Substitution of fish meal with Madagascar cockroach (Gromphadorhina portentosa) meal in diets for juvenile Nile tilapia (Oreochromis niloticus): Effects on growth, nutrient assimilation and nitrogen turnover rates.

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Research Article

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Abstract

Among the wide variety of alternative ingredients aimed to substitute fish meal in aquafeeds, insect meals have been recently proposed as novel, nutritionally good dietary components. In the present study,

early juveniles of Nile tilapia (*Oreochromis niloticus*) were fed five isoproteic and isoenergetic experimental diets formulated with varying dietary levels of Madagascar cockroach meal substituting fishmeal on a dietary protein basis (0, 25, 50, 75 and 100%). Diets were supplied for 29 days to eventually compare growth parameters among treatments and to estimate the relative assimilation of the dietary nitrogen supplied by fishmeal and insect meal. Nitrogen stable isotope analyses were applied to diets and fish muscle tissue to explore the isotopic changes elicited by the experimental ingredients and to estimate the nutritional contributions of these ingredients to fish growth. At the end of the bioassay no statistical differences were detected in final mean weight, specific growth and survival rates among treatments. Isotopic changes over time allowed calculating the nitrogen turnover rate in muscle tissue and the time required to reach isotopic equilibrium. The relative proportions of dietary nitrogen supplied by insect and fish meal were similar to the established dietary proportions. Cockroach meal in all mixed diets supplied relatively high proportions of dietary nitrogen (from 16 to 69%) to the biosynthesis of fish muscle tissue. Due to the high growth promoted by the diets, the nitrogen turnover rates in muscle tissue were short and ranged from 4.7 to 6.2 d, except in diet containing 100% cockroach meal (7.8 d).

Introduction

In animal nutrition studies, the estimation of assimilation yields valuable information on diet suitability and can indicate the specific physiological allocation of dietary components. Researchers have applied isotopic measurements at natural abundance levels as biomarkers to estimate the contribution of dietary nutrients to the biosynthesis of tissues (Gamboa-Delgado 2022; Jafari et al. 2020). In the case of nitrogen, the estimation of its assimilation is applied under the principle that the nitrogen stable isotopes values ($^{15}N/^{14}N$, expressed in delta notation as $\delta^{15}N$) of animal consumers reflect the isotopic profiles of the assimilated components of their diets (Phillips 2012). Isotopic data can thus be converted to nutritional contributions by means of mass-balance, isotopic mixing models, that in turn provide an opportunity to estimate the relative contribution of nutrients available in the dietary mixtures, to the growth of farmed species (Zhou and Gu 2020).

Fishmeal often represents the main ingredient used in aquaculture feeds as it offers a balanced source of indispensable amino acids, essential fatty acids, vitamins, and minerals, while also improving the palatability of feeds (Amer et al. 2020). However, the high prices driven by the increasing demand for this resource have fostered the search for sustainable alternative ingredients for the aquaculture industry (Turchini et al., 2019). In the search for new protein sources, insect meals have received increased attention as a raw material due to the positive characteristic such as the fast reproduction rates of insects and the consequent high, continuous production of nutritive meals. However, it has been shown that the insects' life stage and their adaptability to rearing conditions, define their suitability to yield nutrients (Makkar et al. 2014; Gasco et al. 2018). Insects are generally rich in proteins (30–68% on dry matter basis) and have well-balanced amino acid profiles when fed to other animal consumers; insects also represent important sources of energy, lipids, vitamins, and minerals (Finke 2015; Koutsos et al. 2019; Gasco et al. 2020). The use of insects in aquaculture nutrition has been recently implemented with encouraging results in several aquatic species having commercial value, such as rainbow trout (*Oncorhynchus mykiss*) (Belforti et al. 2015), Atlantic salmon (*Salmo salar*) (Belghit et al. 2019), Pacific white shrimp (*Litopenaeus* vannamei) (Motte et al. 2019), European sea bass (*Dicentrarchus labrax* L.) (Mastoraki et al. 2020) and Nile tilapia (*Oreochromis niloticus*) (Alves et al. 2021). To evaluate the nutritional performance of ingredients aimed to replace fish meal, experiments have focused on measuring traditional growth parameters (Mastoraki et al. 2020), digestive efficiency, reproductive performance (Basto et al. 2020; Chemello et al. 2021) and effects of dietary amino acids on metabolism (Fabrikov et al, 2020). In this context, the objective of the present study was to compare the growth parameters and explore the role of the dietary nitrogen supplied by Madagascar cockroach (*Gromphadorhina portentosa*) meal and fish meal in contributing to the somatic growth of juvenile Nile tilapia (*Oreochromis niloticus*).

Materials And Methods

Experimental animals and bioassay conditions

Juvenile Nile tilapia (1.3 ± 0.7 g) were acquired from a commercial hatchery located in Soto la Marina (Tamaulipas, Mexico). Fish were placed in plastic bags containing oxygen saturated water and immediately transported to the Aquatic Production Laboratory of the Veterinary and Zootechnics School (Autonomous University of Nuevo Leon). Fish were allowed to adapt to local conditions for 15 days in glass aquaria kept under a natural photoperiod (12:12 h dark:light). Over the conditioning period, mean water parameters remained as follows: temperature 29.2 ± 0.6°C, pH 8.4 ± 0.2, total ammonia nitrogen 0.09 ± 0.04 mg L^{-1}, nitrite was maintained below detection limits, and mean values for nitrate were 10.7 ± 2.9 mg L^{-1}. To establish known, basal nitrogen isotopic values in fish muscle, during the acclimation period animals were supplied with a previously analyzed, commercial tilapia feed (44% crude protein and 15% crude lipid, NutriPec, Purina®).

Experimental diets

The main ingredients employed to formulate the experimental diets were fish meal (Monterey sardine) and Madagascar cockroach meal, which constituted the only sources of dietary protein in the mixed diets. Both main ingredients were analyzed before the experiment to verify their proximal, elemental, and isotopic profiles. Care was taken to ensure that the conditioning diet and the experimental diets had contrasting isotopic values to promote isotopic changes in muscle tissue. The insect meal was obtained from Madagascar cockroaches grown in plastic boxes fitted with coconut fiber and peat moss substrates (Universidad Autónoma de Aguascalientes, Mexico). Insects were maintained at 26°C and fed a mixture of discarded vegetables and water containing 10% cane sugar. Once grown, insects were sacrificed by freezing, dehydrated (55°C / 96 h) and finally ground to obtain a fine powder.

Five experimental diets were formulated to gradually replace the fish meal-derived protein with insect meal. Diets were isonitrogenous (40% crude protein) and isoenergetic (3.7 kcal/g) and were manufactured as follows: diet 1 contained 100% fish meal as protein source (diet 100F), diets 2 to 4 included fish meal substitutions of 25, 50 and 75% cockroach meal (diets 75F/25C, 50F/50C, 25F/75C). Diet 5 was formulated with 100% insect meal as protein source (diet 100C). Diets 100F and 100C were used as nutritional and isotopic controls to eventually estimate the isotopic differences between diets and fish (isotopic discrimination factors, Δ15N) (Table 1).

Table 1

Nutrient formulation (gr ingredient 1000 gr diet^{-1}) of five experimental diets for juvenile Nile tilapia, which were used to compare the contribution of nutrients supplied by fish meal (F) and Madagascar cockroach meal (C) to fish growth. The proximal and isotopic composition of each diet is presented.

Ingredient	100F	75F/25C	50F/50C	25F/75C	100C
Fish meal[a]	597.9	453.3	305.3	151.7	0.0
G. portentosa meal	0.0	177.3	354.0	520.6	696.9
Wheat starch[b]	286.0	259.3	220.3	180.3	158.7
Fish oil	63.1	55.2	45.0	43.8	32.1
Binder (CMC) [c]	20	20	20	20	20
Cellulose[c]	3.2	5.1	25.3	53.9	62.3
Constant ingredients[d]	30	30	30	30	30
Total	1000	1000	1000	1000	1000
Proximal and isotopic analyses					
Crude protein (g kg^{-1})	397	406	403	398	401
Lipids (g kg^{-1})	123	119	122	124	118
Gross energy (Kcal g^{-1})	3.7	3.6	3.7	3.6	3.7
Moisture (%)	6.2	5.6	5.9	6.4	5.8
Ash (g kg^{-1})	92	85	78	74	65
δ^{15}N (‰)	16.52	15.48	13.07	12.15	8.54
[a]Alimentos Costamar (Sonora, Mexico).					
[b]Almidones y gluten S.A. (Monterrey, Mexico).					
[c]Sigma-Aldrich (St. Louis, MO, USA).					
[d]Mineral and vitamin mix, 20 g; calcium phosphate, 10 g.					

[Table 1.]

Growth parameters and sampling procedures

A total of 160 juvenile Nile tilapia having a mean weight of 1.01 ± 0.2 g were randomly allocated into five duplicate treatments (16 fish per replicate) consisting in 20 L glass tanks. At the onset of the bioassay, the conditioning diet was replaced by the different experimental diets, which were *ad libitum* three times a day (9:00, 13:00 and 17:00 h). Uneaten feed and faces were siphoned out on a daily basis and before the first feeding ration. To determinate the effect of consuming diets on growth, all fish from every replicate tank were individually weighed on the initial and final experimental days. Fish were immobilized with an absorbent cotton cloth and transferred to an analytical balance. Production parameters were estimated as weight gain = [(average final weight – average initial weight)/average individual initial weight] X 100, specific growth rate = [$\log_e$ average final weight – $\log_e$ average initial weight] / (time) (100) and survival = (final number of fish / initial number of fish) × 100. A second sampling scheme was established to collect muscle tissue for stable isotope analysis. Sampling was fitted to the exponential isotopic change frequently observed in rapidly growing aquatic organisms receiving a diet shift (Winter et al. 2019). To this end, fish were sampled on experimental days 0, 3, 7, 14, 21, and 29. In every sampling point, one fish from every replicate was euthanized in ice/water slurry and immediately dissected to obtain muscle tissue. Muscle samples were dried in a convection oven (60°C/24h). Dried samples of muscle, individual ingredients and experimental diets were finely ground with pestle and mortar and stored in desiccators until pretreatment for stable isotope analyses (SIA).

Elemental and stable isotope analyses

Diet and fish muscle samples of 1 ± 0.2 mg were packed in tin microcapsules that were in turn organized in 96-well plastic microplates. Isotope analysis for nitrogen, at natural abundance levels, were conducted at the Stable Isotope Facility, University of California, (Davis, CA, USA) as described in García-Pérez et al. (2020). Reported results were expressed in delta (δ) notation as *per mill* (‰) deviations from the isotopic values of the international standard reference (atmospheric nitrogen, Eq. 1). Instrument precision (SD) was 0.06‰ for $\delta^{15}N$ values, as indicated by the consistency of internal reference materials (*e.g.*, glutamic acid and chitin).

$$\delta^{15}N = (R_{sample}/R_{standard} - 1) \cdot 1000 \quad (1)$$

where $R = {}^{15}N/{}^{14}N$

Nitrogen turnover and half times in muscle tissue

Diet-elicited isotopic changes were registered over time and the isotopic discrimination factors ($\Delta^{15}N$) were estimated at the end of the bioassay. It was considered that $\Delta^{15}N$ values = ± 0.5‰, were indicators of an isotopic equilibrium being reached between fish and their respective diets. Isotopic values determined at different times were introduced into an exponential model (Eq. 2, Hesslein et al. 1993) that allows separating the isotopic change caused by growth (*k*) and metabolic turnover (*m*).

$$C_{SAMPLE} = C_n + (C_o - C_n)e^{-(k+m)t} \quad (2)$$

Where C_{SAMPLE} is the isotopic value in fish tissue at time *t*, C_o is the isotope value of fish tissue in equilibrium with the initial diet (conditioning diet in this study), C_n is the isotope value reached when fish are in equilibrium with a new diet (experimental diets in this study). The growth rate constant, *k*, was obtained by fitting an exponential growth model to observed weight data, k = log (final weight/initial weight)/time(d), while parameter *m* was estimated using iterative non-linear regression. By using the latter two coefficients, provides an indicator of the period necessary for half of the constituent nitrogen to be replaced in muscle tissue (t_{50}, half times, Eq. 3) (MacAvoy et al. 2005).

$$t_{50} = ln2 \ / \ m + k \quad (3)$$

Estimation of nutritional contributions to growth

From the $\delta^{15}N$ values measured in fish meal (source 1), insect meal (source 2) and fish muscle tissue (isotopic mixture), a two-source, one-isotope mass-balance mixing model (Eq. 4; Phillips and Gregg 2001) was used to estimate the relative proportions of dietary nitrogen that were incorporated from both main ingredients into muscle tissue. Underlying assumptions required by isotopic mixing models (Gamboa-Delgado 2022) were met or considered for the calculations. Among these, verification of contrasting isotopic values in the sources, estimation of elemental content in ingredients (N) and verification of $\Delta^{15}N$ values. The latter values were obtained from the isotopic differences observed between the two control diets and the respective control fish (muscle). Instead of using literature values, the use of observed $\Delta^{15}N$ values as correction coefficients tends to increase the precision of results obtained from the isotopic mixing models (Phillips 2012). $\delta^{15}N$ values, sample number and standard errors were introduced into the model to estimate the assimilation proportions and their 95% confidence intervals.

$$f_1 = (\delta^{15}N_{fish\ muscle} - \delta^{15}N_{source2}) / (\delta^{15}N_{source1} - \delta^{15}N_{source2}) \text{ and } f_2 = 1 - f_1 \quad (4)$$

Nitrogen contents in insect meal and fish meal were slightly, but statistically different; therefore, in order to calculate the total amount of dry matter contributed by each feeding source, the following equation (Fry 2006; Eq. 5) was used:

$$f_{total1} = f_1 \cdot W_2 / (f_1 \cdot W_2 + f_2 \cdot W_1) \text{ and } f_{total2} = 1 - f_{total1} \quad (5)$$

where f_{total1}= is the total percent contribution of source 1 in a two-source mixing model, W_1 and W_2 represent the nitrogen content in each of the two sources (main experimental ingredients).

Statistical Analysis

After data homoscedasticity and normality were verified, student's t tests for independent samples were applied to verify significant differences between the isotopic values of experimental ingredients and the conditioning diet. The mean values of the production parameters obtained from every treatment, were compared by one-way ANOVA followed by pair-wise comparisons (Tukey tests). The expected proportions of dietary nitrogen (*i.e.*, established in the formulation of the different diets) and the observed, respective proportions of nutrients assimilated in tilapia muscle tissue were compared by means of Chi-square goodness of fit tests (X^2). All statistical tests were conducted using SPSS 17.0 software (SPSS Inc.) at a significance level of $P < 0.05$.

Results

Growth and survival

At the end of the 29-day feeding period, there were no significant differences in mean final weight (range 9.1 to 11.9 gr), weight gain (794 to 1074%), SGR (7.6 to 8.6) and survival rate (overall survival 97%) among the five dietary treatments. Diet 75F/25C promoted the highest weight gain in fish, while diet 100C elicited the lowest value; however, differences were not statistically significant (Table 2).

Table 2
Mean final wet weight (FW), weight gain (WG) and specific growth rate (SGR) of Nile tilapia O. niloticus reared under experimental diets containing Madagascar cockroach meal (C) and fish meal (F).

Diet	FW (g)	WG (%)	SGR
100F	10.6 ± 2.7	954 ± 273	8.3 ± 0.9
75F/25C	11.9 ± 3.6	1074 ± 358	8.6 ± 1
50F/50C	10.4 ± 2.5	936 ± 252	8.2 ± 0.9
25F/75C	10.4 ± 3.2	941 ± 322	8.1 ± 1.2
100C	9.1 ± 2.7	794 ± 270	7.6 ± 1
Sig.	0.140	0.169	0.167

Data are presented as means ± standard deviations, comparisons were done at a level of significance of 0.05.

[Table 2.]

Isotopic influence of diets and nitrogen half times in muscle tissue

Dietary $\delta^{15}N$ values were rapidly transferred to the muscle tissue of the experimental fish under the different treatments. The basal isotopic value of the preconditioning diet ($\delta^{15}N$=6.9‰) was significantly different to those determined in the experimental ingredients ($\delta^{15}N$=8.5 to 16.5‰) and in consequence, there was a measurable isotopic influence of the diets on fish muscle. The latter, in conjunction with the fast growth of fish, caused conspicuous, exponential isotopic changes in muscle tissue of fish reared on the different diets. By day 21, isotopic equilibrium between diets and fish was apparently reached, or closely approached in all treatments (Fig. 1).

[Figure 1]

Integration of such isotopic values into the exponential model of change indicated that growth (k) was the main component promoting isotopic change (55–85%). In turn, the metabolic turnover rate (m) of elemental nitrogen in tissue was less important in contributing to isotopic change (15–45%). From the *k* and *m* values, elemental half times were estimated for nitrogen (Table 3) and it was found that the half times in tissue ranged from 4.7 to 6.2 days, and there was a slight tendency to increase as the dietary levels of insect meal increased in the dietary treatments.

Table 3
Estimated growth rates (*k*), nitrogen metabolic turnover rates (*m*) in whole bodies and nitrogen half times (t_{50}) in juvenile Nile tilapia *O. niloticus* fed experimental diets with different cockroach meal levels (C) replacing fish meal (F). $\Delta^{15}N$ refers to the observed isotopic difference between diets and muscle tissue after isotopic equilibrium was reached.

Diet	k (d^{-1})	m (d^{-1})*	k vs m	t_{50} (d)	$\Delta^{15}N$ (‰)
100F	0.078 ± 0.010	0.063	55 – 45	4.9 ± 0.6	1.60
75F:25C	0.083 ± 0.014	0.063	57 – 43	4.7 ± 1.1	1.12
50F:50C	0.079 ± 0.009	0.032	71 – 29	6.2 ± 0.6	2.26
25C:75C	0.081 ± 0.010	0.052	61 – 39	5.2 ± 0.7	1.15
100C	0.076 ± 0.013	0.013	85 – 15	7.8 ± 0.9	2.58

**m* values were estimated using iterative non-linear regression to fit expected values on observed values, r^2 = 0.94 to 0.98.

[Table 3]

Nutritional contributions to growth

Isotope values determined in experimental ingredients and fish muscle, and their following integration into an isotopic mixing model, indicated that the dietary nitrogen supplied by cockroach meal in diets 75F/25C, 50F/50C and 25F/75C, contributed similar proportions of dietary nitrogen to fish growth, than those supplied by fishmeal (Table 4). Diet 75F/25C supplied 83.6% of dietary nitrogen from fish meal and 16.4% from the insect meal, while diet 50F/50C respectively supplied 60.1 and 39.9%. Differences were not statistically significant. When the relative contributions to growth were determined on a dry matter basis, the tendency remained similar, although the slightest lower nitrogen content in insect meal implied a concomitant higher assimilation of dry matter from this ingredient.

Table 4. Estimated relative proportions of dietary nitrogen and total dry matter supplied from fish meal (FM) and Madagascar cockroach (*Gromphadorhina portentosa*) meal (CM) contributing to the growth of juvenile Nile tilapia (*O. niloticus*) (mean ± CI, n = 6).

Diet	Expected (Diet)	Observed in fish muscle min.	mean	max.
Nitrogen				
75F/25C				
FM	75[a]	79.4	83.6[a]	87.7
CM	25	12.3	16.4	20.6
50F/50C				
FM	50[a]	55.3	60.1[a]	65.0
CM	50	35.0	39.9	44.7
25F/75C				
FM	25[a]	27.2	31.1[a]	35.0
CM	75	65.0	68.9	72.8
Dry matter[a]				
75F/25C				
FM	72[a]	77.1	81.2[a]	85.3
CM	28	14.7	18.8	22.9
50F/50C				
FM	46[a]	54.1	56.2[a]	58.4
CM	54	41.6	43.8	45.9
25F/75C				
FM	22[a]	26.1	27.8[a]	29.6
GR	78	70.4	72.2	73.9

[Table 4]

Discussion

Growth and survival

The incorporation of insect meal in aquaculture feed has been a widely studied topic, due to the relatively simple production process and the limited need for space required for its manufacture. In addition, insects represent an important source of nutrients that can optimize the growth performance of fish (Henry et al., 2015). Results from the present study indicate that the three combined diets containing cockroach meal and fish meal, performed as well as the fish meal only diet. Total substitution of fish meal also led to similar growth parameters and the latter observation agrees with results reported by Taufek et al. (2016) who conducted a feeding trial with African catfish (*Clarias gariepinus*) fed on diets having high and complete replacement levels of fish meal with cricket meal (*Gryllus bimaculatus*). Diets containing up to 75 and 100% cricket meal improved the body weight gain and specific growth rate in catfish, when compared to 100% FM, although a reduced FCR was observed at higher inclusion levels of insect meal. It is considered that the proximal and amino acid profile of several insect meals can be similar to that found in marine-derived ingredients such as fish meal and squid meal (Belghit et al. 2018). In the present study, there were no significant differences in final weight among treatments at the end of the bioassay. However, fish fed on diet containing only cockroach meal exhibited lower mean final weight. This might be due to the presence of chitin in the *G. portentosa* meal. Chitin is a structural polysaccharide that can have an effect on feed digestibility and might interfere with the physiological use of protein for growth (Longvah et al. 2011; Fontes et al. 2019).

Isotopic influence and half times in muscle tissue

The characteristic fast growth rate of tropical fish promoted a fast transference of the dietary nutrients, and their intrinsic $\delta^{15}N$ values, to the tilapia muscle tissue. Isotopic equilibrium was reached after three weeks of the onset of the experiment, and it was possible to estimate the isotopic discrimination factors or $\Delta^{15}N$. Such values were small between fish and their respective diets (1.12–2.58‰). $\Delta^{15}N$ values were smaller than average values reported in the literature (3.4‰, Post 2002). The latter bibliographic value has been recurrently used as correction factor in nutritional studies when it is difficult or unviable to estimate $\Delta^{15}N$ values; however, several studies have shown that mean literature values are frequently over-relied on and might not be consistent enough to interpret results (Boecklen et al. 2011; Gamboa-Delgado 2022). In the present study, the estimation of $\Delta^{15}N$ elicited by both main ingredients provided specific correction factors for the isotopic mixing model. That is, instead of using the isotopic values of diets, the isotopic values of muscle tissue obtained from control fish were used to estimate the proportional assimilation proportions. If only literature values had been used as correction factors, an overestimation of the contribution of insect meal would have been evident. Previous studies have suggested that high $\Delta^{15}N$ values might indicate nutritional deficiencies, for example, Britton & Busst (2018) demonstrated that omnivorous fish fed only plant-derived proteins having lower dietary protein content and quality had higher discrimination factors for nitrogen ($\Delta^{15}N$=5.4‰) than fish fed on prey items ($\Delta^{15}N$=3.1‰)

In fast growing fish, the main elicitor of isotopic change is tissue accretion (Nahon et al. 2020). The somatic growth (k) implies that the initial isotopic values of fish (established by the conditioning diet) were rapidly “diluted” by the isotopic values of the nutritional components of the different experimental diets. In turn, nitrogen turnover rates in tissue (m) were lower than k and contributed less to the observed isotopic change in muscle tissue. From the latter parameters, it was estimated that the half times (t_{50}) for nitrogen in tissue ranged from 4.7 to 7.8 days. Zhou and Gu (2020) applied similar techniques on the same tilapia species and reported t_{50} values ranging from 10.9 to 12.4 days, and although authors employed fish having similar initial weight (1.2 g), fish were grown for a longer period and up to 30 g, hence explaining the higher t_{50} values.

Nutritional contributions to growth

Results from the present study indicate that the dietary nitrogen supplied by cockroach meal contributed similar, high proportions as the dietary nitrogen supplied by fish meal to tissue growth. The dietary nitrogen supplied by insect meal in the combined diets promoted lower assimilation proportions in comparison to fish meal, however, differences were not significant. In a similar study, Tran et al. (2021) reported that an insect meal (*Tenebrio molitor*) included in diets for perch (*Perca fluviatilis*), contributed nutrients to the biosynthesis of muscle in proportions that were similar to the amounts of insect meal established in the diets. In the present study, results also indicate that the assimilated proportions of dietary nitrogen were similar to the dietary proportions and, as a result, the biosynthesis of muscle tissue was significantly supported by the available protein in insect meal. When disproportional contributions are observed it is usually due to the presence of ingredients having nutritional imbalances, in particular unsuitable amino acids profiles for the consuming organism. For example, in other aquatic organisms, the presence of plant meals in diets has led to lower nutritional contributions to growth, in comparison to contributions promoted by animal- and microbial-derived proteins (Gamboa-Delgado et al. 2020).

Obtained data indicates a positive nutritional performance of insect meal and allows declaring that the nutrients available in the cockroach meal were consumed, digested, and ultimately assimilated in the muscle tissue of fish. Previous studies have indicated that insects have several advantages in terms of nutritional value and the amino acid composition of their proteins, which generally meet animal requirements for maintaining good growth and health (Gasco et al. 2020). In aquaculture nutrition, the use of plant-derived proteins in aquafeeds is common; however, two of the limiting amino acids in plant meals are represented by lysine and methionine. Studies have demonstrated that both amino acids are present at adequate levels in insect meals derived from black soldier fly larvae, housefly maggot, mealworm, locust and common cricket (lysine 4.7 to 7.0 g per g of protein, methionine 1.4 to 3.0 g per g of protein), as compared to lysine and methionine content in fish meal (8.0 and 2.7 g per g of protein, respectively) (Makkar et al. 2014; Tran et al. 2015). The latter aspects might have contributed to the observed high assimilation of dietary nitrogen from the insect meal in treatments receiving the combined diets.

Conclusion

Results from the present study indicate that the use of an insect meal obtained from Madagascar cockroach (*Gromphadorhina portentosa*) and included in diets for juvenile Nile tilapia, promoted growth, survival, and nutrient assimilation rates comparable to those elicited by a reference diet containing fish meal. Even at high fish meal replacement levels (50 and 75%) the growth parameters remained acceptable, and the isotopic data indicated that relatively high proportions of dietary nitrogen supplied by the insect meal contributed to the biosynthesis of fish muscle tissue. Stable isotope analysis also allowed exploring the time periods required for fish to fully reflect the isotopic and nutritional characteristics of their diets. Complete substitution of fish meal with insect meal is not advisable as it was observed that the growth rate decreased under diet containing only insect meal as protein source. There is a growing interest in using alternative protein sources, and although the commercial production of insect biomass is still developing, preliminary nutritional studies point towards the future viable utilization of this unconventional nutritional resource. The nutrient profile of insects can be modified by specific rearing strategies to improve its characteristics for specific animal consumers, as it frequently occurs with other resources such as plants and microorganisms.

Declarations

Funding and/or 'Competing interests'

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No conflicts of interest are declared by the authors

Compliance with ethical standards

Ethical animal management approval for fish is not required by Mexican regulations. However, the feeding trial was developed adhering to maintenance and euthanasia protocols recommended by Sloman et al. (2019).

Author contributions

All authors contributed to the study conceptualization, review and editing of the manuscript. Oscar Daniel García-Pérez, Carlos Alberto García Munguia and Rosa Maria Sanchez-Casas obtained the biological material and acquired the main experimental ingredients for the nutritional study. David Villarreal-Cavazos and Oscar Daniel García-Pérez participated in the diets formulation, biometric processes, data integration, sample collection and pre analysis of samples. Julián Gamboa-Delgado interpreted the isotopic data and ran the mixing models. Gustavo Moreno-Degollado assisted with funding acquisition, laboratory resources and experimental setup. The first draft of the manuscript was written by Oscar Daniel García-Pérez and Julián Gamboa-Delgado and all authors commented on previous versions of the manuscript. All authors have read and approved the final manuscript.

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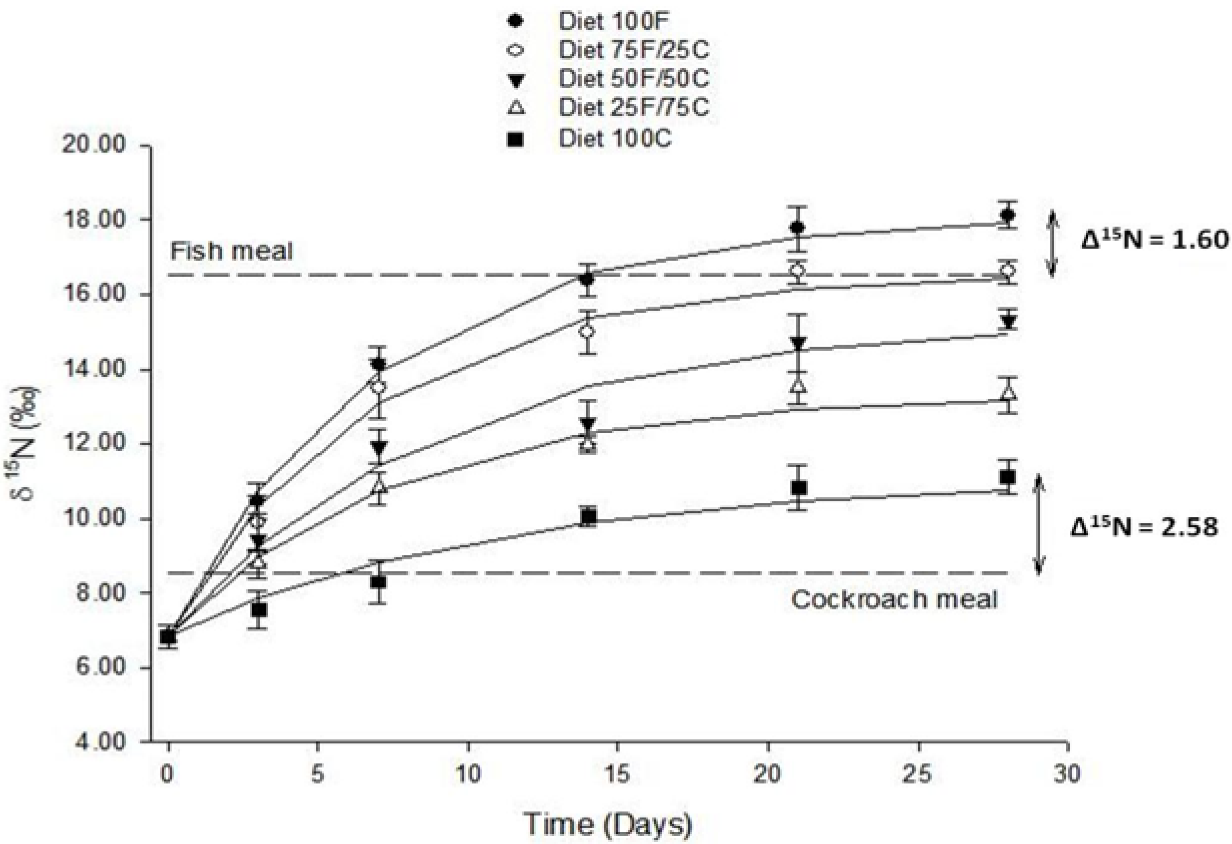
Figures



(Graph created in Sigma Plot 12.0)

Figure 1

Legend not included with this version.