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Research Article

Effect of Feeding in Juvenile Tilapia (*Oreochromis niloticus*) with Diet Contain *Tenebrio molitor* meal (Ordercoleoptera)

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Keywords

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Abstract

This work studies the effect of feeding juvenile Nile tilapia (*Oreochromis niloticus*) a diet based in *Tenebrio molitor* larvae meal (TM). Three different diets were tested: C diet (control, 100% fish meal, FM) and diets I and II, with 25% and 50% of FM replaced with TM, respectively. The experiment was developed in two different stages. In stage 1, the fish were divided into two groups: one group was fed with C diet and the other with diet I. In stage 2, each treatment (C and I) was divided into two treatments, four in total: treatment C→C (fish feed with diet C in both stages), treatment C→II (fish feed with diet C in stage 1follow by diet II in stage 2), treatment I→II (Fish feed with diet I in stage 1 and diet II in stage 2) and treatment I→C (fish feed with diet I in stage 1 and C diet in stage 2).

The administration of a diet based on insect meal at the second stage of the experiment (I→II and C→II fish groups) decreased the growth and nutritional indexes except for daily growth coefficient were similar C-C and I-II. The protease activity is significantly higher in fish fed with C diet at stage 2 but no significant differences were found in fish fed with C and II diet at 90 min of *in vitro* protein hydrolysis. The administration of an insect-based diet at early stages of development (stage 1) do not affect the nutritional indices composition, essential amino acid or polyunsaturated fatty acids in muscle if the fish were feed with diet C in the stage 2.

ABBREVIATIONS

AA: Amino Acids; ADC: Apparent Digestibility Coefficient; ADF: Acid Detergent Fibre; ADIN: Acid-Detergent-Insoluble Nitrogen Content in the Acid-Detergent-Insoluble Residue; ALA: α-Linolenic Acid; ARG: Arginine; AU: Activity Units of Protease; CP: Crude Protein; CPc: Crude Protein corrected; DGC: Daily Growth Coefficient; DHA: Docosahexaenoic Acid; DM: Dry Matter; EAA: Essential Amino Acids; EFA: Essential Fatty Acids; EPA: Eicosapentaenoic Acid; FCE: Feed Conversion Efficiency; FI: Feed Intake; FM: Fishmeal; GE: Gross Energy; GEc: Gross Energy corrected; GLC: Gas-Liquid Chromatography; HIS: Histidine; HUFAs: Highly Unsaturated Fatty Acids; ILE: Isoleucine; LEU: Leucine; LYS: Lysine; MET: Methionine; NEAA: Non-Essential Amino Acids; PER: Protein Efficiency Ratio; PHE: Phenylalanine; PUFAs: Polyunsaturated Fatty Acids; THR: Threonine; TM: *Tenebrio molitor* Larvae Meal; VAL: Valine

INTRODUCTION

Aquaculture presents several challenges, including the conservation of the environment [1,2]. The finfish and crustacean aquaculture sectors are still highly dependent upon marine capture fisheries for sourcing key dietary nutrient inputs, including fishmeal, fish oil and low-value fish [3]. Compared to other conventional animal and plant protein sources, fishmeal is an excellent source of high-quality animal protein, with a well-balanced essential amino acid profile, essential lipids, including omega-3 fatty acids, and a good source of digestible energy [4,5].

In this context, insects have great potential meeting the rising demand in meat products and replacing fishmeal because they are a good source of protein, vitamins, high digestibility [6,7], and they are part of the natural diet of poultry, pigs, fish, and some human communities. Regarding environmental point of view, insects have high growth and feed-conversion rates and a low environmental footprint over their entire life cycle [8].

Some previous experiments with insect inclusion in fish feed for aquaculture has shown good results within certain levels of substitution [9-16]. However, in these same experiments, it has been found that high levels of substitution do not give good

results, which in large part could be due to a lower digestive utilization.

The digestive utilization of diet is partially determined by the digestive enzyme, which activities seem to be influence by protein source of diet to improve digestive efficiency of the protein digestion. In *Rhamdia quelen,* trypsin and chymotrypsin activities were higher in fish fed diets containing animal protein source [17]. The alkaline proteases were negatively affected by dietary soybean meal while amylase activity had greater variation between diets and intestine sections. On the other hand, fish fed meat and bone meal diets showed higher gastric protease activity [17]. In *Penaeus vannamei* larvae, squid meal stimulated significantly chymotrypsin activity while trypsin activity decreased with fish protein soluble concentrate [18].

On the other hand, the inclusion of insects in the fish diets affect to composition of de muscle, especially the fatty acids composition. Fish fed black soldier fly diets low in fish oil had reduced levels of omega-3 fatty acids in their muscle fillets; specifically key fatty acids were affected by diets with proportionally less α-linolenic acid (18:3n-3; ALA), eicosapentaenoic acid (20:5n-3; EPA), and docosahexaenoic acid (22:6n-3; DHA) [13].

The objective of this work was to study if the feeding with insect based-diet during the first month of live of Nile tilapia (*Oreochromis niloticus*) affects the growth, nutritional indices, proximal composition of fish *in vitro* protein hydrolysis, and proteases activities in juveniles fed a diet with partial replacement of fish meal by insect meal (*Tenebrio molitor*, TM) or a fish meal diet

MATERIAL AND METHODS

Diet ingredients and formulation

Tenebrio molitor larvae, fed with cereal bran, were purchased from a pet store (La grillería, Valencia, Spain). The larvae of *T. mollitor* were used in pests feeding with possibility to developed mass-rearing systems to produce tn. In our installations, *T. molitor* were frozen, lyophilized, and homogenized by grinding to assist in diet formulation. Three experimental diets were designed with different rates of substitution of fishmeal with *T. molitor* larvae insect meal (TM) (Table 1):

- Control diet C: without TM.

- Insect diet I: 25% of FM was replaced with TM.

- Insect diet II: 50% of FM was replaced with TM.

The rest of the ingredients for the diets were purchased from specialized suppliers. The different diets were developed by the Technical Services of the University of Almería (Spain). Diets proximal composition, amino acids content, and fatty acid profile are show in Tables 1-3, respectively.

Nile tilapia feeding trial

Animals used in this experiment were juveniles of Nile tilapia (*O. niloticus*), supplied by Valenciana de Acuicultura (S.A.). Tilapia is one of the most cultivated and growing species, and is a kind of fresh water whose natural diet may include insect larvae. The fish were transported from the farm to the aquarium of the University of Almería and place in a tank until 15 days old (one week) during this time fish were feed with the same diet than fish farm and kept in the same condition than fish farm. The experiment was designed in two stages (Figure 1).

Table 1: Ingredients, proximate composition and gross energy on dry matter basis (DM) of the experimental diets C (control diet 100% fish-meal) , I (75% fishmeal: 25% tenebrio meal) and II (50% fishmeal: 50% tenebrio meal).

	Diets		
	C	I	II
Ingredients (g/Kg)			
Fishmeal (FM)[a]	610	450	300
Tenebrio larvaemeal (TM)[b]	-	230	430
Wheatmeal[a]	230	230	200
Fishoil[a]	70	20	-
Vitamin/mineral premix[c]	50	50	50
Cellulose[d]	40	20	20
Analyzedcomposition(g/Kg)			
Drymatter	913.63	898.72	906.27
Ash	147.95	135.13	105.23
Etherextract	117.08	103.74	120.86
CrudeProtein	504.51	484.38	503.75
NFE[e]	193.42	249.67	243.07
ADFom[f]	4.95	17.23	35.02
ADIN[g]	3.48	8.08	17.63
CPc[h]	501.03	476.30	486.12
Gross energy[i] (MJ/kg)	19.62	19.82	20.86

[a]Local supplier (Almería, España). [b]La grillería del sur, Valencia, Spain. [c]Vitamin and mineral mix (values are g/kg 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, 1000000 IU/kg; calciferol, 500 IU/kg; DL-α-tocoferol, 10; menadione sodium bisulphite, 0.8; thiamin hydrochloride, 2.3; riboflavin, 2.3; pyridoxine hydrochloride, 15; cianocobalamin, 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; excpt. to 1000 g (Dibaq-Diproteg). [d]Sigma, Spain.
[e]NFE: Nitrogen-free extractives = 100 – (ash + ether extract + crude protein + gross fibre).
[f]ADFom: Acid detergent fibre expressed exclusive of residual ash.
[g] ADIN: Insoluble nitrogen content in ADFom.
[h]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 ADFom fraction (Nx6.25)).
[i]Gross energy of the diets was calculated according to Jobling (1994) based on an estimated of 23.64 MJ/kg for protein, 39.33 MJ/kg for ether extract and 17.20 MJ/kg for carbohydrates.

Stage 1: 336 15-day-old Nile tilapia divided into two groups (C and I), housed in four 60-l fibre glass tanks (80 X 40 X 31 cm) (two tanks/treatment, 84fish/tank to maintained the same density than fish farm), and were supplied a different diet, control (C) or insect (I) during 34 days. At this stage no fish were weighed due the small size implies a big error in individual weight, On the other hand, they were randomly distributed and consistent

Table 2: Amino acid composition of the three different diets formulated. C (control diet 100% fish meal) , I (75% fish meal: 25% tenebrio meal) and II (50% fishmeal: 50% tenebrio meal).

	Diets		
Amino acid	**C**	**I**	**II**
Arginine*	6.14	5.88	5.35
Histidine*	3.72	4.27	4.12
Isoleucine*	5.13	5.04	5.27
Leucine*	7.40	6.93	7.48
Lysine*	7.42	6.51	5.50
Methionine*	2.57	2.70	2.38
Phenylalanine*	4.34	4.33	4.20
Threonine*	5.05	5.18	5.05
Valine*	6.40	6.49	7.52
Alanine	8.47	9.29	11.02
Asparticacid	8.55	7.87	7.66
Glutamicacid	11.41	9.79	8.14
Glycine	10.51	11.26	10.34
Proline	5.65	6.26	6.99
Serine	4.61	4.94	5.22
Tyrosine	2.63	3.25	3.76
EAA/NEAA[a]	1.15	1.13	1.10

Results show the mean (n=3) in percentage of the total amino acids analyzed. *Essential amino acid. [a]Balance of essential amino acid (EAA) and not essential amino acids (NEAA).

Table 3: Fatty acid composition of the three different diets; C (control diet 100% fish meal), I (75% fish meal: 25% tenebrio meal) and II (50% fishmeal: 50% tenebrio meal).

	Diets		
Fatty acid	**C**	**I**	**II**
14:0	6.93	5.00	3.30
16:0	24.45	21.65	21.35
16:1n-7	7.28	4.30	2.35
18:0	5.63	4.40	4.25
18:1n-7	3.00	1.55	0.60
18:1n-9	12.68	28.35	33.70
18:2n-6	3.05	15.60	26.00
18:3n-3	0.53	0.70	1.35
18:4n-3	1.18	0.70	0.15
20:1n-9	1.50	0.55	0.15
20:4n-6	1.48	0.70	0.10
20:5n-3	9.18	5.15	2.35
22:5n-3	1.95	1.00	0.25
22:6n-3	15.40	7.50	2.60
Others	5.48	2.85	1.50
Monounsaturated	24.78	34.75	36.80
n-3	28.23	15.05	6.70
n-6	4.53	16.3	26.10
Saturated	37.00	31.05	28.90
n-3/n-6	6.26	0.92	0.26

The values show the mean (n=3) in percentage of the total fatty acids analyzed.

in size among treatments. The stage 1 was lasted until the fish were easy and exactly weighed. During stage 1 not growth or nutritional indices were measure. This stage was considered as adaptation to the diet.

After 34 days, the fish were lightly anaesthetized with clove oil (30 mg/l) [19] and weighed and measured to be used in stage 2.

Stage 2: Fish from each group (C and I) of stage 1, were divided in four different groups. Each group was distributed among 6 different 300 L fibreglass (110cm highx 30cm diameter), (in total 12 tanks, three tanks/treatment, 28 fish/tank). The following groups were performed (Figure 1),

1. C→C: Fish fed with diet C Stage 1 and with diet C in stage 2
2. I→II: Fish fed with diet I in stage 1, and with diet II in stage 2
3. I→C: Fish fed with diet I in stage 1 and with diet C in stage 2.
4. C→II: Fish fed with C diet in stage 1 and with diet II in stage 2

The expriment lasted until quadruple the weight (30-40 days). During the experiment the fish were fed with the experimental diets *ad libitum* twice a day, at 08:30 and at 13:00. The intake of each tank was recorded. The feed was weighed before and after feeding the fish; after 15 min, uneaten pellets were removed, dried, and weighed to calculate daily food intake.

The oxygen concentration was maintained at 7 ppm with continuous water renewal. Fish were maintained at natural photoperiod and 30 ± 2°C of water temperature. All procedures were performed in accordance with the guidelines of Council Directive 86/609/EEC (European Communities, 1986) on the protection of animals used for experimental and other scientific purposes.

Fish performance indexes

The nutritional and growth indices were determined according Sánchez-Muros et al., [21].

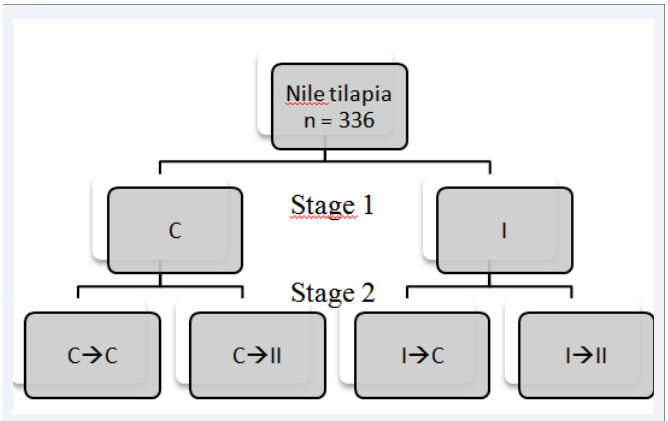

Figure 1 Stages of adaptation to *T. molitor* larvaes meal. Letters C, I, and II indicates the diet suministrated; n it is the initial tilapias's number for the experience. C (control diet 100% fish meal) , I (75% fish meal: 25% tenebrio meal) and II (50% fishmeal: 50% tenebrio meal).

DGC = Daily growth coefficient = [($W_f^{1/3}$- $W_i^{1/3}$)/time] x 100. Being W_f = final weight and W_i = initial weight and "t" the time in days of the experiment.

FI = Feed intake (%) = (Daily feed intake/average body weight*) x 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 x body weight/total length^3.

Sampling

For the first stage, the initial weight in this experimental stage was not recorded because the fish they were randomly distributed and consistent in size among treatments. At the end, the fish were weighed and distributed in twelve tanks to start the second stage.

At the end of the second experimental stage, fish fasted 24 h before the sampling. All fish in each tank were lightly anaesthetised and quickly weighed and measured. From each tank, five fish (15 fish per treatment) were sacrificed by overdose of anaesthesia, and muscle, liver, housing, intestine, stomach, spleen, head, gonads, and perivisceral fat were immediately removed and weighed to monitor changes in the main measurements of morphometric and nutritional indexes. Components of fish fed with each different diet were stored at -20°C for subsequent analysis in muscle, liver and intestine.

Analytical methods

Chemical composition of diets and fish: Dry matter and ash were determined gravimetrically after drying at 105 ± 0.5°C (AOAC, 2000; #934.01) and after combustion at 500°C in a mufla oven ((AOAC, 2000; #942.05), respectively, to constant weight. Content of crude protein was determined by Kjeldahl(AOAC, 2000; #954.01) (Nx6.25), and total lipid was determined by ethyl ether extraction (Soxhlet technique)(AOAC, 2000; #920.39). All analyses were performed in triplicate.

No digestible components (the cuticle) was measured as acid detergent fibre [22,23], according to ANKOM Technology, method 12 (acid detergent fiber in feeds – filter bag technique), solutions as in AOAC [24], method 973.18, and expressed exclusive of residual ash (ADFom). The acid-detergent insoluble nitrogen (ADIN) content in the acid-detergent-insoluble residue was calculated by the Kjeldahl method (AOAC, 2000; #954.01) [21] and used to correct the initial crude protein calculated in feed (crude protein corrected, CPc = CPi - ADIN) and the protein efficiency ratio (PERc).

Gross energy (GE) was calculated according to Jobling [23].

Diets composition, amino acids and fatty acids profile of diet are show in Tables 1,2, and 3 respectively.

Amino acid profiles: The analysis of amino acid profiles was performed according to AOAC [24], method 994.12. Acid digestion of the samples was performed using 2.5 mM aminobutyric acid as an internal marker. All analyses were performed in triplicate.

Fatty acid profiles: For fatty acid analysis, all samples were transmethylated using the method of Lepage and Roy [25] with minor modifications of Venegas-Venegas et al. [26]: 1 ml of freshly prepared transesterification reagents (methanol/acetyl chloride, 20:1, v/v) was added to 50 mg each of freeze-dried meals of TM and FM, the different diets and muscle of fish, or 2 ml for fresh liver with 50 mg of dry matter, in glass tubes, as well as 100 µl of a solution of internal standard (heptadecanoic acid 17:0, 10 mg/ml). The tubes were shaken and then placed in a hot block (100°C for 30 min, or 45 min for livers). Next, the mixture was cooled to room temperature, and 1 ml of distilled water was added to each tube. Samples were shaken again and centrifuged (3,000 rpm, 3 min). The upper hexane phase was collected for gas-liquid chromatography (GLC) analysis. The analyses were performed in triplicate.

Protease activity: To measure the protease activity on intestinal extracts at the end of the experiment, sets of nine intestines of each tilapia groups maintained at -20°C were homogenised with distilled water in a ratio of 100 mg/mL, using a Polytron PT 2100 homogeniser [21]. Soluble protein content was quantified using a fast measurement set, the Pierce™ BCA Protein Assay Kit (Thermo Scientific™). Alkaline protease activity in the pool extracts was measured using the method developed by Kunitz [27, subsequently modified by Walter [28] at pH 9 using casein as substrate. The analyses were performed in triplicate. One unit of protease activity was defined as 1 µg of tyrosine released per minute [20].

***In vitro* protein hydrolysis:** For the study of *in vitro* protein hydrolysis, the released amino acids by intestinal proteases were quantified using the method described by Church et al. [29]. The hydrolysis was measured *in vitro* on the intestines of each experimental group at the end of the second stage for two experimental diets used in this stage (C and II) adding 100 activity units of protease to 40 mg of dietary protein. The analyses were performed in triplicate. The results are expressed as g hydrolysed protein/kg protein in feed after 90 min of incubation.

Statistical analysis

The statistical software used was JMP 7.0.2. The results for weights and lengths of the fish at the end of the first stage and the comparisons between the results of *in vitro* protein digestibility for the two diets within the same fish group were statistically analysed using the Student's t-test. All multiple-comparison analyses were performed using one-way analysis of variance (ANOVA) followed by the Tukey-Kramer HSD test. Results are expressed as the means ± SEM (standard error of mean), with probabilities of $P<0.05$ considered significant.

RESULTS

The initial weigh of the fish in the first experimental stage was not recorder because the fish were very small (15-days-old). At the end of first stage, the weights of C fish (6.04 ± 0.21 g) and fish fed with diet I (5.45 ± 0.19 g) were significantly different (the Student's t-test, t=-2,03407, DF=329, p=0.0427).

The growth performance and feed utilization parameters of Nile tilapia during Stage 2 are shown in Table 4. The final weight of fish fed with control diet (I→C and C→C groups)

was higher respect to insect diet (I→II and CII groups). No significant differences were found among the four treatments for FI. Nevertheless, the FCE and PER were significantly higher in fish fed with C diet than fish fed with TM. I→C fish group had the highest DGC, but no significant lower than control. Neither significant difference were found between the controls, C→C, and I→II. The lowest DGC was for C→I fish.

No significant differences were found for the condition factor and biometric indexes between different treatments (Table 4).

The proximate muscle composition of Nile tilapia fed the different diets (Table 4) showed similar ash content and protein values; however, the group of fish that received TM displayed lower body lipid, with significant differences between the C→C and C→II fish groups.

The essential amino acid composition in muscle (Figure 2) was similar in the four experimental groups, with the exception of arginine, which was significantly lower in fish fed with diet I-II. There were no significant differences among the four groups in the balance of essential and non-essential amino acids (EAA/NEAA), the balance of essential and non-essential amino acids (EAA/NEAA).

The fatty acid profile of muscle of fish reflected the fatty

Table 4: Growth performance, somatic indexes and body composition of Nile tilapia on the second stage of the experience. The treatment are named following the sequence of feeding in stage 1 and 2 being C (control diet 100% fish meal), I (75% fish meal: 25% tenebrio meal) and II (50% fishmeal: 50% tenebrio meal).

	Fish treatment					
	C→C	**C→II**	**I→C**	**I→II**	**SEM**	**P-Value**
Growth performance						
Initial body weight (g)	6.08	6.01	5.47	5.43	0.29	0.25
Final body weight (g)	28.30[a]	23.60[b]	28.85[a]	24.30[b]	0.95	<.0001
DGC[a] (%)	3.17[ab]	2.77[c]	3.42[a]	2.95[bc]	0.08	<0.01
FI[b] (%)	3.15	3.67	3.06	4.00	0.27	0.12
FCE[c]	1.10[a]	0.86[b]	1.16[a]	0.82[b]	0.05	<0.01
PER[d]	2.23[a]	1.70[b]	2.34[a]	1.62[b]	0.11	<0.01
PERc[e]	2.24[a]	1.76[b]	2.35[a]	1.68[b]	0.11	<0.01
Somatic indexes (%)						
Digestive	3.48	4.16	3.75	3.42	0.27	0.21
Gonads	1.45	1.47	1.22	1.37	0.39	0.97
Head	24.54	26.09	25.46	25.13	0.83	0.62
Housing	86.20	82.78	83.64	84.24	2.16	0.72
Intestine	2.87	3.56	3.16	2.89	0.25	0.19
Liver	1.52	1.74	1.37	1.54	0.14	0.36
Muscle	37.85	33.12	37.30	35.60	2.09	0.39
Perivisceral Fat	0.20	0.22	0.10	0.03	0.07	0.22
Spleen	0.13	0.10	0.12	0.15	0.02	0.60
Stomach	0.61	0.60	0.59	0.53	0.06	0.70
Condition Factor [f]	1.70	1.67	1.77	1.62	0.07	0.43
Muscle composition (g/kg)						
Ash	81.64	81.12	79.30	79.57	6.82	0.99
Ether extract	112.77[a]	56.59[b]	61.73[ab]	78.73[ab]	11.40	0.03
Crude Protein	806.88	845.94	835.00	865.94	22.11	0.36

Growth performance: Mean of three replicate tanks (28 fish/tank) at the end of the experiment. Different letters indicate significant differences (P<0.05) based on the Tukey- Kramer HSD test.
[a]DGC: Daily growth coefficient = [(Weight$_f^{1/3}$- Weight$_i^{1/3}$)/time] x 100.
[b]FI: Feed intake = (Feed daily intake/average body weight) x 100
[c]FCE: Feed efficiency = wet weight gain/dry feed intake.
[d]PER: Protein efficiency ratio = wet weight gain/crude protein intake.
[e]PERc: Protein efficiency ratio corrected considering the crude protein corrected –CPc– presented in Table 1.
Somatic indexes: Results show, in % of total fish weight, the mean (n=15); different letters indicate significant differences (P<0.05) based on the Tukey- Kramer HSD test.
[f]Condition Factor = 100 x body weight/total length^3.
Muscle composition: Results show, in g/kg of dry matter of the muscle of Nile tilapia, the mean (n=9); different letters indicate significant differences (P<0.05) based on the Tukey- Kramer HSD test.

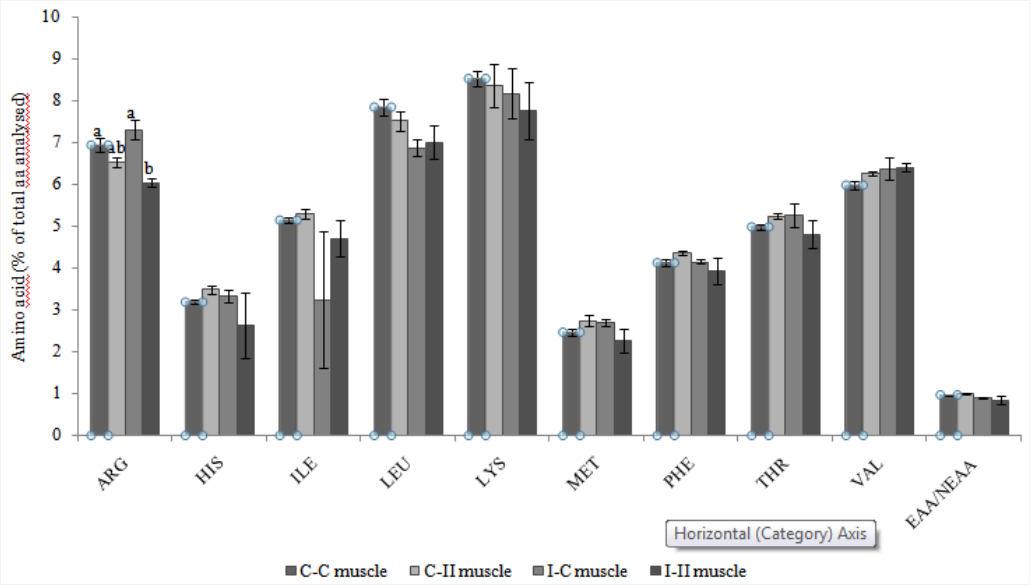

Figure 2 Essential amino acids in the four experimental group fish. Results show the mean ± SEM (n=3) in percentage of total amino acids analysed; different letters indicate significant differences (P<0.05) based on the Tukey- Kramer HSD test. ARG: Arginine; HIS: Histidine; ILE: Isoleucine. LEU: Leucine; LYS: Lysine; MET: Methionine; PHE: Phenylalanine; THR: Threonine; VAL: Valine. EAA/NEAA: Balance of essential amino acids (EAA) and not essential amino acids (NEAA). The treatment are named following the sequence of feeding in stage 1 and 2 being C (control diet 100% fish meal), I (75% fish meal: 25% tenebrio meal) and II (50% fishmeal: 50% tenebrio meal).

acids profile of diet (Table 5). Significant differences were found in the total value of monounsaturated (F=33.5387, DF=11, p<0.0001), saturated (F=29.9357, DF=11, p<0.001), n-3(F=74. 5368, DF=11, p<0.0001) and n-6 fatty acids (F=167.6343, DF=11, p<0.0001) in the four treatments. Fish fed with C diet at the end of the experiment(C→C and I→C) show high level of saturate (33.52 and 35.57% respectively) and n-3 (32.50 and 29.03% respectively) and low levels of n-6(5.35 and 7.65%) than fish fed with diet II (C→II and I→II) (9.48 and 21.63%). The muscle ratio n-3/n-6 shows significant differences among all treatments showing a clear difference between the fish having a diet with insect meal or control diet at second stage.

The values for protease activity (Figure 3) determinate in I→C fish group were the highest (25.15AU/mg soluble protein) with significant differences (p<0.0001) respect to C→C fish group (22.36AU/mg soluble protein); fish fed with II diet showed the lowest values (18.44and 19.92AU/mg soluble protein for C→II and I→II fish group respectively).

The statistical comparison of *in vitro* protein hydrolysis (Table 6) among the four fish groups fed with its corresponding diet at the end of the experiment, did not produce significant differences (p=0.11) for diets C and II.

DISCUSSION

Insect meal as protein source is an interesting new alternative to fishmeal. Nowadays, different insect species (silkworm pupae, *Anaphe infracta*; mealworms, *Tenebrio molitor*; grasshoppers, *Zonocerus variegatus*; termites, *Macrotermes subhyalinus*; black soldier fly, *Hermetia illucens*; domestic housefly, *Musca domestica*) have been used in the feeding of different fish species (common carp, *Cyprinus carpio*; rainbow trout, *Oncorhynchus mykiss*; african catfish, *Clarias gariepinus*; mud catfish, *Hererobanchus longifilis*; channel catfish, *Ictalurus punctatus*; blue tilapia, *Tilapia aurea*; Nile tilapia, *Oreochromis niloticus*) focused to the zoothechnics parameters of growth and nutritive indexes [9-16]. However, the effect of the administration of insect at early stages of development is unknown.

The results of this experiment show that the feeding at early stages of development with TM affects the final growth. At final of stage 1, fish fed with an insect based diet weighed less than fish fed with control diet. Similar results were found at stage 2 (Table 4), with the highest weight correspond to the fish fed with C diet, independently of the diet consumed at stage 1.

The low final weight obtained in fish fed with insect based diet could be due to a low nutritive utilization of diet, as reflect the FCE and PER (Table 4). The poor diet utilization could be mainly due to nutritive imbalance or low digestibility. The experimental diets have similar content in macronutrients and amino acids (Tables 1 and 2). Nevertheless, the fatty acids composition varies among the diets (Table 3) decreasing the n-3 HUFAS in the diet with the inclusion of insect meal. This was reflected in the fatty acids composition of muscle and liver of fish (Table 5).The low HUFAS content in insect-based diet could contribute to lower final weight [30]. These fatty acids have particularly important roles in animal nutrition, reflecting it in critical physiological processes [31].

On the other hand, low digestibility decreased the digestive efficiency that affects nutrients absorption and the growth. The cuticle of insect is composed by chitin, scleroprotein, wax, and other indigestible component that could decrease the diet digestibility. In fact, a decrease of protease activities was found (Figure 3) in fish feed with insect-based diet, in agreement of this a positive correlation (R^2 =0,98) between protease activity

Table 5: Fatty acids profile (% total fatty acids analysed) in muscle and liver of Nile tilapia fed with four different treatments . C→C ,C→II, I→C, I→II which indicates the sequence of feeding in stage 1 and 2 being C (control diet 100% fish meal) , I (75% fish meal: 25% tenebrio meal) and II (50% fishmeal: 50% tenebrio meal).

	Muscle						Liver					
Fatty acid	**C→C**	**C→II**	**I→C**	**I→II**	**SEM**	**P-Value**	**C→C**	**C→II**	**I→C**	**I→II**	**SEM**	**P-Value**
14:00	4.52^{a}	2.27^{b}	4.00^{a}	2.20^{b}	0.33	<0.01	3.98^{a}	2.19^{c}	2.98^{b}	1.87^{c}	0.16	<0.0001
16:00	21.20^{a}	16.13^{b}	22.18^{a}	14.45^{b}	0.78	<0.001	18.10^{a}	12.60^{b}	17.80^{a}	11.50^{b}	0.83	<0.0001
16:1n-7	6.35^{a}	2.60^{c}	4.10^{b}	2.60^{c}	0.23	<0.0001	6.45^{a}	2.89^{c}	5.04^{b}	2.86^{c}	0.30	<0.0001
18:00	7.03^{ab}	5.97^{b}	8.48^{a}	5.38^{b}	0.39	<0.01	8.13^{a}	4.79^{b}	9.55^{a}	5.21^{b}	0.51	<0.0001
18:1n-7	3.52^{a}	1.18^{b}	3.48^{a}	0.87^{b}	0.12	<0.0001	3.83^{a}	0.75^{b}	3.93^{a}	0.91^{b}	0.20	<0.0001
18:1n-9	15.53^{b}	30.93^{a}	16.12^{b}	33.95^{a}	1.08	<0.0001	21.18^{b}	41.06^{a}	20.32^{b}	41.06^{a}	1.06	<0.0001
18:2n-6	2.60^{b}	17.83^{a}	4.80^{b}	19.25^{a}	0.56	<0.0001	2.38^{b}	18.90^{a}	2.68^{b}	17.48^{a}	0.58	<0.0001
18:3n-3	0.23	0.43	0.25	0.63	0.18	0.42	n.d.^{b}	0.42^{a}	0.14^{ab}	0.41^{a}	0.75	<0.01
18:3n-6	n.d.^{b}	n.d.^{b}	n.d.^{b}	0.55^{a}	0.01	<0.0001	n.d.^{b}	0.38^{ab}	n.d.^{b}	0.76^{a}	0.12	<0.01
18:4n-3	0.67^{a}	n.d.^{b}	n.d.^{b}	n.d.^{b}	0.02	<0.0001	0.15	0.11	n.d.	n.d.	0.08	0.52
20:1n-9	1.77^{a}	0.93^{b}	1.08^{b}	0.97^{b}	0.06	<0.0001	1.70^{ab}	1.26^{bc}	1.97^{a}	1.00^{c}	0.12	<0.001
20:4n-3	0.67^{a}	0.15^{ab}	0.32^{ab}	n.d.^{b}	0.12	0.02	0.65^{ab}	n.d.^{c}	0.16^{bc}	1.04^{a}	0.14	<0.001
20:4n-6	1.68^{ab}	1.48^{b}	2.18^{a}	1.37^{b}	0.12	0.01	1.83^{a}	0.86^{b}	2.18^{a}	1.13^{b}	0.11	<0.0001
20:5n-3	3.70^{a}	1.18^{b}	3.15^{a}	0.85^{b}	0.16	<0.0001	2.25^{a}	0.33^{c}	1.46^{b}	0.60^{c}	0.14	<0.0001
22:1n-11	0.60^{a}	n.d.^{b}	n.d.^{b}	n.d.^{b}	0.01	<0.0001	n.d.	n.d.	n.d.	n.d.		
22:5n-3	6.40^{a}	3.00^{c}	5.02^{b}	2.63^{c}	0.18	<0.0001	5.33^{a}	1.15^{b}	5.14^{a}	0.81^{b}	0.36	<0.0001
22:5n-6	1.07^{a}	0.17^{b}	0.67^{ab}	0.47^{ab}	1.18	0.04	0.70^{a}	n.d.^{b}	0.94^{a}	0.09^{b}	0.08	<0.0001
22:6n-3	20.83^{a}	12.73^{b}	20.30^{a}	10.10^{b}	1.10	<0.001	18.88^{a}	5.71^{b}	21.26^{a}	5.78^{b}	0.82	<0.0001
Others	1.89^{bc}	2.67^{ab}	0.61^{c}	3.74^{a}	0.31	<0.01	4.50^{b}	6.61^{ab}	4.44^{b}	7.50^{a}	0.07	<0.01
Monoun saturated	27.77^{b}	35.65^{a}	24.78^{b}	38.38^{a}	1.11	<0.0001	33.15^{b}	45.96^{a}	31.26^{b}	45.83^{a}	1.18	<0.0001
n-3	32.50^{a}	17.50^{b}	29.03^{a}	14.22^{b}	1.02	<0.0001	27.25^{a}	7.72^{b}	28.16^{a}	8.64^{b}	1.22	<0.0001
n-6	5.35^{b}	19.48^{a}	7.65^{b}	21.63^{a}	0.63	<0.0001	4.90^{b}	19.77^{a}	5.80^{a}	18.69^{b}	0.60	<0.0001
Saturated	33.52^{a}	24.53^{b}	35.57^{a}	22.25^{b}	1.20	<0.001	30.70^{a}	19.64^{b}	30.84^{a}	18.76^{b}	1.23	<0.0001
n-3/n-6	6.07^{a}	0.90^{c}	4.00^{b}	0.66^{b}	0.37	<0.0001	5.56^{a}	0.39^{b}	4.89^{a}	0.46^{b}	0.23	<0.0001

Results show the mean (n=9); n.d.: not detected. Different letters indicate significant differences (P<0.05) based on the Tukey- Kramer HSD test.

Table 6: Mean of *in vitro* protein hydrolysis (g hydrolysed protein/kg of DM after 90 min) on the intestinal extracts (n=3) of tilapias fed with the experimental diets at the end of the experiment. C→C ,C→II, I→C, I→II indicate the sequence of feeding in stage 1 and 2 being C (control diet 100% fish meal) , I (75% fish meal: 25% tenebrio meal) and II (50% fishmeal: 50% tenebrio meal).

	Fish group			
	C → C	**C→ II**	**I → C**	**I → II**
C diet	163.81	177.49	146.30	153.05
II diet	171.68	187.12	133.50	163.30
S.E.M	10.00	14.21	6.12	8.89
P-Value	0.63	0.67	0.27	0.50

Differences between the results of two diets in the same fish group were analysed with a Student's t-test significant differences were consider for P-value<0.05.

and DGC were found. The result of feeding tilapia with species of order Coleoptera did not report good results of DGC or final weight, at high levels of fish meal replacement [32,21].

Regarding DGC, it is worth highlighting C→C fish group did not have significant differences in DGC with I→II group that could be a certain adaptation, nevertheless there are not differences between DGC of fish I→II and C→II (Table 4).

The final weigh and DGC of I→C fish group indicated that feeding with insect-based diet at early stages of development does not irreversibly affect growth and enable recover to change to a control diet, showing a compensatory growth. The compensatory growth has been observed in a broad range of teleost once the sub-optimal conditions have been suppressed [33]. The compensatory growth has been observed for various growth-stunting conditions or their combination, including suboptimal

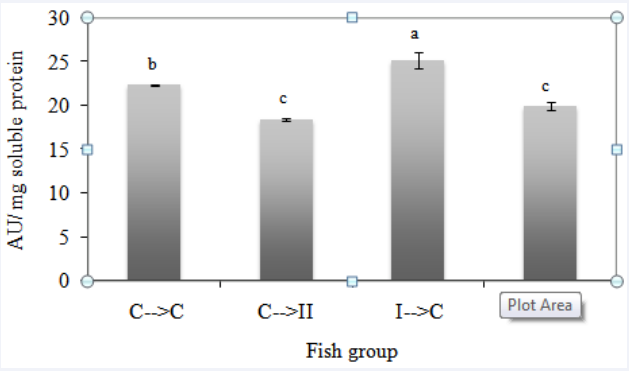

Figure 3 Activity units (AU) of protease in the intestinal extracts of tilapias fed with the experimental diets at the end of the experiment. Results show the mean ± SEM (n=3) of intestine pools (nine intestine for each treatment) of fish fed with the different experimental diets. Different letters indicate significant differences (P<0.05) based on the Tukey-Kramer HSD test. One unit of protease activity (AU) was defined as 1 µg of tyrosine released per minute. The treatment was named following the sequence of feeding in stage 1 and 2 being C (control diet 100% fish meal) , I (75% fish meal: 25% tenebrio meal) and II (50% fishmeal: 50% tenebrio meal).

temperature, crowding, or other stressful environments, being feed restricted, are the conditions most often studied [34]. Regarding muscle composition no significant differences were found in the AAEE/AANE and the amino acid profile in fish muscle, except for arginine (Figure 3).

The lipids content of muscle are similar in the four treatments (Table 4) except for I→C treatment regarding C→ II which could be related to a lower digestibility of dietary lipids due to chitin present in the diets I and II [35]. However, the fish of group I→II show no significant differences in muscle lipids compared to control, which could be due to lower nutritive utilization of diet as reflected the FCR. In agreement with this, Alegbeleye et al. [35], found lower body lipid in African catfish feed with grasshopper (*Zonocerus variegatus*), decreasing as grasshopper meal inclusion in diet increased. They explained it as a lower apparent digestibility coefficient $ADC_{(lipid)}$ in the fish feeding on grasshopper, decreasing with the increase of grasshopper inclusion. Opposite to previous results, in a study of the potential of mealworm in practical diets for African catfish [7], inclusion of TM in diets caused a significant increase in whole-body lipid concentrations in fish at all levels of TM incorporation.

The fatty acids composition of muscle reflects fatty acids diet composition, as it had been demonstrated in other fish species [36-38]. The administration of control diet during stage 2 (I→C) restores the lipids until similar levels to the fish fed with control diet.

Muscular and hepatic (Table 5) fatty acids profiles show major n-3 fatty acids in liver than muscle, specially HUFAs. The reactions of de saturation and elongation occurs in the microsomal fraction of the liver [39], so that a migration from liver to muscle must have happens.

The digestive efficacy had been study through the protease activity and *in vitro* protein hydrolysis. It had been found that the protease activity change with protein source [17,40]. In our case, the activity decreased with inclusion of TM. Similar results have been obtained by Ji et al. [41], in *Cyprinus carpio* var. Jian fed with silkworm pupae.

Pavasovic et al. [40], had found a negative correlation between protease activity and the apparent digestibility coefficients for crude protein in red claw crayfish gut (*Cherax quadricarinatus*). Despite de variations in protease activity (Table 6) not differences were found in *in vitro* protein hydrolysis from the C and II diets for the four different fish groups. The analysis of results *in vitro* hydrolysis and protease activity suggest that the changes in the activity are due to the secretion of proteases, not by changes in the specific activities of proteases.

Indigestible matter as chitin, scleroprotein, wax, etc composes the cuticle of insect. Usually, the cuticle has been determined as ADFom, and it has observed that contain nitrogen [42,43,21]. Our study has shown that the rate of the dietary ADFom and nitrogen content in it (ADIN) (Table 1) had a slight effect on the initial protein value. This coincides with earlier studies by Finke [21] in which the estimate of chitin in raw whole insects showed that the amount of nitrogen in chitin is a relatively small percentage of the total nitrogen in insects.

Shiau and Yu [44] demonstrated that a diet supplemented with chitin and chitosan (polymer obtained from the de-acetylation of chitin) depressed growth in tilapia (*O. niloticus* X *O. aureus*), and also that feed conversion ratio, body weight gains, body lipid content, lipid and dry matter digestibility were lower in animals fed with 2% chitin than control fish, but protein and ash levels in fish were not affected by the diets, which it is in agreement with our results (Table 4).

On the other hand, in Nile tilapia, chitinolytic activity was present in the serum, stomach, and intestine, with the serum having the highest specific activity. In our experiment, the chitinase activity has not been determined, nevertheless the FCE and final weight in fish fed with insect meal (Table 4) indicates low or any activation on the digestive chitinase even in fish fed at stage 1 and 2 with insect meal.

CONCLUSIONS

The inclusion of *Tenebrio* meal at first stages of development of Tilapia decrease the growth and nutritional indices, probably due a deficit of HUFAS n-3 and by decreases in digestive efficiency provoke by cuticle of insect. Nevertheless, the administration at early stages of development does not cause any negative effect in fish performance and they can recuperate the weight and the essential fatty acids profile in muscle (especially in EPA and DHA) at the same values than control fish, after administration of control diet. The authors consider that, although more experiments are necessary and the use of TM requires better understanding of the administration of a diet based on insect meal. However, this finding may have some application in aquaculture. Since the insect meal could be used in the early stages of fish growth, with consequent saving in fishmeal, provided that the fishmeal is used in the later stages of the productive cycle.

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