Aquaculture Nutrition

Aquaculture Nutrition 2015 doi: 10.1111/anu.12313

Nutritional evaluation of *Tenebrio molitor* meal as fishmeal substitute for tilapia (*Oreochromis niloticus*) diet

MªJ. SÁNCHEZ-MUROS[1,2], C. DE HARO[1], A. SANZ[3], C.E. TRENZADO[3], S. VILLARECES[1] & F.G. BARROSO[1]

[1] *Department of Biology and Geology, University of Almería, Almería, Spain;* [2] *Faculty of Agricultural Sciences, Technical University of Machala, Machala, Ecuador;* [3] *Department of Zoology, University of Granada, Granada, Spain*

Abstract

Fishmeal (FM) and soy meal (SM) are the most common protein sources used in fish feed, but they are associated with environmental, economic or production problems. Insects could be an interesting alternative source of protein. This work investigates the nutritive value of *Tenebrio molitor* meal (TM) and the effect of total replacement of SM by TM (TM/FM diet) or partial replacement of FM by TM (TM/FM/SM diet) versus a diet based in SM and FM (FM/SM diet) for tilapia. We studied the chitin content in TM, the *in vitro* protein digestibility, protease activity, oxidative stress and antioxidant defences, growth performance and nutrient utilization. The results indicate that an inclusion up to 500 g kg^{-1} of TM in the diet did not affect feed intake, *in vitro* protein digestibility, muscle amino acid composition or biometric indexes. However, their inclusion reduced growth performance and affected the muscle fatty acid profile. The lowest values for protease activity, elevated FRAP in digestive tracts and ROS level in muscle and higher SOD activity were observed in fish fed with SM/FM diet. The use of TM requires better understanding of the role of chitin in digestion and the study of toxins that might affect fish growth.

KEY WORDS: body composition – metabolism, digestibility – feed, enzymes – metabolism, feeding – feed, nile tilapia – fish – aquatic animals, stress – diseases

Received 5 October 2014; accepted 23 February 2015

Correspondence: MªJ. Sánchez-Muros, Department of Biology and Geology, University of Almería, 04120 Almería, Spain. E-mail: mjmuros@ual.es

Introduction

Feeding in aquaculture is heavily dependent on fishmeal, even with supplementation with other protein sources. Many studies have focused on a nutritional perspective, and the benefits and weaknesses of plant and animal protein sources are reasonably well known to date.

In addition to the nutritional criteria, protein sources should meet certain conditions for their production, such as regular availability in quantity, economic value, non-competition with resources for humans (water, land, or even the same source, as occurs with soy) and environmental sustainability.

Along with their derivative protein concentrates, fishmeal and soy meal are two of the most protein sources used as ingredients in fish feed. Both of them are associated with environmental (overexploitation of natural resources), economic (fluctuation of feedstuff prices) or production (variable quality) problems.

Insects could be an interesting source of protein. Mass rearing of insects could ensure a constant production in quantity, with high quality and stability of supply and price. Other advantages of using insects in fish feed are diverse. Mass rearing of insects does not compete with feed resources or land use and maximizes the benefits of waste management using 'waste nutrients' for insect growth sources. Insects can be grown in industrial plants and do not need much land or water, especially when compared to crops such as soybeans. Use of insects contributes to natural recycling of nutrients and could be a high-quality animal protein feedstuff using environmentally sustainable technology.

Regarding nutritive value, there are several facts supporting consideration of insects as a component of animal feeds: the natural diets of many species, such as poultry, pig, and all cultivated species of fish, include invertebrates such as oligochaetes, crustaceans and insects (Weir 1972; Bell *et al.* 1994; Matthews 1998; Matsuno *et al.* 1999; Khan & Panikkar 2009). Studies on insect body composition show large differences between species. The species

with high crude protein (CP) levels show a value of 650–800 g kg^{-1} such as Chinese grasshopper (*Acrida cinerea*), 654 g kg^{-1} CP (Wang *et al*. 2007); house cricket (*Acheta domesticus*), 620 g kg^{-1} CP (Nakagaki & Defoliart 1991); and migratory grasshopper (*Melanoplus mexicanus*), 770 g kg^{-1} CP (Ramos Elourdy *et al*. 1998).

The amino acid profiles are very different between species. *Acrida cinerea* has 17 g kg^{-1} methionine, 7 g kg^{-1} lysine and 38 g kg^{-1} cysteine, which is similar to or better than fishmeal, with 16 g kg^{-1} methionine, 5 g kg^{-1} lysine and 16 g kg^{-1} cysteine (Wang *et al*. 2007), while the Mormon (*Anabrus simplex*) and house crickets (*Acheta domesticus*) are deficient in methionine (DeFoliart *et al*. 1982; Finke *et al*. 1985; Nakagaki & DeFoliart 1987).

Energy content depends mainly on fat content, which varies widely by species (Cerda *et al*. 1999) and life stage. The caloric value has been determined in several species and is generally high, varying between 16.62 and 25.58 MJ kg^{-1} of DM (Ashiru 1938; Malaisse & Parent 1980).

The fatty acid composition of insect fat shows similar levels of unsaturation to poultry or fish (DeFoliart 1992). However, insect meal is rich in n-6, and fishmeal is rich in n-3 fatty acids. In *Acrida cinerea*, Wang *et al*. (2007) found high levels of polyunsaturated fatty acids (PUFAs), especially for linoleic and linolenic acids, at levels of 31.4%, 40.8% and 12.2% of total fatty acids.

The use in animals was tested in broilers (Wang *et al*. 2007), where it was observed that the inclusion of up to 15% of insect meal did not affect growth. These results are consistent with those obtained by Finke *et al*. (1985), who also found no significant difference in weight gain among chickens fed a corn–insect mixture or a control diet.

Fish are the cultured animals with major exigencies of quality and quantity of protein. Published results about the use of insect meal in fish feeding indicate that their inclusion is possible. However, replacement of high levels of fishmeal, over 25–50%, decreased growth (Wing-Keong *et al*. 2001; St-Hilaire *et al*. 2007b; Alegbeleye *et al*. 2012).

Nevertheless, these studies had focused on production parameters, and the nutritive properties of insect meal and its *in vitro* digestibility and oxidative stress caused by feeding have not been studied in fish.

Tenebrio molitor is a coleopteran widely cultured for use as pet feed. This work addressed the nutritive quality of *T. molitor* larvae in Nile tilapia (*Oreochromis niloticus*) as an alternative to fishmeal and soy meal as a protein source. We studied the meal composition and *in vitro* digestibility of protein. The meal obtained from dried larvae was used to replace fishmeal or soy meal in diets for tilapia, and its effect in nutrient utilization, chemical composition and amino acid profile in muscle, fatty acid profile in muscle and liver, chitin content in diets, digestive protease activity, *in vitro* protein digestibility, oxidative stress and antioxidant defences was evaluated.

Materials and methods

Diet ingredients and formulation

Tenebrio molitor larvae were used as a partial protein replacement of fishmeal (FM) and soy meal (SM) in fish diets. *T. molitor*, fed with cereal brans, was purchased from a pet store called 'La grillería', (Valencia, Spain). In our installations, *T. molitor* were frozen, lyophilized and ground to homogeneity to make *T. molitor* larvae meal (TM). Proximate composition, crude protein (CP), ether extracts, ash and nitrogen-free extractives (NFE) of TM, FM and SM used in this trial were analysed (Table 1) to assist in diet formulation (Table 2). The rest of the ingredients for the diets were purchased from specialized suppliers.

The experimental diets were designed to observe the substitution of FM and SM by TM. The maximum level of substitution was limited by amino acid content to maintain the amino acid balance among the diets. In two experimental diets, 50% of protein from FM was replaced with

Table 1 Proximate composition of protein feedstuffs evaluated in this study, g kg^{-1}

	Feedstuff		
	FM	SM	TM
Dry matter	900.0	879.0	349.6
Ash	177.3	78.4	48.3
Ether extract	81.6	30.4	300.9
Crude protein (Nx6.25)	730.3	504.1	584.2
NFE[1]	10.8	387.2	66.6
ADF[2]	1.1	76.5	65.7
ADIN[3]	n.d.	8.2	23.6
CPc[4]	n.d.	495.8	560.6

Results show the mean (n = 3) in g kg^{-1} of wet matter basis for the result of dry matter and in dry matter basis for the rest.
n.d., Not determined.
[1] NFE = Nitrogen-free extractives = 100 − (ash + ether extract + crude protein).
[2] ADF = Acid detergent fibre.
[3] ADIN = Insoluble nitrogen content in ADF.
[4] CPc = Crude protein corrected = CPi-ADIN (where 'CPi' represents the initial crude protein analysed (Nx6.25) and 'ADIN' the acid detergent-insoluble nitrogen content in ADF fraction (Nx6.25)).

protein from SM (FM/SM diet, used as control) or with protein from TM (TM/FM diet); in other diet, 75% of FM protein was replaced with SM (50%) and TM (25%) (TM/FM/SM). The different diets were made in the Technical Services of University of Almería (Spain).

Table 2 Ingredients, proximate composition and gross energy on dry matter basis (DM) of the experimental diets

	Diets		
	FM/SM	TM/FM/SM	TM/FM
Ingredients (g kg^{-1} DM)			
Fishmeal (FM)[1]	310	150	300
Tenebrio larvae meal (TM)[2]	0	210	430
Soy meal (SM)[3]	500	500	0
Fish oil[1]	85	40	0
Cellulose[4]	20	20	20
Wheat[1]	65	60	230
Premix[5]	20	20	20
Analysed composition (g kg^{-1} DM)			
Dry matter	929.5	913.4	906.3
Ash	129.8	88.1	105.2
Ether extract	107.6	108.8	120.9
Crude Protein (Nx6.25)	492.2	475.6	503.8
NFE[6]	210.3	267.6	243.1
ADF[7]	49.2	61.8	35.0
ADIN[8]	10.6	14.6	17.6
CPc[9]	481.6	461.0	486.1
Gross energy[10] (MJ kg^{-1})	19.5	20.1	20.8
GEc (MJ kg^{-1})[11]	19.4	19.9	20.4

[1] Local supplier (Almería, España).
[2] La grillería, Valencia, Spain.
[3] Bunge Canada (Oakville, ON, Canada).
[4] Sigma, Spain.
[5] Vitamin and mineral mix (values are g kg^{-1} except to those in parenthesis): Premix: 25; Choline, 10; DL-α-tocoferol, 5; ascorbic acid, 5; $(PO_4)_2Ca_3$, 5. Premix composition: retinol acetate, 1 000 000 IU kg^{-1}; calciferol, 500 IU kg^{-1}; DL-a-tocoferol, 10; menadione sodium bisulphite, 0.8; thiamin hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride, 15; cyanocobalamin, 25; nicotinamide, 15; pantothenic acid, 6; folic acid, 0.65; biotin, 0.07; ascorbic acid, 75; inositol, 15; betaine, 100; polypeptides, 12; Zn, 5; Se, 0.02; I, 0,5; Fe, 0.2; CuO, 15; Mg, 5.75; Co, 0.02; Met, 1.2; Cys, 0.8; Lys, 1.3; Arg, 0.6; Phe, 0.4; Tryp, 0.7; except 1000 g (Dibaq-Diproteg).
[6] NFE = Nitrogen-free extractives = 100 − (ash + ether extract + crude protein + gross fibre).
[7] ADF = Acid detergent fibre.
[8] ADIN = Insoluble nitrogen content in ADF.
[9] CPc = Crude protein corrected = CPi-ADIN (where 'CPi' represents the initial crude protein analysed (Nx6.25) and 'ADIN' the acid detergent-insoluble nitrogen contented in ADF fraction (Nx6.25)).
[10] Gross energy of the diets was calculated according to Jobling (1994) based on an estimated of 23.64 MJ kg^{-1} for protein, 39.33 MJ kg^{-1} for ether extract and 17.20 MJ kg^{-1} for carbohydrates.
[11] GEc = Gross energy corrected = CPc × 23.64 MJ kg^{-1} + ether extract × 39.33 MJ kg^{-1} + carbohydrates (take account TM as ADF-ADIN) × 17.20 MJ kg^{-1}.

A proximate analysis was conducted on each diet (Table 2). Gross energy (GE) was also calculated according to Jobling (1994). Finally, the amino acid profiles (Table 3) were analysed in protein sources and calculated for the experimental diets. Fatty acid profiles (Table 4) were analysed in diets and protein sources.

The percentage of chitin inclusion was determined as acid detergent fibre (ADF) (Stelmock *et al.* 1985) and the acid detergent-insoluble nitrogen content in the acid detergent-insoluble residue as ADIN (Finke 2007). The initial protein (CPi) and initial gross energy (GEi) of protein sources and diets were recalculated as crude protein corrected (CPc) and gross energy corrected (GEc), considering the percentage of ADF and ADIN (Tables 1 and 2) (see below *Chemical composition of diets and fish* section). Diets were also analysed for ferric reducing antioxidant power (FRAP) and reactive oxygen species levels (ROS).

Nile tilapia feeding trial

The Nile tilapias were provided by Valenciana de Acuicultura S.A. (Valencia, Spain) at 15 days after hatching. The experimental trial was conducted in an aquarium of the University of Almería, located in south-eastern Spain (36° 49′38.08″N 2° 29′20.96″W). All procedures were conducted according to the guidelines of Council Directive 86/609/EEC (European Communities, 1986) on the protection of animals used for experimental and other scientific purposes.

When fish reached a weight of 32.09 ± 1.42 g (mean ± SD), nine fish were sacrificed. The intestines were immediately removed and kept at -20 °C to determine the *in vitro* digestibility of each experimental diet. The fish were weighed, measured and placed in nine experimental tanks (3 tanks/diet and 11 fish/tank) with a volume of 250 L and replacement rate of 10.4 L h^{-1}. Fish were maintained at 27 °C with a natural photoperiod (12L:12D). Animals were fed with the appropriate experimental diet, *ad libitum* twice per day, at 09:30 and at 13:30 for 42 days. The feed was weighed before and after feeding the fish; after 15 min, uneaten pellets were removed by aspiration, dried and weighed to calculate the daily feed intake.

Fish performance indexes

DGC = Daily growth coefficient = $[(W_f^{1/3} - W_i^{1/3})/t] \times 100$. Being W_f = final weight, W_i = initial weight and 't' the time in days of the experiment.

Table 3 Amino acid composition (g kg^{-1}) of FM, SM, TM and of the three different diets formulated

	Protein source			Experimental diet		
Amino acids	FM	SM	TM	FM/SM	TM/FM/SM	TM/FM
Arginine[1]	22.99	16.95	18.04	15.60	15.71	14.66
Histidine[1]	13.89	8.18	11.97	8.39	8.68	9.31
Isoleucine[1]	13.77	9.79	16.12	9.16	10.34	11.06
Leucine[1]	29.45	22.05	31.39	20.15	22.03	22.33
Lysine[1]	31.21	19.68	22.87	19.52	19.32	19.20
Methionine[1]	13.88	2.70	5.12	5.65	4.50	6.36
Phenylalanine[1]	18.01	18.30	14.28	14.73	14.85	11.54
Threonine[1]	15.66	11.27	16.17	10.49	11.38	11.65
Valine[1]	17.10	11.62	24.35	11.11	13.49	15.60
Alanine	29.80	16.48	41.30	17.48	21.38	26.70
Aspartic acid	38.07	37.68	31.66	30.64	31.20	25.03
Glutamic acid	51.86	60.38	44.45	46.27	47.30	34.67
Glycine	27.44	15.50	24.72	16.26	17.06	18.86
Proline	17.88	17.12	29.67	14.10	17.47	18.12
Serine	16.87	17.91	21.39	14.18	15.98	14.26
Tyrosine	13.59	7.32	22.65	7.87	10.45	13.82
EAA/NEAA[2]	0.90	0.70	0.74	0.78	0.75	0.80

Results of protein source show the mean on a dry matter basis of three replications. The values of the experimental diets are obtained theoretically with the percentage of inclusion of FM, SM and TM and without to take account the rest of the ingredients.
[1] Essential amino acid.
[2] Balance of essential amino acids (EAA) and not essential amino acids (NEAA).

Table 4 Fatty acid composition of FM, SM, TM and of the three different diets

	Protein sources			Experimental diets		
Fatty acid	FM	SM	TM	FM/SM	TM/FM/SM	TM/FM
14:0	7.85	n.d.	2.20	5.75	3.55	3.30
16:0	22.95	15.05	16.60	24.40	21.55	21.35
16:1n-7	7.90	n.d.	2.20	6.20	2.90	2.35
18:0	5.30	4.80	3.40	5.55	4.80	4.25
18:1n-7	4.00	0.80	n.d.	2.50	1.15	0.60
18:1n-9	8.40	14.30	43.55	13.95	25.70	33.70
18:2n-6	1.05	48.50	30.35	10.80	23.60	26.00
18:3n-3	0.20	6.85	1.10	1.70	2.05	1.35
18:4n-3	1.90	n.d.	n.d.	1.05	0.50	0.15
20:1n-9	0.30	n.d.	n.d.	1.50	0.85	0.15
20:4n-6	1.40	n.d.	n.d.	1.25	0.60	0.10
20:5n-3	14.05	n.d.	n.d.	6.60	3.25	2.35
22:5n-3	2.65	n.d.	n.d.	1.45	0.75	0.25
22:6n-3	16.05	n.d.	n.d.	12.65	6.45	2.60
Others	6.00	5.60	0.60	4.45	2.30	1.50
Monounsaturated	20.60	15.10	45.75	24.35	30.60	36.80
n-3	34.85	6.85	1.10	23.45	13.00	6.70
n-6	2.45	48.50	30.35	12.05	24.20	26.10
Saturated	36.10	23.95	22.20	35.70	29.90	28.90
Total PUFAs	37.30	55.35	31.45	35.50	37.20	32.80
n-3/n-6	14.22	0.14	0.04	1.95	0.54	0.26

The values show the mean in percentage of the total fatty acids analysed. All analyses were performed by triplicate.
n.d., Not detected.

FI = Feed intake (%) = (Daily feed intake/average body weight*) × 100. *Average between final and initial weight.

FCE = Feed conversion efficiency = wet weight gain/dry feed intake.

PER = Protein efficiency ratio = wet weight gain/crude protein intake.

Condition factor = 100 × body weight/total length^3.

Sampling

At the end of the experiment, fish were fasted for 24 h before sampling. All fish of each tank were lightly anaesthetized and quickly weighed and measured. Nine animals/diet were sacrificed by overdose of anaesthesia (a solution of clove oil 100 mg L^{-1}), and immediately, eviscerated carcass, liver, intestine, stomach, spleen, gonads, head and muscle were removed and weighed to monitor changes in the main morphometric indexes. Different components of fish fed with each different diet were stored at -20 °C (intestine, liver, muscle and stomachs) and -80 °C (intestine, liver and muscle) for subsequent analysis.

Analytical methods

Chemical composition of diets and fish All analyses for proximate composition were performed according to the Association of Official Analytical Chemists (AOAC 2000). Dry matter (DM) and ash were determined gravimetrically after drying at 105 ± 0.5 °C and after combustion at 500 °C in mufla oven, respectively, until they reached a constant weight. Content of crude protein (Nx6.25) was determined by the Kjeldahl method, and total lipid content was determined by ethyl ether extraction (Soxhlet technique). All analysis was performed in triplicate.

Amino acid profiles The amino acid profiles of FM, SM and TM and in the muscle of the fish fed with the experimental diets were obtained through the Protein Chemistry Service of Biological Research Centre (CSIC, Madrid, Spain), following the basic principle of operation developed by Spackman *et al.* (1958) via continuous flow chromatography (Biochrom 30 series). Tryptophan was not detected. The asparagine and glutamine are deaminated and detected as aspartic and glutamic acid, respectively. The analyses were performed in triplicate. The amino acid composition for the three different diets was calculated theoretically from the percentage of FM, TM and SM inclusion.

Fatty acid profiles For fatty acid analysis, all samples were transmethylated by the method described by Lepage & Roy (1984), with minor modifications from Venegas-Venegas *et al.* (2011): 1 mL of freshly prepared transesterification reagents (methanol/acetyl chloride, 20:1, v v^{-1}) was added to 50 mg of each sample. The hexane phase was collected for gas–liquid chromatography (GLC) analysis. The analyses were performed in triplicate.

Chitin content Chitin was determined using the method described by Stelmock *et al.* (1985) as ADF, and the ADIN content in the acid detergent-insoluble residue for feedstuff and diets was calculated by the Kjeldahl method (AOAC 2000) and was used to correct the initial crude protein (CPi) and protein efficiency ratio (PER) and to recalculate the gross energy (GE) in diets. All analyses were performed in triplicate.

CPc = crude protein corrected = CPi-ADIN

'CPi' represents the initial crude protein analysed (Nx6.25) and 'ADIN' the acid detergent-insoluble nitrogen contained in the ADF fraction (Nx6.25).

PERc = Protein efficiency ratio corrected = wet weight gain/crude protein intake corrected with CPc value.

GEc = Gross energy corrected = CPc ×23.64 MJ kg^{-1} + Ether extract × 39.33 MJ kg^{-1} + carbohydrates (take account TM as ADF-ADIN) × 17.20 MJ kg^{-1}.

Protease activity The samples of intestines and stomachs were homogenized with distilled water in a ratio of 250 mg mL^{-1}, using a Polytron PT 2100 homogenizer, before being centrifuged and diluted 5 times in distilled water for the determination of acid protease activity. For assaying alkaline protease activity, centrifuged samples were not diluted. Soluble protein content of the supernatant for each tissue was calculated using a fast measurement set, Pierce BCA ™ Protein Assay Kit (Thermo Scientific™, Rockford, IL, USA).

The acidic protease activity of stomach extracts was measured by a modification of the method of Anson (1938), at pH 2 using haemoglobin as a substrate (Díaz-López *et al.* 1998). Alkaline protease activity in the gut extract was measured using the method developed by Kunitz (1947), subsequently modified by Walter (1984), at pH 9 using casein as substrate. All these analyses were performed in triplicate. One unit of protease activity was defined as 1 μg of tyrosine released per minute.

In vitro *protein digestibility* For the study of *in vitro* protein digestibility of the diets, the released amino acids were quantified during the hydrolysis of the protein by intestinal proteases using the method described by Church *et al.* (1983). *In vitro* digestibility was measured in intestines of tilapias fed with only the commercial diet (Le gouessant aurea-miet AL1, Lamballe, France). The results are expressed as g hydrolysed protein kg^{-1} DM after 90 min of *in vitro* hydrolysis.

Oxidative stress For ROS, FRAP and enzymatic assays, tissue samples were homogenized in ice-cold buffer (100 mMTris–HCl, 0.1 mM EDTA, and 0.1% triton X-100 [v v^{-1}], pH 7.8) at a ratio of 1:9 (w v^{-1}). Homogenates were centrifuged at 30 000 ***g*** for 30 min, and the supernatant was collected and frozen at −80 °C until analysis.

The analysis of ROS was performed as previously described by Hayashi *et al.* (2007), based on the joining of free radical of peroxide to the chromogenic solution. The absorbance was measured at 505 nm. The calibration curve was calculated and expressed in terms of equivalent levels of hydrogen peroxide (1 unit = 1.0 mg H_2O_2 L^{-1}).

The FRAP test was performed as previously described by Çakatay & Kayali (2006).The absorbance change is translated into a FRAP value (in μM) by relating the change in absorbance at 593 nm of the test sample to that of a standard solution of known FRAP value (500–1200 μmol L^{-1}).

All enzymatic assays were carried out at 25 ± 0.5 °C using a Power Wavex microplate scanning spectrophotometer (Bio-Tek Instruments, Winooski, VT, USA) in duplicate in 96-well microplates (UVStar®; Greiner Bio-One, Frickenhausen, Germany). The enzymatic reactions were started by the addition of the tissue extract, except for superoxide dismutase, SOD (EC 1.15.1.1), where xanthine oxidase was used. Catalase, CAT (EC 1.11.1.6), activity was determined by measuring the decrease of H_2O_2 concentration at 240 nm, according to Aebi (1984); SOD activity was measured spectrophotochemically by the ferricytochrome *c* method using xanthine/xanthine oxidase as the source of superoxide radicals. One activity unit was defined as the amount of enzyme necessary to produce a 50% inhibition of the ferricytochrome *c* reduction rate measured at 550 nm (McCord & Fridovich 1969); glutathione peroxidase, GPX (EC 1.11.1.9), activity was measured following the method of Flohé & Günzler (1984); glutathione reductase, GR (EC 1.6.4.2), activity was assayed as described by Calberg & Mannervik (1975), with some modifications, by measuring the oxidation of NADPH at 340 nm; glutathione S-transferase, GST (EC 2.5.1.18), activity was determined by the method of Habig *et al.* (1974), adapted to microplates. GST activity was monitored at 340 nm by the formation of glutathione-CDNB-conjugate; DT-diaphorase, DTD (EC 1.6.99.2), activity was measured as described bySturve *et al.* (2005). DTD activity was defined as the difference between sample and control dichlorophenolindophenol DCPIP reduction. Except for SOD, for which the arbitrary units have already been mentioned, one unit of activity is defined as the amount of enzyme required to transform 1 μmol of substrate per minute under the above-mentioned assay conditions. The protein content of the supernatant solutions was determined by the Bradford (1976) method, using bovine serum albumins as a standard.

Statistical analysis

The statistical software used was JMP 7.0.2. The results were analysed using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer HSD test. The results are expressed as means ± SEM (standard error of mean) with probabilities of $P < 0.05$ considered significant.

The correlation of n-3/n-6 ratio between the diet and muscle and liver was quantified using Spearman rank order correlation. The significance of the Spearmen rank order correlation can be assessed for data with any underlying distribution and was thus calculated using untransformed variables.

Results

The proximate composition showed significant differences among raw sources (Table 1). TM has the lowest and highest values of ash and ether extract, respectively. The crude protein content of TM was lower than FM and higher than SM. The NFE extract content of TM was lower than SM.

The amino acid composition analysis (Table 3) shows higher percentages of isoleucine, leucine, threonine, valine, proline, serine and tyrosine for TM. The balance of essential amino acids and non-essential amino acids (EAA/NEAA) was similar for SM and TM and among the diets (0.78 for FM/SM, 0.75 for FM/TM/SM and 0.80 for TM/FM).

Compared to FM, the fatty acid analysis of TM (Table 4) showed elevated levels of monounsaturated (especially 18:1n-9) and n-6 fatty acids (especially 18:2n-6) and lower levels of n-3 fatty acids (especially 20:5n-3 and 22:6n-3). The ratio of the n-3/n-6 series was much higher in FM than TM and SM, and the latter showed a slightly higher ratio than TM. The fatty acid profile of the diets (Table 4) reflects the TM inclusion.

The ADF included lignin and minerals and the chitin of exoskeleton of insect. The results (Table 1) show the highest values for SM (due to lignin), followed by TM (due to chitin); in FM, the percentage was small and based on minerals. The ADF in diet reflects the inclusion of SM and TM, as well as the wheat meal or cellulose. Considering the proportion of ADF in TM and its inclusion within each experimental diet, this represents 13.7 g kg^{-1} for the TM/FM/SM

diet and 28.2 g kg^{-1} for the TM/FM diet, with an ADIN inclusion of 4.9 g kg^{-1} and 10.1 g kg^{-1} from TM, respectively. The correction of values CPi affects TM, which decreased the CP content by 23.6 g kg^{-1} and energy.

The values of *in vitro* protein digestibility were 32.6 ± 1.4% of DM for FM/SM diet, 23.8 ± 2.8% of DM for TM/FM/SM diet and 27.2 ± 2.3% of DM for TM/FM diet. No significant differences were detected for *in vitro* digestibility values among the diets at 90 min.

The highest values for protease activity in stomach and intestine (Table 5) were observed in fish fed with less fishmeal (TM/FM/SM diet), followed by fish fed with the TM/FM diet.

To evaluate the nutritive effect of TM, the growth performance, nutrient utilization and morphometric parameters related to fish production were determined. No differences were found in feed intake levels (Table 6), but DGC, FCE and PER were lower in fish fed with diets with TM than in fish fed with the control diet (FM/SM) (Table 6). PER corrected with CPc data improved values slightly in all groups, but significant differences were maintained ($P < 0.05$).

No significant differences in morphometric indexes were found among the treatments, except for the hepatosomatic index, which is higher in fish fed with diets TM/FM (Table 6).

No differences in the macronutrient composition of muscle were observed among the experimental diets (Table 6). Regarding essential amino acids in muscle (Fig. 1), the composition, expressed as a percentage of total amino acids analysed, was similar in fish fed with the three experimental diets, except for higher values of histidine and arginine and lower values of leucine in fish fed on diet FM/SM.

The fatty acid profile of muscle (Table 7) reflects the fatty acid profile of the diet (Table 4) with higher levels of 18:1n-9 and 18:2n-6 in fish fed with TM. The fish fed with control diet (FM/SM) presented higher levels of eicosapentaenoic acid (20:5n-3; EPA) and lower levels of n-6 fatty acids than fish fed with TM diets. The levels of docosahexaenoic acid (22:6n-3; DHA) were only significantly lower for fish fed with TM/FM diet. Fish fed with TM also had the highest percentages of monounsaturated fatty acids and the lowest levels of saturated fatty acids.

Regarding fatty acids in liver (Table 7), the profile was similar to that of muscle, but there were major differences between fish fed with FM/SM and fish fed with FM/TM. The results also show significant differences for DHA with the TM/FM/SM diet that did not appear in muscle, and EPA was not found in the liver of the fish fed with the TM/FM diet.

The n-3/n-6 ratio in muscle was significantly different between fish fed with the FM/SM and FM/TM diets, reflecting the higher level of n-3 in fishmeal, but no differences were found in fish fed with FM/TM/SM (Table 7). In liver, however, the n-3/n-6 ratio was significantly different among the three different diets (Table 7). There is a significant and positive correlation of the n-3/n-6 ratio between the diet and muscle ($y = 1.5431x + 0.4288$ $R2 = 0.9949$) and liver of the fish ($y = 1.1985x + 0.041$ $R2 = 0.979$).

The total oxidant and antioxidant power of diet and oxidant and antioxidant response of muscle and digestive tracts have been determined via ROS and FRAP (Fig. 2a and b). No differences were observed in FRAP content in diet or FRAP production in muscle. The highest FRAP production was obtained in the digestive tracts of fish fed with the FM/SM diet. ROS levels (Fig. 2a), however, were higher in the FM/SM diet and in muscle of fish fed with this diet.

The results of liver antioxidant enzyme activities (Table 8) showed significant differences in SOD only between fish fed with and without TM, with the highest value for fish fed with the FM/SM diet. The other enzyme activities assayed showed no significant differences.

Table 5 Activity units (AU) of protease in Nile tilapias fed with three experimental diets at the end of the experiment

	Diets		
AU mg $prot^{-1}$	FM/SM	TM/FM/SM	TM/FM
Stomach	12.46 ± 1.01[c]	44.28 ± 2.93[a]	27.64 ± 0.99[b]
Intestine	17.00 ± 2.58[c]	54.87 ± 1.19[a]	47.05 ± 0.71[b]

Mean ± SEM of three intestine and stomach replicates of fish fed with the different experimental diets. Different letters indicate significant differences ($P < 0.05$) based on the Tukey–Kramer HSD test. One unit of protease activity (AU) was defined as 1.0 μg of tyrosine released per minute.

Discussion

The analysis of the composition of TM (Table 1) reveals a protein percentage higher than SM but lower than FM and higher in fat than either SM or FM. The amino acid composition (Table 3) is worse than FM but better than SM, showing high percentages of all essential amino acids for fish except phenylalanine. The values of two non-essential amino acids, glutamic acid and aspartic acid, were also

Table 6 Growth performance, somatic indexes and body composition of Nile tilapia fed a control diet (FM/SM) and experimental diets (TM/FM/SM and TM/FM)

	Diet		
	FM/SM	TM/FM/SM	TM/FM
Growth performance			
FBW[1] (g)	84.73 ± 4.40[a]	60.24 ± 3.20[b]	60.2 ± 1.33[b]
DGC[2] (%)	2.74 ± 0.18[a]	1.84 ± 0.16[b]	1.83 ± 0.06[b]
FI[3] (%)	1.98 ± 0.08	2.05 ± 0.03	2.05 ± 0.03
FCE[4]	1.03 ± 0.03[a]	0.74 ± 0.06[b]	0.74 ± 0.02[b]
PER[5]	2.08 ± 0.06[a]	1.55 ± 0.13[b]	1.46 ± 0.03[b]
PERc[6]	2.13 ± 0.07[a]	1.60 ± 0.13[b]	1.51 ± 0.03[b]
Somatic indexes (%)			
Carcass	86.85 ± 0.57	79.4 ± 4.75	86.68 ± 1.97
Liver	1.47 ± 0.20[ab]	1.32 ± 0.16[b]	1.92 ± 0.15[a]
Intestine	3.29 ± 0.33	3.71 ± 0.60	2.80 ± 0.28
Stomach	0.82 ± 0.14	0.67 ± 0.13	0.48 ± 0.05
Spleen	0.11 ± 0.03	0.06 ± 0.01	0.10 ± 0.03
Head	23.18 ± 0.93	23.16 ± 1.21	22.91 ± 0.44
Muscle	34.17 ± 2.57	32.14 ± 2.76	33.26 ± 1.27
Gonads	2.26 ± 0.59	2.58 ± 0.74	2.99 ± 0.61
Condition Factor[7]	1.72 ± 0.05	1.63 ± 0.06	1.58 ± 0.02
Body composition (g kg^{-1})			
Dry matter[8]	977.7 ± 4.4	970.6 ± 8.3	971.1 ± 2.7
Ash	64.8 ± 1.1	72.7 ± 4.4	68.2 ± 0.9
Ether extract	42.3 ± 12.2	45.9 ± 12.2	50.3 ± 6.9
Crude protein (Nx6.25)	877.2 ± 10.5	880.5 ± 9.7	873.3 ± 7.2

Grow performance: Mean ± SEM of three replicate tanks (11 fish/tank) at the end of the experiment. Different letters indicate significant differences ($P < 0.05$) based on the Tukey–Kramer HSD test.

Somatic indexes: Results show, in % of total fish weight, the mean ± SEM (n = 9); different letters indicate significant differences ($P < 0.05$) based on the Tukey–Kramer HSD test.

Body composition: Results show, in g kg^{-1} of dry matter of the muscle of Nile tilapia, the mean ± SEM ($n = 6$); different letters indicate significant differences ($P < 0.05$) based on the Tukey–Kramer HSD test.

[1] FBW (final body weight).

[2] DGC = Daily growth coefficient = $[(W_f^{1/3} - W_i^{1/3})/t] \times 100$.

[3] FI (feed intake) = (feed daily intake/average body weight) × 100.

[4] FCE (feed conversion efficency) = wet weight gain/dry feed intake.

[5] PER (protein efficiency ratio) = wet weight gain/crude protein intake.

[6] PERc = Protein efficiency ratio corrected considering the crude protein corrected – CPc – presented in Table 2.

[7] Condition factor = 100 × body weight/total length^3.

[8] Dry matter sample of lyophilized muscle.

lower in TM than in SM. The results obtained caused an EAA/NEAA balance in TM lower than FM but similar to SM. The differences in amino acids limited the percentage of substitution at a maximum of 50% of fishmeal to maintain the amino acid balance. Nevertheless, this level of substitution affects the fatty acid profile of the experimental diet, decreasing n-3 and increasing n-6 with the decrease in fishmeal inclusion because the composition of fatty acids (Table 4) is quite different, with SM or FM being TM richer in 18:2n-6 and lower in 18:3n-3 than FM.

Insect exoskeletons contain chitin. Chitin content has been determined as ADF, which also includes lignin from vegetable sources. The ADF level of SM is due to lignin, while it is due to chitin in TM. Both TM and SM are higher in ADF than in FM (Table 1), in which ADF is zero. The β1,4 bond in chitin is indigestible for several fish species (Rust 2002). However, chitinase activity has been described in the digestive tracts of some species of fish, with the chitinase produced by the fish (Smith *et al.* 1989) or by digestive bacteria (Krogdahl *et al.* 2005). Nevertheless, the presence of chitinase and the ability to digest it are discussed in several species. In red tilapia (*O. niloticus x O. hornorum*) and Nile tilapia, dietary fishmeal can be replaced with shrimp meal, which is rich in chitin, at levels of 50% or 100% without significant retardation in weight gain and feed efficiency (El-Sayed 1998; Mansour 1998). Conversely, in other species of tilapia (*O. niloticus* **x** *O. aureus*), the inclusion of chitin depresses growth (Shiau

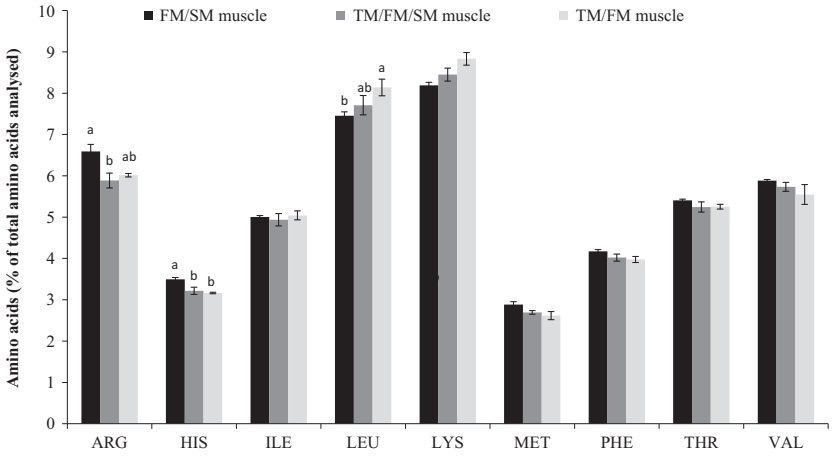

Figure 1 Essential amino acids in fish's muscle fed with three different diets. Results show the mean ± SEM ($n = 6$) of percentage of total amino acids analysed; different letters indicate significant differences ($P < 0.05$) based on the Tukey–Kramer HSD test. ARG: Arginine; HIS: Histidine; ILE: Isoleucine. LEU: Leucine; LYS: Lysine; MET: Methionine; PHE: Phenylalanine; THR: Threonine. VAL: Valine.

Table 7 Fatty acids profile (% total fatty acids analysed) in muscle and liver of Nile tilapia fed a control diet (FM/SM) and experimental diets (TM/FM/SM and TM/FM)

	Muscle			Liver		
Fatty acid/Diet	FM/SM	TM/FM/SM	TM/FM	FM/SM	TM/FM/SM	TM/FM
14:00	3.08 ± 0.61	2.03 ± 0.24	2.43 ± 0.30	3.05 ± 0.05	2.4 ± 0.65	2.43 ± 0.07
16:00	22.5 ± 0.36	18.92 ± 0.77	18.72 ± 1.35	21.80 ± 2.10^{x}	16.5 ± 1.89^{xy}	10.70 ± 0.51^{y}
16:1n-7	3.55 ± 0.86	1.72 ± 0.3	1.93 ± 0.47	3.90 ± 0.70	2.87 ± 0.55	2.90 ± 0.23
18:00	8.90 ± 0.8	8.42 ± 1.03	6.85 ± 1.08	11.70 ± 0.20	7.53 ± 1.01	4.67 ± 0.70
18:1n-9	13.03 ± 1.35^{b}	17.9 ± 2.40^{ab}	26.65 ± 3.03^{a}	15.45 ± 3.75^{y}	25.27 ± 3.29^{y}	39.03 ± 2.02^{x}
18:1n-7	3.42 ± 0.65	2.60 ± 0.23	1.58 ± 0.30	3.20 ± 0.60^{x}	1.77 ± 0.41^{xy}	0.87 ± 0.26^{y}
18:2n-6	6.47 ± 0.56^{b}	15.55 ± 1.98^{a}	19.05 ± 1.46^{a}	6.25 ± 3.55^{z}	12.87 ± 0.23^{y}	18.53 ± 1.07^{x}
18:3n-3	0.53 ± 0.17	0.48 ± 0.34	0.33 ± 0.22	0.25 ± 0.25	0.50 ± 0.06	0.50 ± 0.06
18:3n-6	*n*.d.	n.d.	n.d.	n.d.	0.30 ± 0.3	0.60 ± 0.15
18:4n-3	0.20 ± 0.2	0.07 ± 0.07	0.08 ± 0.08	n.d.	n.d.	n.d.
20:1n-9	1.25 ± 0.06^{a}	0.58 ± 0.07^{b}	0.57 ± 0.11^{b}	1.45 ± 0.15	0.67 ± 0.35	0.87 ± 0.07
20:4n-3	n.d.	n.d.	n.d.	n.d.	0.67 ± 0.67	n.d.
20:4n-6	2.72 ± 0.43	2.40 ± 0.38	2.32 ± 0.51	3.50 ± 0.40^{x}	1.67 ± 0.63^{xy}	1.00 ± 0.15^{y}
20:5n-3	2.83 ± 0.12^{a}	1.23 ± 0.15^{b}	0.47 ± 0.26^{b}	1.50 ± 0.40^{x}	0.30 ± 0.06^{y}	n.d.
22:1n-11	n.d.	n.d.	n.d.	n.d.	0.17 ± 0.17	n.d.
22:5n-3	4.85 ± 0.32^{a}	3.68 ± 0.19^{ab}	2.88 ± 0.46^{b}	1.25 ± 1.15	1.07 ± 0.19	0.40 ± 0.10
22:5n-6	n.d.	n.d.	n.d.	0.45 ± 0.45	0.33 ± 0.18	n.d.
22:6n-3	22.77 ± 2.15^{a}	18.22 ± 2.38^{ab}	11.70 ± 1.81^{b}	21.00 ± 2.20^{x}	10.67 ± 2.65^{y}	3.27 ± 0.28^{y}
Others	3.90 ± 0.08^{b}	6.20 ± 0.56^{a}	4.67 ± 0.63^{ab}	5.25 ± 2.45^{y}	14.47 ± 1.91^{x}	14.23 ± 1.55^{x}
Monounsaturated	21.25 ± 1.55	22.8 ± 2.51	30.73 ± 3.38	24.00 ± 5.20^{y}	30.73 ± 3.94^{xy}	43.67 ± 2.22^{x}
n-3	31.18 ± 1.71^{a}	23.68 ± 2.30^{ab}	15.47 ± 1.94^{b}	24.00 ± 0.90^{x}	13.20 ± 2.86^{y}	4.17 ± 0.23^{z}
n-6	9.18 ± 0.45^{b}	17.95 ± 1.60^{a}	21.37 ± 1.15^{a}	10.20 ± 0.01^{z}	15.17 ± 0.43^{y}	20.13 ± 0.90^{x}
Saturated	34.48 ± 0.12^{a}	29.37 ± 1.48^{b}	28.00 ± 2.12^{b}	36.55 ± 1.85^{x}	26.43 ± 2.34^{y}	17.80 ± 1.05^{z}
Total PUFAs	40.37 ± 1.57	41.63 ± 0.79	36.83 ± 0.87	34.20 ± 0.90	28.37 ± 3.29	24.30 ± 0.86
n-3/n-6	3.42 ± 0.29^{a}	1.37 ± 0.26^{b}	0.74 ± 0.13^{b}	2.35 ± 0.09^{x}	0.86 ± 0.17^{y}	0.21 ± 0.02^{z}

Results show the mean ± SEM ($n = 6$); different letters indicate significant differences ($P < 0.05$) based on the Tukey–Kramer HSD test with 'a' or 'b' for muscle and 'x', 'y' or 'z' for liver.
n.d., Not detected.

& Yu 1999). Chitinolytic activity was present in the serum, stomach, and intestine of Nile tilapia, with the serum having the highest specific activity (Molinari *et al*. 2007). In this experiment, diets were formulated without considering chitin and its nitrogen content, but these were both determined to help understand the growth and nutritive utilization rates. The ADIN was higher in TM, due to the nitrogen atoms present in the chitin molecules. However, that nitrogen did not significantly affect the protein content initially valued in the diets (Table 2) or PER (Table 6).

The *in vitro* digestibility of protein was similar for the three sources of protein analysed. Low digestibility of soy

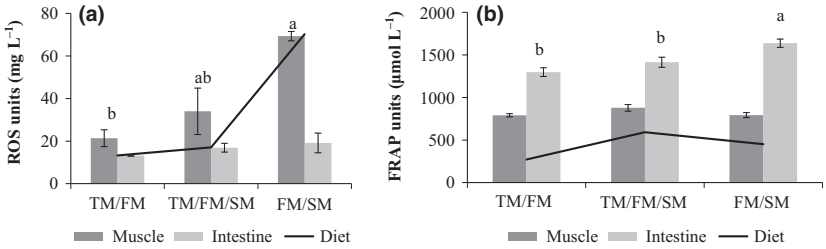

Figure 2 ROS (a) and FRAP (b) levels in the three experimental diets and muscle and intestine of the fish fed with each diet. Results show the mean ± SEM (n = 6); different letters indicate significant differences ($P < 0.05$) based on the Tukey–Kramer HSD test.

Table 8 Effects of different experimental diets based in TM on liver antioxidant activity in Nile tilapias

	Diets		
Antioxidant enzymes in liver	FM/SM	TM/FM/SM	TM/FM
SOD[1]	467.56 ± 37.39[a]	368.31 ± 17.15[b]	383.47 ± 28.08[b]
CAT[1]	228.77 ± 20.67	215.82 ± 15.57	200.10 ± 22.50
GPX[2]	48.77 ± 3.38	45.79 ± 3.07	48.42 ± 1.64
GR[2]	6.19 ± 0.69	5.98 ± 0.50	4.85 ± 0.59
GST[2]	179.95 ± 29.76	147.44 ± 13.82	205.01 ± 23.57
DTD[2]	21.36 ± 4.06	11.67 ± 2.88	16.90 ± 4.03

Results show the mean ± SEM (n = 9); different letters indicate significant differences ($P < 0.05$) based on the Tukey–Kramer HSD test for superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPX), glutathione transferase (GST) and DT-diaphorase (DTD).
[1] U mg^{-1} protein
[2] mU mg^{-1} protein.

protein has been described due to the presence of protease inhibitors (Rodiles *et al.* 2012); nevertheless, in this experiment, the *in vitro* digestibility of protein is less in SM but is not significantly different from TM and FM. This, together with the high values of alkaline and acid proteases observed (Table 5) in fish fed with the TM-based diets, could indicate a high potential for digesting dietary proteins, but we cannot affirm a high digestibility of TM because the digestibility was determined in diet but not in meal.

Current practice has achieved a high percentage of substitution of FM with plant sources. El-Sayed *et al.* (2003) reported that growth performance was not significantly impaired in tilapia fed with a diet in which 100% of the dietary protein came from a complex mixture of vegetable sources. Nevertheless, feeds based on individual plant proteins have generally resulted in a decrease in fish growth performance (Mbahinzirek *et al.* 2001; Sklan *et al.* 2004), which has been attributed to the presence of antinutritional factors in plant protein, particularly soybean meal (Bureau *et al.* 2000). Currently, commercial fish diets have a high percentage of soy meal. Nevertheless, a loss of integrity of the villus tips and increased inflammatory cell infiltration into the lamina propia have been observed upon feeding diets containing 600–700 g kg^{-1} of soybean meal to rainbow trout (Burrells *et al.* 1999), causing the inclusion of soybean meal to be limited. The experimental diets replaced 50% of the fishmeal with SM, for a 500 g soy kg^{-1} diet in TM/FM/SM and FM/SM diets, and the intestine histology has not analysed. However, the growth rate in fish fed with the control diet is similar to that described for this species (Edwards *et al.* 2000).

The experimental diets were well accepted, with similar feed intake levels among the three experimental diets (Table 6); the inclusion of TM at the percentages tested in this experiment did not affect the palatability of the diet. Nevertheless, tilapias fed with TM were observed to decrease their nutritive utilization and growth index compared to fish fed the diet without TM (FM/SM). Similar results were obtained for *Ictalurus punctatus* (cat fish), and *Oreochromis aureus* (blue tilapia) fed with *Hermetia illucens* (black soldier fly) prepupa ate a 34% substitution (Bondari & Sheppard 1981, 1987; Newton *et al.* 2005).

The lower growth (Table 6) in fish fed with insect meal could be due to the chitin content in the diet. In tilapia (*O. niloticus x O. aureus*) fed diets with chitin, a growth

decrease has been described (Shiau & Yu 1999). Similarly, in Atlantic salmon, the inclusion of chitin, at a percentage of 1% or higher, decreased fish growth (Shiau & Yu 1999; Gopalakannan & Arul 2006; Olsen *et al*. 2006). In our study, the proportion of ADF evaluated as chitin in TM and its inclusion within each experimental diet represented 1.37% for the TM/FM/SM diet and 2.82% for the TM/FM diet, which might explain the cause of decreased growth and nutrient utilization in tilapia fed TM-containing diets versus the fish that were fed with the control diet (FM/SM). Low lipid utilization and the low lipid retention can be attributed to the presence of chitin and its negative influence on lipid digestibility (Kroeckel *et al*. 2012). However, we note that the growth and nutritive indexes worsened independently of the percentage of inclusion of the insect meal, suggesting a negative effect of some compounds in *T. molitor*. These could be chitin, which exceeds 1% in the experimental diets, or it may be some other toxin. For example, the endogenous production of 1, 4 benzoquinone has been described in *Tenebrio sp*. (El-Kashlan *et al*. 1996).

The biometric indexes (Table 6) show normal development of fish, with no differences among diets except for the hepatosomatic index (HI), which is higher in the TM/SM diet than the TM/FM/SM diet, but there are no significant differences from the control diet (FM/SM). This is not due to the presence of TM because the TM/FM/SM diet also contains *T. molitor* and induced a lower HI than the control.

The muscle proximate composition (Table 6) was not affected by any of the experimental diets. In *Clarias gariepinus,* the replacement of FM with TM shows a slight increase in the total lipid level without significant changes in protein levels compared to control fish (Wing-Keong *et al*. 2001). The results of our experiment show no change in body composition, but our data are only for muscle. In rainbow trout (*Onchrynchus mykiss*) fed with diets containing housefly (*Musca domestica*) or black soldier fly (*Hermetia illucens*), the body protein is also unchanged, while lipids decrease in fish fed with diets containing black soldier fly (St-Hilaire *et al*. 2007a).

The amino acid composition of muscle is similar for all experimental diets, and the amino acids profile seem to be a characteristic of the each species rather than a consequence of feeding. However, the muscle fatty acid composition (Table 7) reflects the fatty acid composition of the diets, and the percentage of n-3 fatty acids was significantly lower in fish fed with TM. Nevertheless, from a human nutritional perspective, n-3 fatty acids (15.47%) and n-3/n-6 ratio (0.74) from muscle of fish fed with TM/FM diet are higher than that obtained for chicken breast (1.19% of n-3 and 0.05 n-3/n-6 ratio) (Rule *et al*. 2002) but with lack in content of EPA and DHA. The percentages of EPA and DHA in muscle of fish fed with TM are lower than that observed from the control diet. The data for liver fatty acids suggest a greater use and/or mobilization to the muscle of these fatty acids because at 42 days of experimentation, no EPA or DHA was detected in the liver of fish fed the diet with more TM (TM/FM). This effect is less evident in fish fed with a lower percentage of substitution (TM/FM/SM), which may be a consequence of the inclusion of fish oil in the diet formulation. On the other hand, synthesis of highly unsaturated fatty acids (HUFA) from precursors such as 18:3n-3 has been described in some freshwater fish species, such as tilapia, which are able to convert the C18 essential fatty acid (EFA), 18:3n-3 and 18:2n-6, to the longer chain, more unsaturated and physiologically important, HUFA. EPA, DHA and arachidonic acid (20:4n-6; AA) are synthesized via a series of fatty acid desaturations and elongations, so they only require the C18 PUFA (Henderson & Tocher 1987; Sargent *et al*. 1989, 1995).

The oxidative stress and antioxidant defence results (Fig. 2a and b) show that FRAP in muscle and intestine of fish (Fig. 2b) matches the increased SOD activity in liver (Table 8), as well as the main antioxidant enzymes that initiate the cascade of reductive reactions, indicating that the animals fed with a greater amount of FM (FM/SM) are those who need to increase their antioxidant defences. Both circumstances, along with the highest level of ROS found in the muscle of animals fed with FM/SM diet and FM (Fig. 2a), indicate that the probable cause is the higher level of PUFAs and HUFAs, indicating a high response capacity of these two organs, as it has been described under multiple stress conditions (Sánchez-Muros *et al*. 2013).

The possible presence of toxins could have resulted in increased activity of GST and DTD. Nevertheless, no significant changes were found in DTD and GST activities among the diets.

In summary, the results of this work indicate that TM has a composition comparable to SM and that the partial replacement of FM or total replacement of SM with TM does not affect feed intake, *in vitro* protein digestibility, the amino acid composition of the muscle or biometric indexes. However, the inclusion of TM at either level tested (25 and 50%) decreased growth by approximately 29% and affected the fatty acid profile of muscle. TM is a protein source that cannot be used at high rates of substitution,

and need to improve the lipid profile. The use of TM requires better understanding of the role of chitin in digestion and better detection of toxins that might affect fish growth.

Acknowledgements

The authors are very grateful to the Consejería de Innovacion y Ciencia (project AGR5273), European Regional Development Funds (FEDER funds), Campus de Excelencia Internacional Agroalimentaria (CEI3) Campus de Excelencia Internacional del Mar (CEImar), Ministerio de Educación (Spain) and Secretaria Superior de Ciencia Tecnología e Investigacion, SENESCYT (Ecuador).

References

Aebi, H. (1984) Catalase "*in vitro*". *Methods Enzymol*., **105**, 121–126.

Alegbeleye, W.O., Obasa, S.O., Olude, O.O., Otubu, K. & Jimoh, W. (2012) Preliminary evaluation of the nutritive value of the variegated grasshopper (*Zonocerus variegatus* L.) for African catfish *Clarias gariepinus* (Burchell. 1822) fingerlings. *Aquac. Res*., **43**, 412–420.

Anson, M.L. (1938) The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. *J. Gen. Physiol*., **22**, 79–89.

AOAC (2000) Official Methods of Analysis of the Association of Official Analytical Chemists International. AOAC International, Gaithersburg, MD, USA.

Ashiru, M.O. (1938) The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. *J. Gen. Physiol*., **22**, 79–89.

Bell, J.G., Ghioni, C. & Sargent, J.R. (1994) Fatty acid compositions of 10 freshwater invertebrates which are natural food organisms of Atlantic salmon parr (*Salmo salar*): a comparison with commercial diets. *Aquaculture*, **128**, 301–313.

Bondari, K. & Sheppard, D.C. (1981) Soldier fly larvae as feed in commercial fish production. *Aquaculture*, **24**, 103–109.

Bondari, K. & Sheppard, D.C. (1987) Soldier fly, *Hermetia illucens* L., larvae as feed for channel catfish, *Ictalurus punctatus* (Rafinesque), and blue tilapia, *Oreochromis aureus* (Steindachner). *Aquac. Res*., **18**, 209–220.

Bradford, M.M. (1976) Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem*., **72**, 248–254.

Bureau, D.P., Harris, A.M., Bevan, D.J., Simmons, L.A., Azevedo, P.A. & Cho, C.Y. (2000) Feather meals and meat and bone meals from deferent origins as protein sources in rainbow trout (*Oncorhynchus mykiss*) diets. *Aquaculture*, **181**, 281–291.

Burrells, C., Williams, P.D., Southgate, P.J. & Crampton, V.O. (1999) Immunological, physiological responses of rainbow trout to increasing dietary concentrations of soybean proteins. *Vet. Immunol. Immunopathol*., **72**, 277–288.

Çakatay, U. & Kayali, R. (2006) The evaluation of altered redox status in plasma and mitochondria of actue and chronic diabetic rats. *Clin. Biochem*., **39**, 907–912.

Calberg, L. & Mannervik, B. (1975) Purification and characterization of the flavoenzyme glutathione reductase from rat liver. *J. Biol. Chem*., **250**, 5474–5480.

Cerda, H., Martínez, R.N.B., Pizzoferrato, L., Hermoso, D. & Paole, M. (1999) Cría, análisis nutricional y sensorial del picudo del cocotero *Rhynchophorus palmarum* (coleoptera: curculionidae), insecto de la dieta tradicional indigena amazónica. *Ecotropicos*, **12**, 25–32.

Church, F.C., Swaisgood, H.E., Porter, D.H. & Catignani, G.L. (1983) Spectrophotometric assay using o-phthaldialdehyde for determination of proteolysis in milk and isolated milk proteins. *J. Dairy Sci*., **66**, 1219–1227.

DeFoliart, G.R. (1992) Insects as human food: gene DeFoliart discusses some nutritional and economic aspects. *Crop Prot*., **11**, 395–399.

DeFoliart, G.R., Finke, M.D. & Sunde, M.L. (1982) Potential value of the mormon cricket (Orthoptera: *Tettigoniidae*) harvested as a high protein feed for poultry. *J. Econ. Entomol*., **75**, 848–885.

Díaz-López, M., Moyano-López, F.J., Alarcón-López, F.J., García-Carreño, F.L. & Navarrete del Toro, M.A. (1998) Characterization of fish acid proteases by substrate-gel electrophoresis. *Com. Biochem. Physiol. Part B*, **121**, 369–377.

Edwards, P., Lin, C.K. & Yakupitiyage, A. (2000) Semi-intensive pond aquaculture. In: Tilapias: Biology and Exploitation (Beveridge, M.C.M. & McAndrew, B.J. eds), pp. 377–403. Kluwer Academic Publishers, Dordrecht, the Netherlands.

El-Kashlan, I.H., Helal, E., El-Hamid, A. & Abdellatif, M.A. (1996) Teratogenicity and embryotoxicity of 1,4- benzoquinone and wheat flour infested with Tenebrionid beetles on mice. *J. Saudi Soc. Agric. Sci*., **8**, 121–133.

El-Sayed, A.-F.M. (1998) Total replacement of fishmeal with animal protein sources in Nile tilapia (*Oreochromis niloticus*) feeds. *Aquac. Res*., **29**, 275–280.

El-Sayed, A.F.M., Mansour, C.R. & Ezzat, A.A. (2003) Effects of dietary protein level on spawning performance of Nile tilapia (*Oreochromis niloticus*) broodstock reared at different water salinities. *Aquaculture*, **220**, 619–632.

Finke, M.D. (2007) Estimate of chitin in raw whole insects. *Zoo Biol*., **26**, 105–115.

Finke, M.D., Sunde, M.L. & DeFoliart, G.R. (1985) An evaluation of the protein quality of mormon cricket (*Anabrux simplex* H.) when used as a high protein feedstuff for poultry. *Poult. Sci*., **64**, 708–712.

Flohé, L. & Günzler, W.A. (1984) Assay of glutathione peroxidase. *Methods Enzymol*., **105**, 115–121.

Gopalakannan, A. & Arul, V. (2006) Immunomodulatory effects of dietary intake of chitin, chitosan and levamisole on the immune system of *Cyprinus carpio* and control of *Aeromonas hydrophila* infection in ponds. *Aquaculture*, **255**, 179–187.

Habig, W.H., Pabst, M.J. & Jakoby, W.B. (1974) Glutathione S-transferases – the first enzymatic step in mercapturic acid formation. *J. Biol. Chem*., **249**, 7130–7139.

Hayashi, I., Morishita, Y., Imai, K., Nakamura, M., Nakachi, K. & Hayashi, T. (2007) High-throughput spectrophotometric assay of reactive oxygen species in serum. *Mutat. Res*., **631**, 55–61.

Henderson, R.J. & Tocher, D.R. (1987) The lipid composition and biochemistry of freshwater fish. *Prog. Lipid Res*., **26**, 281–347.

Jobling, M. (1994) Fish Bioenergetics. Volumen 13 de Chapman & Hall Fish and Fisheries Series Fish & Fisheries Series. Chapman & Hall, London.

Khan, M.F. & Panikkar, P. (2009) Assessment of impacts of invasive fishes on the food web structure and ecosystem properties of a tropical reservoir in India. *Ecol. Model*., **220**, 2281–2290.

Kroeckel, S., Harjes, A.G.E., Roth, I., Katz, H., Wuertz, S., Susenbeth, A. & Schulz, C. (2012) When a turbot catches a fly:

Evaluation of a pre-pupae meal of the Black Soldier Fly (*Hermetia illucens*) as fish meal substitute — Growth performance and chitin degradation in juvenile turbot (*Psetta maxima*). *Aquaculture*, **364–365**, 345–352.

Krogdahl, A., Henre, G.-I. & Mommsen, T.P. (2005) Carbohydrates in fish nutrition: digestion and absorption in postlarval stages. *Aquac. Nutr.*, **11**, 103–122.

Kunitz, M. (1947) Crystalline soybean trypsin inhibitor II. General properties. *J. Gen. Physiol.*, **30**, 291–310.

Lepage, G. & Roy, C.C. (1984) Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *J. Lipid Res.*, **25**, 1391–1396.

Malaisse, F. & Parent, G. (1980) Les chenilles comestibles du Shaba meridional (Zaire). *Naturalistes Belges*, **61**, 2–24.

Mansour, C.R. (1998) Nutrient requirements of red tilapia fingerlings. MSc Thesis, Fac. Sci. University of Alexandria, Alexandria, Egypt, 121 pp.

Matsuno, T., Ohkubo, M., Toriiminami, Y., Tsushima, M., Sakaguchi, S., Minami, T. & Maoka, T. (1999) Carotenoids in food chain between freshwater fish and aquatic insects. *Comp. Biochem. Physiol. B*, **124**, 341–345.

Matthews, W.J. (1998) Structure of Fish Assemblages. In: Patterns in Freswater Fish Ecology, Vol. **2** (Matthews, W.J. ed.), pp. 30–84. Kluwer Academic Publishers, Massachusetts, USA.

Mbahinzirek, G.B., Dabrowski, K., Lee, K.J., El-Saidy, D. & Wisner, E.R. (2001) Growth, feed utilization and body composition of tilapia (*Oreochromis sp.*) fed with cottonseed meal-based diets in a recirculating system. *Aquac. Nutr.*, **7**, 189–200.

McCord, J.M. & Fridovich, J. (1969) Superoxide dismutase: an enzymatic function for erythrocuuprein (hemocuprein). *J. Biol. Chem.*, **244**, 6049–6055.

Molinari, L.M., Bocchi Pedroso, R., Oliveira Scoaris, D., Ueda-Nakamura, T., Vataru Nakamura, C. & Prado Filho Dias, B. (2007) Identification and partial characterisation of a chitinase from Nile tilapia, *Oreochromis niloticus*. *Comp. Biochem. Physiol.*, **146**(B), 81–87.

Nakagaki, B.J. & DeFoliart, G.R. (1987) Protein quality of the house cricket *Acheta domesticus* when fed to rooster chicks. *Poult. Sci.*, **66**, 1367–1371.

Nakagaki, B.J. & Defoliart, G.R. (1991) Comparison of diets for mass-rearing *Acheta domesticus* (Orthoptera: Gryllidae) as a novelty food, and comparison of food conversion efficiency with values reported for livestock. *J. Econ. Entomol.*, **84**, 891–896.

Newton, L., Sheppard, C., Watson, D.W., Burtle, G. & Dove, R. (2005) Using the black soldier fly, Hermetia illucens, as a value-added tool for the management of swine manure. Waste Management Programs. North Carolina State University. http://www.cals.ncsu.edu/waste_mgt/smithfield_projects/phase2report05/cd,web%20files/A2.pd

Olsen, R., Suontama, J., Langmyhr, E., Mundheim, H., Ringo, E., Melle, E., Malde, M. & Hmere, G. (2006) The replacement of fish meal with Antarctic krill, *Euphausia superba* in diets for Atlantic salmon, *Salmo salar*. *Aquac. Nutr.*, **12**, 280–290.

Ramos Elourdy, J., Pino M., J.M. & Cuevas Correa, S. (1998) Insectos comestibles del Estado de México y determinación de su valor nutritivo. Anales del Instituto de Biología, serie Zoología, **69**, 65–104.

Rodiles, A., Santigosa, E., Herrera, M., Hachero-Cruzado, I., Cordero, M.L., Martínez-Llorens, S., Lall, S.P. & Alarcón, F.J. (2012) Effect of dietary protein level and source on digestive proteolytic enzyme activity in juvenile Senegalese sole, *Solea senegalensis* Kaup 1850. *Aquacult. Int.*, **20**, 1053–1070.

Rule, D.C., Broughton, K.S., Shellito, S.M. & Maiorano, G. (2002) Comparison of muscle fatty acid profiles and cholesterol concentrations of bison, beef cattle, elk, and chicken. *J. Anim. Sci.*, **80**, 1202–1211.

Rust, M.B. (2002) Nutritional physiology. In: Fish Nutrition (Halver, J.E. & Hardy, R.W. eds), pp. 368–446. Academic Press, New York, USA.

Sánchez-Muros, M.J., Villareces, S., Miranda-de la Lama, G., de Haro, C. & Barroso, F.G. (2013) Effects of chemical and handling exposure on fatty acids, oxidative stress and morphological welfare indicators in gilthead sea bream (*Sparus aurata*). *Fish Physiol. Biochem.*, **39**, 581–591.

Sargent, J.R., Henderson, R.J. & Tocher, D.R. (1989) The lipids. In: Fish Nutrition (Halver, J.E. ed), pp. 153–218. Academic Press, New York, USA.

Sargent, J.R., Bell, J.G., Bell, M.V., Henderson, R.J. & Tocher, D.R. (1995) Requirement criteria for essential fatty acids. *J. Appl. Ichthyol.*, **11**, 183–198.

Shiau, S.-Y. & Yu, Y.-P. (1999) Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus*×*O. aureus*. *Aquaculture*, **179**, 439–446.

Sklan, D., Prag, T. & Lupatsch, I. (2004) Apparent digestibility coefficients of feed ingredients and their prediction in diets for tilapia *Oreochromis niloticus* & #xB7; *Oreochromis aureus* (Telepstei Cichlidae). *Aquac. Res.*, **35**, 358–364.

Smith, J.L., Opekun, A.R., Larkai, E. & Graham, D.Y. (1989) Sensitivity of the esophageal mucosa to pH in gastroesophageal reflux disease. *Gastroenterology*, **96**, 683–689.

Spackman, D.H., Stein, W.S. & Moore, S. (1958) Automatic recording apparatus for use in the cromatography of amino acids. *Anal. Chem.*, **30**, 1990–1206.

Stelmock, R.L., Husby, F.M. & Brundage, A.L. (1985) Application of van soest acid detergent fiber method for analysis of shellfish chitin. *J. Dairy Sci.*, **68**, 1502–1506.

St-Hilaire, S., Cranfill, K., McGuire, M.A., Mosley, E.E., Tomberlin, J.K., Newton, L., Sealey, W., Sheppard, C. & Irving, S. (2007a) Fish offal recycling by the black soldier fly produces a foodstuff high in omega-3 fatty acids. *J. World Aquaculture Soc.*, **38**, 309–313.

St-Hilaire, S., Sheppard, C., Tomberlin, J.K., Irving, S., Newton, L., McGuire, M.A., Mosley, E.E., Hardy, R.W. & Sealey, W. (2007b) Fly prepupae as a feedstuff for rainbow trout, *Oncorhynchus mykiss*. *J. World Aquaculture Soc.*, **38**, 59–67.

Sturve, J., Stephensen, E. & Forlin, L. (2005) Effects of redox cycling compounds on DT diaphorase activity in the liver of rainbow trout (*Oncorhynchus mykiss*). *Comp. Hepatol.*, **4**, 4.

Venegas-Venegas, E., Rincón-Cervera, M.A. & Guil-Guerrero, J.L. (2011) Triarachidonoyl glycerol purification process. *J. Am. Oil Chem. Soc.*, **88**, 323–328.

Walter, H.E. ed. (1984) Proteinases: Methods With Haemoglobin, Casein and Azocoll as Substrates, pp. 270–277. Verlag Chemie, Weinheim, Germany.

Wang, D., Zhai, S.-W., Zhang, C.-X., Zhang, Q. & Chen, H. (2007) Nutrition value of the Chinese grasshopper *Acrida cinerea* (Thunberg) for broilers. *Anim. Feed Sci. Technol.*, **135**, 66–74.

Weir, J.S. (1972) Diversity and abundance of aquatic insects reduced by introduction of the fish. *Biol. Conserv.*, **4**, 169–175.

Wing-Keong, N., Liew, F.-L., Ang, L.-P. & Wong, K.-W. (2001) Potential of mealworm (*Tenebrio molitor*) as an alternative protein source in practical diets for African catfish, *Clarias gariepinus*. *Aquac. Res.*, **32**, 273–280.