Levels of fat for potential consumption of juvenile *Ambystoma mexicanum* (Shaw & Nodder, 1798) axolotls: Lipid levels

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ABSTRACT

Objective: The present study focused on the effect of different lipid levels on growth (weight and length) and survival in juvenile *Ambystoma mexicanum*.

Design/methodology/approach: Four diets with the same 45% protein level and different lipid levels: 6, 8, 10 and 12% were tested for a period of 81 days. For the preparation of the diets, two key ingredients were used, such as fishmeal and fish oil, these as protein base and lipid source. The experiment consisted of placing six organisms per experimental unit in tubs with 40 L of water for a period of 81 days; period during which four biometrics were performed, the organisms were fed every 48 hours to the weight of their biomass. The digestibility of the diets, initial and final height, initial and final weight, weight gained, weight gained per day, specific growth rate, survival, Fulton index and protein efficiency rate were recorded.

Results: At the end of the experiment, significant differences ($p>0.05$) were observed in the growth and survival of the axolotls in the diets of 8, 10 and 12% lipids.

Limitations on study/implications: A wider range of lipid levels could not be tested, due to the number of individuals available for the experiment.

Findings/conclusions: According to the data obtained in this study, it is recommended to include a level of 45% protein and 8% lipids in the diets of juvenile *Ambystoma mexicanum*, for their better development and nutrition.

Keywords: nutrition, lipid, feeding, axolotl, amphibian, caudata.

INTRODUCTION

Today aquaculture is the fastest growing activity worldwide in terms of the production of aquatic organisms (Garzón *et al.*, 2019) and within this activity is the aquarium hobby

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that is based on the hobby of keeping in ornamental aquariums or ponds whether they are fish, crustaceans, mollusks, echinoderms, amphibians and reptiles (Wabnitz *et al.*, 2003; Lango-Reynoso *et al.*, 2012). To meet the needs of these farming systems, it is important to recognize the needs of the species for their development (Sicuro, 2021). The foregoing includes their digestive physiology, life stage, feeding, as well as the particle size of the food, texture, palatability, buoyancy or sinking among others. The clear objective of the industry dedicated to the manufacture of artificial food is to formulate mixtures in adequate quantities and using quality ingredients that correspond to the nutritional profiles of the species (Garzón *et al.*, 2019). The correct formulation of the food supplied to an ornamental organism improves the quality of the water and reduces excessive maintenance costs (Velasco-Santamaría, 2011). Thus, fulfilling the basic requirements for both survival and growth.

The main source for obtaining energy for an animal comes from the macronutrients consumed in its diet, among which lipids and carbohydrates stand out, taking into account that proteins are the main source of muscle growth (Evers *et al.*, 2019; Huaspa Kana, 2019). Lipids are one of the most studied nutrients in fish and crustaceans since their metabolic function is based on energy distribution in the body and cell structure. It has been determined that aquatic organisms fed low-lipid diets have slow growth, low survival, and unfavorable physical-metabolic changes (Khalili & Sampels, 2018). Lipids are the most efficient macronutrients for a good energy intake, maximizing their storage (Tocher, 2003). The knowledge of the food and nutritional requirements for each organism that is used in the aquarium market, allows to position a healthy product with high economic potential (Velasco-Santamaría, 2011). Therefore, the objective of this work was to evaluate the optimal requirement of lipid content in diets supplied to juvenile *Ambystoma mexicanum* (Shaw & Nodder, 1798) axolotls and the effect on their growth and survival.

Most studies related to the nutrition of organisms are focused on mammals and birds and in the case of aquaculture, the most popular species with high commercial value. There are few studies that address breeding methods and the development of experimental diets that improve the growth of amphibians. For the particular case of the genus *Ambystoma*, there are no studies that address the development of inert diets for maintenance in captivity and some are limited to giving general recommendations (McWilliams, 2008) or establish eating habits in free life or in captivity, based on organisms typical of their habitat (zooplankton) (Chaparro-Herrera *et al.*, 2011). The possibility of having a specific diet for this species, considered in danger of extinction, is of priority importance to develop adequate management plans.

MATERIALS AND METHODS

Axolotl obtention

This study was carried out in the Laboratory of Water Quality and Experimental Aquaculture (LACUIC) belonging to the University of Guadalajara, located in Puerto Vallarta, Jalisco. A total of 72 *A. mexicanum* specimens (with an average weight of 16.4±3.2 g and an average length of 11.8±1.6 cm) were obtained by donation from the Axos-PIMVS production center located in the city of Tepic, Nayarit, Mexico. The juveniles

were transferred in individual bags and placed in Styrofoam boxes with ice to maintain a constant temperature (18.0 °C) during their transfer to LACUIC. Once in the laboratory, they were placed in plastic containers with a capacity of 500 mL per individual for a quarantine process, (40 days) while they were given a prophylactic treatment with the product Azoo Diesase Treatment® at a concentration of 1 mL per 10 L. During the quarantine they were supplied with silver cup El Pedregal food every 48 hours (55% protein and 12% lipids). Throughout the trial they were provided with a controlled environment at a stable temperature of 18.0±1.0°C. All applicable international, national and/or institutional guidelines for the care and use of animals were followed by the authors

Preparation of diets

The elaboration of the diets was based on the results obtained by Manjarrez-Alcívar *et al*., (2022). The ingredients used to prepare the four isoproteic diets (Table 1) were weighed on an analytical scale (Nimbo NBL®; d=0.0001 g) and mixed in a food processor (Kitchen Aid®) until a homogeneous mixture was achieved, which was processed in an extruder (Kitchen Aid®) to remove excess water and form a food into 3.0 mm diameter pellets, which were allowed to dry in an oven (NOVATECH®) at 65 °C for a period of approximately 24 hours. To determine that the diets met the theoretical amounts of protein and lipids necessary to develop the experiment, proximal analyzes were performed according to the protocols established by the A.O.A.C (1995). The determination of the nitrogen content was carried out by the micro Kjeldahl method and a factor of 6.25 was used for the calculation of total protein. Total lipid analyzes were performed using the Soxhlet method using hexane as carrier solvent. The ash content was determined by gravimetric difference with the calcination method in a Thermolyne muffle at 550 °C for a period of 8 h. The determination of the extract free of nitrogen (ELN) was calculated by the difference of the dry matter, with the formula

$$ELN = 100 - (\%\ crude\ protein + \%\ total\ lipids + \%\ ash)$$

Experimental design

After the quarantine period (40 days), the axolotls were randomly placed in plastic tubs, with a capacity of 80 liters, with a working volume of 25 L. Four diets with the same 45% protein level and different lipid levels: 6, 8, 10 and 12% were tested for a period of 81 days (Four treatments in triplicate). Six organisms were placed per tub (one axolotl for every 4.5 L of water) and they were fed every 48 hours at 4% of their initial biomass, after this time, the uneaten food was removed from the tubs with a net and the tub was completely cleaned to feed again. During the 81 day trial four measurements were performed in which the size and weight of the axolotls were recorded.

Response variables

The response variables were: final weight, final length, total weight gain, weight gained, weight gained per day, specific growth rate, and survival. Survival was calculated using the formula:

$$SE(ti) = SP(ti) \times SP(t2) \times SP(t1)$$

where $SP(ti)$=mean population survival in the interval ($ti-1$, ti), The specific growth rate (SGR) was calculated with the formula

$$\left(SGR\ \%\ increase\ in\ weight\ per\ day\right) = \left[\left(\ln Wf - \ln Wi\right) / t\right] \times 100$$

where Wf=final weight (g), Wi=initial weight (g), and t=time (days) and the

$$size\ heterogeneity = CV\ Wf\ /\ CV\ Wi$$

was calculated; where Wf=final weight, Wi=initial weight, and CV=coefficient of variation.

Statistical analysis

Shapiro Wilk normality and homoscedasticity tests were performed for each of the measured variables. A one-way analysis of variance (ANOVA) was carried out to compare among the treatments the average of: survival, initial and final length (cm), initial and final wet weight (g), weight gain and length gain, coefficient of variation (g), size heterogeneity (fish body weight g), and final biomass. In all cases a significance level of $P<0.05$ was used. Tukey's post-hoc test was used to identify differences between treatment means using the statistical program SPSS version 17.0.

RESULTS AND DISCUSSION

Table 1 shows the proximal composition of the diets made with different lipid levels and a protein level, in which the data obtained in the determination of these are similar to those necessary to carry out the experiment. Total, protein levels were 45.0±0.5% and lipid levels presented a maximum difference of 0±0.4%.

Figure 1 shows the growth of juvenile axolotl *A. mexicanum*. The final weight obtained from the juveniles for each of the diets per axolotl was: P45/L6 with 35.7±0.8 g, P45/L8 35.8±4.9 g, P45/L10 with 37.1±0.8 g and P45/L12 with 37.4±2.8 g.

According to what was obtained (Table 2) in the initial weight and initial length, total weight gain, weight gained, weight gained per day and specific growth rate, the axolotls did not present statistical differences between the treatments except in survival, where was observed a decreased survival on the P45/L6 diet ($P<0.05$).

Despite the fact that in this experiment a trend of greater biomass was observed in the juveniles of the 12% lipid treatment, when performing the statistical analysis of the evaluated variables there were no significant differences in: weight, length, total weight gain, weight gained, weight gained per day and specific growth rate (Table 2). This suggests that *A. mexicanum* has an adaptability to lipid intake, coinciding with Garzón *et al.* (2019) where it was reported for other ornamental fish species such as: *Poecilia latipinna*, *Trichogaster trichoptrus* and *Pterophyllum scalare* that the nutritional requirements necessary for their best

Table 1. Formulation and proximal composition in the experimental diets of juvenile Axolotls *A. mexicanum* with a protein level of 45.0% and different lipid levels: 6, 8, 10 and 12% during a period of 81 days.

Ingredients	P45/L6	P45/L8	P45/L10	P45/L12
Fish meal	59.1	59.1	59.1	59.1
Cornmeal	16.0	16.0	16.0	16.0
Fish oil	5.3	4.9	4.3	3.6
Cornstarch	33.8	26.8	19.9	12.9
Gelatin	5.0	5.0	5.0	5.0
Vitamins and minerals	3.0	3.0	3.0	3.0
Vitamin C	0.5	0.5	0.5	0.5
Sodium benzoate	0.2	0.2	0.2	0.2
Alpha tocopherol	0.01	0.01	0.01	0.01
Total (g)	100.0	100.0	100.0	100.0
Proximal composition				
Crude protein (%)	45.5±0.8	45.0±0.1	45.01±0.2	45.2±0.5
Total lipids (%)	6.5±0.2	8.5±0.1	10.2±0.1	12.2±0.4
Ashes (%)	11.3±0.1	11.6±0.5	11.1±0.4	11.3±0.2
Nitrogen free extract	36.5	34.7	33.6	31.1
Kcal consumables	4.701	4.815	4.901	4.998

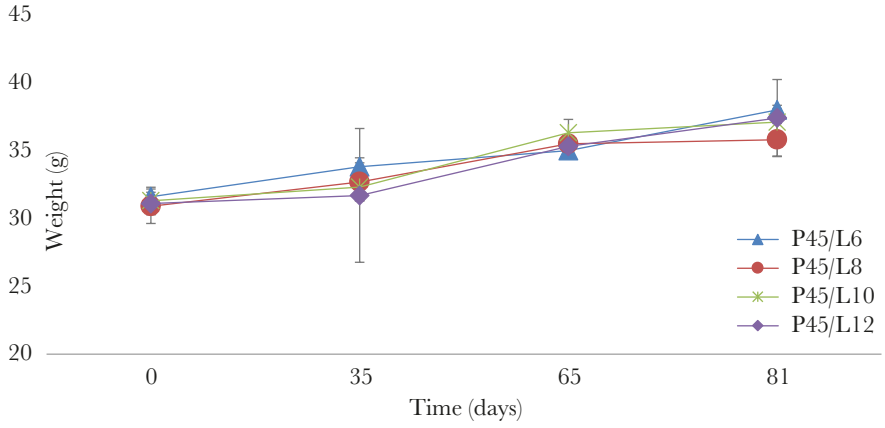

Figure 1. Weight gain in juvenile axolotls *A. mexicanum* fed with a 45% protein level and different lipid levels: 6, 8, 10 and 12% during a period of 81 days.

performance are in a range of 40 to 50% protein and 6 to 8% lipids. In other cold-water fish such as salmon, it was estimated that a high lipid intake between 15 and 20% contributes to a 13% decrease in the amount of protein in diets, from 48% to 35% without altering their growth, obtaining the same protein efficiency and better energy utilization, attributing this to its energy density (Akoh, 2017).

Regarding survival, this was greater than 80% in all treatments. The treatments of 8 and 12% of lipids obtained 100%, this means that the animals were kept in adequate conditions, the above coincides with what was reported by: Bolasina and Fenucci (2007) for the fish *Urophycis brasiliensis* where three different diets with 42% protein and different

Table 2. Effect of four diets with a protein level of 45% and different lipid levels 6, 8, 10 and 12% on: weight, length, survival, weight gained per day, weight gained per day, Fulton's K and rate. growth factor (SGR) and feed conversion factor (FCA) in juvenile axolotls *A. mexicanum* cultured for 81 days.

Response variables	Treatments			
	P45/L6	P45/L8	P45/L10	P45/L12
Survival (%)	83.3±2.8 [b]	100±0.0 [a]	94.4±9.6 [a]	100±0.0 [a]
Initial weight (g)	31.6±0.1 [a]	30.9±0.7 [a]	31.3±0.3 [a]	31.1±0.3 [a]
Final weight (g)	35.7±0.8 [a]	35.8±4.9 [a]	37.1±0.8 [a]	37.4±2.8 [a]
Initial length (cm)	15.4±0.0 [a]	15.4±0.4 [a]	14.7±0.9 [a]	15.3±0.1 [a]
Final length (cm)	16.4±0.1 [a]	16.3±0.6 [a]	16.2±0.2 [a]	16.4±0.6 [a]
Total weight gain (%)	20.5 [a]	15.6 [a]	18.3 [a]	20.33 [a]
Weight gained (g)	6.5±0.9 [a]	4.9±4.5 [a]	5.7±0. 5 [a]	6.3±3.1 [a]
Weight gained per day (g/day)	0.1±0.0 [a]	0.1±0.0 [a]	0.1±0.1 [a]	0.1±0.0 [a]
Specific growth rate	0.1±0.1 [a]	0.1±0.0 [a]	0.1±0.0 [a]	0.1±0.0 [a]
Fulton's K	0.80±0.04 [a]	0.82±0.02 [a]	0.87±0.04 [a]	0.84±0.01 [a]
Food convertion factor (%)	2.7±0.3 [a]	2.8±0.5 [a]	2.4±0.5 [a]	2.7±0.7 [a]

levels of crude lipids 5, 8 and 11% lipids for 40 days where the best survival was recorded in the 8% lipid diet. Toledo *et al*., (2014) reported a survival greater than 98% in diets with 8, 10 and 12% lipids in *Calyptocephalella gayi*.

CONCLUSIONS

According to the results obtained in this study, it is recommended to include a level of 45% protein and 8% lipids in the diets of juvenile *Ambystoma mexicanum*. These results contribute to the formulation of specific diets for the optimal development and nutrition of the species, as well as to improve management and cultivation plans.

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