Fish Nutrition Research in Asia

Proceedings of the Fifth Asian Fish Nutrition Workshop

Edited by S.S. De Silva

Asian Fisheries Society Special Publication No. 9

International Development Research Centre

Fish Nutrition Research In Asia

Proceedings of the
Fifth Asian Fish Nutrition Workshop

Edited by
S.S. De Silva

1994

Published by the Asian Fisheries Society
in association with the
International Development Research Centre (Canada)

Fish Nutrition Research in Asia

Proceedings of the Fifth Asian
Fish Nutrition Workshop

Edited by

S.S. DE SILVA

1994

De Silva, S.S., editor. 1994. Fish nutrition research in Asia.
Proceedings of the Fifth Asian Fish Nutrition Workshop.
Asian Fish Soc. Spec. Publ. 9. Asian Fisheries Society,
Manila, Philippines.

Copyedited, typeset and printed at Deakin University, Victoria, Australia

ISBN 971-8709-57-6

Contents

Introduction

The Asian Fish Nutrition Network is very pleased that in January 1993 it was able to conduct the Fifth Workshop on Fish Nutrition Research in Asia at Udorn Thani in north-east Thailand. Former workshops were held in a rural setting and this time Udorn Thani was chosen because it is an area where small-scale aquaculture is beginning to take off. This volume details the proceedings of the workshop.

The proceedings reflect current ongoing research in nutrition in relation to finfish culture. Over the years, through the efforts of the network, there has been a gradual change in research strategy: emphasis has turned to on-farm research in relation to semi-intensive culture systems, which is by far the most predominant form of culture in the region and for that matter in the world. It is hoped that in the ensuing years a healthy mix of basic and applied research into fish nutrition in the region will benefit the fish farming community, particularly rural, small-scale farmers, for whom aquaculture provides not only an additional source of income but also represents a very significant source of animal protein in their diet.

I am grateful to all sessional chairpersons, rapporteurs and referees, and in particular to Dr Santosh P. Lall, Dr Kok Leong Wee and Dr (Ms) Mali Boonyaratpalin for acting as resource people. A special vote of thanks is extended to Dr Armaratne Yakkupitiyage for taking care of the organizational logistics and Mr Mick Taylor and his team of the Aquaculture Outreach Programme of the Asian Institute of Technology for overseeing the logistics in Udorn Thani.

SENA S. DE SILVA

Summary of the Discussions

The workshop was divided into eight main sessions in which review papers were presented and which also included original papers in that area of research. This summary details the main points that were discussed.

1. Problems of Translation of Laboratory Findings into Practice

   It was evident that the problem of putting laboratory findings into practice mostly centred around diet development, and were variable from country to country. In most instances the laboratory-developed diets were not adopted by farmer/feed manufacturers and generally when adopted there was a time-lag of 6–10 years.

   It was agreed that the degree of translation and the problems associated thereof varied according to the:
   (a) social conditions;
   (b) intensification of aquaculture in the country/region; and
   (c) reputation of the institution.

   It was also felt that, more often than not, the direct beneficiaries of nutrition research, particularly in diet development, were feed millers. The need to test developed diets 'on-farm' was stressed.

   There was general reiteration by the workshop that for semi-intensive culture practices the most logical approach to diet development would be a 'bottom-up' approach whereby diet development is made around existing farming practices, using ingredients that are familiar to the community.

2. On-Farm Nutrition Research

   It was agreed that there is a serious dearth of on-farm nutritional research in the region. As a consequence our knowledge of some of the common farming practices, and hence ways of improving and sustaining such practices in the long-term may become a problem. Particular areas in which we need to further research actively are:
   (a) effect of fertilizers on nutrient limitation in semi-intensive systems;
   (b) nutritional input from natural/endogenous food to target species; and
   (c) nutrition requirements of target species under varying supplementary feeding and fertilization regimes.

   The scope of methodology for execution of on-farm nutrition research in semi-intensive systems was considered in outline and major problems encountered in respect of such methodologies were discussed.

   The group was of the view that a manual encompassing these methodologies needs to be published which will ensure to some degree at least standardization of the techniques and

therefore comparison of findings from different farming systems, or similar farming systems in different countries made more meaningful.

3. Farm-Made Feeds

The role of farm-made feeds in rural aquaculture in Asia was discussed. It was pointed out that this topic was addressed at a recent workshop conducted by FAO-RAPA/AADCP in Bangkok in December 1992, when the extent and effectiveness of on-farm made feeds in Asian aquaculture was dealt with. The participants noted that research effort needs to be expended on on-farm made feeds, which are essentially supplemental feeds, and that initially such research should concentrate on the degree of utilizability of such feeds by target species and quantify the direct nutritional gain from such feeds to target species. It was felt that some of this research can be linked to that dealt with earlier, particularly diet development based on existing farming practices and effectiveness of supplementary feeds in semi-intensive aquaculture.

(The proceedings of the workshop on on-farm made aquafeeds have since been published, and the reference is:
New, M.B., A.G.J. Tacon and I. Csavas (eds.), 1992. *Farm-Made Aquafeeds. 14–18 December 1992*, Bangkok, Thailand: FAO-RAPA/AADCP.

4. Laboratory-based Research

The discussion endorsed that in general there had been an improvement in methodologies adopted, and that the findings of different researchers are becoming comparable. The group also noted that there had been a significant increase in the number of fish nutrition publications in peer-reviewed journals, and the positive role played by the nutrition network in this regard was acknowledged. It was evident that nutrition researchers in the region are embarking increasingly on energetics studies of commonly cultured tropical species; this trend is welcome. However, the group noted that laboratories embarking on such studies should not only be properly equipped but also the researchers should have a sound background/training in fish energetics. The group did not think that this was a major problem and noted that such research in the region was perhaps going through a metamorphosis as much as nutrient requirement studies and digestibility studies were about a decade ago.

5. Workshop Format

All participants were in agreement that holding the workshops in a 'rural aquaculture' setting was good, and that it should be continued. The general format of the workshop, including having the field trip midway through the workshop, was endorsed. However, participants pointed out that time allotted for discussion of individual presentations should be extended, and each session should be followed up with a general discussion on that specific area of research.

The plan to have a training programme immediately following the workshop was welcomed. It was suggested that such workshops should endeavour to bring together 'new' nutrition researchers of the host country, as much as possible, so that such researchers would not only gain from the course but also from the opportunity to interact more closely with senior and experienced nutrition researchers of the region as well as the resource persons.

Evaluation of Free Essential Amino Acid in Muscle of Nile Tilapia (*Oreochromis niloticus*), as a Basis of Amino Acid Requirement for Growth

C.B. SANTIAGO[1]
R.T. LOVELL[2]

[1] *SEAFDEC Aquaculture Department, Binangonan Freshwater Station, Binangonan, Rizal, Philippines*
[2] *Department of Fisheries and Allied Aquacultures, Auburn University, AL 36849, U.S.A.*

SANTIAGO, C.B. and R.T. LOVELL. 1994. Evaluation of free essential amino acid in muscle of Nile tilapia (*Oreochromis niloticus*) as a basis of amino acid requirement for growth. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop*. Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 1–7.

Abstract

The free essential amino acid levels in muscle of young Nile tilapia after feeding graded amounts of a corresponding test amino acid were measured in nine separate experiments to determine if free amino acids indicate requirements for growth. Data were analyzed by the broken line regression method. Breakpoints for free threonine and free isoleucine were near the threonine and isoleucine requirements for growth. Breakpoints for free lysine, free histidine, and free valine were much higher than the lysine, histidine, and valine requirements for growth, respectively. Levels of other free essential amino acids in muscle behaved erratically or increased linearly as the dietary test amino acid increased. Amino acid requirements as estimated by levels of free essential amino acids in muscle did not consistently confirm amino acid requirements for growth of Nile tilapia.

Introduction

The amino acid requirements of most warmwater fishes studied so far were determined by feeding amino acid test diets for a certain length of time and measuring growth response (Harding et al. 1977; Wilson et al. 1977, 1978, 1980; Nose 1979; Robinson et al. 1980a, 1981; NRC 1983; Santiago and Lovell 1988; Borlongan and Benitez 1990; Borlongan 1991; Ravi and Devaraj 1991). Serum or plasma free amino acids were also measured to confirm the requirements for some essential amino acids in fish (Harding et al. 1977, Wilson et al. 1978; Hughes et al. 1983) in the same manner as levels of plasma free amino acids were used as indicators of amino acid requirements of the chick (Zimmerman and Scott 1965; Robbins et al 1977), pig (Mitchell et al. 1968) and rat (Stockland et al. 1970).

The purpose of the present study was to determine if muscle free essential amino acid levels are indicators of amino acid requirements for growth of Nile tilapia (*Oreochromis niloticus*).

Materials and Methods

Experimental diets, design and feeding

Ten feeding experiments were conducted over an 18-month period. Each experiment consisted of seven dietary treatments, representing levels of the test amino acid, with three replicates each in a completely randomized design. Diet composition, preparation, and storage were as reported by Santiago and Lovell (1988). Vitamin-free casein and gelatin, the natural protein sources, were supplemented with crystalline L-amino acids to provide an amino acid profile similar to that of 28% whole egg protein except for the test amino acid. The lowest level of the test amino acid came solely from the intact proteins.

Young fish (13–87 mg body weight) were stocked randomly in 60-L aquaria at 45–100 per aquarium depending on the size and availability of fish for each of the experiments. Equal number of fish per aquarium and fish of the same size were used in each experiment. The fish were fed as much as they would consume four times daily (at 0730, 1030, 1300, and 1600 hours) up to eight weeks. Growth was measured and amino acid requirements based on growth were determined (Santiago and Lovell 1988).

Preparation of tissue extract for estimation of free amino acids

Approximately 15–33 fish in each replicate were killed 12–15 hours following the final feeding and the laterodorsal muscle on each side of the fish was removed. An equal number of fish was taken from each aquarium in the experiment. Muscle samples from all fish fed the same diet were pooled. A portion of the samples was dried for moisture determination and the remaining portion was used for muscle free amino acid analysis.

Muscle tissues were prepared by modifying various procedures (Tallan et al. 1954; Gerritsen et al. 1965; Clark et al. 1966; Quinn and Fisher 1977). A known weight of muscle was frozen for about one hour, minced and homogenized. Ten percent 5-sulfosalicylic acid was added to the samples. Samples were centrifuged twice in a refrigerated centrifuge for 30 minutes at 15 000 rpm. Supernatants of some samples were analyzed directly for free amino acids after dilution with 0.2N citrate buffer of pH 2.2. Supernatants of other samples too dilute for direct analysis were freeze-dried and the residue was dissolved in an appropriate volume of the dilution buffer before analysis of free amino acids. Free amino acids were analyzed in a single-column automatic amino acid analyzer (Liquimat III) using Dionex DC–6 amino acid analysis resin and lithium citrate buffer system. Flow rate of buffers through the column was 20 mL/hr. Separation of acidic, neutral and basic amino acids was performed at temperature settings of 34, 59 and 67°C, respectively. Free amino acid corresponding to the test amino acid was identified on the basis of its position on the chromatogram and the amount was based on the area under the curve.

Free amino acid levels were measured in muscle instead of blood because the amount of blood that could be collected from fish at the end of the experiments was too small for analysis. In land animals, there is evidence that substantial exchange of plasma and muscle free amino acids occurs with no net changes in uptake or output of plasma amino acids by muscle (Lindsay and Buttery 1980). Kaushik (1979) obtained a similar pattern of changes in free arginine levels, in blood and muscle of rainbow trout fed different levels of dietary arginine. Thus it was presumed that the same is true for Nile tilapia.

Statistical methods

Data on free essential amino acids were subjected to one-way analysis of variance and the means were compared by Duncan's new multiple range test. Regression of muscle free amino acid level on dietary amino acid level was done using the broken line regression model (Brookes et al. 1972; Robbins et al. 1979; Zeitoun et al. 1976). Linear and quadratic regressions were also tried to determine the best fitted lines.

Results and Discussion

Lysine

There was an increase in muscle free lysine as dietary lysine increased (Table 1). Marked increases occurred at 1.6 and 1.9% dietary lysine. Statistical analysis by the broken line regression method showed a breakpoint at 1.83% lysine which is higher than the requirement for growth (1.43% of the diet or 5.12% of dietary protein; Santiago and Lovell 1988).

Lysine requirement of channel catfish (*Ictalurus punctatus*) based on growth was at first confirmed by serum free lysine (Wilson et al. 1977) but, in a subsequent study, serum free lysine provided little indication of the lysine requirement (Robinson et al. 1980b). Free lysine in the plasma of growing rats remained at low levels when lysine in the diet was growth-limiting, but when dietary lysine was in excess of that required for maximum growth, plasma free lysine accumulated in a rapid and linear manner (Stockland et al. 1970). A similar pattern of changes in free lysine was likewise observed in the chick (Zimmerman and Scott 1965).

Table 1. Free lysine, arginine and histidine in muscle (μmoles/g dry muscle) of young Nile tilapia fed varying levels of lysine, arginine and histidine*

LYSINE		ARGININE		HISTIDINE	
% dry diet	Free Lys	% dry diet	Free Arg	% dry diet	Free His
1.1	3.48^{d}	0.6	0.41^{c}	0.2	2.71^{e}
1.3	4.04^{d}	0.8	0.62^{bc}	0.3	3.87^{d}
1.4	5.00^{cd}	1.0	0.63^{bc}	0.4	4.70^{c}
1.5	6.60^{c}	1.2	0.84^{bc}	0.5	5.06^{c}
1.6	9.73^{b}	1.4	1.12^{ab}	0.6	5.95^{b}
1.7	11.01^{ab}	1.6	1.28^{ab}	0.7	5.23^{c}
1.9	12.30^{a}	1.8	1.70^{a}	0.8	9.64^{a}

* Column means followed by a common superscript are not significantly different ($p>0.05$).

Arginine and histidine

Free arginine in muscle increased linearly with increasing dietary levels of the amino acids and did not indicate requirements for growth (Table 1). The breakpoint for free histidine was at 0.59% of the diet which is higher than the requirement for growth (0.48% of the diet or 1.72% of the protein) as reported by Santiago and Lovell (1988). Similarly, serum free arginine and histidine were not good indicators of arginine and histidine requirements in channel catfish (Robinson et al. 1981; Wilson et al. 1980). However, Kaushik (1979) reported earlier that the biochemical estimation of the variations of free arginine levels corroborates the growth studies on arginine requirement of rainbow trout. A more recent study showed that plasma and muscle free arginine levels did not increase markedly when the arginine requirement was met based on growth response (Kaushik et al. 1988). In the chick, Robbins et al. (1977) observed that plasma free histidine concentration was an indicator of the histidine requirement for growth.

Threonine

Free threonine increased up to 1.0% dietary threonine, decreased slightly at 1.2% and increased at 1.6% (Table 2). The broken line method showed a breakpoint at 1.1% threonine which is near the requirement of Nile tilapia for growth (1.05% of the diet or 3.75% of protein; Santiago and Lovell 1988). Threonine requirement of channel catfish determined by growth was likewise confirmed by serum free threonine (Wilson et al. 1978).

Table 2. Free threonine, phenylalanine and tyrosine, and methionine in muscle (μmoles/g dry muscle) of young Nile tilapia fed varying levels of threonine, phenylalanine, and methionine*

THREONINE		PHENYLALANINE**			METHIONINE***	
% dry diet	Free Thr	% dry diet	Free Phe	Free Tyr	% dry diet	Free Met
0.2	1.50^{f}	0.6	0.28^{c}	0.23^{b}	0.15	trace
0.4	2.38^{e}	0.8	0.36^{b}	0.24^{b}	0.35	0.14^{d}
0.6	3.22^{d}	1.0	0.38^{b}	0.26^{ab}	0.55	0.23^{c}
0.8	5.32^{c}	1.2	0.40^{b}	0.26^{ab}	0.75	0.38^{b}
1.0	6.93^{b}	1.4	0.40^{b}	0.29^{ab}	0.95	0.53^{a}
1.2	6.74^{b}	1.6	0.44^{b}	0.25^{ab}	1.15	0.40^{b}
1.6	7.57^{a}	1.8	0.57^{a}	0.30^{a}	1.35	0.41^{b}

* Column means followed by a common superscript are not significantly different ($p>0.05$).
** Dry diet contained 0.5% tyrosine.
*** Dry diet contained 0.15% cystine.

Phenylalanine and methionine

Free phenylalanine and tryosine levels increased linearly as the dietary level of phenylalanine increased (Table 2) and did not reflect phenylalanine requirement. Free methionine levels did not indicate methionine requirement for growth. Free cystine was hardly detectable, probably due to the low dietary cystine level. It is possible that, due to the interrelationships of phenylalanine and tyrosine and of methionine and cystine, requirements for these amino acids may not be easily predicted using data on free amino acids. In growing rats, plasma free tyrosine was a much better indicator of dietary adequacy of phenylalanine plus tyrosine than plasma free phenylalanine alone (Stockland et al. 1971). In channel catfish, neither free phenylalanine nor free tyrosine in the serum could be used to predict requirement for phenylalanine plus tyrosine (Robinson et al. 1980a). However, methionine requirement of channel catfish as estimated by analysis of serum free methionine was similar to the level required for growth (Harding et al. 1977).

Table 3. Free leucine, isoleucine and valine in muscle (μmoles/g dry muscle) of young Nile tilapia fed varying levels of leucine and isoleucine*

LEUCINE				ISOLEUCINE			
% dry diet	Free Leu	Free Ile	Free Val	% dry diet	Free Ile	Free Leu	Free Val
0.6	0.25^{d}	0.31^{d}	1.06^{bc}	0.4	0.09^{d}	0.26^{d}	0.46^{b}
0.7	0.35^{c}	0.60^{b}	1.66^{a}	0.5	0.36^{bc}	0.38^{c}	0.49^{b}
0.8	0.36^{c}	0.76^{a}	1.58^{a}	0.6	0.32^{c}	0.43^{c}	0.58^{b}
0.9	0.27^{d}	0.26^{d}	1.18^{b}	0.7	0.41^{abc}	0.57^{b}	0.84^{a}
1.0	0.49^{b}	0.42^{c}	1.27^{b}	0.8	0.47^{ab}	0.75^{a}	0.96^{a}
1.1	0.26^{d}	0.23^{d}	0.90^{c}	0.9	0.45^{ab}	0.60^{b}	0.84^{a}
1.2	0.58^{a}	0.28^{d}	0.91^{c}	1.1	0.51^{a}	0.78^{a}	0.99^{a}

* Column means followed by a common superscript are not significantly different ($p>0.05$)

Leucine, isoleucine and valine

Muscle free leucine levels changed erratically with increasing dietary leucine (Table 3). This is unlike channel catfish in which serum leucine remained constant regardless of dietary leucine intake (Wilson et al. 1980), but like channel catfish in that free isoleucine and valine were low initially and then increased considerably up to 0.8% dietary leucine in the present study and then decreased at higher levels of the test amino acid.

Free isoleucine in the muscle increased with the levels of isoleucine in the diet (Table 3). Statistical analysis by the broken line method showed breakpoint at 0.8% dietary isoleucine. The breakpoint is near the isoleucine requirement of Nile tilapia (0.87% of the diet or 3.11% of dietary protein) determined by growth (Santiago and Lovell 1988). Free leucine and free valine levels also showed breakpoints at 0.89 and 0.85% dietary isoleucine, respectively, which are near the isoleucine requirement for growth. In channel catfish, serum free isoleucine, free leucine, and free valine increased with increasing dietary isoleucine and the data did not confirm the requirement based on growth (Wilson et al. 1980).

As dietary valine increased, there was a corresponding increase in muscle free valine in Nile tilapia but no considerable change in muscle free isoleucine (Table 4). However, changes in free leucine were variable. Tuttle and Balloun (1976) also observed that dietary valine caused a significant linear increase in plasma valine but no effect on plasma isoleucine in turkey poults. In channel catfish, serum free valine remained statistically the same over the range of dietary valine tested and free leucine and free isoleucine concentrations paralleled that of free valine (Wilson et al. 1980). Plasma free valine has been reported to indicate the requirement of lake trout for valine (Hughes et al. 1983). Regression analysis of muscle free valine in the present study showed a breakpoint at 1.1% dietary valine which is much higher than the requirement for growth (0.78% of the diet or 2.80% of protein) (Santiago and Lovell 1988).

Table 4. Free valine, leucine and isoleucine in muscle (µmoles/g dry muscle) of young Nile tilapia fed varying levels of valine*

VALINE			
% dry diet	Free Val	Free Ile	Free Leu
0.4	0.39^{f}	0.49^{a}	0.74^{b}
0.6	0.68^{e}	0.49^{a}	0.76^{b}
0.8	0.73^{d}	0.43^{a}	0.54^{e}
0.9	0.78^{c}	0.48^{a}	0.74^{b}
1.0	0.96^{b}	0.45^{a}	0.67^{c}
1.2	0.98^{b}	0.41^{a}	0.59^{d}
1.4	1.05^{a}	0.45^{a}	0.83^{a}

* Column means followed by a common superscript are not significantly different ($p>0.05$)

Tryptophan

Free tryptophan could not be measured in the present study. Nevertheless, Walton et al. (1984) were unsuccessful in estimating the tryptophan requirement of rainbow trout on the basis of plasma free tryptophan levels.

General Discussion

It appears from the foregoing that measurement of the levels of free amino acids in serum and muscle tissue as a basis for estimating amino acid requirements has given variable results with fish. The method has been used to confirm results of growth trials but not as the sole basis for estimating the required levels of essential amino acids. It is probable because a number of factors influence free amino acid level in muscle and plasma, such as dietary level (Morrison et al. 1961a&b), dietary predecessors (Kaushik 1979; Morrison et al. 1961b; Plakas et al. 1980; Yamada et al. 1982), duration of experiment (Mitchell et al. 1968), feeding methods (Stockland et al. 1970), the time interval between the last feeding and sampling (Mitchell et al. 1968; Plakas et al. 1980), and interactions among amino acids (Tuttle and Balloun 1976).

In carp fed casein and amino acid diets, plasma free essential amino acids generally reached maximum levels four hours after feeding and then declined below the prefeeding values by 16 hours (Plakas et al. 1980). In Nile tilapia fed a casein diet, Yamada et al. (1982) found that free

essential amino acids reached maximum levels 4 hours after feeding and then declined to fasting levels within 24 hours. On the other hand, free essential amino acids in plasma of fish fed an amino acid diet attained their peak levels at 2 hours and returned to fasting levels in 4–8 hours. In the present study, Nile tilapia were fed diets consisting of a mixture of casein, gelatin, and crystalline amino acids and the fish were sacrificed 12–15 hours after the last feeding. Thus within this period the free amino acids may have increased initially, then subsequently decreased to fasting levels.

Among the free essential amino acids measured, only free threonine and free isoleucine indicated the amino acid requirements for growth of Nile tilapia.

References

Borlongan, I.G. 1991. Arginine and threonine requirements of milkfish, *Chanos chanos* (Forsskal) juveniles. *Aquaculture* 93: 313–322.

Borlongan, I.G. and L.V. Benitez, 1990. Quantitative lysine requirement of milkfish (*Chanos chanos*) juveniles. *Aquaculture* 87: 341–347.

Clark, A.J., Y. Peng and M.E. Swendseid, 1966. Effect of different essential amino acid deficiencies on amino acid pools in rats. *J. Nutr.* 90: 228–234.

Gerritsen, T., M.I. Rehberg and H.A. Waisman, 1965. On the determination of free amino acids in serum. *Anal. Biochem.* 11: 460–466.

Harding, D.E., O.W. Allen and R.P. Wilson, 1977. Sulfur amino acid requirement of channel catfish: L-methionine and L-cystine. *J. Nutr.* 107: 2031–2035.

Hughes, S.G., G.L. Rumsey and M.C. Nesheim, 1983. Dietary requirements for essential branched-chain amino acids by lake trout. *Trans. Am. Fish. Soc.* 112: 812–817.

Kaushik S. 1979. Application of a biochemical method for the estimation of amino acid needs in fish: Quantitative arginine requirements of rainbow trout in different salinities. In Halver, J.E. and K. Tiews (eds.) *Finfish Nutrition and Fishfeed Technology, Vol. 1.* Berlin, Heeneman and Co., pp. 197–207.

Kaushik, S.J., B. Fauconneau, L. Terrier and J. Gras, 1988. Arginine requirement and status assessed by different biochemical indices in rainbow trout. *Aquaculture* 70: 75–95.

Lindsay, D.B. and P.J. Buttery, 1980. Metabolism in muscle. In Buttery, P.J. and D.B. Lindsay (eds.) *Protein Deposition in Animals*. London, Butterworths, pp. 125–146.

Mitchell, J.R. Jr., D.E. Becker, A.H. Jensen, B.G. Herman and H.W. Norton, 1986. Determination of amino acid needs of the young pig by nitrogen balance and plasma free amino acids. *J. Anim. Sci.* 27: 1327–1331.

Morrison, A.B., J.M. McLaughlan, F.J. Noel, and J.A. Campbell, 1961. Blood amino acid studies III. Effects of amount and quality of dietary protein and length of test period on plasma free lysine levels in the rat. *Can. J. Biochem. Physiol.* 39: 1681–1686.

Morrison, A.B., E.J. Middleton, and J.M. McLaughlan, 1961a. Blood amino acid studies II. Effects of dietary lysine concentration, sex and growth rate on plasma free lysine and threonine levels in the rat. *Can. J. Biochem. Physiol.* 39: 1675–1680.

National Research Council, 1983. *Nutrient Requirements of Warmwater Fishes and Shellfishes*. Washington DC. Academy Press. 102 p.

Nose, T. 1979. Summary report on the requirements for essential amino acids for carp. In Halver, J.E. and K. Tiews (eds.) *Finfish Nutrition and Fishfeed Technology, Vol. 1.* Berlin, Heenemann and Co., pp. 145–156.

Plakas, S.M., T. Katayama, Y. Tanaka and O. Deshimaru, 1980. Changes in the levels of circulating plasma free amino acids of carp (*Cyprinus carpio*) after feeding a protein and an amino acid diet of similar composition. *Aquaculture* 21: 307–322.

Quinn, M.R. and H. Fisher, 1977. Effect of dietary histidine deprivation in two rat strains on hemoglobin and tissue concentration of histidine-containing dipeptides. *J. Nutr.* 107: 2044–2054.

Ravi, J. and K.V. Devaraj, 1991. Quantitative essential amino acid requirements for growth of catla, *Catla catla* (Hamilton). *Aquaculture* 96: 281–291.

Robbins, K.R., D.H. Baker, and H.W. Norton, 1977. Histidine status in the chick as measured by growth rate, plasma free histidine and breast muscle carnosine. *J. Nutr.* 107: 2055–2061.

Robbins, K.R., H.W. Norton and D.H. Baker, 1979. Estimation of nutrient requirements from growth data. *J. Nutr.* 109: 1710–1714.

Robinson, E.H., R.P. Wilson and W.E. Poe, 1980a. Total aromatic amino acid requirement, phenylalanine requirement and tyrosine replacement value for fingerling channel catfish. *J. Nutr.* 110: 1805–1812.

Robinson, E.H., R.P. Wilson and W.E. Poe, 1980b. Re-evaluation of the lysine requirement and lysine utilization by fingerling channel catfish. *J. Nutr.* 110: 2313–2316.

Robinson, E.H., R.P. Wilson and W.E. Poe, 1981. Arginine requirement and apparent absence of a lysine-arginine antagonist in fingerling channel catfish. *J. Nutr.* 111: 46–52.

Santiago, C.B. and R.T. Lovell, 1988. Amino acid requirements for growth of Nile tilapia. *J. Nutr.* 118: 1540–1546.

Stockland, W.L., Y.F. Lai, R.J. Meade, J.E. Sowers and G. Oestemer, 1971. L-phenylalanine and L-tyrosine requirements of the growing rat. *J. Nutr.* 101: 117–184.

Stockland, W.L., R.J. Meade and A.L. Melliere, 1970. Lysine requirement of the growing rat. Plasma free lysine as a response criterion. *J. Nutr.* 100: 925–933.

Tallan, H.H., S. Moore and W.H. Stein, 1954. Studies on the free amino acids and related compounds in the tissues of the cat. *J. Biol. Chem.* 211: 927–939.

Tuttle, W.L. and S.L. Balloun, 1976. Leucine, isoleucine and valine interactions in turkey poults. *Poul. Sci.* 55: 1737–1743.

Walton, M.J., R.M. Coloso, C.B. Cowey, J.W. Adron and D. Know, 1984. The effects of dietary tryptophan levels on growth and metabolism of rainbow trout (*Salmo gairdneri*). *Br. J. Nutr.* 113: 2568–2577.

Wilson, R.P., O.W. Allen, E.H. Robinson and W.E. Poe, 1978. Tryptophan and threonine requirements of fingerling channel catfish. *J. Nutr.* 108: 1595–1599.

Wilson, R.P., D.E. Harding and D.L. Garling, 1977. Effect of dietary pH on amino acid utilization and the lysine requirement of fingerling catfish. *J. Nutr.* 107: 166–170.

Wilson, R.P., W.E. Poe and E.H. Robinson, 1980. Leucine, isoleucine, valine and histidine requirements of fingerling catfish. *J. Nutr.* 110: 627–633.

Yamada, S., Y. Tanaka, T. Katayama, M. Sameshima and K.L. Simpson, 1982. Plasma amino acid changes in *Tilapia nilotica* fed a casein and a corresponding free amino acid diet. *Bull. Jpn. Soc. Sci. Fish.* 48: 1783–1787.

Zeitoun, I.H., D.E. Ullrey, W.T. Magee and W.G. Bergen, 1976. Quantifying nutrient requirements of fish. *J. Fish. Res. Board Can.* 33: 167–172.

Zimmerman, R.A. and H.M. Scott, 1965. Interrelationship of plasma amino acid levels and weight gain in the chick as influenced by suboptimal and superoptimal dietary concentrations of single amino acids. *J. Nutr.* 87: 13–18.

A Comparative Study on the Nutritive Value of Some Essential Fatty Acids for Chinese Prawn (*Penaeus chinensis*)

JI WENJUAN
XU XUELIANG
Yellow Sea Fisheries Research Institute,
Qingdao,
P.R. China

WENJUAN, J. and X. XUELIANG, 1994. A comparative study on the nutritive value of some essential fatty acids for Chine prawn (*Penaeus chinensis*). In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish Soc. Spec. Publ. 9. Manila, Philippines. Asian Fisheries Society, pp. 9–14.

Abstract

A feeding experiment on juvenile Chinese prawn (*P. chinensis*) was conducted for a 32-day period with four experimental diets added with 1% highly purified ω-3 and ω-6 fatty acids (Linoleic 18:2ω6, Linolenic 18:3ω3, arachidonic 20:4ω6, and docosahexenoic acid 22:6ω3) respectively and a control diet which contained 5% palmitic acid (16:0) and oleic acid (18:1ω9) mixture. Prawns fed the diet containing 1% of docosahexenoic acid (22:6ω3, DHA) with 4% of 16:0, 18:1ω9 mixture had the highest survival rate, molting frequency and weight gain. The results indicate that DHA has the highest EFA value for juvenile Chinese prawn. Comparisons were made between wild and cultured Chinese prawn for fatty acid composition. There are large proportions of ω-3 polyunsaturated fatty acids in both groups suggesting that supplementation with oils rich in DHA in artificial diets is likely to improve production.

Introduction

World shrimp culture has developed rapidly during the past two decades. In China, marine shrimp culture has developed rapidly since 1978. Chinese prawn (*Penaeus chinensis*) is a preferred species for mariculture in China, and China has become the leading shrimp producer in the world. With this increasing development, well-balanced formulated shrimp feed becomes increasingly important for future shrimp farming operations.

From a nutritional point of view, it has been shown that some marine fish and crustaceans lack the ability to synthesize ω-6 and ω-3 series fatty acids *de novo* to maintain their biological and physiological functions (Castell et al. 1972; Watanabe et al. 1974; Fujii and Yone 1976; Kanazawa et al. 1979; Yu and Shinnhuber 1977). It has been demonstrated that both ω-6 and ω-3 series fatty acids are essential for normal growth and survival in several penaeid species (Bottino et al. 1980; Colvin et al. 1976; Kayama et al. 1980).

The purpose of this study was to evaluate relative EFA value of 18:2ω6, 18:3ω3, 20:4ω6 and 22:6ω3 for the prawn *Penaeus chinensis*.

Materials and Methods

Prawn

Juvenile Chinese prawn were obtained from Jiao Nan hatchery station in Jiao Nan county. On arrival in the laboratory, they were acclimated for 10 days and reared on clam meat, and the prawns (0.4–0.5 g in body weight, 2.5–3.0 cm in length) were divided into 30 groups and maintained on one of five purified diets for 32 days.

Test diets

The five experimental diets were formulated and prepared to contain the same nutrients except for 1% of the fatty acid that was to be tested. The basal diet ingredients are shown in Table 1. The composition of supplementing fatty acids added to the basal diet is shown in Table 2. All lipid-containing ingredients, such as crab protein concentrate, gelatin, corn starch, dextrin and cellufil were solvent-extracted prior to making the basal mixture to remove any trace of lipids. Each diet was sealed in a plastic bag flushed with nitrogen and frozen at –20°C until used for feeding. The fatty acid levels of the experimental diets, as fed, are given in Table 3.

Table 1. Basal diet ingredients

Ingredient	% of dry diet
Crab protein concentrate	40
Gelatin	10
Corn starch	15
Dextrin	5
Cellufil	17.3
Mineral mix (as in HFX CRD)	4
Vitamin mix (as in HFX CRD)	2
Cholesterol	0.5
Vitamin E acetate	0.2
Choline chloride (70%)	1.0
Experimental Lipid Mixture	5.0

Table 2. Composition of supplementing fatty acids incorporated into the basal diets

Diet No	18:2ω6	18:3ω3	20:4ω6	22:6ω3	16:0 + 18:1ω9
1	1	0	0	0	2 + 2
2	0	1	0	0	2 + 2
3	0	0	1	0	2 + 2
4	0	0	0	1	2 + 2
5	0	0	0	0	2.5 + 2.5

Table 3. Fatty acid analysis of five experimental diets (%)

Diet No	18:2ω6	18:3ω3	20:4ω6	22:6ω3	16:0 + 18:1ω9
1	25.4	0	0	0	74.5
2	0	24.6	0	0	75.3
3	0	0	22.7	0	74.1
4	0	0	0	22.8	76.0
5	0	0	0	0	100.0

Feeding method

After acclimation, the juvenile prawns were randomly selected and divided into 30 groups. Each group consisted of 4 individual prawns in a specially made experimental cage with perforated dividers to prevent cannibalism. Thirty cages were distributed in 10 troughs (90 × 60 × 60 cm) supplied with running sea water at the rate of 150 litres per day. The sea water was filtered through quartz sand, 4 high density fibre cartridge filters and activated carbon. There were six replicates (6 cages, 24 individual prawns) for each dietary treatment. Each experimental group was fed 3 times daily, at 10% of their body weight initially, and reduced to 5% of the body weight by the end of the experiment. After the 32-day feeding, the weights and survival rate were determined for each treatment. Molting was recorded twice daily.

Determination of fatty acids

Fatty acids of the hepatopancreas, muscle and ovary of wild and cultured prawn were analyzed. The five experimental diets and the prawn carcasses after feeding trials were analyzed for fatty acid compositions. The total lipids were extracted by the method of Bligh and Dyer (1959). Methylation of fatty acids was made by the improved method of Maeda et al. (1987). The mixture of the methylesters was analyzed using gas chromatography. GC was performed with a HP 5890II-GC fitted with a flame ionization detector and capillary column (0.32 mm × 25 M Carbowax).

Results

The fatty acid composition of wild and cultured prawn is shown in Table 4. The major saturated fatty acid in *P. chinensis* is palmitic acid (16:0), the major monounsaturated fatty acids are oleic acid (18:1ω9) and palmitoleic acid (16:1ω7). There are large proportions of ω-3 polyunsaturated fatty acids in both wild and cultured prawn. But compared to cultured prawn, there is a higher proportion of DHA and a lower proportion of linoleic acid in the fatty acid compositions of wild prawn. The variation is likely to be due to different food items.

Table 4. A comparison of fatty acid compositions between wild and cultured prawn (*Penaeus chinensis*)

Fatty acids	Ovary			Hepatopancreas		
	Cultured after wintering	Cultured III*	Wild III	Cultured after wintering	Cultured III	Wild III
C14:0	1.3	1.3	1.6	2.3	2.7	2.6
C16:0	17.3	16.9	16.8	20.3	19.0	17.6
C16:1ω7	16.5	14.9	20.2	18.3	14.1	18.9
C18:0	3.3	4.1	2.5	5.3	5.1	2.4
C18:1ω9	20.7	19.4	23.5	21.9	20.0	23.0
C18:2ω6	5.5	4.1	1.2	4.1	1.9	0.8
C18:3ω3	0.9	1.0	1.0	1.6	1.1	0.5
C20:1ω9	1.1	2.7	2.9	1.8	3.8	9.0
C20:4ω6	4.0	2.8	1.4	ND**	3.0	3.0
C20:5ω3	18.3	18.0	13.1	14.5	17.0	9.4
C22:6ω3	1.8	6.6	7.0	3.0	3.6	3.8
C22:6ω3/C18:2ω6	0.327	1.609	5.833	0.732	1.894	4.75

* The brookstock fed clam meat for 44 days after wintering, GSI = 8.0

** Not Detected

After the 32-day feeding trial, the effects of dietary 18:2ω6, 18:3ω3, 20:4ω6, and 22:6ω3 on survival rate, molting frequency and growth rate of Chinese prawn are shown in Tables 5 and 6 and Figures 1 and 2. Prawns fed the control diet containing only 16:0 and 18:1ω9 mixture with no EFA supplementation had poor survival, lower molting frequency, and did not grow. Addition of 1% of 18:2ω6 to the diet improved the weight gain and survival rate of the prawns ($p<0.05$), but the molting frequency was not improved. Prawns fed the diet supplemented with 1% of 18:3ω3 showed remarkably improved growth, molting and survival rate ($p<0.01$). Tables 5 and 6 and Figures 1 and 2 also show that additional 1% of 20:4ω6 to the diet improved survival, molting and growth rate significantly. Prawns fed the diet containing 1% of 22:6ω3 (DHA) had the best survival rate, molting frequency and weight gain among the four fatty acids supplemented in the diets ($p<0.01$).

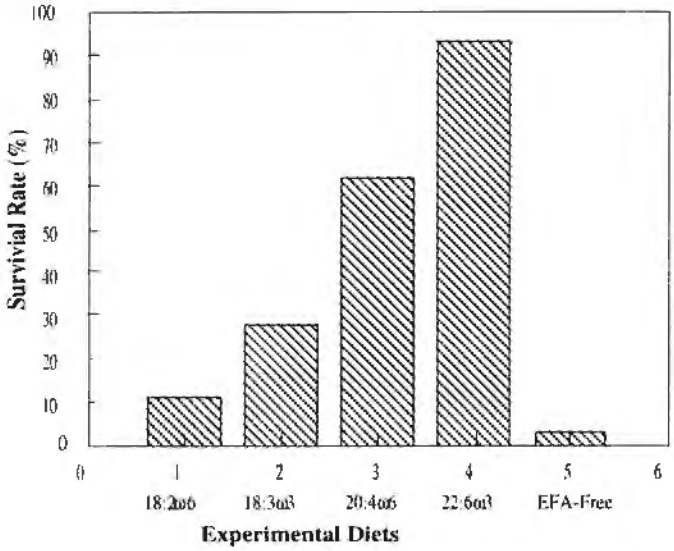

Figure 1. The effect of ω-3 and ω-6 fatty acids on the survival rate of *Penaeus chinensis*

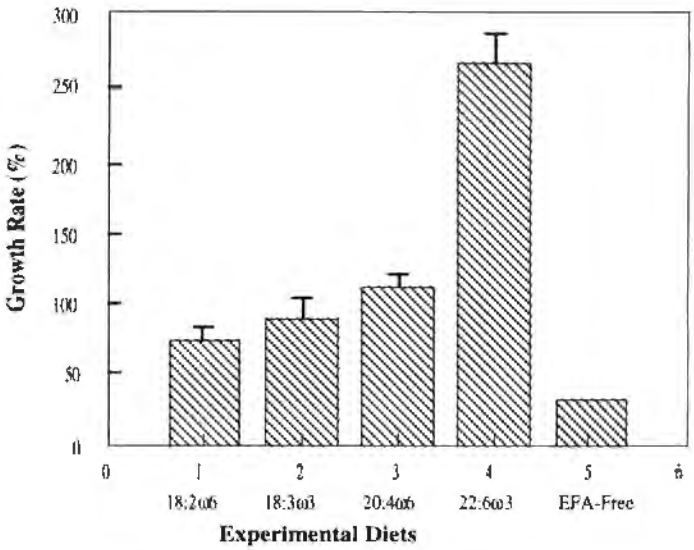

Figure 2. The effect of ω-3 and ω-6 fatty acids on the growth rate of *Penaeus chinensis*

Table 5. Effect of dietary ω-3 and ω-6 fatty acids on survival rate and molting frequency of Chinese prawn (*Penaeus chinensis*)

Diet No	Survival rate (%)	Molting frequency
1	12.5	3
2	29.2	4
3	63.0	5
4	92.0	6
5	4.0	3

Table 6. Effect of dietary ω–3 and ω–6 fatty acids on the growth rate (% average increase per day) of Chinese prawn (*Penaeus chinensis*), average weight per prawn

	Weight (g)		Growth rate (%)
Diet No	Initial	Final	
1	0.37 ± 0.27	0.67 ± 0.03	81 ± 8.02^{c}
2	0.38 ± 0.10	0.74 ± 0.14	95 ± 21.2^{bc}
3	0.45 ± 0.19	0.99 ± 1.15	120 ± 6.44^{bc}
4	0.45± 0.17	1.62 ± 0.23	260 ± 18.4^{a}
5	0.45 ± 0.10	0.62 ± 0.00	38 ± 0.00^{d}

Values with the same superscript in a column are not significantly different ($p>0.05$ or $p>0.01$)

Discussion

Kanazawa et al. (1977, 1978, 1979) have reported that crustaceans such as *P. japonicus* lack the ability for *de novo* synthesis of 18:2ω6, 18:3ω3, 20:5ω3 and 22:6ω3. The results of the present study also indicate that juvenile Chinese prawn fed the control diet containing 5% of 16:0 and 18:1ω9 mixture with no EFA supplementation suffered high mortality, did not grow and molted less frequently. Once the ω-3 and ω-6 HUFA from exogenous food sources become unavailable certain important physiological and biochemical processes are inhibited, consequently resulting in high mortality.

Kanazawa et al. (1979) demonstrated that the addition of 18:2ω6 and 18:3ω3 to the diet for *P. japonicus* significantly improved the weight gain and that 18:3ω3 of ω-3 series was more effective. In Chinese prawn addition of 1% linoleic acid (18:2ω6) to the diet improved survival and growth rate ($p<0.05$), but did not stimulate the molting frequency. However, the addition of 1% linolenic acid (18:3ω3) to the diet significantly improved survival, growth rate and, as well, the molting frequency ($p<0.01$). The results indicate that linolenic acid (18:3ω3) has more EFA value than linoleic acid (18:2ω6) for juvenile Chinese prawn.

It is also evident that in Chinese prawn 1% of arachidonic acid (20:4ω6) significantly improved survival and molting frequency, but the weight gain was only slightly better than that of prawns fed the diet containing 1% of 18:3ω3. These results may indicate that 20:4ω6 might play an important role in promoting the molting mechanism of crustaceans, but is not specifically growth stimulating.

The present study confirmed that the ω-3 and ω-6 series fatty acids are essential for the normal growth and survival of juvenile Chinese prawn and that the ω-3 family of fatty acids have a greater EFA value than the ω-6 family of fatty acids in their diet. The nutritive value of DHA is the best among these four supplementing fatty acids, suggesting that supplementation of some oils rich in DHA may benefit Chinese prawn production.

References

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem.* Physiol. 37: 911–917.

Bottino, N.R., J. Gennity, M.L. Lilly, E. Simmons and G. Finne, 1980. Seasonal and nutritional effects of the fatty acids of three species of shrimp *Penaeus setiferus, P. aztecus, P. duorarum*. *Aquaculture* 19: 139–148.

Castell, J.D. and J.C. Kean, 1989. A standard reference diet for crustacean nutrition research I. Evaluation of two formulations. *J. of the World Aqua. Soc.* 20 (3): 93–99.

Castell, J.D., R.O. Sinnhuber and J.H. Wales, 1972. Essential fatty acids in the diet of rainbow trout (*Salmo qairdneri*) growth, feed conversion and some gross deficiency symptoms. *J. Nutr.* 102: 77–86.

Castell, J.D. and J.F. Covey, 1976. Dietary lipid requirements of adult lobster (*Homarus americanus*). *J. Nutr.* 106: 1159–1165.

Castell, J.D. 1982. Fatty acids metabolism in crustaceans. *Proc. Second Intl. Conf. on Aquaculture Nutrition*. pp. 124–145.

Colvin, P.M. 1976. The effect of selected seed oils on the fatty acid composition and growth of *Penaeus indicus*. *Aquaculture* 8: 81–89.

Fujii, M. and Y. Yone, 1976. Studies on nutrition of red sea bream-XI. Effect of dietary linolenic and n–3 polyunsaturated fatty acid on growth and feed conversion *Bull. Japan. Soc. Sci. Fish.* 42: 583–588.

Guary, J., M. Kayama, Y. Marakami and H.J. Cecaaldi, 1976. The effects of a fat-free diet and compounded diets supplemented with various oils on molt, growth and fatty acid composition of prawn *Penaeus japonicus* bati. *Aquaculture* 7: 245–254.

Kanazawa, A., S. Teshima and Ono Kazuo, 1979. Relationship between EFA requirement of aquatic animal and capacity for bioconversion of linolenic acid to highly unsaturated fatty acids. *Comp. Biochem. Physiol.* 63B: 295–298.

Kanazawa, A. and S. Teshima, 1977. Biosynthesis of fatty acids from acetate in the prawn *Penaeus japonicus*. *Mem. Fac. Fish.*, Kagoshima Univ. 26: 49–53.

Kanazawa, A., S. Teshima and S. Tokiwa, 1979. Biosynthesis of fatty acids from palmitic acid in the prawn, *Penaeus japonicus*. *Mem. Fac. Fish.*, Kagoshima Univ. 28: 17–20.

Kanazawa, A., S. Tokiwa and M. Kayama, 1977. Essential fatty acids in the diet of prawn–I, Effects of linoleic and linolenic acids on growth. *Bull. Japan. Soc. Sci. Fish.* 43(9): 1111–1114.

Kanazawa, A., S. Teshima, M. Endo and M.Kayama, 1978. Effects of eicosapentaenoic acid on growth and fatty acid composition of the prawn, *Penaeus japonicus*. *Mem. Fac. Fish.* Kagoshima Univ. 27(1): 35–40.

Kanazawa, A., S. Teshima, S. Tokiwa, M. Kayama and M. Hirata, 1979. Essential fatty acids in the diet of prawn–II. Effect of docosahexaenoic acid on growth. *Bull. Japan. Soc. Sci. Fish.* 45(9): 1151–1152.

Kayama, M., M. Hirata, A. Kanazawa, S. Tokiwa and M. Saito, 1980. Essential fatty acid in the diet of prawn–III. Lipid metabolism and fatty acid composition. *Bull. Japan. Soc. Sci. Fish.* 46: 483–488.

Maeda, Y. et al. 1987. Sample analysis method for fatty acids in food samples. *Japan. J. Food Hygiene Soc.* 28(5): 384–389.

Read, G.H.L. 1981. The response of *Penaeus indicus* to purified and compounded diets of varying fatty acid composition. *Aquaculture* 24: 245.

Watanobe, T., C. Ogino, Y. Koshishi and T. Matsunaga, 1974. Requirement of rainbow trout for essential fatty acids. *Bull. Japan. Soc. Sci. Fish.* 40: 493–499.

Xu Xueliang, Wenjuan Ji, J.D. Castell and R.K. O'Dor, 1992. Effect of dietary ω-3 and ω-6 fatty acids on survival, molting and growth of juvenile shrimp (*Penaeus chinensis*). *Marine Fisheries Research* (13): 21–28.

Yu, T.C. and R.O. Shinnhuber, 1977. Effect of dietary n–3 and n–6 fatty acids on growth and feed conversion efficiency of coho salmon (*Oncorhychus kisutch*). *Aquaculture* 16: 31–38.

Quantitative Dietary Ascorbic Acid Requirement of Indian Major Carp, *Labeo rohita*, Fingerlings

M.R. HASAN[1]
D.K. SHAHA[1]
P.M. DAS[2]
M.A. HOSSAIN[3]

[1]*Department of Fisheries Biology and Limnology*
[2]*Department of Pathology*
Bangladesh Agricultural University, Mymensingh 2202
[3]*Freshwater Station, Fisheries Research Institute, Mymensingh 2201*
Bangladesh

HASAN, M.R., D.K. SHAHA, P.M. DAS and M.A. HOSSAIN, 1993. Quantitative dietary ascorbic acid requirement of Indian major carp, *Labeo rohita*, fingerlings. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 15–21.

Abstract

A 98-day growth trial was conducted to determine the quantitative dietary ascorbic acid requirement of Indian major carp, *Labeo rohita* (Hamilton), fingerlings (mean weight 6.66 g; SD ± 0.10) by feeding five test diets containing graded levels of L-ascorbic acid (0, 10, 20, 50 and 100 mg/100 g dry diet). The performances of fish fed diets with and without ascorbic acid (ASA) were evaluated on the basis of their growth, survivability, food conversion, protein utilization, body composition, haematological parameters such as haematocrit, haemoglobin, total erythrocyte and leucocyte counts, ascorbic acid level and histopathological change in the liver. There was no significant ($p>0.05$) variation in growth, food conversion and protein utilization of fish fed different levels of ASA. Haemoglobin (Hb) content varied between 7.95 and 9.92 g/dL and was not significantly ($p>0.05$) different. Haematocrit (PCV), total erythrocyte count (TEC) and total leucocyte count (TLC) varied significantly ($p<0.05$) and ranged between 31.88 and 40.25%, 2.25 and 3.69 million/µL and 20.29 and 31.69 thousand/µL respectively. There were decreasing trends of PCV, TEC and TLC values with increasing levels of ASA in the diets. No remarkable histopathological change was observed in the liver of test fish fed with diets containing various levels of ASA. No noticeable variation in the ascorbic acid level was observed in the liver. None of the fish died during the experimental period and fish fed ASA free diet did not exhibit any deficiency signs. The results of this study therefore indicate that this major carp species may not require any ascorbic acid in their diet and may be capable of synthesizing it.

Introduction

Ascorbic acid or vitamin C is considered to be an essential nutrient for animal life. It is involved in many biological functions and its primary role is to act as a reducing agent (Masumoto et al. 1991). A large number of animals are able to synthesize this vitamin and therefore, do not require this vitamin in their diets. However, the need for dietary ascorbic acid has been overwhelmingly demonstrated in most of the fish species studied (Tacon 1991). A review of the literature reveals that most of these studies are done on salmonids, channel catfish and Japanese shrimp. So far very little work has been done on fish species of the Indian region except on green

snakehead (Mahajan and Agrawal 1979, 1980b; Agrawal and Mahajan 1981) and Indian major carp, *Cirrhinus mrigala* (Mahajan and Agrawal 1980a).

Quantitative requirements of ascorbic acid by fish for optimum growth vary among species and sizes of fish (Boonyaratpalin et al. 1989). The objective of the present study was therefore to investigate the quantitative dietary ascorbic acid requirement of Indian major carp, *Labeo rohita* (Hamilton), fingerlings.

Materials and Methods

Five isonitrogenous and isoenergetic test diets were prepared with graded levels (0, 10, 20, 50 and 100 mg/100 g dry diet) of L-ascorbic acid (BDH Chemicals Ltd, Poole, England) to determine the quantitative dietary ascorbic acid requirement of *L. rohita*. Caesin was used as the dietary protein source and dextrin and soluble starch (1:0.8) were used as the source of dietary carbohydrate.

All the diets were formulated to contain 28% protein, 9% lipid and 9% crude fibre. The formulation and proximate composition of the experimental diets are presented in Table 1. The required quantity of ascorbic acid was dissolved in distilled water and sprayed into the dietary ingredients. The ingredients were then thoroughly mixed and the required quantity of water was added. The diets were prepared as described previously (Hasan et al. 1989) in batches to minimize the loss of ASA during storage.

Table 1. Diet composition (% dry weight) with various levels of L-ascorbic acid (mg/100 g diet)

Diets	1	2	3	4	5
Caesin	36	36	36	36	36
Dextrin	20	20	20	20	20
Starch (soluble)	16	16	16	16	16
Soybean oil	6	6	6	6	6
Cod liver oil	3	3	3	3	3
Vitamin premix [a]	2	2	2	2	2
Mineral premix [b]	4	4	4	4	4
α–cellulose	9	9	9	9	9
Binder [c]	4	4	4	4	4
L-ascorbic acid	0	10	20	50	100
Analyzed composition					
Crude protein	28.06	27.78	28.13	27.93	27.22
Crude lipid	9.12	9.14	8.26	8.52	8.07
Ash	5.27	5.02	5.55	5.94	5.12

[a] Embavit-GS (ascorbic acid free vitamin and trace mineral mixture, Rhône-Poulenc Bangladesh Ltd)

[b] g/100 g mix: sodium chloride 4.35, magnesium sulphate 13.70, sodium biphosphate 8.72, potassium phosphate (dibasic) 23.98, calcium biphosphate 13.58, ferric citrate 2.97 and calcium lactate 32.70

[c] Sodium carboxymethyl cellulose.

An indoor laboratory static rearing system was used for the growth trial. Ninety, six-litre capacity rectangular glass aquaria containing 80 litres of water were used as experimental tanks. In each tank a 200-W thermostatic heater (Aquarium System, Ohio, USA) and artificial aeration were used to maintain the required temperature and dissolved oxygen concentration respectively. The major carp fingerlings were purchased from a local fish vendor. Prior to the experiment, the fingerlings were acclimated to laboratory conditions for 30 days. Towards the end of the acclimation period, fish fingerlings were fed the control test diet (diet 1) to accustom them to a purified diet.

Fish fingerlings (mean weight 6.66 g; SD ± 0.10) were randomly distributed at the rate of twenty fish per tank with two replications for each treatment. All fish were fed twice daily at 0800 and 1700 hrs at a feeding rate of 3–5% body weight per day during the entire experimental period of fourteen weeks. The feeds were slowly administered into the test tanks and the feeding behaviour and acceptability of the diets were observed. The total biomass of the fish from each

tank was determined at weekly intervals and the feeding rates adjusted accordingly. In order to maintain good water quality, water in each tank was changed daily. On termination of the experiment, the fish were weighed individually and the mean weight for each tank calculated.

Feed ingredients, experimental diets and fish samples were analyzed for their proximate composition by the methods given by the Association of Official Analytical Chemists (1970). Ascorbic acid content of the fish liver was determined colorimetrically after Sakariah (1980). The method was based on the oxidation of ascorbic acid to dehydroascorbic acid and then to diketogulonic acid, followed by coupling with 2,4 dintrophenyl hydrazine to give red coloured osazones.

Water quality parameters were monitored from each test tank throughout the experimental period. Temperature and pH were measured by Jenway microprocessor pH meter (Model 3100) and dissolved oxygen by Jenway oxygen meter (Model 9070). The mean and the ranges of the water quality parameters were temperature 28 ±0.5°C; pH 7.69 (7.62–7.95); dissolved oxygen 5.12 (3.00–7.52) mg/L.

At the end of the experimental period, some haematological parameters were studied from fish from each tank. Fish were anaesthetized with 5 ppm quinaldine (Sigma Chemical Co., USA) (Hossain and Shariff 1992) before collecting blood. Samples were collected from the caudal vein of fish using 1 mL disposable hypodermic syringes and 23 G needles rinsed with anticoagulant. Five per cent heparin (Leo Pharmaceutical, Ballerup, Denmark) was used as anticoagulant. Collected blood samples were pooled from 6–8 fish from each tank.

Blood haemotocrit (PCV) value was determined by microhaematocrit method after Schalm et al. (1975). Haemoglobin (Hb) level was determined spectrophotometrically by the cyanhaemoglobin method after Makarem (1974). Total erythrocyte count (TEC) and total leucocyte count (TLC) were performed using the New Improved Neubaurer haemocytometer (Siddiqui and Naseem 1979). In both cases, modified Dacie's fluid (Blaxhall and Daisley 1973) was used as dilutant. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were calculated from the values of PCV, Hb and TEC (Schalm et al. 1975).

At the end of the trials, fish samples were fixed in 10% buffered formalin and routine histopathological examinations of the liver were carried out to observe the changes, if any, in fish under different treatments.

Specific growth rate (SGR), weight gain (%), food conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU) were calculated after Castell and Tiews (1980). Comparison of treatment means was carried out by one-way analysis of variance (ANOVA) and Duncan's Multiple Range Test using the MSTAT-C statistical package in an ALR PowerFlex Plus microcomputer.

Results

The growth, food conversion and protein utilization data of *L. rohita* fed different levels of ascorbic acid are presented in Table 2. There was no significant difference ($p>0.05$) between different diets in terms of weight gain (%), specific growth rate, food conversion ratio, protein utilization ratio and apparent net protein utilization. It is interesting to note that although there was no significant ($p>0.05$) difference between performances of different diets due to the large variations within replicate values, diet 5 (100 mg ASA/100 g diet) showed the poorest performance.

There was no fish mortality and fish were found healthy throughout the experimental period. The fish fed an ASA-free diet (control) also did not exhibit any deficiency signs. Histological examinations of the liver of fish fed an ASA-free diet and diets containing different levels of ASA did not reveal any noticeable change. There was no noticeable variation in the ascorbic acid concentration in the liver of fish fed different levels of ASA, the values ranging between 158.65 µg/g to 165.18 µg/g.

The proximate carcass composition of fish fed a diet deficient in ASA and diets containing graded levels of ASA were analyzed. Carcass composition of fish fed different diets did not show any significant differences and were within the limits of the normal health conditions.

Table 2. Growth, food conversion, protein utilization and carcass composition (% fresh weight) of *Labeo rohita* fingerlings fed different levels of ascorbic acid

Diets		1	2	3	4	5	±SE*
Initial weight (g)		6.68	6.62	6.58	6.75	6.70	0.074
Final weight (g)		18.63	16.59	16.83	19.13	15.02	1.243
Weight gain (%)		178.9	150.7	156.1	183.6	124.3	18.8
SGR (%)		0.98	0.88	0.89	0.99	0.77	0.067
SGR as % of control		100	89.8	90.8	101	78.6	
FCR		2.75	3.22	3.38	2.79	3.98	0.276
PER		1.30	1.12	1.07	1.28	0.93	0.107
ANPU (%)		22.63	18.94	17.60	23.49	16.20	2.793
Carcass composition							
	Initial						
Moisture	74.83	72.10	72.35	72.82	71.12	72.46	0.933
Crude protein	14.58	16.32	16.01	15.73	16.94	16.23	0.450
Crude Lipid	5.92	8.24	7.88	7.57	9.05	7.91	0.544
Ash	4.01	3.42	3.55	3.56	3.30	3.68	0.167

Figures in the same row without any superscripts are not significantly different ($p>0.05$; ANOVA test); *Standard error of treatment mean, calculated from residual mean square in the analysis of variance.

Results of haematological examination of fish from different treatment groups are presented in Table 3 and presented graphically in Figure 1. Haemoglobin (Hb) content varied between 7.95 g/dL (diet 5) and 9.92 g/dL (diet 1) and was not significantly ($p>0.05$) different. Haematocrit (PCV) and total erythrocyte count (TEC) varied significantly ($p<0.05$). The highest PCV value (40.25%) was recorded for diet 2 (10 mg ASA) and the lowest value (31.88%) for diet 5 (100 mg ASA). A similar trend was also observed for TEC values (Table 3).

The total leucocyte count (TLC) varied significantly ($p>0.05$), the highest value (31.69 thousand /μL;) being recorded for diet 1 (0 ASA) and the lowest value 20.20 thousand/μL) for diet 5, although it was not significanlty ($p>0.05$) different from those of diets 2, 3 and 4. In general the values of different blood parameters decreased with the increasing level of ascorbic acid incorporation in the diet (Figure 1).

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) values were calculated using the PCV, Hb and TEC values. There was no significant ($p>0.05$) difference within these parameters for different diets (Table 3). The MCV values ranges between 109.56 and 141.76 10^{-18}L and the values increased with the increase of ASA level in the diet. The MCH values ranged between 25.90 and 36.16 pg. The MCHC values ranged between 23.61% and 27.92%. Although the values of individual parameters were not significantly different among them, the lowest value was nevertheless recorded for diet 2 (10 mg ASA) for all the parameters calculated.

Table 3. Haematological values and liver ascorbic acid concentration of *Labeo rohita* fingerlings fed different levels of ascorbic acid

Diets	1	2	3	4	5	±SE**
Haematocrit (%)	37.88^{ab*}	40.25^{a}	35.00^{bc}	35.88^{abc}	31.88^{c}	1.43
Haemoglobin (g/dL)	9.92^{a}	9.50^{a}	9.19^{a}	9.13^{a}	7.95^{a}	0.47
TEC (million/μL)	3.27^{a}	3.69^{a}	2.58^{b}	2.60^{b}	2.25^{b}	0.17
TLC (thousand/μL)	31.69^{a}	22.35^{b}	22.50^{b}	20.96^{b}	20.29^{b}	1.70
MCV (10^{-18}L)	117.89^{a}	109.56^{a}	137.24^{a}	139.70^{a}	141.76^{a}	8.59
MCH (pg)	30.89^{a}	25.90^{a}	34.51^{a}	36.16^{a}	35.16^{a}	2.96
MCHC (%)	26.20^{a}	23.61^{a}	26.18^{a}	27.92^{a}	24.87^{a}	3.33
Liver ASA (μg/g)	158.65^{c}	161.45^{b}	160.47^{b}	164.24^{a}	165.18^{a}	0.52

TEC = total erythrocyte count; TLC = total leucocyte count; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration.

* Figures in the same row with different superscripts are significantly different ($p<0.05$; Duncan's test)

** Standard error of treatment mean, calculated from residual mean square in the analysis of variance.

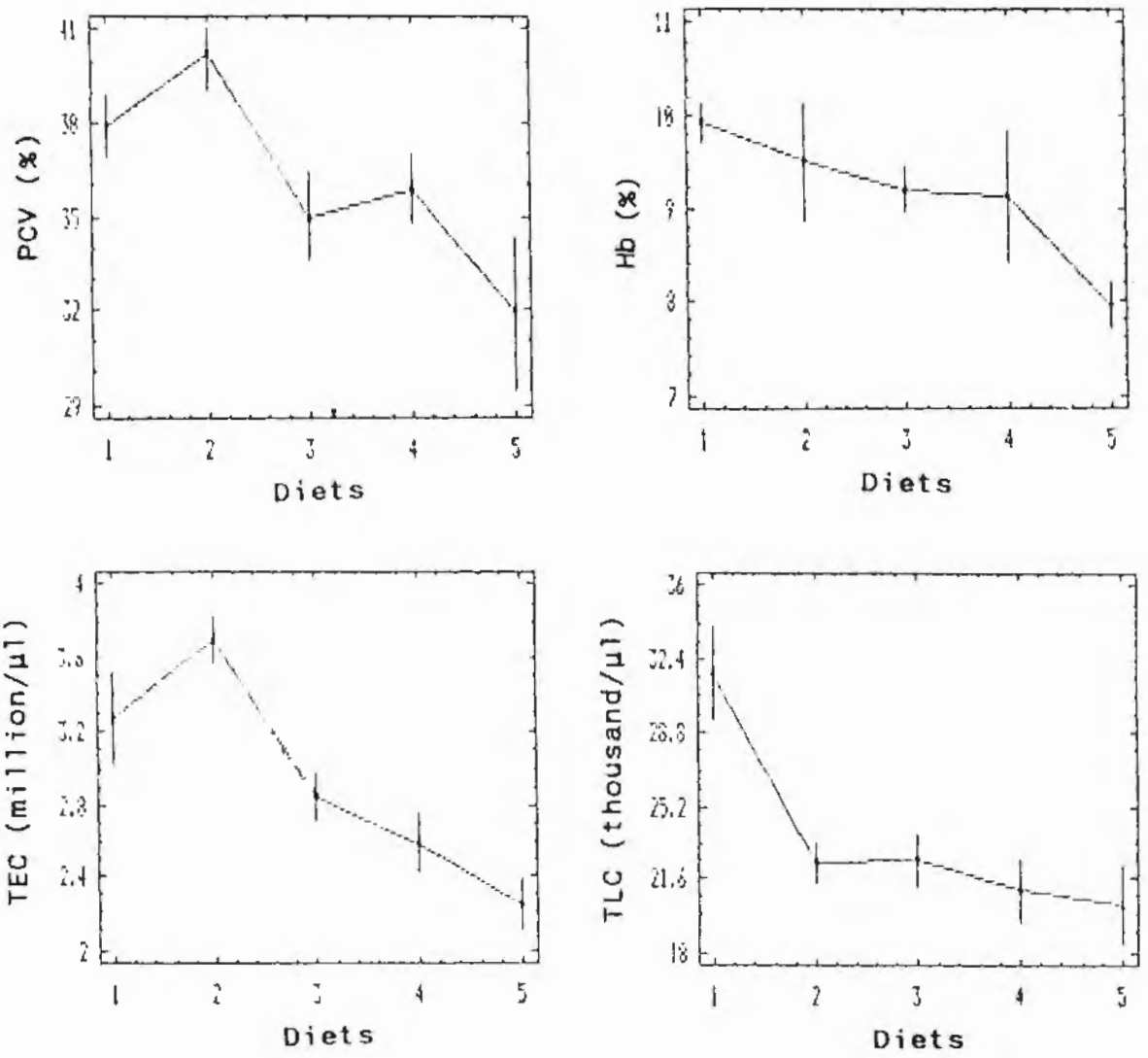

Figure 1. Changes in haematocrit (PCV), haemoglobin (Hb), total erythrocyte count (TEC) and total leucocyte count (TLC) of *L. rohita* for different diets. Diet 1: 0 mg ASA (control); diet 2: 10 mg ASA; diet 3: 20 mg ASA; diet 4: 50 mg ASA; diet 5: 100 mg ASA. Vertical bars represent standard error of mean.

Discussion

The results of the present investigation show that the growth, food conversion and protein utilization of *Labeo rohita* fingerlings were not affected by the level of ascorbic acid (ASA) in the diet. The most common clinical vitamin C deficiency symptoms (e.g., lordosis, scoliosis, lethargy, haemorrhagic exophthalmia etc.) found in fish (Halver 1985) were also not observed in the ASA deficient group in the present study.

The dietary requirement of ASA has been demonstrated in most of the fish species studied, e.g., rainbow trout, *Oncorhynchus mykiss* (Kitamura et al. 1965, Halver et al. 1969), coho salmon, *O. kisutch* (Halver et al. 1969), channel catfish, *Ictalurus punctatus* (Lovell 1973; Andrews and Murai 1975), green snakehead, *Channa punctatus* (Mahajan and Agrawal 1979), major carp, *Cirrhinus mrigala* (Mahajan and Agrawal 1980a), Asian catfish, *Clarias batrachus* (Butthep et al. 1985), Nile tilapia, *Oreochromis niloticus* (Soliman et al. 1985, 1993), sea bass, *Lates calcarifer* (Boonyaratpalin et al. 1989). However, a large number of land animals including ruminants and poultry are able to synthesize this vitamin to meet their physiological needs. Among fish, common carp has been reported to be able to synthesize ascorbic acid (Ikeda and Sato 1964).

The haematological findings showed that PCV and TEC values were highest in fish fed 10 mg ASA (diet 2) followed by 0, 20, 50 and 100 mg ASA containing diets. This difference was statistically significant ($p<0.05$). However, no significant difference was observed in respect of Hb values at various levels of ASA inclusions. Moreover, the derived haematological indices such as MCV, MCH and MCHC values in various groups were not significantly ($p>0.05$) different, although an increasing trend was observed in MCV values in fish fed a higher level of ASA.

This study indicates that a higher inclusion of ASA reduces the PCV and TEC values and increases the MCV values. Slight anaemia observed in fish fed higher inclusions are macrocytic in nature. The Hb, MCH and MCHC values indicate that the anaemia is normochromic. Macrocytic normochromic anaemia is usually observed in vitamin B_{12}, cobalt and folic acid deficiencies. As no histopathological change was observed in the liver, the deficiency of the erythrocyte maturing factor produced from the liver may be excluded from the etiology of macrocytic normochromic anaemia.

The TLC values indicate that it decreases at higher inclusion levels, i.e., the body defensive mechanisms related to leucocytes decrease gradually. However, with the exception of TLC, the data of the present study are similar to the values of haematological parameters of *L. rohita* from open waters (Siddiqui and Naseem 1979). The slight variation in PCV, Hb, TEC could have been due to size, sex, season (Siddiqui and Naseem 1979) and encaptivity stress of test fish compared to pond-reared fish (Klontz and Smith 1968). Higher TLC is the index of defense mechanism (Ellis 1977). The decrease in TLC with the higher level of ASA in the diets indicates that ASA requirement in the diets of *L. rohita* is minimum (<10 mg/100 g diet). The higher level of ASA in diets could have acted as a chronic stressor which weakened the defense mechanism slowly. Similar trends were also observed by Rehulka (1990) when rainbow trout were fed hydrolytically changed and oxidized fat.

The findings of the present study therefore indicate that the optimum level of ascorbic acid requirement for *Labeo rohita* fingerlings lies between 0 to 10 mg/100 g diet. However, a further study should be conducted to detect the L-gulonolactone oxidase enzyme activity in the liver and kidney of this species to find whether it is capable of synthesizing L-ascorbic acid.

References

Agrawal, N.K. and C.L. Mahajan, 1981. Effect of ascorbic acid deficiency on the uptake of iodine by thyroid and non-thyroid tissues of an air-breathing freshwater fish *Channa punctatus* Bloch. *J. Fish Biol.* 18: 411–416.

Andrews, J.W. and T. Murai, 1975. Studies on the vitamin C requirements of channel catfish (*Ictalurus punctatus*). *J Nutr.* 105: 557–561.

Association of Official Analytical Chemists, 1970. *Official Methods of Analysis of the Association of Official Analytical Chemists*, Washington DC, AOAC, 1015 p.

Blaxhall, P.C. and K.W. Daisley, 1973. Routine haematological methods for use with fish blood. *J. Fish Biol.* 5: 771–781.

Boonyaratpalin, M., N. Unprasert and J. Buranapanidgit, 1989. Optimal supplementary vitamin C level in sea bass fingerling diet. In M. Takeda and T. Watanabe (eds.) *The Current Status of Fish Nutrition in Aquaculture*, Proc. Third Int. Symp. on Feeding and Nutr. in Fish. Tokyo, Laboratory of Fish Nutrition, Tokyo University of Fisheries, pp. 149–157.

Butthep, C., P. Sitasit and M. Boonyaratpalin, 1985. Water soluble vitamins essential for the growth of *Clarias*. In C.Y. Cho, C.B. Cowey and T. Watanabe (eds.). *Finfish Nutrition in Asia: Methodological Approaches to Research and Development*. Ottawa, Ont., Canada, IDRC, pp. 118–129.

Castell, J.D. and K. Tiews (eds.), 1980. Report of the EIFAC, IUNS and ICES Working Group on Standardization of Methodology in Fish Nutrition Research EIFAC/T36, EIFAC/FAO, p. 24.

Ellis, A.E. 1977. The leucocytes of fish: a review. *J. Fish Biol.* 11: 453–491.

Halver, J.E. 1985. Recent advances in vitamin nutrition and metabolism in fish. In C.B. Cowey, A.M. Mackie and J.G. Bell (eds.) *Nutrition and Feeding in Fish*. London, Academic Press Inc. (London) Ltd., pp. 414–429.

Halver, J.E., L.M. Ashley and R.M. Smith, 1969. Ascorbic acid requirements of coho salmon and rainbow trout. *Trans. Am. Fish. Soc.* 98: 762–771.

Hasan, M.R., M.G.M. Alam and M.A. Islam, 1989. Evaluation of some indigenous ingredients as dietary protein sources for the catfish (*Clarias batrachus*, Linnaeus). In E.A. Huisman, N. Zonneveld and A.H.M. Bouwmans (eds.) *Aquaculture Research in Asia: Management Techniques and Nutrition*. Wageningen, Pudoc, pp. 125–137.

Hossain, M.A. and M. Shariff, 1992. Effect of copper on humoral immune response in goldfish, *Carassius auratus Linnaeus*. In M. Shariff, R. Subasinghe and J.R. Arthur (eds.) *Diseases in Asian Aquaculture*. Manila, Fish Health Section, Asian Fisheries Society, pp. 371–377.

Ikeda, S. and M. Sato, 1964. Biochemical studies on L-ascorbic acid in aquatic animals—III. Biosynthesis of L-ascorbic acid by carp. *Bull. Japan. Soc. Sci. Fish.* 30: 365–369.

Kitamura, S., S. Ohara, T. Sawa and K. Nakagawa, 1965. Study on the vitamin requirements of rainbow trout, *Salmo gairdneri*. I. On the Ascorbic acid. *Bull. Japan. Soc. Sci. Fish.* 31: 818–826.

Klonz, G.W. and L.S. Smith, 1978. Methods of using fish as biological research subjects. In *Methods of Animal Experimentation III*. London, Academic Press Inc. (London) Ltd., pp. 323–385.

Lovell, R.T. 1973. Essentiality of vitamin C in feeds for intensively fed caged channel catfish. *J. Nutr.* 103: 134-148.

Mahajan, C.L. and N.K. Agrawal, 1979. Vitamin C deficiency in *Channa punctatus*. Bloch. *J. Fish Biol.* 15: 613–622.

Mahajan, C.L. and N.K. Agrawal, 1980a. Nutritional requirement of ascorbic acid by Indian major carp, *Cirrhina mrigala*, during early growth. *Aquaculture* 19: 37–48.

Mahajan, C.L. and N.K. Agrawal, 1980b. The role of vitamin C in calcium uptake by fish. *Aquaculture* 19: 287–294.

Makarem, A.H. 1974. Haemoglobins, myoglobins and haptoglobins. In R.J. Henry, D.C. Cannon and J.W. Winkelman (eds.) *Clinical Chemistry: Principles and Technics*. Maryland, Harper and Row Publishers, Inc., pp. 1111–1214.

Masumoto, T., H. Hosokawa and S. Shimeno, 1991. Ascorbic acid's role in aquaculture nutrition. In D.M. Akiyama and R.K.H. Tan (eds.) *Proceedings of the Aquaculture Feed Processing and Nutrition Workshop*. Singapore, American Soybean Association, 541 Orchard Road, Singapore, pp. 42–48.

Rehulka, J. 1990. Effect of hydrolytically changed and oxidized fat in dry pellets on the health of rainbow trout, *Oncorhynchus mykiss* (Richardson). *Aqua. Fish. Manage.* 21: 419–434.

Sakariah, D. 1980. Determination of Vitamin C. In *Practical Manual (Biochemistry & Nutrition) for M.Sc. Course in Food Technology*. Mysore, India, Food Technological Research Institute.

Schalm, O.W., N.C. Jain and E.J. Carroll, 1975. *Veterinary Hematology*. 3rd ed. Philadelphia, Lea and Febiger.

Siddiqui, A.R. and S.M. Naseem, 1979. The haematology of rohu, *Labeo rohita*. *J. Fish Biol.* 14: 67–72.

Soliman, A.K., K. Jauncey and R.J. Roberts, 1985. Qualitative and quantitative identification of L-gluconolactone oxidase activity in some teleosts. *Aqua. Fish. Manage.* 16: 249–256.

Soliman, A.K., K. Jauncey and R.J. Roberts, 1993. Water soluble vitamins requirement of tilapia. 4–Ascorbic acid (vitamin C) requirement of Nile tilapia *Oreochromis niloticus* (Linnaeus). *Aqua. Fish. Manage. (in press)*.

Tacon, A.G.J. 1991. Vitamin nutrition in shrimp and fish. In Akiyama, D.M. and R.K.H. Tan (eds.) *Proceedings of the Aquaculture Feed Processing and Nutrition Workshop*. Singapore. American Soybean Association, 541 Orchard Road.

Pantothenic Acid Requirements of Sea Bass

MALI BOONYARATPALIN
JARURATT WANNAGOWAT
KRASINDHU HANGSAPREURKE
Feed Quality Control and Development Division
National Inland Fisheries Institute
Jatujak, Bangkok 10 500, Thailand

BOONYARATPALIN, M., J. WANNAGOWAT and K. HANGSAPREURKE, 1994. Pantothenic acid requirements of sea bass. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 23–30.

Abstract

Juvenile sea bass of average weight 2.7 g were fed semipurified diets containing five levels of supplemental pantothenic acid (0, 15, 30, 60 and 90 mg/kg) in a flowthrough sea water system with a flow rate 0.7 L/min. The fish were weighed and counted every two weeks over a 12-week period. The fish were then sampled for haematological tests, histological examination and accumulated pantothenic acid in the liver. Fish fed the unsupplemented pantothenic acid diet (0 mg/kg) showed lack of appetite, low feed efficiency, haemorrhages, clubbed gills and total mortality in five weeks. Others fed 15, 30, 60 and 90 ppm supplemental pantothenic acid diets showed no deficiency signs. The fish fed with 90 mg/kg pantothenic acid showed the best growth ($p<0.05$) at four weeks and the highest amount of pantothenic acid in the liver. Dietary pantothenic acid requirement for maximum growth and survival of sea bass reduced to 60 ppm as fish grew larger than 6.5 g.

Introduction

Lates calcarifer (Bloch), commonly called the giant sea perch or sea bass, is an economically important food fish in the tropical and subtropical regions of south east Asia and the Pacific. It is raised commercially in Thailand, Malaysia, Singapore, Indonesia, Hong Kong and Taiwan, in both brackishwater cages and freshwater ponds.

Pantothenic acid is a constituent of coenzyme A (CoA) and phosphopantetheine. CoA mediates energy transfer via the TCA cycle from carbohydrate, lipid and protein, while phosphopantetheine participates in fatty acid synthesis. The importance of pantothenic acid in the diets of trout (Phillips et al. 1945; Mclaren et al. 1947; Kitamura et al. 1967); salmon (Halver 1958); carp (Ogion 1967); eels (Arai et al. 1972); channel catfish (Murai and Andrews 1979); clarias (Buthep et al. 1985); Pirot fish (Ikeda et al. 1988); sea bass (Boonyaratpalin et al. 1993) have been demonstrated. The deficiency signs of pantothenic acid are growth retardation, anorexia, abnormal swimming behaviour, club gills, and mortality. Gill histopathological examinations have been experimentally applied for assessment of pantothenic acid deficiency in fish (Karges and Woodward 1984). Pantothenic acid measurement by radioimmunoassay (RIA) has been applied in fish (Masumoto 1991).

The present study was designed to determine the quantitative pantothenic acid requirement in juvenile sea bass and record histopathological changes in deficient fish.

Materials and Methods

Facilities and fish

Fifteen 30 × 60 × 30 cm glass aquaria with a flowthrough sea water system, located in a wet laboratory at the National Institute of Coastal Aquaculture were used in a 12-week feeding experiment. Juvenile sea bass obtained from the National Institute of Coastal Aquaculture hatchery were acclimated, graded and used in the study. Sea bass fry of average weight 2.7 g was stocked at a density of 10 fish per aquarium.

Experimental diet

Semipurified diets with five levels of pantothenic acid, 0, 15, 30, 60 and 90 mg/kg, were used. An unsupplemented feed (0 mg/kg) was used as the control diet; the composition of the experimental diets is shown in Table 1.

The ingredients of the semipurified diets were well mixed prior to adding water then passed through a meat grinder. The spaghetti-like strands were then broken into small pellets depending on the size of the fish and kept frozen until they were fed to experimental fish.

Management

Juvenile sea bass from the hatchery were acclimated to the semipurified diet. Only fish which accepted the semipurified diets were selected for the experiment. Each diet was fed ad libitum to three replicates, twice a day at 0830–1030 hrs and 1530–1700 hrs. The fish were weighed and counted every two weeks and observed for gross signs of vitamin deficiency every day during feeding. At the termination of the experiment fish were sampled for haematological studies (Hasser 1960), histological examination (Humason 1967) and accumulative pantothenic acid in the liver using a conventional microbioassay (Walsh et al. 1979).

Table 1. Composition of experimental diet (g/kg)

	Diet Number				
	1	2	3	4	5
Pantothenic acid (mg/kg diet)	0	15	30	60	90
Casein (vitamin free)	390	390	390	390	390
Gelatin	78	78	78	78	78
Cod liver oil	70	70	70	70	70
Soybean oil	80	80	80	80	80
Dextrin	50	50	50	50	50
Alpha-Cellulose	151	151	151	151	151
Na C. M.C	50	50	50	50	50
Vitamin C (fat coated)	1	1	1	1	1
Vitamin mixture[1]	20	20	20	20	20
Mineral mixture[2]	40	40	40	40	40
Amino acid mixture[3]	70	70	70	70	70

[1]Vitamin mixture (mg/kg diet): Thiamin HCl 25; riboflavin, 100; pyridoxine, 25; nictonic acid, 375; inositol, 1 000; biotin, 2.5; folic acid, 7.5; vitamin B_{12}, 0.05; menadione, 20; alphatocopherol acetate, 200; vitamin A (IU), 5 000; vitamin D_3 (IU), 1 000; BHT 4; made up to 20 g by dextrose monohydrate.

[2]Mineral mixture (units/100 g mineral mixture) calcium lactate, 32.70 g; K_2HPO_4, 23.98 g; $CaHPO_4.2H_20$, 13.58 g; $MgSO_4.7H_20$, 13.20 g; $Na_2HPO_4.2H_20$, 8.72 g; NaCl 4.35 g; ferric citrate, 2.97 g; $ZnSO_4.7H_2O$, 0.3 g; $CoCl_2.6H_2O$, 100 mg; $MnSO_4.H_2O$, 80 mg; KI, 15 mg; $AlCl_3.6H_2O$, 15 mg; $CuCl_2$, 10 mg. Total 100 g.

[3]Amino acid mixture (g/100 g dry diet): L-Phenylalanine, 0.6; L-Arginine HCl, 1.3; L-Cystine, 0.7; L-Tryptophan, 0.2; L-Histidine HCl H_2O, 0.2; DL-Alanine, 1.3; L-Aspartic acid Na, 1.0; L-Valine, 0.7; L-Lysine HCl, 0.6; Glycine, 0.4 (Yone 1976).

Results and Discussion

Sea bass fed the diet without supplementary pantothenic acid had a significantly lower percent weight gain ($p<0.05$) in the first two weeks and had negative percent weight gain thereafter. The fish fed diet supplemented with 90 mg pantothenic acid/kg showed the best percent weight gain and in the first two weeks were significantly higher than treatments with 15 and 30 mg/kg. After two weeks, the difference was not significant. However, the overall percent weight gain of fish fed 90 ppm supplementary pantothenic acid was significantly higher than in other treatments (Table 2, Fig. 1). There was no significant difference ($p<0.05$) in growth among the three treatments of 15, 30 and 60 ppm supplementary pantothenic acid diets during the 12-week experiment (Table 3).

Table 2. Average weight gain (%) of sea bass fed experimental diets with various pantothenic acid levels for 12 weeks. Values with the same superscript in each column are not significantly different ($p>0.05$)

Pantothenic	Experimental period (week)						
acid (ppm)	1–2	3–4	5–6	7–8	9–10	11–12	0–12
0	77.50 ± 7.92^{a}	-1.18 ± 2.19^{a}	—	—	—	—	—
15	122.56 ± 2.26^{bc}	56.76 ± 10.66^{b}	56.77±16.96	20.67±2.98	19.94±3.14	25.96±3.38	892.34 ± 65.87^{a}
30	119.19 ± 3.79^{b}	58.50 ± 5.91^{b}	54.55±3.64	20.22±0.45	24.84±3.75	22.87±1.06	889.29 ± 36.24^{a}
60	129.20 ± 6.21^{cd}	57.58 ± 1.84^{b}	55.25±2.07	25.47±2.85	21.66±3.13	22.57±1.35	948.24 ± 25.58^{a}
90	137.98 ± 2.07^{d}	65.44 ± 7.88^{b}	60.78±3.83	26.20±4.15	25.27±4.18	23.75±1.57	1142.01 ± 145.46^{b}

Feed efficiency (Table 3) of the diet without pantothenic acid supplementation was significantly lower than the supplementary groups ($p<0.01$) by the end of week two and decreased dramatically (negatively) afterwards. The feed efficiency at the end of 12 weeks of diets with 60 and 90 ppm supplementary pantothenic acid was significantly higher than diets with 15 and 30 ppm pantothenic acid supplementation ($p<0.01$).

Table 3. Effect of supplementary pantothenic acid levels in the diet on growth, feed efficiency and survival of sea bass after a 12-week feeding trial

Pantothenic	Av. body wt. (g)			Survival	Feed
acid (ppm)	Initial	Final	Gain(%)	(%)	Efficiency (%)
0	2.71^{a}	0.00^{a}	-100.00^{a}	0.00^{a}	0.00^{a}
15	2.70^{a}	26.83^{b}	892.34^{b}	88.66^{bc}	71.49^{b}
30	2.70^{a}	27.20^{b}	889.29^{b}	84.45^{b}	71.56^{b}
60	2.71^{a}	28.49^{b}	948.24^{b}	95.53^{c}	83.18^{c}
90	2.67^{a}	35.26^{c}	1142.01^{c}	95.53^{c}	84.72^{c}

By week four, the survival of fish fed diets without supplementary pantothenic acid was significantly lower than the supplementary treatments (Fig. 2). There were 82.22, 100, 100, 100 and 100% survival rate for treatments with 0, 15, 30, 60 and 90 ppm pantothenic acid supplementation.

By the end of week five all the pantothenic acid deficient fish had died. By the end of 12 weeks, the survival rate of fish fed diets with 15 and 30 ppm pantothenic acid supplementation was significantly lower than treatments with 60 and 90 ppm. There was no significant difference in survival between fish which received 60 ppm and 90 ppm pantothenic acid.

Signs of pantothenic acid deficiency were evident after two weeks in fish fed diets without supplementary pantothenic acid. Fish gradually developed poor appetite, poor growth, haemorrhage operculum and skin, ventral and anal fin erosion, club gills and total mortality in five weeks. Histopathological examination showed that pantothenic acid-deficient fish exhibited gill hyperplasia, which appeared on the gill filament at the base of the lamellae (Fig. 3). Fusion of lamellae began at the distal end of the filament and progressed proximally (Fig. 4). This phenomenon had been reported by Wood and Yasutake (1957) for salmonids. Moreover, sea bass showed live cells vacuolated by fat (Fig. 5) and muscle degeneration (Fig. 6).

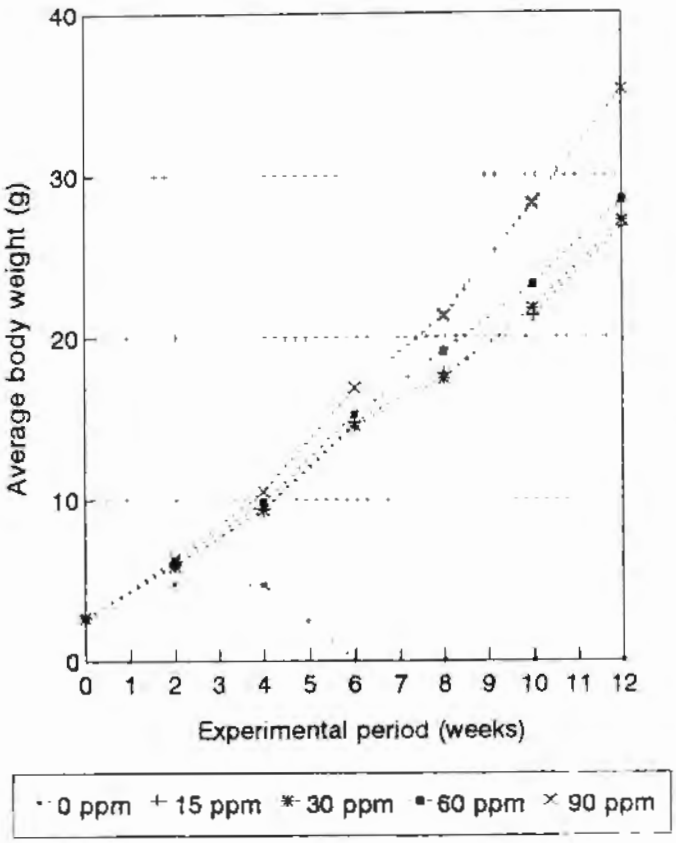

Figure 1. Average body weight (g) of sea bass fed experimental diets with various levels of pantothenic acid.

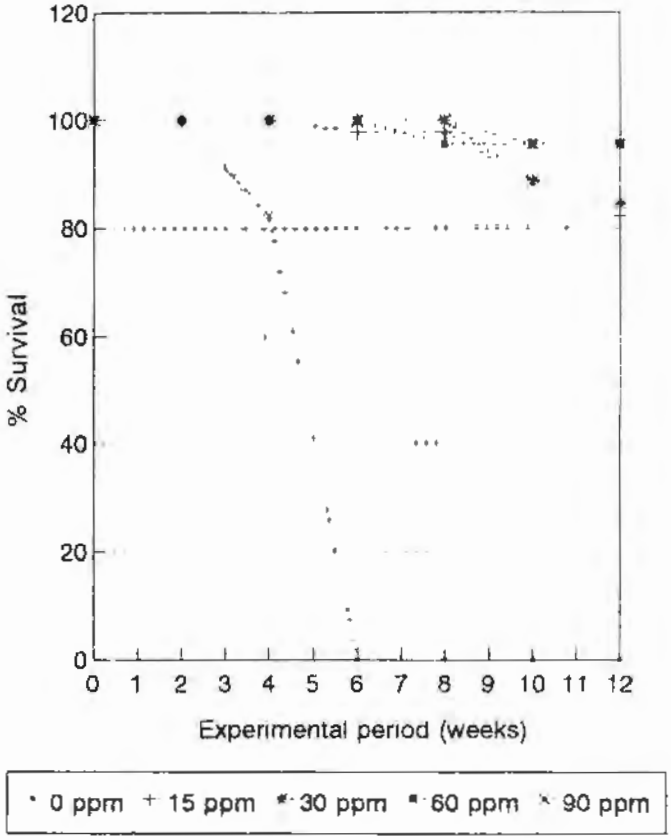

Figure 2. Percent survival of sea bass fed experimental diets with various levels of pantothenic acid.

Haematological parameters and liver pantothenic acid of deficient fish were not established due to their small size. The number of red blood cells, white blood cells and percentage haematocrite, haemoglobin and plasma protein were not significantly different among pantothenic acid supplemented groups ($p>0.05$). Sea bass fed diets supplemented with 90 ppm pantothenic acid showed significantly higher liver pantothenic acid content than fish fed 15, 30 or 60 ppm diets ($p<0.01$).

Sea bass were very sensitive to pantothenic acid deficiency as evident from the short time of two weeks required for the development of deficiency signs and total mortality in five weeks. The pantothenic acid requirement for sea bass (60–90 ppm) was high compared to values reported by NRC (1981), Ogino (1967), and Murai and Andrew (1979) for trout (40–45 ppm), carp (30–45 ppm) and channel catfish (10 ppm) respectively. Karges and Woodward (1984) fed 0.7 g rainbow trout with a pantothenic deficient diet and found that the time required for the development of deficiency signs was only 12 days whereas Masumoto (1991), using 150 g fish, found that the time required for the development of deficiency signs was 14 weeks. The data from the present study indicates that the pantothenic acid requirement could be reduced from 90 ppm to 60 ppm as fish grow from 2.7–6.5 to 6.5–30 g. However, studies on the quantitative requirements for larger sea bass need to be carried out.

The reasons pantothenic acid-deficient sea bass had total mortality in such a short experimental period (five weeks) might be explained by the problems localized in gill tissue. Fish gills play important roles in acid-base and water balance, ammonia excretion, osmoregulation and respiration. Passage of water through a fused gill filament might make respiration difficult, probably causing death.

Figure 3. Gill hyperphasia, appearing on the gill filament at the base of the lamellae and fusion of lamellae began at the distal end.

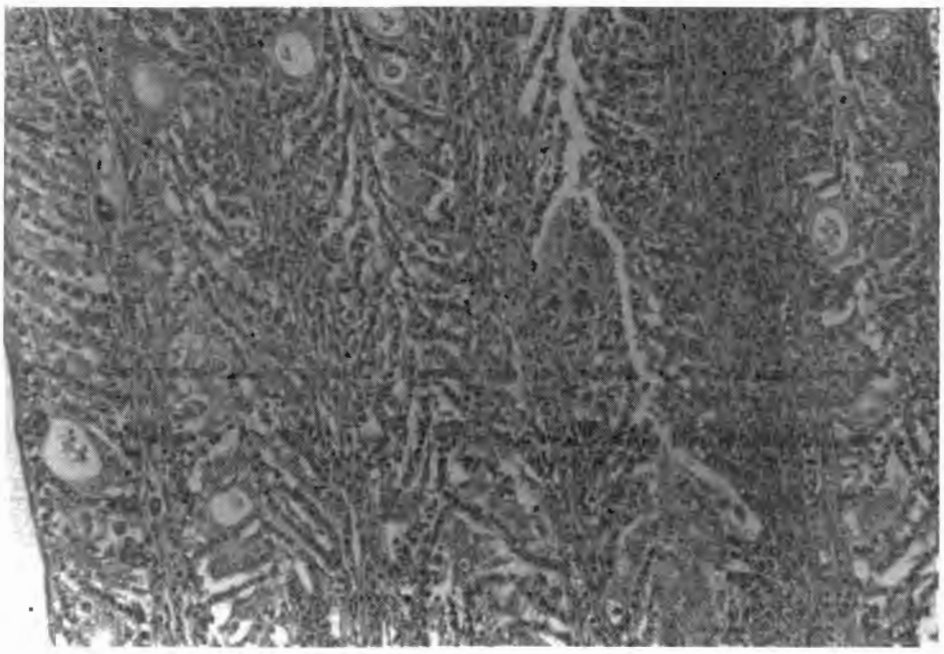

Figure 4. Severe gill hyperphasia caused fusion of primary and secondary lamellae in fish fed diet without pantothenic acid supplementation.

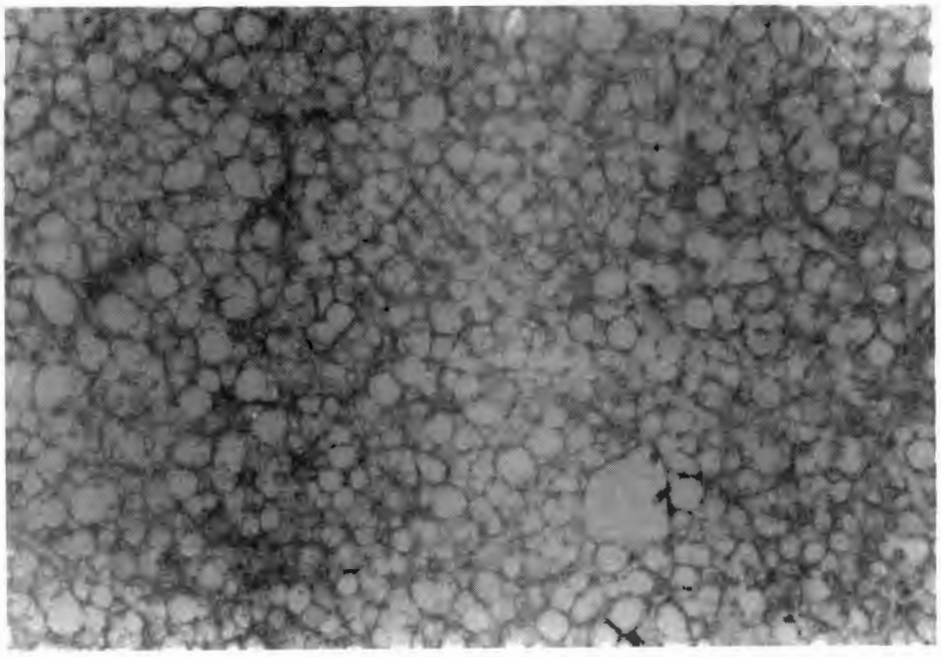

Figure 5. Liver cells of pantothenic acid-deficient fish showing accumulation of large amount of lipid droplets.

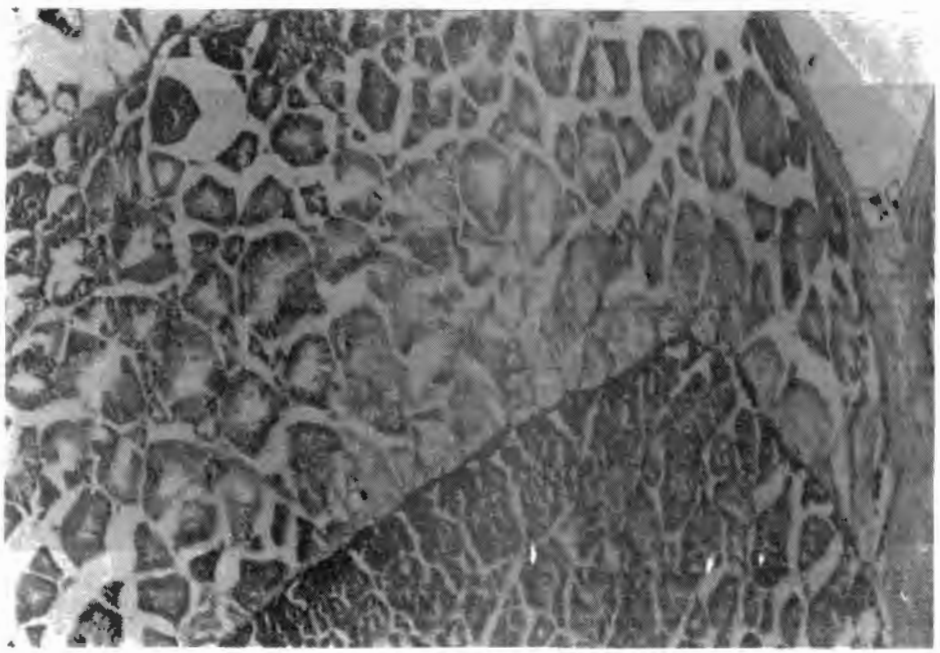

Figure 6. Light micrograph showed muscle degeneration of pantothenic acid-deficient fish.

Conclusions

1. Pantothenic acid is essential for growth, health and survival of sea bass.
2. The minimum requirement for maximum growth of 2.7–6.5 g sea bass was 90 ppm.
3. The minimum requirement for maximum growth and survival of 6.5–35 g sea bass was 60 ppm.
4. The pantothenic acid requirement reduces as fish grow.
5. The pantothenic acid deficiency signs are growth retardation, anorexia, abnormal swimming behaviour, haemorrhage operculum and skin, ventral fin and anal fin erosion, gill hyperphasia, liver cell vaecuolation by fat, muscle degeneration and total mortality.

References

Arai, S., T. Nose, and Y. Hashimoto, 1972. Qualitative requirements of young eels (*Anguilla japonica*) for water-soluble vitamins and their deficiency symptoms. *Bull. Freshwater Fish. Res. Lab.* 22(1): 63–69.

Boonyaratpalin, M., J. Wannagowat and C. Borisut, 1993. Effect of vitamin B_1, B_2, pantothenic acid and inositol deficiencies in juvenile sea bass, *Lates calcarifer*. National Institute of Coastal Aquaculture, Department of Fisheries, Thailand. *Technical paper No.1/1993*, (in Thai).

Buthep, C., P. Sitasit and M.Boonyaratpalin, 1985. Water-soluble vitamins for the growth of *Clarias*. *Finfish Nutrition in Asia: Methodological Approaches to Research and Development*. International Development Research Center, pp. 118–135.

Hesser, E.F. 1960. Methods of routine fish hematology. *Prog. Fish.-Cult.* 22: 164–171.

Halver, J.E., 1958. Water soluble vitamin requirements of chinook salmon. *J. Nutr.* 62: 225–243.

Humason, G.L., 1967. *Animal tissue techniques*. 2nd ed. San Francisco, W.H. Freeman.

Ikeda, S., Y. Ishibashi, O. Murata, T. Nasu and T. Harada, 1988. Qualitative Requirements of the Japanese Parrot Fish for Water-soluble Vitamins, *Nippon Suisan Gakkashi*. 54(11): 2029–2035.

Karges, R. G. and B. Woodward, 1984. Development of lamellar epithelial hyperplasia in gills of pantotheic acid deficient rainbow trout, *Salmo gairdneri* Richardson. *J. Fish Biol.* 25: 57–62.

Kitamura, S., T. Suwa, S. Ohara, and K. Nakagawa, 1967. Studies on vitamin requirements of rainbow trout II. The deficiency symptoms of fourteen kinds of vitamin. *Bull. Japan. Soc. Sci. Fish.* 1120–1125.

Masumoto, T. 1991. Pantothenic acid metablolism and deficiency mechanism in rainbow trout (*Oncorhynchus mykiss*). Dissertation, University of Washington. 108 p.

McLaren, B.A., E. Keller, D.J. O'Donnell and C.A. Elvehjem, 1947. The nutrition of rainbow trout. I. Studies of vitamin requirements. *Arch. Biochem. Biophys.* 15: 169–178.

Murai, T. and J.W. Andrews, 1979. Pantothenic acid requirements of channel catfish fingerlings. *J.Nutr.* 109: 1140–1142.

National Research Council (NRC), 1981. *Nutrient Requirements of Cold Water Species*. Washington, D.C., National Academy Press, p. 14.

Ogino, L. 1967. B vitamin requirements of carp. II. Requirements for riboflavin and pantothenic acid. *Bull. Japan. Soc. Sci. Fish.* 33: 351–354.

Phillips, A.M., A.V. Tunison, H.B. Shaffer, G.K. White, M.W. Sullivan, C. Vincent, D.R. Brockway and C.M. McCay, 1945. The nutrition of trout. The vitamin requirement of trout. *Fish Res. Bull.* 7: 1–31.

Walsh, J.H., B.W. Wyse and R.G.Hansen, 1979. A comparison of microbiological and radioimunoassay methods for the determination of pantothenic acid in feeds. *J. Food Biochem.* 3: 175–189.

Wood, E.M. and W.T. Yasutake, 1957. Histopathology of fish. V. Gill disease. *Prog. Fish.-Cult.* 19: 7–13.

Studies on the Utilization of Carbohydrate-rich Ingredients and Optimal Protein:Energy Ratio in Chinese Bream, *Megalobrama amblycephala* Yih

HE XIQIN[1]
JIA LIZHU
YANG YUNXIA
Institute of Hydrobiology, Academia Sinica, Wuhan, PRC

XU GUOHUAN
Institute of Reservoir Fisheries, Ministry of Water Resources, Wuhan, PRC

XIQIN H., J. LIZHU, Y. YUNXIA and X. GUOHUAN, 1994. Studies on the utilization of carbohydrate-rich ingredients and optimal protein:energy ratio in Chinese bream, *Megalobrama amblycephala* Yih. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ 9. Manila, Philippines, Asian Fisheries Society, pp. 31–42.

Abstract

The optimum protein and energy (P/E) ratio and digestibility of nine carbohydrate rich ingredients in practical diets for Chinese bream fingerlings were determined. The diets contained energy levels of 266, 294 and 324 kcal/100 g at low (22%) and suboptimum (27%) protein levels. Diets providing 22% protein and 266 kcal/100 g of energy or 27% protein and 324 kcal/100 g of energy with a P/E ratio of 83 mg protein/kcal produced better growth rate, feed and protein conversion than high protein diets. SGR, FCR, PER and PPV of bream fingerlings fed the 22% protein diets decreased while those of the fish fed the 27% protein diets increased as dietary energy increased. By providing a balanced P/E ratio in diets, dietary protein level can be reduced from 31% for bream fingerlings to 27% or 22%, and thus 30% or 50% of dietary protein spared. This protein sparing action was obtained by utilizing corn meal, a carbohydrate rich ingredient.

The results showed that among nine carbohydrate rich ingredients, scrap rice and corn meal were well digested in terms of dry matter (87.86% and 69.0%), total energy (87.0% and 70.4%), carbohydrate (88.5% and 74.5%) and protein digestion coefficients (87.2% and 75.5%). Sweet potato was very poorly digested by bream fingerlings.

Introduction

The blunt snout bream, *Megalobrama amblycephala* Yih, is one of the commercially important food fish in China. It has been cultivated in ponds, lakes and reservoirs all over the country. The total annual yield of bream is approximately 180 000 tonnes. However the main limitation for bream production is the availability of fingerlings. Traditionally, raw materials, such a soybean cake, wheat middling and aquatic plants were used to feed the bream fingerlings.

---

[1] Present address: Institute of Reservoir Fisheries
Ministry of Water Resources, Wuhan, PRC.

Protein is the most expensive component of fish feed. The economical efficiency of fish culture depends on feed to a large extent, and especially dietary protein cost. The dietary protein requirement for optimum growth for bream fingerlings has been known to be 26–33% at 20°C and 33–40% at 25–30°C (Shi et al. 1988). It is well known that a supplement of lipids has a sparing effect on dietary protein (Adrom 1976; Watanabe et al. 1979; Shimeno et al. 1980; De Silva et al. 1991). However, animal lipids and plant oil are as expensive as fish meal in China. On the other hand, native cereal, grain and agricultural by-products which contain high carbohydrate are rather cheap and abundant. It is generally believed that carbohydrate is poorly used by fish (Lin et al. 1978; Bergot 1979). As a herbivorous fish, the natural food of blunt snout bream is mainly aquatic vegetation, indicating that the bream probably can accept a diet high in carbohydrate. The aim of this study was to examine whether carbohydrate-rich ingredients could be used in practical diets to spare expensive dietary protein, resulting in a cost saving and increased yields. The optimum protein and energy ratio and apparent digestibility coefficients of locally available carbohydrate rich ingredients for bream fingerlings are reported in this paper.

Materials and Methods

Protein to energy (P/E) ratio experiment

A 68-day feeding experiment was conducted to determine the optimum dietary P/E ratio for bream fingerlings. Six test diets of two dietary protein levels (27% and 22%) were each formulated at three levels of energy (266, 294, 324 kcal/100 g). Accordingly P/E ratios were 66, 74 and 83 mg protein/kcal at 22% protein level and 83, 93 and 105 mg protein/kcal at 27% protein level. In diet no. 7, 31% crude protein and 321 kcal/100 g of energy was provided to meet the nutritional requirements of bream fingerlings as a control. The composition and proximate analysis of the test diets are given in Table 1.

Table 1. Test diet composition and nutrient contents (%)[1]

Diet No.	1	2	3	4	5	6	7
Fish meal	12.0	12.0	12.0	15.0	15.0	15.0	14.3
Soybean cake	20.7	22.1	24.2	30.0	30.0	30.0	26.5
Rapeseed cake	—	—	—	1.6	3.4	5.1	21.3
Corn	59.6	48.7	37.8	46.9	35.7	24.6	31.9
Crude husk	1.7	11.2	20.0	0.5	9.9	19.3	—
Mineral premix[2]	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Vitamin premix[3]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Binder	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Crude protein	21.51	21.72	22.18	26.98	27.20	27.41	30.98
Crude lipid	4.38	4.31	4.24	4.49	4.47	4.44	5.12
Available energy (kcal/100 g)	324	294	266	324	294	266	321
P/E ratio (mg/cal)	66	74	83	83	93	103	97

1 Proximate composition of the ingredients: fish meal 65.6% protein, 5.13% fat; soybean cake 42.73% protein, 4.8% fat; rapeseed cake 36.4% protein, 7.8% fat; corn 7.91% protein, 4.55% fat; crude husk 4.9% protein, 3.7% fat
2 NRC (1983)
3 NRC (1977)

Bream fingerlings of average weight of 8.50 ± 0.16 g were randomly distributed into 21 round plastic tanks of 57 cm diameter with a water volume of 110 L, eight fish per tank. All diets in triplicate were randomly assigned to the tanks. Dechlorinated tap water was supplied at a rate of 0.4 L/min with supplemental aeration. After a period of two weeks acclimation the fish were fed with experimental diets to satiation (ad libitum) at four meals daily. The water temperature ranged from 20°C to 26°C with a mean value of 23°± 1.4°C. The dissolved oxygen content ranged from 6.80 to 7.38 mg/L. The residual choride content was less that 0.02 mg/L.

At the start and end of the experiment, five fish were randomly sampled from each treatment. Moisture, protein, fat and ash contents were determined by the same methods as earlier reported (He 1988). At the termination of the experimental period samples of blood and liver were taken for

the determination of related physiological and biochemical parameters. Haemoglobin (Hb) and red blood cell count (RBC) were measured separately on six individual fish from each treatment. Hb was measured by alkaline spectrophotometric method while RBC content was estimated microscopically. Pooled samples of 4–7 fish from each treatment were taken to measure serum glutamate-pyruvate transaminase (GPT) and glutamate-oxaloacetate transaminase (GOT) by the method of Reitman-Frankel (1957). Pooled samples of liver, each consisting of two fish, were taken from each treatment for the determination of glucose-6-phosphatase activity, hepatic glucagon and lipid content. G-6-Pase activity was assayed by the method of Harper (1965). Each 250 mg sample of fresh liver was homogenized in a glass blender with 9.75 mL citrate buffer. The homogenate was filtered through double layers of gauze. Five mL of filtrate was transferred to the test tube, and kept in a water bath at 27°C. After 5 minutes 0.1 mL G-6-Pase was added and 2 mL 10% TCA added after 30 minutes. After centrifugation at 3500 × g for 5 minutes, the phosphorus concentration of supernatant was determined. The G-6-Pase activity was expressed as µmoles P/min/g liver. For the determination of hepatic lipid content, fresh liver was homogenized in a blender with 3 volumes of water. Then the homogenate was extracted three times with 2 volumes of chloroform/methanol (3/1, v/v). After centrifugation, the residues were kept 24 hours at room temperature to remove the solvent and then dried to constant weight at 60°C. Hepatic glucose content was measured by the method of Carrell et al. (1956).

One way analysis of variance and Newman-Keul multiple range test were applied to evaluate the differences between treatment means. Linear regressions were used where appropriate.

Apparent digestibility coefficients determination

Apparent digestibility coefficients for dry matter, total energy, available carbohydrate and crude protein in nine carbohydrate rich ingredients were determined using the chromic oxide indicator method. The ingredients tested were corn, millet, sweet potato, wheat middling, Cassava, sorghum, potato, scrap rice and beet crumb. The experiment was carried out in cone-shaped plastic tanks specially designed with a screen (1.5 cm mesh) in the middle to separate the fish from faecal materials and with a valve at the bottom to drain faecal material. A 76 cm diameter 330 L volume tank was stocked with 7–8 fish of mean weight 125 g. The water was aerated and renewed daily, average temperature being 24.5°C ± 0.2°C with a range of 21.2°–26.5°C. Fish were acclimated to the reference diet (Table 2) for 20 days. Then the fish were fed either a reference diet or a test diet composed of 70% reference diet and 30% test ingredients (Cho and Slinger 1979). Test diets were fed to satiation twice daily. Two hours after each feeding, the uneaten feed was cleaned out. Faeces were collected by opening the valve and draining them out. Excess water was drained from the faecal materials by gauze. Only the unbroken faeces were taken by forceps. Pooled samples for each treatment were dried at room temperature overnight.

Diets and faeces were then analyzed for dry matter at 105°C for 24 hours, total energy by a Shimadzu CA-4P bomb colorimeter, available carbohydrate by anthrone method and nitrogen content by micro-kjeldahl method. Chromic oxide was determined using the wet-acid digestion technique of Furukawa and Tsukuhara (1966). The apparent digestibility coefficients of test diet were calculated as

$$100 - \left(\frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in diets}} \times \frac{\%\ Cr_2O_3\ \text{in diets}}{\%\ Cr_2O_3\ \text{in faeces}} \times 100\right)$$

and the apparent digestibility coefficient of the test component calculated as

100/30 (digestibility of test diet—70/100 digestibility of ref. diet)

The composition of the reference diet and the nutrient contents of test feedstuffs, reference diet and test diets are presented in Tables 2, 3 and 4 respectively.

Table 2. Composition of reference diet for digestibility determination

Ingredient	Contents (%)
Fish meal	14.3
Soybean cake	26.5
Rapeseed cake	21.3
Corn	30.9
Mineral premix	4.0
Vitamin premix	1.0
Binder	1.0
Cr_2O_3	1.0

Table 3. Nutrient content of test feedstuffs (%)

Test feedstuffs	Crude protein	Crude lipid	Carbohydrate
Corn	9.17	3.5*	74.34
Millet	10.19	2.6*	59.98
Sweet potato	3.62	1.3	83.73
Wheat middling	18.16	3.67	41.89
Cassava	1.41	0.2	88.01
Sorghum	10.53	3.3	63.14
Potato	14.87	0.2	70.15
Scrap rice	10.58	2.0	77.57

* Quoted from 'A table of feedstuffs contents and nutritive values for pig and chicken' (1983)

Table 4. Nutrients contents of reference diet and test diets (%)

Test diets	Crude protein	Crude lipid	Ash	Crude fibre
Reference diet (RD)	32.69	4.86	9.00	4.22
RD + corn	25.60	4.25	7.31	6.17
RD + millet	27.84	4.74	8.13	5.73
RD + sweet potato	25.57	3.32	6.40	3.67
RD + wheat middling	30.52	4.67	8.30	6.04
RD + cassava	25.73	3.50	7.62	3.81
RD + sorghum	27.72	4.36	7.35	4.72
RD + potato	26.96	3.10	8.86	3.76
RDt + scrap rice	26.35	4.18	7.38	3.41
RD + beet crumb	24.24	4.16	—	8.27

Results

Protein and energy (P/E) ratio, specific growth rate (SGR) and feed conversion ratio (FCR)

The growth rate, feed and protein conversion of bream fingerlings fed diets of varying P/E ratio are shown in Table 5. At 22% protein level, the highest growth rate and lowest feed conversion ratio were obtained with diet no. 3, containing an energy level of 266 kcal/100 g and a P/E ratio of 83 mg/kcal. Increasing the energy level from 266 to 324 kcal/ 100 g (lowering P/E ratio from 83 to 66 mg/kcal) resulted in a decreased SGR and an increased FCR. The relationships between SGR and P/E ratio, and FCR and P/E ratio at 22% protein level are shown in Figures 1 and 2. The highest SGR and lowest FCR were obtained with diet no. 4, containing an energy level of 324 kcal/100 g and a P/E ratio of 83 mg/kcal. Increasing the energy level from 266 to 324 kcal/100 g (lowering P/E ratio from 105 to 83 mg/kcal) resulted in increasing the SGR and decreasing the FCR. However, the relationships between SGR and P/E ratio, FCR and P/E ratio at 27% protein level were not significant ($r = -0.8171$, $p = 0.39$ and $r = 0.6367$, $p = 0.56$). The overall mean SGR and FCR of fish fed 22% or 27% protein diets were comparable or even better than in fish fed 31% protein diet.

Table 5. Growth rate, feed and protein conversion of blunt snout bream fingerlings fed diets of varying P/E*

Diet No	Crude protein (%)	P/E	Mean Initial Weight(g)	Mean Final Weight(g)	SGR (%/day)	FCR	PER	PPV (%)
1	21.5	66	8.30±0.18	11.49±0.15	0.48 ± 0.02^{a}	5.82 ± 0.23^{b}	0.80 ± 0.03^{b}	37.6 ± 1.6^{bc}
2	21.7	74	8.85±0.07	13.32±0.37	0.60 ± 0.05^{a}	4.65 ± 0.29^{a}	1.00 ± 0.06^{a}	48.7 ± 3.4^{ab}
3	22.2	83	8.77±0.62	13.96±0.44	0.69 ± 0.05^{a}	3.75 ± 0.18^{a}	1.20 ± 0.05^{a}	55.8 ± 3.4^{a}
4	27.0	83	7.74±0.81	11.80±1.66	0.61 ± 0.06^{a}	4.41 ± 0.56^{a}	0.87 ± 0.11^{b}	25.7 ± 5.2^{cd}
5	27.2	93	8.18±0.23	11.53±0.27	0.51 ± 0.03^{a}	5.68 ± 0.15^{a}	0.65 ± 0.01^{b}	17.2 ± 1.1^{d}
6	27.4	103	9.21±0.17	13.15±0.60	0.52 ± 0.04^{a}	5.23 ± 0.52^{a}	0.63 ± 0.07^{b}	25.5 ± 3.3^{cd}
7	31.0	97	8.51±0.37	12.03±1.28	0.50 ± 0.09^{a}	6.00 ± 1.25^{a}	0.59 ± 0.13^{b}	21.7 ± 6.6^{cd}

* Means with each column not sharing a common superscript letter are significantly different ($p<0.05$)

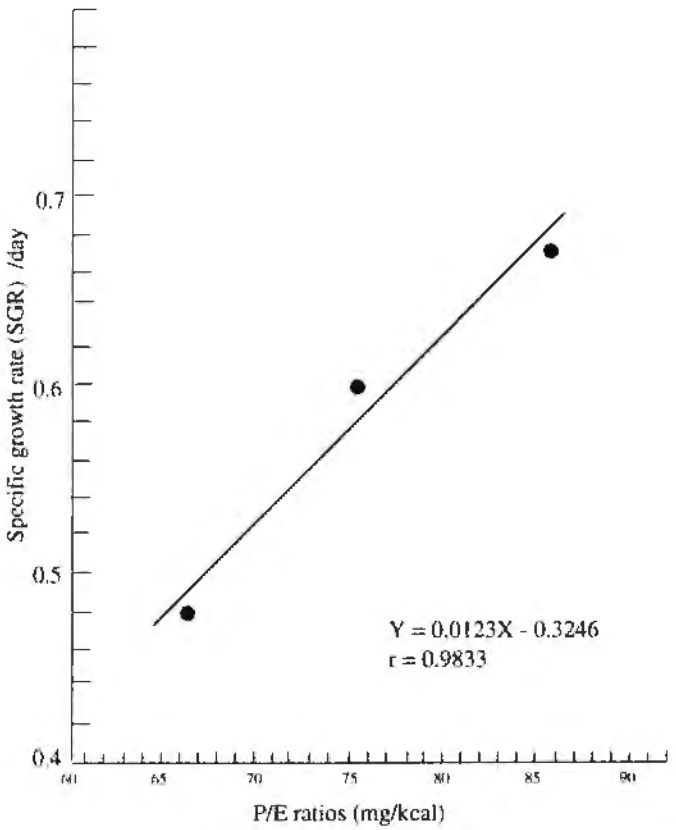

Figure 1. SGR of bream fingerlings as a function of dietary P/E ratio at 22% protein level

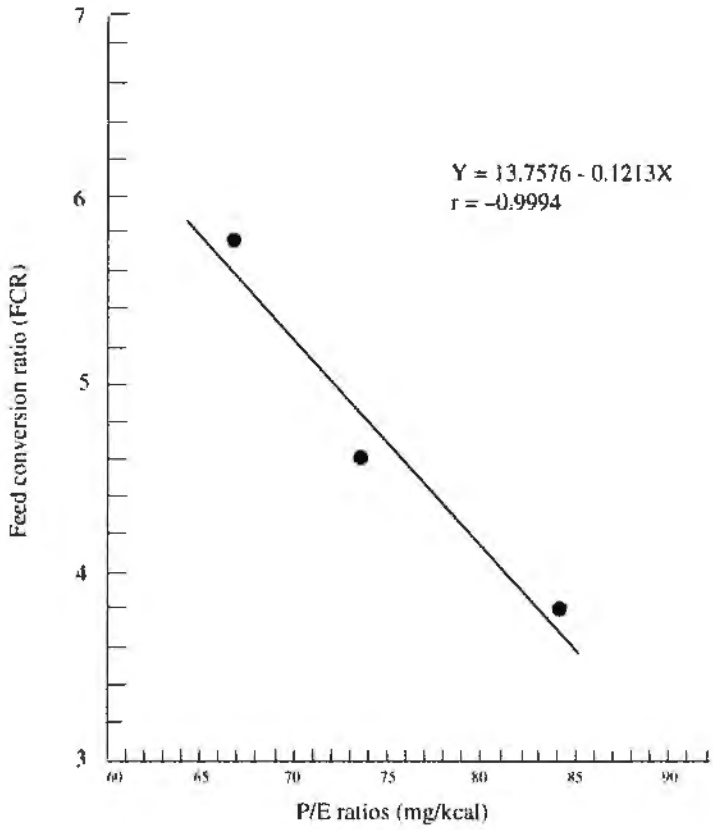

Figure 2. FCR of bream fingerlings as a function of dietary P/E ratio at 22% protein level

Protein efficiency ratio (PER) and protein productive value (PPV)

PER and PPV of the fish fed varying P/E ratio diets showed a similar tendency to those of SGR and FCR. The highest PER and PPV were obtained for fish fed diets no. 3 and 4 at 22% and 27% protein levels respectively. Regression analysis showed that there was a significant correlation between PER and P/E ratio ($r = 0.99$, $p = 0.02$) and a less significant correlation between PPV and P/E ratio ($r = 0.98$, $p = 0.10$) at 22% protein level (Figures 3 and 4). Increasing the energy level at 22% protein level resulted in lowering of PER and PPV, while increasing the energy level at 27% protein level resulted in raising of PER and PPV. However, the relationships between PER and P/E ratio, PPV and P/E ratio at 27% protein were not significant ($r = 0.90$, $p = 0.29$ and $r = -0.02$, $p = 0.99$). The fish fed the 22% protein diets had an overall average PER and PPV of 1.00 ± 0.02 and $47.4 \pm 5.3\%$, which were higher than the fish fed 27% protein diets (overall average PER 0.72 ± 0.08 and PPV $22.8 \pm 2.8\%$ and significantly higher ($p<0.05$) than the fish fed 31% protein diet.

Compared with the high protein diet, a substantial amount of dietary protein was spared when fed 22% or 27% protein diets. Based on the PER data, 167 g of the dietary protein was required for each 100 g weight gain with the 31% protein diet while 118 g and 83 g were required with 27% and 22% protein diets. Thus when fish were fed with the low or suboptimum protein diet instead of the high protein diet, 30% or 50% respectively of dietary protein was spared.

Physiological and biochemical parameters

The physiological and biochemical parameters of the fish fed varying P/E ratio diets are shown in Table 6. Neither significant difference nor any defined trend were found in RBC, Hb content, GOT and GPT activities for fish fed varying P/E ratio diets. A significant negative correlation was found between P/E ratio and G-6-Pase activity at 22% protein level ($r = 0.99$, $p = 0.05$, Fig. 5). At 27% protein level, G-6-Pase activity was lowest in liver of bream fed diet no. 4 which was markedly lower than the fish fed diet no. 5 ($p<0.05$).

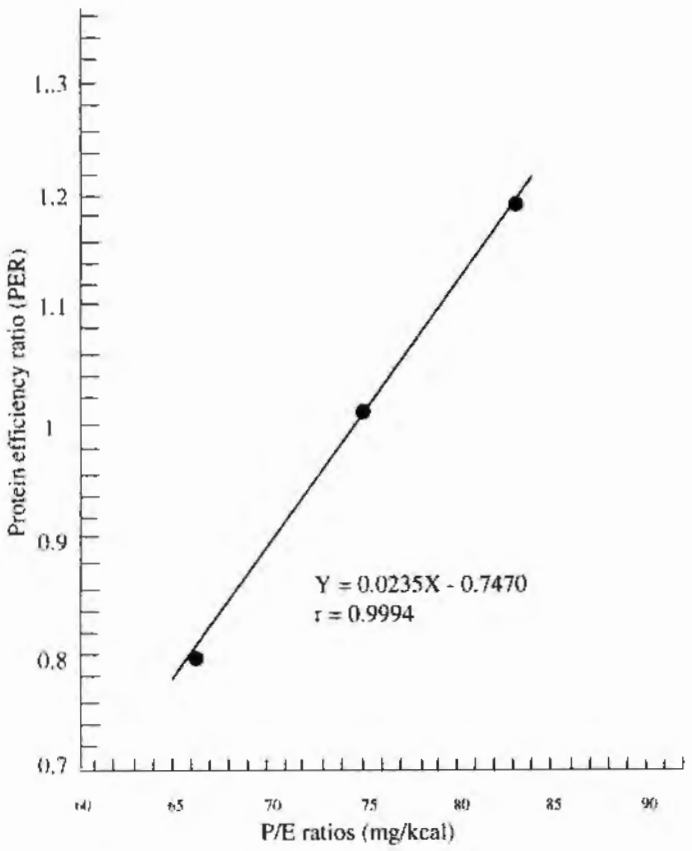

Figure 3. PER of bream fingerlings as a function of dietary P/E ratio at 22% protein level

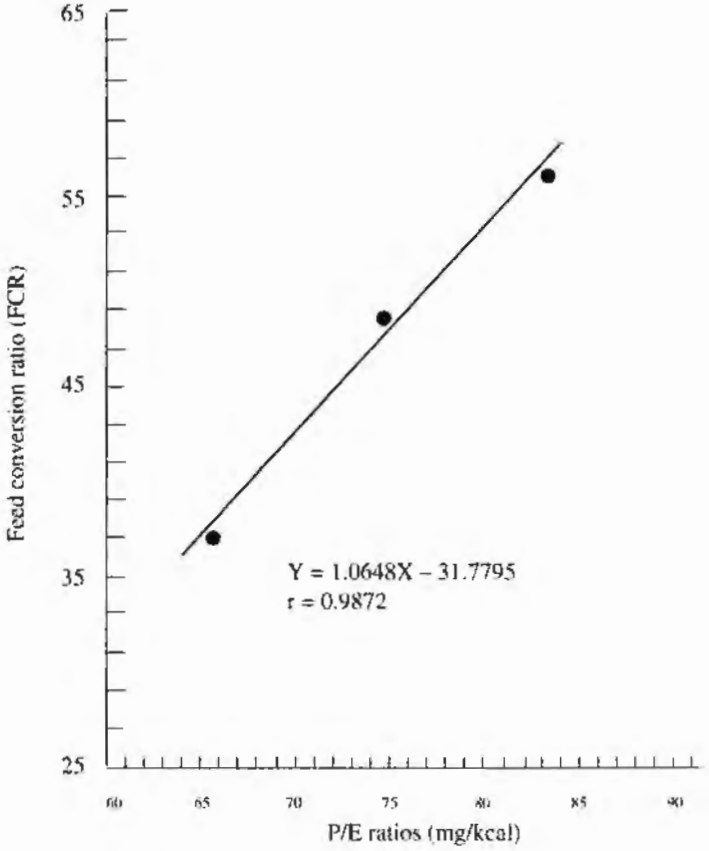

Figure 4. PPV of bream fingerlings as a function of dietary P/E ratio at 22% protein level

However, there was no definite relationship between P/E ratio and G-6-Pase activities ($r = 0.66$, $p = 0.54$) at 27% protein level. The highest hepatosomatic index (HSI) was obtained in fish fed diet no. 3 at 22% protein level, and in fish fed diet no. 4 at 27% protein level. A positive correlation between P/E ratio and HSI at 22% protein level and a negative correlation between P/E ratio and HSI at 27% protein level were found.

The overall average HSI value of 2.14 ± 0.13 at 22% protein level was higher than the overall average HSI value of 1.92 ± 0.15 at 27% protein level and the HSI value of 1.97 ± 0.04 at 31% protein level. The lowest hepatic glucagon content was obtained in fish fed diet no. 3 at 22% protein level and diet no. 4 at 27% protein level. Hepatic glucagon content increased as dietary energy increased at 22% protein level while it decreased as dietary energy increased at 27% protein level, though the correlation coefficients between hepatic glucagon content and P/E ratio at both protein levels were not significant ($r = -0.98$, $p = 0.12$ and $r = 0.93$, $p = 0.24$). The hepatic glucagon content of $0.66 \pm 0.2\%$ protein level and $0.71 \pm 0.14\%$ at 27% protein level were higher than the value of $0.48 \pm 0.15\%$ at 31% protein level. No defined correlation was found between hepatic lipid content and P/E ratio at 22% protein level. However, hepatic lipid content increased as the dietary energy increased at 27% protein level.

Carcass composition

The body composition of the fish fed varying P/E ratio diets is shown in Table 7. All test groups of fish at the end of the experiment had a lower moisture and protein content, and a higher lipid content compared to the initial fish samples. The carcass protein and lipid contents were higher, moisture and ash contents were lower in fish fed 22% protein diet than those of fish fed 31% protein diet. No significant effect of varying P/E ratio diets on the carcass composition of fish at both dietary protein levels was observed.

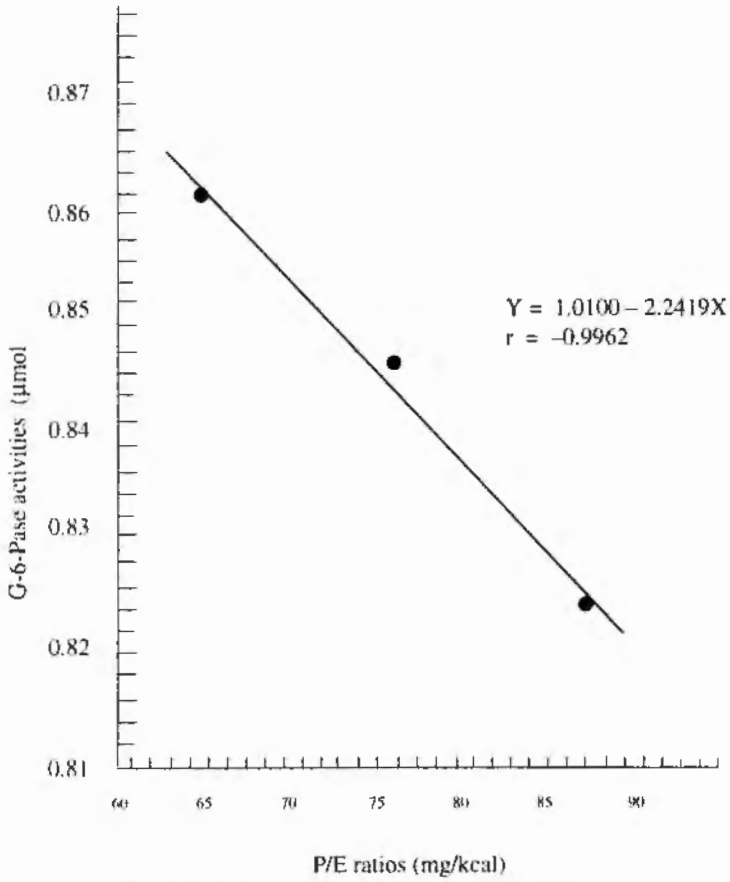

Figure 5. G-6-Pase activities of bream fingerlings as a function of dietary P/E ratio

Table 6. Physiological and biochemical parameters of blunt snout bream fingerlings fed diets of varying P/E ratio*

Diet No.	Crude protein (%)	P/E (mg/kcal)	RBC ($10^4/\mu L$)	Hb(%)	GOT	GPT	G-6-Pase (μmol P/min/g)	HSI (%)	Hepatic glucagon (%)	Hepatic lipid (%)
1	21.5	66	237±10^a	7.07±0.29^a	34.5	85	0.861±0.057^{ab}	1.89±0.16^{ab}	1.03±0.12^a	4.56±1.04^a
2	21.7	74	243±20^a	7.30±0.21^a	110	81	0.846±0.127^{ab}	2.19±0.02^{ab}	0.60±0.18^a	5.44±0.38^a
3	22.2	83	231±21^a	6.83±0.62^a	23.5	51	0.823±0.060^{ab}	2.33±0.25^a	0.35±0.08^a	5.13±0.55^a
4	27.0	83	221±22^a	7.35±0.34^a	98	135	0.730±0.071^b	2.13±0.16^{ab}	0.43±0.11^a	6.94±2.11^a
5	27.2	93	271±30^a	7.31±0.26^a	28	43	1.117±0.111^a	1.99±0.23^{ab}	0.81±0.31^a	4.21±0.22^a
6	27.4	103	216±19^a	7.08±0.68^a	95	154	0.991±0.042^{ab}	1.64±0.12^b	0.88±0.22^a	2.98±0.16^a
7	31.0	97	220±36^a	6.91±0.24^a	56	115	1.015±0.089^{ab}	1.97±0.04^{ab}	0.48±0.15^a	6.04±1.72^a

* Means with each column not sharing a common superscript letter are significantly different ($p<0.05$)

Table 7. Body composition (DM%) of blunt snout bream fed varying P/E ratio diets

	Diet No.	Moisture (%)	Protein (%)	Lipid (%)	Ash (%)
Initial		88.84	56.01	22.52	12.54
Final	1	70.76	53.47	31.17	10.57
	2	71.31	53.60	29.38	11.23
	3	71.03	52.36	30.79	10.89
	4	73.77	47.00	27.13	11.77
	5	63.57	47.45	28.54	10.94
	6	70.91	49.99	25.62	11.18
	7	78.80	50.42	24.48	12.14

Apparent digestibilities

The apparent digestibilities of nine different ingredients are given in Table 8. Apparent digestibility coefficients of carbohydrate were between 74.5–88.5% for scrap rice, wheat middling, beet crumb and corn, and between 53.5–67.2% for sorghum, millet, potato and cassava. The energy and dry matter digestibilities were higher than 70% for scrap rice and corn, followed by sorghum, cassava, potato, wheat middling, millet, beet crumb and sweet potato in decreasing order. The protein digestibility coefficients were higher for scrap rice, wheat middling and corn than other test ingredients. On the whole, scrap rice was the most digestible ingredient for bream fingerlings. More than 87% of total energy, dry matter and nutrients were digested. Wheat middling, one of the popular feedstuffs used by fish farmers, had a high carbohydrate and protein digestibility coefficient (78% and 84% respectively), but a low energy and dry matter digestibiity coefficient (48% and 46% respectively). Sweet potato was poorly digested by bream fingerlings. Energy, dry matter and carbohydrate digestibility coefficients were 3.7%, 1.7% and zero respectively.

Table 8. Apparent digestibility coefficients of feedstuff for blunt snout bream

Feedstuff	Total energy(%)	Available dry matter(%)	Carbohydrate(%)	Protein(%)
Corn	70.4	69.0	74.5	75.5
Millet	45.0	42.6	56.9	70.8
Sweet potato	3.7	1.7	0	58.8
Wheat middling	48.4	46.0	78.5	84.2
Cassava	50.4	52.3	53.5	65.8
Sorghum	54.7	56.0	67.2	66.5
Potato	50.0	51.3	55.3	54.5
Scrap rice	87.0	87.6	88.5	87.2
Beet crumb	—	35.6	75.2	61.5

Discussion

It is generally believed that fish require a high protein diet for maximum growth. However, from economical, nutritional and environmental protection standpoints a high protein fish diet may not necessarily be appropriate. Dietary protein is one of the most costly components of fish diet. Moreover, the excretion of nitrogenous wastes increases the oxygen demand and pollutes the water body. One of the challenges faced by fish nutritionists is to optimise the use of cheap nonprotein energy in order to save the expensive dietary protein, and still provide reasonable fish growth.

Dietary protein and energy are two important factors determining fish growth and nutrient utilization. It has been reported that the level and balance of dietary protein and energy affect weight gain, feed consumption, nutrient efficiency, digestibility and carcass composition of various animals including fish (Page and Andrew 1973). The present study showed that proper balance of dietary protein and energy is extremely important for optimum growth and nutrient efficiency in bream fingerlings. A diet providing 27% protein and 324 kcal/100 g physiological energy or 22% protein and 266 kcal/100 g physiological energy produced better growth performance and nutrient utilization than other diets. Both diets had a P/E ratio of 83 mg/kcal, suggesting that within the acceptable dietary protein range this optimum is quite constant. Shifting away in either direction from this optimum value for bream fingerlings showed detrimental effects with reduced growth rate and nutrient conversion.

Santiago and Laron (1991) also reported that within a certain range of dietary protein concentrations favourable to red tilapia fry, the optimum P/E ratio remains constant but it becomes lower at a marginal protein level. With an increase of dietary energy, growth of bream fingerlings fed the 22% protein diet decreased, but growth of fish fed the 27% protein diet increased (Table 5). The observed growth depression of bream fingerlings fed either low protein (22%), high energy (324 kcal/100 g) or suboptimum protein (27%), low energy (266 kcal/mg) diets might be explained by different mechanisms. It was reported that increasing the level of digestible energy in a low protein (27%) channel catfish diet reduced gain because it caused the fish to eat less and then not obtain their daily protein requirement (Mangalik 1986). Reduced growth was observed in catfish (Prather and Lovell 1974) and yellowtail (Shimeno et al. 1985) fed high protein diets containing

inadequate non-protein energy. In these cases the depression of growth was related to protein deamination and much of the protein was utilized as an energy source.

In general, carnivorous fish require higher P/E ratio than omnivorous or herbivirous fish (Winfree and Stickney 1987). Being a herbivorous fish, the optimum P/E ratio of 83 mg/kcal for bream fingerlings is lower than many other fish. The low P/E ratio for bream fingerlings presumably indicates that bream utilize less protein as an energy source and the potential for nonprotein energy utilization should be great.

On the basis of a balanced P/E ratio, the choice of a suitable energy source becomes an important issue. Lipid and carbohydrate are the two main nonprotein energy sources. Using lipid effectively as an energy source to spare the costly dietary protein has been shown for rainbow trout (Reiniz et al. 1978; Beamish and Medland 1986), channel catfish (Dupree et al. 1979), turbot (Adron et al. 1976) and hybrid tilapia (Shiau and Huang 1990). However, there is controversy on whether carbohydrate could be effectively used as an energy source. Most carnivorous fish cannot utilize dietary carbohydrate properly. Dietary carbohydrate was less utilized as an energy source than dietary lipid in rainbow trout (Ogino et al. 1976), turbot (Adron et al. 1976), and cod (Hemre et al. 1989). In contrast, carp, catfish and tilapia utilize carbohydrate effectively as an energy source to various extents (Ogino et al. 1976; Garling and Wilson 1977; Anderson et al. 1984). It is interesting to note that channel catfish and *Tilapia zillii* can efficiently utilize both carbohydrates and lipids as energy sources if they are substituted for one another at a rate of 2.25:1 commensurate with CHO:L physiological fuel values (Garling and Wilson 1977; El-Sayed and Garling 1988). The difference in carbohydrate utilization between various fish is thought to be related to their natural feeding habits and the structure and function of their digestion systems.

In the present study corn meal was used as a main energy source. The dry matter, total energy and carbohydrate digestibilities for corn meal were found to be 70, 70, and 75% in bream fingerlings respectively (Table 8). The best growth performance was observed for fish fed diet no. 3 containing 38% corn meal and, to a lesser extent, for fish fed diet no. 2 which contained 49% corn meal. However, growth was depressed when 60% corn meal was incorporated in the diet. The PER value of 1.20 at 22% protein level and of 0.87 at 27% protein level implies that 30–50% of dietary protein could be spared by adjusting the proper balance of P/E ratio. These findings are of great interest in that the protein sparing action was obtained by utilizing corn meal, a native carbohydrate-rich component, as a nonprotein ingredient. Corn meal and many other grains are cheaper and more abundant than lipid in China and other developing countries. The possibility of including local cereals at levels of up to 40–50% is meaningful in developing low-cost formulated diets for bream fingerling culture.

One may be concerned about the possible adverse effects on the well-being of farm fish when a high level of carbohydrate-rich ingredients are incorporated in the diet. It was reported that fish fed a high-carbohydrate diet resulted in an increase of the hepatosomatic index (Kaushik 1989), an increase in liver glycogen deposition (Cowey et al. 1975; Hilton and Atkinson 1982) and carcass lipid content (Shimeno et al. 1985) and a stimulation of enzyme activities in the liver (Likimani and Wilson 1982; Degani 1987). Some similar effects were presented in this study. For example, with increasing dietary carbohydrate level hepatosomatic index and hepatic lipid content increased at 27% protein level and the hepatic glycogen deposited and the G-6-Pase activities elevated at 22% protein level. The response in relation to high dietary carbohydrate inclusion seems to differ at different protein levels. In order to illustrate the role of dietary carbohydrate in fish nutrition more research on carbohydrate metabolism in fish is needed.

The evaluation of nutritive values of feedstuffs primarily depends on the digestion coefficient, as nutrients can only be assimilated after digestion. The dry matter and energy digestibility represent the total nutrient digestibility of feedstuffs. They are better estimations of feedstuff digestibility than individual nutrient digestibility. Apart from sweet potato which was very poorly digested by bream fingerlings, the dry matter and energy coefficients of all other test feedstuffs ranged from 35.6–87.6% and 45.0–87.0% respectively. The highest dry matter digestion coefficient of 87.6% for scrap rice and the lowest value of 35.6% for beet crumb corresponds to the lowest crude fibre content for scrap rice and the highest content for beet crumb (Table 4). This finding was in agreement with reports for other fish species (De Silva et al. 1990; Nandeesha et al. 1991).

Overall an average of 68.7%, with a range of 53.5–88.5% of the dietary carbohydrate were digested for all ingredients tested (except sweet potato). These values were in general higher than those reported for trout, 38–55% (Bergot and Breque 1983), yellowtail, 39–57% (Shimeno et al. 1977) and cod, 33% (Hemre et al. 1989) and comparable to that for carp, 17–90% with a mean

value of 56% (Kirchgessmer et al. 1986) and red tilapia of 64–90% (Kamarudin et al. 1989). Obviously the omnivorous and herbivorous fish have a higher carbohydrate digestibility than carnivorous fish.

The variation in dietary carbohydrate digestibility for various feedstuffs rich in native starches is presumably associated with the physical state of starch. Potato and sweet potato starch had an average particle size of 30–33 μm, corn and wheat starch had an average of 15 μm, whereas rich starch had the least particle size of only 3–8 μm (Zhang 1988). Scrap rice and corn meal were well digested in terms of dry matter, total energy, carbohydrate and protein digestion coefficients (Table 8). Both of them are suitable for use in formulated diets for bream as a high efficiency energy-yielding ingredient. Kamarudin et al. (1989) found that among the nine kinds of test feedstuffs the apparent dry matter digestion coefficient was highest in rice bran for red tilapia. However, Law (1986) noted that rice bran was poorly digested by grass carp. The difference might be due to differences in species and test conditions. The sweet potato which is a good feedstuff for pigs, had exceptionally low values for dry matter, total energy and carbohydrate digestion coefficients. The reason for the unexpected low digestibility of sweet potato is not yet apparent.

Acknowledgements

We wish to thank the International Development Research Centre (IDRC) of Canada for financial support of this work. We also would like to acknowledge Dr S.S. De Silva for his useful comments and Dr J.A. Mathias for reviewing the manuscript.

References

Adron, J.W., A. Blair, C.B. Cowey and A.M. Shanks, 1976. Effects of dietary energy level and dietary energy source on growth, feed conversion and body composition of turbot (*Scophthalmus maximus L.*). *Aquaculture* 7: 125–132.

Anderson, J., A.J. Jackson and B.S. Capper, 1984. Effects of dietary carbohydrate and fibre on the tilapia *Oreochromis niloticus (Linn.)*. *Aquaculture* 7: 125–132.

Beamish, F.W.H. and T.E. Medland, 1986. Protein sparing effects in large rainbow trout, *Salmo gairdneri*. *Aquaculture* 55: 35–42.

Bergot, F. 1979. Carbohydrate in rainbow trout diets, effects of the level and source of carbohydrates and the number of meals on growth and body composition. *Aquaculture* 18: 157–167.

Bergot, F. and J. Breque, 1983. Digestibility of starch by rainbow trout: effects of the physical state of starch and of the intake level. *Aquaculture* 34: 203–212.

Cho, C.Yand S.T. Slinger, 1979. Apparent digestibility measurements in feedstuffs for rainbow trout. In Halver, J.E. and K. Tiews (eds.) *Finfish Nutrition and Feed Technology, Vol. II*, pp. 239–247.

Cowey, C.B., J.W. Adron and D.A. Brown, 1975. Studies on the nutrition of marine flatfish. The metabolism of glucose by plaice (*Pleuronectes platessa*) and the effect of dietary energy source on protein utilization in plaice. *Br. J. Nutr.* 33: 219–231.

Degani, G. 1987. Effect of dietary carbohydrate source on soluble protein glucose concentration and enzyme activity (Aldolase) of European eels (*Anguilla anguilla L.*). *Comp. Biochem. Physiol.* 87A: 27–30.

De Silva, S.S., R.M. Gunasekera and K.F. Shim, 1991. Interaction of varying dietary protein and lipid levels in young red tilapia: evidence of protein sparing. *Aquaculture* 95 (3/4): 305–318.

De Silva, S.S., K.F. Shim and A.K. Ong, 1990. An evaluation of the method used in digestibility estimations of a dietary ingredient and comparison on external and internal markers, and time of faeces collection in digestibility studies in the fish *Oreochromis aureaus* (Steindachner). *Report. Nutr. Dev.* 30: 215–226.

Dupree, H.K., E.T.Jr. Gauglitz, A.S. Hall and C.R. Houle, 1979. Effects of dietary lipids on growth and acceptability (flavor) of channel catfish (*Ictalurus punctatus*). In Halver, J.E. and K. Tiews (eds.), *Finfish Nutrition and Feed Technology, Vol. II*, pp. 87–103.

El-Sayed, A.F. and D.L. Garling, 1988. Carbohydrate-to-lipid ratios in diets for tilapia zillii fingerlings. *Aquaculture* 73: 157–163.

Furukawa, A. and H. Thsukahara, 1966. On the acid digestion method for the determination of chromic oxide as an index substance in the study of digestibility of fish feeds. *Bull. Japan. Soc. Sci. Fish.* 32: 502–506.

Garling, D.L. and R.P. Wilson, 1977. Effects of dietary carbohydrate-to-lipid on growth and body composition of fingerling channel catfish. *Prog. Fish.-Cult.* 39: 43–47.

Hemre, G., O. Lie, E. Lied and G. Lambestsen, 1989. Starch as an energy source in feed for cod (*Gadus morhua*): Digestibility and retention. *Aquaculture* 80: 261–270.

He Xiqin, Lizhu Jia, Zhongjie Li and Yang Yunxia, 1988. Nutrient requirements of juvenile allogynogenetic crucian carp, *Carassius auratus gibelio*. In Ryans, R.C. (ed.), *Fish Physiology, Fish Toxicology, and Fisheries Management. Proceedings of an International Symposium*, Guangzhou, PRC, pp. 73–87.

Hilton, J.W. and J.L. Atkinson, 1982. Response of rainbow trout (*Salmo gairdneri*) to increased levels of available carbohydrate in practical trout diet. *Br. J. Nutr.* 47: 597–607.

Kamarudin, M.S., K.M. Kaliapan and S.S. Siraj, 1989. The digestibility of several feedstuffs in red tilapia. In De Silva, S.S. (ed.), *Fish Nutrition Research in Asia. Proceedings of the third Asian Fish Nutrition Network Meeting*, pp. 118–122.

Kaushik, S.J., P. Luquet, D. Blanc and A. Paba, 1989. Studies on the nutrition of Siberian sturgeon, *Acipenser baeri* I. Utilization of digestible carbohydrate by sturgeon. *Aquaculture* 76: 97–107.

Kirchgessner, M., H. Kurzinger and F.T. Schwarz, 1986. Digestibility of crude nutrients in different feeds and estimation of their energy content for carp (*Cyprinus carpio L.*). *Aquaculture* 58: 185–194.

Law, A.T. 1986. Digestibility of low-cost ingredients in pelleted feed by grass carp (*Ctenopharyngodon idella*). *Aquaculture* 51: 97–103.

Lakmani, T.A., and R.P. Wilson, 1982. Effect of diet on lipogenic enzyme activity in activities in channel catfish hepatic and adipose tissue. *J. Nutr.* 112: 112–117.

Lin, H., D.R. Romsos, P.I. Tack, and G.A. Leveille, 1978. Determination of glucose utilization in coho salmon, *Oncorhynchus kisutch* (Walbaum), with (6-^{3}H) and (U-^{14}C) glucose. *Comp. Biochem. Physiol.* 59A: 189–191.

Mangalik, A. 1986. Dietary energy requirements of channel catfish. Ph.D. Dissertation, Auburn, AL, Auburn University, 126 p.

Nandeesha, M.C., G.K. Srikanch, P. Keshavanath and S.K. Das, 1991. Protein and fat digestibility of five feed ingredients. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia, Proceedings of the Fourth Asian Nutrition Workshop*, pp. 75–81.

NRC, 1977. *Nutrient Requirements of Warmwater Fishes.* Washington DC, National Academy of Science, 78 p.

NRC, 1983. *Nutrients Requirements of Warmwater Fishes and Shellfishes.* Washington DC, National Academy of Science, 102 p.

Ogino, C., J.Y. Chiou and T. Takeuchi, 1976. Protein nutrition in fish—VI. Effects of dietary energy sources on the utilization of protein by rainbow trout and carp. *Bull. Japan. Soc. Sci. Fish.* 42: 213–218.

Page, J.W and J.W. Andrews, 1973. Interactions of dietary levels of protein and energy on channel catfish (*Ictalurus punctatus*). *J. Nutr.* 103: 1339–1346.

Prather, E.E and R.L. Lovell, 1974. Response of intensively fed channel catfish to diets containing various protein-energy ratios. *Proceedings of the Annual Conference Southeastern Association of Fish and Wildlife Agencies*, 37: 358–363.

Reinitz, G.L., L.E. Orme, C.A. Lemm and F.N. Hitzel, 1978. Influence of varying lipid concentrations with two protein concentrations in diets for rainbow trout (*Salmo gairdneri*). *Trans. Am. Soc.* 107: 751–754.

Santiago, C.B. and M.A. Laron, 1991. Growth response and carcass composition of red tilapia fry fed diets with varying protein levels and protein to energy ratios. In De Silva, S.S (ed.) *Fish Nutrition Research in Asia, Proceedings of the Fourth Asian Nutrition Workshop*, pp. 55–62.

Shi, W. et al., 1988. Study on the optimum demand of blunt-nose bream (*Megalobrama amblycephala*). In *Aquaculture International Congress and Exposition*, p. 29 (Summary only).

Shiau, S.Y. and S.L. Huang, 1990. Influence of varying energy levels with two protein concentrations in diets for hybrid tilapia (*Oreochromis niloticus x O. aurea*) reared in seawater. *Aquaculture* 91: 143–152.

Shimeno S., H. Hosokawa, H. Hirata, and M. Takeda, 1977. Comparative studies on carbohydrate metabolism of yellowtail and carp. *Bull. Japan. Soc. Sci. Fish.* 43: 213–217.

Shimeno, S., H. Hosokawa, M. Takeda, and H. Kajiyama, 1980. Effects of calorie to protein ratios in formulated diet on the growth, feed conversion and body composition of young yellowtail. *Bull. Japan. Soc. Sci. Fish.* 46: 1083–1087.

Shimeno, S., H. Hosokawa, M. Takeda, H. Kajiyama, and T. Kaisho, 1985. Effect of dietary lipid and carbohydrate on growth, feed conversion and body composition in young yellowtail. *Bull. Japan. Soc. Sci. Fish.* 51: 1893–1898.

Watanabe, T., T Takeuchi, and C. Ogino, 1979. Studies on the sparing effects of lipids on dietary protein in rainbow trout (*Salmo gairdneri*). In Halver, J.E and K. Tiews (eds.), *Finfish Nutrition and Fishfeed Technology, Vol. I*, pp. 113–125.

Winfree, R.A., and R.R. Stickley, 1981. Effects of dietary protein and energy on growth, food conversion efficiency and body composition of *Tilapia aurea*. *J. Nutr.* 111: 1001–1012.

Zhang Litain, 1988. *The Chemistry of Carbohydrate*. Beijing, Light Industry Press, 408 p. (in Chinese).

Studies on the Protein to Energy Ratio (DP/DE) in Diets of Grass Carp, *Ctenopharyngodon idellus* (C. et Val.)*

MAO YONGQING
LIU YONGJIAN
LIN DING
TIAN LIXIA
DENG DONG FANG
Ichthyology Laboratory, Zhongshan University, Guangzhou, P.R. China

YONGQING, M.L., L. YONGJIAN, L. DING, T. LIXIA and D. DONG FANG. 1994. Studies on the protein to energy ratio (DP/DE) in diets of grass carp, *Ctenopharyngodon idellus* (C. et Val.). In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 43–47.

Abstract

Twelve test diets of four levels of protein (21, 29, 37 and 41%) each containing three different energy levels were formulated. Experiments were conducted, in triplicate, in 1 × 0.5 × 0.5 m tanks, each stocked with 70 grass carp fingerlings of average weight 5.2 g. The experiment lasted 60 days. The water temperature was maintained at 28.6± 1.7°C, DO: 5.1–6.6 mg/L. An integrated analysis of the values of SGR and FCR indicated that optimum DP/DE ratio was 104.7 mg/kcal. The optimum protein level was 37%. Dietary protein level was the main factor affecting SGR, FCR, PER and the contents of liver lipid and liver starch.

Introduction

Studies on the protein to energy ratio for a number of fish species have been reported. The objective of these studies was to improve the biological value of dietary protein and the availability of nonprotein energy for the economical use of protein. This study was carried out to determine the optimum dietary protein to energy ratio for grass carp fingerlings.

Materials and Methods

The fish, selected for uniform size for the experiment, were taken from among the fingerlings nurtured on our own fish farm. Having been bathed in 0.02% formaldehyde solution, the fish were acclimated in sterilized tanks for a week so as to get used to the new environment and feeding devices. They were weighed, grouped and given the experimental feed.

Four different dietary protein levels, 21, 29, 37 and 41%, were used in the experiment. For each, three different energy levels were used (Table 1).

* Research was supported by the International Development Research Centre (IDRC) of Canada.

Table 1. Compositions of the experimental diets (% by weight)

Diet No.	Casin (protein)	Potato starch	α – starch	Oil	Cellulose	Vitamin Premix	Test salt-mixture	Gross energy cal/g
1	22(21)	44	5	2	15	2	10	4021
2	22(21)	44	5	4	13	2	10	4137
3	22(21)	44	5	6	11	2	10	4254
4	30.5(29)	36	5	2	14.5	2	10	4161
5	30.5(29)	36	5	4	12.5	2	10	4277
6	30.5(29)	36	5	6	10.5	2	10	4394
7	38.8(37)	28	5	2	14.2	2	10	4298
8	38.8(37)	28	5	4	12.2	2	10	4414
9	38.8(37)	28	5	6	10.2	2	10	4531
10	43(41)	24	5	2	14	2	10	4368
11	43(41)	24	5	4	12	2	10	4484
12	43(41)	24	5	6	10	2	10	4601

Feeding experiments were carried out in tanks of 1 × 0.5 × 0.5 m, each stocked with 70 grass carp fingerlings of average weight 5.2 ± 2 g. Each tank had a double filter system and the water was exchanged every 40 minutes. The faeces collected in each tank were removed and half the water was changed daily. The complete drainage system was cleaned once every week. Also every week the water was disinfected with 2 ppm bleaching powder solution.

The water temperature was recorded at 0900 and 1500 hrs daily and maintained at 28.6 ± 1.7°C through the experiment. The dissolved oxygen, pH and NH_4–N were measured once every week. Their values were 5.1–6.6 mg/L, 7.1–7.4 and 0.002–0.078 mg/L respectively.

The fish were fed at 1000 and 1500 hrs every day. The daily ration was 3.5% of body weight and regulated every week according to a growth rate of 2%. The fish usually completed eating in 30–40 minutes, and the amount of daily feed intake was recorded. The fish in each tank were weighed once every twenty days. At the end of the experiment, each fish was weighed and its body length was measured, and one third of the fish were killed for carcass analysis. The methods used to ascertain the amount of protein, fat, ash and moisture content were Kjeldahl, Soxhlet, for high temperature resistance furnace and constant temperature drying respectively. Somogyi Method was used for sugar analysis. Calorimeter (TR–2800) was used for energy analysis (Lin et al. 1987).

Faecal matter was siphoned and those strands with complete foreskin were dried at 60°C and used for analysis. The digestibility was determined with the indirect method using resistant ash as an indigenous marker.

Feed evaluating formula

$$\text{specific growth rate (SGR)} = \frac{\text{Ln Wt} - \text{Ln Wo}}{t} \times 100$$

$$\text{feed conversion ratio (FCE)} = \frac{\text{Total weight gain}}{\text{Total amount of feed}} \times 100$$

$$\text{protein efficiency ratio (PER)} = \frac{\text{Net body weight gained}}{\text{weight of protein taken}}$$

$$\text{percent nutrient digestibility} = 100 - 100 \left(\frac{\%\ \text{indigestible marker in feed}}{\%\ \text{indigestible marker in faeces}} \times \frac{\%\ \text{nutrient in faeces}}{\%\ \text{nutrient in feed}} \right.$$

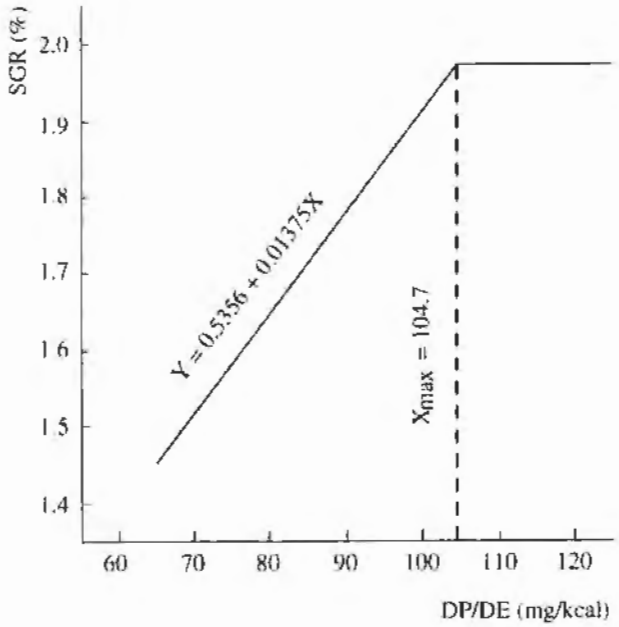

Figure 1. Relationships between dietary DP/DE and SGR

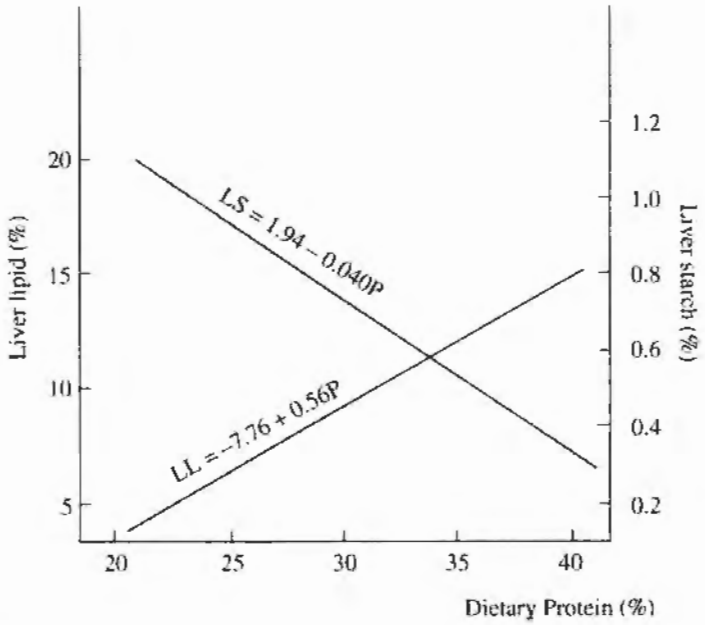

Figure 2. Relationships among dietary protein level, liver lipid and liver starch

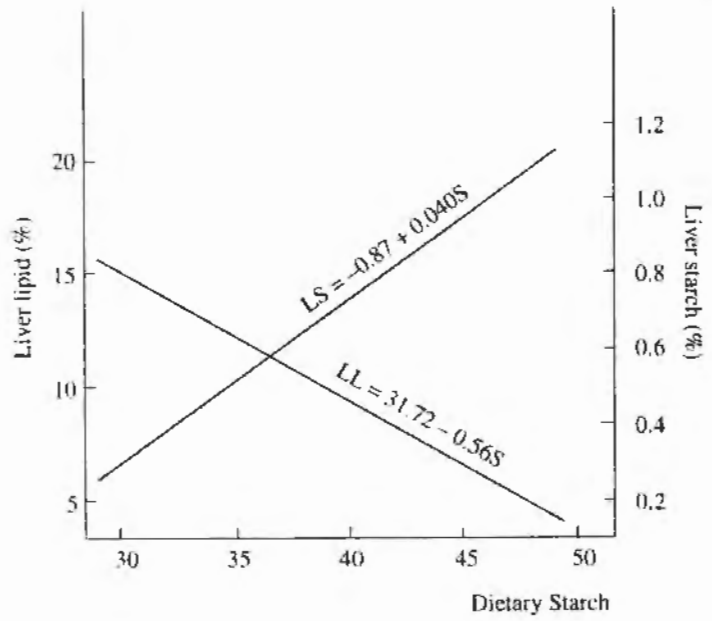

Figure 3. Relationships among dietary starch level, liver lipid and liver starch.

Results

The value of SGR of grass carp was in the range 1.44–2.02%. The DP/DE ratio was in the range 67–101 mg/kcal. SGR increased from 1.44% to 1.99% as the DP/DE ratio increased. Taking the SGR as an index, using the broken line analysis, the optimum of DP/DE ratio was 104.7 mg/kcal (Figure 1). The FCE ranged from 54–83% and the FCE increased from 54% to 81%, in direct proportion to the DP/DE ratio.

With an integrated analysis of the values of SGR and FCR, we came to a tentative conclusion that the optimum DP/DE ratios in grass carp diets should be 105 mg/kcal and the optimum protein level should be 37%.

Adding 2, 4 and 6% lipid in diets at the same protein level, the dietary energy level was increased and SGR and PER did not improve (Table 2). This did not show the protein sparing effect under the conditions of this experiment.

Table 2. Results of feeding experiments

Diet No.	Initial Mean wt. (g/fish)	Final Mean wt. (g/fish)	Survival rate (%)	SGR	FCR	PER	DP/DE* (mg/kcal)
1	5.97	14.58	100	1.49a	58.49a	2.78a	69.3
2	5.34	12.70	98.6	1.44a	54.14a	2.58ab	67.5
3	5.03	12.50	95.2	1.50a	58.20a	2.77a	67.9
4	4.78	13.67	96.2	1.75b	72.48b	2.50ab	95.3
5	4.86	14.39	98.6	1.81bc	72.54b	2.50ab	92.6
6	5.12	14.55	98.6	1.74b	70.83ab	2.44ab	86.4
7	4.92	15.96	91.0	1.96c	82.74b	2.24bc	111.6
8	5.16	17.08	99.5	2.00c	80.08b	2.16bc	102.1
9	5.18	17.10	98.1	1.99c	81.49b	2.20bc	100.6
10	5.14	16.27	99.5	1.92c	76.21b	1.86c	123.6
11	5.37	17.36	96.7	1.96c	78.60b	1.92c	114.6
12	5.26	17.66	99.0	2.02c	81.84b	1.99c	115.4

* The values were determined in the present experiment

Chemical composition of the carcass and liver

There was no remarkable relationship between dietary DP/DE ratios and the chemical composition of the carcass. This, and the chemical composition of the liver of grass carp after the experiment is shown in Table 3. The liver lipid content increased as the dietary protein content increased (Figure 2).

There is a negative relationship between the liver starch content and dietary protein, and a positive relationship between the liver starch content and the dietary starch level (Figure 3).

Discussion

It has been reported that the optimum P/E ratio was about 90 mg/kcal for carp (Ogino 1970). There was much information about the optimum P/E ratios for channel catfish, ranging from 88 to 134 mg/kcal (Garling 1976); for rainbow salmon, plaice and yellowtail, ranging from 125 to 150 mg/kcal (Ringrose 1971, Takeda 1975, Cowey 1972); for Tilapia aurea, 108 mg/kcal; (Winfree 1981); for eel, 110 mg/kcal (Nose 1972); and for grass carp, 110 mg/kcal (Dabrowski 1977), from studying the protein requirement. This is consistent with the results in the present experiment.

Table 3. Carcass and liver proximate composition of grass carp fingerlings reared on different diets. (% on wet basis, average values)

	Body				Liver		
Diet No	Moisture	Protein	Lipid	Ash	Moisture	Lipid	Liver starch
Final							
1	77.68	16.13	2.66	3.53	74.49	5.07	1.11
2	78.28	16.43	2.54	3.34	72.89	4.87	1.51
3	78.09	15.16	3.40	3.26	71.82	6.26	0.84
4	79.60	16.58	2.16	3.06	70.33	6.70	0.46
5	78.63	16.01	2.91	3.21	72.62	6.79	0.77
6	77.81	16.85	3.36	3.20	71.63	6.56	0.80
7	78.13	15.97	2.96	3.37	67.38	13.79	0.24
8	78.89	15.22	2.91	3.21	70.13	9.59	0.63
9	79.95	15.46	3.46	3.18	65.40	13.50	0.57
10	78.45	15.67	2.99	3.27	66.06	14.20	0.30
11	78.16	15.50	3.10	3.17	63.03	16.78	0.25
12	77.56	15.94	4.19	3.08	62.07	19.38	0.37
Initial	80.66	14.31	1.54	3.64			

References

Cowey, C.B., J.A. Pope, J.W. Adron and A. Blair, 1972. Studies on the nutrition of marine flatfish. The protein requirement of plaice (*pleuronectes platessa*). *Br. J. Nutr.* 28: 447–456.

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

Garling, D.L. and R.P. Wilson 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings. *Ictalurus punctatus. J. Nutr.* 106: 1368–1375.

Lin Ding and Yongqing Mao, 1987. *Nutrition and Feed of Fish*, Zhongshan University Press.

Nose T. and S. Atai, 1972. Optimum level of protein in purified diet for eel *Anguilla japonica. Tokyo: Bull. Freshwater Fish Res. Lab.* 22: 145–154.

Ogino, C. and K. Saito, 1970. Protein nutrition in fish. I. The utilization of dietary protein by young carp. *Bull. Japan. Soc. Sci. Fish.* 36: 250–254.

Ringrose, R.C. 1971. Calorie-to-protein ratio for brook trout (*Salvelinus fontinalis*). *J. Fish. Res. Board Canada* 28: 1113—1117.

Takeda, M., S. Shimeno, H. Hosokawa, K. Hedetoshi and T. Kaisyo, 1975. The effect of dietary calorie-to-protein ratio on the growth, feed conversion, and body composition of young yellowtail. *Bull. Japan. Soc. Sci. Fish.* 41: 443–447.

Winfree, R.A. and R.R. Stickney, 1981. Effects of dietary protein and energy on growth, feed conversion efficiency and body composition of Tilapia aurea. *J. Nutr.* 111: 1001–1012.

Studies on the Energy Partitioning in Diet Consumed by Grass Carp, *Ctenopharyngodon idellus* (C. et Val.)*

LIN DING
CHEN YINGFENG
Ichthyology Laboratory, Zhongshan University, Guangzhou, 510275 PRC

DING, L. and C. YINGFENG, 1994. Studies on the energy partitioning in diet consumed by grass carp *Ctenopharyngodon idellus* (C. et Val.). In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines. Asian Fisheries Society, pp. 49–55.

Abstract

A model for partitioning of food energy within the body of grass carp is described. An experimental diet with crude protein, 28%; lipid, 5% and gross energy, 4600 kcal/kg diet (19.3 MJ/kg diet) was used and the intake energy, digestible energy, metabolizable energy and retained energy were measured, and a scheme for the partitioning of dietary energy for grass carp was developed. The results show that 30.5% of the dietary energy was lost in faeces, urine and gill excretions; 36.9% was oxidized to supply the energy needs of the fish and 32.6% was retained within the body.

Introduction

Fish require energy to sustain life processes. It is required for growth, reproduction, swimming and maintenance of vital physiological functions, and is derived from carbohydrate, protein and lipid in the diet. The most efficient utilization of feed is obtained when the diet contains an optimum balance of nutrients and an adequate level of dietary energy.

Bioenergetics is the study of the balance between energy supply in the food and energy expenditure, and requires examination of the physiological processes by which energy is transformed in living organisms. The energy available to fish is dependent upon the chemical composition of the diet, the digestibility of nutrients and the level of food intake. Providing the optimum energy level in diets is important because dietary energy exceeding the requirements can reduce growth rate or may cause excessive fat deposition.

In recent years several reports on the energy budget of salmonid fishes (Cho et al. 1982; Smith 1988) have been published but information on herbivorous fish is limited. Stanley (1974) attempted to determine an energy budget for large grass carp feeding on the aquatic macrophyte, but his estimates were not realistic.

Fischer (1972) made a comparison of energy budgets of grass carp fed with only plant and only animal food and the results showed that the growth rate was lower when the fish were fed plant food than animal. The energy budgets for the two types of foods were different. Fischer's (1973) work suggested that grass carp could assimilate both the types of food given, animal food being better assimilated than plant food.

* This study was supported by a research project from the International Development Research Centre (IDRC) of Canada.

Grass carp is an important species of food-fish. The culture of grass carp in ponds has a long history in China (Lin Ding and Mao Yongqing 1989). In recent years the yield of grass carp in some ponds has reached 7 500 kg/ha. One of the major reasons for this increase in production is the use of pelleted feed (Lin Ding 1991). Detailed studies on nutritional energetics for grass carp are needed, particularly in view of the expanding aquaculture interest in herbivorous fishes.

The present study was designed to develop a model for partitioning food energy utilized by grass carp from a diet containing highly digestible protein. It will provide a theoretical basis of energetics for a practical diet formulation for grass carp.

Materials and Methods

Experiment design

Grass carp were obtained from the fish culture experiment station of the Ichthyology Laboratory, Zhongshan University. The energy values of samples which included diets, faeces, carcasses and so on, were measured using an oxygen bomb calorimeter (model GR–2800). The experimental diet was formulated from semipurifed ingredients (Table 1, no. 1) and contained 28% crude protein, 4.28% fat, 7.29% crude fibre, 6.77% ash and fuel values of energy 4.6 kcal/g (19.32kJ/g).

Before the research the fish were kept in tanks, at a temperature of 22–23°C and were fed experimental diet no. 1. At the beginning of the experiment, grass carp were distributed into duplicate groups of 20 fish weighing 494–533 g. Fish were fed equal amounts of diet twice daily at 1000 and 1600 hrs at a rate of 3.5 to 4% of their body weight for 40 days.

Table 1. Composition of experimental and non-protein diets

Ingredient	No. 1 Test diet (%)	No. 2 Non-protein diet[1] (%)
Whole egg protein[2]	31.6	–
White dextrin	45.4	65.0
Alpha starch	–	20.0
Cellulose	8.0	5.0
Soybean oil	1.5	3.0
Fish liver oil	1.5	2.0
Vitamin mix[3]	2.0	1.0
Mineral mix[4]	8.0	4.0
Carboxymethyl cellulose (CMC)	2.0	–

1. Similar to Ogino et al. (1973)
2. Refer to Ogino et al. (1980)
3. Refer to Halver's formula
4. Refer to USP. XII No. 2 fortified with trace elements

The experiment was conducted in 250-L flow-through aquaria (100 × 50 × 50 cm) with a flow rate of approximately 12 L/min. The following water quality parameters were monitored during the study: temperature, 28°C ± 0.5°C; dissolved oxygen, 6–7 mg/L; carbon dioxide, 5–8 mg/L; ammonia (NH_4–N), 0.1–0.3 mg/L; and pH 7.4–7.6. The survival rate of fish was 100% during the experiment.

Intake of diet energy is the gross energy in the diet consumed. IE is the weight of diet consumed times the gross energy of a unit weight of diet. To determine energy digestibility in the present study we used the indirect method based on the concentration of chromic oxide (Cr_2O_3) used as a marker in the diet and in the faeces. Samples of diet and faeces were analyzed for energy and the marker. The digestibility coefficient of energy of the diet was calculated using the following equation:

$$\text{Digestibility coefficient} = 100 - \left(\frac{\%\ Cr_2O_3 \text{ in diet}}{\%\ Cr_2O_3 \text{ in faeces}} \times \frac{\%\ \text{GE in faeces}}{\%\ \text{GE in diet}} \times 100\right)$$

Digestible energy (DE) and metabolizable energy (ME) values were calculated using the following equations:

DE = IE – FE, where faecal energy (FE) is the gross energy content of faeces.

ME = IE – (FE + UE + ZE), where UE and ZE represent energy lost through urine and gills respectively.

Energy retention and growth was measured according to the method described by Cho et al. (1982). Grass carp were fed a nonprotein diet (Table 1) and metabolic faecal nitrogen and endogenous nitrogen excretion was measured in an apparatus (Figure 1) similar to those described by Ogino et al. (1973) with some modifications.

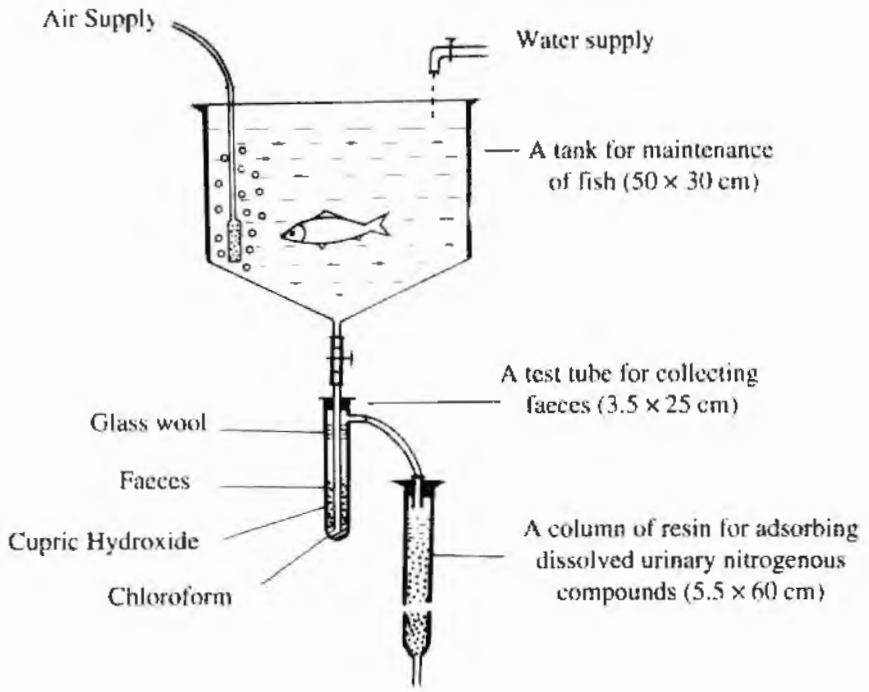

Figure 1. The apparatus used for the separation of faecal nitrogen and urinary nitrogen

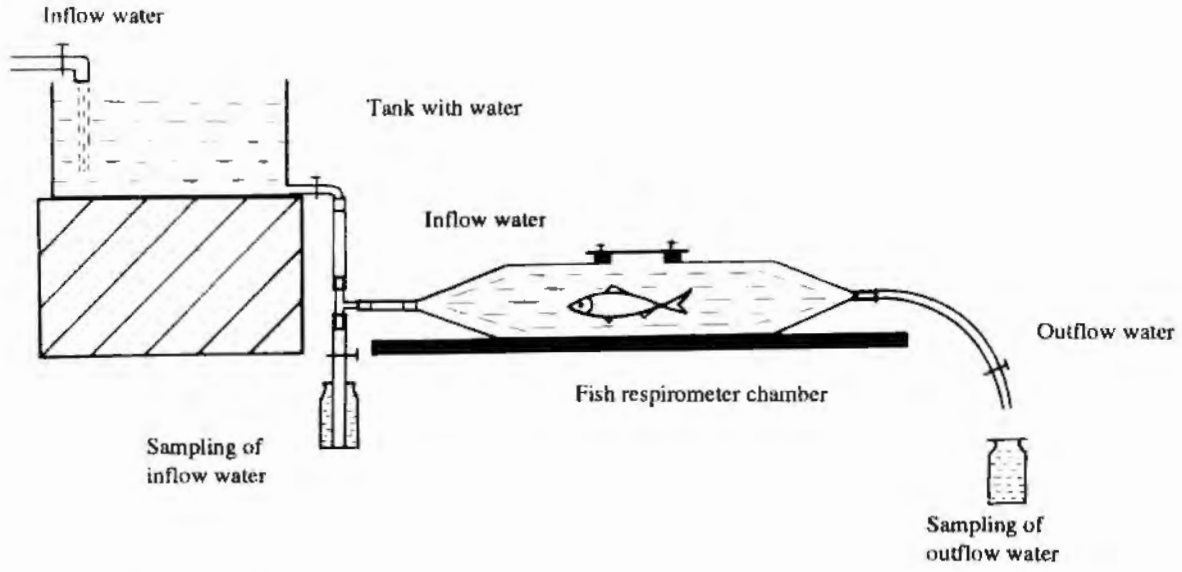

Figure 2. A respirometer used for measuring oxygen consumption rate of fish

Oxygen consumption is the preferred method for measuring and reporting metabolic rate in fish (Fischer 1970; Cho 1982). We designed a respirometer similar to that described by Fischer (1970) to measure oxygen consumption in grass carp (Figure 2). The experiment was carried out on five different size classes of grass carp in the weight range of 5 g to 180 g. While being measured fish were kept in a 3 L glass chamber (10 × 40 cm) with a constant monitored flow. Oxygen consumption of grass carp was measured by the modified Winkler's method in a constant flow (about 6 L per hr). The oxygen consumption by individual fish was measured from 0800 hrs to 1700 hrs at 3-hourly intervals.

The basal metabolism energy was determined by measuring the oxygen consumption rate and calculating heat production from the calorific equivalent value of oxygen consumed (3.3–3.5 kcal/g of oxygen or 13.8–14.7 kJ/g of oxygen). Oxygen consumption rate was calculated at

$$(\text{mg/kg BW/hr}) = \frac{(A_1 - A_2) \times V}{W}, \text{ where}$$

A_1 = the oxygen content of inflow water;
A_2 = the oxygen content of outflow water;
V = the content of running water through the respirometer per hour; and
W = body weight of test fish.

Results

Intake, digestible and retained energy

The intake energy and retained energy were determined for fish fed continuously on test diet no. 1. These results are presented in Tables 2 and 3. Table 2 shows that the intake energy and digestible energy are 19.80 MJ and 15.12 MJ/kg respectively. The digestible energy is 76.50% of intake energy and intake energy lost in faecal energy should be 23.5%.

Table 2. Energy intake and digestible energy content of a test diet measured with grass carp*

Experiment No	Diet intake (g)	Gross energy (MJ/kg)	Intake energy (MJ)	Digestible energy coefficient (%)	Digestible energy (MJ/kg)
1	1009	19.24	19.41	76.5	14.85
2	1045	19.24	20.11	76.5	15.38
Average	1027	19.24	19.76	76.5	15.12

* All results are expressed on a dry matter basis.

Table 3. Growth and energy retained by grass carp fed test diet*

Experiment No.	Initial weight (g)	Final weight (g)	Weight gain (g)	Gross energy content carcass Initial (MJ/kg)	Gross energy content carcass Final (MJ/kg)	Retained energy (RE) (MJ)	RE/IE (%)
1	124.5	368.1	243.6	19.3	24.4	6.58	33.9
2	134.4	362.8	228.4	19.4	24.6	6.30	31.3
Average	129.1	365.5	236.0	19.4	24.5	6.44	32.6
±SD	±5.0	±2.7	±7.6	±0.1	±0.1	±0.20	±1.3

* All results are expressed on a dry matter basis

The retained energy is that portion of the feed energy retained as parts of the body or voided as a useful product. In growing fish part of the retained energy is stored as protein and part as fat, which results in the weight gain of fish. In the current experiment using whole egg protein of a higher biological value in the diet greatly promoted protein deposition within the fish body. The result shows that retained energy reached 32.6% of intake energy.

The nitrogen balance of grass carp

The nitrogen balance was obtained by measuring the metabolic faecal nitrogen (Fo) and endogenous urine nitrogen (Uo) excretions when the fish were fed on a nonprotein diet. The results are listed in Table 4.

Table 4. The metabolic and endogenous nitrogen excretion of grass carp fed test diet

Body weight of grass carp	Metabolic nitrogen (Fo)	Endogenous Nitrogen (Uo)	Total excretion of nitrogen (Fo + Uo)
(g)		mg N/100 g B.W/day	
256.1 ± 50.5	3.44 ± 1.17	8.58 ± 2.48	12.02 ± 1.60

The nitrogen balance (Fo + Uo) for grass carp was 12.02 (mg N/100 g B.W/day). Multiplying the above data with the corresponding calorific values of nitrogen (kJ/gN), the nitrogen maintenance energy of 0.72 kJ was obtained for the grass carp's body weight. This figure is generally small and could be neglected in evaluation of digestible energy and metabolic energy.

Non-faecal excretion energy passed through the urine, gills and body surface is generally about 2–7% of intake energy (Brett et al. 1979; Cho et al. 1979). The present study refers to this data.

Basal metabolism

Oxygen consumption by grass carp was measured by placing different sized fish in a respirometer at 22.0 ± 1.0°C. The results showed a significant correlation between oxygen consumption (Y) and the body weight of the fish (X). Body weight and oxygen consumption were expressed by the following equation: $Y = 0.459\ X^{0.631}$ (Figure 3).

The oxygen consumption for 100 g body weight of grass carp calculated using this equation was 0.2 g O_2 per day. If oxygen consumption is converted to heat production, the oxygen caloric equivalent values of 13.8 kJ/g of O_2 consumed must be used when the dietary sources are protein and carbohydrate in the test diet in this experiment. Therefore, the basal metabolism of fish should be 2.76 kJ (0.2 g O_2 × 13.8 kJ/g O_2) per day. Consequently the total basal metabolism of grass carp measured in the growth experiment (Experiment 1) would be 1.32 MJ or 6.7% of the intake energy.

The partitioning of gross energy of diet consumed by grass carp

The energy intake of grass carp was divided among several functions within the fish body. On the basis of results obtained in this study, a summary of dietary energy utilization by grass carp is described in Figure 4. To illustrate the energy flow in grass carp we have used the energy balance equation I = M + G + E (I, energy ingested; M, metabolism; G, growth; E, excretion) to describe various parametrics:

$100I = 36.9M + 32.6G + 30.5E$

The energy budget shows that about one-third of the energy of the diet fed to grass carp was lost as combustible waste product. This included uneaten food, faeces, and urinary and gill excretions. One-third of the dietary energy was oxidized to supply the energy needs of the fish, and one-third was retained for fish growth. Figure 4 gives a breakdown of intake energy in various fractions. The values in parentheses are the percentages of intake energy.

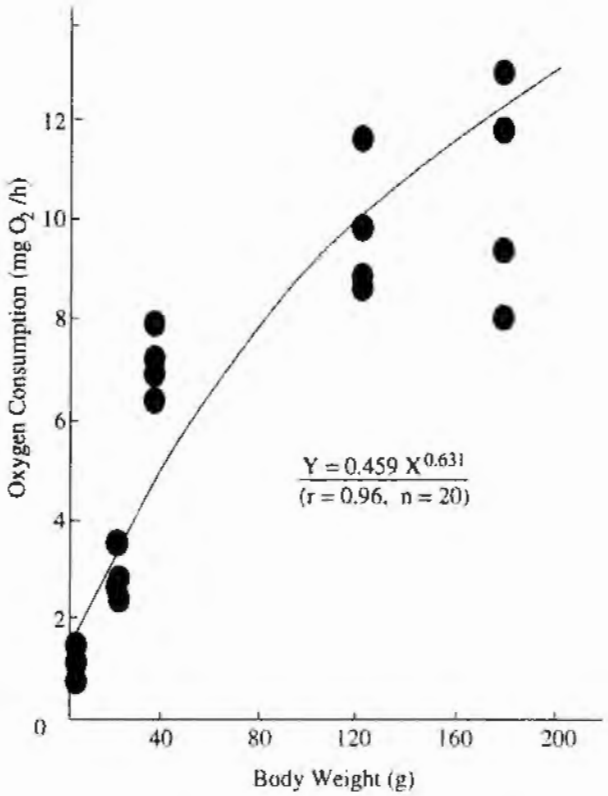

Figure 3. The relationship between body weight and oxygen consumption (basal metabolic rate) in grass carp

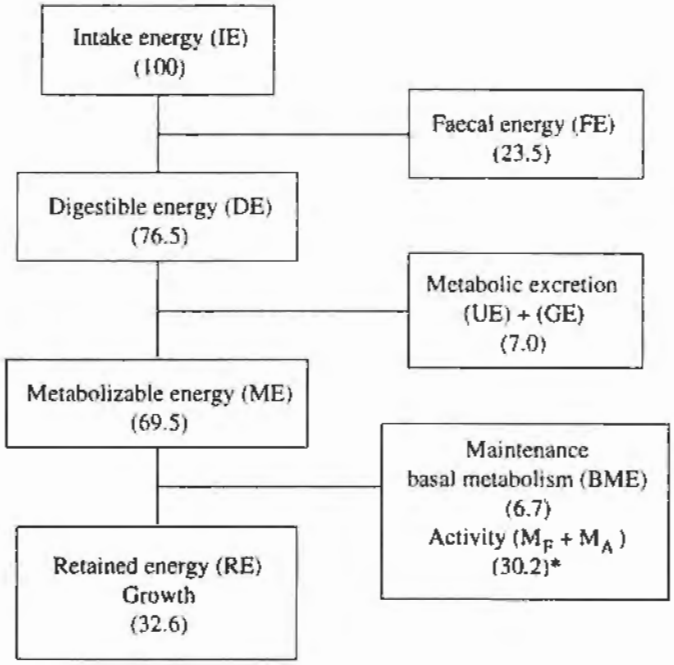

Figure 4. Partitioning of dietary energy for grass carp
* including feeding metabolism, heat increment

Discussion

The oxygen consumption–weight dependence has been widely studied in fish. It can be described by the general equation $Y = aW^b$, where Y is the oxygen consumption, W is the body weight and a is a constant which is dependent on species and temperature. Winberg (1961) gives a formula for all fish species: $Y = 0.307 \times W^{0.81}$. In the present experiment the following regression was obtained for grass carp fed with the test diet: $Y = 0.459 \times W^{0.63}$.

The exponent b deviated greatly from those obtained by Winberg (1961) and recommended by Brett and Groves (1979). However, it is similar to the results given by Fischer (1970) for grass carp. Fischer reported that the following regression was obtained for fish fed with animal food (*Tubificidae*): $Y = 0.300 \times W^{0.77}$ and for fish fed with plant food (*Lactuca saliva*): $Y = 0.487 \times W^{0.61}$. Therefore oxygen consumption is dependent on the ecological characteristic of the species.

Energy budgets for carnivorous fish are more readily available (Brett and Groves 1977; Cho et al. 1982; Smith 1989) than for herbivorous fish. Stanley (1974) attempted to determine an energy budget for large grass carp fed on the aquatic macrophyte *Egeria densa*. Although it was deduced that from a caloric intake of 96 kcal approximately 40 kcal was excreted, 74 kcal deposited in growth and 8 kcal expended in metabolism, there was no net gain in protein (quoted by Brett and Groves 1979). Fischer (1972) reported that when grass carp were fed only on lettuce (*Lactuca satira*) they were hardly able to maintain their body weight.

For fingerlings 40 to 120 g in weight an average energy budget equation at 22°C worked out to be 100I = 16M + 3G + 81E. However, considerable improvement in growth occurred when grass carp shifted to a diet of tubificids, obtaining an average equation of 100I = 23M + 17G + 60E. In our experiment we used whole chicken-egg powder as a major protein source in the diet, and grass carp obtained higher growth energy than in both of the studies mentioned above.

These results suggest that successful fish culture will depend on the provision of diets containing an appropriate balance of nutrients and adequate energy and protein to permit the most efficient growth. Therefore it is necessary to supply 3200 to 3500 kcal/kg of digestible energy in a diet containing 28% crude protein for maximum growth for juvenile grass carp.

References

Brett, J.R. and T.D.D. Groves, 1979. Physiological energetics. In Hoar et al. (eds) *Fish Physiology* Vol VIII. New York. Academic Press, pp. 279–352.

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. *J. Fish. Res. Board Canada* 21: 1183–1226.

Cho, C.Y. and S.J. Slinger. 1979. Apparent digestibility measurement in feedstuffs for rainbow trout. *Proc. World. Symp. On Finfish Nutrition and Fish Feed Technology. Vol. II.* Hamburg, pp. 239–347.

Cho, C.Y. and S.J. Kaushik, 1985. Effects of protein intake on metabolizable and net energy values of fish diets. In Cowey, C.B., A.M. Mackie and J.G. Bell (eds.) *Nutrition and Feeding in Fish.* London, Academic Press. pp. 95–117.

Cho, C.Y., S.J. Slinger and H.S. Bayley. 1982. Bioenergetics of salmonid fishes: Energy intake, expenditure and productivity. *Comp. Biochem. Physiol.* 73B (1): 25–41.

Elliott, J.M. 1976. The energetics of feeding, metabolism and growth of brown trout (*salmo trutta* L.) in relation to body weight, water temperature and ration size. *J. Anim. Ecol.* 45: 923–948.

Fischer, Z. 1970. The elements of energy balance in grass carp (*Ctenopharyngodon idella* Val.). Part I. *Pol. Arch. Hydrobiol.* 17: 421–434.

Fischer, Z. 1972. The elements of energy balance in grass carp (*Ctenopharyngodon idella* Val.) Part II. Fish fed with animal food. *Pol. Arch. Hydrobiol.* 19: 65–82.

Fischer, Z. and V.P. Lyakhnovicj, 1973. Biology and bioenergetics of grass carp (*Ctenopharyngodon idella* Val.) *Pol. Arch. Hydrobiol.* 20: 521–557.

Lin Ding and Yongqing Mao, 1989. Fish nutrition research in China. In Takeda M. and T. Watanabe (eds.) *The Current Status of Fish Nutrition in Aquaculture.* Toba, Japan. The Proceedings of the Third International Symposium on Feeding and Nutrition in Fish. pp. 91–98.

Lin Ding, 1991. Nutrient requirements of grass carp, *Ctenophyaryngodon idella* (C. et V.) In Wilson R.P. (ed.) *Handbook of Nutrient Requirements of Finfish.* CRC Press, London, pp 89–96.

Ogino, C., J. Kakin and M.S. Chen, 1973. Protein nutrition in fish–II. Determination of metabolic faecal nitrogen and endogenous nitrogen excretions of carp. *Bull. Japan. Soc. Sci. Fish.* 39 (5): 519–523.

Ogino, C., H. Kawasaki and H. Nanri. 1980. Method for determination of nitrogen retained in the fish body by the carcass analysis. *Bull. Japan. Soc Sci. Fish.* 46 (1): 105–109.

Smith, R.R. 1971. A method for measuring digestibility and metabolizable energy of feeds. *Prog. Fish.-Cult.* 33: 132–134.

Smith, R.R. 1988. Nutritional energetics. In Halver, J.E. (ed.) *Fish Nutrition.* New York, Academic Press, pp. 1–30.

Dietary Carbohydrate Utilization by Silver Barb, *Puntius gonionotus*

M.A.B. HABIB[1]
M.R. HASAN[2]
A.M. AKAND[3]

[1&2] *Department of Fisheries Biology and Limnology,*
[3] *Department of Aquaculture and Management*
Bangladesh Agricultural University,
Mymensingh 2202 Bangladesh

HABIB, M.A.B., M.R. HASAN and A.M. AKAND, 1994. Dietary carbohydrate requirement of silver barb, *Puntius gonionotus*. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ 9. Manila, Philippines, Asian Fisheries Society, pp. 57–62.

Abstract

A feeding trial was conducted to evaluate the utilization of carbohydrate (CHO) as dietary energy sources by silver barb (tawes), *Puntius gonionotus*. Eight isonitrogenous diets were formulated from purified ingredients with eight different levels of carbohydrate (0, 6, 12, 18, 24, 30, 36 and 42%). Replicate groups of fishes were fed at the rate of 3% of their biomass daily for eight weeks in 15 L aquaria. Based on specific growth rate (SGR), weight gain (%), food conversion ratio (FCR) and protein efficiency ratio (PER), it was observed that the silver barb (*P. gonionotus*) fry grew best on the diet containing 30% dextrin with P/E ratio of 73.8.

Dietary level had significant ($p<0.05$) effect on weight gain, SGR and PER of growth parameters. Analysis of correlation coefficient (R) indicates that the dietary carbohydrate (dextrin) directly correlated with weight gain (%), SGR and PER and inversely correlated with the FCR. Carbohydrate-free diet resulted in reduced carcass crude protein and lipid content and increased moisture and ash contents. The intracorrelation values in growth parameters were all strongly ($p<0.01$) correlated. In the case of intracorrelations in carcass composition, moisture was found to be strongly ($p<0.01$) and inversely correlated with carcass crude protein ($r = -0.857$).

Introduction

The silver barb (tawes), *Puntius gonionotus*, which is locally known as sharpunti, was introduced to Bangladesh about five years ago. It is now a popular farmed fish, preferred for its fast growth and delicacy. However, farmers are facing problems in its culture because of the non-availability of complete feed. It appears that there is some information available on its life history (Sipitakkiat and Leenannond 1984), but there is no information available about the basic nutrient requirements of this fish except for a report on protein requirement (Wee and Ngamsnae 1987).

Information on nutritional requirements of major dietary components such as protein and energy is the prerequisite for the formulation of a least costly and balanced diet for fish. Carbohydrate is one of the major dietary components as it not only supplies the necessary energy but also has a protein-sparing effect in fish. The present study was undertaken to determine the optimum dietary requirement of carbohydrate level for silver barb, *P. gonionotus*.

Materials and Methods

Eight isonitrogenous diets were formulated using casein as the source of protein with eight levels (0, 6, 12, 18, 24, 30, 36 and 42%) of carbohydrate (dextrin) (Table 1). The experimental diets contained a crude protein level of approximately 27.0 to 28.0% and a gross energy level of 2.44 to 4.16 kcal/g. Purified dietary ingredients were mixed and pelleted using a meat grinder. The pellets were dried in an oven at 40°C and stored in a refrigerator at 6°C. The fish were fed twice a day at 1000 and 1700 hrs at the rate of 3% of body weight per day. Fish were weighed every week to determine the ration for each tank. The weighing of fish during and on termination of the experiment was as described by Hasan et al. (1989).

Table 1. Composition of test diets (%)

	Diet No.							
Ingredients	1	2	3	4	5	6	7	8
Casein	35	35	35	35	35	35	35	35
Dextrin	0	6	12	18	24	30	36	42
Lipid[1]	5	5	5	5	5	5	5	5
Mineral mixture[2]	4	4	4	4	4	4	4	4
Vitamin mixture[2]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chromic oxide	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Alpha Cellulose	52	46	40	34	28	22	16	10
Carboxymethyl Cellulose	3	3	3	3	3	3	3	3
Nutrient contents								
Crude protein (%)	27.9	27.6	27.7	27.9	27.1	27.0	28.0	27.3
Crude lipid (%)	4.9	4.8	4.7	4.8	4.8	4.8	4.8	4.9
Gross energy (kcal/100g)	2.44	2.68	2.93	3.17	3.42	3.66	3.91	4.16

[1] Mixture of soybean oil : cod liver oil (2:1)
[2] After Akand et al. (1989)

Twenty-day old induced-bred fry of *Puntius gonionotus* were used in the diet. Fry were maintained on a pelleted artificial feed until used in the experiment. Prior to the initiation of the feeding trial, fish were acclimated to test diets for one week. Each test diet was fed to duplicate groups of fish for eight weeks. Ten fish were stocked in 15 L glass aquaria, provided with aeration. Aquaria were cleaned and refilled with fresh water every morning.

Proximate composition of fish and feed samples were analyzed by the methods of the Association of Official Analytial Chemists (1970). Gross energy of the diets was calculated using kilocaloric values of 5.6/g protein, 9.5/g lipid and 4.1/g carbohydrate (CHO) (Cho et al. 1982). Specific growth rate ($\log_e$ final body weight – $\log_e$ initial body weight/time × 100), food conversion ratio (dry food intake/live weight gain), protein efficiency ratio (live weight gain/protein intake) and survival rate (initial no. of fish/final no. of fish × 100) were calculated.

Variations in weight gain (%), SGR, GCR and PER, and body lipid (%), protein (%), ash (%) and moisture (%) after feeding the test diets were analysed by One Way ANOVA and their mean differences by least significant difference (LSD). Correlations of the proximate composition of feed and carcass composition of the fish were calculated by simple regression analysis.

Results

During the feeding trial the fish readily accepted the diets and no food remained at the bottom of the aquaria. Mortality of fish in all dietary groups was less than 5%. The growth response of the fish under various dietary treatments is presented in Table 2. Growth response of fish in terms of weight gain (%) and specific growth rate varied significantly among the dietary treatments. Weight gain (%) was highest in fish fed diet no. 6 followed by diets no. 5, 7 and 8, respectively.

Table 2. Average initial and final weight, weight gain, Specific Growth Rate (SGR), Food Conversion Ratio (FCR), Protein Efficiency Ratio (PER) and survival rate (%) of fish fed various experimental diets

Diet No.	Initial wt (g)	Final wt. (g)	Weight gain (%)	SGR	FCR	PER	Survival (%)
1	0.55	1.00	80.2de	1.05d	1.78a	0.78e	5
2	0.56	1.04	87.1ce	1.12c	1.65bc	0.84ce	5
3	0.56	1.06	90.1ce	1.15c	1.67b	0.92c	0
4	0.56	1.09	96.6c	1.21c	1.64bc	0.93c	5
5	0.56	1.21	115.3b	1.37b	1.63bc	1.11b	0
6	0.57	1.41	147.8a	1.62a	1.32d	1.38a	0
7	0.58	1.25	115.2b	1.37b	1.60ce	1.09b	0
8	0.57	1.21	112.3b	1.35b	1.58c	1.04b	0

Their significant differences were done after LSD test.
Means followed by the same letter are not significantly ($p<0.05$) different

Similar results were obtained when the growth parameter was expressed in terms of specific growth rate (SGR) (Table 2). SGR varied between 1.05 and 1.62 among dietary groups. The FCR ranged from 1.32 to 1.78 and was found to be significantly different among the groups. FCR reflected the same trend as weight gain (%) and SGR. The PER showed a similar response to those of weight gain (%) and SGR. PER, which ranged between 0.78 and 1.38 was significantly ($p<0.05$) different among the treatment groups.

The proximate composition of fish after feeding the test diets for 56 days is shown in Table 3. After the feeding trial the carcass protein and lipid contents increased and that of ash contents decreased among the fish in all dietary groups. The contents of carcass protein, lipid and ash were found to be significantly different among the treatment groups. With the increase of dietary carbohydrate level, the body lipid content was found to increase in all dietary groups. This is also evident from the correlation coefficient analysis of dietary carbohydrate level with body composition (Table 4). The protein and lipid contents were found to be significantly ($p<0.05$) higher in fish fed diet no. 6 than those fed other diets (Table 3). There was no significant difference in the protein content of fish fed diets no. 1 and 4, and 5 and 7. Similarly there was no difference in the protein content of fish fed diets no. 2, 5 and 7.

Table 3. Average carcass lipid, protein, ash and moisture (% fresh weight basis) content of fish fed various experimental diets

Diet No.	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
Initial	76.87	15.23e	2.18h	3.83a
1	75.12	17.35b	3.46g	2.46e
2	76.97	16.02d	3.61f	2.35h
3	76.82	16.56c	3.72c	2.50d
4	75.69	17.47b	3.66e	2.40g
5	76.65	16.16d	3.69d	2.62b
6	75.26	17.69a	3.85a	2.43f
7	76.71	16.21d	3.82b	2.58c
8	75.69	17.45b	3.72c	2.52d

Means followed by the same letter in each column are not significantly ($p<0.05$) different.

Table 4. Correlation coefficients (r) of proximate composition and carbohydrate content of feed with body weight gain (%), SGR, FCR and PER of fish

	Weight gain (%)	SGR	FCR	PER
Moisture	0.342	0.214	−0.264	0.301
Crude protein	−0.444	−0.429	0.481*	−0.486*
Crude lipid	0.194	0.143	−0.125	0.227
Carbohydrate	0.635**	0.626**	−0.318	0.533*

df =16, *$p < 0.05$ and **$p < 0.01$

The correlation values of weight gain (%), SGR, FCR and PER with dietary carbohydrate level showed that dietary carbohydrate had significant ($p<0.01$) correlations with weight gain per cent ($r = 0.635$, $p<0.01$), SGR ($r = 0.626$, $p<0.01$) and PER ($r = 0.533$, $p<0.05$) (Table 5). The direct and residual effects of proximate composition of feed on the growth parameters of fish is shown in Table 5. Residual effect showed that there were some effects other than proximate composition of feed on the growth of *P. gonionotus*.

Table 5. Direct and residual effects of proximate composition of feed on the growth parameters of *P. gonionotus*

	Direct effect				Residual effect
	Moisture	Crude protein	Crude lipid	Ash	
Weight gain (%)	0.253	-0.454	0.077	0.554	0.588
SGR	0.095	-0.397	-0.068	0.668	0.599
FCR	-0.325	0.552	-0.165	-0.013	0.791
PER	0.245	-0.515	0.109	0.429	0.639

Discussion

On the basis of the weight gain (%), SGR, FCR and PER of fish, it is estimated that 30% dietary carbohydrate (dextrin) is required for maximum growth of silver barb, *P. gonionotus*. The results presented in Tables 2 and 4 indicate that the inclusion of dietary carbohydrate (dextrin) had a positive effect on the growth of silver barb. The weight gain (%), and SGR increased with an increased dietary carbohydrate level from 0 to 30% and declined thereafter at higher levels of carbohydrate (diets no. 7 and 8). The decrease in weight gain (%) and specific growth rate may be due to the higher energy content in the diets (Page and Andrews 1973; Daniels and Robinson 1986). An inverse relationship between growth and dietary energy was reported by Daniels and Robinson (1986) in juvenile red drum (*Sciaenops ocellatus*). Thirty per cent inclusion of dietary carbohydrate seems to be high in comparison to the salmonids which need a much lower amount. Dietary carbohydrate levels of 12% and 20% are recommended for trout (Phillips et al. 1948) and chinook salmon (Buhler and Halver 1961), respectively, as higher amounts usually retard the growth (Austreng et al. 1977). The requirement of a comparatively high amount of dietary carbohydrate (30%) for maximum growth of silver barb may be due to the herbivorous nature of this fish. Herbivorous fish can metabolize carbohydrate better than carnivorous species (Shimeno et al. 1979; Cowey and Sargent 1979; and Furuichi and Yone 1981).

The values of SGRs and FCRs are good at protein/energy ratio of 10.41. This value is a little higher than the value (8 to 9) recommended for channel catfish (NRC 1983) but comparable to the values reported by Akand et al. (1989 and 1991) for stinging catfish, *Heteropneustes fossilis*, and Hasan et al. (1989) for Asian catfish, *Clarias batrachus*. PER values (Table 2) showed a trend similar to SGRs. The values of PERs are high with diets containing 24% and 36% carbohydrate. Low PER values in the fish fed diets no. 1, 2, 3, 4 and 8 may be due either to inadequate or to excess dietary energy (Daniels and Robinson 1986).

The significant correlation of dietary carbohydrate with weight gain (%), SGR, FCR and PER indicates that dietary carbohydrate is essential for better food conversion, protein utilization and maximum growth of silver barb. Direct correlation of weight gain (%) of fish with SGR and PER, and inverse correlation of the same with FCR are usual in fish (De Silva et al. 1989, and Akand et al. 1991).

Although the carcass protein and lipid contents increased after feeding the test diet, there was no appreciable change in body composition of the fish following the treatments. Deposition of high body lipid contents in the fish fed higher amounts of carbohydrate (diets no. 6, 7 and 8) may be due to the availability of enough energy in those diets. Fatty carcass of fish at higher dietary carbohydrate levels were also reported by Wee and Ng (1986). Inverse relationship of body lipid content with protein and moisture contents is a common phenomenon in fish and comparable to the results of Stansby and Olcott (1976). Based on the results of the present investigation, it is estimated that the dietary carbohydrate requirement of *P. gonionotus* is about 30% at 29°C when dextrin is the source of carbohydrate.

Acknowledgements

The authors are grateful to the International Foundation for Science (IFS), Stockholm, Sweden for the partial facilities to conduct this research from Project No. A–1589/1 on 'Culture of *Clarias batrachus* by formulated feed' donated by IFS to the first author. The first two authors gratefully acknowledge the International Development Research Centre (IDRC), Canada for facilitating their participation in the Fifth Asian Fish Nutrition Network Workshop at Udorn Thani, Thailand.

References

Akand, A.M., M.I. Mia and M.M. Hague. 1989. Effect of dietary protein level on growth, food conversion and body composition of shingi (*Heteropneustes fossilis*) (Bloch). *Aquaculture* 77: 175–180.

Akand, A.M., M.R. Hasan and M.A.B. Habib, 1991. Utilization of carbohydrate and lipid as dietary energy sources by stinging catfish, *Heteropneustes fossils* (Bloch). In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop*, Asian Fish. Soc. Spec. Publ. 5. Manila, Philippines, Asian Fisheries Society, pp. 93–100.

Association of Official Analytical Chemists, 1970. *Official Methods of Analysis*. Washington, D.C., AOAC, 1015 p.

Austreng, E., S. Risa, D.J. Edwards and H. Hvidsten, 1977. Carbohydrate in rainbow trout diets. II. Influence of carbohydrate levels on chemical composition and feed utilization of fish from different families. *Aquaculture* 11: 39–50.

Buhler, O.R. and J.E. Halver, 1961. Nutrition of salmonid fishes. IX. Carbohydrate requirements of chinook salmon. *J. Nutr.* 74: 307–318.

Cho, C.Y., S.J. Slinger and H.S. Bayley, 1982. Bioenergetics of salmonid fishes, energy intake, expenditure and productivity. *Comp. Biochem. Physiol.* 73B: 25–41.

Cowey, C.B. and J.R. Sargent, 1979. Nutrition. In Hoar, W.S., D.J. Randall and J.R. Brett (eds.) *Fish Physiology, Vol. VIII*. New York, USA, Academic Press, pp. 1–69.

De Silva, S.S., R.M. Gunasekera and D. Atapattu, 1989. Dietary protein requirement of young tilapia and evaluation of least cost dietary protein levels. *Aquaculture* 80: 271–284.

Daniels, W.H. and E.H. Robinson, 1986. Protein and energy requirements of juvenile red drum (*Sciaenops ocellatus*). *Aquaculture* 53: 243–252.

Furuichi, M. and Y. Yone, 1980. Effect of dietary dextrin levels on the growth and feed efficiency, and the chemical composition of liver and dorsal muscle, and the absorption of dietary protein and dextrin in fishes. *Bull. Japan. Soc. Sci. Fish.* 46: 225–229.

Hasan, M.R., M.G. Alam and M.A. Islam, 1989. Evaluation of some indigenous ingredients as dietary protein sources for the catfish (*Clarias batrachus*, Linnaeus) fry. In: Huisman, E.A., N. Zonneveld and A.H.M. Bouwmans (eds.) *Aquaculture Research in Asia: Management Techniques and Nutrition*. Wageningen, Pudoc Press, pp. 125–137.

National Research Council, 1983. *Nutritional Requirements of Warmwater Fishes and Shellfishes*. Washington D.C., National Academic Press, pp. 2–38.

Page, J.W. and J.W. Andrews, 1973. Interactions of dietary levels of protein and energy on channel catfish (*Ictalurus punctatus*). *J. Nutr.* 103: 139–149.

Phillips, A.M., A.V. Tunison and O.R. Brockway, 1948. *The Utilization of Carbohydrates by Trout*. Fisheries Research Bulletin (NY Conservation Department), USA No. 11, 44 p.

Shimeno, S., H. Hosakawa and M. Takeda, 1979. The importance of carbohydrate in the diet of a carnivorous fish. In Halver, J. and K. Tiews (eds.) *Proceedings of a World Symposium on Finfish Nutrition and Fishfood Technology, Vol. I*. Hamburg, 20–23 June, 1978. Berlin, Germany, Heeneman, pp. 127–143.

Sipitakkiat, P. and Y. Leenannond, 1984. *Life History and Culturing of Pla Ta Pien Khao, (Puntius gonionotus*, Bleeker). Technical Paper No. 39, Bangkok, Thailand, National Inland Fisheries Institute, Freshwater Division, Dept. of Fish., 51 p.

Stansby, M.E. and H.S. Olcott, 1976. Composition of fish. In *Industrial Fishery Technology*. Huntington, N.Y., Robert E. Krieger Pub. Com.

Wee, K.L. and L.T. Ng, 1986. Use of cassava as an energy source in a pelleted feed for the tilapia, *Oreochromis niloticus* L. *Aquaculture and Fisheries Management* 17: 129–138.

Wee, K.L. and P. Ngamsnae 1987. Dietary protein requirement of fingerlings of the herbivorous carp tawes, *Puntius gonionotus* (Bleeker). *Aquaculture and Fisheries Management* 18(2): 121–129.

Leaf Meals as Protein Sources in Diets for Milkfish, *Chanos chanos* (Forsskal)

I.G. BORLONGAN
R.M. COLOSO
Aquaculture Department
Southeast Asian Fisheries Development Center
P.O. Box 256, Iloilo City
Philippines

BORLONGAN, I.G. and R.M. COLOSO, 1994. Leaf meals as protein sources in diets for milkfish, *Chanos chanos* (Forsskal). In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop*. Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 63–68.

Abstract

The potential of partial replacement of fish meal protein with protein from indigenous leaf meals in practical diets for milkfish, *Chanos chanos* (Forsskal) was studied. Five isocaloric (375 kcal/100 g diet), isonitrogenous (40% protein), and isolipidic (10%) diets were formulated to contain leaf meals from either swamp cabbage (kangkong, *Ipomea reptans*), sweet potato (kamote, *Ipomea batatas*), ipil-ipil (*Leucaena leucocephala*), and cassava (kamoteng kahoy, *Manihot esculenta*), or a combination of swamp cabbage, sweet potato and cassava.

The control diet contained fish meal and soybean meal as sources of protein while the test diets contained fish meal, soybean meal, and leaf meals replacing 15% of the fish meal protein. The protein sources were incorporated at levels that gave optimal essential amino acid patterns to the diets. Each diet was fed to triplicate groups of fish (about 0.3 g) maintained at 20 ppt salinity and 29°C in a recirculating system for twelve weeks. Growth, feed conversion ratio (FCR), protein efficiency ratio (PER), and survival of fish fed the various diets were not significantly different from those of the control fish. However, fish fed the diet containing cassava leaf meal showed the best growth, FCR, PER and survival. The data suggest that these leaf meals can be used to partially replace fish meal in a diet for juvenile milkfish if the requirements for essential amino acids are met.

Introduction

The intensification of milkfish culture requires the development of supplementary or complete feeds. Fish meal continues to be the major protein source for fish feed. However, the rising cost, uncertain availability, and fluctuating quality of fish meal has led to the search for alternative protein sources for fish feed to sustain fish production. The use of plant protein sources to completely or partially replace fish meal in fish diets has been studied for many years (Jackson et al. 1982; Tacon et al. 1984; Viola et al. 1984; Wee and Wang 1987; and Wee 1991).

Various leaf meals which are potential protein sources for fish feed are available in the Philippines. These include swamp cabbage or kangkong (*Ipomea reptans*), sweet potato or kamote (*Ipomea batatas*), ipil-ipil (*Leucaena leucocephala*), and cassava or kamoteng kahoy (*Manihot esculenta*). Some have been evaluated as dietary protein sources for other fish species such as Nile tilapia (Wee and Wang 1987; Santiago et al. 1988; Ng and Wee 1989) and Tilapia Mossambique (Jackson et al. 1982). However, there is a scarcity of information on the use of these leaf meals as dietary protein sources for milkfish.

The present study was conducted to evaluate the suitability of indigenous leaf meals as a partial substitute for fish meal in diets for milkfish, *Chanos chanos* (Forsskal) juveniles. The main consideration in the design of the study is for the diets to be balanced with respect to the essential amino acid requirement of juvenile milkfish. Thus, in the formulation, the maximum inclusion level of each of the leaf meals that would give an amino acid profile similar to the amino acid requirement of milkfish was determined.

Materials and Methods

Preparation of leaf meals

The leaves of swamp cabbage, sweet potato, ipil-ipil, and cassava were removed from the stems or stalks of the plant, soaked in water for 24 hours, rinsed, and dried in an air convection oven at 60°C. The leaves were then ground in a Cyclotec grinder (Tecator, Hoganas Sweden) through a 60-mesh screen, and subsequently used as feed ingredient. The proximate and amino acid compositions of the various leaf meals used in the formulation of the experimental diets are given in Table 1.

Table 1. Proximate and essential amino acid composition of the various leaf meals used in the experiment

	Swamp Cabbage	Sweet Potato	Ipil-ipil	Cassava
Proximate Composition (% dry weight)				
Crude protein	27.5	23.2	31.9	23.0
Crude fat	3.0	3.8	5.1	8.6
Crude fibre	11.6	11.1	14.5	15.7
Ash	13.2	11.6	5.0	5.5
Nitrogen-free extract	44.7	50.3	43.5	47.2
Essential Amino Acids (g/100 g protein)[1]				
Arginine	3.31	3.70	5.25	2.10
Histidine	2.66	2.83	1.44	2.19
Isoleucine	3.42	3.70	6.65	5.15
Leucine	6.55	7.90	6.65	4.42
Lysine	4.56	4.39	6.07	6.65
Methionine	1.53	1.82	1.19	1.23
Phenylalanine	5.67	6.47	3.92	4.51
Threonine	3.92	4.45	5.07	2.60
Tryptophan	ND	ND	ND	ND
Valine	5.27	5.85	6.29	3.51
Tyrosine	4.14	6.53	3.45	3.74
Cystine	0.51	0.26	0.63	0.77

[1] Peñaflorida, 1989; ND = not determined

Experimental Diets

The formulation and proximate composition of the experimental diets are presented in Table 2. All diets were formulated to contain 40% protein, 10% lipid, and energy of about 375 kcal/100 g diet. The control diet (diet no. 1) contained fish meal and soybean meal as protein sources. In diets no. 2–5, 15% of the fish meal protein was replaced singly by the different leaf meals while in diet no. 6, the 15% replacement of fish meal protein came from (1:1:1) combination of kangkong, kamote, and cassava.

All test diets were balanced to match the essential amino acid requirement of milkfish juveniles (Borlongan and Coloso, in press) in a 40% protein diet (Table 3). Because of the restriction on protein and amino acid levels, as well as the limiting amino acid content of the leaf meals, the maximum amount of leaf meals incorporated in the diet ranged from 20–27% of the diet. This level of incorporation in the diet is equivalent to about 15% of crude protein. Cod liver oil and breadflour were used as lipid and carbohydrate sources respectively while vitamin and mineral premixes were kept constant in all diets. In addition, breadflour also served as a binder.

Table 2. Composition of experimental diets

Ingredient	Diet No. 1	2	3	4	5	6
	g/100 g diet					
Peruvian Fish meal	53.12	43.75	43.75	43.75	43.75	43.75
Defatted Soybean meal	14.63	14.63	14.63	14.63	14.63	14.63
Swamp cabbage leaf meal	–	23.10	–	–	–	7.69
Sweet potato leaf meal	–	–	27.27	–	–	9.09
Cassava leaf meal	–	–	–	27.27	–	9.09
Ipil-ipil leaf meal	–	–	–	–	20.00	–
Cod liver oil	5.48	5.59	5.27	4.03	5.29	4.96
Vitamin mix[1]	3.00	3.00	3.00	3.00	3.00	3.00
Mineral mix [1]	2.00	2.00	2.00	2.00	2.00	2.00
Butylated hydroxyanisole	0.05	0.05	0.05	0.05	0.05	0.05
Celufil	5.40	–	–	–	–	–
Breadflour	16.32	7.88	4.03	5.27	11.28	5.74
Proximate Analysis (% of dry matter)						
Crude protein	41.2	40.8	40.9	40.7	41.1	40.3
Crude fat	10.2	10.3	10.4	10.5	10.5	10.3
Crude fibre	7.9	6.8	6.8	7.1	7.3	7.2
Ash	10.2	11.5	11.8	10.6	10.4	11.6
Nitrogen-free extract	30.5	30.6	30.1	31.1	30.7	30.6
Estimated energy (kcal/100g diet)[2]	378.6	378.3	377.6	381.7	381.7	376.3

[1]Borlongan and Benitez 1990
[2]Computed based on standard physiological fuel value of 9 kcal g^{-1} for fat and 4 kcal g^{-1} for protein and carbohydrate

Table 3. Calculated level of amino acid in the experimental diets and essential amino acid requirement of milkfish in a 40% protein diet

Amino Acids	Diet 1	2	3	4	5	6	Requirement Level[1]
Arg	2.24	2.11	2.13	2.04	2.20	2.09	2.10 ± 0.10
His	0.80	0.85	0.86	0.82	0.78	0.84	0.80 ± 0.08
Ile	1.67	1.64	1.65	1.74	1.83	1.67	1.68 ± 0.20
Leu	2.83	2.80	2.88	2.68	2.81	2.79	2.04 ± 0.22
Lys	2.85	2.68	2.67	2.81	2.77	2.72	1.60 ± 0.24
Met + Cys	1.42	1.32	1.33	1.32	1.31	1.33	1.30 ± 0.12
Phe + Tyr	2.87	3.04	3.22	2.94	2.90	2.91	2.73 ± 0.18
Thr	1.57	1.57	1.60	1.49	1.63	1.55	1.60 ± 0.18
Trp	0.27	0.23	0.23	0.23	0.23	0.23	0.24 ± 0.08
Val	2.04	2.05	20.8	1.94	2.11	2.03	1.42 ± 0.24

[1] Based on Borlongan and Coloso, in press. Values are expressed as g/100 g dry diet ± SE

Diets were prepared by first mixing all dry ingredients except breadflour, in a Hobart mixer. Cod liver oil (with BHA added) was then blended with the dry ingredient mixture. The breadflour was gelatinized and cooked in 600 mL water and added to the mixture. The semi-moist mixture was then passed through the Hobart food grinder to form 2 mm diameter pellets. The pellets were dried in an air convection oven at 40°C. The dry pellets were then ground, sieved to uniform sizes, and stored at 4°C.

Experimental fish and feeding

Hatchery-bred milkfish fingerlings (0.30–0.40 g) were acclimated for two weeks to 20 ppt salinity and to a dry diet (40% protein) prior to the experiment. The fish were reared in a series of 24 oval, fibreglass tanks (60 L capacity), using a recirculating brackishwater (20 ppt) system. Fish were randomly distributed among tanks at 10 fish per tank. Triplicate tanks per treatment were arranged at random.

The fish were fed twice daily at 0900 hrs and 1300 hrs with half of the daily ration given at each feeding. The feeding rate was 15% of body weight per day for the first six weeks and was reduced to 10% body weight per day for the last six weeks. Fish were weighed and feeding rations were adjusted accordingly at three-week intervals. The experiment lasted for twelve weeks.

The tanks were cleaned daily using a siphon to remove the accumulated faecal matter. Tanks were scrubbed and water quality monitored once a week. The water quality parameters (pH, ammonia–nitrogen, nitrite–nitrogen and dissolved oxygen) recorded during the trial were within acceptable ranges.

Chemical analysis

All feed ingredients and experimental diets were analyzed for proximate composition. Moisture content was determined using a moisture determination balance (Mettler LP15). Crude protein, crude fat, and crude fibre were determined using the Tecator analyzer systems (Tecator, Hoganas, Sweden). Ash was obtained by drying in a furnace at 550°C. Nitrogen free extract (NFE) was calculated from the difference between 100 and content of protein, fat, fibre and ash. Amino acid data on leaf meals and other feed ingredients were derived from Peñaflorida (1989).

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Differences between means were considered significant at $p<0.05$.

Results and Discussion

During the experiment, fish consumed the feed well and the acceptability of all diets was similar. Data for growth, feed conversion ratio (FCR), protein efficiency ratio (PER), and survival are shown in Table 4. Fish fed diet no. 4 containing cassava leaf meal gave the best growth and survival, but this was not significantly different from the other treatments, including the control. Values for FCR and PER also did not significantly differ among the treatments. Results show that leaf meals used in this study could partly substitute for fish meal provided that the essential amino acid requirements of the fish are met and the leaf meals properly processed.

Table 4. Response of milkfish juveniles to the various test diets[1]

Diet No.	Weight Gain (%)	SGR[2] (%/d)	Survival (%)	FCR[3]	PER[4]
1	751.5 ± 28.7[a]	3.06 ± 0.05[a]	67 ± 12[a]	1.6 ± 0.2[a]	1.58 ± 0.2[a]
2	771.9 ± 118.8[a]	3.07 ± 0.22[a]	70 ± 10[a]	1.5 ± 0.4[a]	1.73 ± 0.4[a]
3	755.2 ± 24.8[a]	3.06 ± 0.04[a]	67 ± 6[a]	1.6 ± 0.2[a]	1.58 ± 0.2[a]
4	800.6 ± 88.8[a]	3.13 ± 0.13[a]	87 ± 23[a]	1.4 ± 0.2[a]	1.81 ± 0.3[a]
5	764.4 ± 117.5[a]	3.07 ± 0.19[a]	77 ± 25[a]	1.6 ± 0.4[a]	1.60 ± 0.4[a]
6	755.2 ± 8.6[a]	3.07 ± 0.02[a]	73 ± 15[a]	1.6 ± 0.2[a]	1.58 ± 0.2[a]

[1] Values are means ± SEM. Values in columns having the same superscript are not significantly different at $p<0.05$.

[2] Specific growth rate (SGR) $= \frac{\ln(\text{final wt.} - \text{initial wt.})}{84 \text{ days}} \times 100$

[3] Feed Conversion Ratio (FCR) = total dry feed fed (g)/total wet weight gain (g)

[4] Protein Efficiency Ratio (PER) = wet weight gain (g)/amount of protein fed (g)

One of the factors which limits the incorporation of plant protein sources in fish diets is the presence of anti-nutritional factors. Some processing techniques can be used to remove or reduce the anti-nutritional factors and increase the bioavailability of nutrients. Soaking and drying the leaves prior to use has been shown to reduce the potency of toxic factors present in them. For instance, the mimosine content of ipil-ipil can be reduced by soaking in water for 24–48 hours (Wee and Wang 1987; Peñaflorida et al. 1992). Soaking and drying of cassava leaves also removes any hydrocyanic acid that may have accumulated (Ng and Wee 1989). In sweet potato

leaves, tannins and low levels of trypsin inhibitor may also be eliminated by soaking and drying (Lin and Chen 1985).

With available information on the essential amino acid requirements of milkfish (Borlongan and Coloso, in press) it was possible to find the optimum ratio of fish meal protein to leaf meal protein in the feed intended as a complete diet. At 15% level of replacement, all the essential amino acids were adequately provided. Higher replacement levels would result in deficiency in some essential amino acids and would require supplementation with crystalline amino acids particularly methionine, threonine and tryptophan.

The lack of information on the digestibility of various leaf meals in milkfish has made us assume these values to be more or less equal for all the leaf meals, a limitation in the present study. Studies to determine the digestibility coefficients of various leaf meals for milkfish are necessary. Without more detailed information as to the digestibility of various feedstuffs the conduct of least-cost feed formulations for milkfish can not readily progress.

Since the objective of this study was to evaluate the direct nutritive value of the leaf meals, the experiment was carried out in an indoor clear-water recirculating system. However, milkfish given a low nutritional quality feed in ponds grow better than milkfish given the same feed under controlled laboratory conditions (Sumagaysay et al. 1991). This shows that natural food in ponds contributes significantly to the nutrition of milkfish. Perhaps feed containing leaf meal at 15% replacement may result in higher growth rates of milkfish in ponds.

Supplementary feeds containing leaf meal at levels higher than 15% replacement should also be tested for semi-intensive culture of milkfish in brackishwater ponds. The development of effective and low-cost supplementary feed should encourage fish farmers to intensify milkfish culture in brackishwater ponds.

Acknowledgements

We are grateful to Ms Lucia Jimenez for her technical assistance and Florence Jarder for proximate analyses of feed and feed ingredients.

References

Borlongan, I.G. and R.M. Coloso, 1993. Requirements of juvenile milkfish for essential amino acids. *J. Nutr.* in press.

Jackson, A.J., B.S. Capper and A.J. Matty, 1982. Evaluation of some plant proteins in complete diets for the tilapia *Sarotherodon mossambicus*. *Aquaculture* 27: 97–100.

Lin, S.S.M. and D.M. Chen, 1985. Sweet potato production and utilization in Asia and the Pacific. In Bouwkamp J.C. (ed.) *Sweet Potato Products: A Natural Resource for the Tropics*. Boca Raton, Florida, CRC Press, pp. 139–148.

Ng, W.K. and K.L. Wee, 1989. The nutritive value of cassava leaf meal in pelleted feed for Nile tilapia. *Aquaculture* 83: 45–58.

Peñaflorida, V. Dy, 1989. An evaluation of indigenous protein sources as potential component in the diet formulation for tiger prawn, *Penaeus monodon*, using essential amino acid index (EAAI). *Aquaculture* 83: 319–330.

Peñaflorida, V. Dy, F.P. Pascual and N.S. Tabbu 1992. A practical method of extracting mimosine from ipil-ipil, *Leucaena leucocephala*, leaves and its effect on survival and growth of *Penaeus monodon* juveniles. *The Israeli Journal of Aquaculture-Bamidgeh* 44(1): 24–31.

Santiago, C.B., M.B. Aldaba, M.A. Laron and O.S. Reyes, 1988. Reproductive performance and growth of Nile tilapia (*Oreochromis niloticus*) broodstock fed diets containing *Leucaena leucocephala* leaf meal. *Aquaculture* 70: 53–61.

Sumagaysay, N.S., F.E. Marquez and Y.N. Chiu-Chern, 1991. Evaluation of different supplemental feeds for milkfish (*Chanos chanos*) reared in brackishwater ponds. *Aquaculture* 93: 177–189.

Tacon, A.G.J., J.L. Webster and C.A. Martinez, 1984. Use of solvent extracted sun-flower seed meal in complete diets for fingerling rainbow trout (*Salmo gairdneri* Richardson). *Aquaculture* 43: 381–389.

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

Wee, K.L. and S.S. Wang, 1987. Nutritive value of *Leucaena* leaf meal in pelleted feed for Nile tilapia. *Aquaculture* 62: 97–108.

Wee, L.K. 1991. Use of non-conventional feedstuffs of plant origin as fish feeds—is it practical and economically feasible? In De Silva, S.S. (ed.). *Fish Nutrition Research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop*. Asian Fish. Soc. Spec. Publ. 5, Manila, Philippines. Asian Fisheries Society, pp. 13–32.

Evaluation of Leucaena Leaf Meal as a Dietary Protein Source for Indian Major Carp, *Labeo rohita*, Fingerlings

M.R. HASAN[1]
P.K. ROY[1]
A.M. AKAND[2]

[1] *Department of Fisheries Biology and Limnology*
[2] *Department of Aquaculture and Management*
Bangladesh Agricultural University,
Mymensingh 2202
Bangladesh

HASAN, M.R., P.K. ROY and A.M. AKAND. 1994. Evaluation of leucaena leaf meal as a dietary protein source for Indian major carp, *Labeo rohita*, fingerlings. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop*. Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines. Asian Fisheries Society, pp. 69–76.

Abstract

A 77-day laboratory feeding trial was conducted to evaluate the suitability of leucaena (*Leucaena leucocephala*) leaf meal as a partial substitute for dietary fish meal protein for fingerlings of Indian major carp, *Labeo rohita*. Six experimental diets were formulated to contain 25, 50 and 60% of the total dietary protein as test protein using soaked and unsoaked leucaena leaf meal. All diets were formulated to contain about 30% protein. Evaluation of diets was carried out on the basis of feed acceptability, growth, body composition, feed conversion, protein utilization, digestibility and histopathological changes. Leucaena soaked for 48 hrs has shown significantly ($p<0.05$) better performance than unsoaked leucaena at all levels of inclusion. There was a trend of reduced performance with increasing inclusion of plant protein in the diet for all treatments. However, in terms of cost of feed and economic returns, the diets containing 50% and 25% inclusion of soaked leucaena were better than all other diets. Histopathological examination of the liver revealed that soaked leucaena produced comparatively mild lesions compared with the unsoaked leucaena inclusion. However, lesions were not observed in the liver of fish given 25% inclusion of soaked leucaena. Lesions included congestion of the blood vessels and fatty changes in the hepatocytes; and became more severe with higher levels of inclusion.

Introduction

The use of leaf meal as a possible fish meal substitute to reduce the cost of fish feed is receiving increasing attention by fish nutritionists around the world. Leucaena, a protein-rich tropical leguminous plant, is grown in Bangladesh. It has been widely used in many parts of the tropics as a protein supplement in ruminant and poultry feeds.

Studies have been conducted to evaluate the nutritive value of leucaena leaf meal as a dietary protein source for fish. A review of the literature reveals contradictory results regarding its use as a fish feed. Improved growth performance with leucaena as a dietary protein source has been reported for Java tilapia (Pantastico and Baldia 1979; Ghatnekar et al. 1983), Nile tilapia (Pantastico and Baldia 1980) and Indian major carp (Ghatnekar et al. 1983). On the contrary, reduced performance in fish fed diets containing leucaena has been reported for Java tilapia (Jackson et al. 1982), Nile tilapia (Santiago et al. 1988), common carp (Mohire and Devaraj 1990) and giant tiger prawn (Pascual and Catacutan 1990).

Leucaena leaf contains a toxic non-protein amino acid, mimosine, a lysine derivative (β-N(3-hydroxy-4-oxo pyridine) α-amino propionic acid), which is apparently responsible for the reduced growth in the animal. It has been reported that mimosine can be degraded to a relatively less toxic form through various methods of processing (NAS 1977; Pascual and Peñaflorida 1979; Liener 1980; Gohl 1981; Wee and Wang 1987). Pascual and Peñaflorida (1979) observed that soaking leaves in freshwater was a highly efficient method to extract mimosine from leucaena leaves. Several studies showed that fish fed diets containing soaked leucaena had a higher growth and survival rate than fish fed unsoaked leucaena leaf meal (Pascual and Tabbu 1980; Wee and Wang 1987; Hasan et al. 1990).

The Indian major carp, *Labeo rohita* (Hamilton) is one of the most preferred farmed fish species because of its fast growth and higher acceptability to consumers. However, so far, no appropriate feed has been developed for this species in more intensified culture. Although Hasan et al. (1990) investigated the use of leucaena leaf meals as a dietary protein source for the fry of *L. rohita*, no study has been conducted with fingerlings. The present investigation was designed to evaluate the suitability of leucaena leaf soaked for 48 hours as a partial substitute for dietary fish meal protein for major carp fingerlings.

Materials and Methods

A static indoor rearing system was used for the feeding trial. Ninety-six litre capacity rectangular glass aquaria containing 80 litres of water were used. Artificial aeration was provided to each of the experimental tanks by an air compressor to maintain an adequate level of dissolved oxygen. Fingerlings were purchased from local fish vendors. Prior to the initiation of the experiment, the fingerlings were acclimated to laboratory conditions for at least 15 days. During the acclimation period fish were fed an artificial diet containing fish meal, rice bran, wheat bran and wheat flour (protein content, 30%).

Seven isonitrogenous diets were formulated. The control diet was prepared with fish meal as the sole source of protein. Fish meal (grade A) was purchased from the Bangladesh Fisheries Development Corporation, Dhaka. Leucaena, *Leucaena leucocephala*, leaves were collected from the Bangladesh Agricultural University campus. For soaked leucaena meal, leaves were soaked in tap water at ambient temperature (25–28°C) for 48 hours with frequent stirring and changing of water at six-hourly intervals. Both soaked and unsoaked leaves were sundried until the final product contained about 10% moisture and then ground to make leaf meal.

All dietary ingredients were analyzed for proximate composition prior to the formulation of the diets. The proximate composition (% dry matter) of fish meal, unsoaked leucaena, and soaked leucaena were as follows:

(a) fish meal—crude protein (CP) 65.63, ether extract (EE) 20.92, ash 14.47;

(b) unsoaked leucaena CP 27.23, EE 6.28, ash 6.57, crude fibre (CF) 19.38; and

(c) soaked leucaena CP 29.57, EE 5.34, ash 4.42, CF 18.77.

The mimosine contents (% dry matter) of unsoaked and soaked leucaena were 2.4 and 0.397, respectively. All diets were formulated to contain 30% protein (NRC 1983; Akand et al. 1991), 10–11% lipid (NRC 1983), 10–12% crude fibre (Jauncey and Ross 1982) and 40–42% digestible carbohydrate. Diets were formulated to be isocaloric as far as possible. Each diet contained 0.5% chromic oxide to study protein digestibility.

Soaked and unsoaked leaf meals were tested at three inclusion levels (25, 50 and 60% replacement of fish meal protein). The formulation and proximate composition of the experimental diets are presented in Table 1. The diets were prepared as described previously (Hasan et al. 1989).

Feed ingredients, experimental diets and fish samples were analyzed for their proximate composition (AOAC 1970). The gross energy (GE) content of the diets was calculated using kilocaloric values of 5.5/g protein, 9.1/g lipid and 4.1/g carbohydrate. The metabolizable energy (ME) content of the diets was calculated using kilocaloric values of 4.5 g protein, 8.51/g lipid and 3.49/g carbohydrate (Hasan et al. 1989). The chromic oxide content of the experimental diets and faeces was determined after Furukawa and Tsukahara (1966). Mimosine content of unsoaked and soaked leaf meal was determined colourimetrically (Matsumoto and Sherman 1951). At the end of the trial, fish samples from different treatments were fixed in 10% buffered formalin and histopathological examinations of the gill, liver, muscle, kidney and intestine were carried out.

Table 1. Formulation (% dry weight), proximate composition and cost per kg of the experimental diets (Bangladesh Taka 39 = US$1)

Diet	Control	Unsoaked leucaena			Soaked leucaena		
Test protein (%)	0	25	50	60	25	50	60
Test protein source	–	27.54	55.09	66.10	25.36	50.72	60.87
Fish meal	45.71	34.28	22.86	18.28	34.28	22.85	18.28
Dextrin	41.35	29.92	17.79	11.09	31.14	20.92	15.42
Soybean oil	0.44	1.10	1.76	2.03	1.48	2.51	1.73
Cod liver oil	–	–	–	–	–	–	1.20
Vitamin premix[a]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral premix[b]	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Binder[c]	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Crude fibre[d]	10.00	4.66	–	–	5.24	0.50	–
Chromic oxide	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Cost/kg diet (Taka)	20.46	16.47	12.44	10.19	17.27	14.12	14.33
Proximate composition (% dry matter)							
Crude protein	31.43	29.93	31.58	29.83	30.27	32.03	30.73
Ether extract	9.28	11.21	10.29	10.53	10.05	11.34	11.01
Ash	7.16	6.85	7.14	7.26	6.21	5.49	6.41
Crude fibre[e]	10.00	10.00	10.68	12.81	10.00	10.00	11.43
NFE	42.13	42.01	40.31	39.57	43.47	41.14	40.40
Chromic oxide	0.47	0.45	0.52	0.51	0.44	0.44	0.46
Mimosine[f]	–	0.66	1.32	1.59	0.10	0.20	0.24
Gross energy (kcal/g)	4.30	4.39	4.33	4.22	4.36	4.48	4.35
Metabolizable energy (kcal/g)	3.65	3.73	3.70	3.56	3.68	3.88	3.75
PE ratio[g]	85.64	79.39	85.35	82.40	81.15	83.41	82.39

[a] Embavit-GS (vitamin and trace mineral mixture, Rhône-Poulenc Bangladesh Ltd.);
[b] g/100 g mix: sodium chloride 4.35, magnesium sulphate 13.70, sodium biphosphate 8.72, potassium phosphate (dibasic) 23.98, calcium biphosphate 13.58, ferric citrate 2.97 and calcium lactate 32.70;
[c] Sodium carboxymethyl cellulose;
[d] Acid and alkali digested dry hay;
[e] Calculated values derived from test protein source and other dietary ingredients;
[f] Calculated from the analyzed mimosine content of the soaked and unsoaked leucaena leaf meal;
[g] Protein to energy ratio in mg protein/kcal of metabolizable energy.

Fish (mean weight ± SD = 3.49 ± 0.07 g) were randomly distributed at a rate of twenty fish per tank with three replications for each treatment. All fish were fed four times daily between 0800 and 1700 hours at a fixed feeding rate of 5% body weight per day for the whole experimental period of 11 weeks. The feeding and weighing of fish during and on termination of the experiment were as described by Hasan et al. (1989). In order to maintain good water quality, water in each tank was changed every day throughout the experimental period. Water quality parameters were monitored daily from each test tank. Water temperature ranged from 25–28°C; pH, 6.65–7.68; and dissolved oxygen, 4.8–7.2 mg/L.

Specific growth rate (SGR), weight gain (%), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU) and apparent protein digestibility (APD) were calculated after Castell and Tiews (1980). An economic analysis was performed to estimate the cost of feed to raise a unit biomass of fish following the procedure outlined by Hasan et al. (1991). The approximate cost of each diet was calculated on the basis of the Mymensingh wholesale market price of all the dietary ingredients used and the labour cost for soaking leucaena. Comparison of treatment means was carried out by one-way analysis of variance (ANOVA), followed by testing for pair-wise differences using Duncan's New Multiple Range Test (Zar 1974).

Results

The fingerlings became accustomed to the experimental diets within two to three days of the start of the experiment. The acceptability of all diets was similar up to the third week after which the diets containing unsoaked leucaena were less acceptable than the soaked leucaena. The control

diet was more acceptable than all leucaena diets except the diet containing 25% soaked leucaena which was more or less similar to the control diet.

No mortality of fish occurred during the experimental period. Performance data of carp fingerlings fed diets containing different inclusions of soaked and unsoaked leucaena leaf meal are presented in Table 2. Figure 1 shows that growth was more or less similar in the first three weeks and a clear trend was established from the fifth week. The best specific growth rate was recorded in fish fed diet containing 25% soaked leucaena (SGR 1.41%) followed by fish fed the control diet (SGR 1.24%) and the diet containing 50% soaked leucaena (SGR 1.20%) (Table 2 and Figure 2). The lowest growth was observed in fish fed diet containing 60% unsoaked leucaena. Results show a clear difference between soaked and unsoaked leucaena and a trend of reduced performance with increased inclusion of plant protein in the diets.

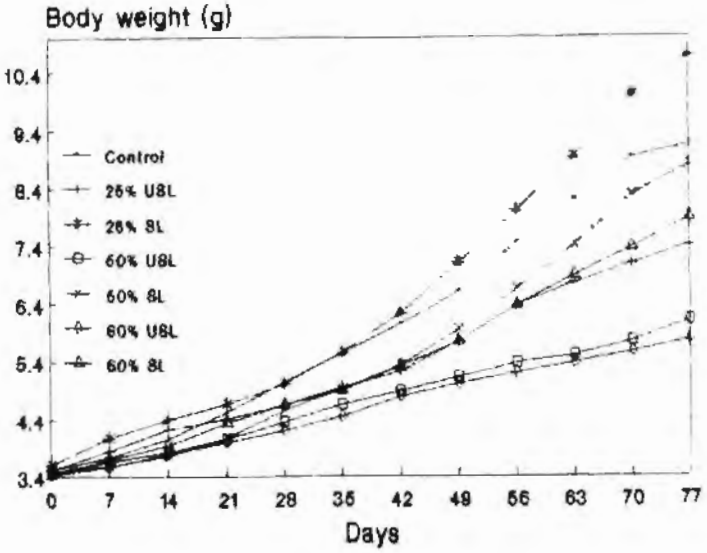

Figure 1 Weekly growth response of *Labeo rohita* fingerlings over a 77-day period.

Feed conversion ratios (FCRs) for the various test diets are presented in Table 2 and Figure 2. Diet containing 25% soaked leucaena gave the best FCR (1.87) followed by 50% soaked leucaena diet (FCR 2.09) and the control (FCR 2.17). For both soaked and unsoaked leucaena, FCRs increased significantly ($p<0.05$) at the higher inclusion level and the soaked leucaena gave better values than unsoaked ones.

Protein utilization was measured in terms of protein efficiency ratio (PER) and apparent net protein utilization (ANPU%). The highest PER (1.64) and ANPU (24.3%) were obtained for 25% soaked leucaena diet and the lowest values (PER = 0.76; ANPU = 9.48%) for the 60% unsoaked leucaena diet. (Table 2 and Figure 2). The trend of PER and ANPU for both soaked and unsoaked leucaena was similar to that for FCRs.

Apparent protein digestibility (APD) values were generally poor in all treatments. Irrespective of the soaking procedure, the APD values decreased with increased inclusion of leucaena in the diets (Table 2).

The proximate carcass composition of fish at the start and end of the trial is presented in Table 2. No definite trend in carcass composition was observed except that the fish fed soaked leucaena had lower moisture and higher lipid contents than the corresponding groups fed unsoaked leucaena diets.

The estimated total cost per kg of feed and the cost of feed to produce a kg weight of fish are given in Tables 1 and 2 respectively. The cost analysis shows that in terms of feed cost, the control diet was the most expensive and the diet containing 50% inclusion of unsoaked leucaena the cheapest. However, in terms of cost of feed per kg weight gain, diets containing 50% and 25% soaked leucaena were significantly ($p<0.05$) better than all other diets including control.

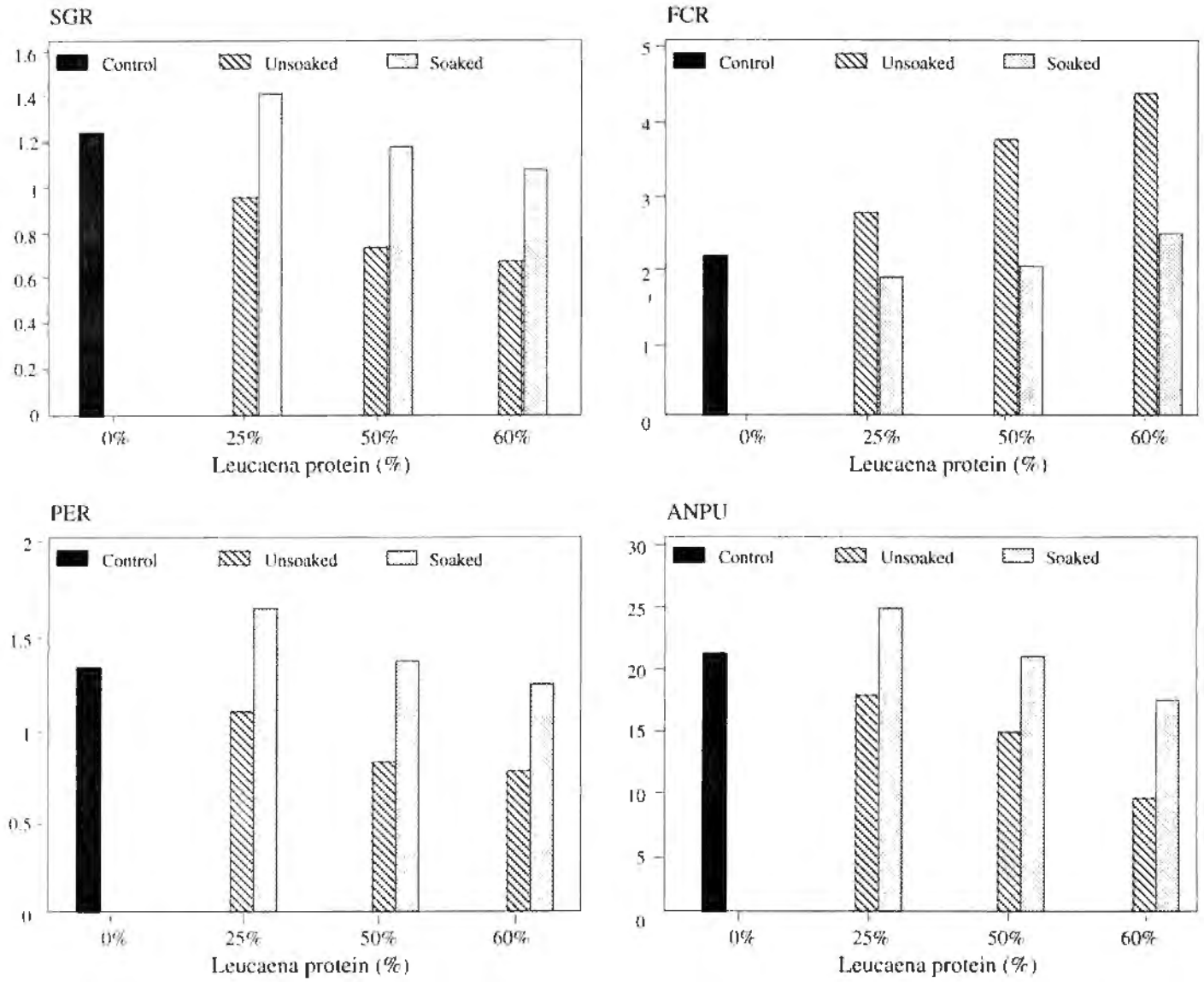

Figure 2. Changes in specific growth rate (SGR), food conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (NAPU) of *Labeo rohita* fingerlings fed different inclusions of leucaena protein.

There was no noticeable change in the histology of gills, muscle, kidney and intestine of fish fed different test diets. Some changes were however found in the histology of liver of fish fed both soaked and unsoaked leucaena leaf meal. Soaked leucaena produced comparatively mild lesions compared with unsoaked leucaena. The lesions included congestion of blood vessels and fatty changes in the hepatocytes which increased in severity with higher inclusions. Thrombus formation was observed in 50% unsoaked leucaena only. Histopathological examinations of fishes fed the control and 25% soaked leucaena however did not reveal any changes.

Discussion

The present results demonstrate the suitability of soaked leucaena as a possible partial substitute for fish meal protein. This study confirms the findings of Pascual and Tabbu (1980) for giant tiger prawn (*Penaeus monodon*), Wee and Wang (1987) for Nile tilapia (*Oreochromis niloticus*) and Hasan et al. (1990) for Indian major carp (*Labeo rohita*). In all these studies, soaking of leucaena improved the growth and other performances of fish compared to the fish fed unsoaked leucaena.

Table 2. Growth, feed conversion, protein utilization and carcass composition (% fresh weight) of *Labeo rohita* fingerlings after feeding for 77 days

Diet	Control	Unsoaked Leucaena			Soaked Leucaena			±SE*
Test protein (%)	0	25	50	60	25	50	60	
Initial weight (g)	3.52a	3.51a	3.46a	3.42a	3.61a	3.48a	3.42a	0.02
Final weight (g)	9.13b	7.41e	6.10f	5.78g	10.66a	8.78c	7.88d	0.10
Weight gain (%)	158.9b	110.7d	76.1e	68.8e	195.6a	151.9b	130.5c	2.90
SGR (%)	1.24b	0.97d	0.74e	0.68e	1.41a	1.20b	1.09c	0.02
SGR as % of control	100.0	78.2	59.7	54.8	113.7	96.8	87.9	—
FCR	2.17d	2.74c	3.77b	4.40a	1.87d	2.09d	2.49c	0.10
PER	1.34b	1.09d	0.81e	0.76f	1.64a	1.36b	1.22c	0.02
ANPU (%)	20.88b	17.75c	14.86d	9.48d	24.33a	20.89b	17.42c	0.32
APD (%)	80.83	72.38	55.19	42.00	74.32	69.17	47.30	—
Cost of diet (kg) weight gain (Taka)	44.47a	45.18a	46.94a	44.80a	32.29c	29.56c	35.64b	1.02
Carcass Composition								
	Initial							
Moisture	78.26	75.41	75.90	75.56	77.13	73.82	74.48	75.17
Crude protein	12.93	14.59	14.87	15.27	12.73	14.21	14.39	13.69
Ether extract	5.51	6.66	5.90	4.87	6.34	9.35	7.16	7.59
Ash	3.23	3.11	3.22	3.18	2.77	2.85	3.12	3.09

Row means followed by a common letter are not significantly different ($p>0.05$); *Standard error of treatment mean, calculated from residual mean square in the analysis of variance.

Mimosine, a non-protein amino acid, comprises about 3–5% of the dry weight of the protein of the leucaena leaf (NAS 1977). Detoxification of mimosine is possible by soaking the leaves in freshwater and then drying or steeping for one week with $FeSO_4$ or storing at 70°C in the presence of moisture (NAS 1977; Pascual and Peñaflorida 1979; Liener 1980; Gohl 1981; Wee and Wang 1987). Pascual and Peñaflorida (1979) reported that more than 96% of mimosine was eliminated from leucaena leaves after 48 hrs of soaking. Wee and Wang (1987) detected no mimosine in the leaf after 48 hrs of soaking in freshwater whereas sundried leaf meal was found to contain 3% mimosine.

In the present study, mimosine content of 0.40% (dry matter basis) was detected in leucaena meal after soaking for 48 hrs, whereas unsoaked leaf meal contained 2.40% mimosine. During soaking, mimosine is presumed to be degraded to a relatively less toxic form, 3, 4-dihydroxy pyridine (DHP) by enzymes present in the leaves (Tangendjaja et al. 1984; Wee and Wang 1987). Therefore, conversion of mimosine to DHP might be the reason for the better performance by the fish fed soaked leucaena meal. Although DHP has been reported to act as a goitrogenic agent in ruminants (Hegarty et al. 1976), there is no such report for fish. In our previous study with carp fry (Hasan et al. 1990) better performance was observed in the soaked leucaena group than in the unsoaked one, but it was significantly lower than that of the control. In the present study, leucaena was soaked for 48 hrs instead of 24 hrs as in the previous study which might have degraded the mimosine to a greater extent (Pascual and Peñaflorida 1979) resulting in better growth.

The poor performance of carp fingerlings fed unsoaked leucaena was also substantiated by histopathological changes in the liver. The congestion of the blood vessels may be due to disturbance in the heart and/or due to local causes. The vascular and fatty changes in the liver might be due to disturbances in metabolism of fat. These disturbances occur mainly in toxemic and anoxic conditions. Although the goitrogenic effect of mimosine in ruminants seems to be well established, the precise mechanism of toxicity in fish and other animals remains obscure (Liener 1980). However, a study of Vogt et al. (1986) reported significant changes in the histology of shrimp, *Penaeus monodon* fed a diet containing unsoaked leucaena leaf meal. The authors recorded that many R-cells of midgut glands become heavily damaged after 20 and 28 days of feeding and postulated that mimosine is probably responsible for these pathological changes.

In the present study, diet containing 25% soaked leucaena showed better performance than the control diet. The reason is not clearly understood. Although the best quality fish meal available was used in the experiment, quality control of fish production may not have been properly maintained.

It is also possible that the high lipid content (20.92% dry matter basis) of fish meal had undergone a certain degree of oxidation resulting in a poor growth response for the control diet.

Apart from excellent growth and feed utilization, liver histology also did not reveal any abnormalities for fish fed 25% soaked leucaena diet. In terms of the cost of feed to produce a kg of fish, both 25% and 50% soaked leucaena inclusions were found to be the cheapest. But considering the abnormalities in the liver, 50% inclusion of soaked leucaena may not be recommended. Therefore, with the present information, 48-hr soaked leucaena leaf meal may be used as dietary protein source for this fish species up to a maximum inclusion of 25% of total protein. Our study indicates that 48-hr soaking eliminates about 83% mimosine from the leaves.

Acknowledgements

This study was conducted as part of a research project on 'Diet formulation for Indian major carp, *Labeo rohita*' funded by the International Foundation for Science (IFS grant no. A/1190–1), Stockholm, Sweden. The International Development Research Centre, Canada is gratefully acknowledged for providing financial assistance to Dr. M.R. Hasan to attend the Fifth Asian Fish Nutrition Workshop.

References

Akand, A.M., M. Soeb, M.R. Hasan and M.G. Kibria, 1991. Nutritional requirements of Indian major carp, *Labeo rohita* (Hamilton) I. Effect of dietary protein on growth, food conversion and body composition. *Agricultures International* 1:35–43.

AOAC, 1970. *Official Methods of Analysis of the Association of Official Analytical Chemists*. Washington, DC, AOAC, 1015 p.

Castell, J.D. and K. Tiews (eds.), 1980. Report of the EIFAC, IUNS and ICES Working Group on Standardization of Methodology in Fish Nutrition Research EIFAC/T36, EIFAC/FAO, 24 p.

Furukawa, A. and H. Tsukahara, 1966. On the acid digestion method for determination of chromic oxide as an indicator substance in the study of digestibility in fish. *Bull. Jpn. Soc. Sci. Fish.* 32: 502–506.

Ghatnekar, S.D., D.G. Auti and V.S. Kamat, 1983. Feeding *Leucaena* to Mozambique tilapia and Indian major carp. In *IDRC Second International Workshop on* Leucaena *Research in the Asian Pacific Region*, Singapore, New York, Unipub, pp. 61–63.

Gohl, B. 1981. *Tropical Feeds*. Rome, Food and Agricultural Organization of the United Nations, 528 p.

Hasan, M.R., A.K. Azad, A.M. Omar Farooque, A.M. Akand and P.M. Das, 1991. Evaluation of some oilseed cakes as dietary protein sources for the fry of Indian major carp, *Labeo rohita* (Hamilton). In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Spec. Publ. 5*, Manila, Asian Fisheries Society, pp. 107–117.

Hasan, M.R., M.G.M. Alam and M.A. Islam 1989. Evaluation of some indigenous ingredients as dietary protein sources for the catfish *(Clarias batrachus*, Linnaeus) fry. In Huisman, E.A., N. Zonneveld and A.H.M. Bouwmans (eds.) *Aquaculture Research in Asia: Management Techniques and Nutrition*. Wageningen, Pudoc, pp. 125–137.

Hasan, M.R., M. Moniruzzaman and A.M. Omar Farooque 1990. Evaluation of leucaena and water hyacinth leaf meal as dietary protein sources of the fry of Indian major carp, *Labeo rohita* (Hamilton). In Hirano, R. and I. Hanyu (eds.) *The Second Asian Fisheries Forum*, Manila, Asian Fisheries Society, pp. 275–278.

Hegarty, M.P., R.D. Court, G.S. Christie and C.P. Lee. 1976 Mimosine in *Leucaena leucocephala* is metabolized to a goitrogen in ruminants. *Aus. Vet. J.* 52: 490.

Jackson, A.J., B.S. Capper and A.J. Matty, 1982. Evaluation of some plant proteins in complete diets for the tilapia *Sarotherodon mossambicus*. *Aquaculture* 27: 97–109.

Jauncey, K. and B. Ross, 1982. *A Guide to Tilapia Feeds and Feeding*. Scotland, Institute of Aquaculture, University of Stirling, 111 p.

Liener, I.C. 1980. Miscellaneous toxic factors. In Liener I.E. (ed.) *Toxic Constituents of Plant Foodstuffs*. New York, Academic Press, pp. 429–467.

Matsumoto, H. and G.D. Sherman, 1951. A rapid colorimetric method for the determination of mimosine. *Arch. Biochem. Biophys.* 33: 195–200.

Mohire, K.V. and K.V. Devaraj, 1990. Supplemental feeds containing sababul leaf meal for rearing common carp. Paper presented at the Second Indian Fisheries Forum held at Mangalore, India from 27–31 May, 1990.

NAS, 1977. *Leucaena, a Promising Forage and Tree Crop for the Tropics*. Washington DC, National Academy of Science, 115 p.

NRC, 1983. *Nutrient Requirement of Warmwater Fishes and Shellfishes*. Washington DC, National Research Council, National Academy of Science, 102 p.

Pantastico, J.B. and J.P. Baldia, 1979. Supplemental feeding of *Tilapia mossambica*. In Halver J.E. and K. Tiews (eds.) *Finfish Nutrition and Fish Feed Technology, Vol. II*. Berlin, FRG, H. Heenemann, pp. 587–593.

Pantastico, J.B. and J.P. Baldia, 1980. Ipil ipil leaf meal as supplement feed for *T. nilotica* in cages. *Fish. Res. J.* Philipp. 5: 63–68.

Pascual, F.P. and M. Catacutan, 1990. Defatted soybean meal and leucaena leaf meal as a protein sources in diets for *Penaeus monodon* juveniles. In Hirano, R. and I. Hanyu (eds.) *The Second Asian Fisheries Forum*. Manila, Asian Fisheries Society, pp. 345–348.

Pascual, F.P. and N.S. Tabbu. 1990. Ipil ipil (*Leucaena leucocephala*) leaves as plant protein source in prawn diets. Aquaculture Department, SEAFDEC. *Q. Res. Rep.* 4 (1) :1–4.

Pascual, F.P. and V. Peñaflorida. 1979. The extraction of mimosine from ipil ipil (*Leucaena leucocephala*) by soaking in water. Aquaculture Department, SEAFDEC, *Q. Res. Rep.* 3: 4–6.

Santiago, C.B., M.B. Aldaba, M.A. Laron and O.S. Reyes. 1988. Reproductive performance and growth of Nile tilapia (*Oreochromis niloticus*) broodstock fed diets containing *Leucaena leucocephala* leaf meal. *Aquaculture* 70: 53–61.

Tangendjaja, B., J.B. Lowry and R.B.H. Wills. 1984. Optimisation of the conditions for the degradation of mimosine in *Leucaena leucocephala* leaf. *J. Sci. Food Agric.* 35: 613–616.

Vogt, G., E.T. Quinitio and F.P. Pascual. 1986. *Leucaena leucocephala* leaves in formulated feed for *Penaeus monodon*: a concrete example for the application of histology in nutrition research. *Aquaculture* 59: 290–234.

Wee, K.L. and S.S. Wang. 1987. Nutritive value of *Leucaena* leaf meal in pelleted feed for Nile tilapia. *Aquaculture* 62: 97–108.

Zar, J.H. 1974. *Biostatistical Analysis*. Englewood Cliffs, N.J., Prentice Hall. 620 p.

Digestibility and Digestible Energy of Protein, Carbohydrate and Lipid in Grass Carp, *Ctenopharyngodon idellus* (C. et Val.)

LIU YONGJIAN
MAO YONGQING
DENG DONG FANG
LIN DING

Ichthyoloy Laboratory
Zhongshan University
Guangzhou
P.R. China

YONGJIAN, L., M. YONGQING, D. DONG FANG and L. DING, 1994. Digestibility and digestible energy of protein, carbohydrate and lipid in grass carp, *Ctenopharyngodon idellus* (C. et Val.). In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Fish Asian Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 77–80.

Abstract

To assess the digestibility and digestible energy of protein carbohydrate and lipid in grass carp, *Ctenopharyngodon idellus*, two different experimental feeds were used; (1) potato starch 40%, casein 37%, and fish liver oil 2%; (2) alpha-starch 40%, and the other ingredients as in (1). Six replications were performed for each kind of feed. Each tank accommodated 10 fish, with an average weight of 131 ± 7 g. The average water temperature was 23.1 ± 0.6, dissolved oxygen was 5–6 mg/L. The experiment lasted four weeks. The results indicated that energy digestibility and digestible energy of casein, potato starch, alpha-starch and fish liver oil in grass carp were 96.62%, 90.34%, 96.22% and 86.68%, and 5.42 kcal/g, 3.68 kcal/g, 3.86 kcal/g, 8.56% kcal/g respectively.

Introduction

Fish utilize dietary proteins, lipids and carbohydrates as energy sources, but the utilization of these nutrients differs remarkably from species to species. To facilitate further research on the utilization of dietary energy in grass carp, this study was conducted to determine the digestibility and digestible energy of casein, fish liver oil, potato starch and alpha-starch in grass carp.

Materials and Methods

Grass carp used for these experiments were taken from our own fish farm. The fish were acclimated to the experimental conditions for a week.

Two kinds of test diets were used: (1) alpha-starch 40%, casein 37%, and fish liver oil 2%; (2) potato starch 40%, and the other ingredients as in (1). The feed sources were: casein (C.P.); potato starch and CMC (B.R.); alpha-starch and cellulose (edible); and fish liver oil used for husbandry. These materials were mixed according to Table 1 and were stored in a refrigerator. Soft pellet feed was used in the experiment.

Table 1. Composition (percentage) of test diet

Ingredients	No. 1	No. 2	Gross energy (cal/g)
Casein	37	37	5669
Potato starch	40		4017
Alpha-starch		40	4012
Fish liver oil	2	2	9880
CMC	2	2	
Cellulose	12	12	
Vitamin mix *	2	2	
Mineral mix **	5	5	
Protein	35.62	35.94	
Lipid	2.14	2.45	
Starch	36.70	36.60	
Ash	4.06	4.01	
Gross energy (cal/g)	4663	4669	

* Vitamin mixture in mg/100 g diet; Vitamin A, 3700 I.U.; vitamin D3, 370 I.U.; vitamin E, 29.6 mg; vitamin B1, 7.4 mg; vitamin B2, 18.5 mg; vitamin B6, 7.4 mg; Vitamin B12, 0.06 mg; calcium D-pantothenate, 37 mg; nicotinic acid, 74 mg; folic acid, 3.7 mg; menadione sodium bisulfite, 7.4 mg; biotin, 0.37 mg; choline, 740 mg; vitamin C, 74 mg, inositol, 74 mg.

** Mineral mixture: prepared according to Huang's method (Yaotong and Yongjian 1989).

Six replications were performed for each diet. Tanks of 100 × 50 × 50 cm accommodated 10 fish. The body weight of the fish was 131 ± 7 g. Dechlorinized tap water was used and each tank had its own filter system. The water temperature was maintained at 23.1 ± 0.6°C. The dissolved oxygen in the water ranged from 5 to 6 mg/L. The pH value was tested once a week, ranging from 7.0 to 7.5 throughout the experimental period. The experiment lasted four weeks.

Fish were fed at 1000 and 1500 hrs every day. The daily feed amounted to 3% of the total body weight of the fish. The tanks were cleaned one hour after feeding. Faeces were collected with a siphon, and those with complete foreskin were dried at 60°C and then kept in a refrigerator.

Gross energy of the feed and faeces was determined with a calormeter, TR-2800. The Kjeldahl method was used for protein determination; the Soxhlet method for lipid; high temperature resistance furnace method (550°C) for ash (Ding and Yongqing 1987); and the Somogyi method for carbohydrate. The starch content was calculated by using the conversion factor of 0.93 (Dept of Biology 1984). The indirect method of resistant ash was used as the indigenous marker for the determination of digestibility (Cho et al. 1985).

The digestibility of each nutrient and the digestible energy of the diet were calculated as follows:

$$\text{Percent Nutrient Digestibility} = 100 - 100 \times \frac{\%\text{ indigenous marker in food}}{\%\text{ indigenous marker in faeces}} \times \frac{\%\text{ nutrient in faeces}}{\%\text{ nutrient in food}}$$

Digestible energy (cal/g) = energy digestibility of nutrient × gross energy of nutrient (cal/g)

Results and Discussion

Digestibility of protein, carbohydrate and lipid

The results of this experiment indicated that the digestibility of casein, fish liver oil, alpha-starch and potato starch was 93.68%–97.72%, 87.36–91.94%, 96.72–97.12% and 91.49–95.28% respectively. No significant difference was found in the digestibility of casein between the two experimental groups. A similar result was obtained for fish liver oil. The digestibility of alpha-starch was higher than of potato starch (Table 2).

Compared with the results obtained for other fish (Ogino 1980) grass carp showed no significant difference in the digestibility of casein and fish liver oil (Table 3); however, digestibility of alpha-starch was higher. One reason could be that the digestibility of alpha-starch depends on the fish used and the experimental conditions.

Table 2. Digestibility of protein, carbohydrate and lipid (%)

	casein	fish liver oil	alpha-starch	potato starch
1st week	93.68 (2.6)	90.46 (0.9)	96.72 (1.9)	91.49 (5.4)
2nd week	96.62 (0.9)	89.49 (0.6)	97.12 (2.0)	92.89 (2.7)
3rd week	97.27 (0.7)	87.36 (1.3)	96.98 (2.0)	95.28 (4.8)
4th week	96.84 (1.0)	91.94 (1.2)	96.95(1.2)	94.85 (0.5)
Average value	96.10	89.81	96.94	93.63
SD (%)	1.60	1.90	0.16	1.80
CV (%)	1.70	2.14	0.17	1.88

Table 3. Digestibility in different fish (%)

	casein	fish liver oil	alpha-starch	potato starch
Grass carp	93–97	87–91	96–97	91–96
Common carp	99	89–93	84–85	
Rainbow trout	96–97	96	26–64	
Yellowtail	98–99	82–97	21–43	
Red sea bream	96–99	97		

Digestibility of energy

The energy digestibility of casein, fish liver oil, alpha-starch and potato starch were 93.60–97.20, 84.98–87.27, 95.91–96.84 and 88.45–91.72% respectively (Table 4). These results showed no significant difference between the digestibility of corresponding nutrients (Table 2) and their energy. This seems to indicate that the lower the digestibility of nutrients, the greater the difference between the digestibility of corresponding nutrients and their energy.

Digestible energy

The digestible energy of casein, fish liver oil, alpha-starch and potato starch based on energy digestibility were 5306–5510 cal/g, 8510–8622 cal/g, 3680–3885 cal/g and 3553–3684 cal/g respectively (Table 5).

Table 4. Digestibility of energy (%)

	casein	fish liver oil	alpha-starch	potato starch
1st week	93.60 (2.1)	86.32 (2.6)	96.84 (1.2)	88.45 (6.1)
2nd week	96.50 (0.6)	86.13 (2.7)	96.17 (2.7)	89.81 (5.2)
3rd week	97.20 (0.1)	86.98 (3.3)	95.97 (2.7)	91.40 (6.4)
4th week	95.19 (0.7)	87.27 (4.1)	95.91 (1.6)	91.72 (4.8)
average value	95.62	86.86	96.22	90.34
SD (%)	1.58	0.54	0.43	1.50
CV (%)	1.66	0.62	0.44	1.68

Table 5. Digestible energy (cal/g)

	casein	fish liver oil	alpha-starch	potato starch
1st week	5306	8528	3885	3553
2nd week	5470	8510	3858	3608
3rd week	5510	8594	3850	3672
4th week	5396	8622	3848	3684
Average value	5420	8564	3860	3629
SD (%)	89.76	53.15	17.06	60.80
CV (%)	1.66	0.62	0.44	1.68

Conclusion

Before the start of the experiment, grass carp were acclimated in the tanks and fed with test diet for a week. Faeces were collected throughout the experiment, and digestible energy was determined. The values showed no significant difference from the beginning to the end of the experiment ($p>0.05$). This seems to lead to the conclusion that the faeces sample collected in the first week can be used for the determination of digestibility of protein, carbohydrate, lipid and energy in grass carp.

References

Cho, C.Y., C.B. Cowey and T. Watanabe, 1985. *Finfish Nutrition in Asia*, pp. 96–102.

Department of Biology, Beijing University, 1984. *Biochemistry Laboratory Manual*, High Education Publishing House, pp. 36–40.

Huang Yaotong and Yongjian Liu, 1989. Studies on the Mineral Requirement in Juvenile Grass Carp, *Acta Hydrobiologica Sinica*, 13(2): 134–151.

Lin Ding and Yongqing Mao, 1987. *Nutrition and Feed of Fish*, Zhongshan University Publishing House, pp. 152–157.

Ogino, C. 1980. *Fish Nutrition and Feed*, pp. 37–60.

Digestibility of Dry Matter and Protein from *Spirulina platensis* by Common Carp, *Cyprinus carpio*, with a Note on Time of Faeces Collection in Digestibility Estimations

N.R. UMESH
K. DATHATHRI
M.C. NANDEESHA
B. GANGADHAR
T.J. VARGHESE
College of Fisheries
University of Agricultural Sciences
Mangalore–575 002
India

UMESH, N.R., K. DATHATHRI, M.C. NANDEESHA, B. GANGADHAR and T.J. VARGHESE, 1994. Digestibility of dry matter and protein from *Spirulina platensis* by common carp, *Cyprinus carpio*, with a note on time of faeces collection in digestibility estimations. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 81–84.

Abstract

Digestibility evaluations of the single cell protein, *Spirulina platensis* were carried out in common carp, *Cyprinus carpio* at different levels of incorporation ranging between 10 and 90%. Between the test diets, a step-wise increase of 10% inclusion of *Spirulina* was adopted. Digestibility of protein was found to be maximum at 50% incorporation. Decline in protein digestibility was observed beyond this level up to 70% incorporation, but thereafter an increasing trend was noticed. However, in terms of dry matter digestibility, there was almost an increasing trend with increasing levels of *Spirulina*. Additional studies conducted to elucidate the difference in the digestibility based on the time of faeces collection, ie between day and night time, showed no significant difference in digestibility values. The results suggest that unlike other plant proteins, *Spirulina* protein is well utilized by common carp even at higher levels of incorporation and this phenomenon could be taken advantage of in the development of diets.

Introduction

Digestibility of individual ingredients in the compounded diet is considered as one of the important factors affecting the growth of fish (Cho et al. 1985; De Silva et al. 1990). Hence, it has been recommended to evaluate the digestibility of each ingredient before its incorporation in the diet. The single cell protein, *Spirulina* has been recognised as one of the potential ingredients for use in aquaculture and successful attempts have been made in the mass culture of this algae (Venkataraman and Becker 1986). The present study was undertaken to evaluate the digestibility of *Spirulina platensis* at different levels of incorporation in common carp, *Cyprinus carpio*. Additional studies were also conducted to determine the effect of time of faeces collection on digestibility estimates.

Materials and Methods

Digestibility evaluation

Standard fish meal diet with a protein content of about 28% was used as the reference diet (Table 1). Test diets were formulated by replacing the same percentage of all the ingredients at which the test ingredient was incorporated. Altogether nine test diets were developed with test ingredient levels varying between 10 and 90% (Table 2). A step-wise increase of 10% of the test ingredient was adopted between the diets.

Digestibility studies were conducted in glass aquaria of 72 × 35 × 30 cm with static water systems. Each aquaria was stocked with 15 fish with a mean weight of 10.9 g (± 3.81 g) and all the aquaria were provided with aeration. For each treatment duplicate tanks were used and water was changed every day. Fish were fed at 3% body weight. For feeding and faecal matter collection, the method described by De Silva et al. (1990) was adopted. Since crude fibre has been found to give better results, it was chosen as the marker in place of chromic oxide (Tacon and Rodrigues 1984); De Silva et al. 1990). Faecal matter collected over a period of two weeks was pooled and analyzed. For determination of variations in the digestibility between day and night, only test diets incorporated with *Spirulina* up to 50% were used.

Table 1. The ingredient composition of the reference diet (RD) and the proximate composition of the individual ingredients (by % dry weight)

Ingredient	Composition						
	RD	DM	Protein (%)	Total lipid (%)	Fibre (%)	NFE (%)	Ash (%)
Fish meal	25	91.40	64.00	7.04	0.3	3.40	16.40
Groundnut oil cake	24	92.00	38.50	6.67	8.2	27.63	11.00
Rice bran	40	91.30	10.63	1.40	31.9	29.87	17.50
Tapioca flour	10	91.51	5.38	2.20	1.0	81.13	1.80
Vitamin and mineral mix	1	—	—	—	—	—	—

Table 2. The proximate composition of the experimental diets (by % dry wt:SP *Spirulina*). The proximate composition of the *Spirulina* by dry matter was 50.5% protein, 1% lipid, 2.1% crude fibre, 26.7 NFE and 11.0% ash

Diet		Protein (%)	Total lipid (%)	Fibre (%)	NFE (%)	Ash (%)
Reference diet (RD)	93.05	28.26	4.15	12.72	26.71	21.21
RD + 10% SP (D_1)	94.19	29.63	3.81	12.83	27.70	20.22
RD + 20% SP (D_2)	93.16	32.62	3.64	12.22	25.64	19.04
RD + 30% SP (D_3)	93.83	34.52	3.50	9.92	29.26	16.63
RD + 40% SP (D_4)	92.68	37.74	3.40	9.13	26.97	15.44
RD + 50% SP (D_5)	93.19	39.71	3.00	7.08	29.19	14.21
RD + 60% SP (D_6)	91.36	45.22	2.53	4.51	25.92	13.18
RD + 70% SP (D_7)	93.10	48.78	2.15	4.72	23.30	14.15
RD + 80% SP (D_8)	91.14	49.62	2.05	3.21	23.38	12.88
RD + 90% SP (D_9)	90.90	51.43	1.89	2.63	22.29	12.66

Analytical methods

Crude protein, crude fat and crude fibre were estimated using Kjeltech, Soxtech and Fibretech (Tecator, Sweden). Ash was determined by burning the sample at 550°C in a muffle furnace. Moisture was estimated by drying the sample at 80°C for more than twelve hours. Apparent digestibility values were calculated employing the formula described by Cho et al. (1985). Statistical analysis was carried out following Duncan's multiple range test (Duncan 1955).

Results and Discussion

The proximate composition of the ingredients used in the reference diet is presented in Table 1. Fish meal was of good quality with a high protein content of 64%. Rice bran had a higher fibre content of 31.9%. The protein content of *Spirulina* used in the present study (Table 2) was lower than the level reported for the same species (Venkataraman and Becker 1986). The proximate composition of the test diets was largely influenced by the level of *Spirulina* incorporation, particularly in respect of protein and fibre. While there was an increasing protein percentage with increasing *Spirulina*, the fibre content declined.

Dry matter digestibility of the reference diet remained significantly lower as compared to the test diets. However, with the test diets, there was a progressive increase in the digestibility with increasing levels of *Spirulina*. It is not clear whether the dry matter digestibility was influenced by the level of fibre in the diet. In respect of protein too, the digestibility was found to increase with increasing levels of *Spirulina*, excepting for a small decline at 60 and 70% inclusion (Table 3). Fibre is known to influence the digestibility of ingredients (NRS-NAS, 1977; De Silva et al. 1990). In the case of carps, the upper limit of fibre in the diet is not established, although it is generally recommended to keep less than 8%. In respect of individual ingredient digestibility, while there was an increasing trend in the digestibility of dry matter, the protein digestibility was maximum at 50% inclusion. This possibly indicates that it would be appropriate to incorporate this ingredient at or around this range for better digestibility.

Table 3. Total dry matter (%) and protein digestibility (%) of the reference diet and test diets and the dry matter and protein digestibility of *Spirulina* estimated on faeces voided once a day using crude fibre as marker in *C. carpio* fed diets containing different levels of *Spirulina*.

Diet	DMD digestibility	Protein digestibility	Ingredient D.M. digestibility	Ingredient protein digestibility
RD	49.33 ± 0.86^{a}	79.92 ± 1.7^{a}	—	—
RD + 10%	51.12 ± 1.2^{ab}	79.50 ± 0.8^{a}	67.23±12.8	70.72±8.6
RD + 20%	53.47 ± 0.7^{ab}	80.70 ± 0.9^{ab}	70.03±2.7	83.84±3.3
RD + 30%	57.74 ± 1.0^{bc}	82.68 ± 1.4^{ab}	77.38±3.5	89.13±2.4
RD + 40%	62.13 ± 2.0^{c}	85.72 ± 3.0^{abc}	82.11±5.9	93.16±6.4
RD + 50%	70.44 ± 2.1^{d}	88.23 ± 0.8^{abc}	87.39±1.2	96.55±1.8
RD + 60%	72.81 ± 3.2^{de}	83.10 ± 1.9^{bcd}	88.46±5.3	85.22±3.4
RD + 70%	76.18 ± 2.3^{e}	84.28 ± 0.6^{bcd}	87.67±3.2	86.14±0.9
RD + 80%	81.01 ± 2.2^{e}	89.68 ± 1.2^{d}	89.10±2.8	92.11±1.5
RD + 90%	83.25 ± 2.9^{e}	90.15 ± 1.8^{d}	89.02±2.3	92.24±1.9

Values with same or without superscript in each column are not significantly different from each other at 5% level.

Studies conducted earlier with other plant protein sources such as *Gliricidia maculata*, *Colocasia esculenta*, *Leucaena leucocephala* and *Eichhornia crassipes* in an Indian major carp, *Catla catla* indicated declining protein and dry matter digestibility when they were incorporated beyond 15% in the reference diet. De Silva et al. (1990) also observed a similar trend in the decline of digestibility with leaf meal in *Oreochromis aureus*. This difference possibly indicates the quality of ingredients and nature of protein. De Silva et al. (1990) while reviewing the work of Cho et al. (1982), suggested 15–20% inclusion of leaf meal as the most appropriate test level instead of the generally recommended level of 30% by Cho et al. The present study also indicates that adoption of any one level may not be appropriate with all ingredients, particularly in respect to ingredients which have a good nutrient profile. *Spirulina platensis* is known to contain a balanced level of amino acids, adequate quantities of vitamins and minerals, besides a low level of nucleic acids (Becker and Venkataraman 1984). Few of the field trials conducted with *Spirulina*-based diets in Indian major carps have given encouraging results (Nandeesha et al. 1993).

Additional studies conducted showed no variation in digestibility of both protein and dry matter collected during day time and night time in test diets and test ingredients (Table 4). Possompes (1973) reported that leaching of nitrogenous compounds stabilizes after one hour. Bacterial action, if any, could not have manifested since both the night and day faeces had remained in the water for almost same length of time. De Silva et al. (1990) also observed a similar non-existence of difference in digestibility during day and night in tilapia.

The results clearly indicate the superiority of *Spirulina platensis* in terms of digestibility. Commercial production of *Spirulina* has been taken up by a major company in India. Efforts are also being made to produce this alga using agricultural wastes. While the cost may hinder its usage in commercial diets of fish with a low market price, its use could be exploited in larval diets.

Table 4. Total dry matter (%) and protein digestibility (%) of the reference and test diets and the dry matter and protein digestibility of *Spirulina* estimated on faeces voided in the day and in the night using crude fibre as marker in *C. carpio* fed diets containing different levels of *Spirulina*

Diet	Total DM digestibility		Protein Digestibility		Ingredient DM digestibility		Ingredient protein digestibility	
	Day	Night	Day	Night	Day	Night	Day	Night
D	49.19±1.9^{a}	47.48±1.0^{a}	80.15±2.0^{ab}	81.21±0.22^{ab}	—	—	—	—
D + 10%	51.63±2.3^{a}	51.46±0.8^{a}	78.52±3.5^{a}	79.39±2.50^{a}	63.64±12.5	87.33±8.3	73.9±14.6	68.05±10.60
D + 20%	57.13±0.2^{b}	54.66±1.4^{bc}	82.63±0.8^{abc}	81.78±1.60^{ab}	88.91±9.0	83.38±7.2	90.57±2.1	84.08±8.10
D + 30%	61.08±0.3^{bc}	60.24±2.2^{c}	84.23±2.0^{bcd}	83.25±0.70^{ab}	88.82±4.2	90.02±7.8	91.72±4.9	88.02±2.31
D + 40%	63.08±1.0^{cd}	65.89±2.4^{d}	87.23±0.9cd	86.53±0.70^{bc}	83.74±2.4	92.25±4.8	87.79±8.0	94.50±1.70
D + 50%	68.59±1.7^{d}	71.65±2.7^{d}	89.21±1.1^{d}	88.95±0.60^{c}	88.0±2.3	93.83±5.4	96.73±2.6	95.69±3.40

Values with same or without superscript in each column are not significantly different from each other at 5% level

Acknowledgements

M.C. Nandeesha is grateful to the International Foundation for Science, Sweden, for the financial assistance provided (A/1003–2).

References

Becker, E.W. and L.V. Venkataraman, 1984. Production and utilisation of blue-green alga, *Spirulina* in India. *Biomass* 4: 105–125.

Cho, C.Y., S.J. Slinger, and H.S. Bayley, 1982. Bioenergetics of salmonid fishes, energy intake, expenditure and productivity. *Comp. Biochem. Physiol.* 73B: 25–41.

Cho, C.Y., C.B. Cowey, and T. Watanabe, 1985. *Finfish nutrition in Asia. Methodological Approaches to Research and Development.* Canada, IDRC, 154 p.

De Silva, S.S., K.F. Shim, and A.K. Ong, 1990. An evaluation of the method used in digestibility estimations of a dietary ingredient and comparisons on external and internal markers, and time of faeces collection in digestibility studies in the fish *Oreochromis aureus* (Steindachner). *Reprod. Nutr. Dev.* 30: 215–226.

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

Nandeesha, M.C., G.K. Srikanth, P. Keshavanath, and S.K. Das, 1991. Protein and fat digestibility of five ingredients by an Indian major carp, *Catla catla* (Ham.). In De Silva S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spl. Publ. 5, Manila, Philippines. Asian Fisheries Society, pp. 75–81.

Nandeesha, M.C., B. Gangadhar, L.V. Venkataraman, D.K. Murthy, K. Dathathri, N.R. Umesh and T.J. Varghese, 1993. *Growth performance of two Indian major carps, catla (Catla catla) and rohu (Labeo rohita) fed with diets containing different levels of Spirulina platensis.* Bioresource Technology (in press).

Possompes, B.P. 1973. Influence de la temperature sur les besions en proteines, le transit alimentaire et la digestibilite chez la truite arc-en-ciel (*Salmo gairdneri* R.). These de 3^{e} cycle, Universite de Paris VI. 70 p.

Tacon, A.G.J. and A.M.P. Rodrigues, 1984. Comparison of chromic oxide, crude fibre, polyethylene and acid-insoluble ash as dietary markers for the estimation of apparent digestibility coefficient in rainbow trout. *Aquaculture* 43: 391–395.

Venkataraman, L.V., and E.W. Becker, 1986. *Biotechnology and Utilisation of Alga—the Indian Experience.* New Delhi, India, Department of Biotechnology, 259 p.

Influence of Sardine Oil on Growth and Flesh Quality of Common Carp, *Cyprinus carpio* (Linn.)

P. KESHAVANATH
B.R. JAGADEESH
College of Fisheries
University of Agricultural Sciences
Mangalore–575 002, India

KESHAVANATH, P. and B.R. JAGADEESH, 1994. Influence of sardine oil on growth and flesh quality of common carp, *Cyprinus carpio* (Linn.). In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 85–91.

Abstract

Two experiments were conducted to determine the effect of three different levels of sardine oil in a practical diet containing 20% protein on growth and flesh quality of common carp fry (av. wt. 0.4 g) and juveniles (av. wt. 150 g). Fish fed the highest level of oil showed the best growth, yielding an increase of 92.5% and 120.9% over the control and low lipid diets respectively in fry following 126 days of rearing, while in juveniles, it was 75.1% and 99.9% respectively after 112 days. Flesh quality was not affected as a result of sardine oil incorporation in the diet. The results indicate the advantage of reducing the protein content of the diet by increasing the lipid level.

Introduction

The protein sparing effect of lipids has been an area of active investigation in the past few years, with coldwater fish attracting the most attention (Tabacheck 1986; Davies 1989). Since nearly 2 000 000 tonnes of sardine is landed annually in India, a sizeable quantity of which goes for reduction, producing a large quantity of oil as a by-product, it was of interest to study the influence of sardine oil on fish growth and flesh quality, employing common carp, one of the species of carps used in composite fish culture practice in India.

Dietary requirements of fish are known to be size dependent and hence, two size classes, fry and juveniles, were used in this study to test the effect of reducing fish meal from the control diet and supplementing different levels of sardine oil on growth and flesh quality.

Materials and Methods

Diets

The fish meal based diet with 30% protein (Varghese et al. 1976) was used as the control. Four test diets containing 20% protein (T_1,T_2,T_3, and T_4) were prepared separately and supplemented with 0, 5, 10 and 15% sardine oil respectively (Table 1). Diets were prepared by mixing the ingredients with water in the ratio 1:0.8; and the resultant dough was heated in an aluminium container at 80°C for 30 minutes. Sardine oil was added after mixing the vitamin

mineral mixture with the cooled dough of the test diets before pelleting in a mechanical pelletizer. The pellets were dried to a moisture level below 10% and stored in air-tight plastic jars.

Table 1. Ingredient proportion and proximate composition of the diets (%)

Ingredients	Diets				
	C	T_1	T_2	T_3	T_4
Fish meal (40.1% C.P.)	25	10	10 (9.5)*	10 (9.1)	10 (8.7)
Groundnut cake (53.4% C.P.)	24	29	29 (27.6)	29 (26.4)	29 (25.2)
Rice bran (14.5% C.P.)	40	45	45 (42.9)	45 (40.8)	45 (39.1)
Wheat flour (13.6% C.P.)	10	15	15 (14.3)	15 (13.6)	15 (13.0)
Vitamin-mineral mix**	1	1	1 (1.0)	1 (1.0)	1 (0.9)
Sardine oil	0	0	5 (4.7)	10 (9.2)	15 (13.7)
	100	100	105	110	115
Proximate composition					
Dry matter	91.5±0.11***	91.6±0.12	92.2±0.09	91.8±0.17	90.9±0.13
Crude protein	29.9±0.54	20.1±0.29	19.0±0.23	18.1±0.12	17.9±0.23
Crude fat	5.8±0.12	4.9±0.09	8.9±0.12	14.0±0.09	18.9±0.21
Crude fibre	14.3±0.75	15.9±1.39	15.1±1.29	14.7±1.33	14.1±0.83
Ash	20.6±0.22	22.4±0.06	21.9±0.12	19.7±0.14	19.0±0.17
Calorie (kj/g)	11.9	10.8	11.9	13.3	14.4

* Figures in parantheses indicate values based on covariance analysis.

** Supplevite-M (Sarabhai Chemicals, India).
250 g Supplevite-M provides

Vitamin A	500 000 IU	Choline chloride	15 g
Vitamin D_3	100 000 IU	Calcium	75g
Vitamin B_2	0.2 g	Magnesium	2.75 g
Vitamin E	75 units	Iodine	0.1 g
Vitamin K	0.1 g	Iron	0.75 g
Cal. pantothenate	0.25 g	Zinc	1.5 g
Nicotinamide	1.0 g	Copper	0.2 g
Vitamin B_{12}	0.6 mg	Cobalt	0.045 g

*** Mean of 3 values ± S.E.

Experimental design

All the treatments were conducted in triplicate in cement cisterns of 25 m^2 each (5 m × 5 m × 1 m) without any soil base. The duration of the experiments was 126 days for fry and 112 days for juveniles. Water level in the cisterns was maintained at 65 ± 5 cm throughout the experimental period. Stocking was done at the rate of 20 fry (av. wt. 0.4 g) or 6 juveniles (av. wt. 150 g) per cistern. Feed was provided at 5% body weight once daily, readjusting the quantity after every fortnightly fish sampling. Water quality was monitored on fish sampling days, measuring temperature, pH, dissolved oxygen, carbon dioxide, dissolved organic matter, total alkalinity and wet weight of plankton (Jhingran et al. 1969). To avoid deterioration in quality, water was partially changed at fortnightly intervals.

A short-term experiment of six weeks duration was conducted in the laboratory to study the digestibility, conversion efficiency (FCE) and protein efficiency (PER) of the different diets. The study was carried out in duplicate in glass aquaria (1.25 m × 0.5 m × 0.5 m) by maintaining 10 fry or 2 juveniles per aquarium. Water level in the aquaria was kept at 30±2 cm. Feeding was done at 5% body weight. Fishes were acclimated to the respective diets prior to the start of the experiment and starved for a day. They were exposed to the feeds for only six hours a day; the unconsumed feed was removed, dried and weighed to determine the amount consumed. Faecal matter was collected 24 hours after feeding by siphoning and filtering through No. 30 bolting silk cloth and then dried to determine the dry matter. Dry matter digestibility (feed assimilation) was estimated based on the difference in content in feed and faecal matter. FCE was calculated as follows.

$$\text{FCE (\%)} = \frac{\text{Wet weight gain (g)}}{\text{Dry weight of feed (g)}} \times 100$$

Protein efficiency ratio was calculated as per Osborne et al. (1919).

Fish Growth

Fish were sampled at fortnightly intervals to assess their growth. On termination of the experiment, the cisterns were completely drained, all surviving fish collected and their individual weights recorded. Specific growth rate was calculated as:

$$\text{SGR } (\%) = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

where W_2 = weight at time T_2 and W_1 = weight at time T_1

Growth data were analyzed using two-way analysis of variance (Snedecor and Cochran 1968) and the multiple range test (Duncan 1955).

Body indices

Body indices, viz. hepatosomatic index (HSI) and gonadosomatic index (GSI), were computed using 10 harvested fish from each treatment by the formulae

$$\text{HSI} = \frac{\text{Weight of liver (g)}}{\text{Weight of fish (g)}} \times 100$$

$$\text{GSI} = \frac{\text{Weight of gonad (g)}}{\text{Weight of fish (g)}} \times 100$$

Proximate composition of diets and fish flesh

Feed ingredients, formulated diets and fish flesh samples were analyzed following AOAC (1975) methods for moisture, crude protein, crude fat and ash. Crude fibre was determined by the method of Pearson (1976), while nitrogen free extract (NFE) was calculated by the difference method of Hastings (1976). Energy levels were calculated by employing the respective energy factors for protein, fat and carbohydrate (Hastings 1975; Smith 1976).

Organoleptic evaluation

Five uniform-sized fish were selected from each treatment for organoleptic evaluation by a taste panel consisting of 10 trained panelists. The fish were judged for overall quality both in raw and cooked condition. Cooked meat was prepared by boiling raw flesh in 1.5% salt solution. The individual attributes included for raw fish were colour and glossiness of skin, colour of flesh, odour and texture of flesh, while for cooked meat the attributes were colour, odour, texture and flavour. The grades assigned by the panelists for various attributes were converted into numerical scores and analyzed statistically applying ANOVA to find the difference in overall quality (Udupa and Jayaram 1979).

Results

Water quality monitored was conducive for fish growth. The ranges of parameters in fry and juvenile experiments were: temperature 25.5° to 32°C/27.5° to 32°C, pH 8.0 to 9.2/7.7 to 9.0, dissolved oxygen 6.8 to 11.2 ppm/4.8 to 11.2 ppm, free carbon dioxide nil to 3.0 ppm/nil to 3.0 ppm, dissolved organic matter 1.8 to 16.4 ppm/1.4 to 11.0 ppm, total alkalinity 30 to 84 ppm/36 to 80 ppm and plankton wet weight 12.0 to 182 mg per 100 L water/7.3 to 193 mg per 100 L water.

The best growth of fry as well as juveniles was obtained with diet T_4, followed by T_3, T_2, C and T_1 (Tables 2 and 3). The net weight gain recorded in T_4, T_3 and T_2 treatments was 92.5%, 64.4% and 60.1% higher than C in fry after 126 days of rearing and 75.1%, 69.8% and 44.1%

respectively in the case of juveniles following 112 days of feeding. Compared to the control, a reduction of 12.9% and 12.4% in growth was recorded with diet T_1 in fry and juveniles. Influence of sardine oil on growth was clearly discernible from the 70th day in fry and from the 28th day in juveniles.

Specific growth rate (SGR) showed the same trend as growth. Average SGR and FCE was lower among juveniles compared to fry. Fish survival ranged from 75 to 85% in fry, while it was 100% in control as well as treated juveniles. Sardine oil administration resulted in higher HSI and GSI values. Treated fish recorded higher feed assimilation, FCE and PER. In both fry and juveniles muscle fat was influenced by dietary fat. Statistical analysis of the panel scores on raw and cooked flesh showed no significant difference in flesh quality of fish (raw F = 0.472/0.040, cooked F = 0.416/0.444 for fry/juveniles; $p<0.05$) from different treatments.

Table 2. Effect of feeding different levels of sardine oil on growth, survival, body indices and flesh composition of common carp fry

Parameter	Diets				
	C	T_1	T_2	T_3	T_4
Average initial weight (g)	0.40±0.04[a]*	0.40±0.06[a]	0.40±0.03[a]	0.40±0.04[a]	0.40±0.06[a]
Average final weight (g)	45.79±0.89[a]	39.95±0.67[a]	73.02±0.48[b]	75.03±0.96[b]	87.75±1.18[b]
Average daily increment (g)	0.36±0.007[a]	0.31±0.005[a]	0.58±0.004[b]	0.59±0.007[b]	0.69±0.09[b]
Average specific growth rate (%)	3.76±0.07[a]	3.65±0.06[a]	4.13±0.03[b]	4.15±0.05[b]	4.28±0.06[b]
Feed assimilation (%)	75.88±1.26[a]	75.80±0.84[a]	80.65±1.42[b]	80.74±0.78[b]	80.75±0.63[b]
Food conversion efficiency (%)	39.83±0.58[b]	25.14±0.37[a]	40.43±0.83[b]	41.28±0.46[b]	42.86±0.69[b]
Protein efficiency ratio	1.33	1.25	2.12	2.29	2.40
Survival (%)	75.00±1.32[a]	75.00±2.67[a]	80.00±3.89[a]	85.00±5.78[a]	80.00±3.33[a]
Body indices					
Hepatosomatic index	1.26±0.09[a]	1.24±0.12[a]	1.36±0.06[a]	1.42±0.18[a]	1.52±0.18[a]
Gonadosomatic index	6.01±1.30[ab]	5.02±1.05[a]	8.43±1.78[b]	5.42±0.92[a]	6.36±2.67[ab]
Flesh composition					
Moisture	79.10±0.05[b]	80.12±0.08[b]	78.08±0.06[b]	76.87±0.13[a]	76.1±0.05[a]
Protein	17.46±0.03[b]	16.41±0.05[a]	18.02±0.05[b]	17.80±0.12[b]	17.12±0.04[b]
Fat	0.99±0.06[a]	0.75±0.03[a]	1.46±0.05[b]	2.75±0.03[c]	3.97±0.04[d]
Ash	1.39±0.03[a]	1.81±0.02[b]	1.53±0.03[a]	1.69±0.03[a]	1.71±0.03[ab]
Calorie (kj/g)	3.57	3.26	3.82	4.28	4.67

Diets T_1, T_2, T_3 and T_4 had 0, 5, 10 and 15% sardine oil supplementation.

* Mean±S.E. Values with different superscripts in the same row differ significantly ($p<0.01$).

Discussion

The results clearly show that sardine oil exerts a positive influence on the growth of common carp fry and juveniles. There was a slight reduction in the protein content of the test diets due to oil incorporation (Table 1), despite which they induced significantly higher growth which could be attributed to the protein sparing effect of sardine oil. A clear difference in growth occurred after 70 days in the case of fry and 28 days with juveniles. This shows size-related difference in the utilization of oil from the test diets. However, on termination of the experiment, it was seen that sardine oil had a better effect on fry in terms of specific growth rate, food conversion efficiency and protein efficiency ratio. While survival was 100% with juveniles, oil treatment appears to have marginally improved survival of fry (Tables 2 and 3).

Higher amount of lipid in fish diets has improved growth only in some cases. Some of the positive reports are those of Shimma et al. (1980) in ayu, Gatlin and Stickney (1982) in channel catfish, Degani (1986) in glass eel, Tabacheck (1986) in Arctic charr, Stickney and Wurts (1986) in blue tilapia, Beamish and Medland (1986) and Kim et al. (1988) in rainbow trout, Williams and Robinson (1988) in red drum and De Silva et al. (1991) in red tilapia. Studies of Ogata and Konno (1986) and Takeuchi et al. (1991) indicate protein sparing effect of lipid only when administered through high protein diets which is in contrast to the present findings, while others have shown growth depression due to lipid supplementation of diets (Caceres-Martinez et al. 1984; Murai et al. 1985).

Table 3. Effect of feeding different levels of sardine oil on growth, survival, body indices and flesh composition of common carp juveniles

Parameter	Diets				
	C	T_1	T_2	T_3	T_4
Average initial weight (g)	150.00±2.42[a]*	150.00±1.88[a]	150.00±2.74[a]	150.00±1.36[a]	150.00±0.98[a]
Average final weight (g)	274.67±3.15[a]	259.17±4.12[a]	329.67±5.18[b]	361.17±2.94[b]	368.33±6.26[b]
Average daily increment (g)	1.11±0.01[a]	0.98±0.02[a]	1.60±0.03[b]	1.89±0.02[b]	1.95±0.03[b]
Average specific growth rate (%)	0.54±0.006[a]	0.50±0.008[a]	0.70±0.01[b]	0.78±0.006[b]	0.80±0.01[b]
Feed assimilation (%)	79.82±1.34[a]	80.75±1.42[a]	81.00±0.88[a]	81.43±1.76[a]	81.51±1.44[a]
Food conversion efficiency (%)	16.19±0.35[a]	11.44±0.27[a]	17.55±0.18[b]	19.26±0.49[c]	19.90±0.13[c]
Protein efficiency ratio	0.54	0.57	0.92	1.07	1.11
Survival (%)	100	100	100	100	100
Body indices					
Hepatosomatic index	1.44±0.06[a]	1.32±0.06[a]	1.60±0.03[ab]	1.79±0.03[b]	1.86±0.05[b]
Gonadosomatic index	8.44±0.83[a]	8.63±1.76[a]	15.15±0.87[b]	14.06±1.99[b]	14.18±0.46[b]
Flesh composition					
Moisture	76.08±0.06[ab]	77.27±0.12[b]	75.82±0.04[ab]	74.92±0.06[a]	73.43±0.16[a]
Protein	18.13±0.04[a]	17.77±0.09[a]	18.41±0.12[a]	18.67±0.07[a]	18.47±0.18[a]
Fat	1.80±0.06[a]	1.45±0.06[a]	1.97±0.05[a]	3.04±0.03[b]	5.07±0.06[c]
Ash	1.78±0.03[ab]	1.91±0.02[b]	1.74±0.05[a]	1.71±0.02[a]	1.66±0.03[a]
Caloric (kj/g)	4.20	3.89	4.29	4.67	5.38

Diets T_1, T_2, T_3 and T_4 had 0, 5, 10 and 15% sardine oil supplementation.

* Mean±S.E. Values with different superscripts in the same row differ significantly ($p<0.01$).

Better growth of fish receiving sardine oil incorporated diets appears to be due to improved food conversion and protein efficiency (Tables 2 and 3). The optimum level of lipid supplementation varies with the species. Viola et al. (1981) recorded higher carp yield and energy retention under intensive culture, employing 5% oil-coated pellets. Daozun et al. (1987) observed 6.5% oil to be optimum in *Mylopharyngodon piceus*, while Berger and Halver (1987) found maximum growth of striped bass (*Morone saxatilis*) fingerlings with a diet containing 17% lipid. De Silva et al. (1991) reported a protein sparing effect of up to 18% lipid level in red tilapia, independent of dietary protein content. In the present study, protein sparing could be noticed at all levels of supplementation. Since the treated fish did not differ between themselves, the lowest level could be considered more economical.

Dietary fat had proportionate influence on muscle fat, but did not affect the protein content; diet T_4 induced the highest fat accumulation in both fry and juveniles.

Murai et al. (1985), Degani (1986), Williams and Robinson (1988), Ellis and Reigh (1991) and Hanley (1991) reported an impact of dietary lipid on carcass fat level, while Berge and Storebakken (1991) found no such effect. Higher HSI and GSI values obtained with the treated fish could be related to the influence of sardine oil. Daniels and Robinson (1986) noticed a positive correlation between HSI and dietary lipid and carbohydrate in juvenile red drum, while Shimmino et al. (1980) noticed an increase in HSI with increasing dietary lipid and decreasing dietary protein at constant digestible carbohydrate level in young yellowtail. The influence of sardine oil on GSI was more pronounced in juveniles. This is attributable to their age as on harvesting some of them were found to be mature.

The source and level of lipid used in the diet could alter the quality of fish flesh. Dupree et al. (1979) discovered that channel catfish fed menhaden oil had a lower flavour rating than those administered corn oil. However, Hardy et al. (1987) did not observe any influence of herring oil, menhaden oil, soybean oil or tallow on the organoleptic properties of Atlantic salmon. Even the highest level of sardine oil tested in the present study did not affect organoleptic quality either in raw or cooked condition. This coupled with its protein sparing effect should make sardine oil the natural choice as a lipid source in carp diets.

Acknowledgements

The authors thank Professor P.K. Salian, Director of Instruction of the College, for encouragement and Professor T.J. Varghese, Head of the Aquaculture Department, for providing facilities to conduct this study. The first author is grateful to the International Development Research Centre, Canada for extending financial support to attend the Fifth Asian Fish Nutrition Workshop to present this paper. Thanks are due to Dr Sena S. De Silva for encouragement and Dr Santhosh P. Lall for suggesting improvements in the manuscript.

References

AOAC, 1975. *Methods of Analysis*. Association of Official Analytical Chemists, 12th ed. Washington, D.C., 1094 p.

Beamish, F.H. and T.E. Medland, 1986. Protein sparing effects in large rainbow trout, *Salmo gairdneri*. *Aquaculture* 55: 35–42.

Berge, G.M. and T. Storebakken, 1991. Effect of dietary fat level on weight gain, digestibility and fillet composition of Atlantic halibut. *Aquaculture* 99: 331–338.

Berger, A. and T.E. Halver, 1987. Effect of dietary protein, lipid and carbohydrate content on the growth, feed efficiency and carcass composition of striped bass, *Morone saxatilis* (Walbaum) fingerlings. *Aquacult. Fish. Manage.* 18: 345–356.

Caceres-Martinez, C., M. Cadena Roa and R. Metailler, 1984. Nutritional requirements of turbot (*Scophthalmus maximus*). I. A preliminary study of protein and lipid utilization. *J. World Maricult. Soc.* 15: 191–202.

Daniels, W.H. and E.H. Robinson, 1986. Protein energy requirements of juvenile red drum (*Sciaenops ocellatus*). *Aquaculture* 33: 243–252.

Daozun, W., G. Xizhang and L. Yufang, 1987. Effects of fat content in feeds on the growth of black carp fingerlings. *J. Fish. China* 11: 23–28.

Davies, S.J. 1989. Comparative performance of juvenile rainbow trout, *Salmo gairdneri* Richardson, fed to satiation with simulated 'standard' and 'high energy' diet formulations. *Aquacult. Fish. Manage.* 20: 407–416.

Degani, G. 1986. Dietary effects of lipid source, lipid level and temperature on growth of glass eel (*Anguilla anguilla*). *Aquaculture* 56: 207–214.

De Silva, S.S., R.M. Gunasekera and K.F. Shim, 1991. Interactions of varying dietary protein and lipid levels in young red tilapia: evidence of protein sparing. *Aquaculture* 95: 305–318.

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

Dupree, H.K., E.Z. Gaulitz Jr, A.S. Hall and C.R. Houle, 1979. Effects of dietary lipids on the growth and acceptablity (flavour) of channel catfish (*Ictalurus punctatus*). In Halver, J.E. and K. Tiews (eds.) *Proceedings of the World Symposium on Finfish Nutrition and Fish Feed Technology, Vol. II.* Hamburg, 20–23 June, 1978. Berlin, Heenamann, pp. 87–103.

Ellis, C.S. and R.C. Reigh, 1991. Effects of dietary lipid and carbohydrate on growth and body composition of juvenile red drum, *Sciaenops ocellatus*. *Aquaculture* 97: 383–394.

Gatlin, D.M. III. and R.R. Stickney, 1982. Fall-winter growth of young channel catfish in response to quantity and source of dietary lipid. *Trans. Am. Fish. Soc.* 1: 90–93.

Hanley, F. 1991. Effects of feeding supplementary diets containing varying levels of lipid on growth, food conversion and body composition of Nile tilapia, *Oreochromis niloticus* (L.). *Aquaculture* 93: 323–334.

Hardy, R.W., T.M. Scott and L.W. Harrell, 1987. Replacement of herring oil with menhaden oil, soybean oil or tallow in the diets of Atlantic salmon raised in marine net pens. *Aquaculture* 65: 267–277.

Hastings, W.H. 1975. Indonesia, preliminary testing of artificial feeds manufactured from local ingredients for milkfish and shrimp. A report prepared for the brackish-water shrimp and milkfish culture research and development project. Rome, FAO, 12 p.

Hastings, W.H. 1976. Fish nutrition and fish feed manufacture. Paper presented at FAO Technical Conference on Aquaculture, Kyoto Japan. FIR. Aq/Conf/76/R, 23. 13 p.

Jhingran, V.G., A.V. Natarajan, S.M. Banerjea and A. David, 1969. Methodology on reservoir fisheries investigations in India. *Bull. Cent. Inland Fish. Res. Inst., Barrackpore.* 12: 109 p.

Kim, J.D., M. Pascaud and S.J. Kaushik, 1988. Influence of dietary lipid protein ratios on growth and fatty acid composition of muscles in rainbow trout, *Ichthyophysiol. Acta.* 12: 7–25.

Murai, T., T. Akiyama, T. Takeuchi, T. Watanabe and T. Nose, 1985. Effects of dietary protein and lipid levels on performance and carcass composition of fingerling carp. *Bull. Japan. Soc. Sci. Fish.* 51: 605–608.

Ogata, H. and S. Konno, 1986. Growth response and smolt production of 1 year cherry salmon fed with diet having different protein and lipid levels. *Bull. Japan. Soc. Sci. Fish.* 52: 313–318.

Osborne T.B., L.B. Mendel and E.L. Ferry, 1979. Method for expressing numerically the growth promoting value of proteins. *J. Biochem.* 37: 224–229.

Pearson, D. 1976. *The Chemical Analysis of Food*. London, Churchill, 575 p.

Shimma Y., H. Shimma and K. Ikeda, 1980. Effects of supplemental oils to single cell protein feeds on the growth and fatty acid composition of ahu *Plecoglossus altivelis*. *Bull. Natl. Res. Inst. Aquacult. Japan* 1: 47–60.

Shimmeno, S., H. Hosakawa, M. Takeda and H. Kajiyama, 1980. Effects of calorie to protein ratios in formulated diet on growth, feed conversion and body composition of young yellowtail. *Bull. Japan. Soc. Sci. Fish.* 46: 1083–1087.

Smith R.R. 1976. Metabolizable energy of feed stuffs for trout. *Feedstuffs* 48: 16–21.

Snedecor, G.W. and W.G. Cochran, 1968. *Statistical Methods*. Calcutta, Oxford and IBH Publishing Co., 593 p.

Stickney R.R. and W.A. Wurts, 1986. Growth response of blue tilapias to selected levels of dietary menhaden and catfish oils. *Prog. Fish.-Cult.* 48: 107–109.

Tabacheck, J.L. 1986. Influence of dietary protein and lipid levels on growth, body composition and utilization efficiencies of Arctic charr, *Salvelinus alpinus* L. *J. Fish Biol.* 29: 139–151.

Takeuchi, T., Y. Shiina and T. Watanabe. 1991. Suitable protein and lipid levels in diet for fingerlings of red sea bream *Pagrus major*. *Bull. Japan. Soc. Fish.* 57: 293–299.

Udupa, S. and M.G. Jayaram. 1979. A note on the statistical analysis of panel test data of food products by sensory evaluation. *Curr. Res.* 8: 106–107.

Varghese, T.J., K.V. Devaraj, B. Shantharam and H.P.C. Shetty. 1976. Growth response of the common carp, *Cyprinus carpio* Var. *communis* to protein rich pelleted feed. Paper presented at the Symposium on Development and Utilization of Inland Fishery Resources. Colombo, Srilanka. pp. 408–416.

Viola, S., U. Rappaport, Y. Arieli, G. Amidan and S. Mokady. 1981. The effects of oil coated pellets on carp (*Cyprinus carpio*) in intensive culture. *Aquaculture* 26: 49–65.

Williams, C.D. and E.H. Robinson. 1988. Response of red drum to various dietary levels of menhaden oil. *Aquaculture* 70: 107–120

Effect of Varied Levels of Protein on the Growth and Tissue Biochemistry of Stunted Yearlings of Rohu, *Labeo rohita*, in the Absence and Presence of Natural Food

M. C. NANDEESHA
K. DATHATHRI
D. KRISHNAMURTHY
T.J. VARGHESE
B. GANGADHAR
N.R. UMESH

College of Fisheries
University of Agricultural Sciences
Mangalore –575 002
India

NANDEESHA, M.C., K. DATHATHRI, D. KRISHNAMURTHY, T.J. VARGHESE, B. GANGADHAR and N.R. UMESH, 1994. Effect of varied levels of protein on the growth and tissue biochemistry of stunted yearlings of rohu, *Labeo rohita*, in the absence and presence of natural food. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 93–99.

Abstract

Culture of 6–12 month old 'stunted' Indian major carps has become one of the most popular techniques to increase yield in some parts of India, particularly in Andhra Pradesh, owing to their rapid growth during the second year of life. In order to understand the protein requirements of such fish in the absence and presence of natural food produced through fertilization, this study was undertaken with 12-month old 'stunted' rohu of about 20g. In the absence of natural food, there was a progressive increase in growth with an increasing level of protein derived from fish meal, starting from 5% up to a maximum of 30%; the weight attained by fish fed diets containing 25 and 30% protein did not differ statistically. RNA/DNA ratio was found to be at maximum at 25% protein. Although the weight attained was lower at 20% protein, activity of enzyme groups protease, amylase and lipase and glycogen levels in muscle and liver were found to be maximum with this group. Based on growth and biochemical analysis, it could be concluded that 25% protein and 37% carbohydrate is optimum in the diet for 'stunted' rohu in the absence of natural food.

Trials conducted in earthern ponds with stunted rohu employing three different levels of protein (15, 20 and 25%) and two different rates of poultry manure application (10 and 20 tons/ha/yr) yielded different patterns of growth owing to the varied quantity of natural food. While growth increased with increasing levels of protein at 10 tons/ha/yr, it declined beyond 15% protein in ponds receiving manure at 20 tons/ha/yr. The results clearly suggest the need to consider the contribution of natural food in the nutrition of carp.

Introduction

In Andhra Pradesh, a southern Indian state, major carps, namely, catla (*Catla catla*), rohu (*Labeo rohita*) and mrigal (*Cirrhinus mrigala*) are cultured commercially in more than 50 000 ha area. On average, about 5 350 kg/ha/yr yield is obtained with a maximum recorded yield of 14 620 kg/ha/yr (Veerina et al. 1992). The yield obtained in this area is much higher than the national

average production of 1 660 kg/ha/yr (Srivastava et al. 1991). The previous record yield in India was only 10 700 kg/ha/yr (Sinha et al. 1991), a six-species culture of Indian and exotic carps. Farmers in Andhra Pradesh have excluded exotic carps from the system in view of the poor economic returns as compared to that from Indian major carps. Even among Indian major carps, about 75% of farmers culture only catla and rohu at 2:8 ratio owing to the excellent market demand for rohu. Almost all farmers use 6–12 month old 'stunted' seed of major carps as they grow faster during their second year.

The present study was undertaken to determine the effect of different dietary protein levels on the growth of 'stunted' rohu yearlings either in the absence or presence of natural food produced by pond fertilization.

Materials and Methods

Induced-bred fingerlings of rohu were reared under high density in unfertilized earthen ponds over a period of 12 months, with very little artificial diet provided to obtain 'stunted' rohu.

Two experiments were conducted using these fish. In the first study, five test diets were formulated to contain 5, 10, 20, 25 and 30% protein and corresponding carbohydrate levels of 64, 57, 47, 37 and 31%. Fish meal and tapioca were used as the major sources of protein and carbohydrate (Table 1). Fat and energy levels were kept constant in all the diets by cod liver oil supplementation. The growth trial was conducted over a period of 90 days by stocking 20 'stunted' seed in 25 m^2 cement cisterns without fertilization. Each treatment was duplicated. Fish were fed daily at 5% body weight with one of test diets and the feed quantity was altered based on fish weight recorded at fortnightly samplings.

Table 1. Formulation and proximate analyses of diets used in this study

Ingredients (%)	5%	10%	20%	25%	30%
Fish meal	8.8	17.3	34.3	42.8	51.3
Tapioca	80.0	71.5	54.5	46.0	37.5
Cod liver oil	4.4	3.8	2.8	2.2	1.7
Sugarcane pith	4.8	5.4	6.4	7.0	7.5
Vit. min. mix*	2.0	2.0	2.0	2.0	2.0
Total	100.0	100.0	100.0	100.0	100.0
Proximate analysis (%)					
Moisture	9.7±0.7	9.8±1.3	10.0±1.9	9.9±1.0	10.8±1.2
Crude protein	6.7±1.3	12.3±0.6	22.4±0.9	25.6±1.7	29.6±2.1
Ether extract	5.0±0.6	4.8±0.8	4.4±1.0	4.6±0.1	4.5±0.0
Ash	5.6±0.5	6.8±0.1	9.2±1.0	10.1±1.6	10.9±1.0
Crude fibre	9.1±2.0	9.2±2.1	11.3±1.8	12.8±2.0	13.2±1.6
NFE	63.9±0.9	57.1±2.3	42.7±1.8	37.6±2.2	31.0±2.6
Gross energy (kcal/100 g)	352.9	353.8	363.8	342.0	338.8

Mean ± standard deviation

* 1 kg of Vit. min. mix. contains Vitamin A 20 00 000 I.U., Vitamin D3 4, 10 000 I.U., Vitamin B2 0.52 g, Vitamin E 350 Units, Vitamin K 0.4 g, Calcium pantathenate 1 g, Vitamin B12 3 mg, Choline chloride 6% W/W, Calcium 340 g, Manganese 11 g, Iodine 0.4 g, Zinc 6 g, Copper 0.8 g, and Cobalt 0.18 g.

The second experiment was conducted in 12 earthen ponds of 675 m^2 each. Three different levels of protein (15, 20 and 25%) under two different rates of fertilization with poultry manure (10 and 20 tons/ha/yr), were evaluated using fish from the same stock of 'stunted' rohu, over a period of 90 days. Fourteen days prior to the stocking of fish, ponds were fertilized with 20% of the total manure dosage and subsequently they were fertilized at about 8% of the remaining 80% dosage every month. Feeding was done only with rice bran and groundnut oil cake mixed in different proportions to obtain 15, 20 and 25% protein, keeping carbohydrate constant (Table 2). Fish were stocked at a density of 2000 no/ha and fed at the rate of 5% body weight daily with wet dough of rice bran-oil cake mixture form for the first 45 days. Thereafter, the feeding rate was reduced to 2.5% body weight. The quantity of feed was adjusted based on fortnightly sampling of fishes.

In both experiments, feed was placed in suspended trays to reduce wastage. Water quality was monitored routinely for temperature, pH, oxygen, carbon dioxide and alkalinity (APHA 1985).

Plankton samples were also collected at fortnightly intervals using 60 micron bolting silk cloth. The dry weight of plankton was determined by drying the sample at 100°C for 24 hours. At the beginning and the end of the experiment whole fish were analyzed for proximate composition (AOAC 1975). Fish from the first experiment were used for estimating RNA and DNA (Schneider 1957) and three enzyme groups, namely, protease (Kunitz 1947), amylase (Sumner 1924) and lipase (Naher 1974). All data were subjected to ANOVA and Duncan's multiple range test (1955) to find out significant differences between the treatments.

Table 2. Percentage composition of ingredients and calculated levels of proximate composition

	Dietary protein level(%)		
	15	20	25
Ingredient (%)			
Rice bran	84.2	68.8	50.0
Groundnut oil cake	15.8	31.2	50.0
Proximate composition (%)			
Moisture	8.4	8.8	9.4
Crude protein	13.8	19.1	25.7
Ether extract	6.1	6.4	6.8
Crude fibre	26.6	23.1	19.0
Ash	13.1	11.8	10.2
NFE	32.0	30.8	28.9
Gross energy (kcal/100 g)	266.2	290.1	325.8

Results

In the first trial where growth response of rohu to different levels of protein and carbohydrate was studied, a progressive increase in weight with increasing dietary protein concentration and decreasing carbohydrate level was observed (Figure 1). However, no significant difference was observed in weight gain between fish fed 25 and 30% protein; similarly there was no difference between lower levels ($p<0.05$). Food conversion ratio, protein efficiency ratio and net protein retention did not differ significantly between 20, 25 and 30% protein treatments (Table 3). Body composition data showed no significant ($p<0.05$) difference in the protein and ash content of fish from various treatments (Table 3). A marginal increase in moisture content was noticed with increasing protein level, the highest being at 30% protein. In contrast to these parameters, fat percentage was significantly ($p<0.05$) lower in fish fed 25 and 30% protein diets as compared to the rest.

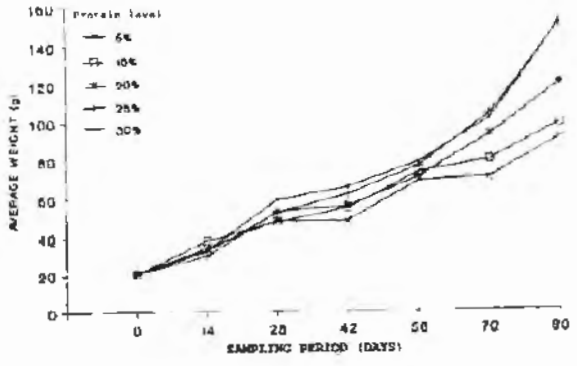

Figure 1. Growth performance of 'stunted' rohu fed with various levels of protein in unfertilized ponds.

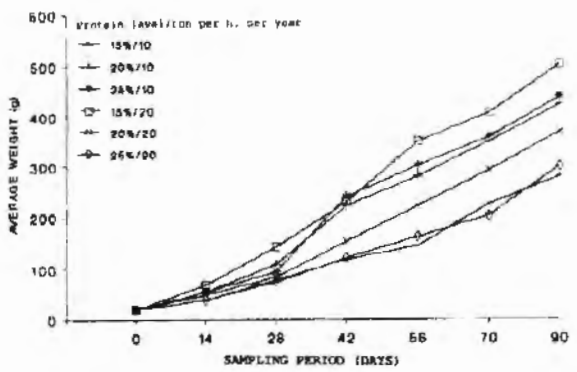

Figure 2. Growth performance of 'stunted' rohu fed with different levels of protein diets in fertilized earthen ponds.

Table 3. Growth, FCR, PER, NPR and proximate composition of rohu fed with different levels of protein

Parameter	Dietary protein level (%)				
	5	10	20	25	30
Initial mean weight (g)	20.2±2.0	20.2±2.0	20.2±2.0	20.2±2.0	20.2±2.0
Final mean weight (g)	90.4±24.0[a]	98.2±18.2[a]	120.0±13.8[b]	150.1±16.3[c]	150.4±2.3[c]
SGR (%)	1.7±0.0[a]	1.8±0.0[a]	2.0±0.0[b]	2.2±0.0[c]	2.2±0.0[c]
FCR	3.0±0.1[c]	2.7±0.1[b]	2.3±0.1[a]	2.0±0.1[a]	1.9±0.0[a]
PER	8.6±0.4[c]	4.6±0.1[b]	2.4±0.3[a]	2.0±0.0[a]	1.7±0.0[a]
NPR (%)	97.0±4.3[c]	54.7±1.5[b]	33.8±1.1[a]	28.6±0.4[a]	24.9±0.0[a]
Carcass composition (%)					
Moisture	70.1±0.9	70.7±0.3	71.0±0.3	72.5±0.2	73.0±0.2
Crude protein	14.1±0.2	14.3±0.2	14.8±0.0	14.2±0.0	14.0±0.3
Crude fat	9.6±0.1[c]	9.7±0.1[c]	9.1±0.0[b]	7.8±0.1[a]	8.0±0.1[a]
Ash	2.9±0.1	2.6±0.1	2.6±0.0	2.8±0.0	3.1±0.0

1.Mean ± standard deviation
2. Figures in the same row having same superscript or without superscript are not significantly different ($p>0.05$)

The highest level of RNA and DNA was recorded in fish fed with 20 and 25% protein diets, their ratio being the highest in the latter treatment. While there was no significant difference in the muscle glycogen level between the treatments, glycogen level in liver was the highest for fish fed 20% protein (Table 4). The total and specific activity of amylase and protease increased up to 20% protein; thereafter, it declined. There was no variation in intestinal lipase activity between different treatments, but hepatopancrease activity was the lowest in fish receiving both the highest and lowest level of protein (Table 5).

In the growth trial conducted in earthen ponds, a progressive increase in weight gain was observed with increasing dietary protein concentration in ponds fertilized with 10 tons/ha of manure. However, under 20 tons/ha manure treatment, growth declined above 15% dietary protein level (Figure 2). The food conversion ratio was best at 25% protein level with 10 tons/ha fertilization. Unfortunately, fish survival was lower in all the treatments due to predation by otters which are highly piscivorous. Analysis of flesh for proximate composition did not show any clear trend. Moisture level remained high at 25% protein at both levels of fertilization (Table 6). The protein content declined with an increasing level of protein at 10 tons/ha, while there was no significant difference ($p<0.05$) in its level between different treatments at 20 tons/ha. Fat content remained higher at 20% protein in both treatments, while ash content was essentially the same in all the treatments.

There was variation in plankton productivity and alkalinity at the two different levels of fertilization (Table 7). Microscopic analysis of plankton samples indicated that throughout the experimental period zooplankton dominated (about 70%) over phytoplankton. Among zooplankton, rotifers were predominant (30%); namely *Brachionus*, *Filinia* and *Keratella*. Other zooplankton encountered were cladocerans, copepodes, protozoans, etc. Among the phytoplankton, Myxophyceae was the most frequent group followed by Chlorophyceae.

Discussion

The protein requirement of fry of Indian major carp is known to vary between 40 and 50% (Sen et al. 1978; Singh et al. 1987; Singh and Bhanot 1988) and that of fingerlings from 30 to 35% (Khan and Jafri 1991). Renukaradya and Varghese (1988) conducted a field study with catla and rohu fingerlings and observed no difference in weight gain of fish fed 30 and 40% protein, and concluded 30% protein to be optimum.

The results of our study clearly demonstrate that in 'stunted' yearlings, 25% protein induces growth equal to that of 30% protein and hence from the view point of reducing production costs, the former level can be considered to be optimum. RNA:DNA ratio is known to provide a dependable indication of growth trends (Buckley 1980; Mustafa and Mittal 1982; Khan and Jafri 1991). In the present study, this ratio is the highest at 25% protein so it is advisable not to use feeds with higher protein level for 'stunted' fish. Although both amylase and protease activity were higher at 20% protein, 25% protein could be considered as adequate and economical based on the final weight gain.

Table 4. RNA, DNA and their ratio in muscle and muscle and liver glycogen in rohu fed with different levels of protein

Treatment	RNA (mg/g)	DNA (mg/g)	RNA/DNA ratio (mg/g)	Muscle Glycogen (mg/g)	Liver Glycogen (mg/g)
5%	0.54±0.02[a]	0.28±0.00	1.89±0.08[a]	0.43±0.01	12.40±0.10[b]
10%	0.70±0.05[a]	0.28±0.00	2.46±0.18[b]	0.46±1.08	13.99±0.23[d]
20%	1.17±0.05[c]	0.35±0.00	3.32±0.14[c]	0.43±0.35	14.92±0.10[e]
25%	1.37±0.03[c]	0.29±0.01	4.55±0.14[d]	0.44±0.12	13.33±0.19[c]
30%	0.89±0.02[b]	0.26±0.02	3.58±0.07[c]	0.45±0.05	8.88±0.05[a]

1. Mean ± standard
2. Figures in the same row having same superscript or without superscript are not significantly different (p>0.05)

Table 5. Total (μ/g tissue) and Specific (μ/g protein) activity at 25°C of amylase, protease and lipase in intestine and hepatopancreas of fish fed with different levels of protein

Treatment		Amylase activity		Protease activity		Lipase activity	
		Intestine	Hapatopancreas	Intestine	Hapatopancreas	Intestine	Hapatopancre
5%	Total activity	14.8±0.2[a]	44.0±1.6[a]	2.3±0.2[a]	13.3±0.5[a]	0.4±0.0	0.4±0.0[a]
	Specific activity	99.2±0.5[b]	194.9±7.1[a]	15.5±1.3[a]	59.0±2.3[b]	0.6±0.0	1.7±0.0[a]
10%	Total activity	22.1±0.6[c]	55.3±1.0[b]	4.0±0.1[b]	24.3±1.2[b]	0.4±0.0	0.4±0.0[b]
	Specific activity	126.6±3.3[c]	265.7±4.6[c]	22.6±0.6[a]	117.0±5.6[c]	2.4±0.2	2.1±0.0[b]
20%	Total activity	26.1±0.7[d]	81.4±0.4[e]	25.9±0.8[c]	45.0±3.1[c]	0.4±0.0	0.5±0.0[c]
	Specific activity	142.8±3.6[d]	360.1±1.8[c]	141.9±4.4[d]	199.0±3.6[d]	2.0±0.2	2.3±0.1[b]
25%	Total activity	17.9±0.2[b]	67.8±0.8[d]	18.1±0.5[c]	13.7±0.7[c]	0.4±0.0	0.5±0.0[c]
	Specific activity	97.9±1.3[ab]	306.2±3.6[d]	99.3±2.6[b]	60.4±1.6[b]	2.1±0.0	2.4±0.1[b]
30%	Total activity	14.2±0.3[a]	61.3±1.0[c]	19.7±1.0[d]	6.6±0.3[d]	0.4±0.0	0.4±0.0[a]
	Specific activity	88.0±1.6[a]	246.1±4.1[b]	122.4±6.0[c]	26.5±1.2[a]	2.4±0.3	1.6±0.0[a]

1. Mean ± standard deviation.
2. Figures in the same column having same superscript or without superscript are not significantly different (p<0.05).

Table 6. Growth and proximate composition of rohu grown in earthen ponds with fertilization and different levels of proteins

Parameter	Dietary protein levels (%)					
	15	20	25	15	20	25
	(Fertilization @ 10 ton/ha/yr)			(Fertilization @ 20 ton/ha/yr)		
Initial mean wt (g)	19.5	19.5	19.5	19.5	19.5	19.5
Final mean wt (g)	280.5±53.1[a]	424.7±66.1[d]	439.2±85.8[e]	502.5±55.63[f]	369.3±89.1[c]	300.3±63.6[b]
Survival (%)	31.9[cd]	21.5[a]	35.6[cd]	25.4[ab]	32.6[cd]	29.6[bc]
FCR	5.4[c]	4.8[c]	2.9[a]	3.5[ab]	3.8[b]	5.21[c]
Meat analysis (%)						
Moisture	77.8b±0.1[c]	77.1±0.1[ab]	79.0±0.3[d]	76.4±0.4[ab]	76.2±0.6[a]	77.6±0.5[b]
Crude protein	19.9±0.1[cd]	18.2±0.0[b]	17.4±0.1[a]	18.5±0.1[c]	18.6±0.0[cd]	18.7±0.0[d]
Ether extract	0.6±0.1[a]	2.2±0.0[c]	1.1±0.6[b]	2.3±0.1[e]	2.3±0.1[c]	0.7±0.1[a]
Ash	1.9±0.3	1.4±0.1	1.6±0.1	1.4±0.1	1.5±0.1	1.7±0.1

1. Mean ± standard deviation
2. Figures in the same row having same superscript or without superscript are not significantly different (p>0.05).

Table 7. Total count of plankton (number/L) in different treatments measured at different intervals in fertilized ponds during the experiment

Fertilization rate and plankton group	Treatment and Days																	
	15% Protein diet						20% protein diet						25% protein diet					
	0	30	55	70	90	Average	0	30	55	70	90	Average	0	30	55	70	90	Average
10 tons/ha/yr																		
Total zooplankton	686	390	168	1218	2406	974	686	640	646	875	1166	803	1031	323	487	938	1133	782
	(44.7)	(36.9)	(58.1)	(39.8)	(78.0)	(53.0)	(44.9)	(83.4)	(40.2)	(51.9)	(58.7)	(53.0)	(76.8)	(36.4)	(32.6)	(47.7)	(61.0)	(51.89)
Total phytoplankton	834	868	293	1844	677	865	843	127	959	812	819	712	312	565	1006	1031	725	728
	(55.1)	(63.6)	(63.6)	(60.2)	(22.0)	(47.0)	(55.1)	(16.6)	(59.8)	(48.1)	(41.3)	(47.0)	(23.2)	(63.6)	(67.4)	(52.4)	(39.0)	(48.2)
20 tons/ha/yr																		
Total zooplankton	3344	688	652	2249	2930	1973	1563	312	234	2874	4090	1815	1405	432	138	2874	4158	1801
	(93.8)	(58.4)	(35.0)	(62.1)	(66.4)	(69.3)	(70.4)	(73.1)	(53.4)	(61.7)	(69.6)	(66.6)	(61.5)	(82.8)	(51.1)	(58.6)	(69.5)	(64.5)
Total phytoplankton	220	90	1212	1375	1481	876	656	115	204	1781	1796	910	881	90	132	2031	1827	992
	(6.2)	(11.6)	(65.0)	(37.9)	(33.6)	(30.8)	(29.6)	(26.9)	(46.6)	(38.3)	(30.5)	(33.4)	(38.5)	(17.2)	(48.9)	(41.4)	(30.5)	(35.5)

Figures in parenthesis indicate percentage occurrence

Herbivorous fish generally possess higher amylase activity as compared to carnivorous fish (Phillips 1969; Das and Tripathi 1991). Its activity is known to exist throughout the intestine in Indian major carps (Das and Tripathi 1991). Efficient utilization of protein and carbohydrate appears to have occurred with the 25% protein and 37% carbohydrate diet. Proteolytic activity tends to vary depending on type of diet (Scherbina et al. 1976). Probably owing to the constant level of fat in all the diets, there was no marked variation in lipase activity between different treatments. Lipase activity is reported to be totally absent in catla, while in rohu and mrigal it is seen only in the anterior 1/5 of the intestine (Dhage 1968).

The results of the field trial involving both fertilization and feeding corroborate the findings of the first experiment, carried out without fertilization. Although there was progressive increase in growth with increasing protein level at 10 tons/ha fertilization, there was no significant difference in fish growth between 20 and 25% protein treatments. However, the increase in fertilization rate resulted in poor growth beyond 15% protein. This variation could be attributed to the level of protein contributed by the natural food produced through fertilization. However, these results require reconfirmation in view of the poor survival of the fish.

Sehgal and Toor (1991) reported that feeding rate in carps should be varied depending on the environmental parameters, particularly in relation to temperature, since feed assimilation and growth are largely dependent on these parameters. In order to ascertain the effect of environmental parameters, particularly temperature, long-term growth trials are essential. Further studies are in progress to determine the nutrient input through feed, taking into consideration the nutritional input through autotrophic, heterotrophic and saprophytic food chains.

Acknowledgements

We are grateful to the International Foundation for Science, Sweden (A/1003–2) and the Indian Council of Agricultural Research, New Delhi for the financial support to undertake this study. Part of the financial assistance was derived through the Indian Branch of the Asian Fisheries Society from a project supported by the International Development Research Centre, Canada.

References

AOAC, 1975. *Official Methods of Analysis, 12th ed.* Washington, DC, Association of Official Analytical Chemists, 1094 p.

APHA, 1985. *Standard Methods for Examination of Water and Waste Water, 16th ed.* Washington, DC, American Public Health Association, 1268 p.

Buckley, L.J. 1980. Changes in ribonucleic acid, deoxyribonucleic acid and protein content during ontogenesis in winter flounder *Pseudopleuronectes amoricauces* and effect of starvation. *US Fish. Bull.* 77: 703–708.

Das, K.M. and S.D. Tripathi, 1991. Studies on the digestive enzymes of grass carp *Ctenopharyngodon idella*. *Aquaculture* 92: 21–32.

Dhage, K.P. 1968. Studies of the digestive system enzymes in the three species of the major carps of India. *J. Biol. Sci.* 11: 63–74.

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

Khan, M.A. and A.K. Jafri, 1991. Protein and nucleic acid concentration in the muscle of the catfish *Clarias batrachus* at different protein levels. *Asian Fisheries Science* 4: 75–84.

Kunitz, M. 1947. Crystalline trypsin inhibitor II. General properties. *J. Gen. Physiology* 30: 297–10.

Mustafa, S. and A. Mittal, 1982. Protein RNA and DNA levels in liver and brain of stored catfish *Clarias batrachus*. *Japan. J. Ichthyol.* 28: 396–400.

Naher, G. 1974. Lipase titrimetric assay. In Bergmeyer H.V. (ed.) *Methods of Enzymatic Analysis. Vol. II, 2nd ed.* New York, Academic Press, pp. 814–817.

Phillips, A.M. 1969. Nutrition, digestion and energy utilization. In Hoar W.S. and D.J. Randall (eds.). *Fish Physiology, Vol I.* New York, Academic Press, pp. 391–432.

Renukaradhya, K.M. and T.J. Varghese, 1986. Protein requirement of the carps, *Catla catla* (Hamilton) and *Labeo rohita* (Hamilton). *Proc. Indian Acad. Sci.* (Anim. Sci.) 95 : 103–107.

Schneider, W.C. 1957. Determination of nucleic acids in tissues by pentose analysis. In Colowick S.P. and N.O. Kaplar (eds.) *Methods in Enzymology, Vol. III.* London, Academic Press, pp. 680–684.

Scherbina, M.A., L.N. Trofimova and O.P. Kazlaskene, 1976. The activity of protease and the intensity of protein absorption with the introduction of different quantities of fat into the carp *Cyprinus carpio*. *J. Ichthyol.* 16: 632–636.

Sehgal, H.S. and H.S Toor, 1991. Comparison of feeding strategies for common carp based on biomass and biomass pond interactions. In De Silva S.S. (ed.) *Fish Nutrition Research in Asia.* Manila, Philippines, Asian Fisheries Society, pp. 181–193.

Sen, P.R., N.G.S. Rao, S.R. Ghosh and M. Rout, 1978. Observations on the protein and carbohydrate requirement of carps. *Aquaculture* 13: 245–255.

Singh, B.N. and K.J. Bhanot, 1988. Protein requirement of *Catla catla* (Ham.). In Mohan, J. (ed.) *The First Indian Fisheries Forum Proceedings*. Mangalore, Asian Fisheries Society, Indian Branch, pp. 77–78.

Singh, B.N., V.R.P. Sinha and K. Kumar, 1987. Protein requirement of Indian major carp, *Cirrhinus mrigala* (Ham.). *Intl. J. Acad. Ichthyol.* 8: 71–75.

Sinha, V.R.P. 1991. Aquaculture in Asian region. In Sinha, V.R.P. and H.C. Srivastava (eds.) *Aquaculture Productivity*. New Delhi, Oxford & IBH Publishing Co., pp 33–47.

Srivastava, U.K., B.H. Dholakia, S.S. Rao and S. Vatsala, 1990. *Evaluation of Fish Farmers' Development Agency Programme for Freshwater Aquaculture*. India, Indian Institute of Management, 252 p.

Sumner, J.B. 1992. *J. Biol. Chem.* 62: 287 p. (Cited: Bernfield, 1955). Sumner, J.B. and Somers, H.F. 1947. *Chemistry and Methods of Enzymes, 2nd ed.* New York, Academic Press.

Veerina, S.S., M.C. Nandeesha, K Gopal Rao and S.S. De Silva, 1992. *Status and Technology of Indian Major Carp Farming in Andhra Pradesh, India*. Special Publication No. 9. Asian Fisheries Society, Indian Branch, (in press).

Use of Water Hyacinth (*Eichornia crassipes*) as Supplementary Feed for Nursing Fish in Vietnam

NGUYEN ANH TUAN
NGUYEN QUANG THUY
BUI MINH TAM
VO VAN UT
Faculty of Fisheries
University of Cantho
Ho Chi Minh City
Vietnam

TUAN, N.A., N.Q. THUY, B.M. TAM and V.V. UT. 1994. Use of water hyacinth, *Eichornia crassipes*, as supplementary feed for nursing fish in Vietnam. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Manila, Philippines. Asian Fisheries Society, pp. 101–106.

Abstract

Water hyacinth (*Eichornia crassipes*) has been used in fish culture for many years in many countries. Four experiments using water hyacinth as supplementary feed for nursing fish were conducted in hapa-in-pond conditions in the Mekong Delta, Vietnam. Fingerlings of (1–6 g) of Nile tilapia (*Oreochromis niloticus*), common carp (*Cyprinus carpio*), grass carp (*Ctenopharyngodon idellus*), and puntius carp (*Puntius gonionotus*) were used for this experiment. The fish were stocked in 9 (or 12) hapas, of 1 × 1 × 1 m, with a density of 30 fish/hapa. Chopped water hyacinth mixed with raw rice bran, and fermented rice bran, was used as feed. A treatment using rice bran alone was the control.

Data obtained during five weeks of nursing showed that there was no significant difference among the treatments in grass carp and puntius. However, there was a significant difference between the performance of common carp fed the water hyacinth-rice bran mixture and those fed the control treatment. A significant difference was also found between Nile tilapia fed the treatments of rice bran-water hyacinth mixture and the fermented mixture.

Water quality parameters, natural food populations and some economical aspects of the use of water hyacinth will be discussed in this paper.

Introduction

The rapid expansion of aquaculture in the Mekong Delta, Vietnam is creating a shortage of fish fingerlings and feed supplies. Many family-scale fish hatcheries are in a phase of construction and this together with the privatization of state hatcheries will probably produce a bigger quantity of fingerlings for aquaculture. However, in terms of feed supplies fish farmers are still facing problems. In fact, as in other developing areas, fish culture in the Mekong Delta is based on the technique of low input. Cultured fish are characterized as omnivorous, herbivorous and planktivorous feeders which do not need costly feed. Since agricultural by-products, i.e. rice bran, broken rice and manures are the main input to such cultivation, there is competition between animal husbandry and fish culture for food, and therefore, a shortage of feed supplies sometimes occurs seasonally, reducing fish production. To overcome the problem pellet feeds may be the answer.

However, because of its high price such feeds are suitable only for shrimps or other exported fish. Poor farmers in rural areas must look for cheaper methods to carry out their aquaculture activities.

Water hyacinth (*Eichornia crassipes*), raw, composted or pelleted has been tried as supplementary feed for pond culture (Edwards 1981; Edwards et al. 1983; Kamal 1983; Vareesajja 1984; Kwang 1985; Pongsri 1985; Gopal 1987). Fish production from these trials looked very promising and was comparable to other feeds. Therefore, use of water hyacinth as pond input was suggested for aquaculture in rural areas, particularly in the Mekong Delta where water hyacinth grows freely. Undoubtedly making use of water hyacinth for pond culture is an important way not only to increase fish production for poor farmers but also to expand aquaculture in this region.

In the past few years some case studies where water hyacinth was used for fish culture have been carried out in the Mekong Delta. The following results were obtained from nursing experiments in which water hyacinth in different forms was the main input.

Materials and Methods

Four fish species—common carp (*Cyprinus carpio*), Nile tilapia (*Oreochromis niloticus*), grass carp (*Ctenopharyngodon idellus*) and puntius carp (*Puntius gonionotus*) of fingerling size (1–6 g/fish) were used for the experiments. The fish were supplied by the fish hatchery of Cantho University. Each group was from one batch. They were acclimated for 48 hours for hapa conditions before experimentation.

Twelve hapas, sized 1 × 1 × 1.4 m (1 m immersed in water), each containing 30 fish, were set in ponds for the experiments. All experiments followed complete randomized design and lasted five weeks.

Newly harvested water hyacinth was chopped into small pieces, and used either to feed the fish directly or mixed with rice bran at 1:1 and 1:2 ratios or fermented for other treatments. The proximate composition of different feeding treatments is presented in Table 1.

Table 1. Proximate composition of feeding treatments (g/100 g)

Treatments	Moist	Protein	Lipid	Ash	Fibre	Carbohydrate
(i)						
Rice bran	11.19	11.89	14.93	5.05	50.55	6.41
% dry basis	0.00	13.51	16.96	5.71	56.54	7.28
(ii)						
Water hyacinth	94.18	0.87	0.15	1.98	1.76	1.06
% dry basis	0.00	13.44	2.51	34.06	31.78	18.25
(iii)						
RB + WH (1:1)	55.35	6.03	7.46	3.78	24.05	3.30
% dry basis	0.00	15.50	16.72	8.47	53.99	7.39
(iv)						
RB + WH (1:2)	67.30	5.58	4.98	3.60	16.67	2.90
% dry basis	0.00	14.99	15.24	10.99	51.20	8.59
(v)						
fermented RB + WH (1:2)	68.63	4.74	4.75	3.40	15.60	2.86
% dry basis	0.00	15.11	15.16	10.84	49.74	9.15

RB = Rice bran WH = water hyacinth

Fish were fed to satiation once daily. The growth and survival rates were determined every seven days. On the same day water quality parameters (dissolved oxygen, CO_2, pH, temperature, transparency) and planktonic organisms (phytoplankton and zooplankton) were also checked and recorded.

Statistical comparisons of the results were processed by using analysis of variance. Duncan's multiple range test was used to evaluate the difference between means of individual diets at 0.05 significant level.

Results

Water quality and planktonic organisms

Table 2 presents some important parameters that might affect the growth and survival rates of experimental fish. Attention was paid to the fluctuation of dissolved oxygen that sometimes dropped down to levels less than 1 ppm. However, the low levels of dissolved oxygen occurred only in the morning and did not last long, and fish kills were not recorded. The natural food population in terms of phytoplankton showed a low fertility in the pond water. This is reasonable because the hapas were set in a large canal in a rice field which had not been used for fish culture. In general, the data on water quality and natural food fluctuated during the experimental period, but were still in a suitable range in which the fish could grow normally. Therefore the results are thought to be due to the effects of experimental treatments.

Table 2. Some water quality parameters and planktonic population

Mean Values	Experiment Number/Species			
	1 C. carp	2 N. tilapia	3 G. carp	4 P. carp
Temperature (°C)	32.5	31	30	31.5
pH	7.2	7.3	7.2	7.0
Transparency (cm)	30	34	40	28
Oxygen (mg/L)	1.54 (0.85–2.88)	1.73 (0.73–3.08)	1.46 (0.79–2.05)	1.36 (0.75–2.01)
CO_2 (mg/L)	17.43	18.97	20.82	19.29
Phytoplankton (individual/L)	6900	5800	4600	4200
Zooplankton (body/L)	560	530	330	290

Experiment 1

Common carp (*Cyprinus carpio*) were stocked in hapas for this experiment. They were fed one of three diets:

Diet (i): Rice bran
Diet (iv): Rice bran + Water hyacinth, ratio 1:2
Diet (v): Rice bran + Water hyacinth, ratio 1:2, fermented

There were significant differences in all of the mean values between fish fed with diet (i) and diet (iv). Fish fed diet (i) (rice bran) performed better compared to those fed raw rice bran and water hyacinth mixture (Table 3). However with diet (v) where rice bran and water hyacinth were mixed at a ratio of 1:2, then fermented, the performance was not significantly different from the other two treatments.

Table 3. Growth performances of common carp fed with different water hyacinth-containing diets

Mean Values	Diets		
	(i)	(iv)	(v)
Initial weight (g)	3.34	3.34	3.34
Final weight (g)	9.59^{a}	8.39^{b}	8.95^{ab}
Percentage weight gain (%)	187.3^{a}	151.4^{b}	186.1^{ab}
Daily weight gain (g/day)	0.17^{a}	0.14^{a}	0.15^{ab}
Specific growth rate (%/day)	2.85^{a}	2.49^{b}	2.66^{ab}
Food Conversion Ratio	13.0a	15.7^{b}	13.8^{ab}

Note: Figures in the same row having the same superscripts were not significantly different ($p<0.05$).

Experiment 2

Nile tilapia (*Oreochromis niloticus*) were used for this experiment where four different diets were applied:

Diet (i): Rice bran
Diet (iii): Rice bran + Water hyacinth, ratio 1:1
Diet (iv): Rice bran + Water hyacinth, ratio 1:2
Diet (v): Rice bran + Water hyacinth, ratio 1:2, fermented

There were no significant differences in all values obtained with Nile tilapia fed the control treatment (diet (i), rice bran) compared to the other three dietary treatments (Table 4). There were also no significant differences between fish fed diet (iv) and diet (v), even though fish on diet (v) performed a little better than fish on diet (iv). At the same time, significant differences were recorded in fish on diet (iii) compared to fish on diets (iv) and (v). Diet (iii) consistently produced better results than other diets.

Table 4. Growth performances of Nile tilapia fed with different water hyacinth-containing diets

Mean Values	Diets			
	(i)	(ii)	(iv)	(v)
Initial weight (g)	1.89	1.89	1.89	1.89
Final weight (g)	10.46^{ab}	11.19^{b}	9.39^{a}	9.76^{a}
Percentage weight (gain)	453.6^{ab}	492.4^{b}	397.0^{a}	416.0^{a}
Daily weight gain (g/day)	0.23^{ab}	0.25^{b}	0.20^{a}	0.21^{a}
Specific growth rate (%/day)	4.61^{ab}	4.81^{b}	4.33^{a}	4.43^{a}
Food Conversion Ratio	7.70^{ab}	6.96^{b}	8.46^{a}	8.16^{a}

Note: Figures in the same row having the same superscripts were not significantly different ($p< 0.05$).

Experiment 3

The experiment was conducted with grass carp (*Ctenopharyngodon idellus*) which were fed one of four different diets.

Diet (i): Rice bran
Diet (ii) Raw water hyacinth
Diet (iv): Rice bran + water hyacinth, ratio 1:2
Diet (v): Rice bran + water hyacinth, ratio 1:2, fermented

There were no significant differences between fish fed the four different diets (Table 5). At the small fingerling stage (1 g) grass carp probably did not prefer plant materials. Therefore, it seemed to be a trend to gain better results from fish fed the control treatment.

Table 5. Growth performances of grass carp fed with different water hyacinth-containing diets

Mean Values	Diets			
	(i)	(ii)	(iv)	(v)
Initial weight (g)	0.75	0.75	0.75	0.75
Final weight (g)	3.28^{a}	2.99^{a}	3.13^{a}	3.13^{a}
Percentage weight gain (%)	337.8^{a}	299.0^{a}	316.9^{a}	316.4^{a}
Daily weight gain (g/day)	0.075^{a}	0.065^{a}	0.069^{a}	0.069^{a}
Specific growth rate (%/day)	4.33^{a}	4.06^{a}	4.18^{a}	4.19^{a}
Food Conversion Ratio	21.9^{a}	24.9^{a}	24.0^{a}	24.0^{a}

Note: Figures in the same row having the same superscripts were not significantly different ($p<0.05$).

Experiment 4

Puntius gonionotus was stocked and fed one of three different diets.

Diet (i): Rice bran
Diet (iv): Rice bran + Water hyacinth, ratio 1:2
Diet (v): Rice bran + Water hyacinth, ratio 1:2, fermented

Values obtained from fish fed with rice bran (diet (i)) were normally higher than those obtained from diets (iv) and (v). However, there were no significant differences between the three diets (Table 6).

Table 6. Growth performances of *Puntius gonionotus* fed with different water hyacinth-containing diets

Mean Values	Diets		
	(i)	(iv)	(v)
Initial weight (g)	1.46	1.46	1.46
Final weight (g)	4.18[a]	3.90[a]	4.10[a]
Percentage weight gain (%)	86.3[a]	163.9[a]	178.8[a]
Daily weight gain (g/day)	0.080[a]	0.070[a]	0.076[a]
Specific growth rate (%/day)	3.09[a]	2.84[a]	3.00[a]
Food Conversion Ratio	25.0[a]	27.5[a]	27.3[a]

Note: Figures in the same row having the sme superscripts were not significantly different ($p<0.05$).

Discussion

On the basis of fish growth, different forms of water hyacinth used as supplementary feed in the nursing of common carp, Nile tilapia, grass carp and puntius carp were comparable to rice bran which has been normally applied to nursing ponds in Vietnam. In terms of percentage weight gain, daily weight gain and specific growth rate, water hyacinth mixed with rice bran at a ratio of 2:1 either raw or fermented could be used to replace rice bran in nursing ponds. In terms of species, only Nile tilapia performed better when fed on water hyacinth. They grew quite fast with a specific growth rate of 4.3–4.8 %/day. At the same time, grass carp which is known as a herbivorous feeder seemed not to perform well with water hyacinth. Grass carp grew faster with rice bran than water hyacinth-containing diets, however, again all of the growth values were not significantly different.

Different species utilized different levels of water hyacinth as their supplementary feed. From the specific growth rate point of view, Nile tilapia increased 4.43%/day, followed by grass carp (4.19%/day), then puntius (3.00%/day) and common carp (2.66%/day) if the fermented mixture of water hyacinth and rice bran (ratio 2:1) was given. Values of the food conversion ratio also indicated differences in water hyacinth utilization. Nile tilapia showed the best results.

Table 7. Cost of feed and unit weight gain with different species (dong/kg)

Species	Diets				
	(i)	(ii)	(iii)	(iv)	(v)
Common carp	9 748	—	—	3 375	3 960
Nile tilapia	5 778	—	2 609	2 115	2 540
Grass carp	16 433	1 245	—	6 020	6 520
Puntius	18 818	—	—	6 790	7 320

Note: Exchange rate was 12 000 dong/USD

It is important to mention that because water hyacinth could be harvested freely, using it kept the feed cost quite low. Cost of diet (iv) and diet (v) was only 35% and 40% of diet (i), and therefore the cost of unit weight gain looks very attractive (Table 7). With Nile tilapia for example, it was 2 115 dong (diet (iv)) and 2 540 dong (diet (v)) compared with 5 778 dong (diet (i)).

In general, data obtained from the above experiments showed the feasibility of using water hyacinth for fish nursing is very good.

References

Edwards, P. (1981). Water weeds as fish feed—a potential weed control technique for Indonesia. A trip report to UNESCO in 1981, 14 pp.

Edwards, P., C. Polprasert, C. Pacharaprakiti, V.S. Rajput and S. Suthirawut, (1983). Compost as fish feed, a practical application of detritivory for the cultivation of tilapia. *Aquaculture* 32: 409–413.

Gopal, B. (1987). *Water Hyacinth—Aquatic Plant Studies 1*. Elsevier Publication, pp. 259–298.

Kamal, M. (1983). Pelleted water hyacinth as fish feed. MSc. Thesis. Bangkok, Thailand, Asian Institute of Technology.

Kwang, L.C. (1985). Water hyacinth (*Eichornia crassipes*) as a pond input for nile tilapia culture. MSc. Thesis. Bangkok, Thailand, Asian Institute of Technology.

Pongsri, C. (1985). Water hyacinth as a protein source in pelleted feed for tilapia. MSc. Thesis. Bangkok, Thailand, Asian Institute of Technology.

Vareesajja, S.C. (1984). Nutritive value of composed water hyacinth in pelleted feed for tilapia. MSc. Thesis. Bangkok, Thailand, Asian Institute of Technology.

Further Studies on Feeding Strategies for Common Carp: Role of Pond Area in Deciding Ration Size

HARJEET S. SEHGAL
H.S. TOOR
Fisheries Research Complex
Department of Zoology
Punjab Agricultural University
Ludhiana 141 004
India

SEGHAL, H. S. and H.S. TOOR, 1994. Further studies on feeding strategies for common carp: Role of pond area in deciding ration size. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 107–114.

Abstract

This study was made to determine the effect of pond size on feeding levels to achieve maximum fish growth (G_{max}). The experiment was based on a 330-day culture of common carp, *Cyprinus carpio* var. *communis* in experimental ponds of 0.002, 0.008, and 0.065 ha area (20 m^2, 80 m^2, and 650 m^2, respectively) manured with 18 tonnes ha^{-1} yr^{-1} of a mixture of cow dung (1 part) and poultry droppings (3 parts) (w/w basis). The supplementary diet fed to the fish consisted of rice bran, groundnut oil-cake and fish meal and had crude protein, total carbohydrate and total lipid contents of 35, 27, and 5.7%, respectively. A direct relationship was observed between pond size and fish growth in terms of average fish biomass (AFB), net weight gain (NWG), average daily weight gain (ADG) and specific growth rate (SGR). The relationship was significant during the middle stanza of growth extending from 135 to 240 days of the culture which corresponded to favourable water quality and plankton production in the culture ponds. However, in ponds which did not receive any supplementary feeding, the increase in the size of pond had no significant effect on fish growth. Similarly, in ponds of minimum area (0.002 ha; 20m^2), differences in the growth of fish fed 0, 1, 3 and 6% of their biomass were nonsignificant. Computer-aided regression models have been developed to predict desired ration size in relation to pond area.

Introduction

The economic viability of an aquaculture system depends to a large extent on reasonable feeding costs. Supplementary feeding is the single highest recurring cost in intensive and semi-intensive culture systems, forming approximately half of the total cost of fish farming. Thus, feeding charts/schedules that will best suit local farming conditions must be based on sound nutritional principles taking into account most of the theoretical aspects of fish nutrition (Hepher 1988).

Several studies done in the recent past to make supplementary feeding cost-effective have been directed either to substitute the high cost fish meal with some less expensive protein sources (Matty and Smith 1978; Beck et al. 1979; Jackson et al. 1982; Winfree and Stickney 1984; Appler 1985; Sehgal and Thomas 1985, 1987; Wee and Wang 1987; De Silva and Gunasekera 1989; Sehgal and Sharma 1991, 1993) or to identify water quality parameters such as temperature and salinity which would allow maximum food consumption and/or conversion efficiency (De Silva and Perera 1985).

There has not been much emphasis on improving feeding practices to lower the cost of supplementary feeding. Traditional carp feeding is by and large based upon the biomass of fish to be fed in a pond. Although pond ecological conditions; physical structure and nutritional value of the feed; and availability and quality of the natural food play an important role in determining consumption rates and digestibility of the feed consumed (Brett 1979; Brett and Groves 1979), these parameters are usually not considered in deciding ration size. Lovell (1977) incorporated temperature, in a rough manner, in the feeding schedules for channel catfish. It was only recently that we made an attempt to incorporate pond ecological conditions, including natural food in stepwise regression models to formulate cost-effective feeding strategies for common carp based on fish biomass and pond ecology, rather than on the basis of fish biomass alone (Sehgal and Toor 1991).

In our continued effort to identify the parameters that have potential roles to play in developing cost-effective feeding charts/schedules for carp under a particular set of farm conditions the present experiment was designed to study the role of pond area in deciding ration size and the possibility of using this parameter in improving the predictive ability of the models developed earlier. Economics of fish biomass based and fish biomass-pond area-pond ecology based feeding strategies for carp, *Cyprinus carpio communis* are compared.

Materials and Methods

The studies were conducted at the Fisheries Research Complex of the Punjab Agricultural University, Ludhiana, India from 10 April 1990 to 5 March 1991 (330 days of culture). The experiments were conducted in twelve rectangular ponds of three different sizes, 20 m^2 (0.002 ha), 80 m^2 (0.008 ha) and 650 m^2 (0.065 ha). A description of these treatments is given in Table 1. Each experimental pond was manured with 18 000 kg ha^{-1} yr^{-1} of cow dung (1 part) and poultry manure (3 parts) (w/w basis) fifteen days before starting the experiment, when one tenth of the manure was applied. The rest of the manure was added in equal instalments twice a week. Thirty-day old common carp, *Cyprinus carpio* raised in our hatchery were stocked to correspond to 10 000 ha^{-1} of the experimental ponds. The mean weight at stocking was 1.55 ± 0.08 g.

Table 1. Description of various treatments used in the present study

Treatment code	Description	
	feeding level*	Pond area (ha)
T1	0	0.002
T2	1	0.002
T3	3	0.002
T4	6	0.002
T5	0	0.008
T6	1	0.008
T7	3	0.008
T8	6	0.008
T9	0	0.065
T10	1	0.065
T11	3	0.065
T12	6	0.065

*Percent dry feed of fresh fish biomass

The supplementary feed used in the present study was prepared by mixing most commonly used ingredients such as rice bran, groundnut oil-cake, and fish meal. The vitamin and mineral premixes were used as recommended by NRC (1983). The proximate composition of the feed was: crude protein (CP)—35%, total carbohydrates (TC)—27%, total lipids (TL)—5.7% and gross energy (GE)—3.62 kcal g^{-1}. The proximate composition of the diet was analyzed by the methods recommended by AOAC (1984). The gross energy was estimated by ascribing CP 5.7 kcal; TC 4 kcal and TL 9.5 kcal g^{-1} of the respective nutrient (Higgs et al. 1985). Modified Winkler's method was used for the estimation of dissolved oxygen (APHA 1976) and pH was recorded with a 'Systronics' pH meter. Phyto-and zooplankton were estimated by the methods recommended by APHA (1976).

Growth was measured in terms of average fish biomass (AFB), net weight gain (NWG), average daily weight gain (ADG) and specific growth rate (SGR). The values of AFB for 0–45 days are based on a combined sample of 15 randomly collected fish from each treatment and on individual weight of 15 fish from the 60th day onwards. The ADG and SGR were calculated according to the following formulae:

$$ADG = \frac{W_T - W_t}{T - t} \qquad SGR = \frac{\text{Log}_e W_T - \text{Log}_e W_t}{T - t} \times 100$$

where W_T is the final weight at time T and W_t is the initial weight at time t.

On the basis of the data collected on growth in experimental ponds of different sizes and associated pond ecological factors, the following four types of statistical analyses were performed.

1. Two-way ANOVA and multiple range test: This test was used to determine the significance of difference between the treatments and the interaction between pond area and ration size with respect to their effects on fish growth.
2. One-way ANOVA and multiple range test: One-way ANOVA was used to determine the significance of difference among the treatments (ration levels and pond area) with respect to NWG, ADG and SGR. The multiple range test was used to find out homogeneous groups of treatments.
3. Polynomial regression: On the basis of the differences among effects of ration size on the growth of fish during different culture periods and in ponds of different areas (as determined by the one-way ANOVA), second order polynomial regression was used to determine maximum and optimum ration sizes during different periods of the culture and in ponds of different areas.
4. Step-wise regression analysis (forward method): Based on the individual relationships of ADG to various water-quality parameters, fish age and pond area, most of which were found highly signficant; step-wise variable selection regression (forward method) was used to develop models for predicting the desired ration size for a particular set of pond area and ecological conditions.

All calculations were made on an IBM compatible PC AT (386) using STATGRAFICS and SIGMAPLOT statistical packages.

Results and Discussion

Ration size-pond area interactions and growth

Two-way ANOVA of the data based on 330 days of culture revealed that both ration size and pond area had significant effects on the growth of the fish. The fish grew best on a ration size of 6% of their biomass followed by the 3, 1 and 0% ration levels. The growth of the fish was also affected by the area of the experimental pond. The best growth occurred in ponds of 0.065 ha (650 m^2) followed by 0.008 ha (80 m^2) and 0.002 (20 m^2) ponds. The differences were statistically significant ($p < 0.0001$). The interaction between ration size and pond area with respect to their effect on fish growth was also significant ($p < 0.001$) suggesting that the fish growth was affected by pond area and ration size rather than by either of the two factors alone.

Exceptionally, however (as determined by one-way ANOVA), there was no significant difference in the growth of fish with respect to an increase in pond area if no supplementary feed was provided. Similarly, in the ponds of smallest area (0.002 ha), an increase in ration level from 0 to 6% did not cause significant differences in fish growth (Table 2).

The results, therefore, suggest that pond area can be used as an important parameter in deciding desired ration levels (in addition to the pond ecological conditions as established in our earlier study, Sehgal and Toor 1991). Since the feeding levels have been found to vary significntly during the three growth stanzas, S_1 (0–135 days), S_2 (135–240 days) and S_3 (240–330 days of the culture) (Sehgal and Toor 1991), determination of ration levels in relation to pond size were also considered separately for these growth stanzas.

Table 2. Growth performance of carp *Cyprinus carpio communis* (L.) on different ration sizes in ponds of different areas

Growth parameter	Pond area (ha)	Ration size (%)			
		0%	1%	3%	6%
0–330 days					
Mean initial weight (g)	0.002	1.5	1.68	1.5	1.65
	0.008	1.56	1.56	1.55	1.56
	0.065	1.42	1.65	1.5	1.5
Mean final weight (g)	0.002	164.04	209.58	232.5	195.99
	0.008	175.98	239.16	429.99	516.36
	0.065	173.02	312.14	584.19	679.4
ADG ($g\ d^{-1}$)	0.002	0.50^a	0.63^a	0.70^a	0.59^a
	0.008	0.53^a	0.72^b	1.36^b	1.32^b
	0.065	0.52^a	0.95^c	1.77^c	2.06^c
SGR (% W d^{-1})	0.002	1.42^a	1.46^a	1.53^a	0.45^a
	0.008	1.43^a	1.52^b	1.70^b	1.76^b
	0.065	1.60^a	1.59^c	1.81^c	1.86^c
0–135 days					
Mean net weight gain (g)	0.002	68.34^a	78.17^a	76.54^a	75.09^a
	0.008	76.02^a	83.30^b	79.09^a	83.43^a
	0.065	72.36^a	82.34^a	85.44^a	83.00^a
ADG ($g\ d^{-1}$)	0.002	0.51^a	0.58^a	0.57^a	0.56^a
	0.008	0.56^a	0.62^a	0.59^a	0.62^a
	0.065	0.54^a	0.61^a	0.63^a	0.61^a
SGR (% W d^{-1})	0.002	2.84^a	2.86^a	2.93^a	2.84^a
	0.008	2.89^a	2.96^a	2.93^a	2.96^a
	0.065	2.83^a	2.91^a	2.98^a	2.98^a
135–240 days					
Mean net weight gain (g)	0.002	73.50^a	108.10^a	130.20^a	104.00^a
	0.008	77.70^a	119.70^b	303.50^b	300.30^b
	0.065	79.80^a	185.90^c	412.70^c	491.40^c
ADG ($g\ d^{-1}$)	0.002	0.70^a	1.03^a	1.24^a	0.99^a
	0.008	0.74^a	1.14b	2.89^b	2.86^b
	0.065	0.76^a	1.77^c	3.93^c	4.68^c
SGR (% W d^{-1})	0.002	0.69^a	0.81^a	0.93^a	0.83^a
	0.008	0.68^a	0.84^a	1.50^b	1.43^b
	0.065	0.70^a	1.12^b	1.58^c	1.84^c
240–330 days					
Mean net weight gain (g)	0.002	20.70^a	20.70^a	23.40^a	15.30^a
	0.008	20.70^a	35.10^b	45.90^b	52.20^b
	0.065	18.90^a	42.30^b	84.60^c	103.50^c
ADG ($g\ d^{-1}$)	0.002	0.23^a	0.23^a	0.26^a	0.17^a
	0.008	0.23^a	0.39^b	0.51^b	0.58^b
	0.065	0.21^a	0.47^b	0.94^c	1.15^c
SGR (% W d^{-1})	0.002	0.15^a	0.12^a	0.12^a	0.09^a
	0.008	0.15^a	0.17^b	0.12^a	0.14^a
	0.065	0.13^a	0.16^b	0.17^b	0.18^c

Values with the same superscript with respect to a single parameter and a single ration size do not differ significantly.

The application of second order polynomial regression analysis to the 330-day culture data on carp growth suggested a significant relationship between ration size and fish growth in ponds of all sizes. The R_{max} values were calculated to be 3.32, 5.54 and 6.71%, in ponds of 0.002, 0.008 and 0.0065 ha, respectively which corresponded to G_{max} of 1.52, 1.76 and 1.86% W d^{-1}, respectively. The R_{opt} values for this period were computed to be 2.58, 2.79 and 2.80% in the ponds of 0.002, 0.008 and 0.065 ha, respectively (Figures 1a to 1c). This relationship was found non-significant on the basis of the data for S_1 (Figures 2a to 2c).

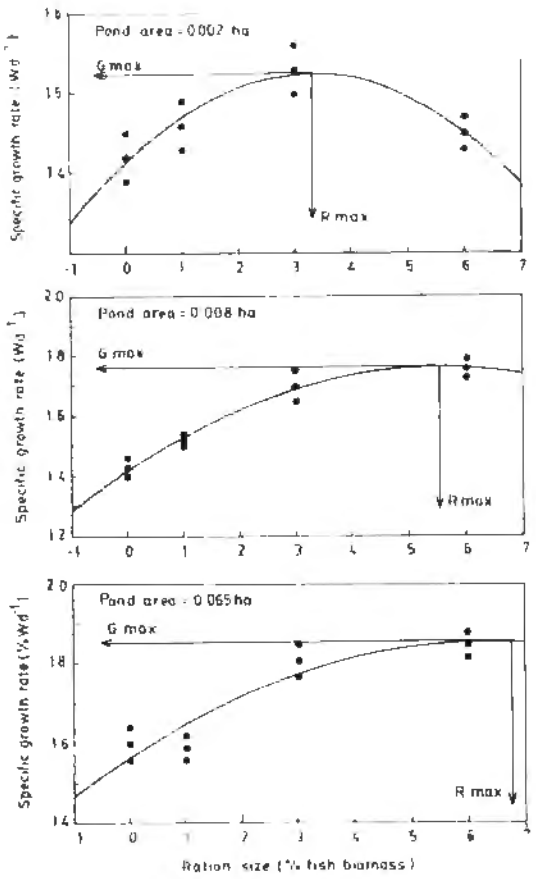

Figure 1. Relationships of carp growth (specific growth rate) to ration size during 0–330 days of the culture. R_{max} and G_{max} refer to maximum ration size and the corresponding maximum growth, wheras R_{opt} and G_{opt} refer to optimum ration size and optimum growth, respectively

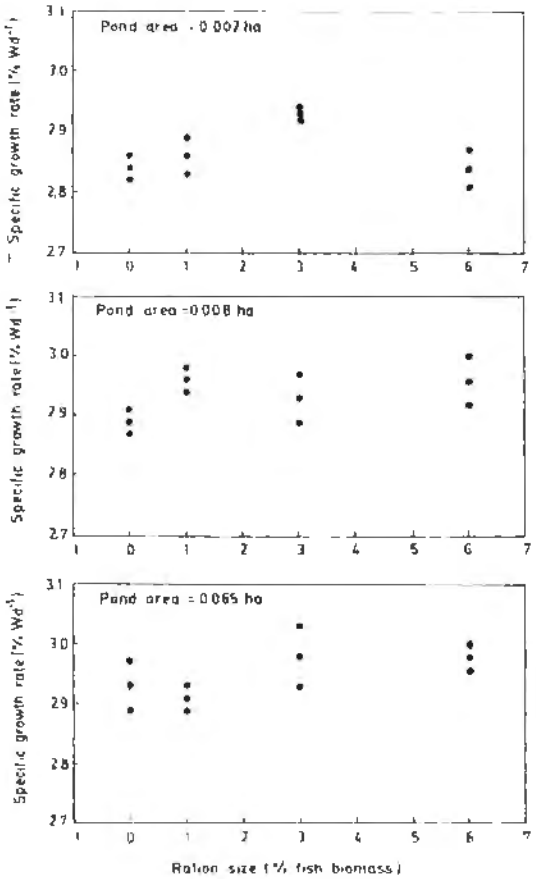

Figure 2. Relationships of carp growth (specific growth rate) to ration size during 0–135 days of the culture. R_{max} and G_{max} refer to maximum ration size and the corresponding maximum growth whereas R_{opt} and G_{opt} refer to optimum ration size and optimum growth, respectively

This suggested that no feeding was required during this period in ponds of any size between 0.002 and 0.065 ha. On the other hand, the growth (SGR)-ration (GR) curves based on the data of S_2 observed steep rises with an increase in the ration sizes in ponds of all sizes (Figures 3a to c). The R_{max} and G_{max} in ponds of 0.002, 0.008 and 0.065 ha were found to be 3.61, 4.57, 5.58% and 0.94, 1.55 and 1.84% W d^{-1}, respectively. Similarly, the R_{opt} and G_{opt} in ponds of these areas were estimated to be 2.76, 3.0, and 2.93% and 0.92, 1.4 and 1.56% W d^{-1}, respectively. The GR curves based on data from the last growth stanza in ponds of 0.002 and 0.008 ha area rather suggested a negative relationship between fish growth and ration size (Figures 4a, 4b). In ponds of bigger area, ie. 0.065 ha, the GR curve showed rise with an increase in the ration size. The R_{max} and G_{max} were calculated to be 4.64% and 0.18% W d^{-1}, respectively and the Ropt and Gopt to be 3.92% and 0.176% W d-1, respectively (Figure 4c).

Hence, it becomes clear that the requirements for rations to attain maximum growth of common carp would vary considerably in ponds of different sizes as well as during periods of culture. The non-significant differences in fish growth during S_1 were possibly due to unfavourable temperatures (29 to 35°C) which probably adversely affected feed consumption and/or were due to the availability of sufficient natural food to satisfy the relatively small fish biomass in the ponds of all sizes. The unfavourable water quality again seems to be responsible for the inability of increased ration size in promoting growth in ponds varying between 0.002 to 0.008 ha area during last growth stanza (S_3). Although the relationship between ration size and fish growth was positive in ponds of 0.065 ha area, the increase in fish growth was too little.

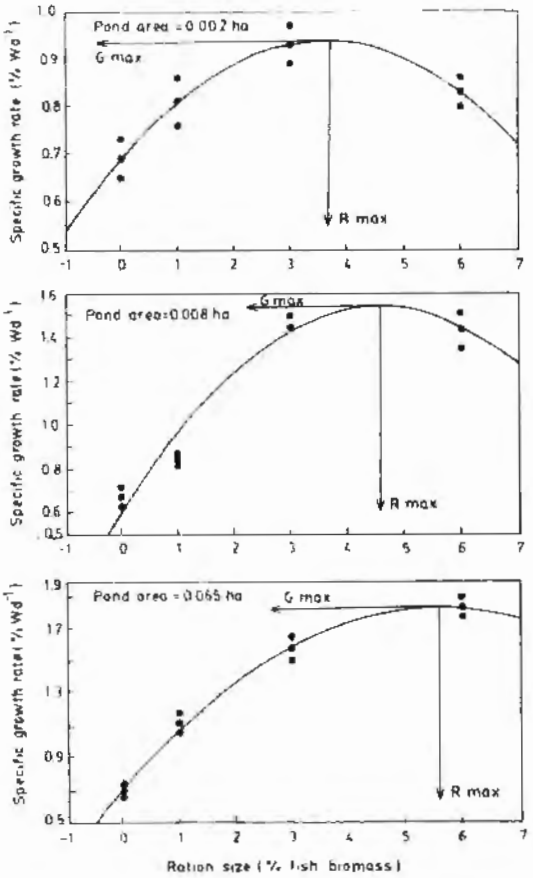

Figure 3. Relationships of carp growth (specific growth rate) to ration size during 135–240 days of the culture. R_{max} and G_{max} refer to maximum ration size and the corresponding maximum growth, wherEas R_{opt} and G_{opt} refer to optimum ration size and optimum growth, respectively

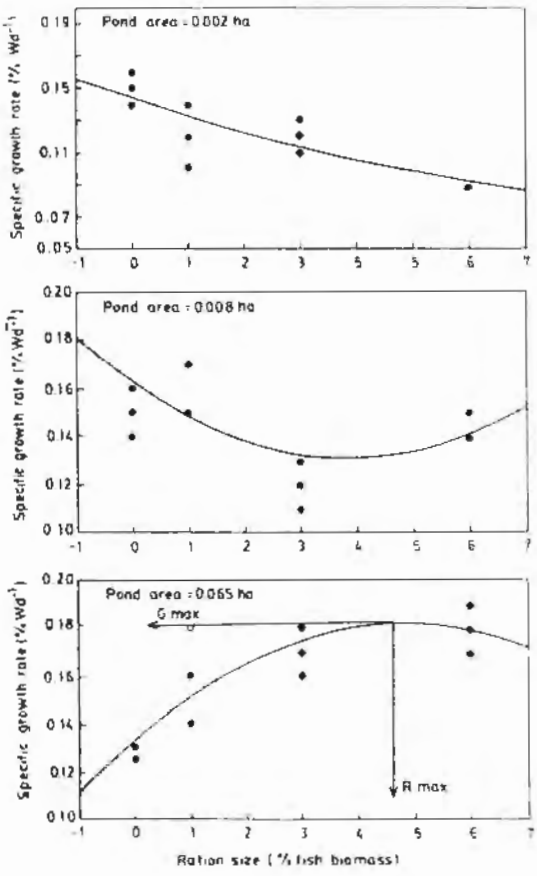

Figure 4. Relationships of carp growth (specific growth rate) to ration size during 240–330 days of the culture. R_{max} and G_{max} refer to maximum ration size and the corresponding maximum growth whereas R_{opt} and G_{opt} refer to optimum ration size and optimum growth, respectively

Pond area and ration size

In addition to many ecological conditions (which are known to influence fish growth) such as temperature (McCormick et al. 1972; Elliott 1975; Sehgal and Toor 1991), dissolved oxygen (Doudoroff and Shumway 1967), Sehgal and Toor 1991), pH, phytoplankton and zooplankton (Sehgal and Toor 1991), the pond area, which showed significant relationship with fish growth, was considered for incorporation in the step-wise regression models. The other parameters considered were the age and biomass of fish which form the basis of traditional fish feeding strategies. The pond area was selected by the regression models only during the middle growth stanza (S_2) when growth rates were maximum due to favourable ecological conditions. During the first growth stanza (S_1), the ration size was suggested to be determined by the dissolved oxygen and total zooplankton count in the pond irrespective of the pond area. It was only during the last growth stanza (S_3) that fish biomass was found responsible for the determination of ration size (Table 3).

Table 3. The multiple (step-wise variable selection) regression relationships between ration size and different parameters

Culture period	Tank condition	Relationship	p<
0–135 days	Manured	Ration size = –0.17 + 0.48 DO – 0.01 TZ	0.001
135–240 days	Manured	Ration size = –4.89 + 10.02 PA + 0.75 DO + 0.01 TZ + 0.15 T	0.01
240–330 days	Manured	Ration size = –2.19 + 0.01 FB	0.001

Ration size = Percent (dry feed) of fresh fish biomass; DO = dissolved oxygen (mg/L); TZ = total zooplankton (no./L); PA = pond area (ha); T = temperature (°C); FB = Average fish biomass (g).

Feeding strategies

In continuation of our earlier effort to quantify the role of pond ecological conditions, besides fish biomass and fish age, in deciding ration size during different phases of fish growth in grow-out ponds (Sehgal and Toor 1991), the role of pond area in deciding rations was studied during the present experiment.

Using the second order polynomial regression analysis, it was found that there were significant relationships between pond area and ration size during the middle growth stanza (135–240 days of the 330-day culture of common carp) when the growth was maximum. Thus, the step-wise regression anaysis was attempted to quantify the role of pond area in addition to the already established role of pond ecological conditions and fish biomass.

The step-wise regression analysis relationships (Table 3) were used to revise the ration sizes and total amount of feed required during the middle growth stanza which we calculated during our earlier study without incorporating pond area into the models (Sehgal and Toor 1991). The desired ration size, with the incorporation of pond area in the model, increased to 3.61% against 3.1% calculated earlier. This accounted for an additional 334 kg of diet which was underfed. Considering a mean FCR of 3.37 for this period (Sehgal and Thomas 1985) a further loss of 99 kg of fish was estimated. The comparison of fish biomass based and fish biomass-pond ecology-pond area based feeding strategies are given in Table 4.

Table 4. Comparison of fish biomass based versus fish biomass-pond area-pond ecology based feeding strategies.

Culture period	Actual amount of feed (kg/ha)[a]	Estimated amount of feed (kg/ha)[b]	Excess amount of feed (kg/ha)[c]	Amount of feed short of the desired (kg/ha)[d]	Cost of feed kg^{-1} (INR/ha)	FCR	Potential additional fish biomass (kg/ha)	Cost of fish kg^{-1}	Estimated loss (INR/ha)
0–135 days (2 April–15 August)	3 820	2 930	890	—	5.25	—	—	—	4 673
135–240 days (15 August–28 November)	4 085	6 614	—	2 529	—	3.37	750	18	13 500 18 173

a = Based on fish biomass; b = based on fish biomass and pond ecology; c = a – b; d = b – a.

Conclusions

The present study revealed that pond area is an important component of fish feeding schedules in addition to pond ecological conditions which vary seasonally. The results suggest that desired ration sizes should be decided on the basis of fish biomass-pond ecology-pond area rather than on the basis of fish biomass or fish biomass-pond ecology alone. Ignoring pond area, particularly during the period of maximum growth, can lead to the underestimation of ration sizes as revealed by the relationships derived by the step-wise regression models resulting in considerable financial losses. It is, therefore, more economical to decide ration sizes on the basis of pond area, pond ecological conditions and fish biomass than on the basis of fish biomass alone or fish biomass and pond ecological conditions.

References

AOAC (Association of Official Analytical Chemists). (1984). Williams, S. (ed.) *Official Methods of Analysis*. Arlington, Virginia, Association of Official Analytical Chemists Inc., 1141 pp.

APHA. 1976. *Standard Methods for the Examination of Water and Waste Water*. Washington, American Public Health Association, 1193 pp.

Appler, H.H. 1985. Evaluation of *Hydrodictyon reticulum* as a protein source in feeds for *Oreochromis (Tilapia) niloticus* and *Tilapia zilli*. *J. Fish Biol.* 327–334.

Beck, H., J. Groop, H. Koops and K. Tiews. 1979. Single cell protein in trout diets. In Halver J.E. and K. Tiews (eds.) *Fin Fish Nutrition and Fish Feed Technology, Vol. 2*. Berlin, Heenemann, pp. 269–280.

Brett, J.R. 1979. Environmental factors and growth. In Hoar, W.S., D.J. Randall and J.R. Brett. (eds.) *Fish Physiology: Bioenergetics and Growth, Vol. 8*. Academic Press, pp. 599–677.

Brett, J.R. and T.D.D. Groves, 1979. Physiological Energetics. In Hoar, W.S., D.J. Randall and J.R. Brett (eds.) *Fish Physiology: Bioenergetics and Growth,Vol 8.* Academic Press, pp. 280–352.

De Silva, S.S. and R.M. Gunasekera, 1989. Effect of dietary protein level and amount of plant ingredient (*Phaseolus aureus*) incorporated into the diets on consumption, growth performance and carcass composition in *Oreochromis niloticus* (L) fry. *Aquaculture* 80: 121–133.

De Silva, S.S. and P.A.B. Perera, 1985. Effects of dietary protein level on growth, food conversion and protein use in young Tilipia nilotica at four salinities. *Trans. Am. Fish. Soc.* 114: 585–589.

Doudoroff, P. and D.L. Shumway, 1967. Dissolved oxygen criteria for the production of fish. *Am. Fish. Soc. Spec. Publ.* No. 4, pp. 13–19.

Elliott, J.M. 1975. The growth rate of brown trout, *Salmo trutta* L. fed on maximum rations. *J. Anim. Ecol.* 44: 805–821.

Hepher, B. 1988. *Nutrition of Pond Fishes.* Cambridge, Cambridge University Press, 388 pp.

Higgs, D.A., J.R. Markert, M.D. Plotnikoff, J.R. McBride and B.S. Dosanjh, 1985. Development of nutritional and environmental strategies for maximizing the growth and survival of juvenile pink salmon (*Onchorhynchus gorbuscha*). *Aquaculture* 47: 113–130.

Jackson, A.J., B.S. Capper and A.J. Matty 1982. Evaluation of some plant proteins in complete diets for the tilapia (*Sarotherodon mossambicus*). *Aquaculture* 27: 97–109.

Lovell, R.T. 1977. Feeding practices In Stickney, R.R. and R.T. Lovell (eds.) *Nutrition and Feeding of Channel Cat Fish.* South Coop. Ser. Bull.

Matty, A.J. and P. Smith, 1978. Evaluation of a yeast, a bacterium and an alga as a protein source for rainbow trout I. Effect of protein level on growth, gross conversion efficiency and protein conversion efficiency. *Aquaculture* 14: 235–246.

McCormick, J.H., K.E.F. Hokanson and B.R. Jones, 1972. Effects of temperature on growth and survival of young brook trout, *Salvelinus fontinalis. J. Fish. Res. Board Can.* 29: 1107–1112.

National Research Council, 1983. *Nutrient requirements of Warmwater Fishes and Shellfishes.* Washington D.C., National Academy Press.

Sehgal, H.S. and J. Thomas, 1985. Efficacy of two newly formulated supplementary diets for carp, *Cyprinus carpio* var. *communis* (Linn.): 1. Effects on survival, growth and yield. *Anna. Biol.* 1(1): 46–55.

Sehgal, H.S. and J. Thomas, 1987. Efficacy of two newly formulated supplementary diets for carp, *Cyprinus carpio* var. *communis* (Linn.): Effects on flesh composition. *Biol. Wastes* 21: 179–187.

Sehgal, H.S. and S. Sharma, 1991. Efficacy of two new supplementary diets for Indian major carp, *Cirrhina mrigala:* Effects on flesh composition. *J. Aqua. Trop.* 6: 25–34.

Sehgal, H.S. and S. Sharma, 1993. A note on evaluation of some wastes and by-products from agriculture and animal husbandry as feed ingredients for (Ham.). *Bioresour. Technol.* 43: (in press).

Winfree, R.A. and R.R. Stickney, 1984. Formulation and processing of hatchery diets for channel cat fish *Aquaculture* 41: 311–323.

Wee, K.L. and S.S. Wang, 1987. Nutritive value of *Leucaena* leaf meal in pelleted feed for Nile tilapia. *Aquaculture* 62: 97–108.

The Effect of Feeding Frequency on the Growth of Catfish (*Clarias* spp.) Reared in a Portable Raceway System

ROSHADA HASHIM
AHYAUDIN ALI
AHMAD ISMAIL
Universiti Sains Malaysia
Penang 11800
Malaysia

HASHIM, R., A. ALI and A. ISMAIL, 1994. The effect of feeding frequency on the growth of catfish, *Clarias* spp., reared in a portable raceway system. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 115–118.

Abstract

A 14-week study was carried out to determine the effects of two feeding frequencies on growth and survival of catfish (*Clarias* spp.) cultured intensively in a limited flow raceway system. Fish were fed twice and four times daily with a commercial diet at a feeding level of 10% body weight for the first four weeks and at 5% body weight thereafter.

Average final weight for fish fed four times daily ranged from 165.6 ± 2.5 g to 177.9 ± 4.0 g and was significantly higher compared with those fed twice daily (133.3 ± 2.4 g). The final weight distribution of the fish fed twice and four times daily was between 30 to 210 g and 80 to 220 g respectively. The narrower final weight spread for fish fed four times a day resulted in a more uniform marketable sized fish, which in Malaysia is between 110 to 130 g.

Introduction

The success of the channel catfish culture industry today has been due to the vast amount of nutritional information made available (Lovell 1988) and the development of effective and appropriate aquaculture techniques relevant to farmers' needs such as feeding and management practices (Shaner et al. 1982). Catfish aquaculture in Malaysia is largely confined to small scale rural farmers who rear them to supplement their income. Intensive catfish culture among these farmers who have limited land resources can be promoted with the introduction of portable raceways. These raceways are easy to set up, economical because the high cost incurred in pond construction is avoided and it also gives the farmer the flexibility to rear fish when and where he desires (Ali 1992). When fish are reared in an intensive culture situation and artificial feed becomes the sole nutrient source, competition for food becomes a growth limiting factor (Magnusson 1962), eventually leading to aggressive behaviour and reduced feed consumption (Refstie 1977). Poor growth can be overcome by increasing food availability and this is achieved by increasing feeding frequency (Hogendoorn 1981, Holm et al. 1990). Since fish growth is dependent on its culture conditions, our objective was to study the effect of feeding frequency on the growth of catfish cultured intensively in a closed, limited water supply system.

Materials and Methods

The experiment was carried out in portable canvas raceways (460 cm × 92 cm × 122 cm) filled with water to a depth of 30 cm. Approximately half the total volume of water was changed daily and no aeration was provided. The temperature throughout the feeding trial ranged from 26°C in the morning to 29°C in the early evening. The average DO (mg/L) and NH_3 (mg/L) for each treatment throughout the experiment is summarised in Table 1.

Table 1. The dissolved oxygen (mg/L) and NH_3 (mg/L) of the raceways in Treatments 1 and 2 (Values obtained are data combined from the replicates)

Treatment	Feeding Frequency	Week	Dissolved Oxygen (mg/L)		NH_3 (mg/L)
			Morning	Evening	
1	2 times/day	1–6	1.6–2.4	16.7–19.5	0.05–0.06
		7–12	2.2–2.3	7.0–8.5	0.05–0.06
2	4 times/day	1–6	1.2–2.4	12.3–20.0	0.06–0.07
		7–12	2.0–2.7	6.0–9.8	0.07–0.5

Catfish (*Clarias* spp.) fry were hatched and reared to stocking size at the School of Biological Sciences, Universiti Sains Malaysia. Six raceways were randomly stocked with 1200 catfish fry per raceway.

The feeding trial was carried out from 10 February 1992 until 25 May 1992. The fry were hand fed a commercial diet containing 30% protein at 10% body weight for the first four weeks and 5% body weight thereafter, at two feeding frequencies. Two replicates of one group were fed twice daily at 0800 and 1600 hrs (Treatment 1), whilst four replicates of the second group were fed four times daily at 0800, 1200, 1500 and 2200 hrs (Treatment 2). Growth and mortalities were recorded weekly and food rations adjusted accordingly. At the end of the feeding trial, the fish were weighed and mortalities recorded. The weight gain, feed conversion ratio and survival rates were calculated. The differences in the growth parameters for each treatment means were tested by Analysis of Variance (ANOVA) followed by the t-Test (Steel and Torrie 1960).

Results and Discussion

In most fish population a situation of hierarchy or ranking arises (Morse 1980, cited in Holm et al. 1990) whereby dominant individuals who have better access to food (Magnusson 1962), consequently attain higher growth rates (Li and Brocksen 1977). Large variations in fish size become more pronounced when fish are cultured in high density, intensive conditions and have to compete for food and space. The ensuing aggressive behaviour and competition which prevails among the population often leads to reduced opportunity to obtain food and thus a reduction in feed consumption among the lower ranking individuals (Refstie 1977). Thus, under these circumstances, availability of food becomes an important criterion in determining growth (Refstie 1977).

In this experiment, an overall significant improvement in growth was observed when fish were fed more frequently (Table 2). Feeding the catfish four times daily resulted in a significantly higher mean final weight of 167.2 ± 2.49 g compared with 133.3 ± 2.44 g for fish fed twice daily (Treatment 1). Thus, when food rations were given at four different times instead of two, food becomes more available, allowing more individuals access to food. The reduction in competition for food in Treatment 2 may explain the better weight gain observed. This is in agreement with a previous study on the relationship between stocking density and feeding frequency in rainbow trout (Holm et al. 1990) which showed that at high stocking densities, a higher frequency of feeding (higher food availability) was important for growth.

Feed conversion ratio was also lower in Treatment 2 but was not significantly different from Treatment 1. Similar studies by Andrews and Page (1975) also showed no difference in feed conversion between fish fed twice and four times daily and suggested that the limiting factor for growth was food intake rather than food utilization.

Table 2. Effect of feeding frequency on weight gain, feed conversion ratio (FCR) and survival of catfish (*Clarias* spp.) after 14 weeks

Feeding Frequency	Initial Weight (g/fish)	Final Weight (g/fish)	Food Taken (g/fish)	Weight gain (g)	FCR	Survival (%)
2 times/day	1.29±0.21	133.3±2.44	252.0	132.01[a]	1.90[b]	56[a]
4 times/day	1.47±0.10	167.2±2.49	285.7	165.73[b]	1.72[b]	57[a]

Means in the same column with different superscripts are significantly different ($p < 0.05$)
FCR = Food consumed (g)/Weight gain (g)

Besides the better growth rate obtained, weight distribution also improved when feed became more available (Figure 1). In Treatment 1, feeding twice daily resulted in a wide and highly uneven fish size distribution ranging from 30 to 370 g. The relative frequency of occurrence was mainly in the lower weight range of 30 to 210 g and fish weighing 90 g had the highest frequency followed by those weighing 30 g. Weight distribution for fish fed four times daily (Treatment 2) was also in the range of 30 to 370 g but fish size was concentrated in the higher weight range and with a narrower spread of 90 to 210 g. Higher frequency of occurrence was observed for fish weighing 170 to 210 g. This observation is probably due to the efficient food distribution as a result of increasing the feeding frequency. Another advantage of feeding four times daily in such a system is the narrow size spread and thus more uniform size fish of between 90–200 g which in Malaysia is within the marketable range of 110–130 g.

The survival rate of approximately 56% for both treatments was low and not significantly different. The high mortalities which occurred in the first three weeks of the feeding trial were more likely attributed to the high stocking density rather than the low DO which occurred towards the end of the trial (Table 1). Ali (1992) reported survival rates greater than 80% when a lower density of 600 catfish fry was cultured using the same portable tank system and at similar DO levels of 2.6 ± 0.40 mg/L. Moreover, being an air breather, the catfish is able to compensate these low DO levels in the water by obtaining atmospheric oxygen.

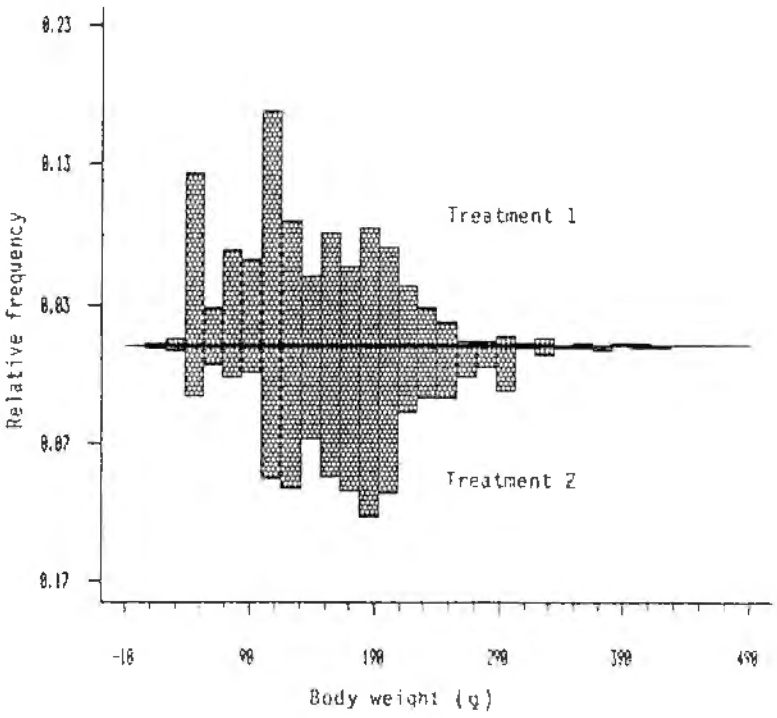

Figure 1. Relative frequency of body weight distribution for catfish fed twice and four times daily respectively after 14 weeks.

Conclusion

The catfish is presently an economically important freshwater fish in Malaysia. Its reported tolerance to a wide temperature range and diseases (Tan 1991) and air breathing characteristics makes it a suitable candidate for culture in such portable canvas tanks. In spite of the constraints in size distribution and survival rates, this method of fish culture provides an alternative for the prospective farmer who has limited land and financial resources. Further studies are currently being carried out to enhance growth by improving the feeding rate and feed quality, and to reduce size variation by selective removal of the smaller sized fish.

Acknowledgement

This work was funded by the Malaysian Government under the Intensification of Research Priority Areas Programme. Ancillary research facilities provided by Universiti Sains Malaysia are gratefully acknowledged. The authors would like to thank Dr S.S. De Silva for the opportunity to attend the workshop and the IDRC for providing the travel support.

References

Ali, A. 1992. Techniques for intensive culture of catfish (*Clarias* spp.) in portable tanks. In Ho, Y.W., M.K. Vidyadaran, N. Abdullah, M.R. Jainudeen and A.R. Bahaman (eds.) *Proceedings of the National IRPA (Intensification of Research in Priority Areas) Seminar (Agricultural Sector)*. 6–11 Jan., Kuala Lumpur, Publ. Ministry of Science, Technology & Environment, Malaysia.

Andrews J.W. and J.W. Page. 1975. The effects of frequency of feeding on culture of catfish. *Trans. Am. Fish. Soc.* 104(2): 317–321.

Hogendoorn, H. 1981. Controlled propagation of the African catfish, *Clarias lazera* (C & V.). IV. Effect of feeding regime in fingerling culture. *Aquaculture* 24: 123–131.

Holm, J.C., T. Refstie and S. Bo, 1990. The effect of fish density and feeding regimes on individual growth rate and mortality of rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 89: 225–232.

Li, H.W. and R.W. Brocksen, 1977. Approaches of the analysis of energetic costs of intraspecific competition for space by rainbow trout (*Salmo gairdneri*). *J. Fish Biol.* 11: 329–341.

Lovell, R.T. 1988. *Nutrition and Feeding of Fish*. Van Nostrand Reinhold, pp. 268.

Magnusson, J.J. 1962. An analysis of aggressive behaviours, growth and competition for food and space in medaka (*Oryzias latipes* (Pisces, Cyprinodontidae)). *Can. J. Zool.* 40: 313–363.

Refstie, T. 1977. Effect of density on growth and survival of rainbow trout. *Aquaculture* 11: 329–334.

Shaner, W.W., P.F. Philip and W.R. Schmehl, 1982. *Farming System Research and Development: Guidelines for Developing Countries*, Boulder, Colorado, USA, West View Press.

Steel, R.G.D. and J.H. Torrie, 1960. *Principles and Procedures of Statistics*. McGraw-Hill Book Company Inc.

Tan, E.S.P. 1991. Improving the quality of fish fry for more profitable aquaculture projects. In Singh, M., T.K. Soon, S. Radhakrishna and T.C. Yap (eds.) *Proceedings of the Third Asian Conference on Technology for Rural Development*, 12–14 August, pp 41–46.

Winfree, R.A. and R.R. Stickney, 1984. Formulation and processing of hatchery diets for channel catfish. *Aquaculture* 41: 311–323.

The Effect of Stocking Density and Growth Promoter on the Performance of Giant Goramy Fingerlings (*Osphronemus gouramy*) in Floating Net Cages

NINGRUM SUHENDA
M. FATUCHRI SUKADI
E. S. KARTAMIHARDJA
R. UTAMI
A. HARDJAMULIA

Central Research Institute For Fisheries
Research Institute For Freshwater Fisheries
Jalan Sempur No. 1 Bogor, Indonesia

SUHENDA, N., M. FATUCHRI SUKADI, E. S. KARTAMIHARDJA, R. UTAMI and A. HARDJAMULIA, 1994. The effect of stocking density and growth promoter on the performance of giant goramy fingerlings, *Osphronemus gouramy*, in floating net cages. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 119–124.

Abstract

An experiment with the objectives of determining suitable feed type and optimal stocking density on growth performance of giant goramy fingerlings (*Osphronemus gouramy*) was conducted at the experimental station of the Research Institute For Freshwater Fisheries located at Cirata Reservoir, Cianjur (West Java).

Completely randomized design was employed with a factorial arrangement of treatments involving feed type (two levels, with and without growth promoter) and stocking density (100, 300 and 500 individuals per cubic metre).

The fish were reared for ten weeks in floating polyethylene net cages (1.18 × 0.85 × 1.1 m), or one cubic metre of water volume. The mean starting weight was 45 g. For the first four weeks the daily feeding rate was 4% of the fish biomass and then 3.5% until ten weeks, the ration being dispensed three times daily. Adjustment of the daily feed allowance was made every two weeks based on sampling. Quixalud (contains halquinol as an active ingredient) was used as the growth promoter at the dosage 25 mg/kg feed. The protein content of feed was 25.5%.

The results of statistical analysis showed that there were no significant interactions between the feed type and stocking density ($p>0.05$) for all parameters.

The different feed types did not result in significant differences ($p>0.05$) for all growth parameters. Increasing stocking densities were accompanied by a decrease in the average weight gain; the higher the stocking densities the higher the feed conversion ratio. The survival rates were the same ($p>0.05$) for all stocking densities.

Although the feed conversion ratio was higher at increased stocking density the most economical was at 500 fish/m^3. The water quality was within the acceptable range for normal life of the fish during the experiment.

Introduction

In Indonesia, fish farming has been geared towards the intensification of production (especially for common carp) where aquatic animals are grown in artificial confinement, for

example, in floating net cages, at high stocking densities. In these types of culture systems the contribution of natural foods to the dietary requirements of the animals becomes too small to promote good growth. Consequently, supplementary feeding is necessary to achieve higher production. The artificial feed that must be supplied, either as supplemental or complete rations, is the single, most critical and expensive item in the entire operation. To get good quality feed, it is essential to have a balanced nutrient content, in protein, fat, carbohydrate and energy. Besides that, using growth promoters is one way to increase growth or production.

Various growth promoters have been used in animal husbandry, among others quixalud. Quixalud contains halquinol as an active ingredient. As a growth promoter, the function of quixalud is to decrease intestinal peristaltic movement so it will increase nutrient absorption, influencing growth and feed conversion ratio. Research on using quixalud in common carp feed was conducted by Hardjamulia, Suhenda and Supriyadi (1987). The results showed that feed containing halquinol gave better growth and feed conversion ratio. However, its utilization in fish feed is limited; one of the reasons being lack of information regarding its efficacy.

Besides feed, fish growth is influenced by stocking density (Vaas van Oven 1957). Higher stocking density will give lower growth (Hickling 1971).

In Indonesia, giant goramy is an economically important fish but its production is still low. Usually, fish farmers culture giant goramy in earthern ponds at a low stocking density. In 1981, the production of giant goramy was 4139 t while in 1987 it was 4495 t. It was indicated that production improvement is still low (Directorate General for Fisheries 1989).

To increase fish production, giant goramy should be cultured in floating net cages at a high stocking density, using good quality feed and growth promoters. The present experiment was conducted with the objective of determing the effect of stocking density and growth promoter on the performance of giant goramy fingerlings in floating net cages.

Materials and Methods

Eighteen floating net cages (1.18 m × 0.85 m × 1.1 m or one cubic metre of water volume), made from polyethylene, located at the experimental station of the Research Institute for Freshwater Fisheries, Cirata Reservoir, Cianjur were used and stocked with giant goramy fingerlings of average weight 45 g. They were acclimated to lake water for two weeks prior to the experiment.

Completely randomized design was employed with a factorial arrangement of treatments involving feed type (two levels, with and without growth promoter) and stocking density (100, 300 and 500 individuals per cubic metre). Diets were formulated containing isoprotein (25.5% crude protein) and isolipid (7.15%). The proximate analysis of experimental feed is given in Table 1.

Table 1. Proximate analysis of experimental feed (as feed basis)

Composition	with quixalud	without quixalud
Water	9.80	9.10
Protein	25.52	25.50
Lipid	7.17	7.13
Ash	13.53	13.50
Crude fibre	2.66	1.87
NFE	41.32	42.90

Note: NFE = Nitrogen Free Extract

A daily feeding rate of 4% of the fish biomass was used for the first four weeks and then (until ten weeks) altered to 3.5% of the fish biomass. Feeding frequency was three times daily. Adjustment of the daily feed allowance was made every two weeks based on sampling. Quixalud was used as a growth promoter at a dosage of 25 mg/kg of feed.

Each parameter was calculated as follows:

Weight gain (Weatherly 1972)

$$b = Wt - Wo$$

b = weight gain (g)
Wt = average final weight (g)
Wo = average initial weight (g)

Net production (Chapman 1967, in Weatherley 1972)

$$P = (B1 - B0) + Bd$$

P = net production (kg)
B1 – B0 = total weight gain (kg)
Bd = total weight of dead animal (kg)

Feed conversion ratio (NRC 1977)

$$K = \frac{F}{(Wt + D) - W0}$$

K = feed conversion ratio
F = total feed fed (kg)
Wt = total weight at the end of the experiment (kg)
D = total weight of dead animal (kg)
W0 = total initial weight (kg)

Survival rate

$$\text{Survival rate} = \frac{\text{Final total number of fish alive}}{\text{Initial total number of fish}} \times 100\%$$

Data were analyzed using their mean differences by least significant difference in completely randomized design.

Results and Discussion

The average weight gain for various treatments is given in Table 2. The result of statistical analysis showed that there was no significant interaction between the feed type and stocking density ($p>0.05$).

Table 2. The average weight gain (g) of goramy for each treatment in the 10-week rearing period

Stocking density (fish/m^3)	Feed		
	GP	WGP	Average
100	121.67	122.78	122.23
300	100.19	94.26	97.23
500	84.78	82.33	83.56
Rata-Rata	102.21	99.79	

Note: GP = growth promoter
WGP = without growth promoter

The different feed types did not result in significant differences ($p>0.05$). It is indicated that using growth promoter did not show a better effect.

Increasing stocking densities was accompanied by a decrease in the average weight gain. Weight gain decreased significantly ($p<0.01$) with an increase of stocking density. The highest weight gain (122.23 g) was obtained at the lowest stocking density (100 fish/m^3). The growth curves for giant goramy fingerlings fed with different feed types (with and without growth promoter) and cultured in different stocking densities for a ten-week period are presented in Figure 1. The relative growth of goramy for various treatments is presented in Table 3. Production of goramy after the ten-week culture period is shown in Table 4.

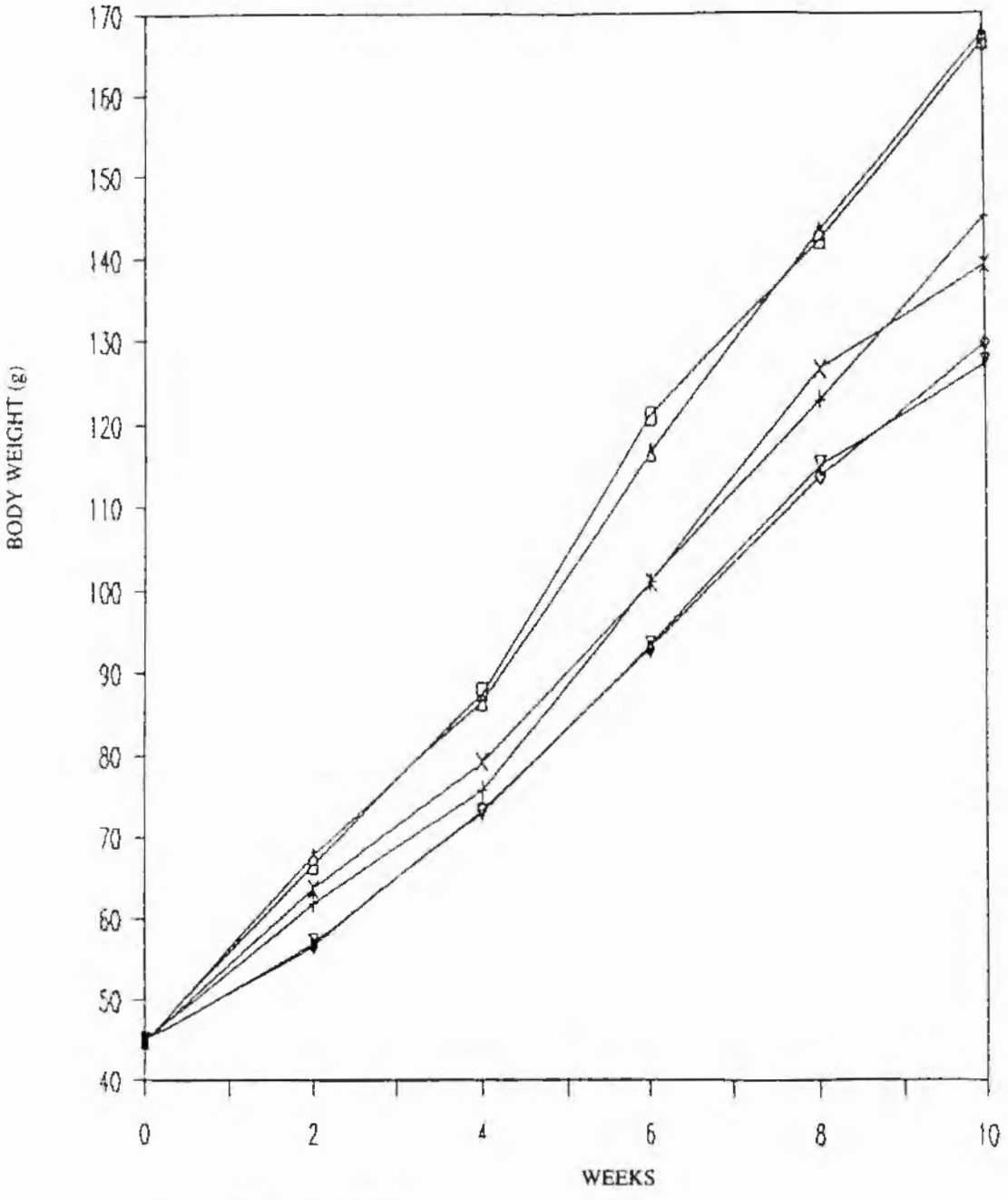

Figure 1. Average body weight of fish by each treatment in ten-week rearing period

Table 3. Relative growth (% of initial weight) in ten-week culture period

Stocking density (fish/m^3)	Feed		
	GP	WGP	Average
100	370.38	372.84	371.61
300	322.64	309.46	316.55
500	288.39	282.96	285.68
Average	327.14	321.75	

Note: GP = growth promoter
WGP = without growth promoter

Table 4. Production (kg) of goramy by each treatment in ten-week rearing period

Stocking density (fish/m^3)	Feed		
	GP	WGP	Average
100	11.485 (255.22)	12.037 (267.40)	11.761 (261.36)
300	29.720 (220.15)	27.647 (204.79)	28.684 (212.47)
500	41.877 (186.12)	40.442 (179.74)	41.160 (182.93)
Average	27.694 (220.50)	26.709 (217.34)	

Note: Values in parentheses are relative net production to initial total weight
GP = growth promoter
WGP = without growth promoter

The lowest stocking density (100 fish/m^3) gave the highest relative growth (371.61%). The lowest relative growth (285.68%) was obtained at the highest stocking density (500 fish/m^3). These values were higher than those obtained in culture in earthern ponds. Fish farmers usually culture goramy in earthern ponds with artificial feeds. In a two-month rearing period, the relative growth of giant goramy was only 100–150%. Tan (1983) indicated that goramy grow faster when it is cultured in a good environment and fed with complete diets. Table 4 indicated that the highest relative net production (261.36%) was obtained for fish which were cultured in the lowest stocking density (100 fish/m^3). It was observed that the higher the stocking densities the higher feed conversion ratio (Table 5). These values are significantly different ($p<0.01$).

Table 5. Feed conversion ratio for each treatment

Stocking density (fish/m^3)	Feed		
	GP	WGP	Average
100	1.86	1.78	1.82
300	1.93	2.11	2.02
500	2.14	2.22	2.18
Average	1.98	2.04	

Note: GP = growth promoter
WGP = without growth promoter

The best feed conversion ratio (1.82) was obtained at the lowest stocking density (100 fish/m^3). Results of statistical analysis showed that there was no significant interaction between the feed type and stocking density ($p>0.05$). The different feed types did not result in significant differences ($p>0.05$) in feed conversion ratio, indicating that the growth promoter did not affect the feed conversion ratio.

The survival rates of giant goramy during the ten-week rearing period are presented in Table 6.

Table 6. Survival rates (%) of goramy by each treatment in the ten-week rearing period

Stocking density (fish/m^3)	Feed		Average
	GP	WGP	
100	91.67	97.00	94.34
300	98.00	95.22	96.61
500	97.67	95.67	96.67
Average	95.78	95.96	

Note: GP = growth promoter
WGP = without growth promoter

There was no significant interaction between the feed type and stocking density ($p>0.05$). The survival rates were the same ($p>0.05$) for all stocking densities and feed types.

The water quality was within the acceptable range for normal life of fish during the experiment. The value of each parameter of water quality was as follows:

- temperature = 29°5 C–31°C
- pH = 7–7.75
- alkalinity = 68.45–133.56 mg/L $CaCO_3$ eq
- DO = 3.57–6.91 ppm
- CO_2 = 5.39–7.79 ppm
- NH_3 = ≤0.037 ppm

The results obtained from this experiment indicated that supplementation of growth promoter in the diets is not necessary. Different feed types (with and without growth promoter) did not result in significant differences ($p>0.05$) for all growth parameters. The stocking density influences the weight gain, and feed conversion ratio. The higher the stocking density the higher the feed conversion ratio.

References

Boyd, C.E. 1979. *Water Quality in Warmwater Fish Ponds*. Auburn University, 354 p.

Directorate General For Fisheries, 1989. *Fisheries Statistics for Indonesia*. Jarkarta, Indonesia.

Hardjamulia, A., N. Suhenda and H. Supriyadi, 1987. The effect of quixalud on the growth of common carp (*Cyprinus carpio*). Collaborative Research Report, 49 p.

Hatimah, S., R Djajadiredja and Machfudz, 1980. Giant goramy cultured in earthern ponds at Depok. Research Report, LPPD, 13 p.

Hickling, C.F. 1971. *Fish Culture*. London, Faber and Faber, 317 p.

Ketola, H.G. 1975. Requirements of Atlantic salmon for dietary phosphorus. *Trans. Am. Fish. Soc.* 104: 548–551.

Lovell, R.T. 1978. Dietary phosphorus requirements of channel catfish (*Ictalurus punctatus*). *Trans. Am. Fish. Soc.* 107: 617–621.

Nose, T. and S. Arai, 1979. Recent advances in studies on animal nutrition of fish in Japan. In Pillay, T.V.R. and W.A. Dill (eds.) *Advances in Aquaculture*. Farnham, Surrey, Fishing News Book Ltd., pp. 584–589.

NRC, 1977. *Nutrient Requirements of Warmwater Fishes and Shell Fishes*. Washington D.C., National Academy of Sciences, 102 p.

Pescod, H.B. 1973. *Investigation of Rational Influent and Stream Standards for Tropical Countries*. Bangkok, Thailand, Asian Institute of Technology, 59 p.

Stickney, R.R. 1979. *Principles of Warmwater Aquaculture*. New York, John Wiley and Sons, 199 p.

Squibb, E.R. (no date) Quixalud feed additive. (Technical information.) E.R. Squibb and Sons Limited, 23 p.

Tan, G.H. 1983. Growth response of *Osphronemus gouramy* (lecepede) Juveniles in cages fed pellet at three levels of crude protein. Final Year Fisheries Project Paper, Selangor, Malaysia, Universiti Pertanian Malaysia, Serdang, 47 p.

Vaas-van Oven, A. 1957. *Research about Stocking Densities for Common Carp*. Bogor, Research Institute For Inland Fisheries, 20 p .

Weatherley, A.H. 1972. *Growth and Ecology of Fish Populations*. New York, Academic Press, 28 p.

Fry Rearing of Mahseer, *Tor Puttitora*, on Different Diets

A.K. RAI
J. D. BISTA
Fisheries Research Centre
Pokhara, Nepal

RAI, A.K. AND J.D. BISTA, 1994. Fry rearing of mahseer, *Tor puttitora*, on different diets. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 125–127.

Abstract

Forty-five-day old fry of mahseer *Tor puttitora* bred at the Fisheries Research Centre, Pokhara were reared for 40 days on different diets. The fry of mahseer fed with plankton and reared in cages in an earthen pond grew at a rate of 24.9%/day, and was followed by those fed on fish meal + boiled chicken egg (5.6%/day), fed on plankton and reared in the tank (3.6%/day) and fed with soybean + boiled chicken egg (2.3%/day), in order. The fry fed soypowder + whole chicken egg showed abnormalities, curvature of the vertebral columns near the caudal fin. The survival rate of fry was above 90% in all treatments except in fry fed soybean + whole chicken egg (85%). However, the growth rate of the fry was enhanced when fed with plankton + soybean + oilcake + egg, reared in a cage in the earthen pond.

Introduction

Mahseer, *Tor* spp. locally called 'Sahar' is a very popular game fish in Nepal. In Nepal 174 fish species are listed, of which two are species of mahseer; *Tor tor* and *Tor puttitora*. Originally, mahseer was a hill stream fish but they also occur accidentally in lowland areas during migration (Shrivastava 1968). Mahseer is considered a delicious food fish and fetches a higher price than other fish in Nepal but the annual catch of this species has declined (Shrestha and Gurung 1987). Over-fishing, damming of rivers and natural calamities (flooding or land sliding) destroy not only the breeding ground of this species but also destroys brood fish including fingerlings, fry, hatchlings and eggs. To maintain or to increase the fish population conservation, as well as the development of technology for breeding, rearing and nursing of fry and fingerlings, is essential, either in culture or as stock in open water bodies.

Mahseer is a carnivorous (Malhotra 1982) intermediate (Macdonald 1948) and its age and growth has also been studied (Pathani 1983) as well as its spawning (Masuda and Bastola 1985; Shrestha et al. 1990). However, the larval rearing of mahseer is not fully developed. This paper presents some preliminary results from a feeding experiment with mahseer fry designed to test the effects of different diets on growth.

Materials and Methods

Six plastic fibre glass tanks (100 L) and cages (50 m^3) of 2 mm stretch mesh made of nylon twine were used for the experiments. Plastic fibre glass tanks were set in a hatchery with 2 replicates for each trial and filled with 80 L of water. Eighty fry of 0.031 g average weight were stocked in each tank. Cages were fixed in an earthen pond which was fertilized with organic

manure (800 kg/ha) and inorganic fertilizer (40 kg/ha), and stocked with 5630 fry/cage. The fry used in the experiments were 45 days old and were bred in the centre. Four different diets were used for the study. Diets used were plankton; fish meal (75%) + whole boiled chicken egg (25%); soybean (50%) + whole boiled chicken egg (50%) for rearing in the tanks; and plankton + soybean (75%) + oilcake (25%) + one whole boiled egg in the cages respectively (Table 1).

The fry in plastic tanks were fed five times a day but those in cages only twice a day. The plankton was collected with a Wisconsin plankton net of 80 µm size from earthen ponds and fed to fry in the tanks. Aeration was provided continuously and water exchanged twice a day (morning and evening). The water temperature in each treatment and secchi disc in the pond were measured daily. The study was carried out for 40 days and growth determined.

Table 1. Mean weight, total length, survival rate and growth rate of fry of mahseer *Tor puttitora* in a plastic tank and in a cage in an earthen pond fed with different diets

	Diets			
	Plankton in tank	Fish meal + whole egg	Soybean + whole egg	Plankton + soybean + oilcake + whole egg
Mean weight (g) at stocking	0.031	0.031	0.031	0.031
Mean total length (mm) at stocking	14.6	14.6	14.6	14.6
Number stocked (no.)	80	80	80	80
Rearing days	40	40	40	40
Mean weight (g) at harvest	0.076	0.101	0.060	0.340
Mean total length (mm) at harvest	20.1	20.2	20.1	30.3
Mean growth rate %/day	3.6	5.6	2.3	24.9
Survival rate	97.5	95.0	85.0	90.5

Results and Discussion

After forty days of rearing fry on different diets, the highest growth (24.9%) was obtained from those fed natural food + soybean + oilcake + whole chicken egg in the cage set in the earthen pond, followed by those fed fish meal + whole egg (5.6%/day), plankton (3.6%/day) and soybean + whole chicken egg (2.3%/day), in order. (Table 1). The highest mean weight of 0.340 g from 0.031 g initial weight was attained from fry reared in the cage in the natural pond fed with natural food + soybean + oilcake + whole egg. Lowest growth occurred when fed on soybean + whole chicken egg (Figure 1).

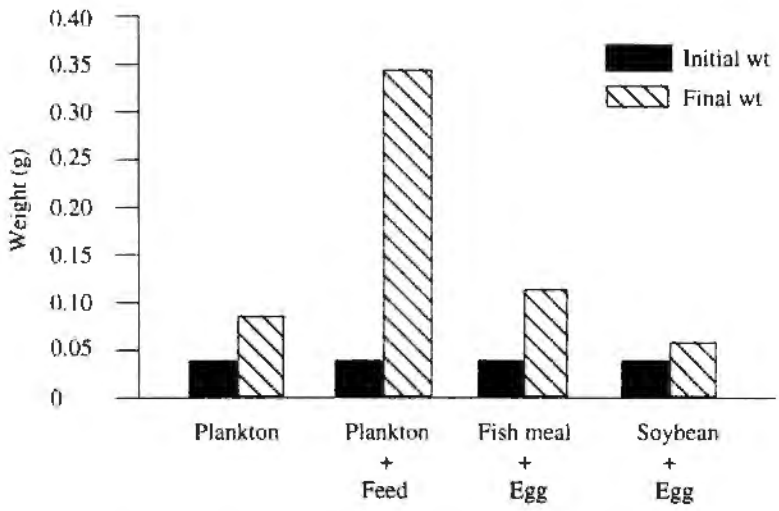

Figure 1. Effects of different diets on the growth of mahseer fry

The survival rate of fry reared on the four different diets was above 85%, rising to 97.5% when fed plankton in the plastic tanks followed by those fed soybean + whole chicken egg (95.0%) and fish meal + whole chicken egg (90.5%). The water temperatures in the tank and the pond were not much different and varied from 22.5°–31.0°C and the secchi disc visibility ranged from 29–31 cm in the pond during the study. Abnormally-bent bodies near the caudal fin were seen in some fry fed with soybean + whole chicken egg which might be due to the lack of animal protein for normal growth.

These results showed that rearing of mahseer fry requires natural pond conditions with sufficient natural food and additional proteinous supplementary feed containing animal protein for better growth. Rai et al. (1992) also reported that larvae of mahseer fed with plankton showed better growth when fed with other supplementary diets. Kulkarni (1970) reported that mahseer fry grew 170–200 mm in length within four months when reared in natural ponds. The fry of katle, *Acrossocheilus hexagonolepis* also showed better growth when fed with 30% protein content feed + plankton (Rai 1990). These results show that natural ponds with enough natural food need to be maintained in addition to supplementary protein-containing feed to promote the growth of mahseer fry.

Acknowledgements

The authors are thankful to the staff of the Fisheries Research Centre, Pokhara for their help in the field work. Special thanks are due to Dr Sena S. De Silva who encouraged us to prepare this paper. We are also very grateful to IDRC for its financial support.

References

Kulkarni, C.V. 1970. Spawning habits, eggs and early development of Deccan mahseer, *Tor khudree* (Sykes). *J. Bombay Nat. Hist. Soc.* 67(3): 510–521.

Macdonald, A. St. J. 1948. Circumventing the mahseer and other sporting fish in India and Burma. *J. Bombay Nat. Hist. Soc.* 114(6): 306.

Malhotra, S.K. 1982. Bionomics of hill-stream cyprinids. Food, parasites and length-weight relationships of Gartwal mahseer, *Tor tor* (Ham.). *Proc. Indian Acad. Sci. (Anim. Sci.)* 91(6): 493–499.

Masuda, K. and K.R. Bastola, 1985. Breeding of Sahar, *Tor putitora*, using naturally matured broods in Tadi river, central Nepal. *J. Nat. Hist. Mus.* 9(1–4): 23–32.

Pathani, S.S. 1983. On the age and growth of mahseer *Tor tor* (Ham.) by opercular bone. *Indian J. Zool.* 11(1): 13–19.

Rai, A.K. 1990. Propagation of katle (*Acrossocheilus hexagonolepis*) and studies on food habits and growth of young ones in different dietary protein levels. A Project Report Submitted to International Development Research Centre (IDRC). Lalitpur, Nepal, Inland Fisheries Dev. Project, Harihar Bhawan, Pulchowk, 22 p.

Shrestha, B.C. and T.B. Gurung, 1987. Declining catches of sahar (*Tor* spp.) from Begnas lake. Paper presented to First Science Congress, RONAST, Nepal.

Shrivastava, G.J. 1968. *Fishes of Eastern Uttar Pradesh and Bihar*. India, Biswa Bidhyalaya Prakashan Varnasi, 163 p.

General Considerations and Recommendations in Fish Nutrition Experimentation

MALI BOONYARATPALIN
Feed Quality Control and Development Division,
National Inland Fisheries Institute
Jutujak, Bangkok 10500, Thailand

BOONYARATPALIN, M. 1994. General considerations and recommendations in fish nutrition experimentation. In De Silva, S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.* Asian Fish. Soc. Spec. Publ. 9. Manila, Philippines, Asian Fisheries Society, pp. 129–134.

Introduction

Nutrition research is essential not only for feed development and profitable aquaculture, but also to help to obtain a better environment which is affected by quality and quantity of feed. A sufficient body of information on nutrient requirements of cultured species would provide culturists with a good basis for devising effective solutions to problems that arise when something goes wrong in the culture system. Compared to livestock husbandry, such as poultry and swine, fish nutrition is a relatively young research area. It is further complicated by concern for water quality; there is loss of nutrients during delivery of nutrients to fish. The loss is inconsistent so that the nutrient requirement level is much lower than the dietary requirement. The requirement level is also affected by water quality or stress induced by poor water quality. Ingredient quality also affects the nutrient requirement level. These factors make comparisons between nutrition research results difficult. Mistakes made during experimentation should be documented so that young researchers will not repeat these, thus saving time, money and other research resources.

This paper describes some important considerations in and offers some recommendations for conducting fish nutrition experiments. This information is mainly derived from our experience, and from Zein-Eldin (1973) and Castell and Tiews (1980).

Ingredients

Quality and quantity

Use the most practical or purified ingredients for nutrient requirements study, otherwise the requirement level might be unreasonably high due to the nutrient quality or because the feed is not well accepted by the test animal. Do not use old ingredients; this often happens in some research laboratories which purchase a large amount of an ingredient and use it over one or two years. This might lead to a wrong conclusion due to the unpalatability or deterioration of some nutrients in the ingredient. This adverse affect might be greater than the effect of the treatment.

The amount of ingredients needed for the entire experiment should be calculated. These ingredients should be procured in bulk and kept in good storage conditions. It is advisable to purchase the whole lot at one time to ensure that the quality of ingredients for the experiment remains constant. When more than one lot of ingredients is used, they are likely to have different qualities, leading to inconclusive or misleading results in some cases.

For example, for a study on the effect of different lipids on the growth and health of sea bass, the protein sources were to have no or very low lipid contents. In this study, casein, gelatine or defatted fish meal was to be used. However, the first two protein sources were not well accepted by sea bass and defatted fish meal is expensive and troublesome to prepare. Therefore, a low fat fish had been identified and prepared as fish meal and used instead of defatted fish meal. Unfortunately, the experiment took longer than expected so that a new batch of fish meal and new test diet had to be prepared and fed to the fish but it was done without realizing that the fat level in this new fish meal was extremely high. The result was that the basal diet had enough essential fatty acid and no significant difference showed among fish fed diet supplemented with palm oil, rape seed oil, soybean oil or cod liver oil. Therefore, if new ingredients are needed for any reason, they should be analyzed before feed formulation and preparation so that adjustments can be made or unsuitable ingredients avoided.

Comparison of qualities

For studies on ingredient quality, such as comparing the growth performance of fish meal from different countries, the researcher must be familiar with the details of processing, sources of raw material, grades of fish meal, criteria for each grade, storage time and conditions and standard of fish meal for each animal feed. Crude protein and ash levels are criteria for grading fish meal in Thailand. There are four grades of fish meal in Thailand, namely, shrimp grade (>68% CP), grade A (>63% CP), grade B (>60% CP), and grade C (>55% CP). The differences are due to the raw materials. There are two types of Danish fish meal; one is processed at low temperature the other under normal temperature. Both have a rather high crude protein content (>72% CP). For a study comparing the performance of Thai fish meal and Danish fish meal for shrimp or sea bass, the Thai fish meal used should be shrimp grade or grade A. The total volatile nitrogen, biogenic amine index, free fatty acid, peroxide value, and anisidine value of fish meal tested should be determined to explain the results obtained, and for fish meal quality improvement.

An erroneous conclusion could be arrived at because of lack of knowledge about the ingredient. For example, a study on soybean meal as a substitute for fish meal in fish diets should consider that (a) fish meal is not only a better protein source for fish but also a fatty acid source for marine fish and that (b) phosphorus in soybean meal is not available therefore, a balance of fish oil and available phosphorus in an isonitrogenous soybean diet is needed. An equal or inferior growth performance to the fish meal diet is to be expected. However, if the experiment shows that soybean meal in fish feed gives a better performance than fish meal as a sole protein source, the fish meal used could have been of poor quality rather than a standard fish meal. In short, the result obtained above is misleading.

Diets

Identification of test diets

The full name of all ingredients used should be given for all prepared feeds. Feed composition with details of vitamins, minerals and additives should be provided. This will clarify the results and provide information for readers to enable them to draw their own conclusions. For example, an advertising document claiming that adding 100 ppm astraxanthin significantly improved growth and immunity in *Penaeus monodon*, without giving details of feed composition, experimental procedure, results and interpretation of results was unclear, as it was not specified whether the pigment sources would have been used as vitamin A (if the diet lacked vitamin A) or whether the effect was due to some impurity in the source which contained 8% pigment only, or whether it was really due to the pigment itself, as claimed.

Reference diets

It is recommended that each fish or shrimp nutrition experiment has a standard reference diet, a positive or negative control diet and treatment diets. The principle of the use of a reference diet is to enable the researcher to determine whether the experimental conditions are optimal for growth, the test animal is in good health, and the positive control diet is as good as the reference diet.

An experiment on vitamin C level and source in *Penaeus monodon* diets carried on for six weeks showed that growth was only double and survival was only 50–60% for all diets tested. Because the reference diet had the same performance this indicated that the poor growth and survival was due to the experimental conditions or poor condition of experimental animals. The condition of experimental animals might have been the main factor because turbid water might contain high nutrients giving a result that is not significant between negative control and treatments but should not cause high mortality.

The reference diet can be the best diet developed by the institution or one of the best commercial diets in the country.

The positive control diet should supply all nutrients required by the test animal and allow optimal growth and survival during the experimental period. The optimal growth and survival rate for each species and experimental conditions need to be established. The optimal growth rate for sea bass in aquaria was 1 to 20 or 30 g in 8 weeks and 95–100% survival. The optimal growth rate for shrimp was 1 to 7 or 8 g in 8 weeks and 75 to 90% survival.

Purified diets

When an amino acid mixture is used as attractant in semi-purified diets, the adjustment or neutralization of pH is required. The optimal dietary pH for fish is 7.5; for shrimp it is 8–8.5. Acid or alkaline diet conditions will affect palatability and nutrient quality.

When a purified diet is used for vitamin requirement study in carnivorous fish, the dietary protein level must be formulated to be higher than the optimal level reported for practical diets. The reason is that fish eat relatively less of the purified diet and thus grow less. Therefore, partial compensation has to be made through a higher dietary protein level which results in higher protein intake and greater growth.

Feeds, Feeding and Experimentation

Feed processing

Mixing may present special problems. The uniform size of ingredients helps achieve a homogeneous mixture. Therefore, all ingredients need to be ground to the same size before weighing and mixing. Preparation of premixtures of microcomponents will facilitate more homogeneous distribution. Homogeneous mixing is very important in diet preparation especially for those micro-ingredients and markers. In a digestibility study where chromic oxide is used as inert marker, if mixing is not homogeneous the dietary chromic oxide level can vary from 0.2 to 0.8 instead of 0.5. This will affect the digestibility of test nutrients.

Another problem in mixing is posed by the vitamin content. An example is adding 30 and 60 ppm of vitamin in diets 1 and 2 using a machine that has a capacity of 2 tonnes/hr. In this case, a sample of 20 kg each was taken for the experiment. From the analysis we found that diet 1 contained 50 ppm and diet 2 had 40 ppm vitamin. A wrong conclusion would have been reached if vitamin contents in the test diets were not analyzed.

Forms of feeds

The physical form of presentation will depend on the preference of the experimental animal, the acceptability being affected by the training of the animal and the facilities available to the researcher. Diets may be presented as microencapsulated or flakes, for fish or shrimp larvae who spend most of their time in the water column. A pellet that sinks to the bottom is given to post-larvae or marketable size shrimp that spend much of their time on the bottom. Shrimp feed should be stable in water for 2-3 hours and attractive since shrimps sense feed by chemo-receptors not by eye and find feed by touch which takes a long time.

Carnivorous fish such as sea bass, grouper, yellowtail and red sea bream prefer moist feed but they can be trained to eat dry feed. A dry slow-sinking pellet is preferred by carnivorous fish; thus

most culturists prefer to give this kind of feed because the fish eat in the water column and soft dry feed is easy to handle and store.

Omnivorous and herbivorous species such as catfish, tilapia and carp accept any form of feed but floating feed allows observation of feeding activity and is less likely to result in over-feeding. However, under-feeding may happen in fish with small stomachs if the feed is too bulky. Fish would not get enough nutrients even if the stomach were full.

The form of feed used in an experiment depends largely on the researcher's facilities. However, powdered feed should be avoided in experiments because of loss of material.

Feeding procedures and frequency

The feeding methods employed in fish nutritional experiments are feeding to satiation, feeding a set amount and pair feeding. Feeding at a set level (% of body weight) will give metabolic efficiency response information only and might result in over-feeding the nutrient-deficient group and under-feeding the nutritionally complete group once appetites are affected by the treatment. Feeding to satiation is more desirable particularly with carnivorous species. By feeding slowly, and carefully watching as the fish eat, the experimenter can feed the fish closer to their maximum rate of consumption without over-feeding and is able to observe possible nutrition deficiency symptoms. Feeding to satiation will provide both feed intake response and metabolic efficiency response information on the test nutrient. Determination of protein retention and fat retention will permit the separation of metabolic efficiency response from the feed intake response. A clearer separation of feed intake response and metabolic efficiency response can be obtained by pair feeding, however, more treatments, facilities and labour are required.

Feeding to satiation is not practical in shrimp nutrition research because shrimp eat very slowly and often. Therefore, feeding at a set level according to their body weight and finely adjusted to the demands of different physiological stages, temperature and water quality is most suitable.

Feeding rate of larvae is different from juveniles or adults. Larvae spend much of their time in the water column. The feed presented not only has to be suspended in the water column but also has to be dense enough so that it is frequently encountered by the larvae and thus ingested. The optimal density of culture animal and feed is very important for artificial feed experiments. The feeding rate has to be based on both volume of water and number of larvae. If stocking rate is low and feeding is based on the volume of water, the water will be polluted and might kill all the shrimp. On the other hand, if we feed according to the number of larvae, the larvae would rarely encounter the feed resulting in high mortality. Concentration of nutrients in the feed also affects the feeding rate. Fish eat to satisfy their energy requirements and would therefore eat more of a low-energy diet than a high-energy diet.

Optimal feeding frequency in fish is influenced by the anatomy of the digestive tract. The size and shape of the stomach as well as the length of the intestine indicate feeding frequency. Stomachless fish have to be fed more than three times a day while fish with large and well-developed stomachs feed only once or twice a day. Feeding once a day or every two days is enough for sluggish fish such as sand goby and grouper in grow-out. Larval fish have a short digestive tract and high metabolic rates so that they need to be fed about every two hours. Shrimp should be fed four to five times a day because of their small stomachs and short intestines. Feeding more often than necessary wastes time and causes size variations, particularly in fish given several small meals.

Starvation control

A starvation control is needed in larval feed experiments for those fish of which little is known about their performance. In a case study with sand goby larvae fed bacteria for 14 days, survival rate was 100% up to 14 days. The conclusion was made that red bacteria is an appropriate feed for newly hatched larvae under 14 days old. The researcher is a microbiologist who did not realize that sand goby larvae can survive for 14 days without feeding. A starvation control is also needed for some outdoor pond experiments where natural food is present. This enables the researcher to differentiate the effect or contribution of natural food from the treatments. A non-protein feed is a treatment required in a nitrogen balance study for obtaining metabolic faecal nitrogen.

Experimental conditions

For micronutrient requirement studies conditions should be such that the water or experimental tanks should be completely devoid of natural food. Therefore, the supply needs to be filtered and tanks have to be in the dark or covered.

The choice of experimental conditions that are standard, reproducible and optimal is the first problem for nutritionists. Aquatic animals pose an additional problem for nutritionists who must know how the fish or shrimp grow well, have a knowledge of optimal temperature, salinity, pH and stocking density, as well as be able to provide adequate water, aeration and effective removal of wastes.

The optimal experimental conditions for sea bass nutrition research based on the experiences at the National Institute of Coastal Aquaculture is as follows: stocking density of 15, 2.5 cm fish per 45 L aquarium with flow-through filtered seawater at a flow rate of 0.7 L/min and adequate aeration to provide dissolved oxygen at 4.5–5.0 mg/L; pH 7.8–8.5; temperature 28–29°C. Final biomass is 300 g/aquarium in 8 weeks. Some unfavourable conditions might have greater effect on growth than the treatments, therefore the effect of the treatments would not be clear. This would result in an erroneous conclusion.

Zein-Eldin and Meyers (1973) reported that a recirculation system with bottom filters and sterilized water is more successful in promoting the growth of juvenile brown shrimp *Penaeus aztecus* than a cleaner flowing water system. However, a suitable water system depends on quality and quantity of the water supply in an area. Therefore, the suitable experimental conditions in each laboratory need to be established.

Experimental animals

It is impossible for an investigator to make an intelligent comparison between groups of animals without an adequate knowledge of the normal variability within the experimental population. Differences in growth rates among individual shrimp and sea bass seem to be greater than in omnivorous or herbivorous species. This inherent variability makes interpretation of data even more difficult when comparisons are made between groups of only 10–15 animals; the mean may be markedly affected by the presence of one rapid- or one slow-growing shrimp or fish. This can be solved by acclimatizing the animal to a control feed, and grading the fish or shrimp twice in twenty days to get the uniform size which accept an artificial feed. Size variability in carnivorous fish and shrimp is a problem which must be recognized and this variable must be duly considered before starting an experiment.

Test animals should be deliberately selected for maximum homogeneity and then randomly distributed among treatment groups in appropriate numbers relevant to the hypothesis of the study to fulfil the biological and statistical requirements of the experiment.

Differences in the number or weight of experimental animals at the start of an experiment should be avoided. However, if these variables were not observed before starting the experiment, statistical test of slope can be used to evaluate the difference instead of testing the means.

Replication

The number of replicates depends on the variability of the test animals, the desired accuracy of the experimental results and the degree of difference expected between treatments. For a small scale experiment in aquarium or tank, three replicates are desirable for fish and five replicates for shrimp. Replication is of immense importance because it enables the experimenter to perform reliable statistical analysis.

The number of animals per replicate will depend on homogeneity, size and limitations of the system and should include at least the minimum number of animals for statistical analysis. The maximum number will depend upon the carrying capacity of the system.

Experimental period

An experiment should be continued until there has been a ten- or twenty-fold increase in fish size or at least a four-fold increase in shrimp size or until a significant difference is observed or deficiency signs occur.

Growth measurement

Growth or total weight in each aquarium or tank should be determined at the start, the end and at least twice in between, or at the break point if it is known. Weighing of each individual should be avoided as it causes too much stress. Nevertheless, at the end of the experiment individual weights or lengths can be determined for size distribution study, and for estimating the health status of fish. The inherent errors in wet weight determination must be recognized. The recommended procedure is to net, anaesthetize, and blot dry the fish with a cloth and then count them in a prepared plastic box with a cover before weighing. The total weight and number for each aquarium should be recorded in absolute terms. However, the specific procedure used for weighing must be stated.

Growth measurement in between the start and end of the experimental period will provide information on what exactly happens during each subperiod in addition to the overall comprehensive result. This allows for a more precise conclusion. However, unnecessary handling of test animals should be avoided.

Growth has frequently been presented as per cent gain either in mean weight or biomass (total weight). The interpretation of this value is complicated when comparing groups of animals of varying initial weights, as per cent increase is inversely related to initial weight. Since growth is a function of time, the use of a simple method of data presentation is recommended, i.e. graph of mean weight or biomass versus elapsed time. Growth data by mean weight should be presented along with a survival graph.

References

Castell, J.D. and K. Tiews, 1980. *Standardization of Methodology in Fish Nutrition Research*. EIFAC Technical Paper No. 36, 24 p.

Yakupitiyage, A., P. Edward and K. Wee, 1991. Supplementary feeding of fish in a duck-fish integrated system. I. The effect of rice-bran. In De Silva S.S. (ed.) *Fish Nutrition Research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop*. Manila, Philippines, Asian Fisheries Society and International Development Research Centre of Canada. pp. 143–157.

Zein-Eldin, Z.P. 1973. General consideration of problems in shrimp nutrition. In *Proceedings of the World Mariculture Society, Vol. 4*. pp. 299–317.

Workshop Recommendations

1. The workshop recommended that every effort be made to continue the network activities as they have contributed significantly to enhancing the quality of fish nutrition research in Asia, particularly in changing the direction of research to a more applied, systems approach rather than a commodity approach.

2. There was general agreement that more research effort should be expended on understanding the nutrient requirements of cultured species in semi-intensive systems, and the effectiveness of utilization of supplementary feeds in such systems.

3. The workshop recommended that a training course be conducted on methodologies of determining the supplementary feed/nutrient requirements of target species in semi-intensive culture systems. The workshop noted that in view of the varying nature of the prevalent culture practices in semi-intensive systems that standardisation of the ensuing results would be difficult. However, adoption of similar methodologies throughout the region will still permit useful comparisons to be made which would have a beneficial effect in increasing production and sustaining growth of the systems.

4. The workshop commended the initiation of the network in its efforts to compile reference lists of research findings published in national languages.

   The workshop recommended that this activity be continued and extended further, if resources permit, to provide researchers with English translations of selected articles. It was expected that members of the network would do the translations on an honorary basis.

   The workshop felt that activity associated with information on the literature is an important function of the network, and that all attempts should be made to retain this activity, in spite of financial constraints.

5. The workshop welcomed that a training course was to be held immediately afterwards. It expressed the view that the planned format and structure of the training course is relevant to fish nutrition researchers in the region.

   The workshop recommended that all efforts be made to organise such training courses, ideally following workshops so that the costs could be reduced, and that these training courses should be publicised extensively in the host country to attract resident young nutrition researchers to such courses.

6. The workshop recommended that the thrust in farm-based nutrition research, and that in semi-intensive systems should be continued. It also noted that the change in the attitude of nutrition researchers to adopt a more 'bottom-up' approach was welcome and should be encouraged further.

Accordingly the workshop re-endorsed that priority for research support and funding be directed to:

(a) areas of research on the role of supplementary feeds and feeding in semi-intensive aquaculture systems;
(b) areas of research concerned with feed costs saving (direct or indirect); and
(c) fish nutrition research under 'on-farm' conditions.

The workshop was of the view that research on the basic nutrient requirements of those species on which little information is available should be encouraged. Because of the long-term nature of such research, cost and often difficulty of acquiring proper purified ingredients, the major support for research funds for basic nutrient requirement studies must be sought through the country's own government research agencies. The workshop felt that research on nutrient systems should be given priority and the relevant methodologies be developed, reiterating recommendations made earlier. Applied fish nutrition is considered more useful for aquaculture development in the region because findings from such research have direct applications in diet formulation and feed production.

7. The workshop noted that fish farmers, especially in semi-urban areas, are increasingly being induced to use commercial feeds which are not specifically formulated for the species cultured or for semi-intensive systems. Such activity incurs higher feed costs and over-burdens the systems with excess nutrients. The workshop therefore recommended that governments be urged to introduce more and correct information on the packaging.

8. The participants noted that use of commercial feeds in semi-intensive culture practices, particularly in coastal and semi-urban areas has increased over the years. This trend has also resulted in the establishment of small-scale feed mills in some countries which are attempting to fill a particular market niche. These small-scale manufacturers however, often do not have R & D components. The workshop recommended that fish nutrition researchers initiate collaborative research with commercial feed manufacturers, as they have done with farmers, which will be beneficial to small-scale manufacturers and to the industry at large.

9. The participants were of the view that the present workshop has been very useful, as were the previous ones, in providing a forum to discuss their research findings and future research openly and uninhibitedly. The workshop also has enabled them to adopt proper techniques or to improve on techniques already used, and most of all to obtain first-hand knowledge of ongoing related work in the region.

The workshop therefore recommends that future meetings of this nature should be held at regular intervals.

List of Participants
Fifth Asian Fish Nutrition Workshop
January 1993

Ms Ilda G. Borlongan
AQD/SEAFDEC
PO Box 256
Tigbauan
Iloilo City
Philippines 5000

Dr M. Boonyaratpalin
Feed Quality Control and Development
Division
National Inland Fisheries Institute
Jatujak
Bangkok 10500
Thailand

Dr Sena S. De Silva
School of Aquatic Science and Natural
Resources Management
Deakin University
Warrnambool
Victoria 3280
Australia

Mr Lin Ding
Icthyology Laboratory
Zhongshan University
Guangzhou
China

Ms Deng Dong Fang
Ichthyology Laboratory
Zhongshan University
Guangzhou
China

Mr Xu Guohuan
Institute of Reservoir Fisheries
Ministry of Water Resources
Wuhan, Hubei 430072
China

Dr M.A.B. Habib
Dept of Fisheries Biology & Limnology
Bangladesh Agricultural University
Mymensingh 2202
Bangladesh

Dr Roshada Hashim
School of Biological Science
USM
Minden
11800 Penang
Malaysia

Dr M.R. Hassan
Dept of Fisheries Biology and Limnology
Bangladesh Agricultural University
Mymensingh 2202
Bangladesh

Prof. Xiqin He
Institute of Reservoir Fisheries
Ministry of Water Resources
Wuhan, Hubei 430072
China

Mr Ngan Heng
Freshwater Fish Culture Station
Batit
Cambodia

Dr P. Keshavanath
College of Fisheries
Mangalore 575 002
Karnataka
India

Dr Jawaman Khajarern
Department of Food Science
Khon Kaen University
Khon Kaen
Tnailand

Dr Santosh Lall
Fisheries Laboratory
PO Box 550
1707 Lower Water Street
Halifax, Nova Scotia
B3J 2S7 Canada

Dr M.C. Nandeesha
(Cambodia)
Mailing Address:
Padek-Phnom Penh
GPO Box 2420
Bangkok 19501
Thailand
Fax: (855) 23-26224

Dr Suhenda Ningrum
Research Institute for Inland Fisheries
Jalan Sempur No 1
Bogor
Indonesia

Mr A.K. Rai
Fisheries Development Division
Department of Agriculture
Harihar Bhawan
Lalitpur
Nepal

Dr C. Santiago
AQD/SEAFDEC
Binangonan
Rizal
Philippines

Dr H. Sehgal
Department of Zoology
Punjab Agricultural University
Ludhiana 141 004
Punjab
India

Mr Khat Sokhorn
Fisheries Research Station
Phnom Penh
Cambodia

Dr Nguyen Anh Tuan
Department of Hydrobiology
Faculty of Fisheries
University of Cantho
Hau Giang
Vietnam

Mr Jaruratt Wannagowat
Feed Quality Control and Development
Division
National Inland Fisheries Institute
Jatujak
Bangkok 10500
Thailand

Dr Kok Leong Wee
Aquaculture Key Centre
University of Tasmania
PO Box 1214
Launceston
Tasmania 7250
Australia

Dr Ji Wenjuan
Yellow Sea Fisheries Research Institute
19 Laiyang Road
Qindao 266003
China

Dr Amararatne Yakupitiyage
Division of Agriculture & Food Engineering
Asian Institute of Technology
GPO Box 2754
Bangkok 10501
Thailand

TITLES IN THE ASIAN FISHERIES SOCIETY SPECIAL PUBLICATION SERIES

- **Fish Quarantine and Fish Diseases in South and Southeast Asia: 1986 Update.** 1987. Edited by J.R. Arthur. Asian Fish. Soc. Spec. Publ. 1, 86 p. Price: Members, free. Nonmembers, US$5.
- **Fisheries Education and Training in Asia: Workshop Proceedings.** 1988. Asian Fish. Soc. Spec. Publ. 2, 162 p. Price: Members, free. Nonmembers, US$8.
- **Exotic Aquatic Organisms in Asia.** 1989. Edited by S.S. De Silva. Asian Fish. Soc. Spec. Publ. 3, 154 p. Price: Members, free. Nonmembers, US$8.
- **Fish Nutrition Research in Asia. Proceedings of the Third Asian Fish Nutrition Network Meeting.** 1989. Edited by S.S. De Silva. Asian Fish. Soc. Spec. Publ. 4, 166 p. Price: Members, free. Nonmembers, US$8.
- **Fish Nutrition Research in Asia. Proceedings of the Fourth Asian Fish Nutrition Workshop.** 1991. Edited by S.S. De Silva. Asian Fish Spec. Publ. 5, 207 p. Price: Members, free. Nonmembers, US$15.
- **Asian Fisheries Science: A Profile.** 1991. A.C. Aquisap, M.A. Carigma, P.B. Cariño, V.M.J. Castrillo, F.C. Gayanilo, Jr., M.E.S. Guzman, C.C. Janagap, J.L. Maclean, E.T. Tech and R.M. Temprosa. Asian Fish. Soc. Spec. Publ. 6, 45 p. Price: Members, free. Nonmembers, US$10.
- **Resource Allocation to Fisheries Research in Asia.** 1993. Edited by F.B. Davy. Asian Fish. Soc. Spec. Publ. 7, 172 p. Price: Members, free. Nonmembers, US$15.
- **Fisheries Education and Training in Asia: Second Workshop Proceedings.** Asian Fish. Soc. Spec. Publ. 8, 293 p. Price: Members, free. Nonmembers, US$10.
- **Fish Nutrition Research in Asia. Proceedings of the Fifth Asian Fish Nutrition Workshop.** 1994. Edited by S.S. De Silva. Asian Fish. Soc. Spec. Publ. 9, 138 p. Price: Members, free. Nonmembers, US$15.

ASIAN FISHERIES SOCIETY

The Asian Fisheries Society seeks to promote interaction and cooperation among Asian Fisheries Scientists and technicians: to propagate an awareness of the importance and the ways of sound conservation and use of aquatic resources; and to join in these goals with similar societies. The Society consists of 2,500 scientists (full members), primarily from Asia, as well as many persons (associates) and organizations (institutional and sustaining members) interested in the Society's objectives. If you would like more information or wish to join the Society, please contact the Executive Officer, Asian Fisheries Society, MCPO Box 2631, Makati, Metro Manila 0718, Philippines.

INTERNATIONAL DEVELOPMENT RESEARCH CENTRE

The International Development Research Centre is a public corporation created by the Parliament of Canada in 1970 to support research designed to adapt science and technology to the needs of developing countries. The Centre's activity is concentrated in five sectors: agriculture, foods and nutrition sciences; health sciences; information sciences; and communications. IDRC is financed solely by the Parliament of Canada; its policies, however, are set by an international Board of Governors. The Centre's headquarters are in Ottawa, Canada. Regional offices are located in Africa, Asia, Latin America and the Middle East.