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# **Feeds and feeding strategies for *Colossoma macropomum* (Cuvier 1818)**

**fish growth as related to dietary protein**

**M. B. van der Meer**

# **FEEDS AND FEEDING STRATEGIES FOR *COLOSSOMA MACROPOMUM* (Cuvier 1818)**

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Magnus B. Van der Meer

## Stellingen

1. Kannibalisme is vooral voor herbivore vissen een efficiënte manier om de eiwitkwaliteit van het voedsel te optimaliseren (dit proefschrift).
2. In de huidige discussies rond duurzame visteelt zijn voerverliezen een onderschatte factor (dit proefschrift).
3. *Colossoma macropomum* lijkt in nutritioneel opzicht meer op een varken dan op een kalf.  
P. Bikker, 1994, Protein and lipid accretion in body components of growing pigs: effects of body weight and nutrient intake, PhD thesis, Wageningen Agricultural University, 203 pp.; W.J.J. Gerrits, 1996, Modelling growth of preruminant calves, PhD thesis, Wageningen Agricultural University, Wageningen, 195 pp.; Dit proefschrift.
4. De voeropnamecapaciteit en de verteringssnelheid worden bij *C. macropomum* medebepaald door het tijdstip van de dag waarop gevoerd wordt (dit proefschrift). De huidige modellen zouden voor het simuleren van de groei van *C. macropomum* dan ook tijdstappen van minimaal één dag moeten aanhouden.
5. Vismeel is geen onontbeerlijk ingrediënt in visvoer (dit proefschrift).
6. Een snelle groei en een variabele lichaamssamenstelling maken *C. macropomum* tot een ideaal proefdier voor nutritioneel onderzoek.
7. Zowel de "compensatoire groei" na een periode van vasten, als ook de aanpassing van de lichaamssamenstelling aan leeftijd, voerhoeveelheid en voersamenstelling berusten voornamelijk op verschillen in groeisnelheid van verschillende delen van het lichaam.
8. De praktische en wetenschappelijke waarde van groeiproeven is gering, wanneer niet in minstens één behandeling de maximaal haalbare groei benaderd wordt.
9. Ondanks dat de indeling van handboeken vaak anders doet vermoeden, zou de voeding van vissen wel eens meer overeenkomsten kunnen hebben met de voeding van de landbouwhuisdieren dan met die van garnalen.
10. Terwijl armoede vroeger meestal ontstond omdat mensen geen werk hadden, ontstaat de "nieuwe armoede" (geen tijd om de kinderen op te voeden) juist bij mensen met teveel werk.  
De Volkskrant.
11. Hoewel ook vissen zwemmen zonder diploma, is het niet hebben van een zwemdiploma voor een visteeltkundig onderzoeker toch een zekere garantie dat hij de gepaste afstand tot zijn onderwerp bewaart (deze promovendus).

12. Models cannot be validated, they can be only invalidated.

J.L. Black, 1995, The testing and evaluation of models, p. 23-31 *in*: Modelling growth in the pig (ed. by P.J. Moughan, M.W.A. Verstegen and M.I. Visser-Reyneveld), European Association for Animal Production.

Stellingen behorende bij het proefschrift :

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### Abstract

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*Colossoma macropomum* is an indigenous fish species from the Amazon region. The amino acid profile of its body protein proved to be similar to that of other fish species. Soya meal and fish meal have, based on their amino acid profiles, a comparable protein quality. This hypothesis was confirmed in a feeding trial. As soya meal is less palatable than fish meal, more soya in the diet tends to decrease feed uptake and growth rate, but increases protein utilization efficiency. Growth of *C. macropomum* is fast and requires a dietary protein content of approximately 43%. Fish growing at maximal speed displayed a protein utilization efficiency of only 50% of the maximal possible efficiency. In *ad libitum* fed *C. macropomum* 20 - 30% of the feed remains uneaten. In spite of this wasted feed, the feed uptake/weight gain ratio ranged between 0.57 and 0.71 with a high quality feed. Increasing the feeding frequency from one to five meals per day increased feed uptake and growth. Fish displayed a clear daily bio-rhythm with maximal feed uptake in the late afternoon. Lipid addition to the diet increased the protein utilization efficiency less than reported for salmon. An increased dietary protein content increased the body protein content and decreased the body lipid content. Adaptation of the size of the internal organs to the characteristics of the diet is possibly the cause that diet composition affects the body composition of fish. Accumulated data were analyzed by an empirical and an explanatory model. Both methods identified protein ration as the major variable determining fish growth. In describing the data, the average errors of both methods were similar. However, the empirical model produced more outlying values. It was concluded that the explanatory model with some minor improvements can be turned into a useful tool for research and practical management. Fast growth and a flexible body composition make *C. macropomum* a suitable study object for the development of improved fish growth models.

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# Feeds and feeding strategies for *Colossoma macropomum* (Cuvier 1818): fish growth as related to dietary protein.

## Table of contents

<b>Chapter 1.</b>	<b>General Introduction.</b>	<b>1</b>
<b>Chapter 2.</b>	<b>The amino acid composition of the dietary protein as a constraint to growth.</b>	
I.	Comparison of amino acid profiles of feeds and fish as a quick method for selection of feed ingredients: a case study for <i>Colossoma macropomum</i> (Cuvier). M.B. van der Meer & M.C.J. Verdegem (1996) Aquaculture Research 27, 487-495.	9
II.	Feed consumption, growth and protein utilization of <i>Colossoma macropomum</i> at different dietary soya meal/fish meal ratios. M.B. van der Meer, E.A. Huisman & M.C.J. Verdegem (1996) Aquaculture Research 27, 531-538.	19
<b>Chapter 3.</b>	<b>The effect of protein ration on growth and protein utilization.</b>	
I.	The effect of dietary protein level on growth, protein utilization and body composition of <i>Colossoma macropomum</i> (Cuvier). M.B. van der Meer, M.A.M. Machiels & M.C.J. Verdegem (1995) Aquaculture Research 26, 901-909.	29
II.	Effect of feeding level on feed losses and feed utilization of soya and fish meal diets in <i>Colossoma macropomum</i> (Cuvier). M.B. van der Meer, R. Faber, J.E. Zamora & M.C.J. Verdegem (1997) Aquaculture Research, 28: 391-403.	41
III.	Effect of number of meals and frequency of feeding on voluntary feed intake of <i>Colossoma macropomum</i> (Cuvier). M.B. van der Meer, H. van Herwaarden & M.C.J. Verdegem (1997) Aquaculture Research, 28, 419-432.	57
<b>Chapter 4.</b>	<b>The effect of dietary energy on growth and protein utilization.</b>	
	Effect of dietary lipid level on protein utilization and the size and proximate composition of body compartments of <i>Colossoma macropomum</i> (Cuvier). M.B. van der Meer, J.E. Zamora & M.C.J. Verdegem (1997) Aquaculture Research, 28, 405-417.	75
<b>Chapter 5.</b>	<b>Modelling the growth of <i>Colossoma macropomum</i>.</b>	
	Modelling growth of <i>Colossoma macropomum</i> (Cuvier): comparison of an empirical and an explanatory model. M.B. van der Meer & A.A. van Dam (1997) Aquaculture Research, submitted.	93
<b>Chapter 6.</b>	<b>General discussion.</b>	<b>119</b>
<b>Summary</b>		<b>129</b>
<b>Samenvatting</b>		<b>131</b>
<b>Resumen</b>		<b>133</b>
<b>Dankwoord</b>		<b>135</b>
<b>Curriculum vitae</b>		<b>137</b>

# Chapter 1

## General introduction

### BACKGROUND

Fish culture is one of the fastest growing food production activities in the world (FAO 1996). While in 1984 only 4.9% of the world fish production was produced by fish culture (the rest being produced by fisheries), in 1994 this was 23.3%. All relevant recent aquaculture developments depend mainly on manufactured feeds. Examples are the shrimp industry in Latin America and Asia, the salmon industry in Norway, Scotland and Chili, the eel industry in Europe and Japan, the channel catfish industry in the USA and the sea bass and sea bream industry in the Mediterranean sea. In these intensive fish culture industries, expenses for feed are the major production cost. Nutritional research leading to decreased feed costs contributed to the fast expansion of the channel catfish industry in the 1980s (Wilson & Lovell 1991).

In The Netherlands, intensive fish culture production developed in the sixties and early seventies when increased labor costs made extensive production methods unattractive (Huisman 1974). The Organisation for Improvement of Inland Fisheries (OVb) identified a lack of expertise in intensive fish culture and initiated 'a study on optimal rearing conditions for carp' (Huisman 1974; Table 1). One of the conclusions of the study was that growth and feed utilization efficiency could be improved by rearing the fish in warm water.

Recognizing the high potential of increased temperatures for fish production, Hogendoorn (1983) focussed research on the warm water species *Clarias gariepinus*, the African catfish. Based on the principles outlined by Huisman, Hogendoorn developed a bioenergetic model for the growth of African catfish. His study had two important impacts: (1) the accumulated knowledge initiated a small industrial production of African catfish in the Netherlands, and (2) the idea of modelling growth of fish had been born.

Hogendoorn's model was descriptive and 'only representative for conditions prevailing in the experimental setup' (Machiels 1987). An 'explanatory' model, however, can be used beyond the range of experimental data. Till today, fully explanatory fish growth models do not exist and models still contain descriptive parts to describe mechanisms which are not yet understood.

The explanatory model called the 'Fish Growth Simulator' or FGS of Machiels (1987) simulated growth and body composition of fish based on the biochemical pathways of dietary protein, fat and carbohydrate during the metabolic processes. Effects of different feed compositions on growth and body composition of the African catfish were adequately predicted by the model. Because the FGS has descriptive components, calibration of the FGS to other species requires experimental data of those species. The FGS was successfully used to simulate the growth of trout and tilapia (Van Dam 1995).

**Table 1.** Fish nutrition research projects of the Wageningen Agricultural University (WAU): species, variables studied and models used.

Author *)	Huisman	Hogendoorn	Machiels	van Dam	vd Meer
Year	1974	1983	1987	1995	1997
<b>Fish</b>					
species	<i>C. carpio</i>	<i>Cl. gariepinus</i>	<i>Cl. gariepinus</i>	<i>O. niloticus</i> / <i>O. mykiss</i>	<i>C. macropomum</i>
origin	Asia, Europe	Africa	Africa	Africa, Asia / North America	South America
opt. temperature	cool	warm	warm	warm / cold	warm
feeding habit	omnivorous	omnivorous	omnivorous	planktivorous/carnivorous	herbivorous
<b>Main explaining variables</b>					
temperature	X	X			
feeding level	X	X	X	X	X
feeding regimes	X	X			X
feed nutrients			X	X	X
feed ingredients			X		X
body weight		X			X
body composition			X		
dissolved oxygen				X	
<b>Main dependent variables</b>					
oxygen consumption		X	X	X	
growth	X	X	X	X	X
feed utilization	X	X	X	X	X
energy deposition	X	X			
protein deposition			X		X
lipid deposition			X	X	X
<b>Model type</b>					
descriptive	X	X		X	X
explanatory			X	X	X

\*) For Huisman, Hogendoorn, Machiels and van Dam: see References; vd Meer: this study.

In nutritional studies, common variables relate to feed quantity or feed quality (the explaining variables), while growth is mostly the measured response (Table 1). The way to describe growth has changed in time. Twenty years ago growth of fish was considered a process of converting dietary energy in biomass energy. In this view, dietary energy is either retained for growth (this is: energy retained through deposition of protein and lipid), or lost for maintenance and physical activities (Huisman 1974; Hogendoorn 1983). However, Machiels (1987) pointed out that weight gain of fish was mainly a matter of protein gain. Increased body energy content is not accompanied by weight increase if the energy is retained as lipid, because lipid deposition is generally followed by an approximately equal loss of body water and therefore not affecting body weight. Results of Van Dam (1995) confirmed this view.

The present explanatory growth models are based on metabolic principles generally valid in animals. If fully explanatory, a model developed for one type of fish requires only minor adaptations to make it suitable for other types. This was also proven by the fact that a model elaborated for African catfish (Machiels 1987) could be adapted for catfish larvae (Conceição et al. 1993), tilapia and trout (Van Dam 1995).

Considering the relative species independent character of nutritional research, knowledge gained using modelling can be applied to other fish species. When the Universidad Nacional in Costa Rica asked the Department of Fish Culture and Fisheries of WAU (Wageningen Agricultural University) to jointly work out the nutritional requirements and feeding strategies of *Colossoma macropomum* (Cuvier), a herbivorous fish species, it was decided to explore first the general requirements of the species. Finally the acquired species specific knowledge would be used to adapt the FGS to this species. It was expected that a study on *C. macropomum*, with different feeding habits than carp (omnivorous), African catfish (omnivorous), tilapia (planktivorous) and trout (carnivorous), could make the model more generally applicable.

*C. macropomum* had been identified as a promising candidate for aquaculture development in Latin America (Lovshin 1980; Saint-Paul 1985; Saint-Paul 1986) and as such fitted in the objectives of Costa Rica to develop its own aquaculture industry. A fish species used for aquaculture activities should ideally possess the following characteristics (Huet 1975):

*(1) Adapts well to the temperature.*

*C. macropomum* is an indigenous fish species from the Amazon region. The water temperature in this area remains close to 29°C during the whole year (Lowe-McConnell 1987). Therefore, it might be expected that the optimal temperature for *C. macropomum* is close to 29°C. Maximal growth of 0.5 g fish was obtained at 32°C, while at the lowest experimental temperature (24°C) growth was seriously impaired (Van der Meer, unpublished data). Poor growth of *C. macropomum* at temperatures below 25°C has also been reported in ponds (Merola & Pagán-Font 1988; Teichert-Coddington 1996).

*(2) Grows fast.*

Some species can be excluded for aquacultural use because they never reach a commercial size or it takes them too long to reach that size. *C. macropomum* is reported to have a maximum size of approximately 30 kg (Saint-Paul 1985), while its commercial size is 1 to 1½ kg. Günther & Boza (1993) found that *C. macropomum* grows faster than well-known aquacultural species like tilapia and African catfish.

*(3) Has a short food chain.*

In ponds where no supplemental feed is available, fish have to feed low in the food pyramid to attain an acceptable production level. Hence, in extensive pond culture the 'best' fish species are omnivorous, able to feed on plankton, macrophytes and detritus. Adult *C. macropomum* is reported to be herbivorous and consume considerable amounts of terrestrial seeds and fruits (Saint-Paul 1985). However, *C. macropomum* is not an efficient exploiter of plankton and grows poorly in ponds where no supplemental diet is available (Van der Meer & Martínez 1993; Teichert-Coddington 1993).

*(4) Reproduces easily under conditions of cultivation.*

Techniques for hormone induced reproduction of *C. macropomum* under culture conditions have already been applied successfully for a long time (FAO 1984). However, it seems that even nowadays these techniques are not completely reliable. Moreover, larval rearing needs considerable

improvement: survival seldomly exceeds a few percentages over the period between hatching and weaning (Van der Meer, unpublished results). However, these problems are no constraint for the development of large scale production of *C. macropomum* as one female may produce over one million eggs per spawning.

(5) *Accepts readily artificial feed.*

Except the difficulty of weaning *C. macropomum* from life feed (e.g. artemia) to dry feed (usually when they attain an individual wet body weight of 60 - 80 mg), *C. macropomum* dwells extremely well on dry, formulated feed. *C. macropomum* seems to learn easily to eat from self-feeders (FAO 1984). Huet (1975) explicitly says that 'it is necessary that reared fish accept an abundance of cheap, artificial food'. *C. macropomum* is reported to accept many kinds of unconventional fish feeds as 'whole grain corn', oil palm seed, chicken feed etc. (FAO 1984). Also wild fruits and seeds have been fed to *C. macropomum* with some success (Roubach & Saint-Paul 1994).

(6) *Satisfies the consumer's demand.*

In its native area *C. macropomum* commands a good price as a consumption fish (Lovshin 1980). However, its intramuscular spines makes the fish less appreciated by people never exposed to fish with similar meat. In Costa Rica, outside its native area, the fish was readily accepted by locals from Asian origin, but the acceptability under the rest of the population was poor (Raymon van Anrooy, MSc thesis, Wageningen Agricultural University).

(7) *Resists high stocking densities.*

Fish should be social and exhibit schooling behaviour to keep them free of stress and allow optimal growth under high stocking densities. As far as known, the relationship between density and growth of *C. macropomum* has never been described adequately. Observations in the field showed that *C. macropomum* in ponds is a social fish, swimming most of the time in schools. However, in aquaria also solitary fish remained apparently unstressed and showed even some degree of domestication by starting to eat from the hands of the person in charge (personal observation).

(8) *Resists diseases and handling.*

*C. macropomum* resists handling well (Lovshin 1980). However, heavy mortalities due to invasions of *Saprolegnia* sp or *Ichthyophthirius multifiliis* are reported in eggs and fish when handling causes damages (Bermúdez 1980). Columnaris disease has been mentioned as a constraint in intensive culture systems of *C. macropomum* (Ravelo & Conroy 1994). Information about other diseases of *C. macropomum* is scarce. Possibly this is due to the few number of intensive production units of this species, but it might also point to a strong resistance against diseases.

*C. macropomum* possesses an extendable underlip allowing to exploit a very thin surface layer of well-oxygenated water for respiration. This helps the fish to survive low dissolved oxygen concentrations (Braun 1983) and could be an important characteristic of the fish under less favourable culture conditions.

Resuming the above 'postulates of Huet', it can be said that *C. macropomum* is a promising species for aquaculture because it is hardy, fast growing, easy to handle and it readily accepts artificial diets. However, reproduction and larval rearing techniques need improvement. The major factor limiting general acceptance of *C. macropomum* might be its intermuscular spines. Although supplemental feeds seem indispensable for good production figures, the costs of feeding might be modest as *C. macropomum* readily eats and grows well on cheap vegetal materials.

## THE OBJECTIVE OF THE STUDY

The general objective of this thesis was to determine the relationships between feed quality and quantity on growth and body composition of *C. macropomum*. Accumulated data and gained insights during this thesis must allow for formulating feeds and conceptualizing feeding regimes appropriate for the species.

Recognizing the importance of the protein metabolism for fish culture from a biological and economical point of view, the present thesis focussed on optimizing the conversion of dietary protein into fish body protein. Effects of dietary protein quality, protein quantity and lipid quantity on this conversion efficiency were studied and compared with such effects in other fish species. The information obtained was used to parameterize an explanatory growth model for *C. macropomum*.

## INTRODUCTION TO THE FOLLOWING CHAPTERS

The ultimate objective of fish culture is fish biomass gain. Biomass gain requires a proportional increase of body protein. Animals can synthesize body protein only from dietary protein. Proteins are composed from amino acids (AA's). AA's that cannot be synthesized by the animal are called "indispensable amino acids" (IAA's). As the AA composition of body protein is considered constant, the lack of only one IAA halts the synthesis of protein and impedes growth. The correct AA composition of a diet is therefore very important if maximum growth or a minimal protein intake/protein gain ratio is required. Once AA compositions of fish and feed ingredients are known, it can be calculated which mix of ingredients will result in the best feed protein quality (Chapter 2-I). This method was applied to select feed ingredients for substitution of fish meal as the main source of protein in fish diets. Selection of alternative ingredients for fish meal is needed to reduce feed costs and to anticipate expected fish meal shortages in the future.

Testing of diets in growth experiments remains necessary, as alternative ingredients might contain substances which interfere in the (protein) metabolism, by that inhibiting a good dietary protein quality from being expressed in good growth. Presuming that at low protein rations fish tend to improve the protein utilization efficiency, it was expected that growth will best reflect differences in dietary protein quality at low protein rations (Chapter 2-II).

Not only protein quality, but also protein quantity can affect its utilization efficiency. It is generally accepted that fish use dietary protein less efficiently at higher feeding levels. This might be caused by either higher feed losses at higher feeding levels, or by a lower feed digestibility when the digestive tract becomes overloaded (Henken, Kleingeld & Tijssen 1985). Feeding restricted protein rations improves the protein utilization efficiency, but the limited amount of available protein will reduce growth. At extremely low protein rations, protein utilization efficiency will decrease as

most of the protein will be spent for maintenance, rather than for increasing the amount of body protein. On these grounds an optimal protein utilization efficiency was expected at a growth rate somewhere between zero and maximum growth. The relation between protein ration and protein gain was studied by applying isocaloric diets with different protein concentrations (Chapter 3-I).

During the experiments described in Chapter 2-II an increased turbulence of the water suggested that substantial amounts of feed were lost in the water. Indications were found that feed losses increased with protein ration (Chapter 3-I). Therefore, an experiment was designed to reveal the relationship between feed ration and feed losses (Chapter 3-II). Feeding levels were set at *ad libitum* and at approximately 60 and 80% of the *ad libitum* level. At sampling days feed marked with chromic oxide was applied at one of the three daily feedings. Chromic oxide recuperation from the digestive tract shortly after the last daily feeding was used to estimate feed losses and feed evacuation rate in the digestive tract in dependence of feed ration.

Feed uptake can be manipulated not only by applying *ad libitum* or restricted feeding levels, but also by varying the feeding regime. Different feeding regimes with one to five meals per day and with intervals of 3 to 24 hours between meals were tested (Chapter 3-III). Feed rations were recorded per meal. Chromic oxide marked feed was used to estimate feed losses and feed evacuation velocity depending on the feeding regime.

In Chapter 4 the effect of the dietary energy level on protein utilization efficiency was studied to evaluate the importance of the "protein sparing action" of dietary lipid in *C. macropomum*. Fish were dissected to study the distribution of fat deposition over intestines, head and trunk.

In Chapter 5 the accumulated data of the previous chapters were analyzed. Just like the data of the separate experiments, the accumulated data were analyzed by a linear method (regression analysis). However, the data were also used to calibrate the "Fish Growth Simulator" model (Machiels 1987; Van Dam 1995). Conclusions and accurateness from both analyses were compared. Finally, in Chapter 6, recommendations are given for the optimalization of the culture of *C. macropomum* under controlled conditions and suggestions for further research are made.

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## Chapter 2

### THE AMINO ACID COMPOSITION OF DIETARY PROTEIN AS A CONSTRAINT TO GROWTH

#### I

### Comparison of amino acid profiles of feeds and fish as a quick method for selection of feed ingredients: a case study for *Colossoma macropomum* (Cuvier)

#### ABSTRACT

The amino acid profile of *Colossoma macropomum* was determined. Levels of all indispensable amino acids were found to lie within limits found in other fish species. Suitability of local, Costa Rican, ingredients as protein sources for *C. macropomum* was evaluated based on their protein content and indispensable amino acid profile. It was calculated that in a standard diet (40% protein, 25% fish meal) soya meal, blood meal and tankage can substitute more than 80% of the fishmeal. More than 40% of the fish meal can be substituted by cow pea, pigeon pea, cotton meal, peanut oilcake, water hyacinth, shrimp meal, meat meal or poultry by-product in the diet. As differences between amino acid profiles between fish species seem to be small, promising alternative ingredients for *C. macropomum* might be equally interesting for formulation of feeds for other species. Further research is needed to determine if the mentioned dietary concentrations of these ingredients will cause other constraints (digestibility, production and processing costs, presence of anti-nutritional factors etc.) which could limit their use in fish feeds.

## INTRODUCTION

*Colossoma macropomum* (Cuvier) has a considerable potential for aquaculture in Latin America (Saint-Paul 1985). Expenses for feed are the main operating costs in intensive aquaculture (ADCP 1983). Protein is the most expensive macro-ingredient of commercial feeds (Pillay 1990). Van der Meer, Machiels & Verdegem (1995) showed that protein gain was linearly related to protein gift at feeding levels up to those permitting maximal growth. This leaves little space to economize on protein gift, as a decreasing gift is followed by a proportional decrease in (protein) growth. Thus, economizing the production of *C. macropomum* has to be sought in using cheaper sources of dietary protein, rather than in using less protein.

In aquaria growth rates above  $50 \text{ g kg}^{-0.8} \text{ day}^{-1}$  were obtained with fish meal as the main dietary protein source (Günther & Boza 1993; Van der Meer et al. 1995). Substitutes of fish meal in the diet must contain sufficient high quality protein. An important indicator of protein quality is the indispensable amino acid profile. Tacon & Cowey (1985) suggest that to obtain maximum growth each of the indispensable amino acids must be available at equal, or higher, levels than in the fish body. In other words, the required indispensable amino acid profile of the dietary protein is identical to the indispensable amino acid profile of the fish.

In this study the amino acid composition of *C. macropomum* protein was determined and compared with amino acid compositions of other fish species. Based on their protein content and amino acid profile, the suitability of local available ingredients as dietary protein sources for *C. macropomum* was evaluated. Promising substitutes for partly or complete substitution of fish meal in practical diets were indentified.

## MATERIALS AND METHODS

From a *C. macropomum* population with an average weight of 12 grams, two samples of 4 fishes were taken. The whole fishes were freeze dried and subsequently grounded. The part not passing through a 1 mm mesh after 3 times five minutes of grounding was weighed and separated from the sample.

The Indispensable Amino Acid (IAA) which had the lowest  $\text{IAA}_{\text{feed}}/\text{IAA}_{\text{body}}$  ratio (where:  $\text{IAA}_{\text{feed}} = \% \text{ IAA in protein of ingrediend}$  and  $\text{IAA}_{\text{body}} = \% \text{ IAA in body protein of } C. \text{ macropomum}$ ) was considered to be the first limiting IAA. This ratio multiplied by 100 was defined as the Chemical Score (CS) of the feed (Hepher 1988). As in other animals, arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine were considered to be the IAA's (Tacon & Cowey 1985). The non-indispensable amino acids cystine and tyrosine can only be synthesised by the fish from methionine and phenylalanine respectively (Tacon & Cowey 1985). Therefore, the methionine and phenylalanine requirement of the fish will partially depend on the cystine and tyrosine content of the diet. For the calculation of the CS the values of methionine and cystine were summed and taken as one IAA in both fish and ingredient. The same was done with phenylalanine and tyrosine. Tryptophan was not considered in this calculation, as it was not determined in *C. macropomum*.

Dietary protein is needed for maintenance and growth of body protein. We assumed that both processes require that each IAA is available in the same percentage (of the total amount of amino

acids available) as in the body protein. As de novo synthesis of IAA's is not possible, the CS indicates which part of the dietary protein can be converted into fish protein. Therefore, the percentage of dietary protein that can be used for growth and maintenance of body protein ( $P_{\text{grow}}$ ) was calculated as:  $P_{\text{grow}} = (\text{CS} * \% \text{ dietary protein}) / 100$ . Feeds with an equal  $P_{\text{grow}}$  were considered to be of equal quality.

**Table 1.** The amino acid composition of *Colossoma macropomum*.

	Sample 1	Sample 2		
# of fish	4	4		
wet weight (g)	55	56		
dry weight (g)	11.0	12.7		
particles< 1 mm (g)	9.88	11.25		
particles> 1 mm (g)	1.10	1.42		
Amino acid composition.				
	in gram/100 gram dry matter			in g/100 g
	sample 1	sample 2	avg	bodyprotein
indispensable amino acids				
arginine	4.11	4.11	4.11	6.75
histidine	1.46	1.44	1.45	2.38
isoleucine	2.78	2.77	2.78	4.56
leucine	4.65	4.67	4.66	7.66
lysine	5.05	5.10	5.08	8.34
methionine	1.56	1.53	1.55	2.54
phenylalanine	2.52	2.55	2.54	4.16
threonine	2.81	2.78	2.80	4.59
tryptophan	n.d. <sup>1)</sup>	n.d.		
valine	3.14	3.13	3.14	5.15
SUB-TOTAL	28.08	28.08	28.08	46.12
non-indispensable amino acids				
cystine	0.58	0.56	0.57	0.94
tyrosine	1.89	1.87	1.88	3.09
asparaginezuur	5.89	5.94	5.92	9.72
serine	2.82	2.82	2.82	4.63
glutaminezuur	9.39	9.48	9.44	15.50
glycine	4.81	4.77	4.79	7.87
alanine	4.25	4.26	4.26	6.99
proline	3.14	3.12	3.13	5.14
TOTAL	60.85	60.90	60.88	100.00

<sup>1)</sup> n.d. = not determined.

**Table 2.** Indispensable amino acid composition (in % of total protein) of fish species and of some potential protein sources for *Colossoma macropomum*.

Nr	Item <sup>a</sup>	arg	his	iso	leu	lys	met	cys	phe	tyr	thr	try	val	tprot	tIAA	CS	lim. IAA	Refer. <sup>b,c</sup>
<b>FISH SPECIES</b>																		
1	colossoma	6.8	2.4	4.6	7.7	8.3	2.5	0.9	4.2	3.1	4.6	-	5.2	60.9	50.2			1
2	striped bass	6.8	2.5	4.2	7.4	7.9	2.7	-	1.9	1.1	3.7	0.3	5.1	67.9	50.6			8
3	tilapia mossamb.	6.3	2.3	4.2	7.2	7.7	2.1	2.9	5.9	2.5	6.4	0.9	4.9	15.6	54.5			7
4	tilapia nilotica	7.8	3.0	4.9	7.8	10.2	2.4	4.8	4.9	2.9	4.6	1.1	5.6	-	59.8			3
5	catla	5.8	3.5	3.7	6.8	6.3	+	2.6	+	7.8	4.1	-	4.5	-	48.8			2
6	rohu	5.8	3.0	3.4	6.4	7.0	+	1.3	+	7.3	3.7	-	4.1	-	51.5			2
7	mrigala	5.8	3.0	3.7	6.9	7.6	+	1.6	+	8.1	4.0	-	4.3	-	51.7			2
8	sib. sturgeon 45g	6.7	2.0	5.1	6.8	11.7	-	-	4.1	4.0	5.0	-	5.3	-	51.1			10
9	idem, 1000 g	7.7	2.4	5.4	7.4	11.1	-	-	3.8	5.3	5.4	-	6.0	-	50.6			10
10	white sturgeon 17g	6.3	2.3	2.8	7.0	7.9	3.1	-	3.6	3.3	4.3	1.1	2.9	-	45.4			10
11	idem, 993 g	6.5	2.9	2.5	6.3	7.6	2.9	-	3.5	2.8	4.1	1.0	2.7	-	43.4			10
12	hybrid bluegill	5.9	2.1	4.3	7.3	8.3	3.1	0.9	4.1	2.9	4.6	1.4	4.8	-	49.7			6
13	rainbow trout	6.4	3.0	4.3	7.6	8.5	2.9	0.8	4.4	3.4	4.8	0.9	5.1	-	52.0			6
14	coho salmon	6.0	3.0	3.7	7.5	8.6	3.5	1.2	4.1	3.4	5.1	1.4	4.3	-	51.9			6
15	atlantic salmon	6.6	3.0	4.4	7.7	9.3	1.8	1.0	4.4	-	5.0	0.9	5.1	-	49.2			9
16	channel catfish	6.7	2.2	4.3	7.5	8.5	2.9	0.9	4.1	3.3	4.4	0.8	5.2	-	50.6			6
17	common carp	6.2	2.9	3.8	7.2	7.0	+	2.7	+	6.5	4.2	0.9	4.5	-	49.1			2
18	fish meat	6.8	2.8	5.6	8.4	9.8	3.0	1.2	4.2	3.6	5.0	1.2	5.9	-	57.5			11
<b>POTENTIAL PROTEIN SOURCES FOR COLOSSOMA</b>																		
<b>LIFE FOOD</b>																		
19	artemia	7.3	1.9	3.8	8.9	8.9	1.3	0.6	4.7	5.4	2.5	1.5	4.7	52.2	51.5	54.4	threonine	4
20	moina	7.0	2.2	3.4	8.3	8.0	1.4	0.8	4.9	4.5	5.2	1.6	4.4	71.6	51.7	63.3	met&cystine	4
21	rotifer	6.3	2.1	4.8	8.2	8.2	1.2	1.1	5.3	4.2	4.7	1.6	5.5	61.8	53.2	66.2	met&cystine	4
<b>FRUITS</b>																		
22	banana	2.1	2.2	1.5	1.9	1.6	0.5	1.0	1.5	1.0	1.1	0.4	1.5	3.0	16.3	19.2	lysine	5
23	orange	6.9	2.0	2.7	2.4	5.0	2.1	1.1	3.3	1.7	1.6	1.0	4.2	7.2	34.1	31.9	leucine	5
24	pineapple	4.6	2.3	3.3	4.9	6.4	2.8	0.5	3.1	3.1	1.1	1.3	4.1	2.9	39.5	63.6	leucine	5
25	mango	1.8	1.1	1.7	2.9	3.9	0.5	-	1.6	1.0	1.8	0.8	2.5	5.8	19.4	26.3	arginine	5
26	papaya	1.6	0.8	1.3	2.6	4.1	0.3	-	1.5	0.8	1.8	1.3	1.6	5.5	17.7	23.9	arginine	5
27	avocado	3.0	1.5	3.6	6.2	4.7	1.9	1.1	3.4	2.5	3.3	1.1	4.9	7.7	37.0	44.2	arginine	5
28	watermelon	9.5	1.0	3.1	2.9	10.0	1.0	0.3	2.4	1.9	4.4	1.1	2.6	7.3	40.2	37.1	met&cystine	5
<b>CURRENT FISH FEED INGREDIENTS</b>																		
29	menhaden	5.8	2.3	4.6	7.6	7.7	2.9	0.9	3.9	3.2	4.0	1.1	5.3	61.3	49.4	86.3	threonine	4
30	sardina	5.0	2.9	4.8	6.8	8.5	3.0	1.2	3.6	3.5	4.2	0.9	5.6	65.0	49.9	74.1	arginine	4
31	tuna	5.8	3.0	4.1	6.5	6.8	2.5	0.7	3.7	2.9	3.9	0.9	4.7	59.0	45.6	82.1	lysine	4
32	anchovy	5.6	2.3	4.6	7.6	7.8	3.0	0.9	4.0	3.3	4.3	1.2	5.4	65.3	50.0	83.2	arginine	4
33	herring	6.3	2.4	4.4	7.3	7.8	2.9	1.0	3.8	3.1	4.1	1.1	6.0	72.7	50.2	90.2	threonine	4
34	soya	7.8	2.5	4.8	7.0	6.2	1.3	1.6	4.5	3.0	3.6	1.4	5.1	49.0	50.8	76.4	lysine	4
35	tankage	6.0	3.1	3.2	8.5	6.3	1.3	0.8	6.4	2.2	3.9	1.1	6.6	60.0	49.3	59.5	met&cystine	4
36	blood meal	3.9	5.2	1.1	13.2	7.5	1.2	1.5	7.0	2.7	4.8	1.3	8.6	81.5	58.0	24.0	isoleucine	4
37	corn meal	4.3	2.1	3.2	10.1	2.1	1.6	1.6	4.1	3.5	3.2	0.7	3.9	10.9	40.3	25.2	lysine	4
38	wheat	4.0	1.8	3.5	5.9	2.6	1.3	2.1	4.3	2.9	2.5	1.1	3.9	13.7	36.7	30.8	lysine	4
<b>ALTERNATIVE FEED INGREDIENTS</b>																		
39	rice bran	5.7	2.0	3.5	8.5	4.1	1.7	0.8	3.5	4.9	3.4	0.8	5.3	12.7	44.3	49.2	lysine	4
40	broken rice	7.2	1.9	4.0	6.8	3.3	1.9	1.3	4.2	5.7	3.1	1.3	5.7	10.6	46.4	39.0	lysine	4
41	wheat middlings	5.8	2.4	3.5	5.8	3.7	1.3	1.7	3.5	2.6	3.0	1.3	4.5	19.4	39.1	44.8	lysine	4
42	wheat bran	5.9	2.2	3.9	6.5	3.4	1.4	1.9	4.0	2.0	3.1	1.8	4.6	17.9	40.5	40.8	lysine	4
43	corn gluten	3.3	2.2	5.1	16.3	1.9	2.4	1.8	6.9	3.2	3.5	0.6	5.1	41.0	52.4	22.5	lysine	4
44	barley	5.6	2.4	7.0	11.9	4.1	2.0	1.5	6.7	6.0	4.3	1.6	7.0	24.8	60.1	49.5	lysine	4
45	adlay	4.4	2.3	4.0	14.4	1.9	3.0	1.7	4.8	4.2	3.1	-	5.6	12.5	49.4	22.8	lysine	12
46	cow pea	6.0	3.0	3.6	6.6	6.4	1.1	1.0	4.8	2.4	3.4	1.0	4.2	22.2	43.6	59.9	met&cyst	4
47	pigeon pea	4.7	3.6	3.0	6.1	7.5	0.5	0.9	8.1	2.0	2.8	0.6	3.5	21.2	43.3	41.5	met&cyst	4
48	cotton seed	13.1	3.2	3.8	6.9	5.2	1.5	1.8	6.1	3.4	3.8	1.5	5.4	19.9	55.6	61.7	lysine	4
49	cotton meal	11.0	2.7	3.4	5.6	4.1	1.4	1.9	5.6	2.2	3.4	1.3	4.6	41.3	47.2	49.2	lysine	4
50	coffee pulp	4.7	3.7	4.0	7.3	6.4	1.3	0.9	4.7	3.4	4.4	-	7.0	13.6	47.8	63.4	met&cystine	5
51	cocoa	3.6	1.5	2.8	4.2	4.6	0.6	0.5	3.0	2.4	3.3	-	4.1	13.7	30.6	31.7	met&cyst	12
52	peanut oilcake	10.4	2.4	3.6	6.9	3.3	1.0	1.5	5.2	3.7	2.8	1.0	4.7	46.3	46.3	38.9	lysine	4
53	water hyacinth	7.1	2.5	6.0	10.6	7.3	2.1	0.3	6.4	4.6	5.8	-	7.0	9.5	59.7	69.3	met&cyst	4
54	cocos	11.6	2.0	3.0	6.1	2.9	1.6	1.0	4.1	2.5	3.0	1.0	4.5	16.5	43.2	34.2	lysine	4
55	cocos oilcake	12.6	1.7	3.4	6.3	2.9	1.8	1.5	4.2	2.5	3.2	1.1	4.4	14.1	45.5	34.5	lysine	4
56	mulberry	5.8	2.2	4.4	7.7	4.0	1.9	-	5.4	3.8	3.4	-	5.5	21.4	44.0	47.9	lysine	4
57	shrimp meal	6.2	2.5	4.2	6.7	5.4	2.0	1.5	3.9	3.2	3.5	1.0	4.4	36.1	44.3	65.0	lysine	4
58	meat meal	6.9	1.9	3.4	6.3	5.9	1.4	1.3	3.5	1.8	3.3	0.7	4.8	47.7	41.2	70.4	lysine	4
59	poultry by-prod.	6.7	1.8	4.2	7.1	5.1	1.9	1.6	3.3	1.7	3.4	0.8	5.1	62.7	42.6	61.4	lysine	4

**Legend of Table 2:**

% prot = percentage protein in dry matter; IAA = indispensable amino acid; CS = chemical score; lim. IAA = first limiting indispensable amino acid; - = not known/not determined; + = value of methionine included in cystine; phenylalanine included in tyrosine. In the calculation of CS and %IAA, missing values indicated by a "-" in the table were ignored.

**<sup>1</sup> Scientific names and additional information per item:**

FISH SPECIES: 1: *Colossoma macropomum*; 2: *Morone saxatilis*; 3: *Oreochromis massambicus*; 4: *Oreochromis niloticus*; 5: *Catla catla*; 6: *Labeo rohita*; 7: *Cirrhinus mrigala*; 8,9 : *Acipenser baeri*; 10,11: *Acipenser transmontanus*; 12: *Lepomis cyanellus* x *Lepomis macrochirus*; 13: *Oncorhynchus mykiss*; 14: *Oncorhynchus kisutch*; 15 *Salmo salar*; 16: *Ictalurus punctatus*; 17: *Cyprinus carpio*; 18: average of data of 32 fish species.

ZOOPLANKTON: 19: *Artemia salina*, newly hatched nauplii; 20: *Moina* spp.; 21: *Brachionus plicatilis*.

FRUITS: 22: *Mussa* spp., yellow without skin; 23: *Citrus* spp., raw, all commercial varieties; 24: *Ananas comosus*, raw; 25: *Mangifera indica*, raw; 26: *Carica papaya*, raw; 27: *Persea americana*, raw, all commercial varieties; 28: raw. CURRENT FISH FEED INGREDIENTS: 29: *Brevoortia tyrannus*; 30: Pilchard; 31: *Thunnus* spp., offal; 32: *Engraulis ringens*; 33: *Clupea harengus*; 34: Glycine max; 35: meat meal with blood; 36: -.

ALTERNATIVE FEED INGREDIENTS: 39 and 40: *Oryza sativa*; 41 and 42: *Triticum aestivum*; 43 = *Zea mays*, amino acid composition taken from 41% protein gluten (Tacon, 1987), in Costa Rica available at protein concentrations of 20 and 60% (Vargas, 1984); 44 = brewers grains, dehydrated, *Hordeum vulgare*, H. distichum; 45 = *Coix lacrymajobi* (Job's tears); 46 = *Vigna sinensis*, V. unguiculata; 47 = *Cajanus cajan*, red gram; 48 = *Gossypium hirsutum*, whole kernel; 49 = idem, seed meal; 50 = *Coffea arabica/robusta*, dehydrated; 51 = *Theobroma cacao*, shell; 52 = *Arachis hypogaea*, oilcake, mechanically extracted, without hulls; 53 = *Eichornia crassipes*; 54 = *Cocos nucifera*; 55 = *Elaeis guineensis*; 56 = *Morus alba*, fodder, amount of IAA in protein arbitrarily set at 44%; 57 = process residue, dehydrated; 58 = -; 59 poultry by-products, 5-03-798.

<sup>2</sup>) References: 1 = this study; 2 = Mohanty & Kaushik, 1991. 3 = Teshima & Kanazawa, 1988. 4 = Tacon, 1987. 5 = data base of the Animal Nutritionist, version 2.5, 1987. N-Squared Incorporated 5318 Forest Ridge Road, Silverton, Oregon 97381 & Durango Software, P.O. Box 2783, Durango, Colorado 81302. 6 = Tidwell, Webster & Clark, 1992. 7 = Jauncey, Tacon & Jackson, 1983. 8 = Hughes, Lemm & Herman, 1992. 9 = Wilson & Cowey, 1985. 10 = Hung, 1991; 11 = Hephher, 1988; 12 = Hossain & Jauncey, 1989; 13 = De Silva, Keembiyehetty & Gunasekera, 1988; 14 = Göhl, 1991.

Amino acid profiles of fish species and of feed ingredients were taken from literature. Ingredients available in Costa Rica with more than 10% protein on a dry matter basis (Vargas 1984) were evaluated as substitutes for fish meal in the diet. The latter was done by calculating how much fish meal could be removed from a standard diet (40% protein, 25% fish meal) by incorporating 30% of an alternative ingredient, without affecting the  $P_{\text{grow}}$ .

## RESULTS AND DISCUSSION

### Comparison with other fish species

The amino acid composition of *C. macropomum* is given in Table 1. Table 2 reviews the IAA profile of various fish species and feed ingredients. The values found for *C. macropomum* fall within the ranges reported for other species.

Hephher (1988) reviewed the IAA composition of fish muscle from 32 species. The averages calculated (Table 2, nr 18) are higher than the averages for whole body protein (Table 2, nr 1 through 17). In consequence, the use of high portions of non-muscle protein in fish meal will lower the percentage of IAA's. This could explain why tuna meal made of processing wastes (Table 2, nr 31) shows lower levels of IAA's than the other fish meals (Table 2, nrs 29, 30, 32 and 33).

## Food items and feed ingredients

### *Life feeds*

*Moina* spp. (Table 2, nr 20) is an important natural food for *C. macropomum* (Van der Meer & Martínez 1993; Carvalho 1984). Its protein level and CS compares well with values reported for rotifers (Table 2, nr 21). Compared to these natural feeds, artemia (Table 2, nr 19) has a low protein percentage and CS. The superb quality of artemia compared to rotifers and artificial feeds in larval culture of *C. macropomum* (Van der Meer, unpublished data) is apparently not due to the protein quality but to other properties, such as digestibility, catchability etc.

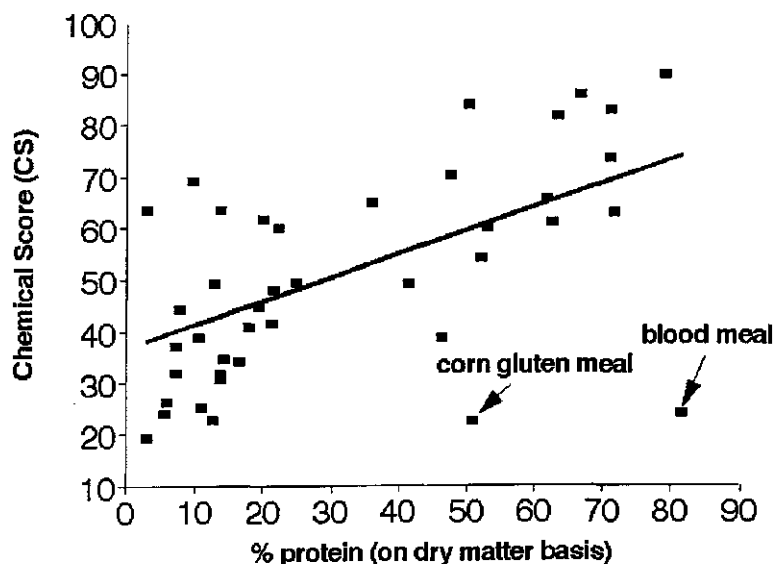
Enrichment of artemia with threonine through bioencapsulation (Sorgeloos, Lavens, Léger, Takaert & Versichele 1986), increases its CS from 54 to 79. This means an increase of  $P_{\text{grow}}$  with 46%. Such an improvement of the protein quality of an expensive food like artemia, could be well worth while.

### *Fruits*

Dried fruits (Table 2, nr 22 through 28) contain less than 8% protein. The CS values indicate that the protein quality varies between extremely low (papaya, CS = 9.3) to moderate (pineapple, CS = 63.6). Although adult *C. macropomum* is reported to forage in nature on fruits (Saint-Paul 1985), their low protein content coupled with a poor protein quality, make it little likely that fruits can sustain substantial growth. Castelo, Amaya & Strong (1980) observed that in their natural habitat *C. macropomum* deposited body fat when fruits were available. Increased amounts of body fat are accompanied by an almost equal decrease in body water content (Günther & Boza 1993; Van der Meer et al. 1995). In summary, feeding fruits will result in slow growth and will yield fatty fish. For aquaculture, favouring fast growth and lean fish, feeding fruits is no option.

### *Feed ingredients*

Our data suggest that ingredients (Table 2, nr 29 through 59) with an higher protein content tend to have an higher CS (Figure 1; regression line:  $CS = 36.7 + 0.463 \cdot \% \text{ protein}$ ,  $r^2 = 0.26$ ,  $P < 0.01$ ). Although this relation lacks a theoretical basis, it might be useful to keep the rule in mind when making a first selection between ingredients. Exceptions from this rule are blood meal (high in protein, but low in CS probably due to the fact that it is made up of only one organ and not of a complete organism) and corn gluten (high in protein but with the low CS of whole corn).

**Figure 1.** The relation between protein content and CS of feed ingredients.

The  $P_{\text{grow}}$  of all fish meals (Table 2, nr 29 through 33) is above 48. Of the alternative ingredients available in Costa Rica (Table 2, nr 39 through 59), only meat meal and poultry by-product have an  $P_{\text{grow}}$  above 30, while cotton meal and shrimp meal have a  $P_{\text{grow}}$  above 20.

### Alternative feed formulations

Table 3 evaluates the possibilities of various ingredients to substitute 30% of the dry matter of a reference diet for *C. macropomum*. Substitutes were incorporated in expense of fish meal and corn flour. The goal was to replace as much as possible of the dietary fish meal, while maintaining  $P_{\text{grow}}$  constant. Ingredients ranked high in Table 3 (blood meal, soya meal, tankage, poultry by-product) can replace most of the fish meal, while the lowest ranked ingredient (adlay) can only substitute corn meal without affecting dietary  $P_{\text{grow}}$ .

Due to its high protein content blood meal not only can replace all the fish meal, but also almost 50% of the soya meal can be replaced by corn meal. However, a limited suitability of blood meal for diets of *C. macropomum* is suggested by Eckmann (1987). Reduced growth due to high blood meal levels in the diet is possibly caused by intoxication by histidine, leucine and/or valine, which are in surplus up to two times the requirement.

Soya meal might be the currently used ingredient with the highest potential to substitute fish meal completely. Validity of the assumption that  $P_{\text{grow}}$  strongly predicts fish growth will be tested in future experiments, starting with substitution of fish meal with soya meal.

**Table 3.** Amount of fish meal needed in alternative diets to maintain  $P_{\text{grow}}$  on the the standard diet level.

Alternative	INGREDIENTS				APROXIMATE COMPOSITION					PROTEIN CHARACTERISTICS		
	Alter-native	Fish meal	Corn flour	Others*)	Prot.	Fat	Carb.	Fibre	Ash	CS**) $P_{\text{grow}}^{***}$	lim. IAA	
STANDARD DIET	0.0	25.0	30.0	45.0	40.0	5.8	40.1	2.6	11.5	65.9	26.3	lysine
blood meal	30.0	0.0	25.0	45.0	49.7	4.1	36.8	2.4	7.0	65.0	32.3	isoleucine
poultry by-prod.	30.0	2.1	22.9	45.0	44.7	10.6	36.0	2.4	6.3	59.0	26.4	lysine
soya meal	30.0	2.6	22.4	45.0	41.2	4.3	42.9	3.6	8.0	63.9	26.3	lysine
tankage	30.0	4.8	20.2	45.0	40.4	7.9	31.9	2.5	17.4	65.3	26.3	lysine
meat meal	30.0	4.8	20.2	45.0	41.3	7.9	32.4	2.5	16.0	63.8	26.3	lysine
shrimp meal	30.0	9.3	15.7	45.0	40.1	4.8	29.3	7.0	18.8	65.7	26.4	lysine
pigeon pea	30.0	9.8	15.2	45.0	35.9	4.1	47.2	4.3	8.5	73.5	26.3	lysine
cotton meal	30.0	10.9	14.1	45.0	42.4	5.4	36.9	5.7	9.6	62.2	26.4	lysine
cow pea	30.0	11.7	13.3	45.0	37.1	4.0	46.4	3.6	8.9	71.1	26.3	lysine
peanut oilcake	30.0	11.6	13.4	45.0	44.2	4.9	35.0	6.0	9.9	59.7	26.3	lysine
water hyacinth	30.0	14.4	10.6	45.0	34.5	4.7	39.5	7.2	14.0	76.3	26.4	lysine
cotton seed	30.0	15.1	9.9	45.0	38.1	9.9	32.3	9.8	9.8	69.2	26.4	lysine
coffee pulp	30.0	15.2	9.8	45.0	35.6	4.6	38.6	9.5	11.6	73.9	26.3	lysine
barley	30.0	15.9	9.1	45.0	39.8	6.8	34.5	9.0	9.9	66.1	26.3	lysine
corn gluten	30.0	16.4	8.6	45.0	45.1	4.8	35.6	4.3	10.3	58.4	26.3	lysine
mulberry	30.0	17.3	7.7	45.0	39.5	5.4	37.5	5.4	12.2	66.7	26.3	lysine
cocoa	30.0	18.4	6.6	45.0	37.7	6.3	35.1	8.0	12.9	69.8	26.3	lysine
wheat middlings	30.0	18.5	6.5	45.0	39.5	5.7	39.4	4.6	10.9	66.7	26.3	lysine
wheat bran	30.0	19.8	5.2	45.0	39.6	5.7	37.6	5.5	11.6	66.5	26.3	lysine
rice bran	30.0	19.8	5.2	45.0	38.1	9.1	34.5	4.9	13.3	69.0	26.3	lysine
cocos	30.0	20.6	4.4	45.0	40.4	8.6	35.5	4.1	11.4	65.2	26.3	lysine
cocos oilcake	30.0	22.2	2.8	45.0	39.6	5.8	39.5	3.8	11.3	66.5	26.3	lysine
broken rice	30.0	22.4	2.6	45.0	38.7	5.7	42.3	2.3	11.0	68.0	26.3	lysine
wheat	30.0	23.1	1.9	45.0	39.9	4.9	41.3	2.8	11.1	66.1	26.4	lysine
adlay	30.0	25.0	0.0	45.0	40.5	7.4	30.3	6.7	15.1	65.1	26.4	lysine

\*) Others: soya meal 30%, tankage 5%, blood meal 5% and wheat flour 5%.

\*\*) CS = Chemical Score.

\*\*)  $P_{\text{grow}} = \text{CS}/100 \times \text{protein \%}$ ; more detailed information about ingredients can be found in tabel 2.

## CONCLUSION

The indispensable amino acid profile of *C. macropomum* is similar to those found in other fish species. Based on their protein content and amino acid profile, several local available ingredients were identified as possible substitutes of most of the fish meal in currently used laboratory diets. These potentially interesting ingredients for *C. macropomum* feeds should be investigated further on (protein) digestibility, production and processing costs, palatability and presence of anti-nutritional or toxic substances. As differences between amino acid profiles between fish species seem to be small, promising alternative ingredients for *C. macropomum* might be equally interesting for formulation of feeds for other species.

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## Chapter 2

### THE AMINO ACID COMPOSITION OF DIETARY PROTEIN AS A CONSTRAINT TO GROWTH

## II

### Feed consumption, growth and protein utilization of *Colossoma macropomum* (Cuvier) at different dietary fish meal/soya meal ratios

#### ABSTRACT

The effect of a gradual substitution of dietary fish meal protein by soya meal protein on growth, feed uptake and protein utilization of 1 gram *C. macropomum* was studied at two different dietary protein levels. Growth rates of fish fed ad libitum 20 and 45% protein diets fluctuated between 41 and 49 and between 60 and 68 g kg<sup>0.8</sup> day<sup>-1</sup>, respectively. Fish incorporated between 31 and 47% of the dietary protein in their body. Increased amounts of soya meal in the diet led to decreased feed uptake, higher body protein levels, lower ash levels and increased NPU values. If the low ash contents in the fish fed 100% soya diets are not prejudicial for health and growth of the fish in the long term, soya meal must be considered a superior protein source for *C. macropomum*. The high growth and the efficient use of the dietary protein indicate that *C. macropomum* is able to utilize soya protein more efficiently than other fish species.

## INTRODUCTION

Under laboratory conditions juvenile *Colossoma macropomum* (Cuvier) can attain growth rates up to 52 - 55 g kg<sup>0.8</sup> day<sup>-1</sup> (Günther & Boza 1993; Van der Meer, Machiels & Verdegem 1995). These high growth rates require the use of dietary protein levels above 40% (Van der Meer et al. 1995). Because protein is the most expensive macro-nutrient in artificial fish diets (Pillay 1990), and because feeds are the major production cost for intensive culture, the use of cheaper dietary protein sources could play a key role in minimizing costs of *C. macropomum* culture.

Normally, fish meal is the main protein source in experimental diets for *C. macropomum*. Fish meal combines the properties of good taste and high protein quality, but it is also relatively expensive (Lovell 1989). Efforts to find cheaper dietary protein sources start normally with substitution of the fish meal with soya meal. Soya meal is generally considered the best vegetable protein source, having a relative high protein content (between 44 and 49% (Tacon 1987)), an appropriate amino acid profile and a high protein digestibility (Lovell 1991).

In tilapia, channel catfish and rainbow trout it was found that protein of solvent extracted soya meal was equal or better digestible than fish meal protein (Lovell 1991). However, to judge protein quality one has not only to consider protein digestibility, but also the amino acid composition. The Chemical Score (CS), gives an indication of the resemblance between the Indispensable Amino Acid (IAA) profile of dietary protein and body protein and, as such, can be used to judge the amino acid composition of a diet. Based on CS's and assuming equal digestibility of soya and fish meal Van der Meer & Verdegem (1996) calculated that 90% of the fish meal of a "standard diet" could be substituted by soya meal without affecting growth of *C. macropomum*.

Substitution of fish meal by soya meal as the main protein source has been investigated in carp (Viola, Mokady, Rappaport & Arieli 1982), channel catfish (Mohsen & Lovell 1990), African catfish (Machiels 1987), rainbow trout (Dabrowski, Poczyczynski, Köck & Berger 1989) and shrimp (Lim & Dominy 1990). The general conclusion from the above mentioned experiments is that the effect of a partial substitution of fish meal by soy meal depends on the species studied; however, complete substitution resulted in significantly reduced growth rates in all cases. Once the IAA compositions of fish and dietary ingredients are known, the CS of a diet can be calculated without additional experimental data. However, for the definitive determination of dietary protein quality, biological testing taking feed uptake and growth into account is recommended (Steward Anderson, Lall, Anderson & McNiven 1993).

The generally poor performance of plant protein in comparison to fish meal protein is not necessarily due to an inferior protein quality, but could also be caused by presence of anti-nutritional factors or toxic substances, a high fiber content and a lower palatability (Lim & Dominy 1991). In this context, adult *C. macropomum* is special because it feeds mainly on fruits and seeds in nature (Saint-Paul 1985). Such a preference has not been reported for any of the other fish species mentioned. Therefore, we supposed that *C. macropomum* would be less susceptible to the mentioned constraints linked to the use of plant protein.

In this study we determined the effects of substitution of fish meal by soya meal on feed uptake, growth and protein utilization to test the assumption that *C. macropomum* is able to use soya and fish meal protein equally well. Diets in which soya meal substituted between 0 and 100% of the fish meal (more than 90 % fish meal, rest being tankage, further referred to as fish meal), but with

similar CS's, were tested at two dietary protein levels. Diets with the highest protein level (45%) were expected to allow maximal growth. Former experiments indicated that protein levels below 30% restricted growth of *C. macropomum* (Van der Meer et al. 1995). Therefore, more pronounced differences between treatments were expected with low protein diets (20%) than with high protein diets (45%).

## MATERIALS AND METHODS

Fry of *C. macropomum* were obtained through hormone induced spawning and subsequent artificial incubation. Larvae started to feed on artemia 5 - 6 days after hatching, and were weaned to dry feed when attaining 80 - 100 mg. Ten days before the experiments started, at a weight of about 0.5 grams, the fish were transferred to the experimental units. At the start of the experiments fish weighed 1.1 gram (Table 2). The experiments were executed in thirty 45-l aquaria. Aquaria belonged to a recirculation unit equipped with a heating device, a sedimentator and a biological filter. Water losses due to evaporation and cleaning were compensated daily.

Ten diets, made of locally available ingredients (Table 1), were tested in two feeding experiments. In experiment 1, five 45% protein diets (diets 1 through 5) and in experiment 2, five 20% protein diets (diets 6 through 10) were tested in six fold. Feed consumption for each aquarium was monitored daily. Fish meal protein was gradually substituted by soya meal protein in diet 1 to 5 and in diet 6 to 10. Diets within one protein level are further referred to as 0, 15, 43, 77, and 100% soya diets, depending on the proportion of soya in the diet calculated as: % soya meal protein / (% soya meal protein + % fish meal protein) \* 100. Within each experiment diets were formulated to be isocaloric and isoproteic.

Each experiment lasted 37 days. Fish were weighed at days 0, 14, 28 and 37. Fish were fed *ad libitum* at 8.30, 12.00 and 16.30 h. At sampling days fish were not fed.

Water temperature was measured at 8.00 and 17.00 hours and maintained between 26.7 and 31.8 °C. Water temperature averaged 28.3 and 28.8°C in experiment 1 and 2, respectively. Dissolved oxygen levels were maintained above 4.3 ppm and nitrite levels below 0.05 ppm.

Standard proximate analyses were used to determine the composition of feeds and fish. At the start of each experiment a sample of about 75 fishes was taken for the determination of the initial body composition. At the end of the experiment two samples per treatment, each comprising the fish of 3 aquaria, were taken to determine the final body composition. Carbohydrates and fibre are quantitatively not important in *C. macropomum* (Günther & Boza 1993; Van der Meer et al. 1995) and were therefore ignored in the body composition.

Amino acid profiles of *C. macropomum* and ingredients were taken from Van der Meer & Verdegem (1995). Dietary protein quality was evaluated with the Chemical Score (Hepher 1988). The minimal differences in CS's between diets of one experiment (Table 1), allowed to test the hypothesis that *C. macropomum* use soya and animal protein equally well.

The biological parameters used to evaluate dietary protein quality were Metabolic Ration (MR in g kg<sup>0.8</sup> day<sup>-1</sup>), Metabolic Growth Rate (MGR in g kg<sup>0.8</sup> day<sup>-1</sup>), the Feed Conversion (FC in g dry feed/g wet weight gain) and the Net Protein Utilization (NPU in g protein gain/g feed protein \* 100).

Feed uptake, growth, feed utilization and body composition parameters of each experiment

were submitted to one-way ANOVA. Significance of differences among diets within experiments were determined by Tukey-test (Statistix 1990).

Trends between the different treatments within each experiment were studied through a quadratic regression analysis:  $Y = \beta_0 + \beta_1 * X_i + \beta_2 * (X_i)^2 + \epsilon$ , where:  $Y$  = average value per treatment;  $\beta_0$ ,  $\beta_1$  and  $\beta_2$  = regression coefficients; and  $X_i$  = the soya proportion in diet  $i$ , ( $i = 0, 15, 43, 77, 100$ ). The quadratic component  $\beta_2 * (X_i)^2$  was only included if it improved the adjusted- $r^2$  of the regression (Statistix 1990).

Body protein content is affected by fish size (Shearer 1994) which was also shown for *C. macropomum* (Günther & Boza 1993). To separate the effect of body weight ( $W_f$ ) from that of diet composition (% soya), we performed a multiple linear regression analysis:  $BP = \alpha + \beta * (\% \text{ soya}) + \gamma * (W_f) + \epsilon$ , where:  $BP$  = average body protein percentage;  $\alpha$ ,  $\beta$  and  $\gamma$  = regression coefficients;  $\epsilon$  = error term.

**Table 1.** Diet composition, expressed in % of dry matter.

Diet	1	2	3	4	5	6	7	8	9	10
<b>INGREDIENTS*</b>										
Fish meal	49.7	42.2	28.1	11.3	0.0	16.6	14.1	9.4	3.8	0.0
Tankage	3.4	2.9	1.9	0.8	0.0	1.2	1.0	0.7	0.3	0.0
Blood meal	1.0	0.8	0.6	0.2	0.0	0.3	0.3	0.2	0.1	0.0
Corn meal	44.3	37.6	25.1	10.0	0.0	65.2	63.0	58.8	53.8	50.4
Soy meal	0.0	13.4	38.5	68.6	88.7	0.0	4.4	12.9	22.9	29.6
Wheat flour	0.0	0.7	2.1	3.8	4.9	8.4	8.6	9.1	9.6	10.0
Soya oil	0.0	0.4	1.1	1.9	2.5	3.4	3.5	3.8	4.0	4.2
Fish oil	0.0	0.4	1.1	1.9	2.5	3.4	3.5	3.8	4.0	4.2
Salt	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Premix	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
<b>PROXIMATE ANALISES</b>										
Protein	45.9	44.3	43.5	43.8	42.4	22.0	21.5	22.4	22.4	19.8
Lipids	7.1	7.3	5.6	6.0	4.7	9.1	8.9	12.6	14.1	12.8
Carbohydrates	28.8	29.9	32.2	32.9	35.1	58.1	60.3	55.7	54.5	60.0
Fibre	2.8	4.0	6.9	7.8	9.2	3.2	2.6	3.0	3.8	2.7
Ash	15.4	14.6	11.8	9.6	8.6	7.7	6.7	6.3	5.4	4.7
Energy (kJ/gram)**	18.5	18.4	17.9	18.3	17.3	18.8	18.9	19.8	20.2	20.0
% soya***	0	15	43	77	100	0	15	43	77	100
CS****	76.0	76.3	77.1	78.0	78.6	61.5	61.7	62.2	62.7	63.1
limiting IAA*****	lys	lys	lys	lys	lys	lys	lys	lys	lys	lys

\* Ingredients: fish meal = brown tuna meal (66.2% protein, 8.8% fat, 0.2% carbohydrates, 4.2% crude fibre, 20.5% ash); tankage = bone and meat meal; blood meal = mechanically dried; corn meal = precooked white meal; soy meal: hexane extracted (43.7% protein, 5.1% fat, 38.2% carbohydrates, 8.1% crude fibre, 6.8% ash); pre-mix: "Vitamelk peces A", Roche S.A., containing per kg:  $3.48 \times 10^6$  IU vit A;  $0.70 \times 10^6$  IU vit D3; 11,000 IU vit E; 2.17 g vit K3; 4.35 g vit B1; 8.70 g vit B2; 43.5 g vit B3; 17.4 g pantothenic acid; 4.35 g vit B6; 74 mg vit B8; 1300 mg folic acid; 8.70 mg vit B12; 130 g vit C; 130 g choline; 17.4 g magnesium; 17.4 g iron; 8.70 g zinc; 2.17 g copper; 435 g iodine; 43.5 mg selenium; 43.5 mg cobalt.

\*\* Energy content: gross energy content calculated using the following energy values: protein, 23.4 kJ/g; lipid, 39.8 kJ/g and carbohydrates, 17.2 kJ/g (Cho, Slinger & Bayley, 1982).

\*\*\* % soya = proportion of soya meal diet in the diet (see text).

\*\*\*\* CS = chemical score (see text).

\*\*\*\*\* IAA = indispensable amino acid; lys = lysine.

## RESULTS AND DISCUSSION

## Feed consumption, growth and feed utilization

No significant differences were found between the metabolic rations of fish fed 0, 14 or 43% soya diets ( $P>0.05$ ; Table 2; Figure 1a). However, the MR administrated with the 100% soya diets was significantly lower than for the 14 or 43% soya diets. The reduced uptake of diets containing more than 43% soya could be caused by a lower palatability of soya compared to fish meal. Decreased palatability of diets containing soybean meal was also reported in salmonids (Lovell 1991).

The growth rates obtained in experiment 1 with the 45% protein diets (between 60.6 and 67.8 g kg<sup>0.8</sup> day<sup>-1</sup>; Table 2) were comparable to maximum growth rates reported before (Günther & Boza 1993; Van der Meer et al. 1995). The average growth rate obtained in experiment 2 (44.3 g kg<sup>0.8</sup> day<sup>-1</sup>) was comparable to growth rates obtained formerly in our laboratory with similar low protein diets (Van der Meer et al. 1995). The 43% soya diet tended to promote better growth than 0 and 100% soya diets (Figure 1b). However, in both experiments no significant differences in growth rate were found between treatments. Mohsen & Lovell (1990) also found that in channel catfish substitution of about 50% of the fish meal protein by soya meal protein gave a slight, but not significant, growth increase.

The lack of significant differences in growth rates within protein levels confirmed the hypotheses that *C. macropomum* can grow equally well on soya and on fish meal protein. In this respect *C. macropomum* differs from carp (Viola et al. 1982), trout (Dabrowski et al. 1989), African catfish (Machiels 1987), channel catfish (Mohsen & Lovell 1990) and shrimp (Lim & Dominy 1990) which all grew better on fish meal diets. In African catfish (Machiels 1987) and channel catfish (Andrews & Page 1974) addition of cristaline amino acids could not prevent reduced growth with 100% soya diets. Possibly this indicates that also in those species the amino acid profile of soya was not the cause of its poorer performance compared to fish meal.

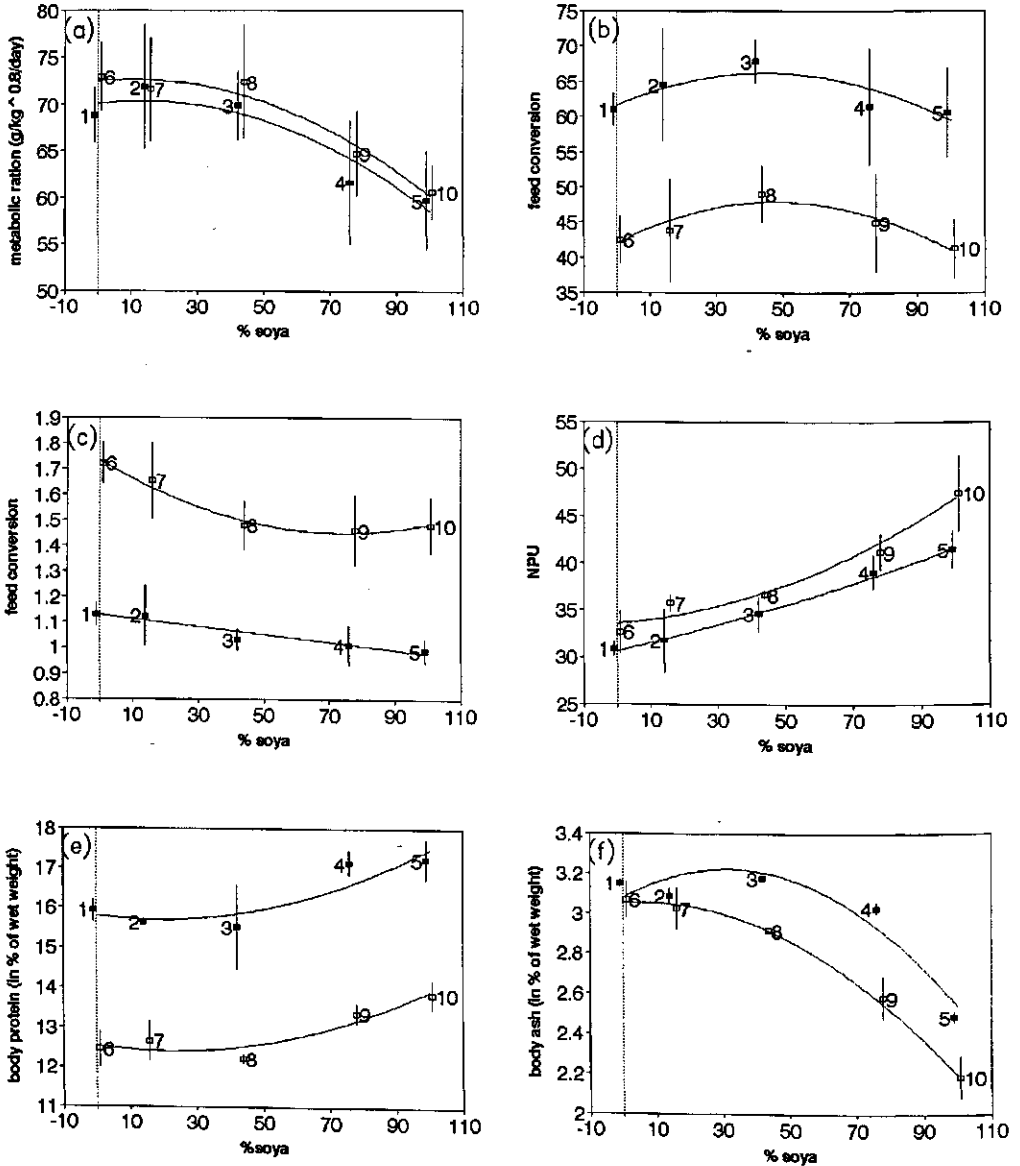
Table 2. Weight increase, feed consumption and feed conversion.

Experi- ment	Diet	% soya	Rep	Fish weight		Growth rate g kg <sup>0.8</sup> day <sup>-1</sup>	Feed consumption MR g kg <sup>0.8</sup> day <sup>-1</sup>	Feed conversion g/g
				initial g/fish	final g/fish			
1	1	0	6	1.19(0.06)	43.5(2.1)a	61.0(2.5)a	68.8(3.2)abc	1.13(0.05)a
	2	15	6	1.14(0.07)	46.5(8.8)a	64.5(8.7)a	71.9(7.3)a	1.13(0.13)a
	3	43	6	1.12(0.08)	49.6(4.5)a	67.8(3.3)a	69.9(4.0)ab	1.03(0.04)ab
	4	77	6	1.13(0.07)	42.9(11.3)a	61.4(9.0)a	61.7(7.3) bc	1.01(0.08)ab
	5	100	6	1.14(0.07)	42.0(9.3)a	60.6(6.9)a	59.8(5.8) c	0.99(0.05) b
2	6	0	6	1.13(0.04)	23.9(3.2)a	42.5(3.7)a	73.0(4.0)a	1.72(0.09)a
	7	15	6	1.11(0.02)	25.1(7.1)a	43.9(8.0)a	71.6(6.0)a	1.66(0.16)ab
	8	43	6	1.10(0.04)	29.4(4.2)a	49.0(4.3)a	72.4(6.6)a	1.48(0.10) b
	9	77	6	1.13(0.05)	26.4(7.3)a	44.9(7.5)a	64.8(4.9)ab	1.46(0.15) b
	10	100	6	1.13(0.04)	22.9(4.0)a	41.3(4.6)a	60.6(3.2) b	1.48(0.12) b

MR = metabolic ration; Numbers between parenthesis refer to standard deviation. Averages within an experiment and column followed by identical letters were not significantly different (Tukey-test,  $P<0.05$ ).

**Figure 1.** Feed uptake, growth, feed utilization and body composition in dependence of the soya protein proportion in the diet: (a) feeding; (b) growth; (c) feed conversion; (d) net protein utilization; (e) body protein content; (f) body ash content.

X-axis: % soya = proportion of soya meal in the diet (see text). Markers: (■) experiment 1, 45% protein diets; (□): experiment 2, 20% protein diets. Numbers near the markers indicate diet number (see table 1); vertical lines indicate the standard deviation.



Both feed conversion (Table 2; Figure 1c) and NPU (Table 3; Figure 1d) tended to improve with increasing soya proportions in the diet. The feed conversion of 43, 77 and 100% soya diets were significantly lower than those of the 0% soya diets. However, the NPU of the 100% soya meal diet was only significantly higher than the 0% soya diet in experiment 2.

### Body composition

In both experiments no significant differences were found in body protein content between treatments (Table 3; Figure 1e). The regression ( $BP = 9.51 + 0.0176\% \text{ soya} + 0.119 \cdot Wf$ ;  $r^2 = 0.62$ ,  $P < 0.001$ ) revealed that not only body weight ( $Wf$ ;  $P < 0.01$ ), but also % soya ( $P < 0.05$ ) increased the body protein level. Van der Meer et al. (1995) indicated that an increasing dietary protein level increases the body protein level. It is plausible that an increased protein digestibility caused by the replacement of fish meal by soya meal, has a similar effect on body protein content as an increased dietary protein concentration.

Body lipid level was mainly determined by the dietary lipid level. The high lipid/low protein diets of experiment 2 resulted in much fatter fish than the low lipid/high protein diets of experiment 1 (Table 3). Within experiments, diets had similar lipid levels and this resulted in fish with similar body lipid content. Possibly due to the high growth rates achieved in this experiment, fat deposition did not reach the 23% of fresh body weight as reported before with similar diets (Van der Meer et al. 1995).

**Table 3.** Body composition in % of wet body weight and net protein utilization.

Experi- ment	Diet	Sub- ratio	Rep	BODY COMPOSITION				PROTEIN UTILIZATION NPU
				Moist	Protein	Lipid	Ash	
1	start			77.3	14.8	6.3	2.84	
1	1	0	2	70.5(0.6)a	16.0(0.4)a	10.2(0.7)a	3.18(0.07)ab	30.9(1.1)a
1	2	14	2	71.0(0.0)a	15.6(0.1)a	10.1(0.1)a	3.09(0.07)ab	31.8(4.8)a
1	3	43	2	71.4(0.8)a	15.5(1.6)a	10.0(0.6)a	3.20(0.00) b	34.7(2.6)a
1	4	77	2	72.6(1.2)a	17.1(0.4)a	8.6(1.0)a	3.03(0.07)a	39.2(2.5)a
1	5	100	2	71.5(1.0)a	17.2(0.7)a	9.8(0.2)a	2.49(0.00) c	41.5(2.8)a
2	start			78.7	14.3	3.7	3.13	
2	6	0	2	66.8(0.5)a	12.4(0.6)a	16.3(0.1)a	3.07(0.13)a	32.7(3.2)a
2	7	14	2	66.5(0.5)a	12.7(0.7)a	17.4(0.2)a	3.03(0.15)a	35.7(1.3)ab
2	8	43	2	67.5(0.2)a	12.2(0.2)a	16.5(0.3)a	2.92(0.14)a	36.7(0.5)ab
2	9	77	2	67.0(0.1)a	13.4(0.4)a	16.8(0.3)a	2.59(0.15)ab	41.2(2.7)ab
2	10	100	2	66.7(0.0)a	13.8(0.5)a	17.7(1.0)a	2.19(0.15) b	47.4(5.7) b

NPU = Net Protein Utilization. Each repetition contained the pooled samples of three aquaria. Numbers between parentheses refer to standard deviation. Averages within an experiment and column followed by identical letters were not significantly different (Tukey-test,  $P < 0.05$ ).

Body ash content decreased significantly with increasing soya levels (figure 1f). In fish the body ash content is low when fed diets low in phosphorus (Sakamoto & Yone 1978; Ogino, Takeuchi, Takeda & Watanabe 1979). Phosphorus is the most critical mineral in soybean meal (Lovell 1991). Lim & Dominy (1990) found that in *Pennaeus vannamei* 100% soya diets resulted in significant lower body phosphorus levels than 0% soya diets. Addition of 2-3% of bicalcium phosphate to diets containing soybean meal could support the same growth of tilapia as 0% soya diet (Viola, Zohar & Arieli 1986). In earlier experiments with *C. macropomum* minimum fresh body ash

contents of 2.62% (Günther & Boza 1993) and 2.25% (Van der Meer et al. 1995) were found in fish exhibiting highest growth rates. In the present experiments minimum ash contents were similar: 2.19 and 2.50% of fresh body weight in experiments 1 and 2, respectively. Fish showed no signs of mineral deficiencies and growth rates were high. Brown, Jaramillo Jr & Gatlin III (1993) found in sunshine bass that bone mineralization was negatively affected at levels below 0.54% available P in the diet, but growth and feed utilization only reduced at dietary P levels below 0.46% available P. Experiments with a longer duration are needed to detect if the low body ash content in *C. macropomum* caused by 100% soya diets are the first symptoms of a mineral deficiency or must be seen merely as a consequence of healthy, fast growth. If growth is not affected in the long run, a 100% soya diet should be preferred above a diet containing fish meal, as much of the P in fish meal is not used by the fish and is a severe cause of water pollution (Watanabe, Satoh & Takeuchi 1988).

### Comparisons between experiments

The reduced growth together with low body protein levels and high NPU values indicate that the dietary protein level was limiting growth in experiment 2. As expected, the relative differences between the treatment means of MGR, FC and NPU were higher in experiment 2. However, only in the case of the NPU the decreased protein level transformed non-significant differences in significant ones. The increasing NPU with increasing soya proportion in experiment 2 demonstrated that soya protein is better utilized by *C. macropomum* than fish meal protein. Possibly this is due to a higher digestibility of soya, as Lovell (1991) states that in several fish species soya protein is equal or better digestible than fish meal protein.

Although CS's indicated that dietary protein quality was best in experiment 1, NPU's were higher in experiment 2. We suggest that the NPU in experiment 1 was negatively affected by the high protein allowance. Reduced NPU's due to high protein allowances, being caused by either high feed allowances (Günther & Boza 1993) or high dietary protein levels (Van der Meer et al. 1995), are well described in *C. macropomum*. While the NPU is an extremely important parameter in practical aquaculture, its use for evaluating dietary protein quality is limited. One might expect a positive correlation between CS and the "maximum-possible-NPU", but not between CS and NPU if experimental conditions are not exactly the same. Having a theoretical basis and being constant for one diet, the CS can be recommended to evaluate dietary protein quality for *C. macropomum*.

### CONCLUSION

The high protein quality of soya as suggested by its high CS was confirmed by the growth and protein utilization efficiency of the fish. Soya protein showed to be of equal quality to *C. macropomum* than fish meal protein. Considering the high similarity in amino acid profiles between fish species, the reduced growth reported in other fish species when fed 100% soya diets, must be attributed to other factors than its amino acid profile. Before 100% soya diets can be recommended for the culture of *C. macropomum*, their long term effect on body ash content and growth should be studied. Under practical aquacultural conditions inclusion of small amounts of fish meal in diets can be recommended because this tended to increase feed uptake and growth.

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## Chapter 3

### THE EFFECT OF PROTEIN RATION ON GROWTH AND PROTEIN UTILIZATION

#### I

#### The effect of dietary protein level on growth, protein utilization and body composition of *Colossoma macropomum* (Cuvier)

##### ABSTRACT

Three size groups of *Colossoma macropomum* were submitted to a 4-week growth trial. Five nearly isocaloric ( $18.8 - 21.0 \text{ kJ g}^{-1}$ ) diets with protein concentrations ranging between 17 to 64 % were administrated at a fixed, near satiation level. Maximum growth was  $6.6, 3.6$  and  $1.9 \text{ g protein kg}^{-0.8}\text{day}^{-1}$  for 5, 50 and 125 g fish, respectively. The protein requirement to achieve maximum growth decreased from  $28.9 \text{ g protein kg}^{-0.8} \text{ day}^{-1}$  for 5 g fish to  $11.7 \text{ g kg}^{-0.8} \text{ day}^{-1}$  for 125 g fish. Possibly due to its high growth rate *C. macropomum* needs a slightly higher dietary P/E ration ( $25.4$  to  $27.9 \text{ mg protein kJ}^{-1}$ ) to obtain maximal growth than most other fishes. The relation between protein ration and protein gain was studied by a quadratic regression model. In fish receiving protein rations equal or below rations resulting in maximal growth, protein ration and protein gain were almost linearly related. The model showed that the portion of the dietary protein which is digested decreases with increasing protein ration. Body protein content increased and body lipid content decreased with feed protein level. Fish fed a 17% protein diet deposited as much as 18% lipid.

## INTRODUCTION

*Colossoma macropomum* (Cuvier) is a native omnivorous species of the Amazon region which can reach a final weight of 30 kg (Saint-Paul 1985). Recently, Günther & Boza (1993) achieved growth rates up to  $53 \text{ g kg}^{-0.8} \text{ day}^{-1}$ . Growing fast and being hardy, *C. macropomum* is considered a promising candidate for the development of fish culture in Latin America (Saint-Paul 1986).

Merola & Pagán-Font (1988) attributed 69 % of the costs of semi-intensive pond culture of *C. macropomum* in Brazil to the cost of the feed. Reducing feeding costs could be a key factor for successful development of *Colossoma* culture. Protein is generally considered the most expensive macro-nutrient of fish feeds (Pillay 1990).

Tacon (1987) recommends for omnivorous fish a crude protein level varying from 42 % (fry) to 35 % (growing adult fish). This recommendation reflects the generally accepted views that the feed protein requirement decreases 'with increasing fish size' (Pillay 1990), 'with decreasing growth rates' (Norman-Boudreau 1981) or 'from early to later phases of growth' (Lovell 1989).

Under laboratory conditions no maximal growth was found when administering feed with protein levels ranging between 24.6 and 36.8 % (Eckmann 1987). Merola & Cantelmo (1987) could not detect any significant differences in growth between cage-reared *C. macropomum* fed 30.9, 34.8 and 40.0 % protein feeds. Thus, accurate recommendations on the dietary protein requirement of *C. macropomum* are lacking. Günther & Boza (1993) showed that body composition of *C. macropomum* depends on fish weight and feeding level. The effect of feed composition on body composition has not yet been studied in this species.

This study looked at the effect of the dietary protein level on growth, protein utilization and body composition of *C. macropomum*. Dietary protein levels in the range from 20 to 60% were used to ensure covering a dietary protein range wide enough to reveal growth differences. Fish of different weight ranges were used to test the assumption of a decreasing protein requirement with increasing fish weight.

Feed ration and growth were expressed in terms of protein ration and protein deposition, respectively. The model used for analyzing protein ration and protein deposition was based on the assumption that protein intake correlates positively with protein retention, as found in farm animals (Simon 1989) and fish (Rychly 1980). The model allows for reduced rates of protein deposition in case of over-nutrition (Simon 1989). The model was used to estimate growth, protein retention and protein digested in function of protein ration.

## MATERIALS AND METHODS

### Fish and feed

Fry of *C. macropomum* were obtained through hormone induced spawning and subsequent artificial incubation. Size groups with an initial average individual weight of 1.44, 30.5 and 96.0 gram, were submitted to separate feeding trials. The groups are further referred to by their average geometrical weights ( $\exp\{(\ln W_f + \ln W_i)/2\}$ , with  $W_f$  and  $W_i$  being final and initial weight) of 5, 50 and 125 gram, respectively.

**Table 1.** Ingredients and chemical composition of experimental diets (in % of dry weight).

diets:	20	30	40	50	60
<b>ingredients *)</b>					
fish meal	3.0	15.0	20.1	24.3	40.0
soya meal	2.5	5.0	19.7	21.8	38.5
tankage	1.0	7.5	13.1	13.5	10.0
blood meal	1.0	2.5	4.2	15.0	16.0
corn flour	50.0	35.0	26.3	7.7	1.0
wheat flour	35.0	30.0	13.5	15.0	2.0
soya oil	1.0	1.0	1.0	1.0	0.0
cod liver oil	5.0	2.5	0.6	2.5	0.0
iodized NaCl	1.0	1.0	1.0	1.0	1.0
premix	0.5	0.5	0.5	0.5	0.5
<b>Proximate analysis of feed dry matter: size group 5&amp;50/size group 125 **)</b>					
crude protein	17.0/17.6	28.5/28.9	40.0/39.7	48.1/50.8	61.8/64.3
fat	11.3/14.7	9.5/12.1	8.2/10.5	9.2/11.2	2.7/ 4.5
carbohydrates	65.9/63.9	51.3/49.7	35.7/34.1	25.0/26.7	19.0/17.5
crude fibre	2.4/ 0.9	2.4/ 0.9	3.4/ 3.6	4.1/ 3.9	5.0/ 4.5
ash	3.4/ 3.3	8.3/ 8.5	12.7/12.2	13.7/ 7.4	11.6/ 9.2
energy***)	19.8/21.0	19.3/20.1	18.8/19.3	19.2/20.9	18.8/19.9

\*) Fish meal: brown sardine meal (65.0% Crude Protein); soya meal: oilmeal, solvent extracted (49.0% CP); tankage: meat and bone meal (60.0% CP); blood meal: drum dried blood meal (81.5% CP); corn flour: pre-cooked (10.9% CP); wheat flour: raw (13.7% CP), premix: "Vitamelk peces A", Roche S.A., a commercially available mineral & vitamin premix for trout.

\*\*) Diets of size group 1 and 2 were made of the same stock of ingredients. Moisture content of the diets fluctuated between 2.5 and 8.1%.

\*\*\*) Gross energy (in kJ/g) was calculated using the following energy values: protein, 23.4 kJ/g; lipid, 39.8 kJ/g and carbohydrates, 17.2 kJ/g (Cho, Slinger & Bailey 1982).

The composition of the experimental feeds used is given in Table 1. Only locally available ingredients were used. Except for diet 20, the theoretical composition of the diets fulfilled the amino acid requirements of omnivorous fish as given by Tacon (1987). Protein levels were about 20, 30, 40, 50 and 60 % of dry matter and are referred to as diet 20, 30, 40, 50 and 60, respectively (Table 1). Caloric value of the diets ranged between 18.8 and 21.0 kJ/g.

### Experimental facilities and procedures

Experiments were performed in 2 recirculation units consisting each of a biofilter and sixteen 45-l aquaria. For each unit, a continuous exchange flow of about 0.4 l/min of tap water was maintained.

Diet 40 was tested in 8-fold (4 aquaria in each unit), the other four diets in 6-fold (3 aquaria in each unit). Each size group was submitted separately to a 4-week feeding trial. Fish were weighed at weekly intervals.

At least 14 days before the start of the experiment the fish were put in the aquaria to acclimatize. During that period the fish were fed ad libitum with a 45 % protein diet made of the same ingredients as the experimental diets. In the second week feed consumption and growth were recorded. The growth coefficients (Hogendoorn 1980) were 0.70, 0.60 and 0.45 and the feed conversion values (g dry feed/g wet weight gain) were 1.05, 1.10 and 1.25 for size groups 5, 50 and

125, respectively. These growth coefficients were used to estimate daily weight gain during the experiments. Daily feed rations were calculated by multiplying the estimated daily weight gain with the appropriate feed conversion value. Feeding rations were corrected weekly in accordance to the measured weight of the fish. The daily ration was divided into three equal portions, which were administrated at 9.00, 13.00 and 17.00 h. On sampling days fish were weighed at 8.00 h and fed at 12.00, 16.00 and 20.00 h.

Water temperature was maintained between 26 and 32 °C and averaged 29.1, 29.1 and 29.4 °C for de 5, 50 and 125 size group, respectively. Dissolved oxygen levels were maintained above 4.3 ppm and nitrite levels below 0.05 ppm.

Faeces and feed rests were removed every morning before the first feeding. Pellets remained stable in the water and feed rests of all diets could still be detected after one night. During the last 5 days of each experiment, before cleaning the aquaria, the presence (or absence) of feed leftovers per aquarium was recorded.

### Chemical analysis

Chemical body composition of the fish was analyzed at the start and at the end of each experiment. Moisture, crude protein, crude fat, ash and crude fibre were determined by standard proximate analysis. Carbohydrates were determined by the anthrone method. Fishes undergoing the same treatment were supposed to have similar chemical body compositions and were therefore pooled. Each sample was analyzed in triplicate.

### Data analysis

For each size group, overall feed ration ( $\text{g dry feed fish}^{-1} 28 \text{ days}^{-1}$ ), daily feed ration ( $\text{g dry feed kg}^{-0.8} \text{ day}^{-1}$ ), protein ration ( $\text{g protein kg}^{-0.8} \text{ day}^{-1}$ ), growth rate ( $\text{g kg}^{-0.8} \text{ day}^{-1}$ ) and protein growth rate ( $\text{g protein kg}^{-0.8} \text{ day}^{-1}$ ) were calculated for each treatment.

The relation between protein ration (Rp) and protein growth rate (GRp) was described by the second order polynomial  $\text{GRp}_i = \alpha + \beta \cdot \text{Rp} + \gamma_{i=5,50,125} \cdot \text{Rp}^2$  with  $\text{GRp}_i = \text{GRp}$  of size group  $i$ ;  $\alpha$ ,  $\beta$  = regression coefficients (equal for all 3 size groups); and  $\gamma_{i=5,50,125}$  = regression coefficient of the quadratic term ( $\text{Rp}^2$ ) of size group  $i$ . The equation was used to estimate - for each size group - the maintenance protein ration (Rp at  $\text{GRp} = 0$ ), the protein ration resulting in optimum protein retention ( $\text{GRp/Rp} = \text{maximum}$ ), and the protein ration resulting in maximum protein growth ( $\text{GRp} = \text{maximum}$ ).

The percentage of dietary protein retained ( $P_{\text{ret}}$ ) was calculated as  $\text{GRp/Rp} \cdot 100$ , while the percentage of the dietary protein digested ( $\text{PP}_{\text{diges}}$ ) was estimated as  $(\text{GRp}_i - \alpha)/\text{Rp} \cdot 100$ , with  $\alpha$  being equal to the estimated protein loss (in  $\text{g kg}^{-0.8} \text{ day}^{-1}$ ) at  $\text{Rp} = 0$  and  $i$  standing for size group. This way of estimating the percentage of digested protein ignores an increased use of protein as a source of energy at higher protein levels, and does not consider the possible use of protein for fat synthesis. Therefore, the estimate will possibly be somewhat lower than the real amount of protein digested.

The effect of size group and diet composition on (a) fish body composition and (b) feed leftover frequency was estimated through analysis of variance. The following model was used:  $X_{ijk} = \mu + A_i + B_j + \epsilon_{ijk}$ , with  $X_{ijk}$  = observed value (values were submitted to an arc sine transformation

before performing the analysis, as is recommended for binomial proportions (Snedecor & Cochran 1989));  $\mu$  = overall mean;  $A_i$  = deviation due to size group  $i$ ;  $B_j$  = deviation due to diet  $j$ ; and  $\varepsilon_{ijk}$  = error term. Means showing significant difference were separated by Tukey-test.

## RESULTS

### Feed ration, feed leftovers and growth

The average feeding rations were 57.3, 32.4 and 22.6 g kg<sup>-0.8</sup> day<sup>-1</sup> for size group 5, 50 and 125, respectively. Feeding rations were higher ( $P < 0.05$ ) for treatments exhibiting slowest growth (Table 2). However, within size groups, the average feeding ration per treatment never deviated more than 4.5 % from the group's average.

The feed leftover frequency was not significantly different between size groups ( $P > 0.05$ , Table 3A). Feed leftover frequency with diet 60 was significantly higher ( $P < 0.01$ ) in diet 60 (average 50%) than in the other diets which had averages fluctuating between 9 and 20% (Table 3B).

The best growth rates were 54.9, 22.7, and 15.3 g kg<sup>-0.8</sup> day<sup>-1</sup> for size groups 5 (diet 50), 50 (diet 40) and 125 (diet 40), respectively. Within size groups, the best protein gain was achieved with the same diets and amounted to 6.6, 3.6 and 1.9 g kg<sup>-0.8</sup> day<sup>-1</sup>, respectively (Figure 1a).

Table 2. Results of the experiment.

Size group	Diet	Wet body weight		Growth		Feed allowance		leftover frequency (%)
		initial (g/fish)	final (g/fish)	weight (g/kg <sup>0.8</sup> /day)	protein (g protein/kg <sup>0.8</sup> /day)	(g/fish)	(g/kg <sup>0.8</sup> /day)	
5	20	1.47	10.9 a	27.9 a	2.67 a	20.1 a	59.5 a	10
	30	1.42	22.9 b	47.8 b	5.16 b	26.0 b	58.0 ab	7
	40	1.44	27.6 cd	53.7 cd	6.06 c	27.8 b	57.4 abc	8
	50	1.48	29.1 d	54.9 d	6.80 d	27.8 b	55.5 c	20
	60	1.49	24.2 bc	48.4 bc	6.30 cd	26.2 b	56.3 bc	50
50	20	30.6	58.0 a	12.2 a	0.53 a	73.0 a	32.9 a	20
	30	31.0	88.5 b	21.7 b	2.95 b	86.1 b	32.6 a	0
	40	30.1	90.2 b	22.7 b	3.65 c	85.8 b	32.7 a	18
	50	29.9	86.0 b	21.6 b	3.48 bc	79.5 ab	31.0 b	17
	60	29.7	78.3 b	19.5 b	3.24 bc	81.4 b	32.9 a	67
125	20	95.8	141.9 a	9.2 a	0.66 a	115.0 a	23.0 a	10
	30	96.9	155.5 ab	11.1 ab	1.76 b	119.8 ab	23.0 a	20
	40	95.9	181.8 b	15.3 b	2.00 b	126.4 b	22.9 a	11
	50	96.1	170.1 ab	13.6 ab	1.85 b	119.2 ab	22.1 b	23
	60	95.6	149.7 ab	10.6 a	1.64 b	113.7 a	22.2 b	33

Means within a size group and in the same column are different when they are not followed by the same letter (Tukey-test,  $P < 0.05$ ).

### Protein utilization

Both the protein ration showing optimal protein retention (Figure 1b) and the protein ration resulting in maximal protein gain (Figure 1a) decreased with fish size. According to the model, the maintenance protein ration (2.5 g/kg<sup>0.8</sup>/day) was independent of fish size (Figure 1a). The maintenance protein ration constitutes an increasing portion of the total protein ration at decreasing feeding levels. Because the maximum protein ration decreases as fish size increases (Figure 1a), large fish use a larger share of the protein ration for protein maintenance than small fish. This effect

in part explains the reduced percentage of retained protein when fish size increases (Figure 1b). In addition, the percentage of protein digested decreased with increasing fish weight and increasing protein ration (Figure 1b). A theoretical 54.6 % is digested at a protein ration close to zero.

At protein rations resulting in maximum protein gain and optimum protein retention, the percentage of protein retained was 32.6 and 22.8%, 26.6 and 20.2%, and 20.1 and 16.4% for size groups 5, 50 and 125, respectively (Figure 1). Optimum protein retention was reached at a protein ration lying between the rations resulting in no protein growth (protein maintenance) and maximum protein growth (Figure 1). Starting at the optimum and proceeding to the left, the percentage of dietary protein retained decreases rapidly while the percentage of protein digested increases; here maintenance needs restrict protein retention. Proceeding to the right, starting at the optimum, both the percentage of dietary protein retained and the protein digested decrease; here the limited amount of protein digested limits protein retention.

**Table 3.** Effect of fish size and feed composition on leftover frequency and body composition.

**Table 3A** F-values of the analyses of variance.

Source of variation	d.f	Leftover frequency	Final body composition			
			Protein	Lipids	Ash	Dry matter
Size group	2	0.39	7.41 #	13.85 ##	9.47 #	27.45 ##
Feed	4	8.39 ##	5.71 #	16.23 ##	1.30	15.23 ##
Error	8					
TOTAL	15					

#P<0.05; ##P<0.01; all values refer to arc sin transformations of the fractions (e.g.: 10% = 0.10, arcsin 0.10 = 0.1001674).

**Table 3B** Mean values.

		Leftover frequency	Body composition			
			protein	lipids	ash	dry matter
Size group	5	19.0 a	11.5 a	11.0 a	2.56 a	25.9 a
	50	24.4 a	13.6 b	13.4 a	3.00 b	31.0 b
	125	19.4 a	14.1 b	6.8 b	2.98 b	24.3 a
Diet number	20	13.3 a	10.4 a	18.4 a	2.80 a	32.7 a
	30	9.0 a	13.2 ab	10.5 b	2.93 a	27.2 b
	40	12.3 a	13.5 ab	8.1 b	2.67 a	24.6 b
	50	20.0 a	13.9 b	8.3 b	2.97 a	25.6 b
	60	50.0 b	14.4 b	6.8 b	2.87 a	25.0 b

Means within a frame are different when they are not followed by the same letter (Tukey-test, P<0.05).

### Body composition

Fish size and diet composition influenced fish body composition (Table 3A and 3B). Body protein content increased with increasing fish size and with increasing dietary protein level ( $P < 0.05$ ). Contrary, body lipid content decreased with increasing fish size and dietary protein level ( $P < 0.01$ ). Body ash content did not alter ( $P > 0.05$ ) with dietary protein level. Because the body lipid content fluctuated in a much wider range than body protein content (fish fed diet 20: 18.4% lipid and 10.4% protein; fish fed diet 60: 6.8% lipids and 14.4% protein), fluctuations in body dry matter content were merely determined by body fat content.

## DISCUSSION

### The model

It is not possible to change the protein content of a (dry) diet without changing the levels of one or more of the other nutrients. Our high protein diets were for example lower in lipids to keep them isocalorical. However, the model we used predicts growth and feed utilization from dietary protein content only. Results of this experiment legitimate the use of the model because the correlation coefficient ( $r^2=0.93$ ) deviated from 1 (one) principally due to within treatment deviations.

### Feed rations, feed leftovers and growth

The settings of the feeding levels were based on observed growth and feed consumption of a 45% protein diet during the pre-experimental period. For the weight range covered in our experiment, the growth rates compared favorable with those reported for *C. macropomum*: Herculano (1987), Luna (1987), Merola & Pagán-Font (1988), Merola & Cantelmo (1987) and Merola & de Souza (1988) and previous work resumed by Saint-Paul (1986). Günther & Boza (1993) reported identical growth rates in 5-gram fish and slightly higher growth rates in 50 - 125 g fish.

The wide gap between protein rations of optimum and maximum protein gain indicate that reasonable growth rates can be achieved under a wide range of protein rations. The best feeding ration, from an economical point of view, lies between the optimum and maximum protein ration. If operational costs (not including feed costs) are high in relation to feed costs, a feeding ration resulting in maximum growth has to be chosen; if feeding costs are predominant than a feeding ration resulting in optimum protein retention is preferred.

Within size groups all fish were submitted to the same feeding regime. The high number of aquaria with rests of diet 60 indicate that the fish of this treatment were fed in excess. Reduced feed intake due to high feed protein levels was reported for *C. macropomum* (Castagnolli 1991), but also with *Ictalurus punctatus* (Page & Andrews 1973; Mangalik 1986). As feed rests were not measured quantitatively, it remains uncertain whether the sub-maximal growth of the fish fed diet 60 must be attributed to the feed composition or mainly to a reduced feed intake.

### Protein requirement and protein utilization

Hepher (1988) summarized experiments in which the optimum protein to energy ratio was calculated. The optimum P/E (crude protein/gross energy) ratios of the feeds for 17 species varied between 16.0 and 37.8 and averaged 24.6 mg protein/kJ (SD=4.9). In the present experiment, using maximal growth as the criterion, the optimum P/E ratio for *C. macropomum* was estimated to be in the range of 25.4 to 27.9 mg protein/kJ. This is slightly above the average mentioned by Hepher (1988), but is not surprising as fast growing fish tend to have a higher protein requirement (Tacon & Cowey 1985). Günther & Boza (1993) found that *C. macropomum* grows faster than most other species.

Figure 1. The relation between dietary protein ration and growth rate of body protein.

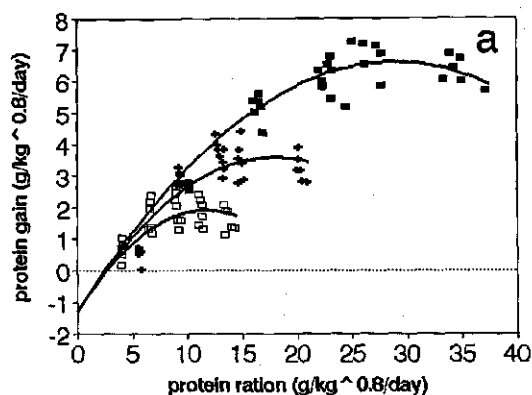


Figure 1a:

X-axis: Rp in g/kg<sup>0.8</sup>/day; Y-axis: GRp in g/kg<sup>0.8</sup>/day.  
Closed squares = size group 1; crosses = size group 2;  
open squares = size group 3.

The regression line:

$$GRp_i = \alpha + \beta * Rp + \gamma_{i=1,2,3} * MRP^2,$$

with:

Size group (i)	5	50	125
$\alpha$	-1.279	-1.279	-1.279
$\beta$	0.546	0.546	0.546
$\gamma_i$	-0.00946	-0.01530	-0.02333
$r^2$	0.93.		

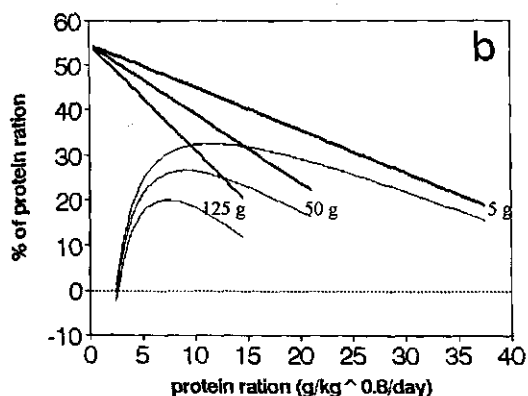


Figure 1b:

X-axis: Rp in g/kg<sup>0.8</sup>/day; bold line: digested protein; thin line: retained protein.

The efficiency of protein deposition declines with age in farm animals (Simon 1989). Reduced protein utilization with increasing fish weight as we found in this experiment, has also been demonstrated in *Clarias lazera* (Henken, Machiels, Dekker & Hogendoorn 1986).

The percentage of digested dietary protein ( $PP_{digest}$ ) was estimated as  $(GRp - \alpha)/Rp * 100$ . As  $GRp = \alpha + \beta * Rp + \gamma * Rp^2$ ,  $PP_{digest} = (\beta * Rp + \gamma * Rp^2)/Rp * 100 = (\beta + \gamma * Rp) * 100$ . If  $GRp$  reaches its maximum then the first order differential equation of  $GRp = \alpha + \beta * Rp + \gamma * Rp^2$  is equal to zero:  $\delta GRp / \delta Rp = \beta + 2 * \gamma * Rp = 0$ , from which follows that  $Rp = -\beta / 2\gamma$ . Therefore, when  $GRp$  is maximal, then  $PP_{digest} = \{\beta + \gamma * (-\beta / 2\gamma)\} * 100 = \frac{1}{2} \beta * 100$ . However, if  $Rp = 0$  then  $PP_{digest} = \beta * 100$ . Thus, the use of our model implies that at maximum growth the dietary protein digestibility is only 50% of the theoretical maximum digestibility at  $Rp=0$ . The strong dependence of the percentage of

protein digested on the protein allowance indicates that digestibility data are only valid at the feeding level under which they were determined.

The coefficient  $\beta$  reflects the conditions under which experimental results were obtained.  $\beta$  will be higher when experimental conditions (diet composition, stress, temperature etc.) are more favourable. Data of Günther and Boza (1993), using the estimated negative growth rate of body protein at a zero protein ration in our experiment, indicate that *C. macropomum* is able of digesting 35 - 42% of the dietary protein at maximal growth. Thus,  $\beta$  during their experiment was in the range 0.70 to 0.84. This is well above the  $\beta$  of our experiment (0.56), but probably below results mentioned by Castagnolli (1991). He reported a dietary protein digestibility in *C. macropomum* of 68 to 86% using 14 to 26% protein diets (as no feeding levels are reported  $\beta$  could not be calculated). Our experimental conditions differed little from those described by Günther and Boza (1993). We suggest that in our high protein diets the high portions of blood meal and tankage were the main cause of the reduced percentage of dietary protein digested. Eckmann (1987) mentioned the limited value of bloodmeal as dietary protein source for *C. macropomum*.

An additional cause for reduced protein utilization efficiency might be sought in the wasteful way of feed intake. *C. macropomum* fed at a near satiation level spit out feed particles while eating. Although apparently all particles are eaten again, feed losses are probable. Incomplete feed collection at a satiation feeding rate due to regurgitation was also reported in turbot (Bromley 1980).

Further optimization of *Colossoma* culture could be obtained by manipulating simultaneously dietary protein levels and feeding rations. The almost linear relation between protein ration and protein gain at feed protein levels below the optimum protein ration (Figure 1a) suggests that feeding costs cannot be reduced by employing sub-optimal dietary protein levels. In intensive culture systems it could be advantageous to use diets with a high protein level (for example 50 - 55% protein), but using lower feeding rations than used in this experiment. Reduced costs of ingredients not contributing to the feed protein level and production of leaner fish could be advantages of this strategy.

### Body composition

Feed composition was well reflected in body composition of the fish. Fish fed diet 20 accumulated up to 20% lipid (Table 3B), which was almost double the amount of fat accumulated in fish fed the other diets. As *C. macropomum* fasts during its annual 3 - 4 months migration (Woynarovich 1986), fat accumulation is necessary to overcome the long periods of starvation. Common carp fed prolonged periods with feeds with a high P/E-ratio can accumulate up to 30% lipids (Hepher 1988).

Body protein content not only increased with fish size as reported by Günther & Boza (1993), but also with increased dietary protein level. Shearer (1994) argues that in many cases so called effects of diet composition on body protein content were due to size differences of the fish. However, in our experiment the diet effect is significant even when fish size effect is removed: the highest protein diets resulted in smaller fish but with higher body protein content (Table 3B). The use of deposited protein as a reserve energy source, as might happen in turbot (Bromley 1980) and salmonids (Shearer 1994), seems of minor importance for *C. macropomum*, as lipid has a much higher energy value as protein and showed to be the most flexible component of the body.

## CONCLUSION

The model ( $GR_p = \alpha + \beta \cdot R_p + \gamma \cdot R_p^2$ ) used to describe the relation between protein ration and protein gain fitted well to the data. The model assumes that at low protein rations the relation between protein ration and protein gain is almost linear. Results of this experiment support this assumption.

General use of this model in aquaculture experiments is recommended. The model allows to estimate on the basis of protein allowance: (a) the protein gain, (b) the percentage of protein retained, (c)  $\alpha$ , the protein maintenance metabolism and (d) the percentage of digested protein. In addition, the theoretical maximum digested percentage of the dietary protein ( $\beta \cdot 100$ ) can be used as a measure for the quality of dietary protein and experimental conditions.

*C. macropomum* needed a dietary P/E level in the range of 25.4 to 27.9 mg protein  $\text{kJ}^{-1}$  to obtain maximal growth. This protein requirement, which lies above the requirement of most other fish species, should be attributed to its fast growth rate. The percentages of dietary protein retained and digested in this experiment are below the maxima reported elsewhere. Possibly, these differences can be attributed to one or a combination of the following factors: (a) the sub-optimal feed quality, which limits protein digestibility; (b) the inability to differentiate between feed ration and feed intake; and (c) the high feed levels needed for maximal growth reduced protein digestibility. Concerning the first factor we suggest to limit the use of non-fish animal protein sources in *C. macropomum* experimental diets. Factors (b) and (c) suggest that studying the relation between protein ration and protein gain from an economical point of view, will help to further improve *C. macropomum* culture practices. The importance of this relation is further demonstrated by the fact that our growth model predicts that, at feeding levels associated with maximum growth, dietary protein digestibility is only 50% of the theoretical maximum.

## ACKNOWLEDGEMENTS

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## Chapter 3

### THE EFFECT OF PROTEIN RATION ON GROWTH AND PROTEIN UTILIZATION

## II

### Effect of feeding level on feed losses and feed utilization of soya and fish meal diets in *Colossoma macropomum* (Cuvier)

#### ABSTRACT

Juvenile, 1 gram *Colossoma macropomum* were fed two different diets: one with fish meal and an other with soya meal as the main dietary protein source. Both diets were provided at *ad libitum* feeding level, and at two restricted feeding levels of approximately 80 and 60% of the *ad libitum* level. The experiment was performed in 30 aquaria, each stocked with 12 fish. Of each treatment (2 diets x 3 feeding levels), there were 5 replicates. Fish were fed three times daily at 9, 13 and 17 h. At sampling days (day 14, 29 and 44) in each aquarium in one of these meals chromic oxide marked feed was used. Shortly after the last meal, fish were weighed and of each aquarium 4 fishes were taken for determination of the chromic oxide content in their digestive tract. Fish fed the fish meal diet attained a higher weight but had a lower body protein content. At the *ad libitum* feeding level, feed intake of the fish meal diet was higher, but feed and protein utilization efficiency were lower than with the soya diet. However, statistical analysis of the data of both the *ad libitum* and the two restricted feeding levels revealed that the reduced voluntary feed intake of the soya diet was the cause of its better utilization with *ad libitum* feeding. There is no evidence that soya protein is more freely available than fish meal protein. The chromic oxide data showed that 15 minutes after feeding all of the feed (99.8%) could be traced back in fish fed the lowest feeding level, while at the *ad libitum* feeding level the recovery was only 72%. These data confirmed the hypothesis that at high feeding levels considerable amounts of feed remain uneaten. Feed losses were not significantly different between the two diets. The chromic oxide recovery data of the 9 and 13 h meals showed that the feed passage rate in the digestive tract was similar for the three different feeding levels. Due to the high feed losses at high feeding levels, the relation between feed ration and feed utilization is mainly determined by the percentage of feed losses at the different feed rations.

## INTRODUCTION

Growth of *Colossoma macropomum* (Cuvier), a fast growing fish from the Amazon region, is mainly determined by the protein ration (Van der Meer, Machiels & Verdegem 1995). Based on amino acid composition, soya meal protein was presumed to be a good substitute for the commonly used fish meal protein (Van der Meer & Verdegem 1996). Van der Meer, Huisman & Verdegem (1996) confirmed this in a feeding experiment. They found that the net protein utilization (NPU) was higher with soya meal diets than with fish meal diets. However, the *ad libitum* feed intake tended to decrease with increasing soya ratio in the diet. NPU in *C. macropomum* decreases with increasing protein ration (Günther & Boza 1993; Van der Meer et al. 1995). Therefore, the effects of protein ration and protein source (soya or fish meal) could not be distinguished when feeding to satiation.

Interactions between feeding level and feed utilization efficiency are common. Therefore Anderson, Lall, Anderson & McNiven (1993) suggested to use the relationship between protein ration and protein growth when comparing protein quality of diets. One of the mechanisms by which the feeding level can affect NPU is through digestibility. Restriction of the feeding level, starting at the *ad libitum* feeding level, increased the digestibility maximally by 28% for the dry matter and with 16% for protein in African catfish (Henken, Kleingeld & Tijssen 1985), with 2.5% for protein in rainbow trout (Storebakken & Austreng 1987) and no effect of feeding level on digestibility was found in eel (Heinsbroek, Thoor & Elizondo 1989).

Starting with feeding levels close to the *ad libitum*, feed uptake of *C. macropomum*, gradually reducing the feeding level improved the feed conversion (FC) with 46-57% and the NPU with 53-62% (Günther & Boza, 1993). Van der Meer et al. (1995) found that decreasing protein rations could improve FC and NPU by 40-52% and 42-65%, respectively. This improvement of FC and NPU cannot be explained by the maximum improvement of 28% of the digestibility by reducing feeding levels, as found in African catfish by Henken et al (1985). An explanation for this discrepancy might be that lowering feeding levels not only improves digestibility, but also diminishes feed losses. The frequency of feed leftovers found in aquaria suggested increased feed losses with higher protein rations (Van der Meer et al. 1995). High water turbidity in an experiment in which fish were fed *ad libitum* (Van der Meer, Huisman & Verdegem 1996) was thought to be an other indication of high feed losses at high rations. Studies describing the relationship between feeding level and feed losses in fish are not known.

Even under well defined culture conditions, accurate measuring of feed losses (might also be called 'feed rests', 'uneaten feed', 'feed spillage' or 'wasted feed') seems not yet possible (Nijhof 1994). However, feed losses can also be calculated as the difference between administered feed ration (might also be called 'feed gift' or 'feed allowance') and feed intake (the amount of feed entering in the digestive tract). Several techniques to estimate feed amounts in the digestive tract of fish have been described. X-raying of fish fed with feed marked with glass beads (Jobling, Baardvik & Jorgensen 1989; McCarthy, Carter & Houlihan 1992) or iron markers (Talbot & Higgins 1983) has a limited precision because marker density in the feed must be low to allow counting of the markers. Radioactivity measurements of feed marked with radioactive isotopes (e.g. Storebakken & Austreng 1988; Langar & Guillaume 1994) requires expensive equipment. Recovery of colored feed from the digestive tract (Johnston, Atkinson & Glanville 1994) requires a laborious manual separation of the different colored feed rests. In this experiment we used chromic oxide to mark the

feed, because it is fast, cheap and has a well established protocol for its use in fish nutrition studies.

The present experiment was conducted to determine the separate effects of protein ration and protein source (fish meal versus soya meal) on protein intake and protein utilization in *C. macropomum*. To create different protein rations both the soya meal diet and the fish meal diet were provided at three feeding levels: one group of fish was fed to satiation, one was fed a low fixed feeding level and a third group was fed at an intermediate fixed level. Chromic oxide ( $\text{Cr}_2\text{O}_3$ ) marked diets were used to quantify feed intake and feed passage rate in the digestive tract. To clarify the relevance of employing different protein rations when comparing utilization efficiencies of different diets, conclusions based on the whole dataset were compared with conclusions based on the data of the *ad libitum* fed fish only.

## MATERIAL AND METHODS

### Fish and experimental facilities

Larvae of *C. macropomum* were obtained through artificial reproduction. Fish were weaned to an artificial diet when weighing 80 to 100 mg. One week prior to the start of the experiment fish were transferred to two experimental units, each equipped with sixteen 45-L aquaria, a sedimentation unit and a biofilter.

One day before the start of the experiment 30 aquaria (15 aquaria in each unit) were stocked with 12 fish each. Individual wet weight of the fish varied between 0.85 and 1.15 g. The experiment lasted 44 days. Bottoms of the aquaria were cleaned daily before first feeding. On days 15 and 30 the aquaria and sedimentators were cleaned thoroughly and fish were restrained from feed. Average water temperature during the experiment was  $29.1 \pm 0.7^\circ\text{C}$ .

### Feeds and feeding

In one experimental diet soya meal was the main protein source, in the other fish meal (Table 1). Diets contained comparable amounts of dietary protein (35.0 and 31.5% in the soya and fish meal diet, respectively), dietary energy (19.77 and 19.84 kJ g<sup>-1</sup>, respectively) and  $P_{\text{growth}}$  being the portion of the dietary protein with the same indispensable amino acid profile as *C. macropomum* body protein (21.7 and 23.7% of the diet respectively; for full explanation see Van der Meer & Verdegem 1996). Although the fish meal diet had a lower protein to energy ratio, this was not expected to affect protein utilization efficiency (Van der Meer, Zamora & Verdegem 1997).

Fish were manually fed seven days a week at 9, 13 and 17 h with a maximum duration of each feeding of one hour. The three feeding levels applied were *ad libitum* and two restricted feeding levels, abbreviated as 'adlib', '60%al' and '80%al', respectively. *Ad libitum* fed fish were fed to apparent satiety during at least four feeding sessions at each feeding. Fish submitted to restricted feeding levels were fed equal portions each feeding. The restricted feeding levels aimed to be approximately 60 and 80% of the *ad libitum* feeding level. Based on findings of Günther & Boza (1993) initial feeding levels of fish submitted to the 60%al and 80%al treatments amounted 22 and 30 g kg<sup>-0.8</sup> day<sup>-1</sup>, respectively. Feeding levels were adapted daily, assuming feed conversions (dry feed in wet body weight gain) of 1.5 and 1.0 for 80%al and 60%al, respectively. If in more than 80%

of the aquaria submitted to treatment 80%al feed rests were found at 18 h, the feeding levels of all restricted feeding regimes were not increased the next day.

**Table 1.** Ingredients and proximate composition of the diets (in % of the feed dry matter).

		soya meal diet	fish meal diet
INGREDIENTS*)	fish meal	0	32.8
	tankage	0	2.3
	blood meal	0	0.7
	corn flour	24.6	54.9
	soya meal	59.0	0
	wheat flour	8.3	4.9
	soya oil/cod oil (50/50)	6.7	2.9
	salt (NaCl)	1.0	1.0
	premix	0.5	0.5
NUTRIENTS	dry matter**)	93.7	94.2
	crude protein	35.0	31.5
	lipids	7.4	12.4
	ash	5.0	10.0
	carbohydrates	50.2	43.8
	fibre	2.4	2.4
PROTEIN QUALITY	energy, calculated***)	19.77	19.84
	CS****)	62.0	75.3
P <sub>growth</sub> ****)		21.7	23.7

\*) Feeds were made of ingredients of the same stock as those used by Van der Meer et al. (1996).

\*\*) Feed dry matter as a percentage of the diet as fed.

\*\*\*) Gross energy content calculated using the following energy values: protein, 23.4 kJ/g; lipid, 39.8 kJ/g and carbohydrates, 17.2 kJ/g (Cho, Slinger & Bailey 1982).

\*\*\*\*) CS = Chemical Score, the estimate of the percentage of dietary protein having the appropriate amino acid profile for protein deposition; P<sub>growth</sub> = amount of protein with an identical indispensable amino acid profile as *C. macropomum* body protein. P<sub>growth</sub> is expressed as a percentage of the feed dry matter and calculated as CS/100 \* dietary protein %. For further explanation see Van der Meer & Verdegem 1996.

### Experimental procedures

The six treatments (2 diets, 3 feeding levels) were randomly assigned to the aquaria, with 5 replicates per treatment. Experimental diets marked with 1% chromic oxide (Cr<sub>2</sub>O<sub>3</sub>) were used on sampling days (days 14, 29 and 44). Of each treatment two aquaria were fed labelled feed at 9 h, two at 13 h and one at 17 h (Table 2). In a pre-experimental trial (fish of the same size, same water temperature and the same diet) it was found that within 25 minutes after finishing a meal with marked feed, faeces with Cr<sub>2</sub>O<sub>3</sub> could never be detected. Feed losses were estimated from the chromic oxide recovery data of 6 aquaria (one replicate per treatment) fed marked feed at 17 h. These 6 aquaria were sampled before 18h20. The subsequent sampling of fish fed marked feed at 9 or 13 h was completed around 18h45. Due to a feeding error, on day 44 only in 22 aquaria chromic oxide determinations could be done.

Table 2. Chromic oxide sampling schedule.

TIME SCHEDULE					
Feeding time labelled feed			9h	13h	17h
Feeding time unlabelled feed			13h, 17 h	9h, 17 h	9h, 13 h
Sampling time			18.20-19.00 h	18.20-19.00 h	18.10-18.20 h
Interval between feeding & sampling			8.5 h	4.5 h	15 min
REPLICATES	day 14	60% al	2	2	1
		80% al	2	2	1
		adlib	2	2	1
		TOTAL			15
	day 29	60% al	2	2	1
		80% al	2	2	1
		adlib	2	2	1
		TOTAL			15
	day 44	60% al	1	1	1
		80% al	1	1	2
		adlib	1	2	2
		TOTAL			11

Feeding times refer to the start of the feeding; each feeding lasted one hour.

Table 3. Parameters and their abbreviations.

Parameter	Units	Abbreviation	Formulas and observations
Fish weight	g	Wi, Wf	Initial (day=1) and final (day=44) wet weight
Weight gain	g	WG	Wf-Wi
Body protein gain	g	PG	$Wf * \{(\text{final body protein } \%) - Wi * (\text{initial body protein } \%)\} / 100$
Geometric mean weight	g	Wgeo	$\exp\{(\ln(Wi) + \ln(Wf)) / 2\}$
Growth rate	$\text{g kg}^{-0.8} \text{ day}^{-1}$	GR	$WG / \{(Wgeo / 1000)^{0.8}\} / 44$
Protein growth rate	$\text{g kg}^{-0.8} \text{ day}^{-1}$	GRp	$PG / \{(Wgeo / 1000)^{0.8}\} / 44$
Feed ration	g	$F_{\text{ration}}$	dry weight
Protein ration	g	$P_{\text{ration}}$	$F_{\text{ration}} * (\text{feed protein } \%) / 100$
Metabolic feed ration	$\text{g kg}^{-0.8} \text{ day}^{-1}$	FR	$F_{\text{ration}} / \{(Wgeo / 1000)^{0.8}\} / 44$
Metabolic protein ration	$\text{g kg}^{-0.8} \text{ day}^{-1}$	PR	$P_{\text{ration}} / \{(Wgeo / 1000)^{0.8}\} / 44$
Protein maintenance requirement	g	PM	$\{(Wgeo / 1000)^{0.8}\} * 1.279 * 44 \#)$
Cr <sub>2</sub> O <sub>3</sub> in fish	g	Cr <sub>fish</sub>	{ methodology of Mink, Schefman-van Neer & Habets (1969)
Cr <sub>2</sub> O <sub>3</sub> in feed	%	Cr <sub>FEED</sub>	
Cr <sub>2</sub> O <sub>3</sub> recovery	%	Cr <sub>RECOV</sub>	$(Cr_{\text{fish}} / (Cr_{\text{FEED}} / 100 * F_{\text{ration}})) * 100$
Feed loss	%	F <sub>LOSS</sub>	$100 - Cr_{\text{RECOV}} \#)$
Apparent feed conversion	g/g	FC <sub>A</sub>	F/WG
Real feed conversion	g/g	FC <sub>R</sub>	$FC_A * (Cr_{\text{RECOV}} / 100)$
Apparent net protein utilization	g/g	NPU <sub>A</sub>	PG/P <sub>ration</sub>
Real net protein utilization	g/g	NPU <sub>R</sub>	$(NPU_A / Cr_{\text{RECOV}}) * 100$
Net protein utilization for protein synthesis	%	NPU <sub>SYNT</sub>	$((PG + PM) / (P_{\text{ration}} * (100 - F_{\text{LOSS}} / 100))) * 100$

#) Maintenance protein metabolism estimated in 1.279 g protein  $\text{kg}^{-0.8} \text{ day}^{-1}$  (Van der Meer et al., 1995).

##) only data from fish fed chromic oxide marked feed at 17 h were used for the calculation of F<sub>LOSS</sub>.

During sampling, the two fishes with the highest and the two with the lowest individual weight were removed and stored at  $-20^{\circ}\text{C}$ . After thawing the fish, the complete digestive tract with its contents was removed and dried for 8 hours at  $105^{\circ}\text{C}$ . Subsequently it was grinded and burned to ash (12 hours at  $550^{\circ}\text{C}$ ). Chromic oxide in the ash was determined by spectrophotometry (Mink, Schefman-Van Neer & Habets 1969). The amount of chromic oxide recovered from the digestive tract ( $\text{Cr}_{\text{RECOV}}$ ) was calculated as a percentage of the chromic oxide in the ration (Table 3). Feed provided during a meal but not found back in the digested tract 0 to 20 minutes after finishing the meal was considered uneaten feed. Hence, feed losses ( $F_{\text{LOSS}}$ ; Table 3) were calculated as being 100 minus the  $\text{Cr}_{\text{RECOV}}$  of fish fed marked feed during the 17 h feeding.

### Proximate analysis

Triplicate proximate analyses were made of the fish at the start of the experiment (one sample), at the end of the experiment (one sample per aquarium) and of the feed (one sample of each diet). In fish, protein, lipids (ether extract) and ash were determined. In the feeds also fibre and carbohydrates were determined. The chemical analyses were made using standard proximate analysis procedures. Carbohydrates were determined by the anthrone method. Chemical analyses of the fish refer to fish of which the digestive canal had been extracted.

### Data analysis

Parameters concerning feed losses, growth and feed utilization, together with their respective abbreviations, can be found in Table 3. Data concerning the percentage of chromic oxide recovered from the digestive tract were examined by analysis of variance (ANOVA). DIET (soya versus fish meal diet), TIME (time between providing marked feed and sampling fish for the determination of the chromic oxide content of the digestive tract which was 8½h, 4½h and 15 minutes for the 9, 13 and 17 h feedings, respectively), FEEDLEVEL (the three feeding levels, being 60%al, 80%al and adlib, respectively) and SAMPLEDAY (referring to the sampling days 14, 29 and 44, respectively) were factors in the ANOVA.

Growth and feed utilization data were analyzed by ANOVA using two models. The first model, further referred to as the ADLIB model, used only the *ad libitum* treatments, with DIET being the sole explaining variable. The second model, further referred to as the COMPLETE model, included data from all treatment groups, using DIET as the variable and  $P_{\text{ration}}$  as the covariable. If the DIET effect was found to be significant it was checked if the slope of the regression line differed between the two diets. Results of the ADLIB and the COMPLETE model were compared.

## RESULTS

### Chromic oxide recovery data

The percentage of the dietary chromic oxide recovery from the digestive tract was significantly affected by TIME, FEEDLEVEL and SAMPLEDAY, but not by DIET (Table 4). The significant effect of SAMPLEDAY on the chromic oxide recovery data was mainly due to higher recovery

figures of sampling day 44. SAMPLEDAY did not interfere with the other effects and data of the three sampling days were pooled. Recovery of chromic oxide in the digestive tract 15 minutes after feeding tended to increase with decreasing feed ration and amounted 71.5, 79.8 and 99.8% on average for the treatments adlib, 80%al and 60%al, respectively (Figure 1). At the end of the day the amount of chromic oxide of previous meals still present in the digestive tract was estimated in 30.6% for the fed fish adlib (6.5% of the 9 h feeding and 24.1% of the 13 h feeding), 49.2% for the fish fed 80%al (13.1 and 36.1%, respectively) and 49.7% for the fish fed 60%al (14.7 and 35.0%, respectively; Figure 1). After correction for non-eaten feed, the percentage of chromic oxide recovered from the digestive tract ( $Cr_{RECOV}$ ) showed an identical decline in time for the three feeding levels. The decline of  $Cr_{RECOV}$  in time fitted well to an exponential evacuation model:  $Cr_{RECOV} = Ae^{-\alpha(t-0.25)}$ , with  $A = 100$ ,  $\alpha = 0.233$  and  $t$  = time lapse (h) between feeding and sampling (Figure 2).

**Table 4.** Analyses of variance of the chromic oxide recovery data.

Source of variation	DF	Mean square	F value	P
Main effects				
DIET	1	0.006360	0.61	0.6909
TIME	2	6.025451	151.02	0.0001
FEEDLEVEL	2	0.398650	9.99	0.0001
SAMPLEDAY	2	0.155131	3.89	0.0248
Remainder	74	0.039899		

Chromic oxide recovery was expressed in % of the amount of  $Cr_2O_3$  in the marked feed ration. Recovery data of chromic oxide were transformed to arc sine as is recommended for proportions (Snedecor & Cochran 1989).

\*) DIET: soya versus fish meal; TIME: time lag between feeding the fish with marked feed and sampling: 8.5 h, 4.5 h and 15 min. for the 9, 13 and 17 h feedings, respectively; FEEDLEVEL: adlib, 80%al and 60%al, respectively; SAMPLEDAY: days 14, 29 and 44, respectively.

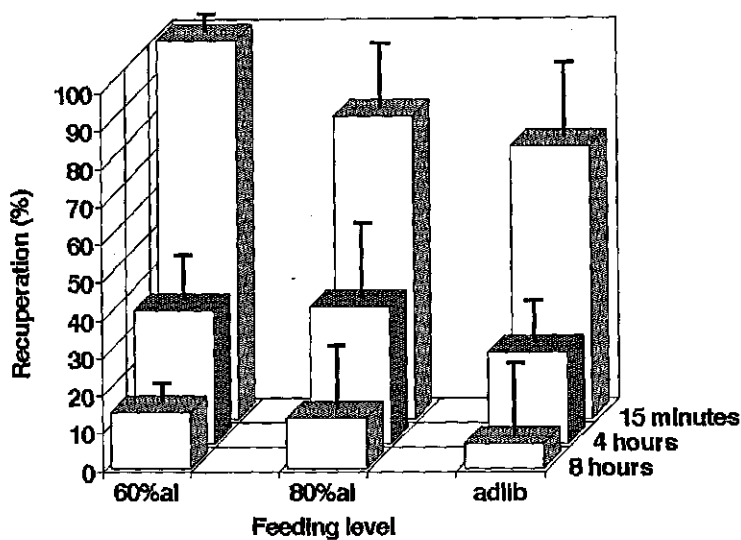
### Growth, body composition and feed utilization

Results concerning feed ration and growth are summarized in Table 5. *Ad libitum* fed fish consumed significantly more when fed the fish meal diet (Table 6, ADLIB model). The fish meal diet resulted also in a higher final weight ( $P=0.09$ ), but growth rate did not differ between diets (Table 6, ADLIB model). However, the COMPLETE model revealed that both final weight and GR differed significantly between diets (Table 6), with the lower final weight and GR found in the soya meal diet (Figures 3a, 3b). The GRp obviously increased with the protein ration, but was not significantly different between diets (Table 6; Figure 3c).

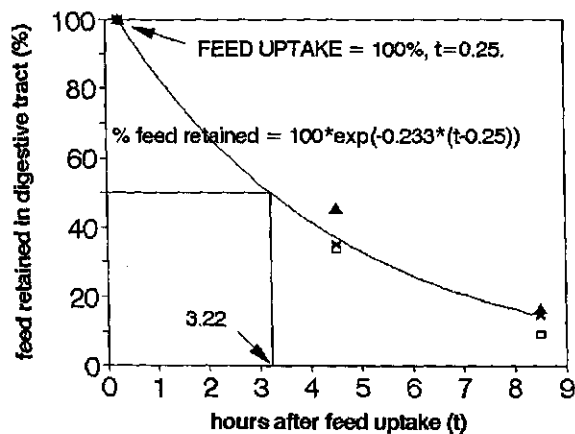
In the ADLIB model body ash ( $P<0.01$ ) and moisture ( $P<0.1$ ) were significantly affected by the diet composition (Table 6). However, the COMPLETE model revealed that not only ash (Figure 3i: higher in the fish meal diet) and moisture (Figure 3f: higher with the soya diet and decreasing with increasing protein ration), but also protein (Figure 3g: higher with the soya diet) were affected by diet composition. Body lipid content (Figure 3h) was not affected by diet composition nor protein ration. As the slopes of the regression lines never differed significantly ( $P>0.10$ ) between the diets the PR(DIET) interaction was removed from the COMPLETE model (Table 6).

Due to the lower feed rations with the soya diet and the non-significant differences in

**Figure 1.** Recovery figures of chromic oxide provided with the feed 15 minutes, 4½ hours or 8½ hours before sampling, as affected by the feeding level. Bars indicate averages, lines standard deviation.



**Figure 2.** Percentage of feed passed through the digestive tract in dependence of time and feeding level.



growth between diets at *ad libitum* feeding level, the ADLIB model indicated a significantly better  $FC_A$  and  $NPU_A$  with the soya diet (Tables 5 and 6). However, the COMPLETE model attributed this phenomenon to differences in the protein ration and no significant diet effect on  $FC_A$  and  $NPU_A$  (Figures 3d and 3e) was found. While the  $FC_A$  and  $NPU_A$  got worse with increasing feeding level,  $FC_R$  and  $NPU_R$  improved with the feeding level (Table 7).

## DISCUSSION

### Amounts of uneaten feed

The wasteful way of feed consumption of *C. macropomum* was mentioned as a possible cause of decreased feed utilization with *ad libitum* feeding (Van der Meer et al. 1995). Fifteen minutes after feeding, only 71.5% of the chromic oxid in the *ad libitum* feed ration was found in the digestive tract of the fish. This indicates that 28.5% of the feed was not eaten. Significant amounts of feed leftovers were never found in the aquaria. Thus, even when fish are fed carefully and no feed rests can be found in the aquaria, there might be a substantial difference between ration allotment and real intake.

Storebakken & Austreng (1987) found feed losses of 35% or higher at the feeding level required for maximal growth of rainbow trout. Rainbow trout in aquaria seemed to eat only 40-60% of the ration, even after correction for observed uneaten feed (Van Weerd, Verástegui & Tijssen 1995). Thorpe, Talbot, Miles, Rawlings & Keay (1990) found feed losses of 19% in salmon sea cage culture. In balance trials with African catfish '25% of the feed energy was not accounted for either as settleable materials or as energy gained or metabolized' (Hogendoorn 1983). This author hypothesized that feed or faecal energy went into solution. Nijhof (1994), using 'an extruded, very water stable eel diet' found that 30% of the protein and 35% of the lipid of uneaten pellets and faeces could not be found back in the settled sludge. As in many studies feed losses are neglected, underestimation of the amount of uneaten feed might be a generally occurring phenomenon in fish nutrition studies.

Feed losses were eliminated by reducing the feeding level to an approximate 60% of the *ad libitum* feeding level. Therefore, the  $FC_A$  and  $NPU_A$  were best at the lowest feeding level. However,  $FC_R$  and  $NPU_R$  were best in the fast growing fish of the *ad libitum* feeding level (Table 7). This makes sense, since slower growing fish need a higher portion of the available nutrients for maintenance. Van der Meer et al. (1995), estimated a maintenance protein metabolism (this is: the dietary protein requirement to maintain the body protein biomass exactly equal) of 1.279 g protein  $kg^{-0.8} day^{-1}$  for *C. macropomum*. In consequence, the protein expenses for maintenance varied between 13.5 (adlib) and 20.1% of the protein ration (60%al; Table 7). Postulating the protein synthesis as the sum of the protein gain (PG, g/fish) and the protein maintenance metabolism (PM, g/fish), the NPU for protein synthesis ( $NPU_{SYNT}$ ) was computed as:

$$NPU_{SYNT} = \{(PG+PM)/\{(P_{ration} * (100-F_{LOSS})/100)\}\} * 100.$$

**Table 5.** Results of the experiment (averages of 5 aquaria  $\pm$  std)\*.

Feeding level	Fish meal diet			Soya meal diet		
	60%al	80%al	adlib	60%al	80%al	adlib
<b>FEED RATION</b>						
FR	26.4 $\pm$ 0.7	37.0 $\pm$ 0.5	53.3 $\pm$ 7.4	26.2 $\pm$ 1.3	33.6 $\pm$ 2.8	36.9 $\pm$ 5.5
PR	8.3 $\pm$ 0.2	11.6 $\pm$ 0.2	16.8 $\pm$ 2.3	9.2 $\pm$ 0.4	11.8 $\pm$ 1.0	12.9 $\pm$ 1.9
<b>GROWTH AND FEED UTILIZATION</b>						
Wi	0.95 $\pm$ 0.09	1.02 $\pm$ 0.10	0.98 $\pm$ 0.07	1.00 $\pm$ 0.11	0.97 $\pm$ 0.09	0.98 $\pm$ 0.10
Wf	17.3 $\pm$ 1.3	26.6 $\pm$ 2.8	33.2 $\pm$ 7.2	17.3 $\pm$ 1.0	20.6 $\pm$ 2.5	25.9 $\pm$ 4.4
GR	30.5 $\pm$ 1.9	39.0 $\pm$ 1.8	45.6 $\pm$ 7.3	29.8 $\pm$ 2.5	34.0 $\pm$ 3.5	39.1 $\pm$ 5.2
GRp	4.28 $\pm$ 0.37	5.52 $\pm$ 0.46	6.47 $\pm$ 1.08	4.35 $\pm$ 0.41	4.93 $\pm$ 0.51	5.75 $\pm$ 0.54
FC <sub>A</sub>	0.87 $\pm$ 0.06	0.95 $\pm$ 0.05	1.17 $\pm$ 0.07	0.88 $\pm$ 0.05	0.99 $\pm$ 0.05	0.95 $\pm$ 0.08
NPU <sub>A</sub>	0.516 $\pm$ 0.051	0.474 $\pm$ 0.042	0.385 $\pm$ 0.020	0.475 $\pm$ 0.040	0.419 $\pm$ 0.026	0.449 $\pm$ 0.041
<b>BODY COMPOSITION</b>						
moisture	72.9 $\pm$ 0.9	72.2 $\pm$ 1.0	72.0 $\pm$ 0.7	74.1 $\pm$ 0.7	73.5 $\pm$ 0.8	72.7 $\pm$ 0.4
protein	13.8 $\pm$ 0.5	14.0 $\pm$ 0.7	14.1 $\pm$ 0.3	14.4 $\pm$ 0.6	14.3 $\pm$ 0.4	14.6 $\pm$ 0.7
lipids	10.9 $\pm$ 1.0	10.7 $\pm$ 1.0	11.2 $\pm$ 1.7	10.2 $\pm$ 1.0	10.8 $\pm$ 1.4	10.7 $\pm$ 0.5
ash	3.39 $\pm$ 0.33	3.20 $\pm$ 0.15	3.08 $\pm$ 0.19	2.24 $\pm$ 0.15	2.29 $\pm$ 0.20	2.33 $\pm$ 0.12

\* For explanation of feed and growth parameters: see Table 3. Body composition given as percentage of wet weight.

**Table 6.** Mean squares (MS) of the analyses of variance of growth, feed utilization and body composition parameters.

explaining variables df	ADLIB model#)			COMPLETE model##)				
	DIET		Remainder	PR###	DIET		Remainder	
	1		8	1	1		27	
	MS	P	MS	MS	P	MS	P	MS
<b>RATION</b>								
FR ###)	671	***	42.4					
PR	37.5	**	4.6					
<b>GROWTH AND FEED UTILIZATION</b>								
Wf	132	*	35.7	1000	***	47.1	**	6.64
GR	109		40.3	1021	***	36.4	**	7.07
GRp	1.29		0.727	20.7	***	0.150		0.195
FC <sub>A</sub>	0.128	***	0.00540	0.221	***	0.00600		0.00539
NPU <sub>A</sub>	0.0103	**	0.00104	0.0452	***	0.00404		0.00156
<b>BODY COMPOSITION</b>								
moisture	1.52	*	0.308	7.95	***	6.46	***	0.576
protein	0.807		0.329	0.000568		1.76	**	0.299
lipids	0.778		1.56	3.75	*	0.468		1.14
ash	1.39	***	0.0260	0.00543		6.64	***	0.0423

\* =  $P < 0.10$ ; \*\* =  $P < 0.05$ ; \*\*\* =  $P < 0.01$

#) One-way Analysis of Variance, only *ad libitum* fed fish were considered,  $n=10$  (2 diets, each with 5 replicates).

##) Analysis of Covariance with the 2 diets as the treatments and the protein ration as the covariable; all data included,  $n=30$  (2 diets, 3 feeding levels, 5 replicates).

###) For explanation of feed and growth parameters: see Table 3.

**Table 7.** Feed losses and feed utilization parameters.

Feeding level	F <sub>ration</sub> *)	F <sub>LOSS</sub> *)	F <sub>cons</sub> **)	P <sub>MAINT</sub> ***)	Feed utilization parameters*)				
					FC <sub>A</sub>	NPU <sub>A</sub>	FC <sub>R</sub>	NPU <sub>R</sub>	NPU <sub>SYNT</sub> *)
60%al	14.2	0.2	14.2	20.1	0.87	0.50	0.87	0.50	69.8
80%al	21.8	21.2	17.2	16.0	0.97	0.45	0.76	0.57	77.0
adlib	30.6	28.5	21.9	13.5	1.06	0.42	0.76	0.58	77.2

\*) For meaning and calculation of parameters: see Table 3.

\*\*) F<sub>cons</sub> is the feed uptake in g/fish and was calculated as: F<sub>ration</sub> \* (100-F<sub>LOSS</sub>)/100.

\*\*\*) P<sub>MAINT</sub> is the protein maintenance metabolism, expressed as a percentage of the protein ration.

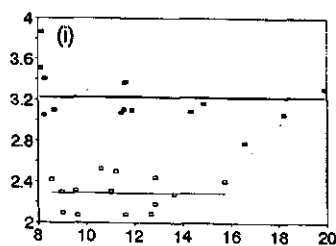
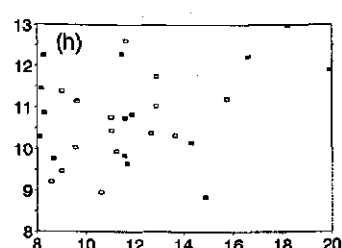
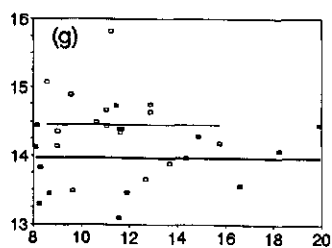
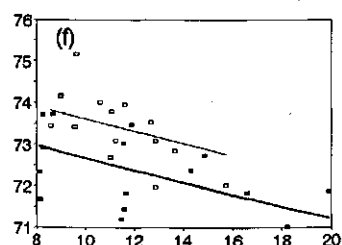
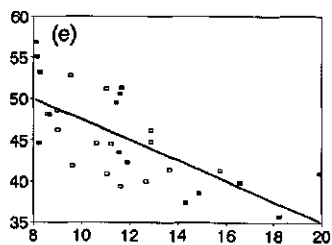
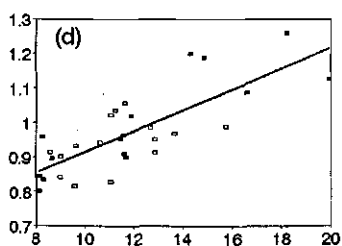
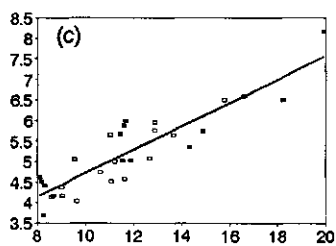
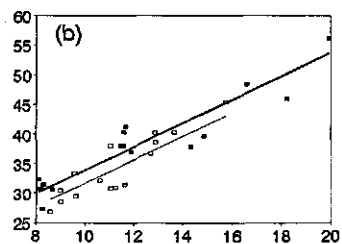
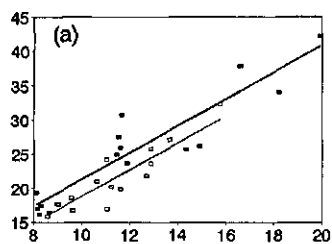
The NPU<sub>SYNT</sub> is the percentage of dietary protein used for protein synthesis, while the Chemical Score represents the percentage of dietary protein available for protein synthesis (Van der Meer & Verdegem 1996). NPU<sub>SYNT</sub> varied between 69.8 (60%al) and 77.2 (adlib; Table 7), which was slightly higher than the average CS of the diets (62 and 75.3 for the soya and the fish meal diet, respectively). This disparity indicates that feed losses or protein maintenance metabolism were somewhat overestimated or that the CS underestimated the amount of dietary protein available for protein synthesis.

The chromic oxide recovery percentages of the 9 h and 13 h feedings were apparently not affected by the feeding level (Figure 2). Hence, the feed passage rate through the digestive tract does not depend on the feeding level. If feed passage rate and digestibility of the feed are (negatively) correlated, as suggested by Henken et al. (1985), digestibility of the feed in *C. macropomum* varies little with the feed uptake level. A substantially decreasing digestibility with increasing feeding level in this experiment is unlikely, as FC<sub>R</sub> and NPU<sub>R</sub> improved with the feeding level (Table 7). We conclude that the decreasing feed utilization (FC<sub>A</sub> and NPU<sub>A</sub>) with increasing feeding level is mainly caused by higher feed losses.

As feed losses increase with feeding level in *C. macropomum* culture, apparent feed utilization (FC<sub>A</sub> and NPU<sub>A</sub>) is bound to decrease when feeding approaches the level necessary for maximal growth. Economically rational aquaculture practices require a balance between optimizing feed utilization and maximizing growth. This implies the application of feeding levels below the *ad libitum* feeding level. Therefore, Cho (1992) argues that restricted feeding levels are needed to attain 'a lasting cohabitation of sustainable aquaculture and a cleaner environment'. However, if feed losses can be reduced to zero, the *ad libitum* feeding level will result in the smallest pollution, because of the better nutrient retention as shown by NPU<sub>R</sub> and FC<sub>R</sub>. Mechanisms reducing feed losses at *ad libitum* feeding could be highly efficient for improvement of the profitability and sustainability of *C. macropomum* culture.

The chromic oxide data suggest that 3.2 hours after feeding 50% of the consumed feed is already evacuated (Figure 2). Although the time feed remains in the digestive tract is short, *C. macropomum* obtains higher feed utilization efficiencies than many other fish species (Günther & Boza 1993). The high feed passage rate stresses the need to sample the digestive tract contents as soon as possible after feeding if feed consumption is to be measured.

The significant effect of SAMPLEDAY on the chromic oxide recovery data suggests that our data were affected by some factor(s) not represented in the ANOVA. Possibly the non-random sampling at days 14 and 29 might have biased our data. Sampling fish by removing the extreme



**Figure 3.** The effect of the protein ration and protein origin on growth, feed utilization and body composition.

Legend: □ soya meal diet; ■ fish meal diet. X-axis: protein ration (PR) in g protein kg<sup>-0.8</sup> day<sup>-1</sup>. Y-axis per graphic:

(a) Wf, final wet weight (g); (b) GR, growth rate (g kg<sup>-0.8</sup> day<sup>-1</sup>); (c) GRp, protein growth (g protein kg<sup>-0.8</sup> day<sup>-1</sup>); (d) FC<sub>A</sub>, feed conversion in g dry feed/g wet weight gain; (e) NPU<sub>A</sub>, ratio of dietary protein retained by the fish. Body composition in percentages of wet weight: (f) moisture; (g) protein; (h) lipid; (i) ash.

weights kept the population of each aquarium homogeneous and (apparently) stress free. If fish weight and feed intake are not linearly related, this procedure might result in biased estimates of the chronic oxide recovery percentages. However, the homogeneous fish weights make it unlikely that this phenomenon was the only factor responsible for the significant SAMPLEDAY effect.

### Soya versus fish meal

The ADLIB model confirmed earlier observations (Van der Meer, Huisman & Verdegem 1996) that the use of soya diets results in reduced appetite (as shown by the lower *ad libitum* feed ration) and a better FC<sub>A</sub> and NPU<sub>A</sub> (Tables 5 and 6). However, the COMPLETE model revealed that differences between diets were not significant (Table 6), while the negative effect of protein ration on FC<sub>A</sub> and NPU<sub>A</sub> (Figures 3d and 3e, respectively) was very significant ( $P < 0.01$ ). Thus, the reduced feed intake of the soya diet was the cause of its better feed utilization at the *ad libitum* feeding level. As equal protein rations result in equal protein growth rates (Figure 3c), there is no reason left to assume that soya meal protein is better available to *C. macropomum* than fish meal protein.

In the present experiment the NPU<sub>A</sub> depended on the protein ration, while protein origin had no significant effect. This demonstrates that the use of the *ad libitum* feeding level is not appropriate to compare the quality of different diets if voluntary feed intake levels are not identical.

### CONCLUSIONS

Although substantial feed rests were never observed, about 25% of the feed ration remained uneaten at the *ad libitum* feeding level. Feed losses can be reduced to almost zero by decreasing the feeding level, but this also decreases growth rates. Studies to improve feed utilization should focus on identifying feeding strategies which reduce feed losses at high feeding levels. Notwithstanding the high feed losses, feed utilization efficiency in *C. macropomum* compares well to those in other fish species. Therefore we think that high feed losses at high feeding levels might be a generally overlooked problem in fish nutrition.

The chronic oxide determination in fish, as described in this article, allows the quantification of feed losses and feed intake. But it also can be used to determine feed passage rates, which might help to understand what happens with the feed, once inside the digestive tract. Further refinement can turn this method into a powerful tool to optimize feeding regimes.

Suitability of soya as the substitute of fish meal as the main protein source of *C.*

*macropomum* diets was confirmed. However, the better protein utilization efficiency with soya diets with *ad libitum* feeding was due to reduced feed intake and not to a superior protein quality. It was demonstrated that conclusions about dietary protein quality based on results of *ad libitum* fed fish might be confusing when the voluntary feed intake between diets differ. The use of *ad libitum* feeding can give valuable information about maximum feed intake and growth with one specific diet. Nevertheless, if comparison of the feed utilization efficiencies of different diets is the aim, an experimental design including fixed, identical feeding levels is required.

## ACKNOWLEDGEMENTS

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## Chapter 3

### THE EFFECT OF PROTEIN RATION ON GROWTH AND PROTEIN UTILIZATION

#### III

#### Effect of number of meals and frequency of feeding on voluntary feed intake of *Colossoma macropomum* (Cuvier)

##### ABSTRACT

Juvenile, 0.87 gram *Colossoma macropomum* were submitted to different *ad libitum* feeding regimes. Feeding frequency ranged from 1 to 5 meals per day and intervals between meals from 3 to 24 hours. Higher feeding frequencies resulted in higher feed uptake, a higher growth rate and a decreased feed utilization efficiency. Feed consumption was higher at 19 h than at 7 h. The amount of feed from different meals persisting in the digestive tract was determined with chromic oxide marked feed. This method revealed that with *ad libitum* feeding about 21% of the feed remained uneaten. Feed losses did not differ significantly between feeding regimes. Feed accumulated in the digestive tract in the morning and early afternoon. In the late afternoon most feed rests disappeared from the digestive tract and feed consumption increased. It is suggested that feed uptake and growth of *C. macropomum* can be improved by extending the feeding period after 19 h.

## INTRODUCTION

Protein growth of *Colossoma macropomum* (Cuvier) increases with protein ration (Van der Meer, Machiels & Verdegem, 1995). However, at high rations the protein utilization efficiency decreases and protein deposition slows down. The main cause of this phenomenon are increased feed losses at increased rations (Van der Meer, Faber, Zamora & Verdegem, 1997). In trout, feed losses are affected by the feeding regime (Boujard, Gelineau & Corraze 1995).

To achieve the highest growth rate, feed uptake has to be maximized (Vahl, 1979). Voluntary feed uptake is generally considered to be determined mainly by stomach fullness. Therefore, models have been elaborated to estimate the daily ration of fish based on stomach contents and stomach evacuation rate (Brett, 1979; Boisclair & Marchand, 1993). The effect of feeding frequency on feed uptake in *C. macropomum* has not yet been determined.

Activities of free-living animals are not equally distributed over the day. However, data from chronobiological research are seldom implemented in aquaculture (Parker 1984; Bergheim, Aabel & Seymour 1991). Nielsen (1984) proposed a model in which light regime and temperature explain daily activity rhythms of animals. In goldfish (Noeske, Erickson & Spieler 1981; Noeske & Spieler 1984) and African catfish (Hogendoorn 1981) the time-of-the-day at which the animals receive feed affected feed uptake and growth.

The objective of this study was the identification of optimal feeding regimes for *C. macropomum*. Effect of time-of-the-day, frequency of feeding and interval between meals on feed uptake and growth of *ad libitum* fed *C. macropomum* is described. The daily rhythm of the fish was studied by recording the feed uptake per meal. It was verified whether feed uptake in *C. macropomum* could be explained by existing feed uptake models.

## MATERIAL AND METHODS

The experiment was carried out in two recirculation systems of sixteen 45-l aquaria each. Both recirculation systems were equipped with a biological filter, a pump and a heating device. Oxygen levels were maintained above 4.5 mg/l, nitrite below 0.05 mg/l and the water temperature averaged 27.9°C. The light regime was 13 hours light/11 hours dark.

Fry of *C. macropomum* were transferred to the aquaria 14 days before the start of the experiment and fed *ad libitum* three times daily. At the start of the experiment fish were graded and animals with extreme weights were sorted out. Each aquarium was stocked with 12 fishes with an average individual weight of 0.87 g. The experiment had a duration of 14 days.

Treatments consisted of 8 feeding regimes (Table 1), applied four fold and identified by their number of daily meals and the number of hours between meals. If a treatment was repeated at different times of the day a 'M' indicates morning, an 'A' afternoon. Each meal had a duration of maximally one hour. Fish were fed in four to six times (depending on the appetite) until apparent, visual satiety was reached. The amount of feed applied (ration) per one hour meal was recorded. This method of feeding is referred to as *ad libitum* feeding.

**Table 1.** The experimental design.

Treatment	Feeding times (h.)				
	7.00	10.00	13.00	16.00	19.00
1/24/M	X				
1/24/A					X
2/6/M	X		X		
2/6/A			X		X
2/12	X				X
3/6	X		X		X
3/3		X	X	X	
5/3	X	X	X	X	X

**Table 2.** Composition of the experimental diet.

Ingredients	%	Proximate analysis	%
Fish meal	39.3	Protein	39.2
Blood meal	8.1	Lipids	9.1
Extruded wheat meal	41.3	Carbohydrate	41.2
Meat protein isolate	4.0	Ash	10.5
Soya oil	2.6		
Fish oil	2.6		
Salt	1.0		
Premix <sup>a)</sup>	1.0		

<sup>a)</sup>premix: "Vitamelk peces A", Roche S.A., a commercially available mineral & vitamin premix for trout. All percentages refer to dry weight.

A 40% protein diet prepared from high quality ingredients (Table 2) was used. Pellets maintained their shape in the water for more than 12 hours. Dust was removed from the feed before feeding. Part of the feed was marked with chromic oxide ( $\text{Cr}_2\text{O}_3$ ). Blood meal was incorporated in the experimental diet to guarantee that marked and normal (unmarked) feed had the same dark brown color.

Chromic oxide marked feed was fed only during the last day (treatments with two or more daily meals) or last two days (treatments 1/24/A and 1/24/M) of the experiment. Of each treatment 2 aquaria, randomly assigned, were fed marked feed during the last daily meal, while the other aquaria were fed marked feed in all meals except the last daily meal (Table 4). Fish fed labelled feed during the last daily meal were sampled 10 to 20 minutes after termination of the meal. All other fish were sampled within 30 minutes after the last meal. After weighing, fish were put on ice and their digestive tracts carefully removed. Digestive tracts were pooled per aquarium. Chromic oxide in feed and digestive tracts was determined as described by Van der Meer et al. (1997). The amount of uneaten feed ('feed losses',  $F_{\text{LOSS}}$ ) was determined on aquaria with fish fed chromic oxide marked feed during the last meal and calculated as:

$F_{\text{LOSS}} = (Cr_{\text{ration}} - Cr_{\text{fish}}) / Cr_{\text{ration}} * 100$ , where:

$Cr_{\text{ration}}$  = ration of last meal (g) \* chromic oxide concentration in the feed (mg  $Cr_2O_3$ /g feed) and  
 $Cr_{\text{fish}}$  = mg of chromic oxide recovered in the digestive tract (mg) within 15 minutes (0.25 h) after finishing the meal.

Chromic oxide recovery data of previous meals were analyzed by adjusting the exponential evacuation model suggested by Van der Meer, Faber, Zamora & Verdegem (1997):

$Cr_{\text{RECOV}} = 100 * e^{-\alpha(t-0.25)}$ , where:

$Cr_{\text{RECOV}}$  = amount of chromic oxide in the digestive tract as a percentage of the total chromic oxide uptake;

$\alpha$  = the instantaneous rate of feed evacuation from the digestive tract;

$t$  = time lapse in hours between feeding and sampling.

The model was fitted to the data by using linear programming for the determination of the  $\alpha$  which minimized:

$\sum (Cr_{\text{RECOV}_i} - Cr_i)^2$ , where:

$Cr_{\text{RECOV}_i}$  = chromic recovery as predicted by the model for treatment  $i$  ( $i = 1/24/M, 1/24/A \dots, 5/3$ ) and

$Cr_i$  = average observed chromic oxide recovery percentage of previous meals of treatment  $i$ .

The effect of feeding regime on feed ration, growth and body dry matter content was analyzed by a one-way analysis of variance. Means were compared by Tukey-test. Effects of feeding frequency and interval between meals on the feeding ration were analyzed by quadratic multiple regression. Also the effect of feeding level on growth and feed conversion was studied by quadratic multiple regression.

Rations per meal and per day were compared with feed uptake trends as predicted by the models of Vahl (1979) and Haylor (1993). The model of Vahl (1979) shows that voluntary feed intake per meal of salmonids increases in a sigmoid pattern with time after being fed to satiation (Figure 1a). This model implies that the daily feed uptake is maximized at one certain interval between meals. In the model of Haylor (1993) voluntary feed uptake of African catfish larvae increases asymptotically after being fed to satiation (Figure 1b). In this model the daily feed uptake increases consistently with decreasing interval between meals.

## RESULTS

### Ration, growth and feed conversion

Higher feeding frequencies resulted in higher daily rations but in smaller rations per meal (Table 3; Figure 2a). Daily rations ranged from 17.3 to 46.8 g  $kg^{-0.8} day^{-1}$  (treatments 1/24/M and 5/3, respectively) and were significant lower in fish fed once (treatments 1/24/M and 1/24/A) than in those fed twice daily (2/6/M, 2/6/A and 2/12). Feeding the fish three times daily increased the daily

rations further if the feeding interval amounted 6 hours (3/6). The highest daily rations were recorded with the 5 meals per day (5/3), but the difference with the daily rations of treatment 3/6 was not significant. The rations per meal tended to increase with increasing interval between meals (Figure 2b). Within treatments with the same feeding frequency, different intervals between meals never resulted in significant differences in rations per meal (Table 3).

Growth rates fluctuated between 27.6 and 65.6 g kg<sup>-0.8</sup> day<sup>-1</sup> (treatments 1/24/M and 5/3, respectively; Table 3) and showed the same trends as the daily feed ration. The coefficient of variation of the final weights of the fish (CV) tended to decrease with increasing feeding frequency (Table 3). The CV of fish from treatment 1/24/M (0.25) was significantly higher than those fed two or more times per day (0.13 - 0.19). The dry matter content of the fish fluctuated between 23.4 and 24.4% of the wet body weight and was not significantly different between treatments (Table 3).

The feed conversion of fish fed once, twice or three times daily varied between 0.57 and 0.64 (g dry feed/g wet weight gain) and did not differ significantly from each other (Table 3). Fish fed five times daily had a higher feed conversion (0.71;  $P < 0.05$ ) which differed significantly from all other treatments except treatment 3/6.

The positive effect of an increased ration on growth declined at high rations and the feed conversion attained its optimum (minimum value) at 27 g feed kg<sup>-0.8</sup> day<sup>-1</sup> (Figure 3).

**Table 3.** Feed ration and growth data

Treat- ment*)	# of rep	Wf**)	C.V.***)	Growth g/kg <sup>0.8</sup> /day	Rday g/kg <sup>0.8</sup> /day	Rmeal g/kg <sup>0.8</sup> /meal	FC g/g	% body dry matter
1/24/M	4	3.19 a	0.25 c	27.6a	17.3 a	17.3 d	0.63 a	23.5 a
1/24/A	4	4.14 a	0.20 bc	35.1a	21.1 a	21.1 e	0.60 a	24.2 a
2/6/M	4	7.04 b	0.17 ab	53.5 b	30.5 b	15.3 cd	0.57 a	23.4 a
2/6/A	4	6.80 b	0.19 b	52.1 b	31.6 b	15.8 cd	0.60 a	23.7 a
2/12	4	6.66 b	0.18 ab	51.3 b	31.9 b	16.0 cd	0.62 a	24.4 a
3/6	4	8.93 cd	0.13 a	63.7 c	40.6 cd	13.5 bc	0.64 ab	24.2 a
3/3	4	7.55 bc	0.15 ab	56.4 bc	34.7 bc	11.6ab	0.62 a	23.4 a
5/3	4	9.33 d	0.15 ab	65.6 c	46.8 d	9.4 a	0.71 b	24.0 a

Means, within each experiment, were compared by Tukey-test; means which share the same subscript letter are not significantly different ( $P < 0.05$ ).

Rday, Rmeal = daily ration and ration per meal, respectively.

FC = feed conversion (= ration in g dry feed per fish/average wet body weight gain).

\*) treatments are indicated by their number of daily meals (first number)/number of hours between meals (second number); M = morning, A = afternoon; see Table 1.

\*\*) Wf = final wet weight; initial fresh weight: 0.87 gram; duration of the experiment: 14 days.

\*\*\*) C.V.: coefficient of variation of final wet weight (= standard deviation/average weight).

**Figure 1.** Prediction of the *ad libitum* feed uptake at different feeding regimes by two theoretical models.

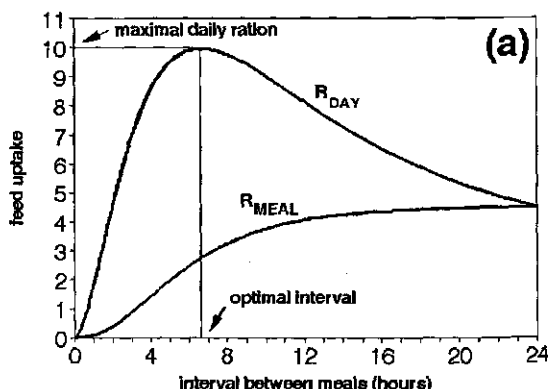
In this figure the rations per day and meal are expressed in % of fresh body weight and denominated  $R_{DAY}$  and  $R_{MEAL}$  (instead of  $R_{day}$  and  $R_{meal}$ , the metabolic rations as used in Figure 2). Note that the rate of gastric evacuation in both models is 0.284. However, the model for the prey eating trout (Figure 1a) requires a much higher maximal  $R_{MEAL}$  as the more constant eating catfish (Figure 1b) to attain the same maximal  $R_{DAY}$ . This figure is based on the papers of Vahl (1979) and Haylor (1993) and does not refer to own experimental data.

**The model of Vahl (1979):**

$$R_{DAY} = \{24/t * S_0 * (1 - \exp(-kt))\} * \{(1 - 2 * \exp(-k(t+a)))^2\},$$

where:  $R_{MEAL}$  = maximum voluntary feed intake at time  $t$ ;  $S_0$  = maximal feed uptake;  $t$  = time after ingesting a maximum meal;  $k$  = instantaneous rate of gastric evacuation;  $a$  = time lag (h) between ingesting a meal and the time at which blood metabolite levels reach its maximum.

$R_{MEAL}$  was calculated as  $R_{DAY} * t/24$ . The figure shows the curves calculated with the model parameters  $S_0$ ,  $a$  and  $k$  set to 4.50%, 2.5 h and 0.284, respectively.

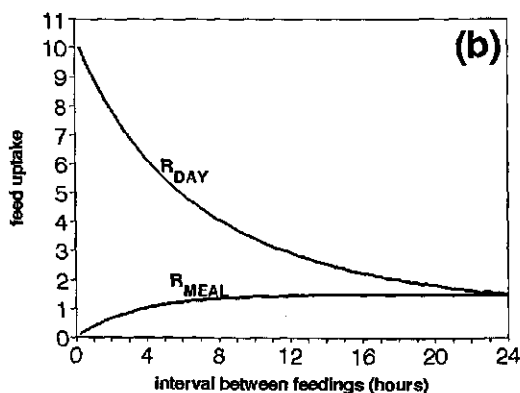


**b The model of Haylor (1993):**

$$R_{DAY} = \{S_0 * (1 - \exp(-(24 - (n-1)*t)*k))\} + \{(n-1)*S_0 * (1 - \exp(-k*t))\} = \{R1\} + \{\sum (R2 \dots n)\},$$

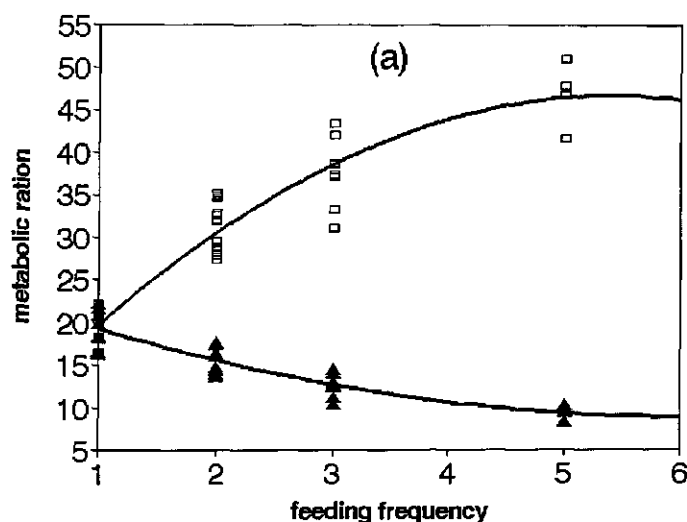
where:  $n$  = number of daily meals;  $R1$  = ration of first daily meal;  $\sum (R2 \dots n)$  = the sum of the rations of subsequent meals. For other parameters and calculation of  $R_{MEAL}$ : see legend Figure 1a.

This model allows for evenly spaced meals during only a part  $(24 - (n-1)*t)$  hours) of the day. When meals are evenly spaced during the whole day (thus:  $n*t=24$ ), then  $R_{DAY} = \{S_0 * (1 - \exp(-(24 - n*t + 1)*k))\} + \{(n-1)*S_0 * (1 - \exp(-k*t))\} = \{S_0 * (1 - \exp(-k*t))\} + \{(n-1)*S_0 * (1 - \exp(-k*t))\} = n*S_0 * (1 - \exp(-k*t))$ . Figure 1b shows the rations per meal and per day if meals are equally distributed over the day (as is required for the model depicted in Figure 1a) and with  $S_0$  and  $k$  set to 1.52% and 0.284, respectively.



**Figure 2.** Effect of feeding frequency (a) and interval between meals (b) on ration per meal ( $R_{\text{meal}}$ ) and ration per day ( $R_{\text{day}}$ ).

□ =  $R_{\text{day}}$ ; ▲ =  $R_{\text{meal}}$ ; feeding frequency (ff) in number of meals per day; interval between meals (t) in hours. Rations were not corrected for feed losses.

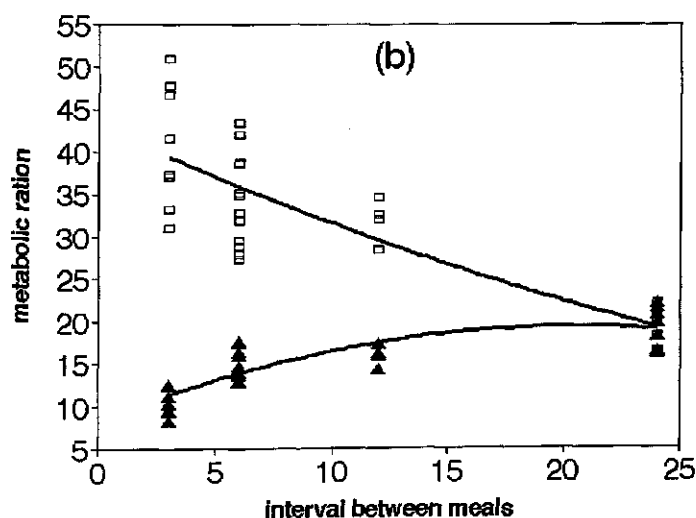


$$R_{\text{day}} = 5.98 + 15.06 \cdot \text{ff} - 1.392 \cdot \text{ff}^2;$$

$$r^2 = 0.89; P < 0.001.$$

$$R_{\text{meal}} = 23.71 - 4.87 \cdot \text{ff} + 0.398 \cdot \text{ff}^2;$$

$$r^2 = 0.72; P < 0.001.$$



$$R_{\text{day}} = 43.10 + 1.28 \cdot t - 0.0120 \cdot t^2;$$

$$r^2 = 0.70; P < 0.001.$$

$$R_{\text{meal}} = 8.35 + 1.07 \cdot t - 0.0261 \cdot t^2;$$

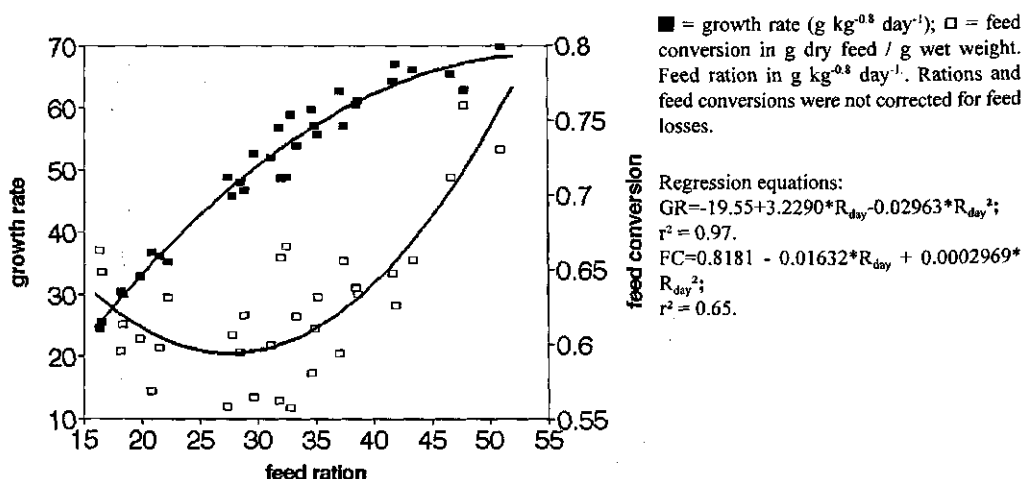
$$r^2 = 0.72; P < 0.001.$$

### Time-of-the-day effect on feed uptake

The ration per meal tended to increase during the day (Figure 4). Except treatment 3/3, all treatments showed the highest average ration at the last daily meal.

Fish fed once daily had a significant higher ration per meal when fed in the afternoon (Table 3) and also showed a notable higher growth rate (+ 27%) and a better feed conversion (- 5%), but those differences were not significant. No significant differences in feed ration, growth, feed conversion or body dry matter content were found between feeding in the morning or afternoon in fish fed twice daily (treatment 2/6/M versus 2/6/A; Table 3).

**Figure 3.** Relationship of growth rate and feed conversion with feed ration.



### Recovery of chromic oxide from the digestive tract

On average 79% of the feed chromic oxide was recovered in the digestive tract 15 minutes after feeding. This percentage varied between 71.6 and 88.9%, but did not differ significantly between treatments (Table 4).

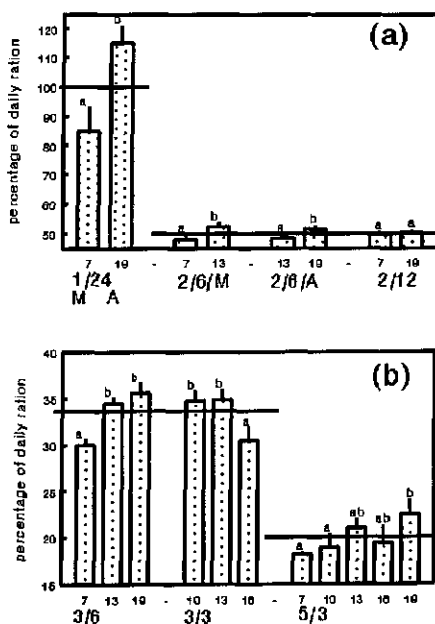
Feed rests of previous meals amounted generally less than 10% of the feed ration. No significant differences were found in percentage of recovered feed after 24 hours (treatments 1/24/M and 1/24/A), after 12 hours (2/12), after 6 hours (3/6 and 2/6/A) and even after 3 hours (5/3). However, in treatments 2/6/M and 3/3 the amount of recovered feed was significantly higher (39.6 and 27.4%, respectively) than in all other treatments, except 2/6/A.

The model which fitted best to the chromic oxide recovery data of the previous meals was:  
 $\text{C}_{\text{I RECOV}} = 100 \cdot e^{-0.267 \cdot (t-0.25)}$

From  $\alpha = 0.267$  it follows that 50% of the feed consumed (calculated as 79% of the ration) was

removed from the digestive tract 2 h and 51 min after feeding. The model underestimated feed rests in the digestive tract when fish were fed once daily (treatments 1/24/A and 1/24/M, Figure 5). Except treatment 2/12, recovery of chromic oxide in all treatments sampled at 19 h was underestimated. The most prominent difference between model output and observation was the much higher amount of chromic oxide recovered from the fish at the 13 h sampling (treatment 2/6/M) than expected from the model (Figure 5).

Figure 4. Distribution of the daily rations over the meals.



On the X-axis are indicated the times when fish were fed (7, 10, 13, 16 or 19 h). Indicated on the Y-axis are the rations per meal, expressed as a percentage (bars) + standard deviation (vertical lines) of the daily ration. The horizontal lines indicate the 'equal meal percentage' (being 100 / number of daily meals). Meals of the same treatment not sharing the same letter do not significantly differ (Tukey test,  $P < 0.05$ ). Meals of treatment 1/24/M and 1/24/A were expressed as a percentage of the average feed uptake of both treatments. They do not share the same letter as their averages were significantly different (Tukey test,  $P < 0.05$ ).

## DISCUSSION

### Growth of the fish

The maximum growth rate of  $65.6 \text{ g kg}^{-0.8} \text{ day}^{-1}$  was similar to results obtained with *C. macropomum* fed *ad libitum* 40–45% protein diets (Günther and Boza, 1993; Van der Meer, Machiels & Verdegem, 1995; Van der Meer, Huisman & Verdegem, 1996). Blood meal is suspected to hamper growth (Eckmann 1987; Van der Meer et al. 1995). Although our experimental diet contained a rather high level of blood meal (8% of the feed dry matter), this did not inhibit high growth rates.

The statement of Vahl (1979) that 'maximal growth requires maximal feed uptake' showed to be valid also for *C. macropomum* (Figure 3). As growth rate is mainly determined by the feed ration, voluntary feed uptake is a good first indicator of the appropriateness of a feeding regime.

**Table 4.** Recovery of chromic oxide marked feed.

treat ment	rep	Feeding times								% of chromic oxide recovered $\pm$ std	
		day before		sampling day						last meal	previous meals
1/24/M	2	x		0							3.0 $\pm$ 0.7 a
	2	0		x						77.0 $\pm$ 13.3 a	
1/24/A	2		x					0			4.8 $\pm$ 0.3 a
	2		0						x	73.8 $\pm$ 1.8 a	
2/6/M	2			x		0					39.6 $\pm$ 12.8 c
	2			0		x				71.6 $\pm$ 1.4 a	
2/6/A	2					x		0			9.2 $\pm$ 0.7 ab
	2					0		x		81.5 $\pm$ 0.2 a	
2/12	2			x				0			3.0 $\pm$ 0.5 a
	2			0				x		80.3 $\pm$ 13.6 a	
3/6	2			x		x		0			4.8 $\pm$ 0.7 a
	2			0		0		x		75.0 $\pm$ 4.2 a	
3/3	2				x	x	0				27.4 $\pm$ 1.9 bc
	2				0	0	x			88.9 $\pm$ 3.7 a	
5/3	2			x	x	x	x	0			4.7 $\pm$ 2.7 a
	2			0	0	0	0	x		85.7 $\pm$ 5.1 a	
Average										79.2 $\pm$ 7.6	

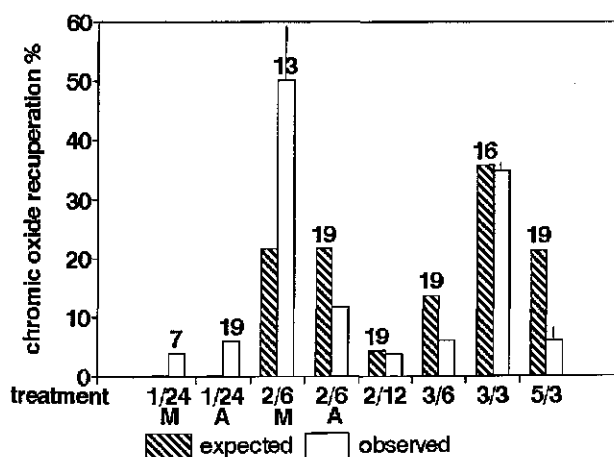
x = labeled feed; 0 = normal feed. Averages of an experiment in one column do not significantly differ from each other when they share the same letter (Tukey-test,  $P < 0.05$ ).

### Feeding frequency

Higher feeding frequencies resulted in higher daily rations, while rations per meal decreased. Ishiwata (1969) found the same phenomenon in several other fish species. The regression analysis of ration on feeding frequency (Figure 2a) suggests that juvenile *C. macropomum* need at least 5 meals per day to maximize feed uptake. This is more frequently than for striped bass (4 times/day; Powell 1972, cited by Charles, Sebastian, Raj & Marian 1984), *Epinephelus tauvina* (3 times/day; Chua & Teng 1978), *Cyprinus carpio* (3 times/day; Charles et al. 1984), *Ictalurus punctatus* (2 times/day; Andrews & Page 1975), *Oncorhynchus mykiss* (2 times/day; Grayton & Beamish 1977), *Salvelinus alpinus* (1 or more times; Jobling 1983), *Channa striatus* (1 time/day; Sampath 1984) and *Heteropneustes fossilis* (1 time/day; Marian, Ponniah, Pitchairay & Narayanan 1982) and *Anguilla anguilla* (3 to 4 times/day (Seymour 1989). The high feeding frequency required by *C. macropomum* is possibly due to the high amount of feed needed to sustain the high growth rates.

A higher feeding frequency resulted in reduced coefficients of variation of the final wet weight (CV's; Table 3). Possibly the higher feeding frequency not only increased feed uptake but also decreased competition between fishes of the same aquarium. Decreased CV's with increasing feeding frequency were also reported in *Salvelinus alpinus* (Jobling 1983).

Figure 5. Observed chromic oxide recovery data and those expected under the assumption of a constant feed evacuation rate.



Vertical lines above bars indicate the standard deviation of the observed values. The numbers above the bars indicate the time of the day at which the chromic oxide samples were taken.

The model used to calculate expected chromic oxide recovery data:

$$\text{chromic oxide recovery} = 100 * e^{-(\alpha * 0.25)}$$

The  $\alpha$  used to predict the expected values of all treatments was 0.267. This model expresses the chromic oxide recovery as a percentage of the feed uptake. Feed uptake was calculated by assuming that 21% of the feed ration remained uneaten. Hence, observed chromic oxide recovery data of this figure were calculated as the % of chromic oxide recovered of previous meals, as given in Table 4, multiplied by  $100/(100-21)$ . See text for further explanation

In several experiments in which fish were fed *ad libitum*, the feeding frequency resulting in the highest daily feed uptake also produced the lowest feed conversion (Marian et al. 1982; Sampath 1984; Charles et al. 1984). However, varying the ration through different feeding frequencies in *C. macropomum*, best feed conversions were found with treatments in which feed uptake was significantly below the maximum feed uptake (Figure 3). The positive effect of a higher feeding frequency on feed utilization is either absent in *C. macropomum*, or overruled by the decreasing feed utilization efficiency with increasing feed uptake (Günther & Boza 1993). Increasing the daily feeding frequency from 3 (treatment 3/6) to 5 (treatment 5/3), growth improved 3% (from 63.7 to 65.6 g kg<sup>-0.8</sup> day<sup>-1</sup>) but feed conversion deteriorated 10% (from 0.64 to 0.71). Therefore, when using feeding regimes with 12 (or less) hours between the first and last meal, it might be not worthwhile to feed more than three times daily.

Hogendoorn (1981) found that *Clarias gariepinus* needs continuous feeding to reach its maximal growth rate. In the present experiment *C. macropomum* maximized both feed uptake and growth with the highest feeding frequency. Possibly also *C. macropomum* consumes more feed and grows best when fed continuously. Figure 3 suggests that increasing the feed uptake above 50 g kg<sup>-0.8</sup> day<sup>-1</sup> hardly improves growth, while the feed conversion deteriorates. However, this relation was obtained by feeding the fish in meals and not by continuous feeding. Boujard et al. (1995) found that in trout a change from meal feeding to feeding continuously significantly deteriorated the feed conversion. We expect that in *C. macropomum* continuous feeding, compared to meal feeding, results in an increased feed uptake, a better growth rate and a less favourable feed conversion. Further experimentation is needed.

### Feed losses

The average feed losses in the present experiment (21%) were slightly lower than the 28% estimated in *ad libitum* fed fish in a former experiment (Van der Meer, Faber, Zamora & Verdegem, 1997). As feeding practices were identical, the somewhat lower losses in the present experiment are probably due to differences in feed characteristics, e.g. palatability, water stability etc.

Feed conversions ranged from 0.60 to 0.71 (Table 3) and compared favorably with those reported before in *ad libitum* fed *C. macropomum* (Günther and Boza, 1993; Van der Meer, Machiels & Verdegem, 1995; Van der Meer, Huisman & Verdegem, 1996). This is possibly due to the high quality ingredients, compared to the local products used in former experiments. However, correction of the ration for uneaten feed improves the feed conversions further to the range of 0.41 - 0.61. This means that in the present experiment 1 g of feed dry matter was converted in 0.58 - 0.40 g of body dry matter. These values show that both feed quality and efficiency at which *C. macropomum* converted feed into body material were excellent. It also implies that uneaten feed is a major factor compromising feed utilization efficiency. It seems worthwhile to study the feeding habits of *C. macropomum* in more detail.

### Feed passage rate

A short average half-life time of the feed in the digestive tract of *C. macropomum* was confirmed (Van der Meer, Faber, Zamora & Verdegem (1997): 3 h 13 min; this experiment: 2 h 51 min). Possibly, adaptations of *C. macropomum* to its natural diet of terrestrial seeds and fruits (Saint-Paul 1986) make this fish digest compounded feeds not only efficiently, but also fast.

An exponential evacuation rate of the digestive tract fitted well to feed rests of previous meals found in the digestive tract of *C. macropomum* (Van der Meer, Faber, Zamora & Verdegem 1997). This model was also fitted to the data of the present experiment. Differences between observed digestive tract contents and those predicted by the model (Figure 5) indicate that the model has at least two shortcomings. Firstly, it ignores time-of-the day effects on feed passage rate. The model assumes a constant evacuation rate during the whole day and this resulted in underestimation of digestive tract contents in the 13 h sample and overestimation in the 19 h samples. Apparently, the feed evacuation rate is not constant, but increases during the day. Secondly, the constant evacuation rate underestimated all recovery data of samples of the fish fed once daily (treatments 1/24/M and 1/24/A). These treatments have the longest time between consumption of marked feed and sampling. The reason for this phenomenon might be that the feed evacuation rate slows down more than proportionally when the digestive tract empties.

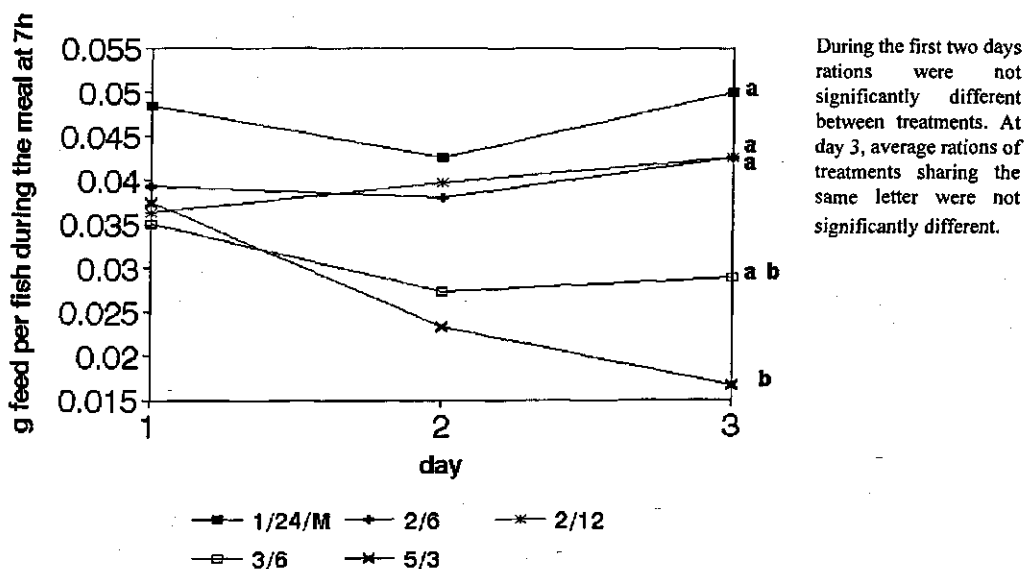
### Time-of-the-day effect on feed uptake

The tendency of increasing rations per meal during the day, as found here in *C. macropomum*, was also reported in marine fish species (Ishiwata 1969). In the present experiment the only exceptions from the rule that rations increased during the day were the 13 and 16 h meals of treatments 5/3 and 3/3, respectively. The more than 25% recovery of chromic oxide of previous meals in fish sampled at 13 or 16 h (treatments 2/6/A and 3/3) show that the feed evacuation rate was reduced in the

morning. Accumulation of feed in the digestive tract could be the cause of decreased rations at 13 and 16 h. The less than 10% of feed recovered of previous meals at 19 h in all treatments indicate that the feed evacuation rate increases after 16 h.

Getachew (1989) found that in *Oreochromis niloticus* stomach fullness increased and stomach pH decreased during the day. He suggests that in *O. niloticus* the digestive processes might be more efficient in the afternoon. While Cho (1992) recommends to feed trout at 'evenly spaced intervals', we think that an optimal feeding regime for *C. macropomum* (and *Oreochromis niloticus*) requires feeding more frequently in the afternoon than in the morning.

Figure 6. Average ration per treatment at 7 o'clock in the morning during the first 3 days of the experiment.



Feed accumulation in the digestive tract (stomach) might be a likely limiting factor when intervals between meals are short. However, even after 5 meals within 12 hours (treatment 5/3) feed uptake did not reduce during the last meal. Hence, at 7 h when fish had been deprived from feed for at least 12 hours, stomach fullness was unlikely to affect negatively feed uptake, irrespective the feeding regime of the previous day. However, already 3 days after the start of the experiment the rations per meal at 7 h of the treatments receiving the highest daily rations were significantly below those receiving a lower daily ration (Figure 6). Vahl (1979) hypothesized that feed uptake depends not only on stomach filling, but also on blood metabolite levels. Both stomach filling and blood metabolite levels are functions of the amount of feed consumed. However, the limiting effect of metabolite levels on feed uptake emerges only some time after feed uptake (Vahl, 1979). Therefore,

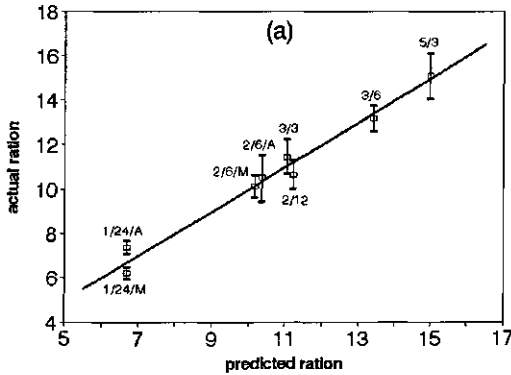
feed uptake at 7 h might be affected by a retarded effect, possibly through blood metabolite levels, of the amount of feed consumed the previous day.

Treatments 1/24/M and 1/24/A only differed in time-of-the-day when fed. Therefore, stomach filling and blood metabolite levels at feeding were expected to be equal in both treatments. The significant higher ration per meal of the fish fed in the afternoon proof that appetite is affected by a time-of-the-day effect. Thus, feed uptake is not only a function of ration and interval between meals (Vahl 1979; Haylor 1993), but also of time-of-the-day at feeding. Nocturnal feeding of *Piaractus brachipomus* (a species very closely related to *Colossoma macropomum*) resulted in higher growth rates and better feed conversions than diurnal feeding (Baras, M  lard, Grignard & Thoreau 1996). The increasing appetite of *C. macropomum* when the night approaches might point to a similar feed uptake preference as *P. brachipomus*. We suggest that when maximal feed uptake is the target, the feeding regime of *C. macropomum* should be extended into the evening and possibly the night.

### Feed uptake models

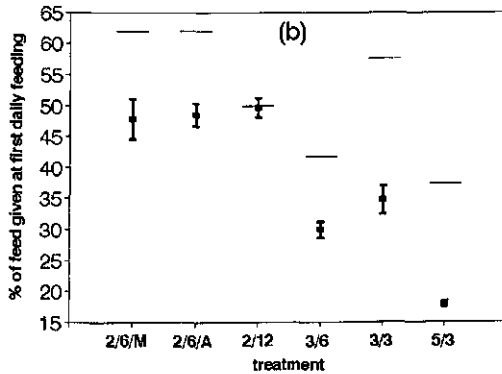
The model of Haylor (1993) was elaborated for African catfish larvae and is based on the assumption that fish eat until their stomach is completely filled. The relation between intervals between meals and *ad libitum* feed ration (Figure 2b) shows a similar pattern as expected from the model of Haylor (1993; Figure 1b). Indeed, assuming a maximum stomach capacity ( $S_0$ ) of 6.95% of the body weight and a gastric evacuation rate ( $k$ ) of 0.137, the daily feed uptake of *C. macropomum* fitted very well to those predicted by the model (Figure 7a). Differences between these values and those obtained by Haylor (1993) with *Clarias gariepinus* larvae ( $S_0 = 21\%$  and  $k = 0.107$ ), might reflect differences in fish size and species.

A fixed relationship between deprivation time, stomach emptying and feed uptake is the basis of the model of Haylor (1993). With long deprivation times during the night and relative short intervals between meals during the day (e.g. treatments 3/3, 3/6 and 5/3) the stomach content is bound to increase during the day. Only in the theoretical case that  $k \rightarrow \infty$  (i.e. the time feed remains in the stomach is zero), the stomach content is equal (zero) at the onset of all meals. Consequently, feed uptake decreases during the day (if  $k < \infty$ ) or remains equal (if  $k = \infty$ ). In fact, reduction of feed uptake by stomach fullness might have never occurred in this experiment due to the high feed passage rate (possibly with the exceptions of the 13 and 16 h meals). Therefore, almost equal rations at all daily meals of one treatment would not have been an unlikely outcome of this study. However, increasing feed uptakes during the day (as observed in treatments 3/6 and 5/3; Figure 4) can never occur according to the model of Haylor (1993). Haylor (1993) explicitly mentions that the model has the potential to predict the voluntary feed intake of individual meals. However, using the 'best fit'  $S_0$  and  $k$  ( $S_0 = 6.95\%$  and  $k = 0.137$ ), almost all feed uptake prediction from the model fall largely outside the 95% confidence limits of the actual feed uptakes per meal (Figure 7b; only feed uptake of first daily meals per treatment were plotted). Therefore, it seems that the model does not represent the main processes determining feed uptake in *C. macropomum* and is not valid under culture conditions as described in this paper.

**Figure 7.** The model of Haylor (1993) fitted to feed uptake data.

a Daily rations.

Predicted (X-axis) and actual (Y-axis) daily ration. Predictions were made with the model of Haylor (1993); see legend Figure 1b. Assumed values of  $S_0$  and  $k$  (6.95% and 0.137, respectively) are based on best fit. Open blocks indicate averages per treatment and bars confidence intervals.



b First meals of the day.

Relationship between actual and predicted feed uptake of the first daily meal. Predictions made with the model of Haylor with values of  $S_0$  and  $k$  as in Figure 8a. Closed blocks indicate the averages per treatment, bars the confidence intervals and horizontal lines the predictions of the model.

The model of Vahl (1979) was developed for salmonids and is based on the assumption that not only a full stomach, but also high blood metabolite levels might reduce feed uptake. As discussed above, the negative correlation between daily feed uptake and feed uptake the next morning (Figure 6) may suggest that high blood metabolite levels can diminish feed uptake in *C. macropomum*. Validity of the model would imply that there is one distinct feeding frequency at which daily feed uptake is maximized. Our data suggest that if there is any distinct interval between meals in *C. macropomum* which maximizes the daily feed uptake, this is less than 3 hours (see Figure 2b). At small intervals between meals the model of Vahl (1979) might not be valid. In the most extreme case of a zero interval (i.e. continuous feeding), the predicted daily feed uptake is zero. This is obviously not the case in practical situations. Therefore, the model of Vahl (1979) is not an appropriate tool to estimate the optimal feeding frequency in *C. macropomum*.

## CONCLUSION

An optimal feeding regime for *C. macropomum* should not only be characterized by a number of daily meals, but also by the times-of-the-day when feeding. Feed uptake of *C. macropomum* increases during the day. This phenomenon is probably related to a lower feed passage rate in the morning and early afternoon. Our data suggest that a maximal feed uptake might require meals after 17 h. Possibly *C. macropomum* requires continuous feeding to maximize feed uptake and growth.

Feed losses were identified as the most important factor affecting feed utilization. At the present state of the art, efforts to optimize culture practices of *C. macropomum* should concentrate on limiting the amount of uneaten feed. The feed uptake models used here are not appropriate for *C. macropomum* and mechanisms determining feed uptake in *C. macropomum* are still to be identified. A major failure of the feed uptake and feed evacuation models is that they do not account for time-of-the-day effects. As in *C. macropomum* the average time the feed remains in the digestive tract is short (less than 3 hours), the time-of-the-day effect on feed uptake and feed evacuation is not only present but also well expressed.

## ACKNOWLEDGEMENTS

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# Chapter 4

## THE EFFECT OF DIETARY ENERGY ON GROWTH AND PROTEIN UTILIZATION

### Effect of dietary lipid level on protein utilization and the size and proximate composition of body compartments of *Colossoma macropomum* (Cuvier)

#### ABSTRACT

Juvenile *Colossoma macropomum* were fed *ad libitum* diets containing either 30 or 40% protein, while the dietary lipid level varied between 5 and 20%. Growth and protein utilization efficiency increased with the dietary lipid level. However, the economical feasibility of the addition of extra dietary lipid to *C. macropomum* diets is questionable, as for every extra gram of protein deposition it was necessary to replace 20 to 25 gram dietary carbohydrates by lipids. Moreover, increased dietary lipid levels resulted in increased lipid deposition. In *C. macropomum* feed uptake was regulated by the dietary protein level and not affected by the dietary lipid level. Dissection of the body in head, viscera and trunk revealed that 45 - 48% of the body lipids were stored in the trunk, independent of diet composition. A positive relation between dietary and body protein level was confirmed in this experiment. However, the dissection of the body revealed that the body protein concentrations in head+viscera and trunk are not changed significantly by the dietary protein level. The higher protein contents of fish fed higher protein diets are due to an increase in the relative weight of the trunk (muscle) to the total body weight.

## INTRODUCTION

High growth rates of *Colossoma macropomum* obtained under laboratory conditions (Günther and Boza 1993; Van der Meer, Machiels & Verdegem 1995) suggest that this species might be a suitable candidate for production under intensive aquaculture conditions. Using fish meal as the main dietary protein source, maximal growth rates required a 42-46% protein diet (Van der Meer et al. 1995). Van der Meer & Verdegem (1996) suggested that several alternative ingredients could substitute most of the fish meal. Experimental data confirmed this for soya meal (Van der Meer, Huisman & Verdegem 1996). However, many alternative ingredients have lower protein concentrations than fish meal and soya meal (Van der Meer & Verdegem, 1996). Hence, diets with high amounts of alternative ingredients will have lower protein levels.

To reduce feeding costs in aquaculture, possibilities to reduce dietary protein levels have been studied extensively. Most studies concentrate on increasing dietary energy levels, or lowering the protein to energy ratio (P/E ratio). At low P/E ratios the use of dietary protein is limited to growth and maintenance of body protein, while at high P/E ratios some protein can be used for energy needs or stored as fat (Winfree and Stickney 1981). Hence, increased dietary lipid levels generally increase the efficiency of the conversion of dietary protein into body protein (net protein utilization, NPU). This so called 'protein sparing action' (PSA) of dietary lipid has, to our knowledge, not yet been studied in *C. macropomum*. Van der Meer et al. (1995) found that maximal growth rates of *C. macropomum* required a dietary P/E ratio of 27 mg protein kJ<sup>-1</sup>. However, optimal protein utilization (NPU) was obtained using a diet with a P/E ratio of 15 mg protein kJ<sup>-1</sup>, while at higher P/E ratios the NPU decreased. If a PSA effect in *C. macropomum* exists, improvement of the NPU is expected when extra energy is added to diets with a PE ratio above 15 mg protein kJ<sup>-1</sup>. However, extra dietary energy normally not only improves the NPU, but also increases the body lipid deposition.

The subject of the present study was the relationship between protein and lipid deposition at different dietary P/E ratios. Our first hypothesis assumed PSA of dietary lipids in *C. macropomum* diets. The second, alternative hypothesis, assumed that protein and energy metabolism do not interfere with each other. This second hypothesis implies that differences in protein deposition are caused by variations in protein ration and differences in non-protein deposition by variations in the non-protein ration.

## MATERIAL AND METHODS

### Fish and experimental facilities.

The experiment was performed in 2 recirculation units of sixteen 45-l aquaria. Each unit was equipped with a heating device, an aerator, a sedimentation tank and a biological filter. In both units a continuous exchange flow of about 0.4 l/min of tap water was maintained.

Juvenile *C. macropomum* were obtained through artificial reproduction. One week before the start of the experiment 20 fish were put in each aquarium. The day before the start of the experiment the five biggest and the five smallest animals of each aquarium were removed. A similar procedure was followed at day 14 to reduce fish density from 10 to 4 fish per aquarium. Average initial weight

of the fish ranged between 1.11 and 1.14 grams (Table 3). The experiment had a duration of 4 weeks. Fish were individually weighed at days 0, 14 and 28.

### Feeds and feeding

The eight experimental diets differed in protein level (30 and 40%) and lipid level (5, 10, 15 and 20%). Diets are referred to by two numbers separated by a '/', the first number being the designed dietary protein percentage, the second number the designed lipid percentage (Table 1). The protein source in all diets was a mix of fish meal (15% of the dry matter), blood meal (1%) and soya meal (84%). The lipid source was a mix of equal amounts of fish oil and soya oil. Salt (1%), a vitamin/mineral premix (1%) and variable amounts of corn starch were used to complete the diets.

Fish were fed to apparent satiation (*ad libitum*) at 8, 12 and 16 h. Each meal lasted maximally one hour. Rations per aquarium were recorded daily. Each diet (treatment) was applied in four aquaria (replicates), two in each recirculation unit.

**Table 1.** Ingredients and chemical composition of experimental diets in % of dry weight.

DIET	30/05	30/10	30/15	30/20	40/05	40/10	40/15	40/20
INGREDIENTS*)								
Soya meal	49.1	49.1	49.1	49.1	64.8	64.8	64.8	64.8
Fish meal	8.8	8.8	8.8	8.8	11.6	11.6	11.6	11.6
Blood meal	0.6	0.6	0.6	0.6	0.8	0.8	0.8	0.8
Starch	36.9	31.8	26.7	21.6	18.9	13.8	8.7	3.6
Soya oil	1.3	3.9	6.4	9.0	1.0	3.6	6.1	8.7
Fish oil	1.3	3.8	6.4	8.9	1.0	3.5	6.1	8.6
Salt	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Premix	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
PROXIMATE ANALYSIS								
protein	31.6	30.5	30.4	30.1	40.4	40.5	39.1	39.0
lipid	4.1	9.4	14.9	20.5	4.8	10.0	15.1	20.4
carbohydrates**)	55.3	52.4	46.0	40.1	42.9	38.4	35.2	28.7
fibre	2.0	2.0	2.4	2.5	3.7	3.1	2.5	3.4
ash	7.0	5.7	6.3	6.8	8.2	8.0	8.1	8.5
energy (kJ/g)***)	18.5	19.8	21.0	22.1	18.7	20.1	21.2	22.2
P/E ratio***)	17.0	15.3	14.5	13.6	21.5	20.2	18.4	17.6
CS****)	63.0	63.0	63.0	63.1	63.1	63.1	63.1	63.1

\*) Soya meal: 51% CP (crude protein), 2.4% EE (lipids, determined as the ether extract); Fish meal: 62% CP, 11.6 EE; Blood meal: 81% CP, 9.7% EE; Starch: 0.7% CP, 0.4% EE; Premix: 'Vitamelk peces A', Roche S.A., a commercial available mineral & vitamin premix for trout.

\*\*) calculated by difference.

\*\*\*) calculated from the proximate composition, see Table 2.

\*\*\*\*) CS (Chemical Score) calculated by the amino acid profiles of the ingredients as given by Van der Meer & Verdegem (1996).

## Chemical analyses

After determination of the final wet weight of the fish, head and viscera were removed. Wet weight and proximate composition of head, viscera and the remaining part of the body (from here on called 'trunk') were determined. Trunks and head+viscera were pooled per aquarium for determination of proximate composition. Amounts of carbohydrates in *C. macropomum* are low (Van der Meer et al. 1995) and were therefore ignored. Carbohydrates in the diets were calculated, supposing that the part not being protein, lipids, fibre or ash consisted of carbohydrates.

## Water quality

Water temperature and dissolved oxygen levels were checked daily at 9, 13 and 17 h. Average temperature in the recirculation units 1 and 2 were 28.9 and 28.8 °C, respectively. Oxygen levels were maintained above 4 ppm. Average dissolved oxygen levels were 5.50 and 6.20 ppm in units 1 and 2, respectively. Nitrite levels were checked weekly and never exceeded 0.05 ppm.

## Data analysis

Parameters with their abbreviations are given in Table 2. Effects of feed composition on ration (= voluntary feed uptake), growth, feed utilization, body composition and body partition in head, trunk and viscera were analyzed by ANOVA:  $Y_{ijkl} = \mu + P_i + L_j + U_k + P_i * L_j + e_{ijkl}$ , where:  $Y_{ijkl}$  = observed value;  $\mu$  = experimental mean;  $P_i$  = effect of the protein lipid level ( $i = 30, 40$ );  $L_j$  = effect of the dietary lipid level ( $j = 5, 10, 15, 20$ );  $U_k$  = effect of the experimental unit ( $k = 1, 2$ );  $P_i * L_j$  = effect of the interaction between dietary protein and lipid levels and  $e_{ijkl}$  = error term (with the subscript 'l' referring to the two repetitions per experimental unit). If required, differences between means were compared by Tukey-test.

If dietary protein is not used for lipid deposition, then the Lipid Energy Gain (LEG) depends on the efficiency of dietary carbohydrate and lipid being converted into body fat. The potential contribution of dietary lipid and carbohydrate to the lipid deposition can be calculated from their caloric equivalents, being 39.8 kJ g<sup>-1</sup> for lipids and 17.2 kJ g<sup>-1</sup> for carbohydrates (Cho, Slinger & Bayley 1982). In similarity with the NPU, which is the ratio protein gain/protein ration, the NLU and NCU (Net Lipid Utilization and Net Carbohydrate Utilization, respectively) were defined as the ratio of the dietary lipid energy and dietary carbohydrate energy deposited as fish body lipids, respectively (see Table 2). Hence, LEG is related to NLU and NCU through the following equation:  $LEG_{(i)} = LR_{(i)} * NLU * 39.8 + CR_{(i)} * NCU * 17.2$ , where:

$i$  = number of the aquarium (1, 2, ... 32); LR, CR = lipid and carbohydrate ration, respectively.

The NLU and NCU which fitted best to the data were calculated with optimization technics available under Quattro Pro, Version 5.00, Borland International Inc., Scotts Valley, USA.

The effect of PSA was illustrated by a linear regression analysis of the NPU, MGR and body lipid content on the dietary P/E ratio. The alternative hypothesis that protein and lipid deposition were independent of each other was tested by (1) comparing the body fat percentage as calculated from the optimized NLU and NCU with the observed body fat percentages, and (2) studying the relationship between protein ration with protein growth by linear regression.

Table 2. Parameters and their abbreviations.

Parameter	Units	Abbreviation	Formulas and observations
feed ration	g	F	dry weight, refers to the total feed gift (days 1 to 28)
protein ration	g	PR	$F * (\text{feed protein } \%) / 100$
lipid ration	g	LR	$F * (\text{feed lipid } \%) / 100$
carbohydrate ration	g	CR	$F * (\text{feed carbohydrate } \%) / 100$
energy ration <sup>a)</sup>	kJ	ER	$F * (\text{feed protein } \% * 23.4 + \text{feed lipid } \% * 39.8 + \text{feed carbohydrate } \% * 17.2) / 100$
non-protein energy ration <sup>a)</sup>	kJ	NPER	$F * (\text{feed lipid } \% * 39.8 + \text{feed carbohydrate } \% * 17.2) / 100$
P/E ratio	mg kJ <sup>-1</sup>	-	PR*1000/ER
fish weight	g	W <sub>0</sub> , W <sub>28</sub>	initial(day=0), final(day=28) wet weight
weight gain	g	WG	$W_{28} - W_0$
geometric mean weight	g	W <sub>geo</sub>	$\exp((\ln(W_0) + \ln(W_{28})) / 2)$
metabolic growth rate	g kg <sup>-0.8</sup> day <sup>-1</sup>	MGR	$WG / \{(W_{\text{geo}} / 1000)^{0.8}\} / 28$
body protein gain <sup>##)</sup>	g	PG	$W_{28} * (\text{body protein } \% / 100) - W_0 * 0.145$
body lipid gain <sup>##)</sup>	g	LG	$W_{28} * (\text{body lipid } \% / 100) - W_0 * 0.05$
body energy gain <sup>a)</sup>	kJ	EG	$PG * 23.4 + LG * 39.8$
body lipid energy gain <sup>a)</sup>	kJ	LEG	$LG * 39.8$
feed conversion	g/g	FC	F/WG
net protein utilization	%	NPU	PG/PR
net lipid utilization	%	NLU	$\{(LR * 39.8) / (LEG - \text{LEGcarboh})\} * 100$ <sup>###)</sup>
net carbohydrate utilization <sup>a)</sup>	%	NCU	$\{(CR * 17.2) / (LEGcarboh)\} * 100$ <sup>###)</sup>
net non-protein energy utilization	%	NNPEU	$(LEG / NPER) * 100$
net energy utilization	%	NEU	$(EG / ER) * 100$

<sup>a)</sup> Energy contents of fish and feeds were calculated by using the following caloric equivalents: protein 23.4 kJ/g; lipid 39.8 kJ/g and carbohydrates 17.2 kJ/g (Cho, Slinger & Bayley 1982).

<sup>##)</sup> For the calculation of protein and lipid gain, the initial wet body of the 1.1 g *C. macropomum* was assumed to contain 14.5% protein and 5% lipids (Van der Meer, Huisman & Verdegem 1996).

<sup>###)</sup> LEGcarboh stands for the lipid deposition (expressed in kJ) originating from the dietary carbohydrates. As LEGcarboh was not determined in this experiment, NLU and NCU were calculated from feed and lipid deposition data (for explanation: see text).

## RESULTS

### Feed consumption, growth and feed utilization

In 28 days the 1.1 g *C. macropomum* attained a weight between 25.1 g (diet 30/10) and 30.8 g (diet 40/15). Feed ration and growth were not affected by the dietary lipid level (Table 3). However, a higher dietary protein level decreased feed consumption ( $P < 0.1$ ) and increased fish growth ( $P < 0.1$ ).

The feed conversion (FC) was significantly affected by the dietary protein level ( $P < 0.01$ ) and dietary lipid level ( $P < 0.01$ ). Average FC's were 0.93 and 0.77 for the 30% and 40% protein diets, respectively. Diets with either 15 or 20% lipids had lower FC's than diets with 5 or 10% lipids.

The 30% protein diets had a significant higher average net protein utilization (NPU) than the 40% protein diets (49.8 and 47.7%, respectively;  $P < 0.01$ , Table 3). The NPU was affected significantly by the dietary lipid level, but only the 10% lipid diets (and not the 5% lipid diets) had

**Table 3.** Feed ration, growth and feed utilization data (avg $\pm$ std).

Diet	W <sub>0</sub>	W <sub>28</sub>	MGR	F	FC	NPU	NNPEU	NEU
30/05	1.11 $\pm$ 0.03	27.3 $\pm$ 3.9	60.0 $\pm$ 6.1	24.5 $\pm$ 3.4	0.937 $\pm$ 0.014	48.8 $\pm$ 1.3	33.8 $\pm$ 2.5	39.8 $\pm$ 1.8
30/10	1.12 $\pm$ 0.07	25.1 $\pm$ 5.8	56.4 $\pm$ 8.0	23.0 $\pm$ 5.1	0.964 $\pm$ 0.022	49.5 $\pm$ 2.0	34.3 $\pm$ 1.1	39.7 $\pm$ 0.8
30/15	1.11 $\pm$ 0.05	28.2 $\pm$ 5.6	60.9 $\pm$ 6.6	24.7 $\pm$ 4.4	0.915 $\pm$ 0.047	50.1 $\pm$ 2.0	39.0 $\pm$ 1.4	42.8 $\pm$ 1.6
30/20	1.12 $\pm$ 0.05	26.2 $\pm$ 2.0	58.2 $\pm$ 3.8	22.9 $\pm$ 1.5	0.913 $\pm$ 0.022	50.9 $\pm$ 1.5	38.8 $\pm$ 1.0	42.7 $\pm$ 1.1
40/05	1.14 $\pm$ 0.06	30.1 $\pm$ 2.6	63.3 $\pm$ 4.3	22.7 $\pm$ 1.2	0.787 $\pm$ 0.031	47.6 $\pm$ 0.9	36.3 $\pm$ 1.4	42.0 $\pm$ 0.6
40/10	1.12 $\pm$ 0.05	27.9 $\pm$ 2.3	60.6 $\pm$ 4.1	21.3 $\pm$ 2.5	0.792 $\pm$ 0.022	44.5 $\pm$ 2.1	40.0 $\pm$ 2.4	42.2 $\pm$ 0.8
40/15	1.11 $\pm$ 0.03	30.8 $\pm$ 1.0	64.9 $\pm$ 2.0	22.5 $\pm$ 1.4	0.759 $\pm$ 0.024	49.3 $\pm$ 2.0	41.2 $\pm$ 2.5	44.7 $\pm$ 1.6
40/20	1.12 $\pm$ 0.05	28.5 $\pm$ 2.8	61.3 $\pm$ 4.3	20.5 $\pm$ 1.8	0.750 $\pm$ 0.009	48.4 $\pm$ 2.6	44.6 $\pm$ 3.5	46.2 $\pm$ 1.3
F-values.								
	d.f.							
DP	1	-	4.16*	3.93*	3.75*	312.10***	14.16***	28.15***
DL	3	-	1.07	1.14	0.91	5.69***	4.21**	14.96***
Unit	1	-	0.88	0.97	1.82	2.10	5.44**	1.44
DP*DL	3	-	0.01	0.02	0.02	0.25	2.05	1.47
Error	23/21#							
Total	32/30#							

Significance and way of calculation of the used parameters can be found in Table 2. DP, DL = levels of dietary protein (30 and 40%, respectively) and lipid (5, 10, 15 and 20%, respectively); DP\*DL = interaction between DP and DL; Unit = recirculation unit (1 and 2, respectively).

\* P<0.1; \*\* P<0.05; \*\*\* P<0.01.

# one head+viscera sample of treatment 40/05 and one trunk sample of treatment 30/20 got lost after determination of the dry matter content; therefore the total number of degrees of freedom for the ANOVA of NPU, NNPU and NEU was only 30.

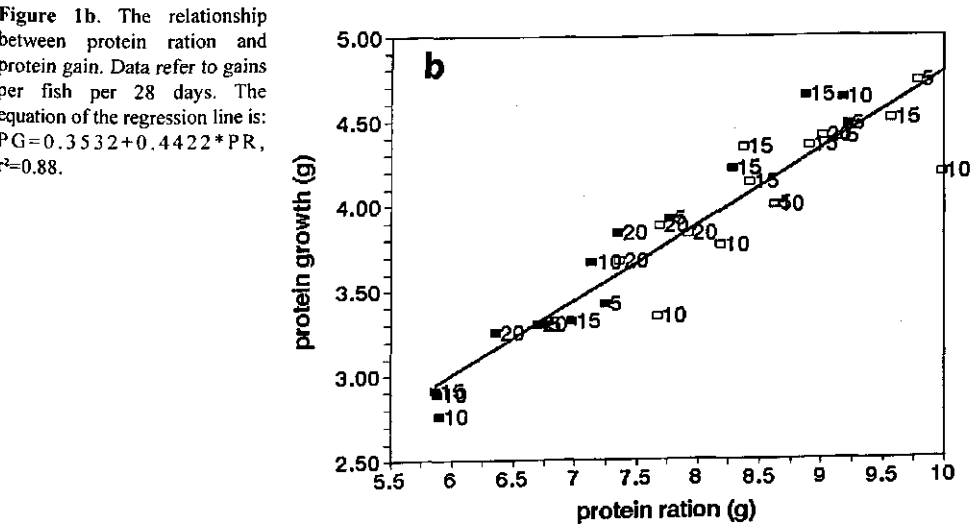
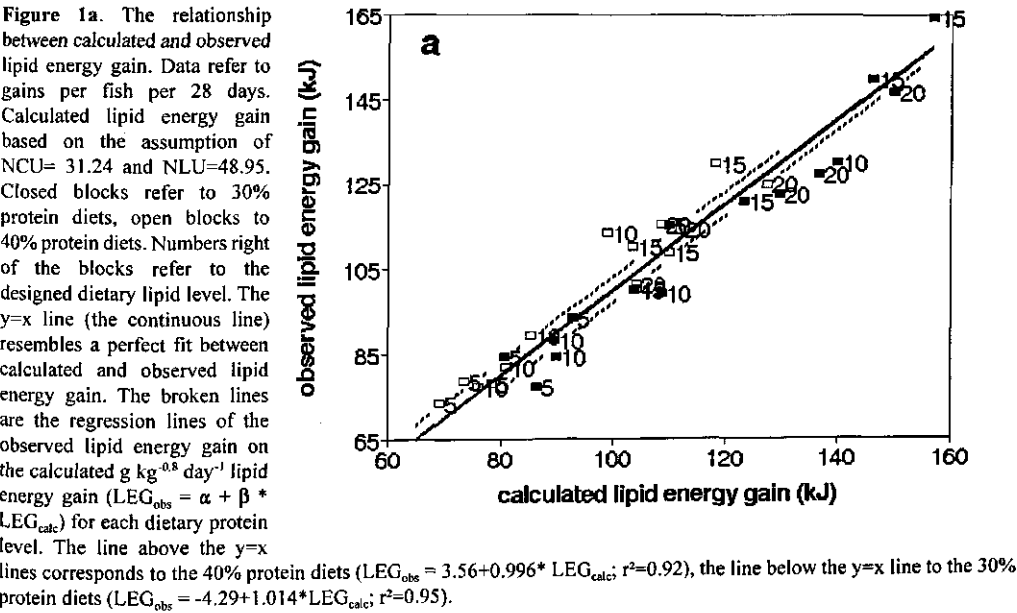
**Table 4.** Relative contribution of trunk, head and viscera to the wet body weight (avg $\pm$ std).

diets	trunk	head	viscera
30/05	61.16 $\pm$ 0.85	27.83 $\pm$ 0.12	11.00 $\pm$ 0.92
30/10	62.18 $\pm$ 1.72	27.81 $\pm$ 0.66	10.01 $\pm$ 1.63
30/15	60.55 $\pm$ 1.73	26.71 $\pm$ 1.86	12.73 $\pm$ 1.19
30/20	60.65 $\pm$ 0.86	27.67 $\pm$ 1.04	11.68 $\pm$ 1.36
40/05	61.90 $\pm$ 1.46	27.74 $\pm$ 1.34	10.36 $\pm$ 0.54
40/10	62.73 $\pm$ 1.34	26.51 $\pm$ 1.84	9.76 $\pm$ 1.16
40/15	62.81 $\pm$ 1.71	26.88 $\pm$ 1.53	10.30 $\pm$ 0.39
40/20	61.88 $\pm$ 0.83	27.43 $\pm$ 0.81	10.69 $\pm$ 0.35
F-values of the analyses of variance			
	d.f.		
DP	1	6.27**	8.34***
DL	3	1.15	3.60**
Unit	1	1.71	0.25
DP*DL	3	0.64	1.60
Error	23		
Total	32		

DP, DL = dietary protein and lipid level; DP\*DL = interaction between DP and DL; Unit = recirculation unit.

\*\* P<0.05; \*\*\* P<0.01.

**Figure 1.** Relationship between non-protein and protein ratios on lipid and protein deposition, respectively.



a significantly lower NPU compared to the 15 and 20% lipid diets (Tukey-test,  $P < 0.05$ ).

The net non-protein energy utilization (NNPEU) increased significantly with both increased dietary protein ( $P < 0.01$ ) and dietary lipid level ( $P < 0.01$ ). Also the net energy utilization (NEU) increased with both dietary protein ( $P < 0.01$ ) and dietary lipid ( $P < 0.01$ ) level.

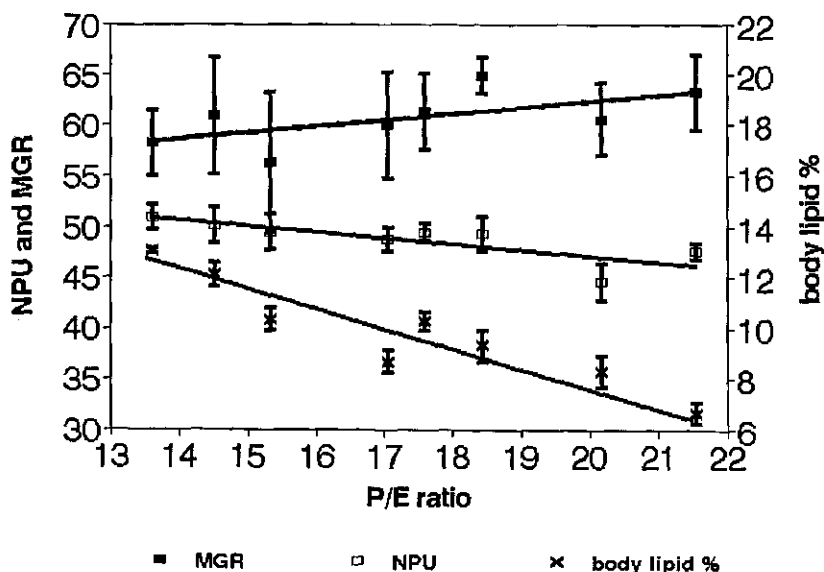
The NLU and NCU which fitted best to our data were 0.490 and 0.312, respectively. With these NLU and NCU lipid percentages of the fish were calculated. Fish fed the 40% protein diets deposited about 3.6 kJ lipids more and those fed the 30% protein about 4.3 kJ less than expected on the basis of the calculated NLU and NCU (Figure 1a). Linear regression analysis showed that protein gain (PG) could be well explained by protein ration (PR):

$PG = 0.3532 + 0.4422 \cdot PR$ ,  $r^2 = 0.88$ ,  $P < 0.01$  (Figure 1b).

### Protein sparing action

As expected under the PSA hypothesis, an increasing dietary P/E ratio was associated with a decreased NPU ( $NPU = 58.79 - 0.5822 \cdot P/E \text{ ratio}$ ,  $r^2 = 0.37$ ,  $P < 0.01$ ) and decreased body lipid percentages ( $BL\% = 21.69 - 0.6863 \cdot P/E \text{ ratio}$ ,  $r^2 = 0.80$ ,  $P < 0.01$ ; Figure 2). The growth rate showed a weak relation ( $P = 0.09$ ) with the P/E ratio ( $MGR = 50.05 + 0.6160 \cdot P/E \text{ ratio}$ ,  $r^2 = 0.09$ ; Figure 2).

**Figure 2.** The effect of the dietary P/E ratio on protein utilization efficiency (NPU), growth (MGR) and body lipid level.



The left Y-axis gives the NPU (%) and the MGR g kg-0. Day-1; the right Y-axis the body lipid level as a percentage of the wet body weight.

Zamora & Verdegem 1997; Van der Meer, Van Herwaarden & Verdegem 1997) and a energetical efficiency of the transformation of amino acids in deposited lipids of 53% (Black 1995). The difference in non-depositable dietary amino acids can therefore account for  $0.8 \times 0.8 \times 0.53 \times 11.8 = 4.0$  kJ. Thus  $4.0/7.9 \times 100 = 51\%$  of the 'extra' lipid deposited with the 40% protein diets might come from dietary protein origin without having any effect on protein deposition efficiency. The additional 49% of the observed lipid deposition might have come from the extraction of protein from protein deposition (a PSA effect). However, if NLU and NCU decrease with carbohydrate and lipid ration, respectively, the fixed NLU and NCU would underestimate the lipid deposition of the high (40%) protein diets. In the present experiment a fluctuation of NLU and NCU from 90 to 110% of their respective averages would have been sufficient to explain 49% of the extra lipid deposition with the 40% protein diets.

### Body partition and body composition

The low protein diets resulted in fish with a higher percentage of viscera ( $P < 0.01$ , Table 4) and a lower percentage of trunk ( $P < 0.05$ ). Dietary lipid levels affected the proportion of viscera ( $P < 0.1$ ), with fish fed the 10% lipid diets having significantly less viscera than fish fed the 15% lipid diets (Tukey-test,  $P < 0.05$ ).

The trunk of the fish was higher in protein and lower in dry matter, lipids and ash than the rest of the body (Table 5). Lower dietary protein levels and higher dietary lipid levels increased dry matter and lipid content in both trunk and head+viscera (all  $P < 0.01$ ). Protein and ash content of the body were slightly higher in the fish fed the 40% protein diets ( $P < 0.01$ , Table 5).

## DISCUSSION

### Growth, feed ration and feed utilization

Data of this experiment confirmed the high growth rates of *C. macropomum* in our laboratory (Günther and Boza 1993; Van der Meer, Machiels & Verdegem 1995; Van der Meer, Huisman & Verdegem 1996). However, feed utilization parameters (FC and NPU) were better than those reported before in fish with similar growth rates. This is probably due to an improved feed formulation: a higher contribution of soya protein to the total dietary protein as suggested by Van der Meer, Huisman & Verdegem (1996) and a lower contribution of blood meal, as suggested by Van der Meer et al. (1995) and Eckmann (1987).

Dietary lipid levels in the range from 5 to 20% did not significantly affect feed ration (Table 3). This is different from the general believe that fish eat to satisfy their energy requirements (Cho & Kaushik 1988). Our data suggest that not lipid but protein is the most important macro-nutrient determining feed ration in *C. macropomum*. A decreased feed ration with increased dietary protein content was also reported in *Piaractus mesopotamicus* (formerly *Colossoma mitrei*; Brenner 1988), channel catfish (Mangalik 1986; Li & Lovell 1992) and in cod (recalculated data from Hemre, Lie, Lied & Lambertsen 1989).

Machiels & Van Dam (1987) suggested that the *ad libitum* feed uptake in African catfish is higher in lean fish than in fatty fish. This seems to contradict with our observations of *C.*

*macropomum*: fish fed the 30% protein diets became fatter (Table 5) and consumed more feed (Table 3). However, in *C. macropomum* the body lipid storage capacity is much higher than in African catfish. While in African catfish the body lipid level never exceeds the body protein level (Machiels & Henken 1986), in *C. macropomum* the body lipid level may surpass the body protein level by 50% (Van der Meer et al. 1995). In the present experiment the body lipid level remained below the body protein level and maximum lipid storage capacity was never reached. We hypothesize that a negative relationship between body lipid content and feed ration will also be found in *C. macropomum* in an experimental design including fish with the (yet unknown) maximum body lipid level. Detection of the effect might be facilitated by the use of diets tending to increase the body lipid level (e.g. diets with a protein level below 20%; Van der Meer et al. 1995).

In *ad libitum* fed *C. macropomum* only 80% of the feed ration is eaten (Van der Meer, Faber, Zamora & Verdegem 1997). Digestibilities of lipid and carbohydrate are assumed to be 80 and 60%, respectively (Van der Meer & Van Dam, Chapter 5). The biochemical efficiencies with which fatty acids and glucose are used for lipid deposition are estimated in 90 and 74% (Black 1995). This means that the NLU could be maximally  $0.8 \times 0.8 \times 0.9 = 0.57$  and the NCU  $0.8 \times 0.6 \times 0.74 = 0.36$ . Our estimates of the NLU and NCU (0.49 and 0.31, respectively) are below these maxima and leave 15% of the available dietary lipid energy and 12% of the available carbohydrate energy for the energy requirements of the animal. The ratios NLU/NCU ( $0.49/0.31 = 1.56$ ) and theoretical maximum NLU/theoretical maximum NCU ( $0.57/0.36 = 1.62$ ) were similar. If the routine metabolism of fish uses energy from lipid and carbohydrate proportionally to their respective availabilities, the observed NLU/NCU ratio is close to the theoretical expected value.

Considering the preference for fruits in its natural diet, Menton (1989) supposed that *C. macropomum* can digest carbohydrates better than most other fishes. However, the estimated NLU and NCU (49 and 31%, respectively) indicated that also in *C. macropomum* lipids are more efficiently used for energy storage than carbohydrates. A negative effect of increased dietary carbohydrate levels on feed digestibility has been reported in Atlantic salmon (Hemre, Sandes, Lie, Torissen & Waagbø 1995). As the fixed NLU and NCU were adequate to explain the variation in NNPEU, any negative effect of increased dietary carbohydrate levels on carbohydrate and lipid digestibility in *C. macropomum* must be of minor importance. A negative effect on protein digestibility cannot be important either, as protein utilization is generally best in high carbohydrate diets (Van der Meer, Huisman & Verdegem 1996; this study).

### The protein sparing action

PSA of dietary energy is described in several trout species: rainbow trout (Watanabe, Takeuchi & Ogino 1978; Beamish & Medland 1986), lake trout (Jayaram & Beamish 1992) and brown trout (Arzel, Lopez, Métailler, Stéphan, Viau, Gandemer & Guillaume 1994). PSA has also been found in African catfish (Machiels and Henken 1987; Henken, Machiels, Dekker & Hogendoorn 1986) and tilapia (Shiau & Peng 1993; El-Sayed and Garling 1988). However, findings in warm water species are not unanimous, as Viola & Arieli (1983) and Hanley (1991) could not find any PSA in tilapia. Also in channel catfish does not show a clear optimum dietary lipid level with regard to PSA (Robinson 1991).

An important effect of PSA for aquaculture practices is improved protein retention

(Kaushik & Cowey 1991). In salmonids the beneficial effect of PSA induced by dietary lipids is well recognized (Kaushik & Médale 1994). Fortification of the diet with 100 grams of lipids increased protein deposition with 38-47 gram and 35-49 grams, in rainbow trout (Cho et al. 1982) and brown trout (Arzel et al. 1994), respectively. Also in *C. macropomum* protein deposition increased with increasing dietary lipid level, but the magnitude of the effect seems to be much lower than in trout. The regression line of the NPU on the P/E ratio indicates that within one dietary protein level the addition of 20% instead of 5% lipid to the diet increased the NPU with 2 - 2.3%. Consequently, the addition of 150 g lipid (in exchange of 150 g carbohydrates) is expected to result in an extra 6 - 7 g of protein deposited per kg feed. It seems unlikely that costs of such a change in diet composition will be sufficiently compensated by improved feed utilization. Doubts about the PSA in other warm water species such as tilapia (Viola & Arieli (1983) and Hanley (1991)) and channel catfish (Robinson 1991), suggest that also in those species the PSA, if present, is not as effective as in trout.

Compared to the 30% protein diets, the 40% protein diets resulted in 7.9 kJ of extra lipid deposition (Figure 1a). More protein used for energy deposition at high P/E ratios was also expected under the PSA hypothesis. However, as the Chemical Score of all experimental diets was 63 (Table 1), 37% of the dietary protein was unavailable for protein deposition (see Van der Meer & Verdegem (1996) for full explanation). Based on the average ration (23.8 and 21.8 g fish<sup>-1</sup> 28 days<sup>-1</sup> for the 30% and 40% protein diets, respectively) and average protein content (30.7 and 39.8%, respectively), the dietary protein not available for protein deposition contained  $23.8 \times 0.307 \times 0.37 \times 23.4 = 63.3$  kJ for the average 30% protein diet and  $21.8 \times 0.398 \times 0.37 \times 23.4 = 75.1$  kJ for the average 40% protein diet. Thus the 40% protein diets contained  $75.1 - 63.3 = 11.8$  kJ more protein energy not available for protein deposition than the 30% protein diets. We assumed a protein digestibility of 80% in *C. macropomum* (Van der Meer & Van Dam, unpublished), 20% feed losses (Van der Meer, Faber, Zamora & Verdegem 1997; Van der Meer, Van Herwaarden & Verdegem 1997) and a energetical efficiency of the transformation of amino acids in deposited lipids of 53% (Black 1995). The difference in non-depositable dietary amino acids can therefore account for  $0.8 \times 0.8 \times 0.53 \times 11.8 = 4.0$  kJ. Thus  $4.0 / 7.9 \times 100 = 51\%$  of the 'extra' lipid deposited with the 40% protein diets might come from dietary protein origin without having any effect on protein deposition efficiency. The additional 49% of the observed lipid deposition might have come from the extraction of protein from protein deposition (a PSA effect). However, if NLU and NCU decrease with carbohydrate and lipid ration, respectively, the fixed NLU and NCU would underestimate the lipid deposition of the high (40%) protein diets. In the present experiment a fluctuation of NLU and NCU from 90 to 110% of their respective averages would have been sufficient to explain 49% of the extra lipid deposition with the 40% protein diets.

In pigs a relative independence of protein and energy metabolism has been noticed. Protein deposition increases linearly with protein intake until the maximum (animal dependent) protein deposition rate at that energy intake level is reached. Only if the energy intake fails to supply the energy required for maintenance and deposition of a minimum amount of lipids, protein deposition will be reduced when the dietary energy content decreases (De Greef & Versteegen 1995). Hence, if protein deposition is limited by protein intake 'protein sparing' is not possible (Bikker, Versteegen & Tamminga 1994). If this is true in fish as well, improvement of low protein diets by lipid addition is virtually impossible, because in practical diets the non-protein portion mainly consists of the energy carrying carbohydrates and lipids. This also would explain why in this study, with dietary

Table 5. Body composition (avg±std).

Diet	Chemical composition of trunk				Chemical composition of head+viscera				Chemical composition of the whole body			
	dry matter	protein	lipids	ash	dry matter	protein	lipids	ash	dry matter	protein	lipids	ash
30/05	24.96±0.39	16.28±0.50	6.97±0.49	2.35±0.18	26.60±0.38	11.57±0.40	11.45±0.88	3.18±0.10	25.60±0.38	14.45±0.44	8.71±0.55	2.68±0.09
30/10	26.10±0.63	16.66±0.54	8.01±0.47	2.27±0.04	29.23±1.10	11.04±0.71	14.19±1.08	3.30±0.26	27.26±0.77	14.53±0.42	10.34±0.52	2.66±0.10
30/15	27.05±0.61	16.13±0.43	9.42±0.69	1.98±0.42	30.72±0.81	10.60±0.39	16.26±1.08	2.82±0.23	28.49±0.59	13.96±0.28	12.11±0.52	2.31±0.19
30/20	27.86±0.66	15.87±0.11	10.08±0.61	2.09±0.10	32.19±0.57	10.95±0.48	17.65±0.45	2.81±0.08	29.57±0.47	14.02±0.04	13.04±0.13	2.39±0.03
40/05	23.92±0.40	16.97±0.42	5.26±0.51	2.40±0.15	25.00±1.23	11.91±0.53	9.15±1.33	3.87±0.36	24.33±0.71	15.14±0.40	6.63±0.51	2.97±0.20
40/10	24.50±0.10	16.29±1.00	6.08±0.24	2.33±0.11	26.83±0.43	10.92±1.20	12.04±2.05	3.20±0.41	25.37±0.12	14.29±0.54	8.30±0.72	2.65±0.20
40/15	24.58±2.10	16.12±1.41	6.99±1.16	2.23±0.15	27.14±3.02	10.61±0.82	12.42±1.87	2.97±0.30	26.51±0.47	14.61±0.24	9.34±0.71	2.62±0.18
40/20	25.99±0.43	16.28±0.39	8.29±0.17	2.01±0.48	28.85±1.40	10.79±1.61	14.67±1.68	2.98±0.47	27.08±0.73	14.47±0.40	10.27±0.43	2.47±0.29
F-values.												
d.f.												
DP	1	18.67***	0.8093	29.70***	0.0557	46.99***	0.714	70.92***	0.8330**	1.198***	46.66***	0.2289**
DL	3	9.45***	0.4142	14.98***	0.1665*	33.73***	1.108**	41.98***	0.4983***	0.499**	24.07***	0.2685***
Unit	1	0.01	0.0617	1.44**	0.0435	0.65	2.565***	0.28	0.0986	0.13	0.058	0.0001
DP*DL	3	0.24	0.4474	0.08	0.0640	1.03	0.194	1.80	0.2030*	0.46	0.370*	0.0491
Error	23#	0.26	0.2952	0.25	0.0644	0.81	0.286	1.55	0.0785	0.33	0.148	0.0345
Total	32#											

DP, DL = dietary protein and lipid level; DP\*DL = interaction between DP and DL; Unit = recirculation unit.

\* P&lt;0.10

\*\* P&lt;0.05

\*\*\* P&lt;0.01.

# one head+viscera sample of treatment 40/05 and one trunk sample of treatment 30/20 got lost; therefore the total number of degrees of freedom for the ANOVA of protein, lipids and ash was only 31 for trunk and head+viscera and 30 for the whole body. Consequently, the number of degrees of freedom of the error for protein, lipids and ash in trunk and head+viscera was 22 and in the whole body 21.

protein levels below the 43% required for maximal growth (Van der Meer et al. 1995), no clear PSA was found. However, a PSA of lipids in *C. macropomum* with dietary protein concentrations above 43% would agree with this concept and must be considered when developing 'nutrient dense' diets.

In the present study the effect of the major variation of dietary lipid level from 5 to 20% and the effect of the relative small variation in dietary protein from 30 to 40%, affected the P/E ratio (Table 1) and the NPU (Table 3) to a comparable extend. Typically, the P/E ratio is more sensible to the dietary protein content than to the lipid content. In practical diets changing the energy content is a gradual process: lipid (39.8 kJ/g) is mostly exchanged by carbohydrates (17.2 kJ/g). In contrast, changing the protein content is an 'all-or-nothing' process: protein (100% protein) is exchanged by either lipids, carbohydrates or ash (all 0% protein). For example, in a diet containing 45% protein and 10% ash, an increase of the dietary lipid from 1 to 44% decreases the P/E ratio from 24.33 to 15.95 mg/kJ. However, in a 10% ash, 10% lipid diet the P/E ratio varies from 21.92 (45% protein) to 8.03 (15% protein). Thus, the P/E ratio decreases to 66% of its initial value by putting 44 times more lipid in the diet, while a reduction of the protein content by only a factor 3 decreases the P/E ratio to 37% of its initial value. This shows that, assuming that the NPU is determined by the P/E ratio, the most efficient approach to assess the maximal NPU is by fluctuating the dietary protein level.

### Body partition and body composition

As demonstrated before in *C. macropomum* (Van der Meer et al. 1995), the body lipid level is highly responsive to variations in the diet composition. As expected, the body lipid level increased with the dietary lipid level. However, despite the relative low variation in dietary protein level (30-40%) compared to the dietary lipid level (5-20%), the negative relationship between dietary protein and body lipid level had a higher significance level (as demonstrated by the F-values in Table 5). Accumulation of large amounts of body fat in *C. macropomum* fed low protein diets has been demonstrated before (Van der Meer et al. 1995). Dietary protein concentrations above the requirement are not likely to cause a significant increase in the body lipid content, as the conversion of amino acids in deposited lipids has an energetical efficiency of only 53% (Black 1995). Fattiness is undesirable in fish cultured for food (Lovell 1989). Increasing the dietary protein level seems to be a secure strategy to produce a lean product.

Castelo, Amaya & Strong (1980) mention viscera and head as the major lipid stores in adult *C. macropomum*. In the present study lipid concentrations were between 70 (30% protein diets) and 80% (40% protein diets) higher in the head+viscera than in the trunk. However, the distribution of body lipid over trunk and head+viscera did not change with increasing lipid levels: in all treatments between 45 and 48% of the body lipid was stored in the meat. Thus, a fatty fish yields fatty fillets.

Van der Meer et al. (1995) reported that the whole body protein content of *C. macropomum* increased when dietary protein levels increased from 20 to 60%. In the present experiment, average protein levels in both trunk and head+viscera were higher in the fish fed the 40% protein diets than in those fed the 30% protein diets (Table 5). However, those differences were not significant. Contrary, the proportion of the protein rich trunk was significantly higher in fish fed the 40% protein diets ( $P < 0.01$ ) and the portion of the protein poor viscera was significantly lower ( $P < 0.01$ ; Table 4). It seems that the main effect of an increased dietary protein level is an increase of the trunk/viscera

ratio, while an increased protein concentration in the different organs is a minor effect. A similar trend might exist in Atlantic salmon (Hillestad & Johnson 1994) and channel catfish (Li & Lovell 1992), where increased dietary protein levels were associated with increased dress-out percentages.

In pigs protein ration was positively correlated with the body protein and body muscle content (Bikker, Verstegen, Campbell & Kemp 1994). However, viscera were the part of the body most responsive to changes in diet composition. Weight of visceral organs increased with increasing protein ration, energy ration or both (Bikker, Verstegen & Tamminga 1994). In pigs, the weight adjustment of metabolic active organs to changes in feed ration or feed composition was the main cause of changes in body composition. In sunshine bass substitution of dietary protein by carbohydrates resulted in fish with more abdominal fat, a higher hepatosomatic index and a lower dress-out percentage (Webster, Tiu, Tidwell, Van Wyk & Howerton 1995). Also in rainbow trout (Lee & Putnam 1973) and cod (Hemre et al. 1989), the exchange of protein by carbohydrates (starch) resulted in an increased hepatosomatic index. Considering the F-values of Table 5, in *C. macropomum*, like in pigs, the effect of diet composition on body composition might be mainly through its effect on the weight of the viscera. Possibly, an increased weight of the viscera caused by the substitution of dietary protein by carbohydrate can be confirmed in future experiments.

## CONCLUSION

In *C. macropomum* the most typical PSA effect was found: addition of dietary energy increases net protein utilization. However, this process was not very efficient and resulted in high lipid contents of the meat.

An effective PSA assumes that protein and lipid deposition interfere with each other. However, in the present experiment protein deposition could be well described by protein ration alone. Equally, lipid deposition could be well described by assuming the same lipid and carbohydrate utilization efficiencies in all diets. Half of the 'extra' lipid deposition with the higher protein diets could be explained by energy supply from dietary protein not containing the required amino acid profile for protein deposition. The effect of PSA in *C. macropomum* might be small because a considerable part of the dietary protein can be used for energy purposes without harming protein deposition efficiency. Possibilities to increase protein deposition by lipid addition might be limited to high protein diets.

Dietary lipid energy was deposited more efficiently than carbohydrate energy. In formulating diets one faces the problem that increasing the dietary lipid level can improve feed utilization, but might economically be unattractive due to higher feed costs (carbohydrate substituted by lipid) and a lower meat quality (higher meat lipid content). On the other hand, increased carbohydrate levels at the cost of the dietary lipid level result in leaner fish, but also in a reduced energy retention efficiency.

Feed ration depended on the dietary protein content rather than dietary energy content. Higher protein rations resulted in fish with more meat. This is the main cause that dietary protein and body protein contents in *C. macropomum* are correlated.

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# Chapter 5

## MODELLING GROWTH OF *COLOSSOMA MACROPOMUM*

### Modelling growth of *Colossoma macropomum* (Cuvier): comparison of an empirical and an explanatory model.

#### ABSTRACT

Accumulated data of growth experiments on the nutrition of *Colossoma macropomum* were analyzed using an empirical (linear regression) and an explanatory model called the Fish Growth Simulator (FGS). Both methods gave satisfactory results. Differences between predicted and observed values fluctuated less by the FGS. However, the linear regression method is simple and results are easily translated to practical advices. The FGS covered a wider range of experimental conditions than the linear regression method. Both linear regression and the FGS stressed the importance of dietary protein in growth of *C. macropomum*. Dietary protein concentration is the main explaining variable of *ad libitum* feed uptake, while protein ration is the main variable determining growth. Dietary protein concentration also affects body protein and body lipid content. Protein utilization efficiency data indicate that substitution of fish meal by soya meal increases dietary protein quality. Although *C. macropomum* can thrive under less than optimal conditions, a high quality, high protein diet is required for maximal growth and optimal feed utilization.

## INTRODUCTION

*Colossoma macropomum* (Cuvier) was identified as a promising species for aquaculture development in Latin America (Saint-Paul 1986). Extensive pond culture seems not feasible, as *C. macropomum* poorly exploits plankton (Van der Meer & Martínez 1993). In intensive pond culture, feed costs accounted for 69% of the total costs (Merola & Pagán-Font 1988). Therefore, optimization of feed composition and feeding regime was considered crucial for the development of an economical sound *C. macropomum* culture. *C. macropomum* was submitted to a series of experiments to estimate its growth potential, to determine its nutritional requirements and to improve the diet composition and feeding regime (Günther and Boza 1993; Van der Meer, Machiels & Verdegem 1995; Van der Meer, Huisman & Verdegem 1996; Van der Meer, Faber, Zamora & Verdegem 1997a; Van der Meer, Van Herwaarden & Verdegem 1997b; Van der Meer, Zamora & Verdegem 1997c).

There are several problems with interpreting the results of dose-response experiments. Application of insights gained through regression analysis of dose-response experiments is limited to the experimental conditions under which the experiments were executed. Also, empirical methods (usually some form of linear method) explain the variation of the dependent variable, but they do not establish cause-effect relationships. In addition, numerous experiments are required, testing many variables at a wide range of levels. Experiments have to be repeated for each species.

Machiels (1987) developed an explanatory dynamic simulation model based on physiological and biochemical principles to simulate growth of *Clarias gariepinus* (Burchell). The explanatory character of the model allowed prediction of growth under a wide range of conditions. Van Dam & Penning de Vries (1995) found that the same model, after reparameterization and calibration, could be used for the simulation of Nile tilapia (*Oreochromis niloticus* L.) and rainbow trout (*Oncorhynchus mykiss* Walbaum). They suggested that the model (called Fish Growth Simulator or FGS) can be used for any other fish species for which the necessary biological information is available.

For the present study a data base was compiled pooling data of different experiments on the nutrition of *C. macropomum*. The data were used to parameterize and calibrate two models: an empirical linear regression model and the FGS. Both models explain how feed ration and feed composition affect growth and body composition. Model results were compared with the conclusions reached after single experiments. The applicability of the models is discussed.

## MATERIAL AND METHODS

### The data base

Data of six nutritional experiments with *C. macropomum* were pooled. Experiments are further referred to by the abbreviations given in Table 1. All experiments were performed in the same experimental units. Average water temperature during the experiments ranged from 27.9 to 29.3 °C. In all experiments initial fish size was close to 1 gram, except experiments FLVEL and PROT in which initial weights up to 108 grams were used. In all experiments ingredients were bought from the same (Costa Rican) suppliers, except in experiment FFREQ, where high quality ingredients were imported

from Europe. Data of FFREQ were not used for estimation of the regression coefficients and calibration of the FGS.

**Table 1.** Experiments comprising the data base.

Exp. number	Most important variable	Abbreviation in text figures		Records	Reference
1	Feeding level	FLVEL	G	16	Günther and Boza 1993
2	Dietary protein percentage	PROT	P	95	Van der Meer, Machiels & Verdegem 1995
3	Dietary soya/fish meal ratio	SOYA	S	62	Van der Meer, Huisman & Verdegem 1996
4	Feeding level	FLVL2	R	30	Van der Meer, Faber, Zamora & Verdegem 1997a
5	Feeding frequency	FFREQ	H	32	Van der Meer, Van Herwaarden & Verdegem 1997b
6	Dietary lipid level	LIPID	L	32	Van der Meer, Zamora & Verdegem 1997c
TOTAL				267	

In FLEVEL each record consisted of the average of the data of 4 aquaria. In all other experiments each record stands for the feed and growth data of one (1) aquarium.

## Calibration of the models

### Linear regression analysis

The general format of a linear regression equation is:

$Y = \alpha + \beta_1 X_1 + \beta_2 X_2 + \dots + \beta_k X_k + \epsilon$ , where:

Y = dependent variable;  $X_{1..k}$  = independent or explaining variables;  $\alpha$  = constant (intercept) and  $\beta_{1..k}$  = partial regression coefficients;  $\epsilon$  = residual.

The objective of the linear regression analysis was to describe *ad libitum* protein uptake, growth and final body composition as a function of variables connected to diet composition, feed ration and body weight (Table 2).

Not all explaining variables could be used, because several were too highly correlated (Table 3). To avoid correlations above 0.5, DPROT and SOYA were the only variables in the model representing diet composition. Because MFR was too highly correlated with fish weight, MPR was chosen to represent feed quantity. Fish weight was represented by average weight ( $W_{geo}$ ) in the growth and feed uptake models, because the different durations of the experiments make initial and final fish weight (WI and WF, respectively) less suitable to represent the effect of fish weight during the experimental period. Final weight (WF) represents fish weight in the final body composition models.

The *ad libitum* protein uptake ( $MPR_{adlib}$ ) was analyzed excluding the data of fish fed under a fixed (restricted) feeding regime. Optional explaining variables for  $MPR_{adlib}$  were DPROT, SOYA and  $W_{geo}$ . However, all fish fed *ad libitum* had a WI close to 1 gram. Consequently, the strong positive relation between  $W_{geo}$  and  $MPR_{adlib}$  was mainly caused by the fact that fish with a higher average weight were the faster growing specimens which had been eating more. Hence,  $W_{geo}$  was removed from the  $MPR_{adlib}$  model, leaving as explaining variables only DPROT and SOYA. As feed rations were expressed per metabolic weight, one can assume only a minor effect of body weight on the *ad libitum* feed uptake.

**Table 2.** Descriptive statistics of the most important variables\*):

	Average	Std	Min	Max	Units	Use **)
<b>Fish weight and growth</b>						
WI	17.5	31.6	0.86	108	g	S
WF	51.2	50.5	1.69	233	g	S, L
Wgeo	26.9	40.7	1.56	156	g	L
MGR	40.5	18.8	-4.52	71.6	g kg <sup>-0.8</sup> day <sup>-1</sup>	L, S
<b>Initial body composition</b>						
BDM	21.1	2.68	15.1	26.4	%	S
BPROT	13.7	1.40	10.7	15.0	%	S
BLIPID	4.20	1.42	2.39	6.44	%	S
BASH	3.10	1.14	2.13	5.96	%	S
<b>Final body composition</b>						
BDM	27.3	3.66	15.9	35.9	%	L, S
BPROT	13.9	1.79	9.05	17.7	%	L, S
BLIPID	10.8	4.29	1.05	22.3	%	L, S
BASH	3.10	0.59	1.87	4.60	%	L, S
BP	8.10	7.49	0.15	32.4	g	L, S
BL	5.43	4.51	0.02	24.7	g	L, S
BA	1.89	1.81	0.08	7.53	g	L, S
<b>Feed ration</b>						
MFR	43.5	17.8	0	81.7	g kg <sup>-0.8</sup> day <sup>-1</sup>	L, S
MFRadlib	48.8	16.5	16.2	81.7	g kg <sup>-0.8</sup> day <sup>-1</sup>	L
MPR	15.6	7.56	0	37.1	g kg <sup>-0.8</sup> day <sup>-1</sup>	L, S
MPRadlib	19.7	6.32	10.6	35.7	g kg <sup>-0.8</sup> day <sup>-1</sup>	L
FLEVEL	91.2	26.4	0	159	%	S
<b>Feed composition</b>						
DPROT	37.0	11.3	17.0	64.3	%	L, S
DLIPID	9.24	3.61	2.70	20.5	%	L, S
DCARBO	41.4	12.7	17.5	65.9	%	L, S
SOYA	31.9	30.9	0	98.4	%	L
P/E ratio	19.2	6.2	8.4	32.8	mg kJ <sup>-1</sup>	S

\*) **Body weight:** WI, WF = initial and final fresh body weight; Wgeo = geometrical mean body weight, calculated as:  $\exp((\ln(WI) + \ln(WF))/2)$ ; MGR = metabolic growth rate, calculated as:  $(WF - WI) / \{(Wgeo/1000)^{0.8}\} / \text{days}$ .

**Body composition:** BDM, BPROT, BLIPID and BASH (body dry matter, protein, lipid and ash content, respectively), all expressed as a percentage of the fresh body weight. BP, BL and BA are expressed in grams per fish and represent the amounts of protein, lipid and ash, respectively.

**Feed ration:** MFR, MPR = feed and protein ration, respectively. FLEVEL = MFR expressed as a percentage of the *ad libitum* dry feed ration. In experiments with fixed rations, the ration resulting in the best growth rate was considered the *ad libitum* ration.

**Feed composition:** DPROT, DLIPID and DCARBO = dietary protein, lipid and carbohydrate content, respectively, all expressed as a percentage of the feed dry matter. SOYA = the percentage of dietary protein originating from soya meal. P/E ratio = dietary protein to energy ratio in mg kJ<sup>-1</sup>; energy calculated assuming the following caloric equivalents: protein 23.4 kJ g<sup>-1</sup>, lipid 39.8 kJ g<sup>-1</sup> and carbohydrates 17.4 kJ g<sup>-1</sup>.

\*\*) L = linear regression; S = fish growth simulator.

**Table 3.** Correlation coefficients of the explaining variables of the linear regression model \*).

	MFR	MPR	DPROT	DLIPID	DCARBO	SOYA	Wgeo
MPR	0.7006						
DPROT	-0.2176	<b>0.4898</b>					
DLIPID	0.0313	-0.3719	-0.5975				
DCARBO	0.1750	-0.5083	-0.9593	0.4229			
SOYA	0.0881	<b>0.1491</b>	<b>0.0968</b>	-0.0294	-0.0360		
Wgeo	-0.6450	<b>-0.4374</b>	<b>0.1982</b>	0.0123	-0.1825	<b>-0.3037</b>	
WF	-0.5562	<b>-0.3127</b>	<b>0.2559</b>	-0.0385	-0.2579	<b>-0.2736</b>	0.9733

\*) Correlation coefficients of variables appearing together in one or more models (see Table 6) appear bold.

**Table 4.** Proximate composition of ingredients (% of dry matter).

Ingredients	Protein	Lipid	Carbohydrates	Ash	Fibre
Fish meal	66.5	10.1	1.5	20.8	1.1
Blood meal	91.0	1.1	1.8	5.4	0.8
Meat&bone meal	53.0	11.1	0.8	32.3	2.8
Soy flour	50.2	1.4	34.6	6.9	6.9
Wheat flour	13.7	1.9	79.6	1.9	2.8
Corn flour	10.9	4.4	80.6	1.7	2.3
Oil	0	99.9	0	0.1	0
Salt	0	0	0	100	0
Premix	0	0	50	50	0

Data are based on proximate composition of ingredients as given by Van der Meer & Verdegem (1996).

Based on the above mentioned considerations, the linear regression consisted of the following five equations:

$$\begin{aligned}
 \text{(a) } MPR_{adlib} &= \alpha_a + \beta_{2a} * DPROT + \beta_{3a} * SOYA + \epsilon_a; \\
 \text{(b) } MGR &= \alpha_b + \beta_{1b} * MPR + \beta_{2b} * DPROT + \beta_{3b} * SOYA + \beta_{4b} * W_{geo} + \epsilon_b; \\
 \text{(c) } BP &= \alpha_c + \beta_{1c} * MPR + \beta_{2c} * DPROT + \beta_{3c} * SOYA + \beta_{4c} * WF + \epsilon_c; \\
 \text{(d) } BL &= \alpha_d + \beta_{1d} * MPR + \beta_{2d} * DPROT + \beta_{3d} * SOYA + \beta_{4d} * WF + \epsilon_d; \\
 \text{(e) } BA &= \alpha_e + \beta_{1e} * MPR + \beta_{2e} * DPROT + \beta_{3e} * SOYA + \beta_{4e} * WF + \epsilon_e;
 \end{aligned}$$

Standardized partial regression coefficients (called  $\beta$ -weights) can be used to compare the effects of variables expressed in different units on a dependent variable (Yamane 1973) and are calculated as follows:  $\beta\text{-weight}_{1,k} = \beta_{1,k} * (sd_{x1,k} / sd_y)$ , with  $sd_{x1,k}$  and  $sd_y$  being the standard deviations of the independent and the dependent variable, respectively.

The variables DPROT and SOYA are percentages. In linear regression analysis transformation of proportions to arc sinus is recommended if variations within one variable are high (Snedecor and Cochran, 1989). Transformation of DPROT and SOYA to their arc sinus equivalents changed slightly the model sum of squares, but did not affect signs nor significance levels of the regression parameters. As regression coefficients are more difficult to interpret when variables are transformed, the untransformed variables were maintained in the regression analysis.

All calculations were executed by a statistical software package (SAS, version 6.04).

### *Fish Growth Simulator*

The FGS model (described by Machiels (1987) and Van Dam & Penning de Vries (1995)) is based on mass balances in which the protein and lipid biomass is calculated from the initial biomass increased with the amount deposited and reduced by the amount oxidized. Synthesis and oxidation rates are based on the stoichiometry of the reaction equations of the intermediary metabolism. The input requirements of the FGS and their settings can be found in Table 5. The main principles which rule the destiny of dietary protein, lipids and carbohydrates are:

1. Based on experimental data (Van der Meer et al 1997a; Van der Meer et al. 1997b), feed losses were calculated by assuming that the percentage of uneaten feed increased linearly from 0 to 25% between 60% and 100% of the *ad libitum* level (Figure 1). For each experiment, the *ad libitum* protein ration was defined as the average protein intake of the treatment resulting in the highest growth rate. All feed given above the *ad libitum* level was assumed to remain uneaten.

2. Protein and fat are synthesized from digested nutrients (amino acids, fatty acids and glucose). Five percent of digested amino acids are deaminated and oxidized.

3. The total metabolic rate consists of the sum of:

- a. routine metabolic rate ( $R_{met}$ ) defined as:  $R_{met} = \alpha \cdot W^{0.8}$  ( $\text{kJ kg}^{-0.8} \text{ d}^{-1}$ ) where  $\alpha$  is a coefficient and  $W$  = fresh body weight. Routine metabolism of *C. macropomum* at 100% oxygen saturation ranged from 349 to 628  $\text{mg O}_2 \text{ d}^{-1}$  in 137-371 g fish (Saint-Paul 1988. We converted these data to an average of 1463  $\text{mg O}_2 \text{ kg}^{-0.8} \text{ d}^{-1}$  or 19.4  $\text{kJ kg}^{-0.8} \text{ d}^{-1}$ .

- b. feeding metabolic rate: the energy cost of feeding, digestion and absorption, assumed to be 4.5% of the gross energy ingested.

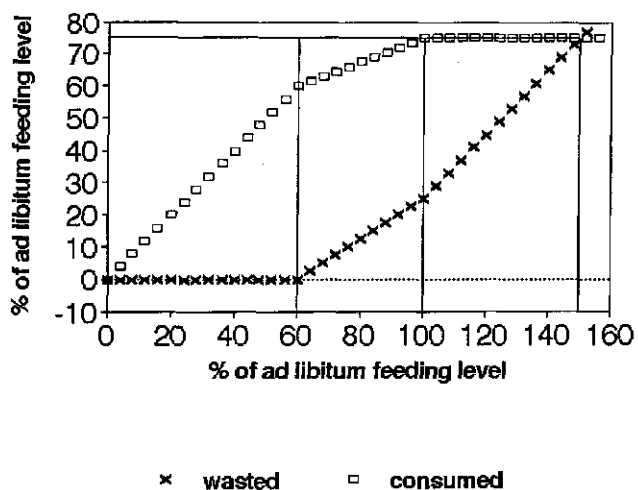
- c. cost of biosynthesis: synthesis of one gram protein costs 5.4 kJ; deposition of one gram lipid costs 1.2 kJ.

4. The part of the energy requirement satisfied by oxidation of body fat (AALIRAT) was related to the protein ration (MPR) and the dietary protein to energy ratio (P/E ratio), using the following equation:  $\text{AALIRAT} = 0.95 - c_1 \cdot \text{MPR} - c_2 \cdot \text{PERATIO} \cdot (\text{MPR})^{0.25}$ , where  $c_1$  and  $c_2$  are constants. At fasting ( $\text{MPR}=0$ )  $\text{AALIRAT} = 0.95$ , which means that 95% of the energy is supplied by oxidation of body fat. At high MPR levels AALIRAT becomes zero and all energy is supplied by protein oxidation. The body lipid level can not drop below 1% of the body fresh weight. Oxidation of protein and lipid yields 19.1 and 39.4 kJ per gram, respectively.

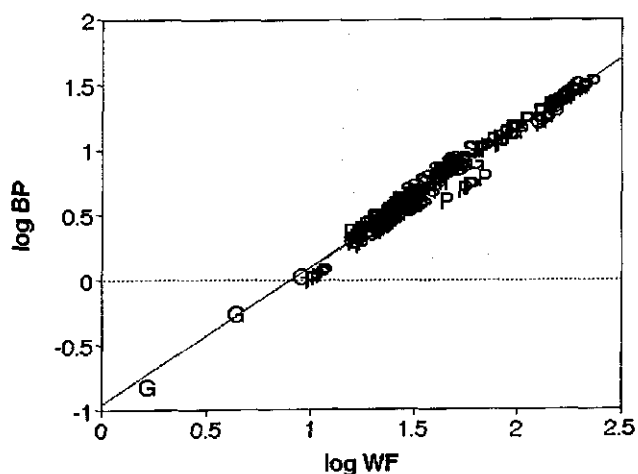
5. Fish fresh weight is computed directly from the protein biomass. Fat deposition is assumed to be accompanied by the loss of an equal amount of body water and does not affect fish weight. From our experimental data the following equation was derived for *C. macropomum* (Shearer 1994):  $\log(\text{BP}) = -0.9613 + 1.0611 \cdot \log(\text{WF})$ ,  $r^2=0.977$  (Figure 2).

After providing the model with the required input data (initial body weight, feeding regime, feed composition, temperature), final fresh weight and lipid biomass were simulated. Calibration of the model consisted of finding values for  $c_1$  and  $c_2$  (in the AALIRAT-equation) that resulted in the best simulation results for fresh weight and lipid biomass. In addition, the model calculated the average metabolic feeding level for the whole culture period and the average AALIRAT.

**Figure 1.** The relationship between feeding level and amounts of feed remaining uneaten as used by the Fish Growth Simulator.



**Figure 2.** The relationship between fish weight and body protein content as used by the Fish Growth Simulator.



Letters in the graphics refer to experiments (see Table 1).

**Table 5.** Input requirements of the Fish Growth Simulator and their sources.

Input (dimension)	Type*)	Source	References**)	Value***)
<i>Fish</i>				
W1	e	observed	1-5	
BPROT (initial)	e	observed	1-5	
BLIPID (initial)	e	observed	1-5	
proportion of digested protein used for gluconeogenesis (-)	e	estimated	7	0.05
proportion of energy supplied by fat, AALIRAT (-)	s	calibrated	this study	variable
routine metabolism, exponent (-)	s	estimated	6	0.8
routine metabolism, constant ( $\text{kJ g}^{-0.8} \text{ day}^{-1}$ )	s	estimated	6	0.081165
cost of protein synthesis ( $\text{kJ g}^{-1}$ )	u	estimated	7	5.7975
cost of lipid synthesis ( $\text{kJ g}^{-1}$ )	u	estimated	7	1.1595
protein digestibility (-)	s, e	calibrated	this study	0.80
lipid digestibility (-)	s, e	calibrated	this study	0.80
carbohydrate digestibility (-)	s, e	calibrated	this study	0.60
minimum body lipid content (% fresh weight)	s	estimated	7	1.0
<i>Feed</i>				
DPROT	e	observed	1-5	
DLIPID	e	observed	1-5	
DCARBO	e	observed	1-5	
MFR	e	observed	1-5	
Feed losses	s	estimated	this study	
<i>Environment</i>				
length of period (days)	e	observed	1-5	
temperature ( $^{\circ}\text{C}$ )	e	observed	1-5	

\*) Types: e, characterizes experiment; s, characterizes species; u, assumed universal to fish.

\*\*) References: 1. Günther & Boza (1993); 2. Van der Meer et al. (1995); 3. Van der Meer et al. (1997a); 4. Van der Meer et al. (1997c); 5. Van der Meer et al. (1995); 6. Saint-Paul (1988); 7. Van Dam & Penning de Vries (1995).

\*\*\*) Information about observed values can be found in Table 2; procedures and considerations to determine the estimated and calculated values are described in the text.

## Performance of the models

### Linear regression analysis

To visualize the results of the linear regression analysis four hypothetical experiments were formulated (Table 7). Feeds were composed of the same ingredients as used in previous experiments (Table 4).

In experiment A fish were fed *ad libitum* with diets containing either 15, 24, 32, 40 or 49% protein. In experiment B the same diets were used, but feed rations were adjusted so that fish of all treatments received the *ad libitum* protein ration of the 15% protein diet. In experiment C all fish received the 40% protein diet, but feed rations varied between approximately 10 and 100% of the *ad libitum* feeding level. In experiment D all diets had identical protein and lipid levels, but the percentage of soya protein varied between 0 and 92.1% of the total dietary protein. For all experiments feed conversion (FC: g dry feed/g wet body weight gain) and net protein utilization (NPU: g protein gain/g protein ration) were calculated.

### *Fish Growth Simulator*

A sensitivity analysis was performed to assess the importance of various input and parameter values of the FGS model. Using the calibrated model, growth of a fish with an initial weight of 1 g, an initial fat percentage of 15%, which was kept at a temperature of 29°C, fed a 35% protein diet at a 25 g kg<sup>-0.8</sup> d<sup>-1</sup> during 28 days was simulated. For each parameter, simulations were performed with parameter values 10% below and 10% above the 'standard setting' and all other parameter values in the 'standard setting'. The effects of these variations on fresh weight and fat biomass were expressed as a percentage of the value of the parameter in the 'standard setting'.

### *Relative errors*

For both models, relative errors (REs) were calculated as:  $RE = 100\% * (FIT - OBS) / ((FIT + OBS) / 2)$ , in which FIT and OBS are the fitted and observed values, respectively.

## RESULTS

### Calibration of the models

#### *Linear regression analysis*

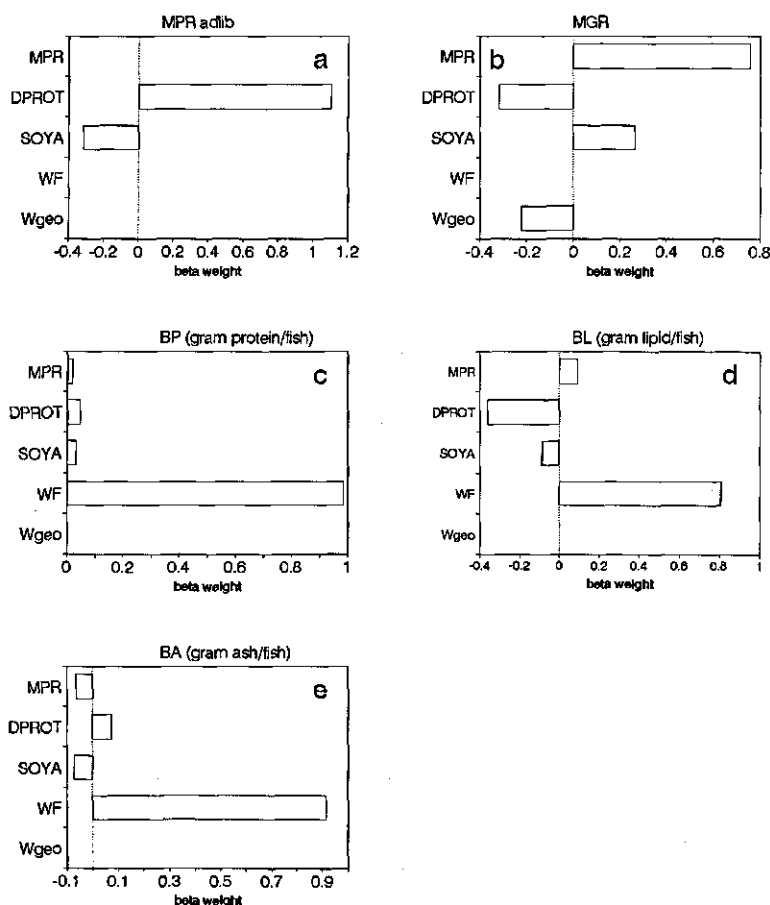
The models for *ad libitum* feed uptake and growth rate had  $r^2$ s of 0.78 and 0.86, respectively (Table 6). Models for BP and BA had  $r^2$ s close to unity, but description of BL was less accurate ( $r^2=0.63$ ). MPR was not significant in the BL model. All other explaining variables contributed significantly ( $P<0.05$ ) to all models.

The  $\beta$ -weights showed that the *ad libitum* protein uptake of *C. macropomum* is mainly determined by the dietary protein content (Figure 3a), while protein ration is the main factor determining growth rate (Figure 3b). Body protein, lipid and ash content were mainly determined by body weight (Figures 3c and 3e, respectively). Body lipid content was also significantly affected by the dietary protein content (Figure 3d).

### *Fish Growth Simulator*

Best fit between simulated and observed values was obtained with digestibilities for protein, carbohydrates and fat of 80, 60 and 60%, respectively. Values of  $c_1$  and  $c_2$  of the AALIRAT equation were 0.06456 and 0.009046, respectively (Figure 8).

Average metabolic protein rations for the whole culture period ranged from 0 (fasting fish in FLVEL) to a maximum of 11.46 g kg<sup>-0.8</sup> day<sup>-1</sup> in SOYA. In all experiments except FLVL2, the maximum MPR was around 11 g kg<sup>-0.8</sup> day<sup>-1</sup>. There was more variability in MPR, ranging from 0 in FLVEL to 41.99 g kg<sup>-0.8</sup> day<sup>-1</sup> in PROT. Average values of AALIRAT ranged from 0.33 in PROT to 0.95 for fasting fish in FLVEL.

**Figure 3.** The beta-weights of the explaining variables of the linear regression equations.**Table 6.** Coefficients ( $\beta$ 's) and sum of squares of the linear regression analysis.

Equation	(1)	(2)	(3)	(4)	(5)
Dependent variable	MPR <sub>adlib</sub>	MGR	BP	BL	BA
Explaining variables					
Intercept	2.164 n.s.	25.24 ***	-1.954 ***	6.145 ***	-0.04903 n.s.
MPR		1.887 ***	0.01868 ***	0.05310 n.s.	-0.01489 ***
DPROT	0.6175 ***	-0.5261 ***	-0.03116 *	-0.1429 ***	0.01190 ***
SOYA	-0.06408 ***	0.1624 ***	0.008059 ***	-0.01259 *	-0.004126 ***
Wgeo		-0.1022 ***			
WF			0.1461 ***	0.07190 ***	0.03283 ***
Mean squares (degrees of freedom)					
Model	1625 (2)	17930 (4)	3242 (4)	739.4 (4)	186.4 (4)
Error	8.994 (101)	56.09 (228)	0.6208 (228)	7.787 (228)	0.06613 (228)
r <sup>2</sup>	0.78	0.85	0.99	0.63	0.98

n.s.  $P > 0.05$  (not significant); \*  $P < 0.05$ ; \*\*  $P < 0.005$ ; \*\*\*  $P < 0.0005$ .

## Performance of the models

### *Linear regression analysis: hypothetical experiments*

Results of the hypothetical experiments can be found in Table 7.

In experiment A increasing dietary protein levels decreased the *ad libitum* feed uptake and increased growth rate. Consequently, the FC improved with the dietary protein level. However, the NPU decreased with increasing dietary protein concentration. Body fat concentration decreased and body protein levels increases with increasing dietary protein levels.

Compared to experiment A, protein rations were lower in experiment B which resulted in lower body protein and lipid contents and better FCs and NPUs. Trends in all these parameters in dependence of the dietary protein concentration were similar to those in experiment A. However, growth rate decreased with increased dietary protein concentration; in this aspect experiments A and B contrast sharply.

Increasing the feed rations (experiment C) resulted in linearly increasing growth rates. Body ash content decreased with increasing growth rate. Feed utilization was extremely efficient at the lowest ration.

In experiment D feed uptake decreased with increasing amounts of soya in the diet, but growth rate was not significantly affected. Therefore FC and NPU improved when animal protein was substituted by soya protein. Addition of soya results in higher body protein levels and lower ash contents.

### *Fish Growth Simulator: sensitivity analysis*

Results of the sensitivity analysis are presented in Table 8.

Fresh weight was affected most strongly by protein digestibility (10% increase led to a 36% increase in weight). Other factors strongly affecting fresh weight were DPROT (+32%), MFR (+29%), temperature (-13%) and cost of protein synthesis (-12%). The parameters defining metabolic expenditure (coefficient and exponent of routine metabolism, coefficient of feeding metabolism) had effects varying from -5 to +9%.

Fat biomass was affected most strongly by MFR (+29%), protein digestibility (+21%) and DPROT (+19%). All other factors caused changes smaller than 10%.

### *Accuracy of the models*

The linear model had a mean RE of 1.1 for the final fresh weight (Table 9). All final weights in experiment FFREQ were underestimated (average RE -65.3). The regression model also underestimated by more than 20% the lipid deposition of the experiments FLVEL and FLVL2. In FLVEL RE's of the body lipid content varied largely, ranging from -200 to +244 (Table 9).

Best simulations of fresh weight with the FGS were obtained with the experiments FLVEL, FLVL2 and SOYA (Figure 4), having mean RE's of less than +/-10% (Table 9). PROT and LIPID had larger mean RE's for fresh weight (+21.4 and -30.1%, respectively). All fresh weights of FFREQ were underestimated by the FGS (average RE -27.5%). The range of RE's was relatively narrow for FLVEL (minimum -13, maximum 15) and FLVL2 (-23, 16). In all other experiments, RE's of the final weight varied between -48 and +48%. In SOYA, the final weights obtained with the 20% protein feed were systematically underestimated while those of the 40% feed were overestimated.

**Table 7.** Effects of variations in feed composition and feed ration on feed uptake, growth and body composition of *C. macropomum*. \*)

A	Dietary protein concentration					B	Dietary protein/non-protein ratio				
	DIET						DIET				
	1	2	3	4	5		1	2	3	4	5
EXPERIMENTAL SETTINGS											
INGREDIENTS						INGREDIENTS					
Fishmeal	4	13	22	31	40	Fishmeal	4	13	22	31	40
Bloodmeal	0.8	2.6	4.4	6.2	8	Bloodmeal	0.8	2.6	4.4	6.2	8
Bone&meat meal	0.4	1.3	2.2	3.1	4	Bone&meat	0.4	1.3	2.2	3.1	4
Soy flour	2	6.5	11	15.5	20	Soy flour	2	6.5	11	15.5	20
Wheat flour	30.9	25.5	20.1	14.7	9.3	Wheat flour	30.9	25.5	20.1	14.7	9.3
Corn flour	58.0	47.8	37.5	27.2	17.0	Corn flour	58.0	47.8	37.5	27.2	17.0
Oil	2.4	1.8	1.3	0.8	0.2	Oil	2.4	1.8	1.3	0.8	0.2
PROXIMATE COMPOSITION (IN % OF FEED DM).						PROXIMATE COMPOSITION (IN % OF FEED DM).					
DPROT	15.2	23.7	32.2	40.7	49.2	DPROT	15.2	23.7	32.2	40.7	49.2
DLIPID	6.0	6.0	6.0	6.0	6.0	DLIPID	6.0	6.0	6.0	6.0	6.0
DCARBO	72.4	61.6	50.7	39.8	29.1	DCARBO	72.4	61.6	50.7	39.8	29.1
DASH	4.0	6.3	8.6	10.8	13.1	DASH	4.0	6.3	8.6	10.8	13.1
SOYA	6.6	13.8	17.2	19.1	20.4	SOYA	6.6	13.8	17.2	19.1	20.4
FEED RATION AND GROWTH (in g/kg <sup>0.8</sup> /day)						FEED RATION AND GROWTH (in g/kg <sup>0.8</sup> /day)					
MPR	11.1	15.9	20.9	26.0	31.2	MPR	11.1	11.1	11.1	11.1	11.1
MFR	73.2	67.2	65.1	64.1	63.5	MFR	73.2	46.9	34.5	27.3	22.6
RESULTS DERIVED FROM THE LINEAR REGRESSION MODEL **)											
MPRadlib	11.1a	15.9b	20.9c	26.0d	31.2e	MPRadlib	11.1a	15.9b	20.9c	26.0d	31.2e
MGR	38.8a	46.5b	52.2c	56.2d	58.5e	MGR	38.8a	35.4ab	31.5b	27.4c	23.1c
BODY COMPOSITION (IN % OF WET WEIGHT).						BODY COMPOSITION (IN % OF WET WEIGHT).					
BPROT	10.1a	11.6b	13.0c	14.4d	15.8d	BPROT	10.1a	11.3ab	12.4bc	13.4cd	14.4d
BLIPID	23.8a	19.9ab	16.2bc	12.7cd	9.1d	BLIPID	23.8a	19.0a	14.3b	9.7bc	5.2c
BASH	3.06a	3.06a	3.10a	3.16a	3.23a	BASH	3.06a	3.32ab	3.64bc	3.99cd	4.34d
BDM	36.9	34.6	32.4	30.3	28.2	BDM	36.9	33.6	30.3	27.1	23.9
FEED UTILIZATION EFFICIENCY.						FEED UTILIZATION EFFICIENCY.					
FC	1.89	1.51	1.30	1.15	1.04	FC	1.89	1.32	1.09	1.00	0.98
NPU	0.35	0.32	0.31	0.31	0.31	NPU	0.35	0.36	0.35	0.33	0.30

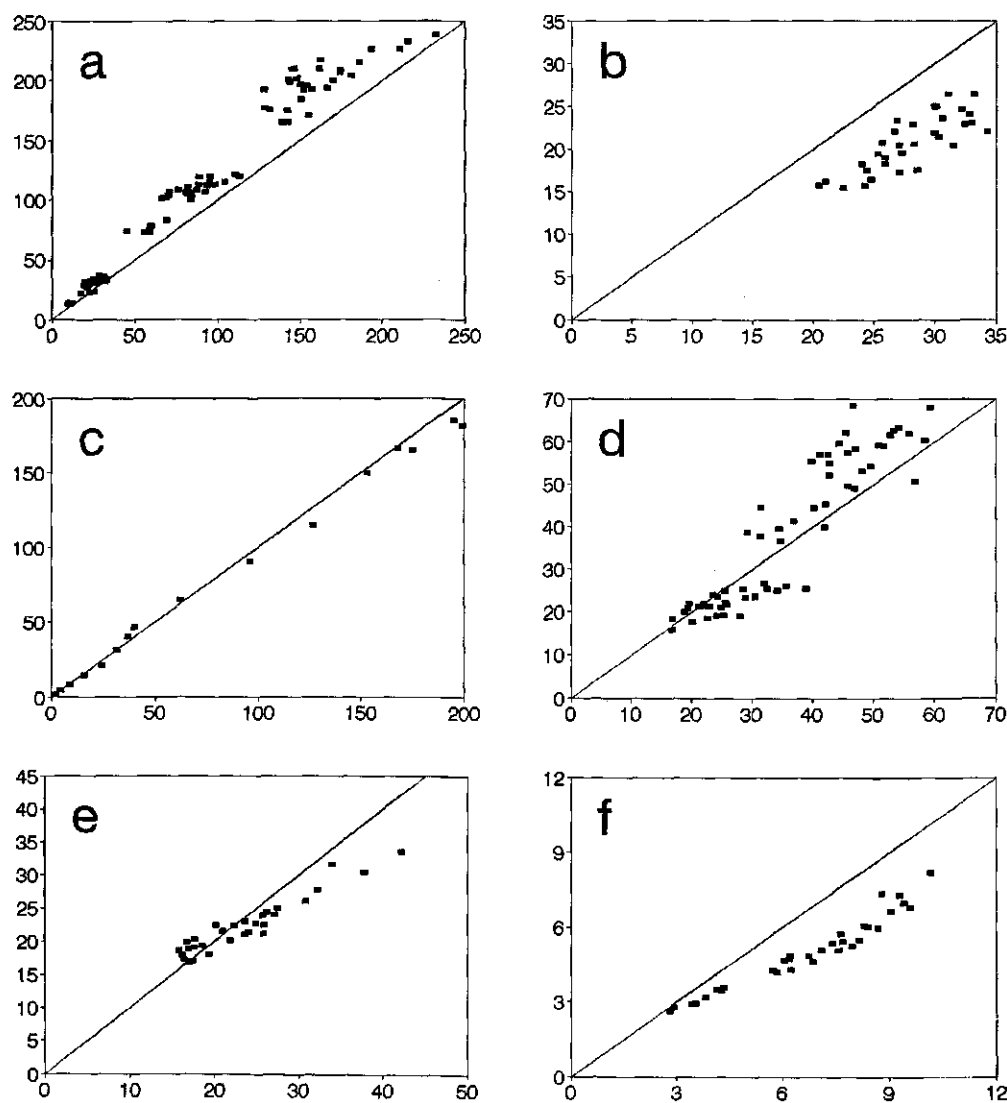
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Table 7 =continued=

C	Feed ration TREATMENT					D	Percentage protein of soya origin DIET				
	1	2	3	4	5		1	2	3	4	5
<b>EXPERIMENTAL SETTINGS</b>											
<b>INGREDIENTS</b>						<b>INGREDIENTS</b>					
Fishmeal	31	31	31	31	31	Fishmeal	40	30	20	10	0
Bloodmeal	6.2	6.2	6.2	6.2	6.2	Bloodmeal	4	3	2	1	0
Bone&meat meal	3.1	3.1	3.1	3.1	3.1	Bone&meat	4	3	2	1	0
Soy flour	15.5	15.5	15.5	15.5	15.5	Soy flour	0	17.5	35	52.5	70.3
Wheat flour	14.7	14.7	14.7	14.7	14.7	Wheat flour	19.4	21.7	22.0	22.3	21.3
Corn flour	27.2	27.2	27.2	27.2	27.2	Corn flour	30.0	21.0	14.0	7.0	1.0
Oil	0.8	0.8	0.8	0.8	0.8	Oil	1.1	2.3	3.5	4.7	5.9
<b>FEED COMPOSITION (IN % OF FEED DM).</b>						<b>FEED COMPOSITION (IN % OF FEED DM).</b>					
DPROT	40.7	40.7	40.7	40.7	40.7	DPROT	38.3	38.3	38.3	38.3	38.3
DLIPID	6.0	6.0	6.0	6.0	6.0	DLIPID	7.3	7.3	7.3	7.3	7.3
DCARBO	39.8	39.8	39.8	39.8	39.8	DCARBO	40.6	41.0	41.5	42.0	42.3
DASH	10.8	10.8	10.8	10.8	10.8	DASH	12.0	10.6	9.2	7.9	6.5
SOYA	19.1	19.1	19.1	19.1	19.1	SOYA	0.0	22.9	45.9	68.9	92.1
<b>FEED RATION AND GROWTH (in g/kg<sup>0.8</sup>/day)</b>						<b>FEED RATION AND GROWTH (in g/kg<sup>0.8</sup>/day)</b>					
MPR	3.0	9.0	15.0	21.0	26.0	MPR	25.8	24.4	22.9	21.4	19.9
MFR	7.4	22.1	36.9	51.6	63.9	MFR	67.4	63.6	59.7	55.9	52.0
<b>RESULTS DERIVED FROM THE LINEAR REGRESSION MODEL **)</b>											
MPRadlib	26.0a	26.0a	26.0a	26.0a	26.0a	MPRadlib	25.8a	24.4ab	22.9bc	21.4cd	19.9d
MGR	12.1a	23.4b	34.7c	46.1d	55.5e	MGR	53.3a	54.2a	55.2a	56.1a	57.1a
<b>BODY COMPOSITION (IN % OF WET WEIGHT).</b>						<b>BODY COMPOSITION (IN % OF WET WEIGHT).</b>					
BPROT	12.8a	13.3a	13.7a	14.1a	14.4a	BPROT	13.6a	14.2ab	14.7abc	15.3cd	15.9d
BLIPID	8.1a	9.3a	10.5a	11.7a	12.7a	BLIPID	14.8a	13.4a	12.0a	10.7a	9.3a
BASH	4.44a	4.10ab	3.71bc	3.44cd	3.17d	BASH	3.37a	3.10ab	2.83bc	2.56cd	2.28d
BDM	25.4	26.7	27.9	29.2	30.3	BDM	31.7	30.7	29.6	28.6	27.5
<b>FEED UTILIZATION EFFICIENCY.</b>						<b>FEED UTILIZATION EFFICIENCY.</b>					
FC	0.61	0.95	1.06	1.12	1.15	FC	1.27	1.17	1.08	1.00	0.91
NPU	0.52	0.35	0.32	0.31	0.31	NPU	0.28	0.32	0.36	0.41	0.46

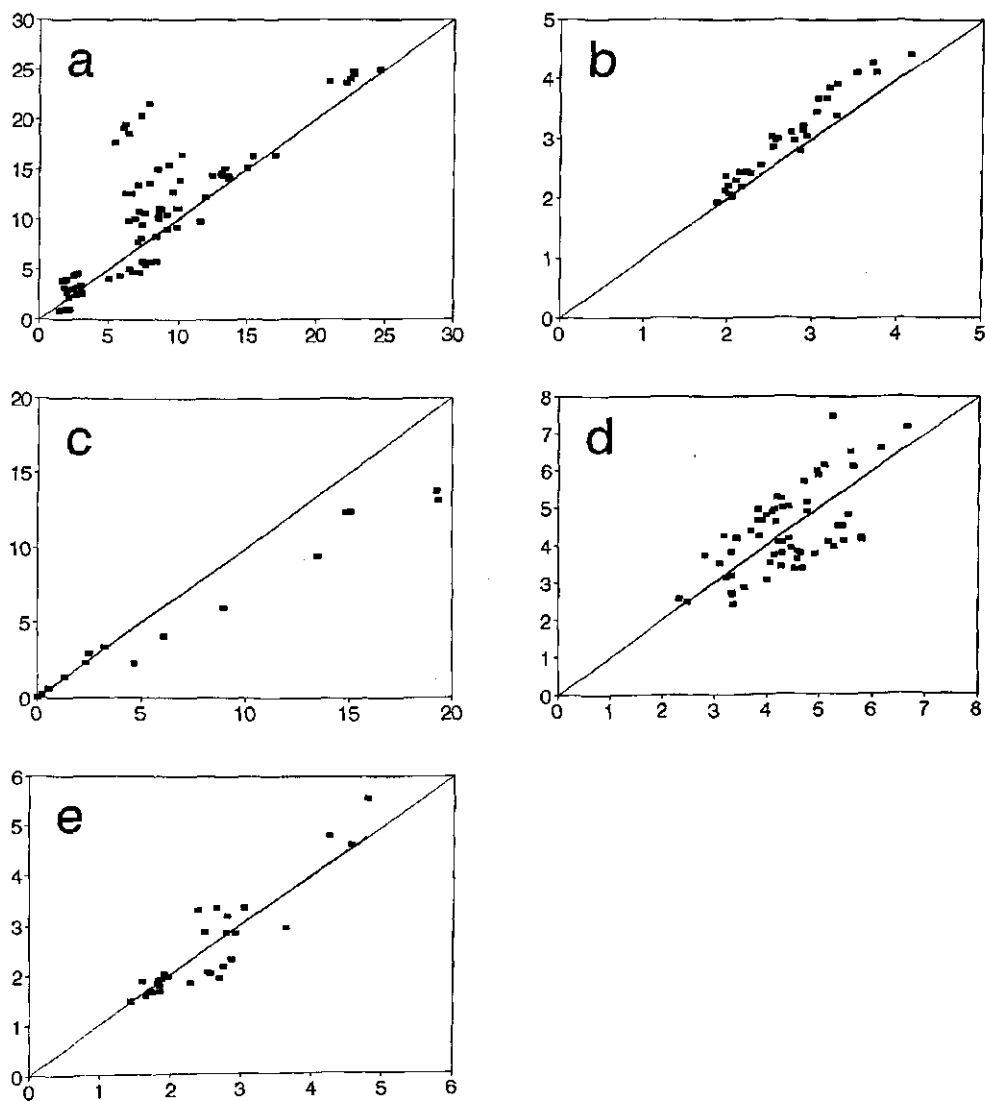
\*) Final weight of all fish was assumed to be 27 grams. All diets contain 1% of salt and 0.5% of a vitamin/mineral premix. The proximate composition of the ingredients as shown in Table 4 was used to calculate the composition of the diets. Subsequently MPRadlib was calculated with equation (1) of Table 6. MPR, the actual protein ration, was set to the MPRadlib level in fish fed *ad libitum* and to below this level when feed was restricted. The growth rate (MGR) was then calculated from equation (2) of Table 6. Amounts of protein, lipid and ash (BP, BL and BA, respectively) in the fish were calculated from equations (3), (4) and (5) of Table 6. BP, BL and BA were then divided by the final weight of the fish and multiplied by 100 to get BPROT, BLIPID and BASH, respectively. Body dry matter content (BDM) was calculated as the sum of BPROT, BLIPID and BASH. Feed conversion (FC) and net protein utilization (NPU) were calculated as:  $FC = F/(WF-WI)$  and  $NPU = (BP-0.12)/(F \cdot DPROT/100)$ , where: F = feed ration in g dry feed fish<sup>-1</sup>. Fish at the start of the experiment were expected to weight 1 gram and contain 12% of protein.

\*\*) MPRadlib, BPROT, BLIPID and BASH were directly calculated from the regression equations of Table 6. These parameters, within one experiment, do not differ significantly when sharing the same *italic* letter. The difference between two estimates was called significant when their 95% confidence intervals did not overlap.

**Figure 4.** Simulation results of the FGS: fresh weight.

Legend: a = PROT; b = LIPID; c = FLVEL; d = SOYA; e = FLVL2; f = FFREQ.

Figure 5. Simulation results of the FGS: lipid biomass.



Legend: see Figure 4.

**Table 8.** Results of the sensitivity analysis of the FGS: variation in the simulated fresh body weight and lipid biomass due to a 10% decrease or increase of several parameters.

Parameter	Fresh body weight *)		Lipid biomass *)	
	-10%	+10%	-10%	+10%
temperature	+12	- 13	+ 7	- 8
MFR	- 23	+29	- 23	+29
DPROT	- 23	+32	- 15	+19
DCARBO	+ 2	- 2	- 5	+ 5
DLIPID	+ 1	- 1	- 3	+ 3
carbohydrate digestibility	0	0	- 6	+ 6
lipid digestibility	0	0	- 3	+ 3
protein digestibility	- 28	+36	- 18	+21
exponent routine metabolism	+ 9	- 9	+ 4	- 4
coefficient routine metabolism	+ 6	- 6	+ 4	- 4
coefficient feeding metabolism	+ 5	- 5	+ 3	- 3
cost of protein synthesis	+14	- 12	+ 8	- 8
cost of lipid synthesis	0	0	0	0
c <sub>1</sub> of AALIRAT equation	+ 1	0	0	0
c <sub>2</sub> of AALIRAT equation	0	0	0	0
Reference value (100%)	20.6 gram		1.86 gram	

\*) Deviations of simulated fresh body weight and lipid biomass from the standard values of 20.6 and 1.86 grams, respectively, are expressed as a percentage of the standard value. The standard values are the simulated results of a fish with an initial weight of 1 g and an initial body lipid content of 10%, fed a 40.7% protein diet (diet 4 of Table 7A) at a ration of 27.5 g kg<sup>-0.8</sup> day<sup>-1</sup> and kept at a temperature of 29°C.

**Table 9.** The relative errors (RE's) of the simulated final weight and the body lipid content.

		LINEAR REGRESSION		FISH GROWTH SIMULATOR	
		avg	( min, max )	avg	( min, max )
Final weight	PROT	11.4	( -39, 74 )	21.4	( -10, 48 )
	FLVEL	-18.0	( -54, 92 )	- 3.6	( -13, 15 )
	FLVL2	- 6.8	( -55, 43 )	- 3.6	( -23, 16 )
	LIPID	-20.5	( -36, 0 )	-30.1	( -48, -14 )
	SOYA	5.1	( -39, 46 )	2.6	( -42, 38 )
	FFREQ	-65.3	( -83, -28 )	-27.5	( -42, - 5 )
	ALL*)	1.1	( -55, 92 )	4.4	( -48, 48 )
Body lipid content	PROT	10.1	( -55, 101 )	16.3	( -72, 106 )
	FLVEL	-22.1	( -200, 244 )	-16.6	( -70, 15 )
	FLVL2	-21.7	( - 7, 56 )	- 2.4	( -33, 32 )
	LIPID	-1.0	( -37, 46 )	9.9	( - 1, 19 )
	SOYA	-8.5	( -27, 54 )	- 0.3	( -33, 35 )
	FFREQ	..... no data .....		..... no data .....	
	ALL	7.6	( -200, 244 )	6.4	( -72, 106 )

\*) Data of FFREQ not included.

Relative errors of the linear regression analysis and the FGS are depicted in Figure 6. Outliers were more frequently produced by the linear regression method than by the FGS. With the regression analysis RE's above +50 were found for non-fed fish (FLVEL), fish fed either the lowest (17%) or the highest (62%) protein diets (PROT). RE's below -50 were found in most records of FFREQ and in some of the low feeding levels of FLVEL. The linear regression model produced RE's for the lipid content above +50 for two groups of fish of PROT. However, the FGS produced similar RE's for the same groups, which might point to irregularities in these records rather than to defects of the models. The regression analysis also produced outliers for the lipid content of fish of FLVEL receiving the lowest feeding level (RE -200) or non-fed fish (RE +244; Figure 6).

Figure 1 consists of two scatter plots, (a) and (b), showing the relationship between the RE of the linear regression model (Y-axis) and the RE of the FGS (X-axis).

Plot (a) is titled "Fresh weight". The Y-axis is labeled "RE of the linear regression model" and ranges from -100 to 100. The X-axis is labeled "RE of the FGS" and ranges from -100 to 100. A rectangular box is drawn around the central cluster of data points, approximately from -40 to 40 on both axes. Several points are labeled with letters: 'G' at the top, 'P' at the top right, 'S' at the top left, and 'H' at the bottom left.

Plot (b) is titled "Body lipid content". The Y-axis is labeled "RE of the linear regression model" and ranges from -120 to 120. The X-axis is labeled "RE of the FGS" and ranges from -120 to 120. A rectangular box is drawn around the central cluster of data points, approximately from -40 to 40 on both axes. Several points are labeled with letters: 'G' at the top, 'P' at the top left, 'S' at the top left, and 'H' at the bottom left. Two specific points are labeled with coordinates: "G (0,244)" and "G (0,-200)".

## DISCUSSION

### Growth and body composition of *C. macropomum*

*Ad libitum* protein uptake of *C. macropomum* is mainly determined by the dietary protein content of the diet (Figure 3a), while protein uptake is the main factor determining growth. The high  $\beta$ -weight of DPROT in the MPRadlib model and of DPROT and MPR in the MGR model (Figures 3a and 3b) with the high coefficients of determination of these models (Table 6) demonstrate that

growth of *ad libitum* fed fish mainly depends on the dietary protein concentration. However, the effect on growth diminishes when DPROT increases. Consequently, higher dietary protein concentrations are associated with lower NPU's (Table 7A), even when protein rations of the different diets are kept equal (Table 7B). Nevertheless, the effect of changes in the dietary protein concentration on the NPU is small compared to effects due to feed restriction (Table 7C) or changes in dietary protein composition (Table 7D). Only feed restrictions severely impairing growth rates improve the NPU (Table 7C). In consequence, optimalization of the dietary protein composition is the best option to improve NPU's when the *ad libitum* feeding level is not surpassed.

*C. macropomum* can realize higher growth rates than many other species (Günther & Boza 1993) and can quickly change its body composition (Van der Meer et al. 1995; Van der Meer et al. 1997c). This makes the fish an interesting object in nutritional studies: differences between treatments become evident within short experimental periods.

#### Body composition

Higher dietary protein concentrations result in fish with a higher body protein concentration (Tables 7A and 7B; Van der Meer et al. 1995; Van der Meer et al. 1996; Van der Meer et al. 1997c). However, the low  $\beta$ -weight of DPROT in the BPROT model suggests that this effect might be easily overshadowed by effects of WF, SOYA and MPR (Figure 3). Shearer (1994) pointed out that many authors fail to distinguish between the effects of diet composition and body weight. He used an allometric analysis in which the logarithm of the weight of a proximate component was regressed against the logarithm of fish weight. He concluded that most claims that diet composition affects body composition cannot be substantiated. However, his method results in high  $r^2$ s even when treatments obviously affect body protein content (e.g. Figure 7b).

The allometric analysis of Shearer (1994) described well our data (model 1 of Table 10). Inclusion of DPROT as the second explaining variable in the allometric model resulted in a small, however significant, increase of the  $r^2$  (model 2 in Table 10). Calculation of the body protein percentage based on model 2 showed that substantial variation in the body protein content was due to DPROT (Figure 7a). SOYA and MPR as well had significant effects when included in the model (model 3 of Table 10). Therefore, in *C. macropomum* not only body weight, but also feed quality (DPROT, SOYA) and feed quantity (MPR) affect the body protein percentage.

Differences in body protein content of fish may be caused by changes in the relative weights of carcass and internal organs (Shearer 1994). Internal organs have a stable protein content (Weatherley & Gill 1983), which is significantly lower than the protein content of the muscle (Van der Meer et al. 1997c). In *C. macropomum* higher dietary lipid levels caused an increased weight of the internal organs and lower body protein contents (Van der Meer et al. 1997c). The 'conflicting opinions concerning the effect of dietary protein level on the body protein' (Shearer 1994) might have their origin in differences between species to adapt the size of their internal organs to changing feed composition and feed quantity. Possibly, fish which in their natural habitat have to cope with large differences in either food availability or food composition (such as *C. macropomum*), adapt easier the size of their internal organs than fish having a more constant natural diet (such as salmonids).

The body lipid content of *C. macropomum* is far more flexible than the protein and ash content: minimum and maximum values had a 1:20, 1:1.8 and 1:2.5 ratio for lipid, protein and ash,

**Table 10.** Models relating the body protein content to body weight, feed quality and feed quantity.

Model	Variable	Coefficient	P	Mean sum of squares (degrees of freedom)		
				Model	Error	r <sup>2</sup>
1	Intercept	-0.9613	0.0001	31.59 (1)	0.00318 (231)	0.977
	log(WF)	1.0611	0.0001			
2	Intercept	-1.0059	0.0001	15.86 (2)	0.00256 (230)	0.982
	log(WF)	1.0391	0.0001			
	DPROT	0.002183	0.0001			
3	Intercept	-1.0806	0.0001	7.99 (4)	0.00178 (228)	0.987
	log(WF)	1.0729	0.0001			
	DPROT	0.001872	0.0001			
	SOYA	0.000887	0.0001			
	MPR	0.001641	0.0006			

Model 1:  $\log(\text{BP}) = \alpha + \beta \cdot \log(\text{WF})$ ; Model 2:  $\log(\text{BP}) = \alpha + \beta \cdot \log(\text{WF}) + \gamma \cdot \text{DPROT}$  and Model 3:  $\log(\text{BP}) = \alpha + \beta \cdot \log(\text{WF}) + \gamma \cdot \text{DPROT} + \delta \cdot \text{SOYA} + \epsilon \cdot \text{MPR}$ ; where: BP = amount of body protein in grams;  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$  = coefficients of intercept, log(WF), DPROT, SOYA and MPR, respectively.

respectively (calculation based on Table 2). Higher dietary protein contents consistently resulted in lower body lipid contents (Van der Meer et al. 1995; Van der Meer et al. 1996; Van der Meer et al. 1997c). Van der Meer et al. (1997c) suggested that the body lipid level is more affected by the dietary protein than by the dietary lipid level. The regression analysis confirmed that after WF, DPROT is the main factor affecting the body lipid content (Figure 3d)

The body lipid content increases with increased feed ration (Table 7C; Figure 3d) but this effect is much smaller than the effect of DPROT (Table 7A and 7B; Figure 3d). Lipid deposition efficiency is little affected by lipid ration and the protein sparing effect of an increased dietary lipid content is small (Van der Meer et al. 1997c). Nevertheless, the extra protein deposition caused by dietary lipid addition is sufficient to prevent the associated extra lipid deposition from increasing the body lipid percentage.

Body ash content of *C. macropomum* was negatively affected by increased amounts of soya meal in the diet (Figure 3; Table 7D; Van der Meer et al. 1996). Low body ash contents might be related to high growth rates (Günther & Boza 1993; Van der Meer et al. 1995). The linear regression analysis confirmed that a higher MPR reduces the body ash content (Table 7C; Figure 3e). However, an increased dietary protein concentration has a limited effect on the body ash content (Table 7A).

### *C. macropomum* compared to other species

We estimated that the maximum metabolic protein ration in *C. macropomum* lies around  $11 \text{ g kg}^{-0.8} \text{ d}^{-1}$ . With a 40% protein feed, the maximum feeding level would be around  $27.5 \text{ g kg}^{-0.8} \text{ d}^{-1}$ , which is comparable to the maximum feeding level in *O. niloticus* (Osman 1988). The higher growth rate of *C. macropomum* compared to tilapia must therefore be caused by a higher feed utilization efficiency, and not by an exceptionally high food consumption.

In the FGS, the species-specific parameters determining protein biomass gain are protein, fat and carbohydrate digestibilities and routine metabolism. The routine metabolism of *C.*

Figure 7 Effect of body weight and diet composition on body protein content.

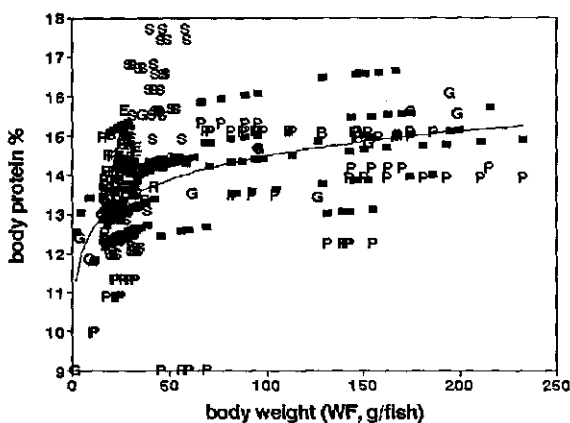


Figure 7a

Legend: The line refers to the allometric analysis of Shearer (1994); model 1 of Table 10; Blocks refer to the same model with dietary protein added as explaining variable: model 2 of Table 10. Letters refer to the experimental data (see Table 1). Note that this figure differs from Figure 2 only in the scale of the axes and the blocks representing model 2.

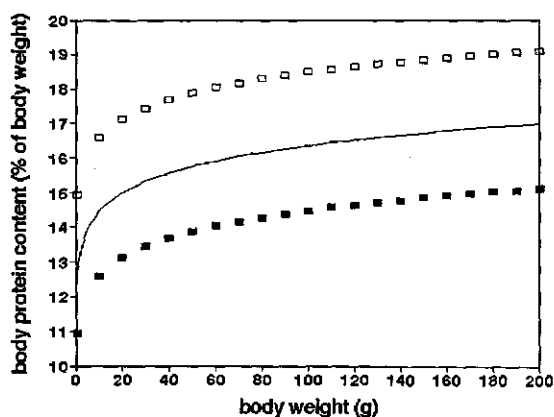


Figure 7b

Closed blocks refer to model 1 of Table 10. Open blocks refer to fish in which the body protein content is increased with 4% of the body fresh weight. The line refers to the model of Shearer (1994) for the pooled data:  $\log(BP) = -0.894 + 1.054 \cdot \log(WF)$ . Although there is a considerable difference between regression line and data (open and closed blocks), the coefficient of determination is high:  $r^2=0.991$ .

*macropomum* is about half that of tilapia and only one third that of trout (Table 11). As routine metabolism can account for 40% of the total metabolism at maximal feeding (Van Dam and Pauly 1995), this represents a major saving for *C. macropomum*. In addition, the FGS suggests that carbohydrate digestibility in *C. macropomum* is relatively high (probably related to its natural diet of fruits and seeds), leading to lower dietary P/E ratios and, therefore, higher protein utilization efficiencies.

The amount of protein used as energy source was calculated from the AALIRAT equation. In this equation the parameter  $c_1$  defines the slope of the linear parts of the curves and  $c_2$  the distance between the curves of different P/E ratios. Thus  $c_1$  can be interpreted as a measure of the efficiency of protein use and  $c_2$  of the protein-sparing effect of extra energy in the feed. In *C. macropomum*  $c_1$

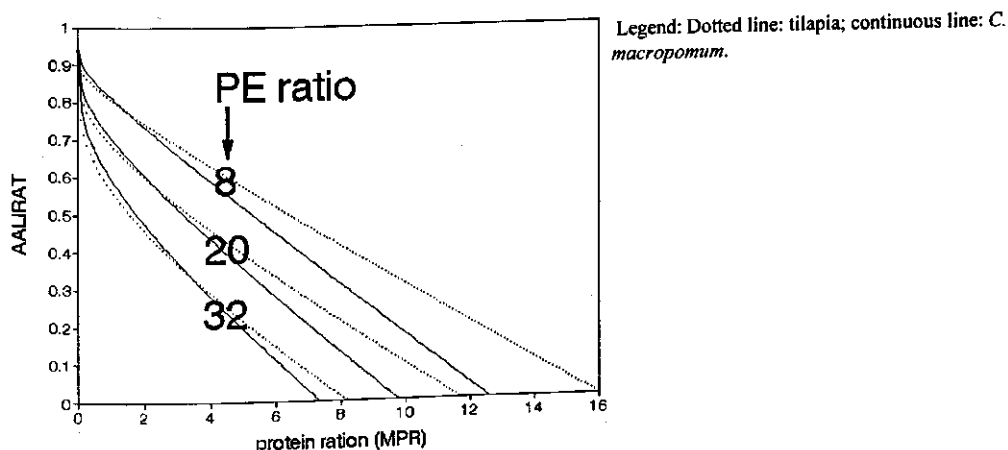
is lower than in tilapia, resulting in more protein oxidation at the same protein ration than in tilapia. The value of  $c_2$  is almost the same in the two species, which means that the effect of extra energy in the feed is similar in both species (Figure 8). The graph suggests that at low protein rations, *C. macropomum* is the more efficient protein user. In *C. macropomum* only a minor part of the protein lost by de-amination can be saved by adding lipids to the diet (Van der Meer et al. 1997c). The protein sparing effect of lipids is more prominent in salmonids (e.g., Kaushik & Médale 1994). Similar differences between species were reported in pigs (Bikker et al. 1994: protein sparing only possible when dietary protein does not limit growth) and calves (Gerrits et al. 1997: even at very low protein rations protein sparing occurs when lipid is added to the diet).

**Table 11.** FGS parameter values of *C. macropomum*, tilapia, trout and catfish.

Parameter	Colossoma	tilapia	trout	catfish
<b>DIGESTIBILITY</b>				
protein	80	80	80	80
lipid	60	50	40	40
carbohydrate	80	50	80	80
<b>ROUTINE METABOLISM</b>				
coefficient	252	452	729	301
exponent	0.8	0.8	0.76	0.8
<b>BODY COMPOSITION</b>				
protein content	$0.109 \cdot W^{1.061}$	0.160	0.156	$0.13 \cdot W^{1.057}$

Parameter values of tilapia (*Oreochromis niloticus*), trout (*Oncorhynchus mykiss*) and catfish (*Clarias gariepinus*) were taken from Van Dam & Penning de Vries (1995).

**Figure 8** AALIRAT in dependence of protein ration and dietary protein to energy ratio



### Protein in *C. macropomum* nutrition

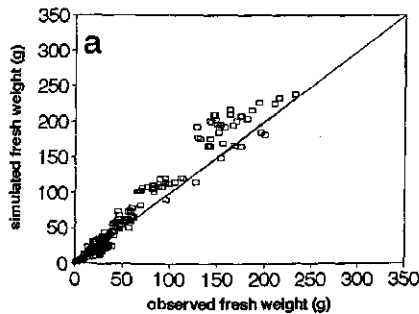
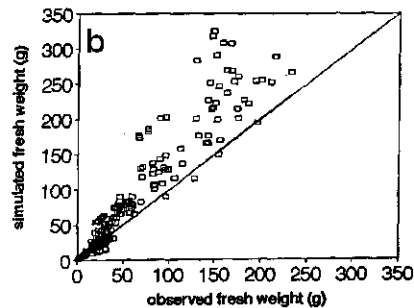
Both the  $\beta$ -weights and the sensitivity analysis showed the importance of protein for growth. An increase in digestible protein, be it through more feed, a higher protein content of the feed or a higher protein digestibility, is the main factor determining changes in protein and fat biomass. We calculated the amounts of protein used as energy source from the protein ration and the dietary P/E ratio, without considering the amino acid profiles of feeds or fish. Presently, many growth models divide the amount of protein used as energy source in 'inevitable' and 'evitable' losses. 'Inevitable' losses are due to differences between the indispensable amino acids profiles of feed and fish (Van der Meer & Verdegem 1996). 'Evisible' protein losses are the fraction of the dietary protein that may or may not be deposited as body protein, depending on the composition of the non-protein part of the diet. Including a module in the FGS to correct the protein availability for these 'inevitable' losses might improve simulation precision and will increase the explanatory character of the model. In African catfish larvae, the division of the dietary protein between deposition and 'losses' was simulated better calibrating the model based on amino acid profiles and protein ration, than based on the P/E ratio (Conceição 1997).

Soya is a good protein source for *C. macropomum*. An increment of the dietary soya decreases feed uptake and increases growth rate (Table 7D). Moreover, it increases the body protein content and decreases lipid and ash content. All these effects of SOYA were very significant ( $P=0.0001$ ; Table 6). Based on the amino acid composition of the protein, soya protein should be of almost equal quality for other fish species as well (Van der Meer & Verdegem 1996; Lovell 1991). However, palatability of soya is higher in *C. macropomum* than in many other species. Its natural diet of fruits and seeds (Saint-Paul 1985) probably facilitates the use of protein of plant origin in artificial *C. macropomum* diets.

### Performance of the models

The FGS followed the general trends in the data set and the principles underlying the model seem to apply also to *C. macropomum*. In earlier versions of the FGS simulations were limited to feeding levels at which the amount of uneaten feed was expected to be negligible (Van Dam & Penning de Vries 1995) or arbitrarily estimated as a percentage of the feed ration (Machiels & Henken 1987). A better estimation of feed uptake is required especially for simulating feed uptake at high feeding levels (Machiels 1987), in ponds (Van Dam 1995) or in fish larvae (Conceição et al. 1997). *C. macropomum* is the first species in which the amount of uneaten feed has been determined experimentally (Van der Meer et al. 1997a; Van der Meer et al. 1997b) and a feed loss module could be formulated. Comparison of the outputs of the model with and without the new module (Figure 9) demonstrates that corrections for feed losses notably improved simulation results. Feed losses in other species might be similar to the losses measured in *C. macropomum* (Van der Meer et al. 1997c). Quantification of feed losses in relationship to the feeding level in other species is required to allow parameterization of the feed loss module for those species.

The calibration procedure was aimed at the best overall simulation result. In PROT, this resulted in overestimation of fresh weight and fat biomass (Figure 4a). The high, fixed feeding levels in this experiment may have led to higher feed losses than for *ad libitum* feeding. It is possible that

**Figure 9** Effect of the correction for feed losses on FGS output.**Figure 9a:** with correction**Figure 9b:** without correction

the amounts of feed specified by the model, even though corrected, still overestimated feed consumption. In FFREQ all simulated fresh weights were systematically too low. For the feeds in FFREQ, high quality ingredients were imported from Europe. The model, calibrated with data from experiments with local Costa Rican ingredients, underestimated the results obtained with high quality ingredients.

In LIPID on the other hand, fresh weight was underestimated whereas fat biomass was slightly overestimated. This suggests a wrong partitioning of energy expenditure over protein and fat oxidation in this experiment. Also in SOYA, weight of all fish fed the 20% protein diet was underestimated, while fat biomass was overestimated. For the 40% protein diet this was reversed. The model, with this equation for AALIRAT, failed to capture the differences in body composition in relation to certain feed compositions.

Deviations between model output and observed values was considerable for both the linear regression analysis and the FGS. For practical use the output of the linear regression method may be improved by introducing more explaining variables. However, such an approach does not help understanding fish growth metabolism. Research to improve the FGS may be more rewarding as it not only gives insight in fish growth, but it also makes the model more generally applicable.

Linear models tend to produce extreme outputs when inputs are far from the 'average data' used to calculate the regression equations. However, also within the scope of variation tested, outliers were more frequently produced by the linear regression method than by the FGS. In most experiments fish were fed to approximately *ad libitum* with a 30 to 40% protein diet made from local ingredients. All obvious outliers were produced with the linear method for records concerning 'non-average data' with respect to either feeding level (FLVEL), protein content (PROT) or origin of the ingredients (FREQ). Thus, the linear regression analysis does not cover accurately the whole range of variable variation as encountered in the database.

The FGS apparently deals with 'extreme' treatments more effectively. Especially predictions of weight reduction and body composition of non-fed fish were very precise (Figure 6). In general,

variation of the RE's within experiments (Table 9) was smaller for the FGS than for the linear regression model.

## CONCLUSION

Description of the data by linear regression analysis was relatively fast, and interpretation of the results to culture practices was easy. Parameterization and calibration of the explanatory FGS model was more complicated and time consuming. The precision of the description of the data by both models was comparable based on average errors. However, the FGS produced less outliers. We think this is a reflection of the more explanatory character of the FGS, compared to the empirical linear regression analysis.

Although linear regression might still be the fastest way for a preliminary analysis of fish growth, its empirical character excludes fundamental improvements in the future. The FGS can already now compete with linear regression methods in describing experimental data. Incorporation of a module considering the amino acid profiles of feeds and fish will reduce the empirical character of the FGS. We expect that this will turn the FGS into a powerful tool to predict growth of *C. macropomum* under controlled culture conditions.

## ACKNOWLEDGEMENTS

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# Chapter 6

## General discussion

### INTRODUCTION

The objective of this thesis was to assess the nutritional requirements of *Colossoma macropomum* (Cuvier). In earlier studies of Machiels (1987) and Van Dam (1995) it had become clear that protein ration is the major factor determining fish growth. Therefore, this thesis was focussed on describing the effects of variations in dietary protein quantity and quality on fish growth.

### A BRIEF DISCUSSION OF THE RESULTS

#### Amino acid profiles

Animals cannot synthesize 'indispensable' amino acids (IAA's). If one IAA's is not available in sufficient amounts, protein deposition is blocked and 'expensive' protein is catabolized. In its natural habitat adult *C. macropomum* feed mainly on terrestrial fruits and seeds (Saint-Paul 1985). However, literature data indicate that many agricultural by-products such as commercial fruits, fruit rests and coffee pulp have not sufficient protein of the required amino acid composition to support acceptable fish growth (Chapter 2-I).

The ideal dietary protein has an amino acid profile identical to that of the protein to be deposited. Not surprisingly, most fish feeds use fish meal as the main protein source. Based on amino acid profile and protein content, soya meal was selected as the most promising substitute for the commercially available fish meal. This was confirmed in a growth trial using diets containing different fish meal/soya meal ratios (Chapter 2-II). Best growth was obtained with diets containing equal quantities of soya and fish meal protein. Growth was not significantly different between all-soya diets and all-fish diets, but the conversion of feed was best in all-soya diets. This proved that in principal soya can substitute fish meal completely in *C. macropomum* diets. As amino acid profiles differ little between fish species, this conclusion should hold also for other species.

The effect of the dietary soya meal/fish meal ratio was determined in 20 and 45% protein diets (Chapter 2-II). In diets with the same soya meal/fish meal ratio, but differing in dietary protein concentration, the amino acid profile of the 45% protein diets was more favourable. However, the protein utilization efficiency of the 20% protein diet was higher. Apparently, also the dietary protein level affects protein utilization efficiency.

In a subsequent experiment all-soya and all-fish meal diets were compared at different feeding levels (Chapter 3-II). When corrected for differences in feed uptake, soya meal protein seemed as efficiently used as fish meal protein. It was concluded that a first selection of feed ingredients can be made based on their protein content and amino acid profile.

### Protein requirement

The relationship between protein ration and growth was studied in an experiment with dietary protein levels ranging from 17 to 63% (Chapter 3-I). Maximal growth of *C. macropomum* required a diet of approximately 43% protein. This contrasts with the supposed low protein content of its natural food, but it is in accordance with the general finding that faster growing fishes require a higher dietary protein concentration (Tacon & Cowey 1985). This experiment also confirmed the conclusion of Günther & Boza (1992) that growth of *C. macropomum* compares favourably with growth of other fish species commonly used in aquaculture.

### Feed losses

Protein utilization efficiency decreased with increasing dietary protein concentration (Chapter 3-I). It was calculated that the protein utilization efficiency of maximal growing fish was only 50% of the maximal possible efficiency (at protein rations close to zero). Increasing percentages of uneaten feed at higher protein rations were suspected to be the main cause.

Even in closed production systems, quantification of feed rests in the water is difficult (Nijhof 1994; Cho, Hynes, Wood & Yoshida 1994). Therefore, an experiment was executed in which fish were fed with chromic oxide marked feed, allowing exact quantification of the feed in the fish. It was found that 20 to 30% of the feed ration remained uneaten by fish fed *ad libitum* (Chapter 3-II). Feed losses were not affected by feeding frequency or feeding time-of-the-day (Chapter 3-III). At feeding levels of around 60% of the *ad libitum* level, fish consumed 100% of the ration (Chapter 3-II).

Feed losses in trout culture were discussed by Cho (1992). He suggests that 'fish feeding' is characterized by improved conversions of feed when feed rations increase, while 'tank feeding' occurs if increasing rations result in decreased conversions of feed. The data of Chapter 3-II fit in this view: after correction of the feed rations with the measured feed losses, the conversion of feed is practically independent from the feeding level. However, other experiments indicate that assuming feed losses increasing from 0 to 30% when feeding levels increase from 60 to 100% of the *ad libitum* level, is not sufficient to explain the differences in conversions of feed completely.

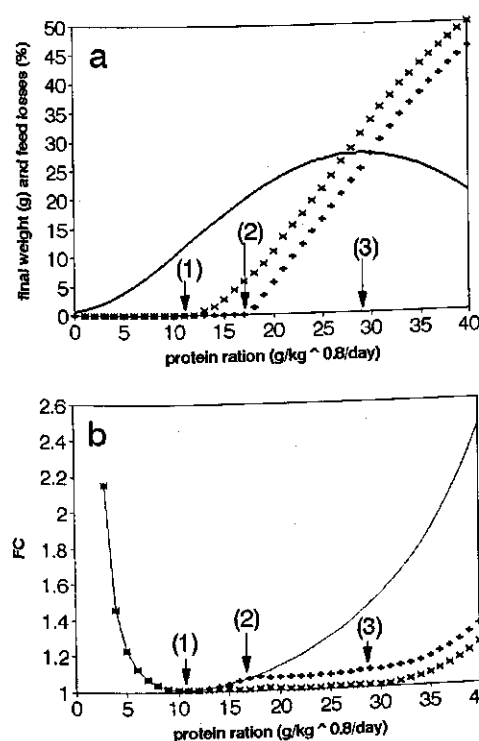
Günther & Boza (1992) found for *C. macropomum* optimal conversion of feed at a feeding level of 25% and 36% of the feeding level required for maximal growth (in 2-40 g fish and 60-200 g fish, respectively). Own experimental results showed that in *C. macropomum* the feeding level required for optimal feed utilization is below 60% of the feeding level required for maximal growth (Figure 1). At feeding levels below 60% of the *ad libitum* feeding level feed losses are negligible (Chapter 3-II). Cho (1992) suggests that the conversion of feed corrected for feed losses remains constant between the feeding level required for maximal feed utilization and the one needed for maximal growth. However, using the data of this thesis, feed losses should be higher than observed in Chapters 3-II and 3-III to explain such a phenomenon. As demonstrated in Figure 1a, it would require that even in fish fed only 60% of the *ad libitum* feeding level 6% of the feed remains uneaten. Also the decreased conversions of feed with increasing feeding level found by Günther & Boza (1992) would require feed losses above 30% for the best growing fish.

Possibly, feed remaining uneaten is not the only cause of increased conversion of feed with

increased feed rations ('tank feeding'). Decreased feed digestibility at high feeding levels has been suggested by Henken, Kleingeld & Tijssen (1985). However, in *C. macropomum* the feeding level did not severely affect the feed evacuation rate in the digestive tract (Chapter 3-II). This makes it unlikely that the feeding level strongly affects feed digestibility. A more than proportional increased cost of growth with increasing growth rate (Conceição 1997) seems a more likely cause of decreased conversions of feed at high rations. This explanation also corroborates with the decreased conversion of feed in the faster growing fish of Chapter 3-III, where all fish were fed *ad libitum* and supposedly suffered similar feed losses.

**Figure 1.** Feed losses and feed conversion in dependence of the feed ration.

Fish are presumed to have an initial weight of 1.5 g and fed a 40% protein diet. Final weight was calculated from the relationship between protein ration and protein gain as given in Figure 1 (size group 5) of Chapter 3-I. Feed rations indicated by (1), (2) and (3) refer to the feeding level required for minimal feed conversion and 60% and 100% of the feeding level required for maximal growth, respectively.



In Figure 1a the continuous line indicates the final weight of the fish. Feed losses indicated with a '+' refer to estimates based on the assumption that between 60% and 100% of the *ad libitum* feeding level feed losses increase from 0 to 25% of the ration. *Ad libitum* is here assumed to be the feeding level associated with maximal growth. Feed losses indicated with a 'x' are calculated on the assumption that the feed conversion corrected for feed losses remains constant between feeding levels (1) and (3). Note that a constant feed conversion in this range of feeding levels requires higher feed losses than those we estimated in *C. macropomum*.

In Figure 1b the continuous line indicates the feed conversion (FC) corresponding to the growth data indicated by the continuous line in Figure 1a. The FC is calculated as: feed ration/wet weight gain (g/g). The markers '+' or 'x' represent feed conversions based on feed losses as indicated by the same symbol in Figure 1a.

Possibly due to the low maintenance requirement of *C. macropomum* (Chapter 5), the feed utilization efficiency favourably compares to other species (Günther & Boza 1992). Nevertheless, the feed losses we found are higher than generally accepted in aquaculture research. It is possible

that measuring feed losses in other species will reveal similar feed losses as in *C. macropomum*. Presently, nutritional research with the goal to improve fish culture sustainability focuses on improved feed formulations (Cho et al. 1994). Our data suggest that concentrating on feeding strategies which diminish feed losses might be as rewarding for sustainability as optimizing feed formulations.

### Feeding regimes

In 1 gram fish maximalization of the daily feed uptake required at least five daily meals (Chapter 3-III). Feed uptake capacity of *C. macropomum* is bigger in the (late) afternoon: even fish fed once daily consumed more when fed in the afternoon than when fed in the morning. In general, feed uptake per meal increased during the day, but in particular feeding regimes feed uptake decreased in the afternoon. This was associated with feed accumulating in the digestive tract. After 13.00 h. feed uptake and feed evacuation rate in the digestive tract increased.

A daily biorhythm in feed uptake similar to the one we found in *C. macropomum* was found in tilapia (Getachew 1987). However, models predicting feed uptake in trout (Vahl 1979) and in African catfish (Haylor 1993) ignore biorhythms and are not suitable to predict feed uptake of *C. macropomum*.

### Dietary lipid content and protein sparing

Lipid deposition in the fish could be well explained by assuming constant lipid and carbohydrate utilization efficiencies (Chapter 4). Consequently, lipid deposition depends mainly on the amounts of lipids and carbohydrate consumed. This relative independence of the protein and lipid metabolism in *C. macropomum* indicates that little protein is used to complement lipids and carbohydrate as energy source. Consequently, a strong 'protein sparing effect' is unlikely. Nevertheless, the consistent higher protein utilization efficiency of diets lower in protein indicates the existence of a protein sparing effect of the dietary energy. Also lipid addition to the diet resulted in limited protein sparing. For *C. macropomum* culture this protein sparing is difficult to exploit because of its minor significance and the associated higher feeding costs and increased body lipid content.

### Body composition and feed uptake

Although the protein sparing effect in *C. macropomum* is small, in our experimental setting it was large enough so that the dietary protein level (and not the dietary lipid level) proved the main explaining variable of the body lipid concentration. Dietary protein content also affects body protein content. Diet composition may also have a significant effect on the relative weights of the head, intestines and trunk. Adaptation of the size of the internal organs (digestive tract, liver etc.; Shearer 1994; Bikker 1994) to the diet composition is the most likely cause of these effects.

Lipid deposition capacity is very large in *C. macropomum*: in Chapter 3-I we found up to 18% lipid in fish fed a 17% protein diet. However, in the other experiments dietary protein levels below 30% were not used and such high body lipid levels were never found again. Therefore, we assume that in these experiments the maximal lipid deposition was not reached. That may be the

reason that in *C. macropomum*, different from salmonids, feed uptake is not determined by the dietary lipid level. Machiels (1987) suggests that not feed composition but body lipid content affects feed uptake in African catfish. If that is true in *C. macropomum* as well, feed uptake will be affected by the dietary lipid content when the body lipid level reaches its maximum of 18% or more.

## Growth models

A complete description of the diet quality requires the use of many parameters. In this study parameters like dietary lipid and protein level were independently varied within experiments. However, nutrients in the diet add up to 100% and correlation between variables, as found in this study, may be a general problem in the analysis of accumulated nutritional data. This limits the number of explaining variables used and the number of effects that can be studied jointly.

Linear regression analysis revealed that dietary protein indeed plays the central role in the nutrition of *C. macropomum*. Dietary protein concentration is the major factor determining feed uptake and dietary protein ration the major factor determining growth. A comparison between the linear regression analysis and the Fish Growth Simulator (FGS) revealed that average errors of both methods were similar. However, the linear regression analysis produced more outliers, especially for data which in one or more aspects differed from the average. The FGS was more precise, but requires more time to be parameterized and calibrated. In principle a model like the FGS only once needs adjustments for a species. However, some components of the FGS are still empirical, which causes the necessity to adjust the model when new data come available. It is expected that in the near future the FGS can be upgraded into a stable and valuable model for practical fish culture. To this effect some research directions are suggested in the following paragraphs.

## RECOMMENDATIONS

### Recommendations for culture practices

Optimization of aquaculture practices requires a balance between maximalization of growth and optimization of the conversion of feed in fish.

#### *Maximal growth*

Maximal growth of juvenile *C. macropomum* requires maximizing body protein deposition. Availability of dietary protein for deposition as body protein depends on feed ration, dietary protein concentration and dietary protein quality. Maximal daily feed uptake of *C. macropomum* requires several meals per day, with the highest feeding frequency in the late afternoon or early night. A high feed uptake will only result in maximal protein uptake if the dietary protein concentration is high. Results obtained with diets made from local (Costa Rican) ingredients indicated that maximal growth requires a diet containing approximately 43% protein.

For most warm water fishes lower dietary protein contents are recommended: e.g. Tacon (1987) recommends for omnivorous fish a dietary protein concentration of 35-42%. Although diets lower in protein are generally cheaper, for *C. macropomum* a dietary protein concentration close to the one required for maximal growth must be recommended as:

(1) Reduction of the dietary protein concentration increases the feed uptake of the fish, apparently as an attempt to maintain protein uptake at the same level. Reduction of the protein level will result in a significant higher consumption of non-protein diet components (such as carbohydrates and lipids) per unit of protein deposited.

(2) Reduction of the dietary protein level not only reduces growth but also increases the body lipid concentration. Lipid accumulation in *C. macropomum* takes also place in the muscle and this may reduce customers' appreciation of the meat.

The dietary lipid concentration has little effect on growth. The small 'protein sparing' effect of lipid addition seems economically of little interest and, moreover, results in extra lipid deposition. The minimal dietary lipid concentration we used in our experimental diets, 4%, is sufficient to assure high growth and results in lean meat.

#### *Optimal feed conversion*

An optimal conversion of feed in *C. macropomum* is found at a feeding level below 60% of the level needed for maximal growth. The high feed losses observed at *ad libitum* feeding suggest that reducing the feeding level is an efficient tool to improve feed conversion efficiency. Restricted feeding levels seem obligatory for maximal sustainability of *C. macropomum* culture.

Soya meal can substitute all the fish meal in *C. macropomum* diets without causing significant growth losses, while protein utilization efficiency increases. However, in diets without fish meal, body ash contents decrease considerably. Therefore, it may be wise to include small amounts of fish meal in *C. macropomum* diets (e.g., 10%), until the long term effects of all-soya diets on growth and health of the fish are known. Moreover, the increased appetite of fish with fish meal diets facilitates feeding practices.

### **Recommendations for future research**

#### *Remaining questions for Colossoma research*

This study on the nutrition of *C. macropomum* has not been completely conclusive and several questions remain unanswered.

Firstly, a more detailed study of the feeding habits of *C. macropomum* may elucidate ways to prevent high feed losses without reducing the feeding level as drastically as we suggest in this study. If the presently measured feed losses at high growth rates cannot be reduced, restriction of the feeding level and high fish densities will be necessary to maximize the economics of *C. macropomum* culture. This would ask for a study on the performance of *C. macropomum* under high fish densities.

Secondly, the use of chromic oxide marked feed showed to be a powerful tool for the detection of feed losses and determination of the rate at which the feed passes through the digestive tract. Feed digestibility can also be studied with chromic oxide marked feed. Our data suggest that feed digestibility in *C. macropomum* is affected by feeding level and ingredient choice. Quantification of these effects would be a valuable addition to the knowledge gained during this thesis.

Thirdly, in the trials of this study fish weights varied between 0.8 and 200 grams and trial durations between 14 and 44 days. Under culture practices it might take 7 months or more from

weaning ( $\pm 60$  mg) to commercial size (1 to 1½ kg in Costa Rica). Experiments of longer duration and covering the whole weight range are required to determine the feasibility of *C. macropomum* culture more exactly.

Fourthly, it could be important for practical *C. macropomum* culture to verify the suggestion made in this study that extension of the feeding regime in the nightly hours increases feed uptake and growth.

#### *Experimental designs in fish nutrition*

A general outline of the nutritional requirements of a new aquaculture species can be given after a short series of well-designed experiments. A prerequisite is that husbandry and fish have been adapted to each other. The growth potential of a species must be tested under conditions which are optimal according to the latest knowledge. Nutritional research can only produce valuable results if variations of the dependent variable (usually growth) are mainly reflections of variations of the studied dietary aspect. Tentative test diets for warm water fish should (1) contain approximately 40% protein, (2) be composed of ingredients tested in similar concentrations in other fish species and (3) be fed several times daily with an interval as large as possible between meals. After having obtained growth data considered being 'maximal for the present state of the art', experiments with a more specific goal can be started according to the table below:

Exp.	Independent variable	Feeding regime	Main objective
1	Dietary protein %	ad libitum	optimum dietary protein %
2	Dietary lipid %	ad libitum	optimum dietary lipid %
3	Feeding regime	ad libitum	maximal feed uptake
4	Feeding level	ad libitum and restricted	maximal feed utilization efficiency
5	Ingredients	practice of industry	optimalization of feed composition and cost

Ideally, each independent variable should be used at a number of different levels so that (1) the extreme levels at both ends result in reduced growth because the independent variable is in respectively shortage or excess, and (2) fish under at least one treatment display maximal growth. In experiments 1 and 2 the required dietary protein and lipid concentrations should be determined. If a substantial interaction between the dietary protein and lipid level is found (e.g. a large protein sparing effect of lipids), the optimal protein concentration found in experiment 1 may need further verification. Experiment 3 should detect preferred feeding times and maximal feed uptake and experiment 4 should elucidate how reduction of the feeding level affects feed utilization and growth. Results of experiments 3 and 4 are of major importance to elaborate optimal feeding strategies. Once commercial production starts, research should start to reduce feeding costs by optimalization of the ingredient composition of the diet.

#### *Model improvement*

This study highlights the effect of protein ration on growth and body composition of *C. macropomum*. Considering only dietary protein as the explaining variable is definitely an oversimplification of the reality as it ignores the important role of lipids, vitamins, minerals etc. in fish nutrition. Nevertheless, growth of *C. macropomum* showed to be highly dependent on the efficient transformation of dietary protein in body protein and, within our experimental setting, most

observed phenomena could be explained adequately by changes in protein quantity and quality.

The FGS could be calibrated for *C. macropomum* in such a way that it followed well the general trends observed in our data set. Predictions proved more consistent than those of an empirical linear regression model. However, the relative errors between observed and predicted values of the FGS leave ample space for improvement. The biggest challenge for future research on the nutrition of *C. macropomum* might be the development of an improved version of FGS.

Improvement of the FGS should not come from a better statistical fitting of the data, but from a better understanding of physiological and physical processes determining fish growth (Machiels 1987). The FGS is not completely 'explanatory' and where theories lack, empirical equations are used. Obviously, these parts require research for basic improvements of the model, e.g.:

- *Feed uptake*

The amount of feed consumed is the most important factor affecting growth rate (Machiels 1987). Composition of feed and fish (Machiels 1987) and available oxygen (Van Dam 1995) were identified as important factors affecting feed uptake. Nevertheless, both authors mention the lack of 'understanding the processes which control feed intake' as a major constraint to improve model output. In *C. macropomum* the amount of uneaten feed can be estimated when the feeding level in relationship to the *ad libitum* feeding level is known. Still, factors causing the considerable fluctuations in *ad libitum* feed uptake of fish on consecutive days are not well studied nor predictable.

- *Digestibility*

Reliable data on the digestibility of feeds and ingredients in *C. macropomum* are not available. Presently used estimates fitted well to our data but they lack confirmation by experimental results. A study of the effect of feed ration on feed digestibility could contribute to a better understanding of the nutrition of *C. macropomum*.

- *Metabolic rates*

Well known biochemical pathways provide the theoretical basis for biosynthesis. Routine metabolism is calculated from the empirical formula of Winberg (1956). The cost of the feeding metabolism is set to a fixed percentage of the feed energy consumed. Upgrading these parts to completely explanatory modules will need extensive physiological research.

- *Substrates for energy supply*

When gluconeogenesis and lipogenesis do not provide sufficient energy to satisfy the demand of the fish, body material has to be oxidized. The part of the model which 'decides' how the energy requirement is satisfied by oxidizing protein or lipid is of major importance for characterizing the growth performance of a fish species. The ratio at which proteins and lipids are oxidized depends on the body composition in fasting fish (Machiels 1987) and on the amount and composition of the food in fed fish (Van Dam 1995). However, there are no theories supporting and integrating these suppositions. This research area may be crucial for the development of a FGS easily adaptable for many fish species. Using the dietary amino acid profile to set maximal theoretical protein utilization efficiencies, as outlined in this thesis and already used in growth models of pigs (de Lange 1995) and fish larvae (Conceição 1997), might be the first step. Determination of the amino acid profiles of different body compartments (muscle, digestive tract, liver, brain, etc.) together with a detailed study how their weight is affected by total body weight and diet composition might be a next step. It not only would improve the FGS, but also yield important information about the (theoretical) amino acid

requirement of *C. macropomum*.

The FGS contains considerable empirical parts which make it not yet reliable enough for general use in practical fish farming. Moughan, Kerr & Smith (1995) describe how growth models are developed for the pig industry to adjust management (specially the dietary ingredient composition and amount of feed) to production aims (growth rates, fat percentage of the meat) and economical constraints (prices and availability of ingredients, prices of the meat). Since the fish industry and its related research are of a 'recent age', model development could be rewarding because present practices might still be susceptible for major improvements. The research presented in this thesis proved that *C. macropomum* grows rapidly and has a flexible body composition allowing to obtain fast and unambiguous responses to relative small variations in treatment and therefore seems a suitable species to develop a further improved fish growth model.

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## SUMMARY

*Colossoma macropomum* (Cuvier 1818) is an indigenous fish species from the Amazon region. When adult, it forages mainly on terrestrial seeds and fruits. The amino acid profile of the body protein of the fish showed to be similar to that of other fish species. Consequently, the amino acid requirement of *C. macropomum* is also similar. Based on literature data, offal products such as coffee pulp, fruits and fruit rests have not sufficient protein of the appropriate amino acid profile for sound growth of fish.

Growing animals require a diet containing sufficient protein with an amino acid profile similar to that of the body protein. Not surprisingly, fish meal is the major protein source of most fish feeds. Based on literature data on amino acid profiles, it was predicted that soya meal could substitute fish meal, which was confirmed in a feeding trial. As soya meal is less palatable than fish meal, more soya in the diet tends to decrease feed uptake and growth rate, but increases protein utilization efficiency.

*C. macropomum* grows fast (1 g/day for 2-30 g fish; 3 g/day for 100-200 g fish) and requires a dietary protein content of approximately 43%. This protein requirement contrasts with the supposed low protein content of its natural food, but is in accordance with the general trend that faster growing fish require higher dietary protein levels.

A model fitted to growth data suggested that protein utilization efficiency of the fish decreases linearly with the protein ration. The model implied that protein utilization efficiency of maximal growing fish is only 50% of the optimum. Using chromic oxide marked feed we found that 20 minutes after feeding, *ad libitum* fed *C. macropomum* contained only 70 to 80% of the ration in their digestive tract. In fish receiving 60% of the *ad libitum* feeding level, 100% of the feed ration was recovered from the digestive tract. Feed losses of 20 - 30% in *ad libitum* fed *C. macropomum* point to uneaten feed as the major cause of decreasing conversions of feed at high feeding levels.

Increasing the feeding frequency from one to five meals per day increased feed uptake and growth of *C. macropomum*. In fish fed in the morning and afternoon, the digestive tract became filled in the early afternoon and appetite reduced. In the late afternoon, feed evacuation rate and feed uptake increased. Except the dip in appetite in the early afternoon, feed uptake per meal increased during the day. Also in fish fed once daily, feed uptake was higher in the afternoon than in the morning. Feed uptake models in other fish species ignored a daily rhythm. Consequently, when applied to *C. macropomum*, they failed to predict feed uptake adequately.

In *C. macropomum* lipid addition to the diet increased the protein utilization efficiency, but less than reported in salmonids. Apparently *C. macropomum* scarcely uses protein to substitute lipid or carbohydrate as a source of energy. An increased dietary protein content increased the body protein content and decreased the body lipid content. Adaptation of the size of the internal organs (digestive tract, liver etc.) to the digestive requirements of the diet may be the cause that diet composition affects the body composition of fish.

Data from all experiments were analyzed with empirical, linear methods. Accordingly, also the accumulated data were analyzed with linear regression. In addition, the same data were analyzed using the Fish Growth Simulator (FGS), an explanatory model describing fish growth. Both methods identified protein ration as the major variable determining fish growth. In

describing the data, the average errors of both methods were similar. However, the regression analysis produced more outliers, both within and between experiments. Due to empirical parts in the FGS, its calibration to new data still requires considerable experimentation time. Development of improved versions of the FGS is recommended. Similar models simulating growth of pigs have proven their usefulness for directing scientific research and as tools for practical farm management. Fast growth and a flexible body composition make *C. macropomum* a suitable study object for the development of the FGS into a valuable tool for both aquaculture research and practice.

## Samenvatting.

*Colossoma macropomum* (Cuvier 1818) is een inheemse vis van het Amazone gebied. Volwassen dieren voeden zich met van het land afkomstige fruit en zaden. De aminozuur samenstelling van de vis bleek veel te lijken op die van andere vissen. De behoefte aan aminozuren van *C. macropomum* zal daarom ook weinig verschillen van die van andere vissen. Uit literatuur gegevens blijkt dat afvalprodukten zoals koffiepulp, fruit en fruitresten niet genoeg eiwit van de benodigde aminozuursamenstelling hebben om voor een aanvaardbare groei van vissen te zorgen.

Groeiende dieren hebben eiwit nodig met een aminozuursamenstelling identiek aan die van hun lichaamseiwit. Het is dan ook niet toevallig dat vismeel de meest gebruikte eiwitbron is in visvoerders. Gebaseerd op data uit de literatuur kon voorspeld worden dat soyameel een goede vervanger van vismeel zou zijn. Experimentele resultaten bevestigden die voorspelling. Aangezien soyameel minder van smaak is dan vismeel, leidt meer soya in het visvoer tot verminderde voeropname en groei, maar neemt de efficiëntie van het eiwitgebruik toe.

*C. macropomum* groeit snel (1 gram per dag op het traject van 2 tot 30 gram; 3 gram per dag op het traject van 100 tot 200 gram). Voor maximale groei heeft de vis een voer nodig met ongeveer 43% eiwit. Deze hoge eiwitbehoefte contrasteert met het lage eiwitgehalte van het natuurlijk voedsel van de vis, maar het is wel in overeenstemming met de algemene trend dat sneller groeiende vissen een eiwitrijker voer nodig hebben.

Een lineair regressie model om groeidata te analyseren gaf aan dat de efficiëntie van het eiwitgebruik lineair daalde met toenemende eiwitgift. Volgens het model is bij maximale groeiende vissen de efficiëntie van het eiwitgebruik maar de helft van de maximale efficiëntie. Met behulp van chroomoxide gemerkt voer werd aangetoond dat in *ad libitum* gevoerde *C. macropomum* 20 minuten na het voeren slechts 70 tot 80% van het gegeven voer aangetoond kon worden in het maagdarmkanaal. In vissen die op 60% van het *ad libitum* voernivo werden gevoerd, werd 100% van het voer in de vis teruggevonden. Voerverliezen van 20 tot 30% in *ad libitum* gevoerde *C. macropomum* wijzen op onopgegeten voer als de belangrijkste oorzaak voor de slechte voerconversies bij hoge voernivos.

Het verhogen van de voerfrequentie van één naar vijf maal per dag verhoogde de voeropname en de groei. Bij de vissen die zowel smorgens als smiddags gevoerd werden vulde het maagdarmkanaal zich in de vroege middag en dat resulteerde in minder honger op dat moment van de dag. Later op de middag werd het maagdarmkanaal minder vol en nam de voeropname weer toe. De voeropname per maaltijd nam toe gedurende de dag (met uitzondering van de verminderde voeropname in de vroege middag). Ook vissen die maar éénmaal daags werden gevoerd vertoonden een hogere voeropname wanneer ze smiddags in plaats van smorgens gevoerd werden. Veel voeropname modellen houden geen rekening met dagritmes in de voeropname. Zulke modellen zijn dan ook ongeschikt om voeropname in *C. macropomum* te voorspellen.

Toevoeging van vet aan het voer van *C. macropomum* verhoogde de efficiëntie van het eiwitgebruik, maar dit effect was minder sterk dan gerapporteerd in zalmachtigen. Blijkbaar gebruikt *C. macropomum* nauwelijks eiwit als energiebron wanneer vet of koolhydraten daarvoor beschikbaar zijn. Een verhoogd eiwitgehalte van het voer verhoogt het eiwitgehalte en

verlaagt het vetgehalte van het lichaam. Aanpassing van het gewicht van interne organen (zoals maag, darm en lever) aan de veranderende eisen die aan het verteringsapparaat worden gesteld met een verandering van de voersamenstelling, zouden wel eens de oorzaak kunnen zijn van het effect van voersamenstelling op lichaamssamenstelling.

De gegevens van alle experimenten werden samengevoegd in één bestand en geanalyseerd met een empirisch, lineair model. Daarnaast werden dezelfde gegevens geanalyseerd met de "Fish Growth Simulator" (FGS), een verklarend model voor het simuleren van de groei van vissen. Beide modellen wezen eiwitgift aan als de voornaamste verklarende variabele voor de groei van *C. macropomum*. De twee modellen waren ongeveer even goed in het beschrijven van de geobserveerde waardes, maar het lineaire regressie model produceerde meer 'uitbijters'. Doordat de FGS niet volledig 'verklarend' is, maar ook beschrijvende, empirische gedeeltes bevat, is aanpassingen van het model aan nieuwe data nog een tijdrovende bezigheid. Het meer verklarend maken van de FGS is dan ook aan te raden. Vergelijkbare modellen hebben in de varkensteelt hun nut bewezen voor het oriënteren van onderzoek, maar ook als instrument voor het management van varkenshouderijen. Een snelle groei en een flexibele lichaamssamenstelling maken *C. macropomum* een geschikt study object om de FGS zodanig te verbeteren dat het een algemeen bruikbaar instrument wordt voor zowel het visteeltkundig onderzoek als de visteelt praktijk..

## Resumen.

*Colossoma macropomum* (Cuvier 1818) es un pez tropical endémico de la región amazónica, donde se le conoce como cachama o tambaqui. De adulto su dieta consiste principalmente de semillas y frutas. Por su gran tamaño, rápido crecimiento y buen sabor, ha sido sujeto de varios estudios tendientes a desarrollar su cultivo.

Para crecer los animales requieren de proteína, no únicamente en cantidad sino en calidad. La composición aminoacídica de la proteína usada como alimento debe de ser idéntica a la proteína a depositar en el cuerpo. A eso se debe que la harina de pescado sea la principal fuente proteica en la mayoría de los alimentos para peces. De acuerdo con la literatura, la harina de soya es el sustituto más prometedor de la harina de pescado. Experimentos con dietas usando harina de soya como fuente de proteína confirmaron esta suposición. Sin embargo, debido a la menor palatabilidad de la harina de soya, un incremento en este ingrediente reduce el consumo total de la dieta así como el crecimiento. Aún así se encontró que la eficiencia en la utilización de la proteína incrementa al usar harina de soya.

*C. macropomum* crece muy rápido (1 gramo por día en el trayecto de 2 hasta 30 gramos; 3 gramos/día para peces de 100 hasta 200 gramos) y requiere una concentración de proteína en el alimento de cerca del 43%. Ese requerimiento de proteína difiere del bajo contenido protéico de su supuesto alimento natural, pero coincide con la tendencia general de que peces con un crecimiento alto requieren una concentración de proteína alta en su dieta.

Se utilizó un modelo de regresión lineal para analizar datos de crecimiento en *C. macropomum*. Este modelo indicó que la eficiencia en el uso de la proteína disminuye al incrementar el porcentaje de la misma en la dieta. De acuerdo con el modelo la eficiencia del uso de la proteína en peces en su etapa de máximo crecimiento es de un 50% del de la máxima eficiencia posible. Usando alimento marcado con óxido de cromo se demostró que 20 minutos después de alimentar *ad libitum* únicamente entre 70 a 80% del alimento se encontraba en el canal digestivo. En peces alimentados con aproximadamente el 60% de la ración *ad libitum*, todo el alimento se encontró en el canal digestivo. El método de alimentación *ad libitum* produce pérdidas en la dieta, que resultan en una baja eficiencia de conversión alimenticia.

Un incremento en la frecuencia de alimentación desde una vez por día hasta 5 veces al día aumentó el consumo de alimento y el crecimiento en *C. macropomum*. En peces alimentados en la mañana y en la tarde se observó que el canal digestivo permanece lleno, resultando en un menor consumo de alimento durante las primeras horas de la tarde. Aproximadamente a las 19 horas se observó una menor cantidad de alimento en el canal digestivo a la vez que el consumo de alimento alcanza su nivel máximo. Con excepción de la tarde, el consumo de alimento tiende a aumentar durante el transcurso del día. También en peces alimentados solamente una vez al día se encontró que el máximo consumo se registró a las 19 horas.

La mayoría de los modelos utilizados hasta ahora para estimar el consumo de alimento en peces no consideran este tipo de bioritmos en el apetito, de ahí que no se consideran apropiados para aplicarse a *C. macropomum*.

La eficiencia del uso de la proteína aumentó poniendo más grasa al alimento de *C. macropomum*; sin embargo, el efecto fue menos pronunciado que el observado en otras especies como salmones. Esto puede indicar que *C. macropomum* no usa proteína como fuente de energía cuando grasa o carbohidratos están disponibles para ese fin. Un aumento de la cantidad de proteína en la dieta disminuye el porcentaje de grasa en el cuerpo e incrementa la proteína. Una probable explicación de esta observación es que el aporte relativo del peso de los órganos (higado, canal digestivo etc.) con respecto al peso total del pez cambia en función de la composición del alimento.

Los datos obtenidos en todos los experimentos realizados fueron agrupados en una sola base de datos y analizados utilizando un modelo de regresión lineal. Estos mismos datos se analizaron también con el Fish Growth Simulator (FGS), que es un modelo mecanístico. Ambos modelos se ajustaron adecuadamente a los datos, pero el modelo de regresión lineal mostró menor calidad en las estimaciones cuando los datos se desviaban mucho del promedio. Se estima que es conveniente continuar desarrollando el FGS en un estilo más mecanístico. Modelos similares, pero utilizados en puercos, ya han probado su valor al indicar la dirección en la cual se debe invertir más esfuerzo de investigación. Debido a su crecimiento rápido y flexibilidad en la composición de su cuerpo, *C. macropomum* se presenta como el sujeto a utilizar en experimentos tendientes a transformar el actual FGS en un instrumento adecuado para la investigación y práctica del cultivo de peces.

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Magnus.

## Curriculum vitae.

Magnus Bjintze van der Meer werd op 28 december 1955 geboren in Terhorne (Friesland). In 1974 haalde hij het atheneum-b diploma aan de Rijks Scholengemeenschap te Heerenveen. Hij vervolgde zijn studie aan de Landbouw Universiteit in Wageningen, alwaar hij in 1982 afstudeerde in de richting Zootechniek, met als hoofdvakken Visteelt & Visserij en Hydrobiologie. Tijdens zijn studie werkte hij voor Euroconsult in Egypte (graskarperproject) en voor Witteveen+Bos (hydrobiologische studie van het Naardermeer). Na zijn afstuderen werkte hij 9 maanden bij de NVVS (Nederlandse Vereniging voor Sportvissers Federaties) in Amersfoort. In 1983 vertrok hij naar Mozambique. Aldaar initieerde en begeleidde hij verschillende visteelt projecten. Op het Ministerie van Landbouw verving hij enige tijd de directeur van het Visteelt Departement. In 1989 gaf hij lessen in de visteelt aan de Veterinaire Faculteit van de Eduardo Mondlane Universiteit en schreef hij een handleiding over de visteelt in Mozambique. In 1990 vertrok hij naar Costa Rica om binnen het Programa UNA-LUW Acuacultura te werken aan verbeteringen in het onderwijs en onderzoek. In 1995 werkte hij acht maanden op een garnalenkweekbedrijf aan de Pacifische kust van Costa Rica. Vanaf september 1995 heeft hij gewerkt aan dit proefschrift.