Turkish Journal of Fisheries and Aquatic Sciences 4: 105-109 (2004)

Use of Duckweed, *Lemna minor*, as a Protein Feedstuff in Practical Diets for Common Carp, *Cyprinus carpio*, Fry

Erdal Yılmaz[1,*], İhsan Akyurt[1], Gökhan Günal[1]
[1] Mustafa Kemal University, Faculty of Fisheries and Aquaculture, 31034, Antakya, Hatay, Turkey.

* Corresponding Author: Tel.: +90. 326 245 58 45/1315; Fax: +90. 326 245 58 17;
E-mail: yilmaze@mku.edu.tr

Received 09 February 2005
Accepted 09 June 2005

Abstract

The use of dried duckweed, *Lemna minor*, as a dietary protein source for *Cyprinus carpio* common carp fry reared in baskets was the topic of investigation in this study. Five diets with similar E:P ratios were fed to common carp fry with an average initial weight of 0.29 g for 90 days. A diet containing 5%, 10%, 15%, or 20% duckweed was substituted for the commercial 32% protein control-group diet, fed in normal rations to common carp. There was no significant difference between the growth performance of fish that were fed diets containing up to 20% duckweed and fish that were fed the control diet (P>0.05), except for the group of fish on the 15% duckweed diet. Also, no significant difference was observed among treatments with respect to feed utilization (P>0.05). While carcass lipid content increased, protein content of the fish fed a diet of 15% duckweed decreased compared to other groups (P<0.05). The results showed that a diet consisting of up to 20% content could be used as a complete replacement for commercial feed in diet formulation for common carp fry.

Key Words: Protein source, feed, replacement, growth, feed utilization.

Introduction

Marine protein sources are often utilized in aquatic feeds because they are an excellent source of essential amino acids, fatty acids, vitamins and minerals, and because they generally enhance palatability (Davis and Arnold, 2000). However, nearly all researchers agree that an alternative ingredient should be used in the aquafeed industry in place of fish meal, whose supplies are limited although demand for it is expected to rise. For this reason, many studies have been conducted on the replacement of expensive marine proteins with lower-cost ingredients. Considerable attention has been devoted to the replacement of fish meal with plant protein sources such as soybean meal (Oliva-Teles *et al.*, 1994; Refstie *et al.*, 1998), mucuna seed meal (Siddhuraju and Becker, 2001), winged bean (Fagbenro, 1999), and various legumes (Hossain *et al.*, 2001; Gouveia and Davies, 1998).

Duckweed, as a natural protein source, has a better array of essential amino acids than most other vegetable proteins and more closely resembles animal protein (Hillman and Culley, 1978). Newly harvested duckweed plants contain up to 43% protein by dry weight and can be used without further processing as a complete feed for fish. Compared with most other plants, duckweed leaves contain little fiber (5% in dry matter for cultivated plants) and little to no indigestible material even for monogastric animals (Chaturvedi *et al.*, 2003). This contrasts with the compositions of many crops such as soy beans, rice, and maize, approximately 50% of whose biomass comprises residues high in fiber and low in digestibility. Duckweed grown on nutrient-rich water has a high concentration of trace minerals, potassium (K), phosphorus (P), and pigments, particularly carotene and xanthophyll, which make duckweed meal an especially valuable dietary supplement for fish (Leng *et al.*, 1995). Fresh duckweed is well suited to intensive fish farming systems with relatively rapid water exchange rates for waste removal (Gaigher *et al.*, 1984), and duckweed is converted efficiently to live weight by certain fish, which include carp and tilapia (Hepher and Pruginin, 1979; Robinette *et al.*, 1980; Van Dyke and Sutton, 1977; Hassan and Edwards, 1992). In most studies, duckweed is used as a protein source in place of fish meal made of other feedstuffs (Wee, 1991). In the current study, duckweed was selected as a protein feedstuff to serve as a complete replacement for commercial carp feed (32% protein) in an original way, investigating effects on growth, feed utilization and body composition of common carp fry over a period of 90 days.

Materials and Methods

Sample Collection, Processing and Diet Preparation

Fresh duckweed was harvested from the raceways in Samandağ, Hatay Province, Turkey. Duckweed collected were identified following the example of Saygıdeğer (1996), separated from other plants, washed, and dried until it reached constant weight. Prior to diet formulation, the proximate composition was determined and amino acid analysis was performed on the duckweed used in this study. Five diets were prepared to provide similar E:P ratios. Dried duckweed and commercial carp feed (32%

protein, Camli Feed Ind.) were finally milled using a laboratory mill. Commercial carp feed was substituted with 5, 10, 15, and 20% dried duckweed diets, with commercial feed that contained no duckweed serving as the control diet. Milled carp feed and dried duckweed were mixed thoroughly using a robotic kitchen device by adding warm water and mixing until a homogeneous dough was obtained. The resulting dough was then passed through a mincer to produce feeds with pellets of 0.5 mm in diameter. After being dried in an oven at +40ºC, the feeds were kept in a refrigerator (+4ºC) until use.

Experimental System and Fish

The feeding trial was conducted at the Topbogazi Research Station of Fisheries and Aquaculture Faculty in Hatay, Turkey on dates between August 21 and December 21 that added up to a total of 3 months. Baskets were located on a pond (3 m width and 1.5 m depth) receiving water from the Topbogazi Reservoir. The pond was partitioned into fifteen areas by constructing baskets (85 cm in diameter and 65 cm in depth) on metal plates, so that each basket could contain 250 liters of water. Common carp fry averaging 0.29 g were obtained from a local fish hatchery and allowed to acclimate to the culture conditions for 10 days before the feeding trial began. Thirty fish and experimental diets were randomly assigned to each basket for each treatment in three replications. In total, 450 fish were used in this experiment. Depending on water temperature, fish were fed in three portions at rates of 10, 5, and 2% body weight/day in the first, second and third months of the experiment, respectively (Celikkale, 1994). All the fish were removed from each basket every 15 days, batch weighed and the amount of feed was adjusted accordingly.

Analytical Procedures and Water Quality Assessment

The dry matter, ash, and protein content of the diets and of the fish carcasses were analysed according to AOAC (1995): dry matter after drying in an oven at 104ºC until constant weight; ash content by incineration in a muffle furnace at 600ºC for 24 h; crude protein by the Kjeldahl method. Total lipid content was determined by choloroform/methanol extraction (Bligh and Dyer, 1959). The amino acid content of the duckweed was analysed with an Eppendorf LC 3000 Amino Acid Analyser (The Scientific and Technical Research Council of Turkey) using the hydrolysis method. Growth performance, feed utilization, and carcass compositions of fish were all subjected to one-way ANOVA to determine if significant differences occured among the dietary treatments. Differences between means were assessed by Duncan’s (1955) multiple range tests. Effects with a probability of $P<0.05$ were considered significant. Water temperature and dissolved oxygen were measured daily using a combined digital YSI-52 model oxygen meter, and pH was monitored weekly using an electronic pH meter (Lutron-206 model).

Results

Amino acid content of duckweed, proximate composition of the experimental diets and water quality criteria measured over the 90 days of the experiment, which occurred between August 21–December 21, were presented at Table 1, 2 and 3, respectively. Water temperature, dissolved oxygen and pH ranged between 25.25–15.75ºC, 9.50–10.90 mg/L and 8.74–7.98, respectively, from August 21 to December 21. Fish fed the pelleted diet containing 5% duckweed had the best final weight and did not differ from fish in the other groups ($P>0.05$) except for the group fed 15% duckweed, with respect to weight gain and spesific growth rate. Also, there were no differences between the groups with respect to feed conversion and protein efficiency ratios at the end of the experiment ($P>0.05$). Live weight gain of fish fed the control diet decreased more dramatically than that of the fish fed diets containing different amount of duckweed (Figure 1). In addition, the highest survival rates were obtained in groups that were fed 5% and 10% duckweed diets (Table 4). The lowest moisture content was obtained in the 10% duckweed group and the highest protein content was obtained in 20% duckweed group. These two groups were found to differ from the group that was fed the 15% duckweed diet, with respect to protein content, and from the groups that were fed 5% and 15% duckweed diets with respect to moisture content ($P<0.05$). Also, carcass lipid content of fish fed the 10% and 15% duckweed diets was higher than that of the others except for that of the control diet. Ash content of the 10% and 20% duckweed fed groups was significantly lower than that of the control group ($P<0.05$) (Table 5).

Discussion

One of the most commonly encountered difficulties, when alternative protein sources are used, is acceptability due to the palatability of the diets fed to fish (Rodriguez *et al.*, 1996). In the current study, no palatability problem was encountered with any of the treatments during the experiment. Growth performances of fish fed diets containing up to 20% duckweed were comparable to that of the fish fed the control diet, except for 15% duckweed group, suggesting that the lower growth recorded was probably not caused by dietary inclusion of duckweed, but may rather have resulted from some effects of initial handling during the weighing and daily management routines in the experimental system. In addition, carcass compositions of the experimental fish did not vary among treatments

Table 1. Proximate and amino acid compositions of duckweed used in the experimental diets

Proximate composition	g 100 g^{-1} dry weight	Requirement for carp*
Crude protein	18.38	
Lipid	2.32	
Ash	23.71	
Amino acids		
Aspartic acid	1.096	
Threonine	0.494	1.32
Serine	0.471	
Glutamic acid	1.216	
Glycine	0.438	
Alanine	0.747	
Valine	0.646	1.16
Methionine	0.179	0.64
Isoleucine	0.518	0.92
Leucine	0.696	1.64
Tyrosine	0.287	
Phenylalanine	0.623	1.16
Histidine	0.224	0.56
Lysine	10.900	2.12

* Data for EAAs requirement of carp from Ogino (1980).

Table 2. Chemical composition (g 100 g^{-1} of dry matter) of diets with duckweed at different levels

Chemical composition	Duckweed substitution rate				
	0% (Diet 1)	5% (Diet 2)	10% (Diet 3)	15% (Diet 4)	20% (Diet 5)
Dry matter	88.0	88.4	88.7	89.2	89.5
Crude protein	31.82	31.09	30.42	29.67	29.00
Crude fibre	6.82	6.93	7.03	7.07	7.21
Ash	15.91	16.34	16.79	17.21	17.65
Lipid	13.64	13.02	12.43	11.82	11.23
NFE	31.82	32.60	33.31	34.22	34.89
Gross energy	435.95	429.09	422.57	416.20	409.53
E:P ratio	13.70	13.80	13.90	14.02	14.12

Gross energy in kcal 100 g^{-1} based on 5.65 kcal g^{-1} protein; 9.45 kcal g^{-1} lipid; 4.0 kcal g^{-1} carbohydrate according to Halver (1972).
E:P ratio: energy to protein ratio in kcal gross energy g^{-1} protein
NFE, nitrogen free extract calculated as 100 – %(crude protein+crude fiber+ash+lipid)

Table 3. Water quality parameters measured over the 90 days feeding periods

Measurements	August-September	September-October	October-December
Water temperature (ºC)	25.25±0.85	23.00±0.57	15.75±0.62
Dissolved oxygen (mg/L)	9.50±0.30	9.70±0.10	10.90±0.60
pH	8.74±0.22	8.54±0.36	7.98±0.04

except for protein content, which may also indicate that the lower growth of fish fed the 15% duckweed diet was due to lower feed (protein) consumption. Fasakin *et al.* (1999) found that the difference in final weights, specific growth rates, feed conversion and protein efficiency ratios of tilapia that were fed diets containing up to 20% duckweed were not significantly different from those of tilapia that were fed the control diet. Hence, it might be advisable to feed common carp fry diets containing up to 20% duckweed alongside with feed utilization similar to that of the experimental fish.

It has been well known that most plant-derived nutrient sources like soybean meal, rapeseed meal, lupin seed meal, pea seed meal, sunflower oil cake, cottonseed meal, leucaena leaf meal, alfalfa leaf meal, mustard oil cake, and sesame meal contain a wide variety of antinutritional substances (Francis *et al.*, 2001). Antinutrients are defined as substances which by themselves, or through their metabolic products arising in living systems, interfere with food utilization and affect the health and production of animals (Makkar, 1993). In an earlier study, common carp that were fed diets containing higher than 13% mucuna meal showed significantly poorer growth and nutrient utilization indices, compared to carp that

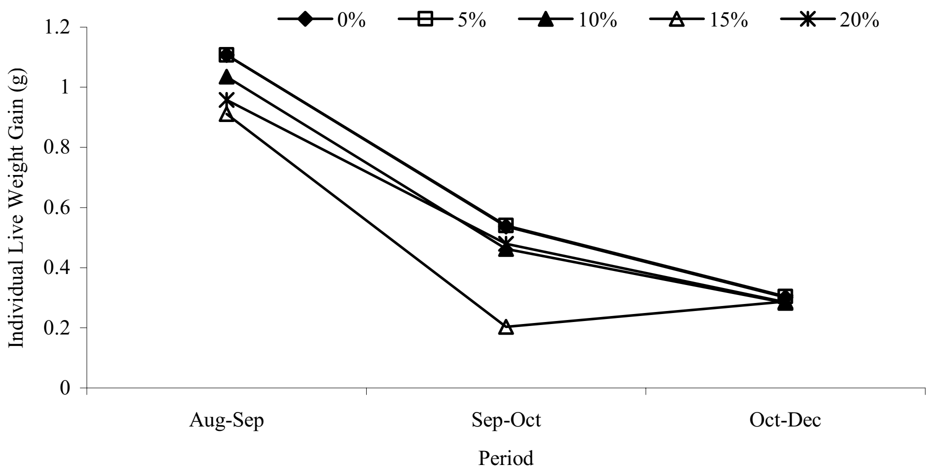

Figure 1. Individual live weight gain for the different substution rates (0 %, 5 %, 10 %, 15 % and 20 %) of duckweed during the experimental period (August 21-December 21).

Table 4. Mean growth and feed utilization parameters of fish fed diets with different duckweed content and control diet for 90 days.

Parameter	Duckweed substitution rate				
	0% (Diet 1)	5% (Diet 2)	10% (Diet 3)	15% (Diet 4)	20% (Diet 5)
IBW (g)	0.295±0.003	0.292±0.005	0.283±0.017	0.286±0.06	0.283±0.013
FBW (g)	2.241±0.094[a]	2.244±0.078[a]	2.063±0.119[ab]	1.687±0.138[b]	2.005±0.132[ab]
BWG (g)	1.946±0.09[a]	1.952±0.07[a]	1.780±0.10[a]	1.401±0.13[b]	1.722±0.14[ab]
SGR (%day^{-1})	2.25±0.04[a]	2.26±0.02[a]	2.21±0.003[a]	1.96±0.08[b]	2.16±0.12[ab]
FCR	2.41±0.11[a]	2.45±0.09[a]	2.45±0.14[a]	2.95±0.26[a]	2.45±0.19[a]
PER	1.49±0.07[a]	1.51±0.05[a]	1.55±0.08[a]	1.35±0.13[a]	1.67±0.14[a]
SR (%)	76.6	86.6	84.4	77.7	74.4

Values are mean ± standard error
Values in the same row with same superscript are not significantly different (p>0.05).
IBW = initial body weight.
FBW = final body weight.
BWG = body weight gain.
SGR, specific growth rate = ((ln FBW – ln IBW)/90 days) x 100.
FCR, feed conversion ratio = feed fed/live body weight gain.
PER, protein efficiency ratio = body weight gain/crude protein fed,
SR = survival rate

Table 5. Body composition (% fresh basis) of the experimental fish fed diets with different levels of duckweed for 90 days.

Components	Duckweed substitution rate (diets)				
	0% (Diet1)	5% (Diet2)	10% (Diet3)	15% (Diet4)	20% (Diet5)
Moisture	78.02±0.26[ab]	79.47±0.21[b]	77.81±0.47[a]	79.41±0.16[b]	77.93±0.64[a]
Protein	15.48±0.59[ab]	14.94±0.28[ab]	16.08±0.53[b]	13.73±0.11[a]	16.60±0.82[b]
Lipid	4.32±0.15[ab]	3.66±0.32[a]	4.92±0.15[b]	4.96±0.15[b]	4.04±0.31[a]
Ash	2.08±0.10[a]	1.93±0.25[ab]	1.19±0.01[c]	1.89±0.21[ab]	1.38±0.09[bc]

were fed a control diet. The diminished growth was attributed to the effects of various antinutrients (tannins, phytates, saponins, L-DOPA, trypsin inhibitor, chymotrypsin inhibitor, phytohaemagglutinating activity) in mucuna seed meal, because the experimental diets used had relatively good acceptability and no essential amino acid (EAA) deficiencies (Siddhuraju and Becker, 2001). Similar results were obtained in another study, in which feeding common carp diets containing more than 12% sesbania meal produced significantly poorer growth and feed utilization compared to fish fed a control diet, despite good feed acceptability and no EAA deficiencies. This was again attributed to the presence of antinutrients such tannins, phytic acid, saponins, trypsin inhibitor and lectins in sesbania meal (Hossain *et al.*, 2001). In contrast, in the current experiment, common carp fry fed diets containing different amounts of duckweed did not show significant differences with respect to growth or feed utilization. These findings support the opinion that duckweed has a higher nutritional value than both mucuna and sesbania meals (Makkar and Becker, 1999), especially considering the fact that the fish used in the present trial were smaller than those used in the two earlier studies and thus were more susceptible to the inhibitory effects of antinutrient content. Therefore, duckweed meal provides an easy, practical and cheaper fish feedstuff because it requires no processing to destroy any antinutrients.

It was interesting to note that fish fed the control diet (commercial carp diet) lost weight in the third month of the experiment due to decreased water temperature, but fish fed diets containing varying amounts of duckweed meal did not. This situation may have resulted from high lysine content of duckweed meal, and it can be hypothesized that high lysine content of duckweed meal could support fish growth and feed utilization at lower temperatures. Fagbenro (1999) found that methionine supplementation in feeds containing winged bean meal provided similar growth compared to control, but methionine deficiency could be compensated for by the cysteine content of the winged bean meal, which was >2.0 g cysteine/100 g protein. Furthermore, Viola *et al.* (1982) determined that when 80% of fish meal was replaced by soybean meal, supplementing the diets with 4–5 g of L-lysine and 100 g oil kg^{-1} helped the carp to achieve similar weight gains and protein utilization rates, compared to those achieved by control-group fish, which were fed a fish meal-based diet. Thus, lysine content of the plant-derived alternative ingredients were of critical importance in sustaining the expected fish growth and feed utilization.

In conclusion, it would be advisable to include up to 20% duckweed in commercial feed for common carp fry in order to produce results similar to those obtained with the control diet with respect to growth, feed utilization, and body composition. These findings also support the use of duckweed in commercial grow-out carp feeds as a dry ingredient. Duckweed meal should be considered for both cold and warm water fish nutrition.

References

AOAC (American Association of Cereal Chemist). 1995. Approved Methods of the American Association Chemist. 9th ed. AACC, St. Paul, MN, USA.

Bligh, G.R. and Dyer, J.N. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Phisiology, 37 (8): 912-917.

Celikkale, M.S. 1994. İçsu Balıkları ve Yetiştiriciliği. Karadeniz Teknik Üniversitesi Basımevi yayın no. 128, Trabzon, 460 pp.

Chaturvedi, K.M.M., Langote, D.S. and Asolekar, R.S. 2003. Duckweed-fed fisheries for treatment of low strength community waste water. WWWTM Newsletter-Asian Insttitute of Technology, India.

Davis, D.A. and Arnold, C.R. 2000. Replacement of fish meal in practical diets for the Pacific white shrimp, *Litopenaeus vannamei.* Aquaculture, 185: 291-298.

Duncan, D. 1955. Multiple range test and multiple *F*-tests. Biometrics, 11: 1-42.

Fagbenro, O.A. 1999. Comparative evaluation of heat-processed Winged bean (*Psophocarpus tetragonolobus*) meals as partial replacement for fish meal in diets for the African catfish (*Clarias gariepinus*). Aquaculture, 170: 297-305.

Fasakin, E.A., Balogun, A.M. and Fasuru, B.E. 1999. Use of duckweed, *Spirodela polyrrhiza* L. Schleiden, as a protein feedstuffs in practical diets for tilapia, *Oreochromis niloticus* L. Aquaculture Research, 30: 313-318.

Francis, G., Makkar, H.P.S. and Becker, K. 2001. Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199: 197-227.

Gaigher, I.G., Porath, D. and Granoth, G. 1984. Evaluation of duckweed (*Lemna gibba*) as feed for tilapia (*Oreochromis niloticus* cross *Oreochromis aureus*) in a recirculating unit. Aquaculture*,* 41: 235-244.

Gouveia, A. and Davies, S.J. 1998. Preliminary nutritional evaluation of pea seed meal (*Pisum sativum*) for juvenile European sea bass (*Dicentrarchus labrax*). Aquaculture, 166: 311-320.

Halver, J.E. 1972. Fish Nutrition*.* Academic Press, London, pp. 713

Hassan, M.S and Edwards, P. 1992. Evaluation of duckweed (*Lemna perpusilla* and *Spirodela polyrhiza*) as feed for Nile Tilapia (*Oreochromis niloticus*). Aquaculture, 104: 315-326

Hepher, B. and Pruginin, Y. 1979. Guide to fish culture in Israel. 4. Fertilisation, manuring and feeding. Foreign Training Dept., Israel. 61 pp

Hillman, W.S and Culley, D.D. 1978. The uses of duckweed. American Scientist, 66: 442-451

Hossain, M.A., Focken, U. and Becker, K. 2001. Evaluation of an unconventional legume seed, *Sesbania aculeata,* as a dietary protein source for common carp, *Cyprinus carpio* L. Aquaculture, 198: 129-140.

Leng, R.A., Stambolie, J.H. and Bell, R. 1995. Duckweed a potential high protein feed resource for domestic animals and fish. Livestock Research for Rural Development, Vol. 7, no. 1, New England.

Makkar, H.P.S. 1993. Antinutritional factors in food for livestock. M. Gill, E. Owen, G.E. Pollot and T.L.J. Lawrence, (Eds.), Animal Production in Developing Countries. Occasional publication No. 16. British Society of Animal Production: 69-85.

Makkar, H.P.S. and Becker, K. 1999. Nutritional studies on rats and fish (carp, *Cyprinus carpio*) fed diets containing unheated and heated *Jatropha curcas curcas* meal of a non-toxic provenance. Plant Food Hum. Nutr., 53: 183-192.

Ogino, C. 1980. Requirements of carp and rainbow trout for essential amino acids. Bull. Jpn. Soc. Sci. Fish., 46: 171-174.

Oliva-Teles, A., Gouveia, A.J., Gomes, E. and Rema, P. 1994. The effect of different processing treatments on soybean meal utilization by rainbow trout, *Oncorynchus mykiss.* Aquaculture, 124: 343-349.

Refstie, S., Strorebakken, T. and Roem, A.J. 1998. Feed consumption and conversion in Atlantic salmon (*Salmo salar*) fed diets with fish meal, extracted soybean meal or soybean meal with reduced content of oligosaccharides, trypsin inhibitors, lectins and soya antigens. Aquaculture, 162: 301-312.

Robinette, H.R., Brunson, M.W. and Day, E.J. 1980. Use of duckweed in diets of channel catfish. Proceedings. 13th Annual.Conference. SE Association. Fish Wildlife Age, 108-114.

Rodriguez, S.M., Olvera, N.M.A. and Carmona, O.C. 1996. Nutritional value of animal by-product meal in practical diets for Nile tilapia, *Oreochromis niloticus* (L) fry. Aquaculture Research, 27: 67-73.

Saygıdeger, S. 1996. *Lemna gibba* L. ve *Lemna minor* L., (*Lemnaceae*)'nin Morfolojik, Anatomik, Ekolojik ve Fizyolojik Özellikleri. Ekoloji ve Çevre Dergisi, 18: 8-11.

Siddhuraju, P. and Becker, K. 2001. Preliminary nutritional evaluation of Mucuna seed meal (*Mucuna pruriens* var. *utilis*) in common carp (*Cyprinus carpio* L.): an assessment by growth performance and feed utilization. Aquaculture, 196: 105-123.

Van Dyke, J.M. and Sutton, D.L. 1977. Digestion of duckweed (Lemna spp.) by the grass carp (*Ctenopharyngolon edella*). Journal of Fish Biology, 11: 273-278.

Viola, S., Mokady, S., Rappaport, U. and Arieli, Y. 1982. Partial and complete replacement of fish meal by soybean meal in feeds for intensive culture of carp (*Cyprinus carpio*). Aquaculture, 26: 223-236.

Wee, K.L. 1991. Use of non-conventional feedstuffs of plant origin as fish feeds. It is practical and economically feasible? S.S. De-Silva (Ed.), Fish Nutrition Research in Asia. Proceedings of the 4th Asian Fish Nutrition Research in Asian Fisheries Society Special Publication Asian Fisheries Society, Manila, Philippines: 13-32.