Aquaculture Nutrition

Aquaculture Nutrition 2014 doi: 10.1111/anu.12154

Evaluation of commercial feeds intended for the Brazilian production of Nile tilapia (*Oreochromis niloticus* L.): nutritional and environmental implications

R. MONTANHINI NETO & A. OSTRENSKY

Department of Animal Sciences, Integrated Group for Aquaculture and Environmental Studies, Federal University of Paraná, Curitiba, Paraná, Brazil

Abstract

This study evaluates the composition and nutritional content of feeds for the commercial production of Nile tilapia (*Oreochromis niloticus* L.) in Brazil. The feeds were assessed from a nutritional standpoint and as potential polluting agents of aquatic environments. The nutritional contents of each feed were calculated by considering the nutritional contributions of its ingredients based on reference tables. These contents were compared to the nutritional recommendations for *O. niloticus*. Altogether, 130 feeds were analysed, including 32 meant for fingerlings, 30 meant for juveniles, 38 meant for the growth stage and 30 meant for the termination stage. The overall digestibility of the feeds in terms of dry matter utilization was estimated to be 68.5%. Phosphorus was the nutrient with the lowest mean digestibility (51.2%), while protein had the highest mean digestibility (86.6%). Calcium and phosphorus levels exceeded their recommended values by 256.2% and 31.3%, respectively. The ratios of methionine, methionine plus cysteine, threonine and tryptophan to lysine were below their recommended values. The indigestible contents of nitrogen and phosphorus were 6.96 (±2.57) and 7.35 (±1.77) g kg^{-1}, respectively. The information obtained here provides a reference for estimating the productive and polluting potential of feeds used in the production of Nile tilapia.

KEY WORDS: aquaculture, composition, diets, digestibility, eutrophication, feedstuffs

Received 16 September 2013; accepted 27 November 2013

Correspondence: R. Montanhini Neto, Department of Animal Sciences, Integrated Group for Aquaculture and Environmental Studies, Federal University of Paraná, Rua dos Funcionários 1540, Curitiba, Paraná 80035-050, Brazil. E-mail: roberto.neto@ufpr.br.

Introduction

The eutrophication of water bodies is an important environmental problem associated with aquaculture (Smith *et al*. 1999; Liu *et al*. 2010). Nutritional strategies to minimize the impact of aquaculture on aquatic environments include manipulating and formulating diets and selecting more digestible raw materials; controlling feed granulation processes; adopting more effective feed management practices for particular fish species; recovering unconsumed feeds; and selecting fish species and strains with higher feed efficiency and better nutrient utilization (Amirkolaie 2011).

Among all the nutrients present in feeds, nitrogen, phosphorus and organic matter are those that generate the most interest with regard to their eutrophying effects in aquatic environments (Schindler 1971; Bureau & Cho 1999). Some authors also cite other feed components (calcium, silicates, nutritional additives, growth promoters, etc.) that could be associated with environmental degradation, but the available information on these components is very variable, inconsistent and/or scarce (Levings 1994; Boyd & Massaut 1999; David *et al*. 2009; Martinez 2009).

Thus, the legislative regulation of feed composition can be an important mechanism to regulate and reduce the environmental impacts of aquaculture. However, such legal frameworks must consider the complexity of the biological processes of fish growth and nutritional requirements as well as limnological and hydrographic factors. Ultimately, these factors determine the eutrophication potential of aquaculture enterprises (Talbot & Hole 1994).

Nutrient discharges from fish farms can be determined retrospectively, in a relatively simple and highly accurate manner, from records of the management practices adopted by farms and of the feed conversion efficiency achieved combined with knowledge about the nutritional content of the feeds and the body composition of the fish (Dosdat

2001). Prospective estimates of nutrient inputs (via feeding) and outputs (via farming wastes) on a daily basis or over longer time periods are an important management and planning tool for producers, researchers and regulatory agencies (Einen *et al.* 1995).

Brazil has extremely favourable conditions for inland fish farming. There are more than five million hectares of freshwater in natural and artificial reservoirs that can be used for the production of aquatic organisms (Bueno *et al.* 2008). Production in cages and pens has become an important part of commercial fish production in Brazil, and the number of enterprises installed in hydroelectric reservoirs has been continuously increasing in recent years (Nunes 2012). According to Brazil (2013), the Brazilian production of Nile tilapia was higher than 250.000 t in 2011, representing almost 50% of the national aquaculture production.

The objective of this study was to evaluate the composition and nutritional content of Brazilian commercial feeds formulated for Nile tilapia. The nutritional adequacy of these feeds and their potential polluting effects on the aquatic environments adjacent to the farms are addressed.

Materials and methods

Collection of information about the commercial feeds

Data on the composition of commercial tilapia feeds were collected for all feeds with active records in the Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA) directorate in the state of Paraná, southern Brazil.

A particular feed was selected for analysis if it was registered as a feed for Nile tilapia and if its record included information about the production stage for which it was intended (fingerlings, juveniles, growth or termination). The centesimal ingredient composition of each feed was recorded in a database and grouped by production stage. The ingredient compositions were input into a software program (Optimix 4.1; Domit Ltd., Curitiba, Brazil.) to calculate the nutritional content of each feed.

Calculation of nutritional contents, digestibility and nutrient ratios

The nutritional contents and digestibility coefficients of each nutrient in the selected feeds were calculated from the reference tables presented by Furuya (2010), NRC (2011) and Rostagno (2011). Every nutrient relevant to the listed ingredients of the selected feeds was recorded.

The content of each ingredient in a given feed was individually determined using the following equation:

$$C_{\mathrm{N}}F_{\mathrm{S}} = \left\{ \sum_{I}^{1\to n} [C_{\mathrm{N}}\mathrm{Ing}_{\mathrm{I}} \cdot \mathrm{Ing}_{\mathrm{I}}^{\%}F_{\mathrm{S}}] \right\}$$

where:

$C_{\mathrm{N}}F_{\mathrm{S}}$ is the total content of nutrient ‘N’ in a feed formulated for stage ‘S’ in grams of the nutrient per kg of feed;

S is the production stage (fingerlings, juveniles, growth or termination);

$\mathrm{Ing}_{\mathrm{I}}$ is the ingredient ‘I’ of the feed;

C_{N} $\mathrm{Ing}_{\mathrm{I}}$ is the content of nutrient ‘N’ in ingredient ‘I’ in grams of the nutrient per kg of the ingredient;

$\mathrm{Ing}_{\mathrm{I}}^{\%}F_{\mathrm{S}}$ is the percentage inclusion of ingredient ‘I’ in a feed formulated for stage ‘S’.

The digestible nutrient contents were determined by multiplying the total calculated nutrient contents by the respective digestibility coefficients of the ingredients for Nile tilapia, as given by Furuya (2010), using the following equation:

$$C_{\mathrm{N}}^{\mathrm{dig}}\mathrm{Ing}_{\mathrm{I}} = (C_{\mathrm{N}}^{\mathrm{tot}}\mathrm{Ing}_{\mathrm{I}} \cdot \mathrm{Dig}_{\mathrm{N}}^{\%}\mathrm{Ing}_{\mathrm{I}})$$

where:

$C_{\mathrm{N}}^{\mathrm{dig}}\mathrm{Ing}_{\mathrm{I}}$ is the digestible content of nutrient ‘N’ in ingredient ‘I’ for Nile tilapia in grams of the nutrient per kg of the ingredient;

$\mathrm{Ing}_{\mathrm{I}}$ is the ingredient ‘I’ of the feed;

$C_{\mathrm{N}}^{\mathrm{tot}}\mathrm{Ing}_{\mathrm{I}}$ is the total content of nutrient ‘N’ in ingredient ‘I’ in grams of the nutrient per kg of the ingredient;

$\mathrm{Dig}_{\mathrm{N}}^{\%}\mathrm{Ing}_{\mathrm{I}}$ is the digestibility coefficient of nutrient ‘N’ in ingredient ‘I’ for Nile tilapia, expressed as a percentage.

The contents of certain nutrients were compared to obtain the ratios between them. The calculated ratios were digestible protein (DP; mg of protein per kg of feed) to digestible energy (DE; kcal of energy per kg of feed) and total calcium (Ca) to available phosphorus (P) ($\mathrm{g\ g^{-1}}$). The ideal protein profile for each evaluated feed was also determined by calculating the ratios (in %) between the digestible essential amino acid (DEAA, including cysteine and tyrosine) contents and the digestible Lysine content.

Estimation of the composition of the indigestible fraction of each feed

From the calculated digestibility coefficients and nutritional content, the indigestible fraction was determined for certain ingredients that could be found in the fishes’ solid excreta if the fish were to consume the analysed feeds. The fractions of each nutrient considered a potential pollutant of the aquatic environment were calculated (Schindler 1971; Bureau & Cho 1999). These faecal composition values were calculated from the difference between the total amount of each nutrient in the feeds and the digestible fraction of the same nutrient for Nile tilapia.

$$\mathrm{IF_N}F_\mathrm{S} = (C_\mathrm{N}^\mathrm{tot}F_\mathrm{S} - C_\mathrm{N}^\mathrm{dig}F_\mathrm{S})$$

where:

$\mathrm{IF_N}F_\mathrm{S}$ is the indigestible fraction of nutrient ‘N’ in the faeces in grams of the nutrient per kg of feed formulated for stage ‘S’;

S is the production stage (fingerlings, juveniles, growth or termination);

$C_\mathrm{N}^\mathrm{tot}R_\mathrm{F}$ is the total content of nutrient ‘N’ in a feed formulated for stage ‘S’ in grams of the nutrient per kg of feed;

$C_\mathrm{N}^\mathrm{dig}F_\mathrm{S}$ is the digestible content of nutrient ‘N’ for Nile tilapia in a feed formulated for stage ‘S’ in grams of the nutrient per kg of feed.

Comparison of feed composition and nutritional recommendations

The feed compositions, digestible fractions and nutrient ratios were compared to the nutritional recommendations for each production stage of Nile tilapia under Brazilian commercial production conditions, as given by Furuya (2010). To obtain the ideal protein profile of each feed, the ratio of DEAA to digestible lysine was compared to the recommendations of Santiago and Lovell (1988). These comparisons revealed whether the commercial formulations met the nutritional standards for each production stage and partly explained the incomplete utilization of the nutrients supplied by the feeds.

Mathematical data treatment and statistical analyses

The data were calculated within a range of possible values so that the estimates would provide an idea of the variability of the results. The lower and upper limits of confidence intervals ($\alpha = 90\%$) were established for the nutritional contents and digestibility coefficients used in the calculations. Thus, the final results considered a range of possibilities relevant to the typical conditions found in commercial farming.

The descriptive statistics (means, standard deviations and confidence intervals) of the calculated centesimal compositions of the commercial feeds, digestibility coefficients and indigestible fractions were determined using the software Statistica 8.0 (Statsoft, Inc., Tulsa, OK, USA).

Results

The calculated nutritional compositions of the feeds formulated for Nile tilapia with active records in the Paraná state MAPA directorate are shown in Table 1. Altogether, 130 feeds met the selection criteria for this study and were selected for analysis. These feeds were distributed among the four production stages as follows: fingerlings, $N = 32$; juveniles, $N = 30$; growth, $N = 38$; and termination, $N = 30$.

Table 2 shows the ingredient sources (animal, plant, mineral or synthetic) and their respective contributions to the crude protein (CP) and total P contents. Plant ingredients were predominant in the feeds for all production stages, with an increasing trend across the production stages (from 630 to 800 g kg^{-1}). Animal products, including bovine, avian and fish meals, were included at lower levels, varying from 170 to 350 g kg^{-1} in a decreasing trend across the production stages. Other mineral (e.g. salts, phosphate, limestone) and synthetic ingredients (e.g. industrial amino acids, vitamins, additives) represented 20–30 g kg^{-1} of the feed compositions.

Plant and animal ingredients contributed similarly (53:57) to the CP content of the fingerling and juvenile feeds, despite their differences in inclusion. For the growth and termination stages, the contribution of plant ingredients was greater than that of animal products (61:39). Supplementation with industrial amino acids contributed 20–50 g kg^{-1} of the CP in the feeds; however, the use and inclusion of these ingredients varied widely among the evaluated feeds.

Conversely, animal ingredients represented the majority of the nutritional contribution of total P in all feeds. In feeds formulated for the initial growth stages, 67% of the P was derived from animal products, 22% from plants and 11% from minerals (in the form of mono-, di- and tricalcium phosphates). In feeds formulated for the grow-out stages, 58% of the P was from animal products, 31% from plant products and 11% from mineral products.

The total essential amino acids (TEAA) (including cysteine and tyrosine) and DEAA compositions of feeds formulated for each production stage are shown in Table 3. By comparing these results to the CP contents, the non-essential

Table 1 Means and standard deviations of the calculated nutritional contents (in g kg^{-1} of feed) of commercial feeds formulated for Nile tilapia (*Oreochromis niloticus* L.) with active records in the Brazilian Ministry of Agriculture, Livestock and Food Supply (N = 130)

Nutrient	Fingerlings (n = 32)	Juveniles (n = 30)	Growth (n = 38)	Termination (n = 30)
Total dry matter	906.0 (±7.7)	904.7 (±6.4)	902.3 (±6.8)	902.2 (±7.2)
Digestible dry matter	628.7 (±32.5)	629.1 (±45.7)	608.5 (±37.8)	611.2 (±44.2)
Total organic matter	777.7 (±34.1)	790.4 (±22.2)	806.8 (±22.5)	803.6 (±27.3)
Digestible organic matter	577.3 (±41.9)	585.6 (±33.0)	578.9 (±35.8)	576.4 (±39.9)
Gross energy (kcal kg^{-1})	4157.1 (±207.8)	4105.0 (±150.1)	4133.6 (±148.9)	4105.7 (±152.7)
Digestible energy (kcal kg^{-1})	3092.6 (±280.9)	3048.4 (±237.9)	3017.1 (±220.4)	3051.2 (±241.4)
Crude protein	399.6 (±47.8)	352.3 (±49.8)	276.7 (±36.8)	240.8 (±38.0)
Digestible protein	347.0 (±34.3)	310.2 (±46.5)	236.8 (±32.3)	207.1 (±36.5)
Protein/Energy[1]	112.2 (±27.4)	101.8 (±25.5)	78.5 (±19.0)	67.9 (±18.8)
Crude fat	54.8 (±19.4)	54.5 (±16.2)	47.3 (±13.6)	50.1 (±16.8)
Non-nitrogen extracts	289.8 (±75.4)	328.8 (±71.1)	440.0 (±54.7)	470.5 (±52.2)
Crude fibre	33.5 (±10.6)	39.5 (±14.1)	42.8 (±11.6)	42.2 (±10.7)
Mineral residue	128.4 (±39.6)	114.3 (±25.6)	95.4 (±26.5)	98.6 (±31.3)
Total calcium	27.4 (±11.6)	24.5 (±8.4)	18.5 (±7.4)	19.6 (±10.5)
Digestible calcium	19.6 (±8.3)	16.8 (±6.1)	13.2 (±5.5)	14.5 (±9.4)
Total phosphorus	16.4 (±5.8)	15.9 (±4.3)	14.5 (±3.8)	13.6 (±4.1)
Available phosphorus	9.7 (±4.0)	8.9 (±2.8)	6.5 (±2.3)	6.1 (±2.4)
Calcium/Phosphorus[2]	2.8 (±1.0)	2.8 (±0.6)	2.9 (±0.8)	3.2 (±1.1)

[1] Ratio of digestible protein (in mg kg^{-1} of feed) to digestible energy (in kcal kg^{-1} of feed).

[2] Ratio of total calcium to available phosphorus (in g g^{-1} of feed).

Table 2 Means and standard deviations of the contributions of selected ingredient sources and their respective nutritional contributions to the calculated crude protein and total phosphorus contents (in g kg^{-1} of feed) of feeds formulated for Nile tilapia (*Oreochromis niloticus* L.) with active records in the Brazilian Ministry of Agriculture, Livestock and Food Supply (N = 130)

	Fingerlings (n = 32)	Juveniles (n = 30)	Growth (n = 38)	Termination (n = 30)
Plant ingredients	631.6 (±190.2)	672.7 (±132.3)	775.3 (±93.6)	796.1 (±82.6)
Animal ingredients	345.4 (±198.1)	310.6 (±135.9)	201.4 (±96.3)	172.9 (±94.0)
Mineral ingredients	20.4 (±36.9)	13.1 (±16.6)	19.7 (±20.9)	27.1 (±33.0)
Synthetic ingredients	2.6 (±1.8)	3.7 (±3.4)	3.6 (±4.1)	3.9 (±4.0)
Plant protein	204.7 (±88.5)	180.5 (±62.4)	158.5 (±49.7)	141.0 (±45.9)
Animal protein	182.7 (±99.7)	164.2 (±77.0)	103.1 (±52.8)	91.1 (±51.5)
Synthetic protein[1]	12.2 (±8.0)	7.7 (±4.9)	15.0 (±8.2)	8.7 (±6.5)
Plant phosphorus	3.5 (±1.4)	3.3 (±0.6)	3.3 (±1.1)	4.6 (±1.4)
Animal phosphorus	11.0 (±6.2)	10.0 (±4.3)	8.5 (±4.5)	7.9 (±4.0)
Mineral phosphorus	1.8 (±1.7)	1.7 (±1.6)	1.9 (±1.8)	1.1 (±0.6)

[1] Derived from industrial amino acids.

amino acid (NEAA) content could be estimated for each stage: fingerlings, 226.1 g kg^{-1}; juveniles, 197.48 g kg^{-1}; growth, 163.92 g kg^{-1}; termination, 133.98 g kg^{-1}. The mean ratio of TEAA to NEAA was 44:56.

The DEAA profiles are shown in Table 4. The ratios of methionine, sulphur amino acids (methionine and cysteine), threonine and tryptophan to lysine were lower than those recommended by the literature. The methionine/lysine ratio was half its recommended value. The other three deficient ratios were 18.3, 22.3 and 28.8% lower than their recommended values, respectively. Among the ratios of other amino acids, the mean values across all stages were 5.3–17.4% greater than the recommended values. The leucine/lysine ratio showed the greatest excess.

The nutritional compliance percentages of feeds formulated for each production stage compared with the respective recommendations are shown in Table 5. The mean DE value calculated for the fingerling stage was only 77.1% of the recommended value; however, DE was close to adequate for the three following stages. The DP content was below its recommended level for the fingerling,

Table 3 Means and standard deviations of the calculated total (Tot.) and digestible (Dig.) essential amino acids (including cysteine and tyrosine) (in g kg^{-1} of feed) in feeds formulated for Nile tilapia (*Oreochromis niloticus* L.) with active records in the Brazilian Ministry of Agriculture, Livestock and Food Supply ($N = 130$)

	Fingerlings ($n = 32$)		Juveniles ($n = 30$)		Growth ($n = 38$)		Termination ($n = 30$)	
Amino acid	Tot.	Dig.	Tot.	Dig.	Tot.	Dig.	Tot.	Dig.
Lysine	25.3 (±7.5)	22.3 (±8.5)	23.3 (±3.8)	20.7 (±4.3)	16.2 (±3.8)	14.1 (±4.1)	14.9 (±5.6)	13.0 (±6.2)
Methionine	6.1 (±2.3)	5.4 (±2.6)	6.4 (±2.1)	5.7 (±2.4)	4.2 (±1.5)	3.6 (±1.6)	3.9 (±1.2)	3.4 (±1.3)
Methionine + Cysteine	12.4 (±4.4)	10.9 (±5.0)	12.2 (±2.6)	10.8 (±2.9)	8.1 (±2.4)	7.0 (±2.6)	8.1 (±2.5)	7.1 (±2.8)
Threonine	14.2 (±4.6)	12.5 (±5.2)	12.7 (±1.8)	11.3 (±2.0)	9.2 (±2.0)	8.0 (±2.1)	9.0 (±2.5)	7.8 (±2.8)
Tryptophan	3.6 (±1.3)	3.2 (±1.4)	3.1 (±0.4)	2.7 (±0.4)	2.4 (±0.5)	2.1 (±0.6)	2.2 (±0.8)	1.9 (±0.9)
Valine	19.9 (±6.5)	17.5 (±7.4)	17.1 (±2.3)	15.2 (±2.6)	13.0 (±2.7)	11.3 (±3.0)	12.2 (±3.5)	10.6 (±3.8)
Isoleucine	16.9 (±5.9)	14.9 (±6.7)	15.0 (±2.1)	13.3 (±2.4)	10.4 (±2.7)	9.1 (±3.0)	10.2 (±3.3)	8.9 (±3.6)
Arginine	27.2 (±8.9)	24.0 (±10.1)	24.3 (±2.8)	21.6 (±3.2)	17.9 (±3.5)	15.6 (±3.8)	17.0 (±4.9)	14.8 (±5.3)
Leucine	32.3 (±11.1)	28.5 (±12.6)	28.4 (±5.5)	25.2 (±6.3)	20.6 (±5.9)	17.9 (±6.4)	20.0 (±8.7)	17.4 (±9.6)
Phenylalanine	18.9 (±6.0)	16.7 (±6.7)	16.5 (±2.3)	14.7 (±2.6)	12.5 (±2.7)	10.9 (±2.9)	11.8 (±3.7)	10.3 (±4.1)
Phenylalanine + Tyrosine	28.6 (±8.7)	25.2 (±9.8)	25.2 (±3.6)	22.3 (±4.1)	19.0 (±4.0)	16.5 (±4.4)	18.0 (±5.8)	15.7 (±6.3)
Histidine	9.2 (±2.9)	8.1 (±3.2)	7.9 (±0.9)	7.0 (±1.0)	6.5 (±1.5)	5.6 (±1.7)	5.7 (±1.6)	5.0 (±1.8)

Table 4 Ideal protein ratios (in %) based on the calculated contents of digestible lysine and digestible essential amino acids (including cysteine and tyrosine) in feeds formulated for Nile tilapia (*Oreochromis niloticus* L.) with active records in the Brazilian Ministry of Agriculture, Livestock and Food Supply ($N = 130$)

Nutrient	Recc.[1]	Fingerlings ($n = 32$)	Juveniles ($n = 30$)	Growth ($n = 38$)	Termination ($n = 30$)
Lysine	100	100	100	100	100
Methionine	52	24	27	26	26
Methionine + Cysteine	63	49	52	50	55
Threonine	73	56	54	57	60
Tryptophan	20	14	13	15	15
Valine	55	79	73	80	82
Isoleucine	61	67	64	64	68
Arginine	82	108	104	110	114
Leucine	66	128	122	127	134
Phenylalanine	73	75	71	77	79
Phenylalanine + Tyrosine	108	113	108	117	121
Histidine	34	36	34	40	38

[1] According to the recommendations of Santiago and Lovell (1988).

growth and termination stages (89.9, 97.4 and 95.0%, respectively); however, DP exceeded its recommended level (115.7%) for the juvenile stage. Due to the lower DE value in feeds for the fingerling stage and the excess DP in feeds for juveniles, these stages exhibited higher DP/DE ratios. The digestible lysine content was adequate in the fingerling, growth and termination stages but was 35.4% greater than its recommended value for the juvenile stage.

Among the calculated macromineral contents, significant Ca excesses were observed, with calculated values up to 256% of the recommended levels. The calculated available P content also showed a mean excess of 31.3%. Despite the concurrent excess P levels, the high calcium contents of the feeds resulted in high Ca/P ratios, ranging from 160 to 183% of the recommended values.

The estimated indigestible fractions of the nutrients in the feeds are shown in Table 6. In terms of mean dry matter utilization, the overall digestibility of the feeds was 68.5%, with no consistent variation among the stages. The evaluated nutrient with the lowest mean digestibility was P (51.2%), which showed higher digestibility in feeds formulated for the initial stages (57.6%) compared with those formulated for the grow-out stages (44.7%). Conversely, protein showed the highest mean digestibility coefficient (86.6%), and its digestibility coefficients were similar between the production stages.

Discussion

One challenge in developing environmentally sustainable aquaculture is the viable replacement of animal meals with

Table 5 Nutritional compliance of the analysed feeds (in %) compared with the recommended levels for major nutrients proposed by Furuya (2010) for feeds formulated for Nile tilapia (*Oreochromis niloticus* L.) with active records in the Brazilian Ministry of Agriculture, Livestock and Food Supply ($N = 130$).

Nutrient	Fingerlings ($n = 32$)	Juveniles ($n = 30$)	Growth ($n = 38$)	Termination ($n = 30$)
Digestible energy	77.2	100.4	98.1	98.0
Crude protein	96.8	118.5	107.0	100.9
Digestible protein	89.9	115.7	97.4	95.0
Protein/Energy[1]	116.5	115.2	99.3	97.0
Digestible lysine	101.3	135.4	102.2	105.6
Total calcium	365.3	359.0	329.8	370.5
Available phosphorus	129.1	137.4	126.5	132.4
Calcium/ Phosphorus[2]	283.0	261.9	260.9	280.1

[1] Ratio of digestible protein to digestible energy.
[2] Ratio of total calcium to available phosphorus.

plant protein sources. The viability of such replacements depends on the nutritional profile and digestibility of the formulated feed (Cyrino *et al*. 2010).

Feed digestibility is defined as the ability of the animal to digest and absorb the nutrients and energy contained in the feed (Pond *et al*. 2005). The greater inclusion of plant ingredients seen here results in greater digestibility of the protein fraction because these ingredients provide more digestible protein to fish compared with animal products, considering the most common feedstuffs used for fish feeds in Brazil (Pezzato *et al*. 2002).

Conversely, the inclusion of plant ingredients is associated with greater P loss, as confirmed by the low mean digestibility value observed for this nutrient. A major problem associated with the use of plant ingredients in fish feeds is the presence of phytate (myo-inositol-1,2,3,4,5,6-hexakisphosphate), which is the main form of P storage in plants (Maga 1982). Up to 80% of the total P content in plant ingredients may be present as phytate (Ravindran *et al*. 1994). This fraction is practically unavailable for monogastric aquatic animals during digestion due to the lack of endogenous enzymes for its efficient hydrolysis (Cao *et al*. 2007). Thus, the majority of phytate-bound P is ultimately excreted in the faeces, potentially contributing to the eutrophication of the aquatic environment (Liebert & Portz 2005). According to Schindler (1971), P is the main nutrient from aquaculture that enriches and pollutes continental aquatic environments.

Aside from losses due to unavailability, the excessive available P content compared with the recommended levels in Nile tilapia feeds may aggravate the polluting potential of this nutrient in aquatic environments adjacent to farms. Even if it is absorbed, this excess P is not metabolically utilized by the fish and is ultimately excreted in the urine (Roy & Lall 2004). Studies indicate that only one-third of the P absorbed is metabolically utilized by the fish and retained in the organism (Bureau & Cho 1999). However, in cases of dietary excess P, as observed in the Brazilian feeds analysed here, the soluble fraction excreted becomes proportionally larger (Sugiura *et al*. 2000). This excretion of soluble P is significantly more polluting to the aquatic environment than the indigestible fraction (phytate-bound

Table 6 Means and standard deviations of the calculated indigestible fractions (in g kg^{-1} of feed) of feeds formulated for Nile tilapia (*Oreochromis niloticus* L.) with active records in the Brazilian Ministry of Agriculture, Livestock and Food Supply ($N = 130$).

Nutrient	Fingerlings ($n = 32$)	Juveniles ($n = 30$)	Growth ($n = 38$)	Termination ($n = 30$)
Dry matter	277.4 (±32.4)	275.6 (±43.9)	293.8 (±37.8)	291.0 (±42.9)
Organic matter	200.3 (±29.3)	204.8 (±27.4)	227.9 (±24.3)	227.2 (±28.5)
Protein	52.6 (±25.6)	42.1 (±11.9)	39.9 (±16.4)	33.7 (±10.6)
Nitrogen	8.4 (±4.1)	6.7 (±1.9)	5.9 (±2.4)	6.8 (±1.9)
Calcium	7.8 (±4.3)	7.7 (±3.2)	5.2 (±2.6)	5.1 (±2.5)
Phosphorus	6.7 (±2.1)	7.0 (±1.6)	8.0 (±1.6)	7.5 (±1.8)

and excreted in the faeces) due to the greater solubility and dispersibility of the phosphorous compounds excreted (Cowey 1995).

Significant excess Ca was also observed in the evaluated feeds. The need for Ca supplementation in feeds for freshwater fishes in tropical environments is usually negligible because the concentration of this macromineral solubilized in water meets the nutritional requirement almost fully (Mayer-Gostan *et al.* 1983; Flik *et al.* 1986). In contrast to terrestrial animals, which are completely dependent upon a dietary supply of minerals, fish can absorb part of their required minerals, especially Ca, directly from the water through the gills or even through the body surface (McCormick *et al.* 1992; Flik & Verbost 1993). However, the excretion of excess Ca is not considered a polluting factor for freshwater environments because the input of aquacultural wastes contributes little compared with the normal limnological concentrations of this mineral (Waters *et al.* 1991).

The high Ca/P ratio observed here may have a negative effect on the digestibility and retention of dietary P. Several studies have suggested that this ratio is as relevant as the individual levels of these minerals (Cheng *et al.* 2006). According to Miranda *et al.* (2000), the recommended ratio for Nile tilapia is approximately 1:1 based on growth performance and the retention of P in the body. In the present study, the Ca/P values of the Brazilian feeds were approximately 2.7:1. The higher proportion of Ca negatively affects P absorption by competing for absorption sites in the intestinal mucosa and/or by forming insoluble compounds with dietary P sources, especially phytate (Vielma & Lall 1998).

In addition to their environmental implications, the excess P and Ca concentrations in the feeds may have a significant economic impact by increasing the costs of the feeds and consequently decreasing the profitability of aquaculture ventures. In recent years, P supplementation in feeds has represented one of the main costs in the formulation of commercial fish feeds (Sugiura *et al.* 2006).

Aside from the disproportionate mineral contents, the feeds analysed in this study showed low compliance with the recommended DEAA profiles. Importantly, despite the higher mean digestibility of the protein fraction from the plant ingredients in the feed compared with the protein from animal ingredients, the amino acid profile of a feed containing more plant protein does not necessarily match the requirements of the farmed fish.

The concept of ideal protein assumes that the metabolic use of dietary amino acids depends upon appropriate ratios between the amino acids, especially for those considered essential (Boisen *et al.* 2000). The ideal protein profile is closely related to the ratios of the amino acids in the body composition of the animals (Akiyama *et al.* 1997; Ngamsnae *et al.* 1999).

Although the crude and digestible protein contents were relatively adequate based on the nutritional recommendations for each production stage, the ratios between the essential amino acids were far from their recommended values. Methionine was the most limiting amino acid in the analysed feeds because it had the lowest proportional compliance. The use of soybean flour as the main protein source reduces the methionine content of the feed due to the well-known deficiency of this amino acid in soybean protein (Sadiku & Jauncey 1995).

Considering the limitation imposed by methionine, which exhibited only half its recommended level in the analysed feeds, it is likely that approximately half the DP in the diet would be excreted following amino acid metabolism. Based on the law of the minimum, the maximum protein synthesis capacity is limited by the most deficient essential amino acid relative to the ideal protein profile (Cowey 1994). For Brazilian Nile tilapia feeds, this high level of N excretion may be even more severe in the fingerling and juvenile stages. The high DP/DE ratios found in feeds formulated for these stages suggest a low digestive supply of carbon chains relative to the amount of N to be metabolized. A large portion of absorbed amino acids are broken up and transformed into other amino acids so that they can be stored as peptides or transformed into other nitrogen compounds with specific metabolic functions. The process of transferring amine groups (called transamination) demands the proportional availability of carbon chains for resynthesis (Li *et al.* 2013). In feeds formulated for juveniles, greater N loss would also result from the high DP content (approximately 15% higher than necessary).

An imbalance among digestible essential amino acids can significantly reduce fish growth, causing animal production and economic losses that are incompatible with the profitability of aquaculture ventures (Kaushik & Seiliez 2010). In addition, all of the amino acids that are not retained after metabolism are excreted into the environment as soluble inorganic nitrogen compounds (Li *et al.* 2009).

In addition to P, nitrogen (N) is a potential polluting nutrient from aquaculture (Schindler 1971). Fernandes *et al.* (2007) have estimated that 88–93% of the N supplied in feeds is released to the environment in the form of waste, and the largest portion (59–64%) corresponds to excretion after absorption and metabolism. Ammonia and urea are the two main nitrogen molecules that result from the metabolism of amino acids and protein residues in fish. These two compounds are responsible for more than 80% of the N excretion from pisciculture (Cowey 1995). These molecules are highly soluble and are promptly available for biological use by phytoplankton (Tanaka & Kadowaki 1995; Páez-Osuna *et al.* 1999).

To avoid AA losses in fish due to unbalanced protein profiles, several authors have studied the effects of supplementing feeds with industrial amino acids (Furuya *et al.* 2004). Adding these supplements makes it possible to more accurately meet individual species' DEAA requirements. In other livestock categories (e.g. birds, pigs and dairy cows), the establishment of minimum CP and DP levels is no longer considered because the DEAA levels are theoretically met (Tuitoek *et al.* 1997; Baker 2009; Lee *et al.* 2012).

However, studies have indicated that fish use industrial amino acids much less efficiently than amino acids derived from peptide bonds (Walton & Wilson 1986; Schuhmacher *et al.* 1997). In addition, the use of these additives depends on the granulation quality of the feed because the additives can solubilize in water and leach out before being consumed by the fish (Zarate & Lovell 1997). A drastic reduction in protein levels associated with the use of industrial amino acids is not recommended because the minimum N contribution from protein must be considered, especially the contribution from NEAA (Gaye-Siessegger *et al.* 2007). To meet this recommendation in fish, the TEAA/NEAA ratio should fall between 40:60 and 50:50 (Mambrini & Kaushik 1994; Peres & Oliva-Teles 2006). Thus, the mean ratio found in the present study appears adequate.

Based on the results of this study, Brazilian commercial feeds formulated for Nile tilapia do not provide adequate nutrition due to nutrient deficiencies, excesses and imbalances. In all cases of inadequacy, the fish cannot fully utilize the nutrients supplied by the feeds, compromising their growth performance and consequently the economic performance of tilapia farms. In parallel, the nutritional wastes resulting from incomplete nutrient utilization, especially nitrogen and phosphorous compounds, will be directly released into the aquatic environment surrounding the farms, potentially causing the eutrophication of adjacent water bodies.

Acknowledgements

We thank the Livestock Inputs Inspection Service of the Brazilian Ministry of Agriculture, Livestock and Food Supply (SEFIP-MAPA) in Paraná state for providing access to the information about actively registered Nile tilapia feeds, which was indispensable for the completion of this study. We also thank the National Council for Scientific and Technological Development (CNPq) for granting funding to Antonio Ostrensky.

References

Akiyama, T., Oohara, T. & Yamamoto, T. (1997) Comparison of essential amino acid requirements with A/E ratio among fish species. *Fish. Sci.*, **63**, 963–970.

Amirkolaie, A.K. (2011) Reduction in the environmental impact of waste discharged by fish farms through feed and feeding. *Rev. Aquacult.*, **3**, 19–26.

Baker, D. (2009) Advances in protein–amino acid nutrition of poultry. *Amino Acids*, **37**, 29–41.

Boisen, S., Hvelplund, T. & Weisbjerg, M.R. (2000) Ideal amino acid profiles as a basis for feed protein evaluation. *Livest. Prod. Sci.*, **64**, 239–251.

Boyd, C.E. & Massaut, L. (1999) Risks associated with the use of chemicals in pond aquaculture. *Aquacult. Eng.*, **20**, 113–132.

Brazil (2013) Statistical Bulletin of Fisheries and Aquaculture - 2011. Ministry of State for Fisheries and Aquaculture, Brasília, Brazil.

Bueno, G.W., Marengoni, N.G., Gonçalves, A.C., Boscolo, W.R. & Teixeira, R.D.A. (2008) Trophic level and bioaccumulation of total phosphorus in cage fish rearing in the aquaculture area at Itaipu reservoir. *Acta. Sci. Biol. Sci.*, **30**, 237–243.

Bureau, D.P. & Cho, C.Y. (1999) Phosphorus utilization by rainbow trout (*Oncorhynchus mykiss*): estimation of dissolved phosphorus waste output. *Aquaculture*, **179**, 127–140.

Cao, L., Wang, W., Yang, C., Yang, Y., Diana, J., Yakupitiyage, A., Luo, Z. & Li, D. (2007) Application of microbial phytase in fish feed. *Enzyme Microb. Technol.*, **40**, 497–507.

Cheng, K.-M., Hu, C.-Q., Liu, Y.-N., Zheng, S.-X. & Qi, X.-J. (2006) Effects of dietary calcium, phosphorus and calcium/phosphorus ratio on the growth and tissue mineralization of *Litopenaeus vannamei* reared in low-salinity water. *Aquaculture*, **251**, 472–483.

Cowey, C.B. (1994) Amino acid requirements of fish: a critical appraisal of present values. *Aquaculture*, **124**, 1–11.

Cowey, C.B. (1995) Intermediary metabolism in fish with reference to output of end products of nitrogen and phosphorus. *Water Sci. Technol.*, **31**, 21–28.

Cyrino, J.E.P., Bicudo, Á.J.D.A., Sado, R.Y., Borghesi, R. & Dairik, J.K. (2010) Fish farming and the environment: the use of environmental friendly feeds in fish culture. *Braz. J. Anim. Sci.*, **39**, 68–87.

David, C.P.C., Sta. Maria, Y.Y., Siringan, F.P., Reotita, J.M., Zamora, P.B., Villanoy, C.L., Sombrito, E.Z. & Azanza, R.V. (2009) Coastal pollution due to increasing nutrient flux in aquaculture sites. *Environ. Geol.*, **58**, 447–454.

Dosdat, A. (2001) Environmental impact of aquaculture in the Mediterranean: Nutritional and feeding aspects. In: Environmental Impact Assessment of Mediterranean Aquaculture Farms (Uriarte, A. & Basurco, B. eds), pp. 23–36. CIHEAM-IAMZ, Zaragoza.

Einen, O., Holmefjord, I., Åsgård, T. & Talbot, C. (1995) Auditing nutrient discharges from fish farms: theoretical and practical considerations. *Aquacult. Res.*, **26**, 701–713.

Fernandes, M., Lauer, P., Cheshire, A. & Angove, M. (2007) Preliminary model of nitrogen loads from southern bluefin tuna aquaculture. *Mar. Pollut. Bull.*, **54**, 1321–1332.

Flik, G. & Verbost, P.M. (1993) Calcium transport in fish gills and intestine. *J. Exp. Biol.*, **184**, 17–29.

Flik, G., Fenwick, J.C., Kolar, Z., Mayer-Gostan, N. & Wendelaabonga, S.E. (1986) Effects of low ambient calcium levels on wholebody Ca^{2+} flux rates and internal calcium pools in the freshwater cichlid teleost, *Oreochromis mossambicus*. *J. Exp. Biol.*, **120**, 249–264.

Furuya, W.M. (2010) Tabelas Brasileiras Para a Nutrição de Tilápias. GMF, Toledo.

Furuya, W.M., Pezzato, L.E., Barros, M.M., Pezzato, A.C., Furuya, V.R.B. & Miranda, E.C. (2004) Use of ideal protein concept for precision formulation of amino acid levels in fish-meal-free diets for juvenile Nile tilapia (*Oreochromis niloticus* L.). *Aquacult. Res.*, **35**, 1110–1116.

Gaye-Siessegger, J., Focken, U., Abel, H. & Becker, K. (2007) Influence of dietary non-essential amino acid profile on growth performance and amino acid metabolism of Nile tilapia, *Oreochromis niloticus* (L.). *Comp. Biochem. Physiol. A Mol. Integr. Physiol.*, **146**, 71–77.

Kaushik, S.J. & Seiliez, I. (2010) Protein and amino acid nutrition and metabolism in fish: current knowledge and future needs. *Aquacult. Res.*, **41**, 322–332.

Lee, C., Hristov, A.N., Cassidy, T.W., Heyler, K.S., Lapierre, H., Varga, G.A., de Veth, M.J., Patton, R.A. & Parys, C. (2012) Rumen-protected lysine, methionine, and histidine increase milk protein yield in dairy cows fed a metabolizable protein-deficient diet. *J. Dairy Sci.*, **95**, 6042–6056.

Levings, C.D. (1994) Some ecological concerns for net-pen culture of salmon on the coasts of the northeast Pacific and Atlantic oceans, with special reference to British Columbia. *J. Appl. Aquacult.*, **4**, 65–141.

Li, P., Mai, K., Trushenski, J. & Wu, G. (2009) New developments in fish amino acid nutrition: towards functional and environmentally oriented aquafeeds. *Amino Acids*, **37**, 43–53.

Li, Y., Bordinhon, A., Allen Davis, D., Zhang, W. & Zhu, X. (2013) Protein:energy ratio in practical diets for Nile tilapia *Oreochromis niloticus*. *Aquacult. Int.*, **21**, 1109–1119.

Liebert, F. & Portz, L. (2005) Nutrient utilization of Nile tilapia *Oreochromis niloticus* fed plant based low phosphorus diets supplemented with graded levels of different sources of microbial phytase. *Aquaculture*, **248**, 111–119.

Liu, J., Wang, Z. & Lin, W. (2010) De-eutrophication of effluent wastewater from fish aquaculture by using marine green alga *Ulva pertusa*. *Chin. J. Oceanol. Limnol.*, **28**, 201–208.

Maga, J.A. (1982) Phytate: its chemistry, occurrence, food interactions, nutritional significance, and methods of analysis. *J. Agric. Food Chem.*, **30**, 1–9.

Mambrini, M. & Kaushik, S.J. (1994) Partial replacement of dietary protein nitrogen with dispensable amino acids in diets of Nile tilapia, *Oreochromis niloticus*. *Comp. Biochem. Physiol. A Physiol.*, **109**, 469–477.

Martinez, J.L. (2009) Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environ. Pollut.*, **157**, 2893–2902.

Mayer-Gostan, N., Bornancin, M., de Renzis, G., Naon, R., Yee, J.A., Shew, R.L. & Pang, P.K.T. (1983) Extraintestinal calcium uptake in the killifish, *Fundulus heteroclitus*. *J. Exp. Zool.*, **227**, 329–338.

McCormick, S.D., Hasegawa, S. & Hirano, T. (1992) Calcium uptake in the skin of a freshwater teleost. *Proc. Natl Acad. Sci. USA*, **89**, 3635–3638.

Miranda, E.C.D., Pezzato, A.C., Pezzato, L.E., Graner, C.F., Rosa, G.J. & Pinto, L.G.Q. (2000) Availability of calcium:phosphorus ratio in diets for Nile tilapia (*Oreochromis niloticus*). *Braz. J. Anim. Sci.*, **29**, 2162–2171.

Ngamsnae, P., De Silva, S. & Gunasekera, R. (1999) Arginine and phenylalanine requirement of juvenile silver perch *Bidyanus bidyanus* and validation of the use of body amino acid composition for estimating individual amino acid requirements. *Aquacult. Nutr.*, **5**, 173–180.

NRC (2011) Nutrient Requirements of Fish and Shrimp. The National Academies Press, Washington, DC.

Nunes, A.J.P. (2012) Tilapia cage farm management in Brazil. *Int. Aquafeed*, **15**, 16–17.

Páez-Osuna, F., Guerrero-Galván, S.R. & Ruiz-Fernández, A.C. (1999) Discharge of nutrients from shrimp farming to coastal waters of the Gulf of California. *Mar. Pollut. Bull.*, **38**, 585–592.

Peres, H. & Oliva-Teles, A. (2006) Effect of the dietary essential to non-essential amino acid ratio on growth, feed utilization and nitrogen metabolism of European sea bass (*Dicentrarchus labrax*). *Aquaculture*, **256**, 395–402.

Pezzato, L.E., Miranda, E.C.D., Barros, M.M., Pinto, L.G.Q., Furuya, W.M. & Pezzato, A.C. (2002) Apparent digestibility of feedstuffs by Nile tilapia (*Oreochromis niloticus*). *Braz. J. Anim. Sci.*, **31**, 1595–1604.

Pond, W.G., Pond, K.R., Church, D.C. & Schoknecht, P.A. (2005) Basic Animal Nutrition And Feeding. John Wiley & Sons, New York.

Ravindran, V., Ravindran, G. & Sivalogan, S. (1994) Total and phytate phosphorus contents of various foods and feedstuffs of plant origin. *Food Chem.*, **50**, 133–136.

Rostagno, H.S. (2011) Tabelas Brasileiras Para Aves e Suínos: Composição de Alimentos e Exigências Nutricionais. Editora da UFV, Viçosa.

Roy, P.K. & Lall, S.P. (2004) Urinary phosphorus excretion in haddock, *Melanogrammus aeglefinus* (L.) and Atlantic salmon, *Salmo salar* (L.). *Aquaculture*, **233**, 369–382.

Sadiku, S.O.E. & Jauncey, K. (1995) Digestibility, apparent amino acid availability and waste generation potential of soybean flour: poultry meat meal blend based diets for tilapia, *Oreochromis niloticus* (L.), fingerlings. *Aquacult. Res.*, **26**, 651–657.

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

Schindler, D.W. (1971) Carbon, nitrogen, and phosphorus and the eutrophication of freshwater lakes. *J. Phycol.*, **7**, 321–329.

Schuhmacher, A., Wax, C. & Gropp, J.M. (1997) Plasma amino acids in rainbow trout (*Oncorhynchus mykiss*) fed intact protein or a crystalline amino acid diet. *Aquaculture*, **151**, 15–28.

Smith, V.H., Tilman, G.D. & Nekola, J.C. (1999) Eutrophication: impacts of excess nutrient inputs on freshwater, marine, and terrestrial ecosystems. *Environ. Pollut.*, **100**, 179–196.

Sugiura, S.H., Dong, F.M. & Hardy, R.W. (2000) Primary responses of rainbow trout to dietary phosphorus concentrations. *Aquacult. Nutr.*, **6**, 235–245.

Sugiura, S.H., Marchant, D.D., Kelsey, K., Wiggins, T. & Ferraris, R.P. (2006) Effluent profile of commercially used low-phosphorus fish feeds. *Environ. Pollut.*, **140**, 95–101.

Talbot, C. & Hole, R. (1994) Fish diets and the control of eutrophication resulting from aquaculture. *J. Appl. Ichthyol.*, **10**, 258–270.

Tanaka, Y. & Kadowaki, S. (1995) Kinetics of nitrogen excretion by cultured flounder *Paralichthys olivaceus*. *J. World Aquacult. Soc.*, **26**, 188–193.

Tuitoek, K., Young, L.G., Lange, C.F. & Kerr, B.J. (1997) The effect of reducing excess dietary amino acids on growing-finishing pig performance: an elevation of the ideal protein concept. *J. Anim. Sci.*, **75**, 1575–1583.

Vielma, J. & Lall, S.P. (1998) Phosphorus utilization by Atlantic salmon (*Salmo salar*) reared in freshwater is not influenced by higher dietary calcium intake. *Aquaculture*, **160**, 117–128.

Walton, M.J. & Wilson, R.P. (1986) Postprandial changes in plasma and liver free amino acids of rainbow trout fed complete diets containing casein. *Aquaculture*, **51**, 105–115.

Waters, D., Jenkins, A., Staples, T. & Donald, A.P. (1991) The importance of hydrological source areas in terrestrial liming. *Water Environ. J.*, **5**, 336–341.

Zarate, D.D. & Lovell, R.T. (1997) Free lysine (l-lysine HCl) is utilized for growth less efficiently than protein-bound lysine (soybean meal) in practical diets by young channel catfish (*Ictalurus punctatus*). *Aquaculture*, **159**, 87–100.