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Palatability of animal oils included in the diet of the Mexican axolotl and its effect on growth and survival

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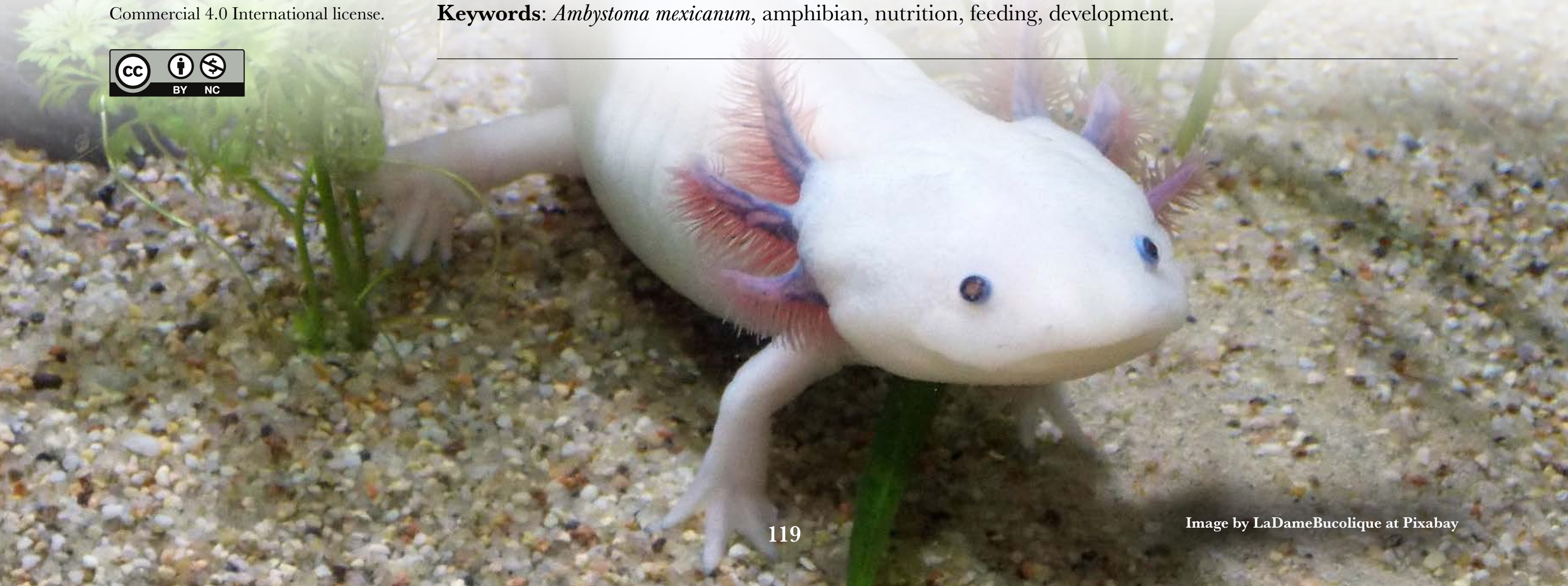
ABSTRACT

Objective: The growth, survival, and condition factor of *Ambystoma mexicanum* juveniles fed with three commercial feed-based diets coated with different animal oils as potential palatability enhancers were evaluated.
Methodology: Three diets were prepared using commercial feed coated with fish, chicken, and krill oil. The experiment lasted 81 days, the food was provided to the axolotls (6 per experimental unit, in triplicate) every 48 h, at 4% of the total biomass. The total weight of each experimental unit was recorded every 15 days, at the end of the experiment individual weight and height and survival were recorded. The following variables were calculated: Fulton's K, specific growth rate, coefficient of variation of final weight and size heterogeneity.
Results: All three treatments showed an increase in total biomass, with a trend towards better performance in chicken and fish oil treatments compared to krill oil. When performing the statistical analysis, it was found that there were no significant differences among the treatments for any of the variables recorded.
Implications: The three oils used in the present study were good palatable agents in the food intake of *A. mexicanum*.
Conclusions: It is recommended to use chicken oil as an attractant additive in the formulation of a specific diet due to its low cost.

Keywords: *Ambystoma mexicanum*, amphibian, nutrition, feeding, development.



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INTRODUCTION

Due to its biological characteristics, such as its regeneration capacity, one of the species that is used as a biological model around the world is *Ambystoma mexicanum* (Shaw & Nodder, 1798), endemic species of Lake Xochimilco, Mexico, which is in danger of extinction due to pollution and the introduction of invasive species that have reduced its populations, in its natural habitat. In contrast, its reproduction in captivity has favored its conservation and wide distribution in the world, either as a biological model or as a pet (Gresens, 2004; Vance, 2017; Gonzalez & Zamora, 2014). One of the biggest challenges of axolotls in captivity is feeding. Especially in its early stages as its diet is mainly live food (zooplankton) due to the size of its mouth being very small (Chaparro-Herrera *et al.*, 2011). When they reach a size of 5 cm in length they can begin to consume pelleted feed, although this transition is difficult as these diets are not very palatable for the animals, so it is recommended to use feed with ingredients that are attractive and palatable (Gresens, 2004). Currently there is no specific food for *A. mexicanum*. There are commercial products that are theoretically designed for the species, but they are not supported by scientific publications or by patents that prove it. In addition, they are difficult to acquire, so commercial fish feed is used conventionally (Gresens, 2004), with the logical nutritional deficiencies by not satisfying the specific requirements for the Mexican axolotl.

Axolotls, being aquatic, base their feeding strategy mainly on smell and taste, so it is important to find ingredients that are attractive and palatable for their food (Farkas & Monaghan, 2015). The palatability of a food is influenced by the ingredients present in it, which stimulate taste and intake. Likewise, the ideal is that these palatable ingredients also provide the nutritional requirements of the species to which the food is directed (Tantikitti, 2014).

Protein ingredients of marine origin have a greater number of low weight molecules, soluble in water, which give them high attractability, (for example fish and krill meal), compared to ingredients of terrestrial origin, so the latter are less used in the production of balanced feed for aquatic animals (Villarreal-Cavazos *et al.*, 2017); however, little has been studied on the effect of oils, insoluble in water, on the palatability of food, in captive axolotls.

The objective of the present study was to determine the palatable capacity of different animal oils added to the food of *A. mexicanum* and their effect on growth and survival, with the intention of designing better specific diets for the culture of the species in captivity.

MATERIALS AND METHODS

Obtaining of the axolotls

The juveniles of *A. mexicanum* were donated by the production center AXOS-PIMVS localizado en Tepic, Nayarit (Bahía de Banderas 62, Lomas de la Cruz, 63037). The specimens were transported in individual bags from the production center to the Laboratory of Water Quality and Experimental Aquaculture (LACUIC), belonging to the University of Guadalajara, located in Puerto Vallarta, Jalisco (20° 40''N; 105° 16''O / 20.667, −105.267). After an acclimatization period of 30 minutes they were placed in a 150 L tank in the laboratory with a controlled environment at a stable temperature of 18±1° C as recommended by Farkas and Monaghan (2015).

Experimental design

For the feeding bioassays, 6 axolotls (29.67±8.20 g) were randomly placed in 80 L tubs (gauged at 35 L) per treatment and in triplicate (each tub represented one experimental unit). 100% water exchanges were carried out every 48 h. Prior to the experiment, the weight and initial size of the axolotls were analyzed with an ANOVA ($P<0.05$).

Diets and food

Diets were prepared using commercial Growfish® brand pelleted feed for developing Tilapia, stage 2 (3.5 mm) (35.0% min. protein, 3.5% min. fat, 5.0% max. ash). The alternative diets evaluated consisted of the commercial feed coated with: krill oil (Simi Krill®) and chicken oil (Proteínas Marinas y Agropecuarias, S.A. de C.V.®). To avoid differences in lipid levels between these diets and the control, the latter was coated with fish oil (Proteínas Marinas y Agropecuarias, S.A. de C.V.®).

1020 g of feed were prepared for each treatment at 3% weight/weight inclusion of the oil (30 g of oil in 990 g of feed). The 990 g of food were placed in trays in an oven at 60 °C for 30 min (in order to remove moisture from the feed and make it better absorb the oil), then it was removed from the oven and placed in airtight plastic bags, for cooling and storage (24 h at 18±1 °C, in a dry room), until use. The feed was placed in a commercial mixer (Kitchen Aid®) to keep it moving while it was sprayed with each attractant oil using the spray method proposed by Oikawa & March (1997) and Kolkovsky *et al.* (2000) using undenatured ethyl alcohol to dilute the oil and make it easier to apply. Once the feed was coated, it was left to dry on a tray at room temperature for 15 min, after which it was placed for 15 min at 60 °C in an oven and finally it was left to dry for 24 h at 18±1 °C to ensure evaporation of the alcohol. After preparing the diets to be tested, proximate analyzes were performed to ensure the levels of proteins, lipids and ash of the treatments were similar between the diets.

The experiment lasted 81 days during which the food was provided to the axolotls every 48 h, at 4% of the total biomass, and the unconsumed food was withdrawn 24 h after being administered. The total weight of the animals in each experimental unit was recorded every 15 days and at the end of the experiment (81 days) a biometry was performed where individual weight and size of each organism were recorded, as well as survival. With the data obtained, the following variables were calculated: Fulton's $K\left(K=\left(W/L^3\right)\times 100\right)$, specific growth rate $\left(SGR\%=\left[\left(\ln W_f-\ln W_i\right)/t\right]\times 100\right)$, coefficient of variation (CV) of the final weight (Kestemont *et al.*, 2003) and size heterogeneity $\left(\text{heterogeneity of sizes}=CV_{wf}/CV_{wi}\right)$; where W_f=final weight (g), W_i=initial weight (g), t=time (days) and CV=coefficient of variation.

To analyze the level of conditioning to the test diets, the food consumed was quantified at the end of the 81 days of the experiment. The axolotls were fed (at 4% of their biomass per experimental unit) once more and the residual food was removed after 24 hours.

Immediately afterwards, it was dried in an oven at 60 °C for 24 hours, placed in airtight plastic bags for cooling and storage (24 h at 18±1 °C, in a dry room), and its weight was recorded.

Statistical análisis

With the data obtained, the Kolmogorov-Smirnov test was performed to verify that the data presented normality and homoscedasticity. After that, an (ANOVA) was performed. In cases where significant differences were found between treatments ($P<0.05$), Tukey's test was performed for comparison. These analyzes were performed using SigmaPlot11 software.

RESULTS AND DISCUSSION

Figure 1 shows the biomass increase of axolotls over 81 days of experimentation using diets with different types of attractants (fish oil, chicken oil and krill oil). All three treatments showed an increase in total biomass, with a trend toward better performance in chicken and fish oil treatments compared to krill oil. However, when performing the statistical analysis, it was found that there were no significant differences between the treatments for any of the variables recorded (Table 1).

The similar consumption of the different diets among the three treatments could be due to the fact that the palatability stimulus caused by the different oils was similar for the axolotls. Which suggests that chicken oil, despite being terrestrial, worked in a similar way to marine oils, which are more expensive. These results coincide with those reported by Sanches-Alves *et al.* (2019) for *Oreochromis niloticus* where hydrolyzed poultry protein was the one that induced a higher feed intake for this species compared to other ingredients, including fishmeal and pork liver.

Farkas & Monaghan (2015) reported that smell is an important sense for feeding in axolotls because they lack an auditory median structure and their vision is very poor and limited to motion detection. But this last characteristic is another reason that could explain the similarity of the results of food consumption, in the present study since the axolotls, when observing the movement of the food at the moment of sinking, reacted quickly

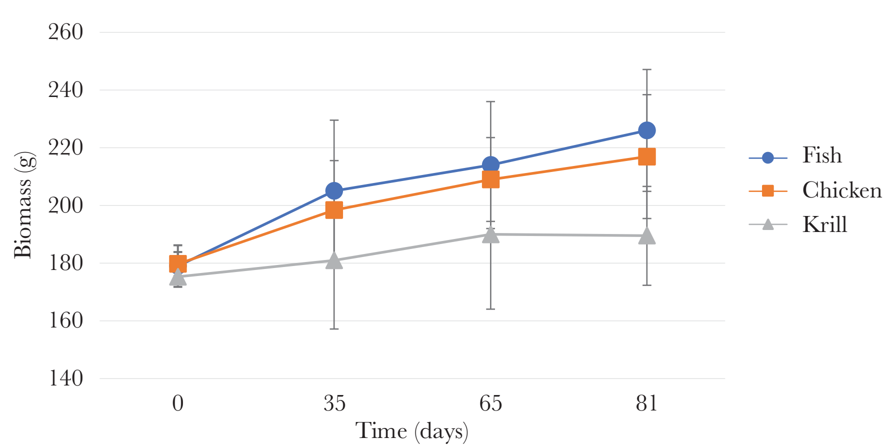

Figure 1. Increase of the total biomass in *A. mexicanum* with different types of attractants added to the diet.

Table 1. Weight, length, survival, size heterogeneity, weight gained per day, Fulton's K, specific growth rate (SGR) and food consumed (mean ±S.D. of three replicates per treatment) in *A. mexicanum* fed three diets for 81 days including different animal oils as attractants.

Diets	Fish	Chicken	Krill
Survival (%)	100±0.00	94.44±9.62	88.89±9.62
Initial weight (g)	29.83±1.20	29.94±0.70	29.22±0.34
Final weight (g)	37.70±3.52	38.38±2.92	35.60±1.34
Coefficient of variation (final weight g)	15.97±2.93	17.85±0.36	18.60±3.95
Size heterogeneity (g)	0.63±0.08	0.61±0.20	0.58±0.16
Initial lenght (cm)	15.19±0.13	15.08±0.14	15.04±0.29
Final lenght (cm)	15.97±0.34	16.20±0.48	16.22±0.38
K- factor	0.92±0.04	0.90±0.03	0.84±0.08
SGR (%)	0.28±0.07	0.30±0.07	0.24±0.05
Weight gained per day (g)	0.10±0.03	0.10±0.03	0.08±0.02
Food consumed (g)	2.60±0.54	2.53±0.52	2.27±0.48

No superscripts were added as no statistically significant differences were found (one-way ANOVA, $P<0.05$).

and indistinctly to capture and ingest it. No rejections due to regurgitation of food were observed in any of the treatments.

Although there were no significant differences between the treatments, a trend was observed that suggests a potential rejection (lower palatability compared to chicken and fish oil) to krill oil, with lower intake results, with negative effects on survival and final weight, compared to chicken and fish oils. The apparent better palatability and intake of feed coated with chicken oil represents an economic advantage in the formulation of a feed since it would not be necessary to add a high-cost oil such as krill oil to the diet of axolotls to obtain a good performance. According to Lee & Meyers (1996) and Nunes *et al.* (2006), the food ingredients obtained from aquatic animals (flours and soluble fractions) have low weight molecules that make them excellent attractants (Smith *et al.*, 2005; Ali *et al.*, 2007), while those obtained from terrestrial animals (oils and poultry meals) do not have this quality (Suresh *et al.*, 2011). Although there are studies in other species that favor the use of krill as an attractant (Oikawa & March, 1997; Kolkovski *et al.*, 2000) in the present work it was not demonstrated it is a more palatable ingredient of primary choice for axolotls since chicken oil obtained better results; chicken oil obtained better results; which coincides with what was reported by Suresh *et al.* (2011) in marine shrimp. In fact, in the only study carried out with the genus *Ambystoma* and specifically with *Ambystoma mexicanum*, by Ocaranza-Joya *et al.* (2021) found no differences in the response of animals to the attractant/palatable effect of krill and chicken oils, concluding that both can be used effectively for this purpose.

The condition index (Fulton's K) showed that the axolotls presented good physical condition between the three treatments, they did not present erratic swimming or diseases after the experiment. The values presented in this study were better than those reported for larvae of the same species ($K=0.79$) in studies that evaluated the development and growth of larvae in three different maintenance media (dechlorinated tap water and enriched with sodium chloride; commercial colloidal solution; Holtfreter's solution (Robles-Mendoza *et al.*, 2009). In relation to survival, these authors report 10% mortality) which is similar to what was observed in this study, since survival was greater than 90% in all treatments.

Although a low heterogeneity of sizes such as the one presented in this study, by itself is not considered an index of condition, in the culture of aquatic animals it is sought that the production be as similar as possible. Especially for cannibal species such as axolotls since the reduction of differences in sizes can reduce type II cannibalism (complete cannibalism) as indicated by Baras *et al.* (2000) and Kestemont *et al.* (2003).

Maya-Monroy (2006) carried out an experiment with *A. mexicanum* larvae with three different diets for a year: a diet with commercial food for turtles ("Tortuguetas"®), a diet based on insects (crickets) and a mixed diet of the previous two. The author found significant differences between these three diets, being the insect-based diet the one that presented the best result in weight and length of the axolotls. The author mentions that despite the fact that the turtle feed has a higher amount of protein per kilo (300 g) compared to a kilo of crickets (205 g), the former was not well accepted by the axolotls, and was even rejected when it was administered to the animals directly in the mouth. This corroborates the importance of developing a palatable and attractive food for the species, which was the main objective of this study.

In that same study (Maya-Monroy, 2006) reports that at the end of the 12 months of experiment the growth curve of the axolotls reached a type of asymptote, where it is slower, unlike the first months of the experiment. This could explain the results of the present study, where the difference between the initial length and final length was not very notable, since it is probable that the animals used in the present study could have been late juveniles in which the exponential growth stage was already had happened.

CONCLUSIONS

In conclusion, the three oils used in the present study were good attractants for the food intake of *A. mexicanum*. However, it is recommended to use chicken oil as a palatable additive in the formulation of a species-specific diet due to its low cost. According to these results, it is suggested to carry out nutritional experiments where different levels of inclusion of chicken oil in the diet are evaluated to determine the best concentration for the species. This study provides knowledge to improve the captive maintenance of this endangered species.

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