response of fermented *Cladophora* containing diet on growth performances and feed efficiency of Tilapia (*Oreochromis sp.*)

A.P.W. Krisna Dewi[1], H. Nursyam[2], A.M. Hariati[3]

[1]*Graduate student, Department of Aquaculture, Faculty of Fisheries & Marine Science, University of Brawijaya, Indonesia*

[2]*Department of Fish Processing Technology, Faculty of Fisheries and Marine Science, University of Brawijaya, Malang, Indonesia*

[3]*Laboratory of Fish Nutrition, Faculty of Fisheries & Marine Science, University of Brawijaya, Malang, Indonesia*

Article published on December 18, 2014

Key words: Filamentous algae, fish diet, feed substitution, essential amino acid index.

Abstract

Feed is still contributed the highest in the cost of aquaculture. Fermented *Cladophora* was applied to replace plant-based protein for tilapia. Five different diets, but all are iso-protein, were formulated, substituting 0% (as control) to 40% of plant-based protein with fermented *Cladophora*. The experiment was set up using 15 aquaria, each of 30 l and stocked with tilapia (initial individual size of 3.65 ± 0.26 g) at density of 1 fish l^{-1}. All the fish was fed at 3% of body-weight day^{-1} and the experiment was ended at day 42^{nd}. The result showed that tilapia fed with 10% of fermented *Cladophora* reached the highest in growth rate, feed efficiency, protein digestibility, and essential amino acid index. The growth rate of this treatment was 30% higher than the control diet (non substitution with fermented *Cladophora*). This means that by changing ingredients in the diet, it may affect in cost reduction or gain in production biomass.

* **Corresponding Author:** A.P.W. Krisna Dewi ✉ ayuputu72@yahoo.com

Introduction

Tilapia is widely cultured in many tropical and subtropical regions of the world. In Indonesia, tilapia production increased for about 134% in the last five years, from 206,904 mt in 2007 to 481,440 mt in 2011 (MMAF, 2011). A High demand of tilapia in Indonesia continues to increase as tilapia is a moderate priced-commodity and therefore it is affordable food fish for domestic consumption. In line with this, the growth of intensive farming in tilapia production is followed by the development of aquafeed or complete diet of this species.

Feed is the single largest expenditure (± 60% of total cost) in semi-intensive and intensive fish culture operations. Traditionally, fishmeal is preferred dietary protein source for many farmed fish and is appreciated for its amino acid balance (NRC, 1993). However, the world fishmeal production is not expected to increase further. Several strategies were applied to develop a sustainable and economical aquaculture system including finding alternate sources of high quality protein. Several studies in tilapia have been conducted to evaluate a variety of alternative protein sources such as fishery and terrestrial animal by-product meals (Hossain *et al.,* 1992; Fagbenro and Jauncey, 1994), oilseed meals and by-products (Jackson *et al.*, 1982; Pantha, 1982; Tacon *et al.,* 1983; Robinson *et al.,* 1984; Shiau *et al.,* 1989; Chiayvareesajja *et al.,*1990; El-Sayed, 1990; Omoregie and Ogbemudia, 1993), aquatic plants (Almazan *et al.,*1986; Klinnavee *et al.,* 1990; El-Sayed, 1992), single-cell proteins (Davies and Wareham, 1988; Chow and Woo, 1990), and legumes and cereal by-product (Jackson *et al.*, 1982; Wee and Wang, 1987; Martinez-Palacios *et al.*, 1988; Santiago *et al.,*1988; Olvera *et al.,* 1990). Most studies were focused on plant by-product meals as they are quite rich in protein and favorable amino acid content. However, they are deficient in one or more essential amino acid and contain various quantities of anti-nutritional factors (NRC, 1993).

There is also a vital need to seek effective ingredients that can either partially or totally replace plant ingredients as protein sources in plant feedstuffs aquafeeds. Algal products can be used to enhance the nutritional value of food and animal feed owing to their chemical composition; they play a crucial role in aquaculture. Green filamentous algae have shown to have low levels of protein (Boyd, 1973), but the very low cost involved in their culturing and harvesting made them potential as cheap sources of protein for fish.

This study aimed to investigate the effect of fermented cladophora formulated in the diets for tilapia on their growth rate, feed conversion efficiency and carcass parameters. The Experiment was set up to test the hypothesis that fish fed with fermented Cladophora-based diets would perform comparable to protein-based diets commonly used in commercial fish diets.

Materials and methods

Fish and experimental conditions

Tilapia (*Oreochromis* sp), with average individual body weight of 3.65 ± 0.26 g were obtained from local hatchery in East Java. The fish were acclimated and maintained in aquaria for approximately 2 weeks. During this period, all fish were given the experimental diets. Upon commencing of the experiment, fish were randomly placed into 15 aquaria (each of 30 l) at an initial density of 1 fish l^{-1}. The fish were manually fed twice a day with the experimental diet at optimum ratio (3% body weight day^{-1}). Every week, fish biomass were weighed to adjust the total feed needed for each aquarium. Water temperature maintained at 27-28°C.

Feed formulation and diet preparation

Prior to its used as ingredient, filamentous green algae, Cladophora, was made clean from contaminants (snails and weeds) and then fermented using EM-4, a local-commercially probiotics. The fermented algal protein source was then dried at 60°C for 48 hours, and ground into powder using a laboratory hammer mill. Five diets, each containing different proportion of fermented Cladophora (Table 1), were prepared by steam-pelleting machine, dried, and stored at -10°C before use. Each diet was assigned randomly to three aquaria, as replicate.

Table 1. Formulation and composition of five experimental diets.

Ingredients	Feed formulation (% fermented Cladophora in the diet)				
	1 (0%)	2 (10%)	3 (20%)	4 (30%)	5 (40%)
Fish meal	30.0	30.0	30.0	30.0	30.0
Soybean meal	21.0	19.0	17.0	15.0	13.5
Fermented Cladophora (FC)	0.0	10.0	20.0	30.0	40.0
Corn starch	14.0	13.0	8.0	5.0	0.0
Rice bran	13.5	8.5	7.0	3.5	0.0
Tapioca flour	10.0	8.0	6.5	5.0	5.0
Fish Oil	4.0	4.0	4.0	4.0	4.0
CMC	3.0	3.0	3.0	3.0	3.0
Vit Mix	2.0	2.0	2.0	2.0	2.0
Min Mix	2.0	2.0	2.0	2.0	2.0
Cr2O3	0.5	0.5	0.5	0.5	0.5
Proximate Analyses (%dm)					
Dry matter	90.6	90.5	90.6	91.4	91.3
Crude Protein	29.9	29.9	29.4	29.2	29.1
Fat	7.2	7.2	7.2	7.1	7.1
Ash	15.0	16.5	18.1	19.8	20.1
Crude Fiber	5.8	5.6	5.6	6.3	6.7
NFE	32.6	31.4	30.3	29.1	28.6
Energy (kcal/g)	3.4	3.4	3.4	3.3	3.3

Sampling and chemical analysis

At the beginning of the trial, a fish was randomly sampled from each aquarium for determination of initial body composition. At the end of the study, all fish were harvested for counting and batch weighing to determine survival rate (SR), final body weight (FBW), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), protein retention (PR), and protein digestibility (DP).

Dry matter analysis was conducted by drying to constant weight at 105°C, crude protein analysis was conducted by Kjeldahl method (AOAC, 1984), and crude ash by drying muffle furnace at 550°C for 4 hours. Lipid analysis was done with diethyl ether extraction method (AOAC, 1984). Energy analysis was determined by bomb calorimeter. Feed digestibility was measured by adding chromic oxide marker into the diet which gave a green appearance to pellet's feeds. Two hours after feeding, the excess feed and fish feces were removed through siphoning. The Essential Amino Acid Index (EAAI) was calculated using the method explained in Labuda *et al.* (1982); Ijarotimi and Keshinro (2011), based on the equation below:

$$EAAI = \sqrt[n]{\frac{100a \; x \; 100b \; x \ldots 100j}{av \; x \; bv \; x \ldots jv}}$$

where:

n = number of essential amino acids, a, b, ... j = represent the concentration of essential amino acids (lysine, tryptophan, leucine, isoleucine, valine, arginine, threonine, phenylalanine, histidine, and the sum of methionine and cystine) in test sample, and av, bv, ... jv = content of the same amino acids in standard protein (%).

Statistical analysis

Results were presented as mean ± standard deviation of three replicates. All data are subjected to one-way ANOVA and Tukey's multiple range tests using statistical software of SPSS v.16 for windows. The differences will be considered significant at $p<0.05$.

Result and discussion

The Mean Body-Weight of Tilapia

The mean body-weight of tilapia fed with various diets were presented in Fig. 1. Growth responses of fish for different diets during the experimental period were shown in Table 2. Diet with 10% of fermented Cladophora resulted in the highest growth response. It also showed significantly the best (lowest) FCR with the other diets. The apparently poor performance of fish fed with diets containing higher inclusion levels of filamentous algae may be due to several factors. Appler (1985) observed that most of the aquatic plants including algae contain 40% or more of carbohydrate, of which only a small fraction consists of mono- and di-saccharides. The 10% fermented Cladophora may reach an optimal ratio between mono- to poly-saccharides that provide optimal growth.

Table 2. Growth responses and feed utilization of tilapia fed at different levels of fermented Cladophora substituting diets.

Parameters	Level (%) of Fermented Cladophora in the diet				
	1 (0%)	2 (10%)	3(20%)	4 (30%)	5(40%)
SR (%)	94.44±1.92[a]	95.56±1.92[a]	88.89±1.92[a]	88.89±1.92[a]	90.00±1.92[a]
SGR (% WG/day)	1.13±0.08[b]	1.51±0.06[c]	1.23±0.08[b]	1.13±0.07[b]	0.99±0.05[a]
FCR	2.21±0.16[b]	1.90±0.04[a]	2.13±0.14[b]	2.28±0.07[b]	2.60±0.10[c]
PER	1.52±0.11[b]	1.76±0.04[c]	1.60±0.10[b]	1.50±0.05[b]	1.32±0.05[a]
Protein Retention (%)	18.22±1.09[b]	22.06±0.41[c]	18.63±0.49[b]	17.39±1.29[b]	14.37±0.46[a]
Protein Digestibility (%)	74.62±1.94[b]	78.99±1.25[c]	74.83±2.22[b]	74.09±1.18[b]	71.05±1.46[a]

Note: symbols after the value indicated statistical difference or P <0.05.

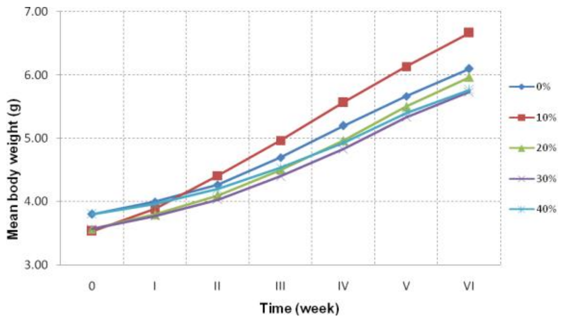

Fig. 1. Mean body-weight of tilapia at the different levels (%) of fermented Cladophora in the diets.

Protein Efficiency Ratio (PER)

Diet added with 10% fermented Cladophora resulted in statistically the highest Protein Efficiency Ratio (PER). However, adding more fermented Cladophora into the formulated diet will reduce the PER, given 40% fermented Cladophora the lowest PER. The more the filamentous algae added into the diet, the more difficulty the fish would utilize the protein from feed. However, the protein utilization reached its optimal level at 10% fermented Cladophora. This means that different sources of plant-based protein can be used and reach effective utilization at certain concentration. Taking into account the price of soy-bean is very high compared to this filamentous algae, a new diet for tilapia can be designed at lower price and with potentially provide higher growth response and PER.

Protein Retention (PR)

The protein retention (PR) revealed similar trends decreasing from diet 2 (10%) to 5 (40%). Diet 2 produced slightly higher values for PER and PR than the control (diet 1) but still lower PR (22.06%) than that of 27% reported for *Cladophora* fed to tilapia (Appler and Jauncey, 1983), which was probably due to the higher protein content of *Cladophora* in the ingredient. Diet 3 and diet 4 produced PER and PR values intermediate between those of diets 1 and 5. This was probably because of its lower protein level (29%) in the diet. Numerous authors have reported increasing PER and PR with decreasing dietary protein level (Ogino and Saito, 1970; Dabrowski, 1977; Mazid *et al.*, 1979; Jauncey, 1981, 1982). The accumulation of reserved lipid was generally well controlled and the reserved lipid was first mobilized into energy prior to muscle protein degradation when fish fed with algae-supplemented diets.

Protein Digestibility

Results indicated that the apparent digestibility for crude protein was significantly affected by the substitution of fermented Cladophora in the ingredients. Protein Digestibility was varied from 71 to 79% for all of the diets. Diet 5 (40% substitution) resulted in the lowest digestibility than other diets. The value of 71% for diet 5 is slightly lower than that of 74% reported for carp fed on diet with an algal meal as the sole protein source (Hepher *et al.*, 1978). Decrease in protein digestibility for tilapia was recorded with increases level of fermented *Cladophora* containing diet. The decreases is, however, very pronounced on substitution with digestibility values recorded being reducing up to 11% for level of substitution reaching 40% fermented *Cladophora*. Due to the presence of indigestible filament algal materials, the more algal content in the diet, the poor the digestibility and consequently the feed utilization for tilapia. Pantastico, *et al.* (1985) reported that newly hatched Nile tilapia fry was able to digest diet from uni-algal cultures of *Euglena elongata* and *Chlorella ellipsoidea*. Both algal culture do not contain celluloses as such in Cladophora.

Essential Amino Acid Index (EAAI) and Biological Value (BV)

The availability index for essential amino acids tended to reflect digestibility coefficients for protein among highly digestible diet. The disadvantage of these well-digested high-protein Cladopora in the diet compared with full soybean meal was that the essential amino acid content, amino acid profile, and amino acid availability of these ingredients were inferior to soybean meal.

The essential amino acid index (EAAI) and biological value (BV) of the fermented Cladophora were 15.66% and 5.4%, respectively. While the control and fermented *Cladophora* containing diets, the EAAIs and BV were presented at Table 3. From the present study it is observed that the BV and EAAI values were generally low; and these could be attributed to the fact that fermented Cladophora is low in protein content and deficient in essential amino acids, tryptophan, compared to that of soybean. Biological value and EAAI more than 80% were stated as a good nutritional quality of the diet (Ijarotimi and Keshinro, 2011).

Table 3. Amino acid composition (mg 100g^{-1} protein) and Nutritional Quality in the Feed and Fermented Cladophora.

Nutritional Quality	Cladophora Fermented Flour	Feed 2 (10%)	Feed 3 (20%)	Feed 4 (30%)	Feed 5 (40%)
Essential Amino Acid					
Arginin	2.39	1.20	1.20	1.19	1.19
Histidin	0.06	0.55	0.53	0.51	0.49
Isoleusin	6.08	0.97	1.02	1.08	1.14
Leusin	2.84	1.43	1.38	1.35	1.31
Lysin	1.87	1.52	1.50	1.47	1.46
Methionin	0.19	0.83	0.80	0.78	0.76
Phenilalanin	1.81	1.13	1.11	1.08	1.06
Threonin	1.75	1.13	1.10	1.08	1.06
Valin	2.39	0.29	0.28	0.27	0.26
Triptopan	Nd*	1.06	1.06	1.06	1.06
Nutritional Quality					
EAAI** (%)	15.66	24.58	24.57	24.56	24.55
BV*** (%)	5.37	15.09	15.08	15.07	15.06

* Nd = not detected   ** Essential Amino Acid Indeks   *** Biological value

Water Quality

During the experiment, water temperature was maintained at the levels of 27-28°C, and with oxygen levels of between 5-6 mg l^{-1}. Webster and Lim (2002) found the temperature for tilapia was between 25-30°C, and with oxygen level should not fall below 3 mg.l^{-1}. So, all water quality during experiment were considered optimal for tilapia growth.

Summarizing all the results, it showed that tilapia fed with 10% of fermented *Cladophora* reached the highest in growth rate, feed efficiency, protein digestibility, and essential amino acid index. The growth rate of this treatment was 30% higher than the control diet (non substitution with fermented *Cladophora*). This means that by changing ingredients in the diet, it may affect in cost reduction or gain in production biomass.

Acknowledgments

The authors appreciated to the support facilities provided by Laboratory of Fish Nutrition and made fully available for authors during the experiment. The author also wishes to express sincere thanks to Siti

Chotipah for her technical support in the laboratory and experimental working platforms. Finally, we all appreciated Mr. D.G.R. Wiadnya, for his guidance in literature review, and his support in the development and final edit of this article.

References

Almazan GJ, Pullin RSV, Angeles AF, Manalo TA, Agbayani RA, Trono MTB. 1986. Azolla pinnata as a dietary component for Nile tilapia Oreochromis niloticus. In First Asian Fisheries Forum, MacLean, J.L., Dizon, L.B. and Hosillos, L.V. (eds), Asian Fisheries Society, Manila, Philippines, pp. 523- 528

AOAC. 1984. Official Methods of Analysis. Association of Official Agricultural Chemists. Washington DC. pp. 1141

Appler HN, Jauncey K. 1983. The Utilization of A Filamentous Green Alga (*Cladophora glomerata* (L) Kutzin) as A Protein Source in Pelleted Feeds for *Sarotherodon* (Tilapia) *niloticus* Fingerlings. Aquaculture **30**, 21-30

Appler HN. 1985. Evaluation of *Hydrodictyon reticulatum* as protein source in feeds for *Oreochromis (Tilapia) niloticus* and *Tilapia zillii*. *Journal of Fish Biology* **27**, 327-334

Boyd CE. 1973. Biotic Interactions between Different Species of Algae. Weed Science, **21(1)**, 32-37

Chiayvareesajja S, Wongwit C, Tansakul R. 1990. Cage culture of tilapia *(Oreochromis niloticus)* using aquatic weed-based pellets. In *Second Asian Fisheries Forum,* Hirano, R. and Hanyu, I. (eds), Asian Fisheries Society, Manila, Philippines, pp. 287-290

Chow CY, Woo NYS. 1990. Bioenergetic studies on an omnivorous fish, *Oreochromis mossambicus,* evaluation of utilization of *Spirulina* algae in feed. In Second Asian Fisheries Forum, Hirano, R. and Hanyu, I. (eds), Asian Fisheries Society, Manila, Philippines, pp. 291 -294

Dabrowski K. 1977. Protein requirements of grass carp fry *(Ctenopharyngodon idella* Val). Aquaculture **12**, 63-73

Davies SJ, Wareham H. 1988. A preliminary evaluation of an industrial single cell protein in practical diets for tilapia *(Oreochromis mossarnbicus* Peters). Aquaculture **73**, 189-196

El-Sayed AFM. 1990. Long-term evaluation of cottonseed meal as a protein source for Nile tilapia *Oreochromis niloticus*. Aquaculture **8(4)**, 315-320

El-Sayed AFM. 1992. Effects of substituting fish meal with *Azolla pinnata* in practical diets for fingerling and adult Nile tilapia *Oreochromis niloticus*. Aquaculture **23**, 167-173

Fagbenro OA, Jauncey K. 1994. Chemical and nutritional quality of dried fermented fish silage and their nutritive value for tilapia *(Oreochromis niloticus)*. Animal Feed Science. Technology. **5(2)**, 167-176

Hepher B, Sandbank E, Shelef G. 1978. Alternative protein sources for warm water fiih diets. In ; J.E. Halver and K. Tiews (Editors), Finfiih Nutrition and Feed Technology, Volume L Heenemann Verlagsgesellschaft mbH, Berlin, pp. 327-342.

Hossain MA, Nahar N, Kamal M, Islam MN. 1992. Nutrient digestibility coefficients of some plant and animal proteins for tilapia *(Oreochromis mossambicus)*. Aquaculture **7**, 257-266

Ijarotimi SO, Keshinro OO. 2011. Determination of Amino Acid, Fatty Acid, Mineral, Functional and Choking Properties of Germinated and Fermented Popcorn (*Zea mays everta*) Flour. European Journal of Food Research & Review **1(2)**, 102-122

Jackson AJ, Capper BS, Matty AJ. 1982. Evaluation of some plant proteins in complete diets for the tilapia *Sarotherodon mossambicus*. Aquaculture **27**, 97-109

Jauncey K. 1981. The effects of varying dietary composition on mirror carp *(Cyprinus carpio)* maintained in thermal effluents and laboratory recycling systems. In, K.Tiews (Editor), Proceedings of the World Symposium on Aquaculture in Heated Effluents and Recirculation Systems, Volume II. Heenemann Verlagsgesellschaft mbH, Berlin, pp. 249-261.

Jauncey K. 1982. The effects of varying dietary protein level on the growth, food conversion, protein utilization and body composition of juvenile tilapias *(Sarotherodon mossambicus)*. Aquaculture **27**, 43-54.

Klinnavee S, Tansakul R, Promkuntong W. 1990. Growth of Nile tilapia *(Oreochromis niloticus)* fed with aquatic plant mixtures. In *Second Asian Fisheries Forum,* Hirano, R. and Hanyu, I. (eds), Asian Fisheries Society, Manila, Philippines. pp. 283-286

Labuda J, Kacerovsky O, Kovae M, Stirba A. 1982. Vyziva a krmenie hospodarskych zvierat. Priroda, Bratislava. 164s

Martinez-Palacios CA, Galvun Cruz R, Olvera Novoa MA, Chavez C. 1988. The use of jack bean *(Canavalia ensiformis* Leguminosae) meal as partial substitute for fish meal in diets for tilapia *(Oreochromis mossambicus* Cichlidae). Aquaculture **68**, 165-175

Mazid MA, Tanaka Y, Katayama T, Asadur-Rahman M, Simpson KL, Chichester, CO. 1979. Growth response of *Tilapia zillii* fingerlings fed isocalonic diets with variable protein levels. Aquaculture **18**, 115-122

MMAF. 2011. Marine and Fisheries in Fig.s 2011 [available in bilingual], Jakarta, Ministry of Marine Affairs and Fisheries, 118p [downloadable from, www.kkp.go.id]

Mune-Mune MA, Minka SR, Mbome IL, Etoa FX. 2011. Nutritional Potential of Bambara Bean Protein Concentrate. Pakistan Journal of Nutrition **10(2)**, 112-119

NRC (National Research Council). 1993. Nutrient Requirement of Fish. National Academy Press. Washington, D.C. 115 pp

Ogino C, Saito K. 1970. Protein nutrition in fish I The utilization of dietary protein by carp. Japanese Society of Fisheries Science **36**, 250-254

Olvera-Novoa MA, Campos GS, Sabido GM, Martinez-Palacios CA. 1990. The use of alfalfa leaf protein concentrate as a protein source in diets for tilapia *(Oreochromis mossambicus)*. Aquaculture **90**, 291 -302

Omoregie E, Ogbemudia FI. 1993. Effects of substituting fish meal with palm kernel meal on growth and food utilization of the Nile tilapia, *Oreochromis niloticus*. *Isr*. Journal Aquaculture Bamidgeh **45(3)**, 113-119

Pantha MB. 1982. The use of soybean in practical feeds for tilapia *(Oreochromis niloficus)*. MSC. Thesis, University of Stirling, UK.

Robinson EH, Rawles SD, Oldenburg PW, Stickney RR. 1984. Effects of feeding glandless and glanded cottonseed products and gossypol to *Tilapia aurea*. Aquaculture **38**, 145-154.

Santiago CB, Aldaba, Laron MA, Reyes OS. 1988. Reproductive performance and growth of Nile tilapia *(Oreochromis niloticus)* broodstock fed diets containing *Leucaena leucocephala* leaf meal. Aquaculture **70**, 53-61

Shiau SY, Kwok CC, Hwang JY, Chen CM, Lee SL. 1989. Replacement of fish meal with soybean meal in male tilapia *(Oreochromis niloticus* X *O. aureus)* fingerling diets at a suboptimal level. Journal World Aquaculture Social **2(4)**, 230-235

Tacon AGJ, Jauncey K, Falaye A, Pantha M, MacGowan I, Stafford EA. 1983. The use of meat and bone meal, hydrolyzed feather meal and soybean meal in practical fry and fingerling diets for *Oreochromis niloticus*. In Proc. 1st. lntl, Symp. on Tilapia in Aquaculture, Fishelson, J. and Yaron, Z. (eds). Tel Aviv University Press, Israel, pp. 356-365

Wee KL, Wang SS. 1987. Nutritive value of leuceana leaf meal in pelleted feed for Nile tilapia. Aquaculture **62**, 97-108

Webster CD, Lim C. 2002. Nutrient Requirements and Feeding of Finfish for Aquaculture. United Kingdom, CABI Publishing. pp.365