Revista Brasileira de Biociências
Brazilian Journal of Biosciences
ISSN 1980-4849 (on-line) / 1679-2343 (print)

ARTICLE

An alternative fish feed based on earthworm and fruit meals for tilapia and carp postlarvae

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Received: December 17 2013 Received after review: November 18 2014 Accepted: February 18 2015
Available online at http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/view/2879

ABSTRACT: (An alternative fish feed based on earthworm and fruit meals for tilapia and carp postlarvae). The search for alternative protein and lipid sources in the nutrition of aquatic organisms in captivity is extremely important for sustainability and ecosystem preservation. We investigated the potential of a new alternative fish feed based on fruits and earthworm and soybean meals for the nutrition of postlarvae of two high-value fish species cultivated worldwide, Nile tilapia (*Oreochromis niloticus* L.) and common carp (*Cyprinus carpio* L.). Postlarvae fed the alternative diet exhibited growth and weight gain similar to those fed a commercial fishmeal-based feed. Although concentrations of minerals and total protein were higher in the commercial than in the alternative feed, lipid concentration was higher in the latter. Tissue concentrations of minerals, protein and lipid, however, did not differ between postlarvae fed the commercial and the alternative feeds. This result suggests a high assimilation efficiency of the alternative feed compared to the commercial feed. The alternative feed tested in our study proved to be efficient in promoting growth and weight gain of carp and tilapia postlarvae. Due to its simple formulation, based on ingredients commonly found in tropical countries, the proposed alternative fish feed offers an economically viable and environmentally sound alternative for fish farmers in these countries.
Key words: sustainable aquaculture, postlarvae nutrition, tropical fish production.

RESUMO: (Uma ração alternativa baseada em farinha de minhoca e frutas para alevinos de tilápia e carpa). A busca por fontes proteicas e lipídicas alternativas na nutrição de organismos aquáticos em cativeiro é de extrema importância à luz da sustentabilidade e conservação de ecossistemas. Nós investigamos o potencial de uma nova ração alternativa, baseada em frutas, farinha de minhoca e soja, na nutrição de alevinos de duas espécies de grande interesse comercial mundial – *Oreochromis niloticus* (L.) e *Cyprinus carpio* (L.), i.e. tilápia e carpa. Os alevinos alimentados com a ração alternativa exibiram crescimento e ganho de peso similares àqueles alimentados com uma ração comercial baseada em farinha de peixe. Embora as concentrações de minerais e de proteínas totais tenham sido maiores na ração comercial do que na ração alternativa, a concentração de lípides foi maior nesta última. As concentrações de minerais, proteínas e lípides nos tecidos dos alevinos, por outro lado, não diferiram significativamente entre os animais alimentados com a ração comercial e a ração alternativa. Este resultado sugere uma elevada eficiência de assimilação da ração alternativa comparada à ração comercial. A ração alternativa testada em nosso estudo provou ser eficiente em promover crescimento e ganho de peso de alevinos de tilápia e carpa. Devido à sua formulação simples, baseada em ingredientes comumente encontrados em países tropicais, a ração alternativa aqui proposta oferece uma alternativa economicamente viável e ecologicamente sustentável para pequenos proprietários e fornecedores de alevinos nestes países.
Palavras-chave: aquicultura sustentável, nutrição de alevinos, piscicultura tropical.

INTRODUCTION

In recent decades, human populations have increased considerably worldwide. Accordingly, the demand for food, especially nutritionally and economically adequate protein sources, has also increased. Fish production has assumed an important role, contributing an alternative and economically viable protein source, especially in developing countries, where other animal-based protein sources are expensive or not available to the general population (Naylor *et al*. 2000).

Between 1970 and 2008, the production of food fish from aquaculture increased at a mean annual rate of 8.3%, reaching an annual production of 30.5 million tons in 2008 (FAO, 2012). In 2010, capture fisheries and aquaculture supplied the world with about 148 million tonnes of fish (FAO, 2012). However, this massive production increase has had profound environmental consequences, such as a reduction in marine fish stocks, mainly caused by the use of fish meal and fish oil in the production of fish feeds (Naylor *et al*. 2000). Additionally, local environmental impacts can occur from the very moment that fishponds are installed, including eutrophication of adjacent natural waters (Smith 2003, Schindler 2006); introduction of exotic species (Pérez *et al*. 2003), parasites and pathogens into natural aquatic communities; as well as impacts associated with the accumulation of harmful substances in freshwater and marine sediments and food webs (Gravningen 2007). Thus, research aiming

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at sustainable fish production is of major importance for human welfare and environmental health.

The development of new methods for fish production that do not use fish meal and fish oil in the production of commercial fish feeds is an important aspect of sustainable aquaculture and should start at the postlarvae production stages. Further, adequate growth and development of cultivated fish postlarvae are crucial for the success of subsequent production stages (Moreira *et al*. 2012). Due to their high growth rates, fish postlarvae require high amounts of amino acids (AAs), highly unsaturated fatty acids (HUFAs) and phospholipids (PLs) as well as mineral nutrients, although very little is known about the exact concentrations required, even for the best-studied species (Conceição *et al*. 2007). Generally, all basic nutritional requirements are met in commercial diets based on fish meal as the main protein, and fish oil as a source of essential fatty acids and sterols. However, as a result of increasing aquaculture, both at regional and national scales, and the increasing susceptibility of ocean fisheries to natural events, such as El Niño and La Niña, the search for alternative diets that are able to sustain fish production and are economically and ecologically viable has become necessary.

In recent decades, different alternative ingredients have been tested, in order to supplement or completely substitute fish meal and fish oil. Alternative sources of amino acids and protein include soy protein byproducts (Shiau *et al*. 1987), corn gluten meal (Yiğit *et al*. 2012), poultry byproduct meal (Samocha *et al*. 2004), and earthworm meal (Tacon *et al.* 1983). Vegetable oils (Bell *et al*. 2003, Figueiredo-Silva *et al*. 2005) and free-living microorganisms (Olsen *et al*. 2000) have been proposed as substitutes for fish oil. Polyunsaturated and highly unsaturated fatty acids, such as linolenic acid (18:3n3), linoleic acid (18:2n6), arachidonic acid (20:4n6), eicosapentaenoic acid (EPA, 20:5n3), and docosahexaenoic acid (DHA, 22:6n3) are considered essential for many fish species (Hertrampf & Piedad-Pascual 2003, Tocher 2010). Although some species, such as tilapia, are able to synthesize essential highly unsaturated fatty acids from dietary precursors (Karapanagiotidis *et al*. 2007), oil supplementation may be necessary in different concentrations for each postlarval stage (Tocher 2010), which increases feed costs and impacts on marine fish stocks.

Nile tilapia (*Oreochromis niloticus* L., 1758) and common carp (*Cyprinus carpio* L., 1758) are the most commercially important freshwater fish species in Brazil and other tropical countries. An alternative fish feed for postlarval stages of these species based on local low-cost farm products may offer an economically interesting and environmentally sound alternative to commercial fish feeds for both small- and large-scale fish farmers in the tropics. In the present study, we tested the potential of an alternative low-cost fish feed based on fruits and earthworm meal, to sustain growth and weight gain of tilapia and carp postlarvae. We hypothesized that the postlarvae of both species, fed with our alternative diet, would show comparable or even superior growth and weight gain compared to postlarvae fed with a commercial balanced diet.

MATERIAL AND METHODS

Composition and preparation of the alternative feed

The composition of the alternative feed differed from that of the commercial feed mainly in its protein and lipid sources (Table 1). As a positive control, we chose the commercial fish feed most commonly used by fish farmers in the region (Vertentes area, Minas Gerais, Brazil) for tilapia and carp postlarvae. In the alternative feed, the main protein sources were earthworm and soybean meals. Lipid sources in the alternative feed were olive oil and, to a small extent, avocado. A higher concentration of oil, and thus total lipids was necessary in the alternative feed than in the commercial feed in order to successfully bind the ingredients into small pellets. No mineral supplement was added, in order to keep the production costs as low as possible. The earthworm meal was prepared using red Californian earthworms (*Eisenia fetida*) raised by a local farmer, and dried for 3 days at 60°C in a circulating-air oven. Fruit ingredients were carefully washed, cut into slices, and dried at 60°C for at least 24 h. The dried ingredients were triturated, homogenized, and immediately used in the feed preparation. The ingredient samples for mineral and biochemical determination were stored at -40°C until analysis. The proportion of each ingredient in the alternative feed was calculated based on its chemical composition and the possible nutritional requirements of postlarvae of the two species (Committee on Animal Nutrition 1999, Hertrampf & Piedad-Pascual 2003; Table 1). The calculations were performed with Super Crac 5.0.

Feeding experiments

One hundred and eighty 60-day-old postlarvae of each species (*O. niloticus* and *C. carpio*) from the same

Table 1. Composition of the commercial diet and the alternative diet fed postlarvae of tilapia and carp.

Commercial Diet*	Alternative Diet (g kg^{-1} dry diet)
Fish meal	Earthworm meal (339)
Soybean meal	Soybean meal (169)
Corn meal	Corn meal (85)
Poultry viscera meal	Pumpkin meal (2)
Poultry liver meal	Guava meal (195)
Dicalcium phosphate	Acerola meal (4)
Corn gluten	Banana meal (4)
Poultry fat	Jatoba meal (2)
Choline chloride	Avocado meal (2)
Rice meal	Olive oil (198)
Fish oil	
Minerals and vitamins	

* Relative concentration of ingredients not provided by the producer.

hatch were randomly chosen and held in 20-L aquaria (20 individuals per aquarium) with oxygenated filtered water, equipped with a thermostat (Elite, Brazil) and a multifunction filter (Master Super II, Brazil). The experiments were conducted at room temperatures between 26 and 28°C; water-quality variables (temperature, pH, electrical conductivity, dissolved oxygen concentration, and oxygen saturation) were monitored with a multiparameter probe (556 MPS, Yellow Springs Instruments, OH, USA). Three feeding treatments were carried out in separate aquaria, as follows: (1) postlarvae were fed three times a day with the alternative feed (experimental feeding treatment) for 21 days, (2) postlarvae were fed a common commercial fishmeal-based feed three times a day (positive control), and (3) postlarvae were held without feeding (starvation treatment). The aim of the starvation treatment was to assess any effects of adult female reserves on the postlarvae tissue. The daily amount of food corresponded to 6% of the larva body weight. Three replicates, i.e. three aquaria per treatment and species, were carried out. The experiment was repeated for different hatches, i.e., two groups of 180 postlarvae each of each species, in order to exclude parental influences. The aquaria were cleaned and refilled with new water twice a week. Total body length, lateral width, and dorsal-ventral height of individuals were measured with a digital caliper (Zaas, USA). The weight of individual postlarvae was measured with a high-precision analytical balance (Shimadzu, USA). Length and weight were measured out before and after each experiment, in order to compare postlarvae growth between the commercial and the alternative feeds, as well as between the feeding and starvation treatments. Based on the results for weight, specific daily growth rates (SGR) were estimated according to the equation

$$SGR = (lnW_f - lnW_i)/t \times 100 \quad (Eq.\ 1)$$

where W_f is the final mean postlarvae weight (in g) after each experiment, W_i is the initial mean weight (in g) before each experiment, and t is the duration of each experiment in days. The feed conversion ratio (FCR; the inverse of the feed efficiency) was estimated as the ratio between the feed supplied (in g d^{-1}) and the mean body weight gain of the postlarvae (in g d^{-1}).

Mineral and biochemical analyses

Three samples of 0.4 g of both the commercial and the alternative feeds as well as samples of each ingredient in the alternative feed were analyzed for mineral composition and concentration, using an Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES, model 720-ES, Varian, USA) equipped with data-analysis software (ICP Expert II version 1.1). Samples were analyzed for the elements P (g kg^{-1}), K (g kg^{-1}), Ca (g kg^{-1}), Mg (g kg^{-1}), S (g kg^{-1}), Cu (mg kg^{-1}), Fe (mg kg^{-1}), Mn (mg kg^{-1}) and Zn (mg kg^{-1}).

Crude protein contents were estimated by determining the Kjeldahl N content of 0.1 g samples and multiplying the values by 6.25. Lipid determination was carried out for fish samples of 1.0 g dry weight (DW) and for 2.5 g DW samples of the alternative and commercial feeds, as well as of each ingredient in the alternative feed, using an automatic lipid extractor (MA-044/491, Marconi, Brazil). Samples were pre-extracted in 50 ml petroleum ether for 4 h at 70 ºC before being extracted in the automatic lipid extractor. After extraction, samples were dried for 30 min at 100 ºC in a bacteriological oven (AL 100/150, American Lab, CA, USA) and weighed again. Total lipid content was calculated by dividing the sample weight after extraction by the initial sample weight.

Total crude fiber content was determined by the acid (ADF) and neutral (NDF) detergent procedure using an automatic fiber analyzer (MA 444/CI, Tecnal, Brazil). For dry weight determination, samples of 2.0 g were dried at 100 ºC (AL 100/150, American Lab, USA). Subsamples of 0.5 g DW were extracted for both ADF and NDF for 1 h at 95º. The crude-fiber content was calculated by dividing the extracted sample DW by the initial sample DW. Cellulose contents were determined by following the same analytical procedure as for fiber analysis and by subtracting the percentage ADF from the NDF content. To determine the ash-free dry mass (AFDM), dried samples were incinerated for 4 h at 550 ºC, and the AFDM was calculated as the weight loss.

In order to estimate the assimilation efficiency of the commercial and the alternative feeds, three postlarvae per species and aquarium were randomly chosen at the end of each experiment, freeze-dried, and stored at -40 ºC until further analysis. As the main goal was to estimate the assimilation efficiency of the individuals, and not the nutritional value of muscle tissue for consumption, the samples were prepared by drying and homogenizing whole individuals. Subsequent analyses of the mineral and biochemical components followed the same analytical procedures as for the feed samples.

Statistical analyses

Growth measurements of postlarvae were compared among feeding and starvation treatments with one-way ANOVAs followed by post-hoc Tukey HSD-tests. The concentrations of minerals and biochemicals in the commercial and alternative feeds, as well as in the tissue of the postlarvae fed with different feeds were tested for differences with Mann-Whitney U-tests. Differences in the daily specific growth rate (SGR) and feed conversion ratios (FCR) between the two diets were tested by Mann-Whitney U-tests separately for each experiment with carp and tilapia. Water-quality variables were tested for differences with Student's t-test. The normality of data distribution and data homoscedasticity were previously tested using Shapiro-Wilk tests and the Bartlett tests, respectively (Zar 2009). All statistical procedures were carried out with Statistica for Windows 7.0 (Statsoft, USA).

RESULTS

Feeding experiments

Tilapia postlarvae given the alternative feed showed statistically significant increases in body weight, length and width in both experiments (Fig. 1). Except for the weight and length increases in the first experiment, tilapia growth did not differ between the fish given the alternative and the commercial feeds (Fig. 1, first experiment in left panels and second experiment in right panels). In the first experiment, the commercial feed supported a slightly higher weight and width increase, compared to the weight and width increases observed for postlarvae fed with the alternative diet (Fig. 1, left panels). In all cases, tilapia showed weight and width reductions in the starvation treatments (Fig. 1). Mortality was negligible in all treatments.

While carp postlarvae fed the alternative feed showed statistically significant increases in body weight, length and width similar to those of postlarvae fed the commercial feed in the first experiment (Fig. 2, left panels), they showed only low and statistically non-significant growth in the second experiment (Fig. 2, right panels). However, postlarvae fed the commercial feed showed significant growth, and postlarvae in the starvation treatment showed a small reduction in dorso-ventral height (Fig. 2, right panels). As body weight showed the largest responses to the feeding experiments, it appears to be the most appropriate and sensitive growth variable to evaluate alternative fish feeds. Therefore, we further evaluated the potential of the alternative feed to sustain postlarvae growth, using two growth variables that are based on body weight gain: the specific daily growth rate (SGR) and the feed conversion ratio (FCR). In general, the alternative feed supported similar or higher SGRs (Fig. 3A; Mann-Whitney U-test) and similar or lower FCRs (Fig. 3B; Mann-Whitney U-test) than the commercial feed. Only in the first experiment with tilapia, the commercial feed supported a higher SGR (Fig. 3A) and a lower FCR (Fig. 3B).

Water-quality variables (data not shown) showed no significant differences among treatments, except for the lower conductivity observed in aquaria of postlarvae fed the alternative feed compared to the commercial feed (Student's t-test, $p<0.05$), and similar to the conductivity in the starvation treatments.

Mineral and biochemical composition of the alternative diet and postlarvae tissues

Except for iron and manganese, the concentrations of all minerals were significantly lower in the alternative than in the commercial feed (Mann-Whitney U-Test, $p<0.05$) (Table 2). The higher iron concentration in the alternative feed was probably due to earthworm meal (Table 3). The earthworm meal, as well as the jatoba meal, also contributed to the slightly higher concentrations of manganese in the alternative feed (Table 3). Jatoba meal, together with other fruit meals, was an important source of K and fibers (Table 3). In particular, the alternative feed had 9.5 times lower Ca concentrations and 1.8 times lower P concentrations compared to those in the commercial feed (Table 2). However, there were no mineral reductions in the postlarvae tissues during the experiments (Mann-Whitney U-test, $p>0.05$), except for P and Ca in tilapia.

The alternative feed had a lower total protein concentration than the commercial feed (Mann-Whitney U-Test, $p<0.05$) (Table 3). Despite the lower protein content of the alternative feed, postlarvae tissue protein concentrations of both species fed the alternative feed did not differ statistically from those of postlarvae fed the commercial feed (Table 3). The relative concentrations of crude fiber and cellulose were higher in the alternative feed than in the commercial feed (Mann-Whitney U-Test, $p<0.05$) (Table 3), although ash-free dry mass was similar in both feeds (Table 3). The lipid concentration was higher in the alternative than in the commercial feed (Table 3). Similarly to the protein concentration, the lipid concentration in postlarvae tissues did not differ between feeding treatments for either species. As no significant difference was detected between the experiments (Mann-Whitney U-Test, $p>0.05$), the data on the mineral and biochemical composition of postlarvae tissue for the same feeding treatment were pooled.

DISCUSSION

Feeding experiments

The alternative feed proposed in this study proved to be efficient in sustaining postlarvae growth. In general, the growth of tilapia postlarvae did not differ between the alternative and the commercial feeds. The carp postlarvae responded less unambiguously to the alternative feed than the tilapia. While the carp postlarvae given the alternative feed showed similar growth to that of postlarvae fed the commercial feed in one experiment, they did not show significant growth in the second experiment. However, the alternative feed supported similar SGRs and generally lower FCRs than the commercial feed. In general, the SGRs and FCRs showed low standard deviations. Accordingly, the feeding experiments can be regarded as valid tests of the alternative feed. However, the SGRs and FCRs exhibited considerable standard deviations in the first experiment with carp. A longer-duration feeding experiment might have increased the reliability of the results.

Interestingly, we observed lower electrical conductivity in aquaria of postlarvae given the alternative feed than in those of postlarvae given the commercial feed. The alternative feed did not dissolve as rapidly as the commercial feed (personal observations). Therefore, the commercial feed may have increased the water turbidity and conductivity by dissolving into the water column. The alternative feed, however, remained in suspension and thus was available for fish feeding longer than the commercial feed, possibly explaining its positive effects on fish growth.

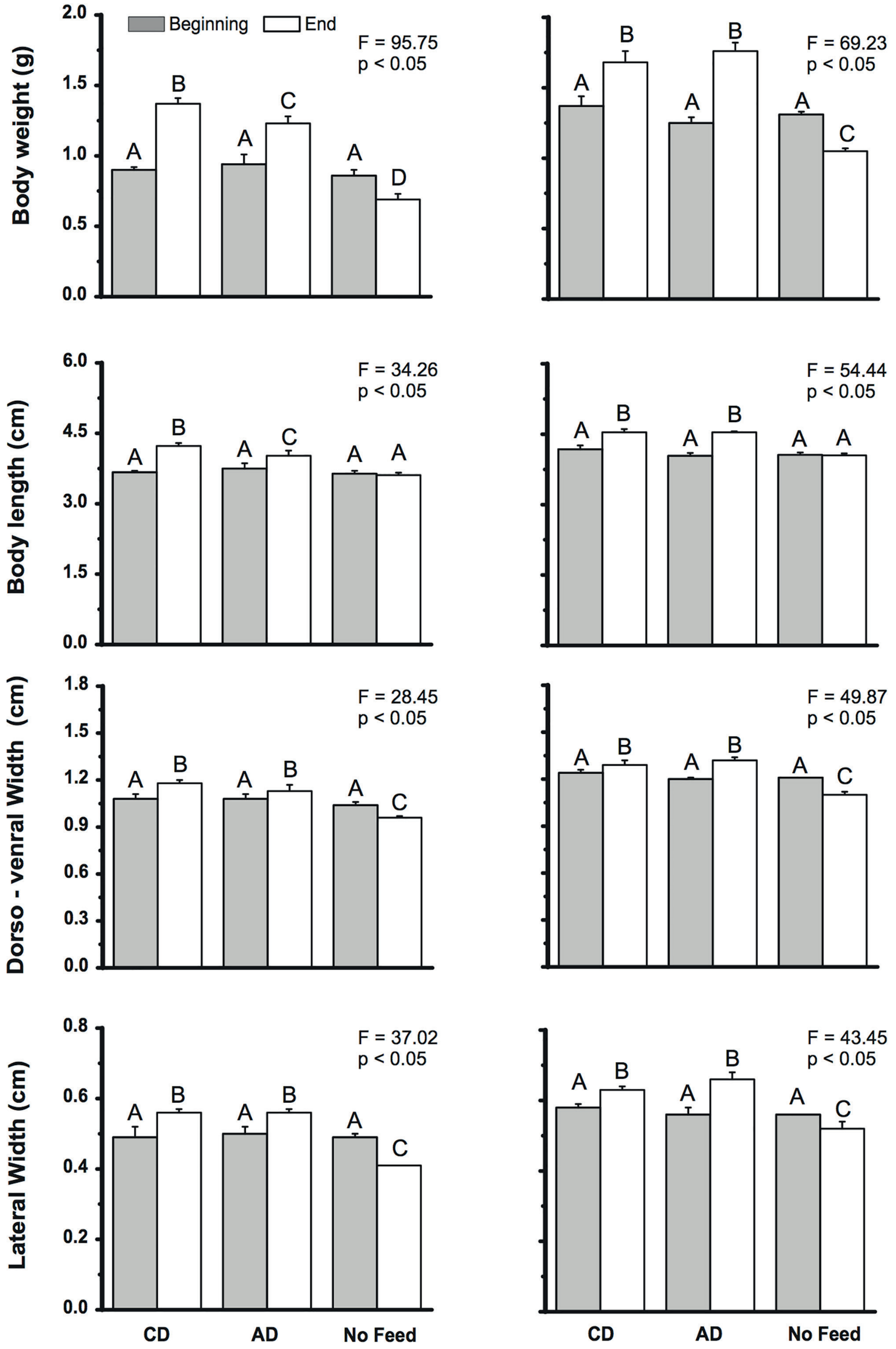

Figure 1. Growth variables measured for tilapia postlarvae (n=60) fed the commercial diet (CD), the alternative diet (AD) and without feeding (no feed), before (white bars) and after (filled bars) the two feeding experiments (first experiment shown on left panels, second experiment shown on right panels). Same letters mean no statistical difference between treatments (one-way ANOVA, followed by HSD-Tukey).

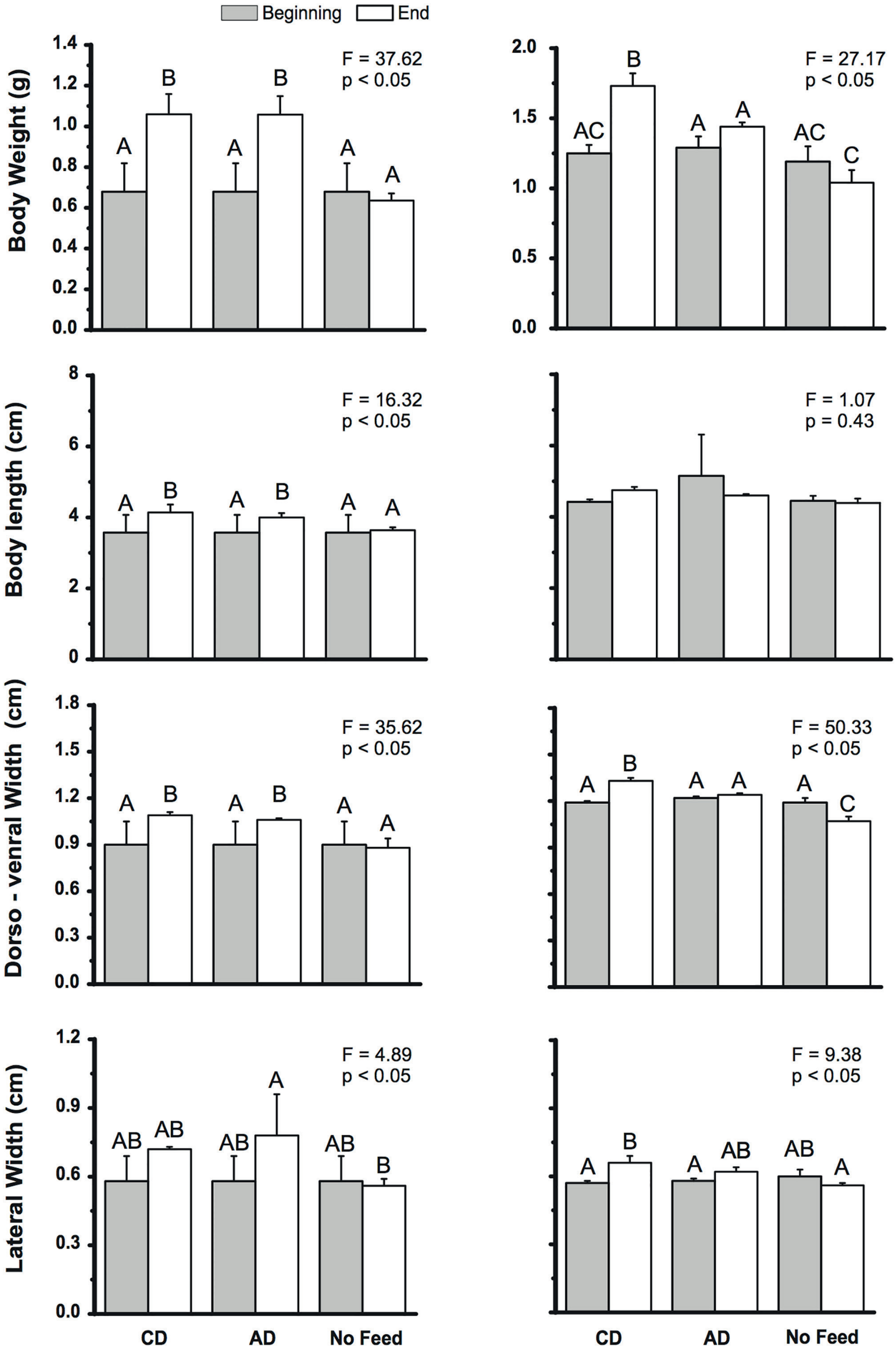

Figure 2. Growth variables measured for carp postlarvae (n=60) fed the commercial diet (CD), the alternative diet (AD) and without feeding (no feed), before (white bars) and after (filled bars) the two feeding experiments (first experiment shown in left panels, second experiment shown in right panels). Same letters mean no statistical difference between treatments (one-way ANOVA, followed by HSD-Tukey).

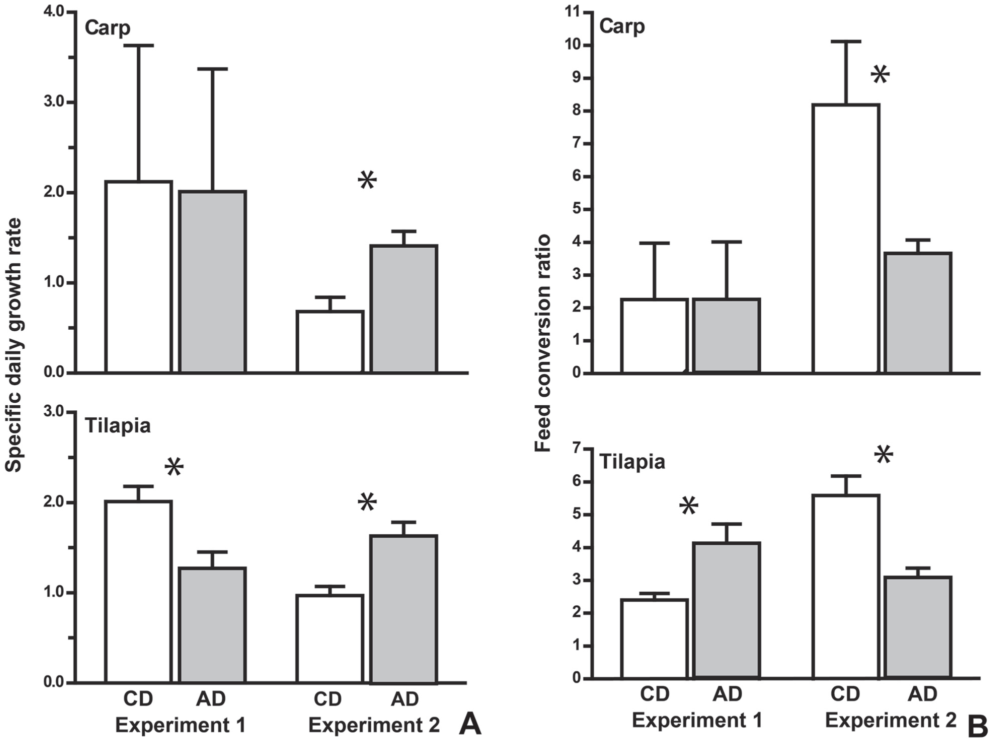

Figure 3. Specific daily growth rate (A; mean, SD) and feed conversion ratios (B; mean, SD) in the first and second feeding experiments with carp (upper graphs) and tilapia postlarvae (lower graphs) fed the alternative diet (AD) and the commercial diet (CD). Significant differences at 95% confidence between the two diets are marked with an asterisk (Mann-Whitney U-test).

Mineral and biochemical composition of the alternative diet and postlarvae tissues

In general, concentrations of all minerals were significantly lower in the alternative than in the commercial feed. In addition to the mineral supplementation in the commercial feed, this result is probably due to its fish-meal component. As our main idea was to produce a diet that can be easily prepared and at low cost for small farmers, no mineral supplements were added to the alternative feed. However, despite the generally lower mineral concentration in the alternative feed, there were no reductions in mineral contents of the tissues of postlarvae given the alternative feed, except for P and Ca in tilapia. This result may suggest a generally high mineral assimilation efficiency of postlarvae fed the alternative feed compared to postlarvae fed the commercial feed, for both species; but also points to the need for P and Ca supplementation of the alternative feed. The lower Ca and P concentrations in the alternative feed compared

Table 2. Mineral concentration (g kg^{-1}) and relative contents (%) of crude fiber, ash-free dry mass (AFDM), cellulose, total nitrogen (TN), total protein, and total lipid in the commercial diet (CD) and the alternative diet (AD) (n=6), as well as in the tissues of tilapia and carp (n=12) fed the commercial (+CD) or the alternative diet (+AD).

Element	CD	Tilapia (+CD)	Carp (+CD)	AD	Tilapia (+AD)	Carp (+AD)
P	15.42±0.04*	23.85±1.29*	15.90±1.55	8.55±0.14	18.60±1.51	17.34±1.24
K	11.16±0.14*	14.40±1.18	11.02±0.74	9.78±0.20	12.76±0.96	11.94±0.94
Ca	22.05±0.12*	38.47±0.56*	20.40±3.73	2.32±0.16	24.58±3.58	21.39±0.93
Mg	1.99±0.01*	1.36±0.18	1.05±0.10	1.41±0.03	1.10±0.10	1.08±0.05
S	5.59±0.06*	8.76±0.18	8.09±0.56	4.03±0.10	8.09±0.61	8.10±0.32
Cu	0.04±0.00*	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00	0.01±0.00
Fe	0.41±0.02	0.14±0.04	0.12±0.05	1.15±0.04*	0.16±0.08	0.10±0.03
Mn	0.05±0.00	0.01±0.00	0.00±0.00	0.07±0.00*	0.01±0.01	0.00±0.00
Zn	0.25±0.00*	0.13±0.01	0.51±0.12	0.08±0.00	0.13±0.02	0.44±0. 70
Crude fibre (%)	7.64±0.77	-	-	12.38±0.45*	-	-
AFDM (%)	90.8±0.44	-	-	90.5±0.24	-	-
Cellulose (%)	61.01±1.59	-	-	71.64±1.66*	-	-
TN (%)	8.61±0.10	11.58±0.15	10.05±0.24	5.68±0.13	10.06±0.29	9.23±0.33
Protein (%)	53.81±0.66	64.10±4.24	63.27±0.88	35.53±0.84*	57.49±3.15	62.82±0.66
Lipid (%)	6.87±0.51	21.40±6.31	29.25±1.20	21.64±0.45*	30.10±5.41	30.13±0.75

* Differences according to Mann-Whitney U-tests, $p<0.05$, between CD and AD, between Tilapia (+CD) and (+AD), as well as between Carp (+CD) and (+AD). Data on postalarvae tissue composition for a same feeding treatment from both experiments were pooled together.

Table 3. Mineral and biochemical composition and concentrations of the ingredients in the alternative diet. AFDM = ash-free dry mass.

Chemical component	Earthworm meal	Soybean meal	Corn meal	Jatoba meal	Pumpkin meal	Banana meal	Acerola meal	Guava meal	Avocado meal
P (g kg-1)	8.6± 0.1	7.2±0.2	0.5±0.0	1.1±0.0	3.2±0.0	1.0±0.0	2.3±0.0	1.3±0.0	1.8±0.5
K (g kg-1)	6.5±0.1	23.9±0.9	0.4±0.0	23.7±0.7	35.4±1.1	15.0±0.4	17.7±0.4	15.5±0.3	19.1±2.3
Ca (g kg-1)	4.6±0.1	2.1±0.0	0.1±0.0	1.3±0.0	1.1±0.0	0.4±0.0	1.92±0.1	0.99±0.0	0.7±0.0
Mg (g kg-1)	1.8±0.0	3.2±0.1	0.1±0.0	1.0±0.0	1.7±0.0	1.6±0.0	1.52±0.0	0.83±0.0	1.4±0.1
S (g kg-1)	8.8±0.1	4.1±0.1	0.9±0.0	1.0±0.0	1.7±0.0	0.4±0.0	1.18±0.0	1.13±0.0	1.5±0.1
Cu (g kg-1)	0.01±0.0	0.02±0.0	0.0 ±0.0	0.01±0.0	0.01±0.0	0.00±0.0	0.00±0.0	0.01±0.0	0.01±0.0
Fe (g kg-1)	2.8±0.1	0.1±0.0	0.04±0.0	0.01±0.0	0.1±0.0	0.02±0.0	0.02±0.0	0.02±0.0	0.03±0.0
Mn (g kg-1)	0.2±0.0	0.03±0.0	0.0±0.0	0.1±0.0	0.02±0.0	0.00±0.0	0.00±0.0	0.01±0.0	0.0±0.0
Zn (g kg-1)	1.2± 0.1	0.1±0.0	0.0±0.0	0.02±0.0	0.02±0.0	0.001±0.0	0.02±0.0	0.01±0.0	0.02±0.0
N (%)	11.2±0.1	9.0±0.2	1.2±0.0	1.6±0.1	2.6±0.1	0.9±0.1	2.3±0.2	1.0±0.1	1.6±0.1
Total protein (%)	70.3±0.6	56.3±1.0	7.6±0.1	9.9±0.5	15.9±0.8	5.8±0.5	14.3±1.2	6.4±0.3	10.1±0.4
Total lipid (%)	6.6±0.2	0.95±0.3	0.5±0.02	0.8±0.4	9.6±0.2	0.1±0.04	1.1±0.4	2.7±0.1	61.9±0.5
Crude fibre (%)	10.9±1.1	7.6±0.7	1.9±0.7	24.2±0.6	11.1± 4.4	4.8±0.8	20.2±0.8	29.9±1.9	10.3±1.3
AFDM (%)	88.3±0.0	99.6±0.0	99.4±0.0	86.8±0.0	86.2±0.0	85.9±0.0	78.8±0.0	96.9±0.0	93.9±0.0
Cellulose (%)	55.9±3.3	78.3±7.5	81.9±15.8	57.9±1.4	82.5±0.9	91.9±1.0	77.1±0.5	65.1±1.9	83.1±2.3

to those in the commercial feed support this conclusion. Viable and low-cost alternatives for Ca and P supplementation still need to be tested, but ground eggshells and black-bean meal as alternative sources of Ca and P, respectively, are promising candidates.

The earthworm and soybean meals were important protein sources in the alternative feed (Table 3), which is the reason why both ingredients are increasingly proposed as alternative protein sources for aquaculture feeds (Tacon *et al*. 1983, Viola & Arieli 1983, Shiau *et al*. 1987). Soy-protein products generally have a good amino-acid profile, and soy meal has one of the best amino-acid profiles of all alternative protein sources, except for methionine and cysteine (Hertrampf & Piedad-Pascual 2003). The alternative feed in our study had a lower total protein concentration than the commercial feed. However, the total protein content was still within the range required by both tilapia (34%, Wang *et al*. 1985) and carp (31 – 38%, Takeuchi *et al*. 1979). Despite the lower protein content of the alternative feed, the postlarvae tissue protein concentrations of the two species given the alternative feed did not differ statistically from those of postlarvae given the commercial feed. This result may also suggest a high protein assimilation efficiency of postlarvae of both species given the alternative feed, which could be associated with both the lower water solubility and the significantly higher crude fiber and cellulose concentrations of the alternative feed than those of the commercial feed. Small amounts of cellulose supplementation have been shown to increase growth and protein utilization efficiency of diets for laboratory fish (Buhler & Halver 1961). Alternatively, the total protein content of the alternative feed may have been sufficient to enable growth and weight gain of postlarvae of both species.

Relative concentrations of crude fiber and cellulose were higher in the alternative feed than in the commercial feed. Although crude-fiber contents up to 8% are normally tolerated by many species, a 12% fiber content, such as in the alternative feed in the present study, may cause digestibility problems and should generally be avoided (Ensminger & Olentine 1980, Hilton *et al*. 1983). Increasing the crude-fiber content up to 8.50% in an experimental diet did not affect growth and survival, but reduced the food-retention time in tilapia juveniles (Meurer *et al*. 2003). However, in our study, digestibility problems did not seem to affect the postlarvae of tilapia and carp, as no growth or weight loss was observed for either species during the experiments.

The lipid concentration was higher in the alternative than in the commercial feed, due to the use of olive oil, and to a lesser extent, avocado meal in the alternative feed. The use of vegetable oils in alternative fish feeds has been tested with success in recent studies (Bell *et al*. 2003). Avocado, although not commonly tested as a lipid alternative in aquaculture studies, has high lipid content and may be a useful alternative to fish oil (Ozdemir & Topuz 2004, this study).

The lipid concentration in postlarvae tissues did not differ between feeding treatments for the two species, suggesting that the postlarvae given the alternative feed efficiently assimilated the alternative plant lipids. Interestingly, a reduction in the protein content of the diet from 48 to 35% (similar to the levels found in our study, when comparing the commercial and the alternative feeds) did not result in growth and weight loss in rainbow trout as long as the lipid content in the diet was increased from 15 to 20% (similar to that observed in our study), suggesting an energy tradeoff between lipid and protein metabolism (Takeuchi *et al*. 1979).

In addition to supporting the growth and weight gain of tilapia and carp postlarvae, the alternative feed also cost less per kilogram than the commercial feed (US$ 1.31, including material and manufacturing costs, for the alternative feed versus US$ 1.57 for the commercial feed), due to the low cost of the locally produced ingredients used in the alternative feed (US$ 0.63 kg^{-1}). The ingredient mainly responsible for elevating the price of the alternative feed was olive oil (US$ 0.59 kg^{-1}

feed). More economical and accessible sources of oil as substitutes for olive oil, such as sunflower or palm oil, should be tested.

In tropical countries, and especially in Brazil, fish production has increased considerably in recent decades, as a result of the increasing demand for food and the popularity of sport fishing, as well as governmental fish-production programs. In these countries, the development of fish meal- and fish oil-free diets is an important component of aquaculture research. The production of easily confected diets, rich in ingredients such as fruits and cereals, and that can be produced locally at low cost by fish farmers themselves, is highly desirable from both the economic and ecological points of view. The fish postlarvae feed proposed in our study may be an economically viable and ecologically sound alternative feed for fish farmers producing carp and tilapia around the globe. Although exotic in Brazil, these are the most commercially important species in the country, and their production certainly contributes to the typical ecological problems associated with the production of exotic fishes.

We conclude that the proposed alternative feed successfully supported growth and body weight gain of tilapia and carp postlarvae. The alternative feed, mainly composed of common local fruits and earthworm meal, may be an economically viable and ecologically sound option for the nutrition of cultivated carp and, especially, tilapia postlarvae. Despite the mineral and biochemical differences observed between the alternative and the commercial feeds, both seemed to provide adequate nutrition for the postlarvae, as evidenced by the similar biochemical and elemental concentrations found in the tissues of postlarvae fed the two diets. Thus, the assimilation efficiency of the alternative feed seems to be as high as or even higher than the assimilation efficiency of the commercial feed tested in our study. Nonetheless, in future formulations of the proposed alternative feed, P and Ca supplements should be added. The protein content of the alternative feed could be increased by using higher concentrations of soy and pumpkin meals, without significant increases in the feed cost.

ACKNOWLEDGEMENTS

The authors thank J.I.D. Machado for assistance with chemical and biochemical analyses, and G. Silva and E. Soares for logistical support during the feeding experiments. This study was supported by a student grant from the Conselho Superior de Desenvolvimento Científico e Tecnológico (PIBIC-CNPq, Brazil) to R.C.C.

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