

# Effect of Shrimp Feed Supplemented with Astaxanthin on Golden Apple Snail *Pomacea diffusa*

## Efeito da Ração de Camarão Suplementada com Astaxantina no Caracol Ampulária Dourada *Pomacea diffusa*

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### Abstract

Known as apple snail, these gastropods are popular as aquarium pets around the world. The bright color of these animals is one of the key points that attract their attention in the ornamental aquaculture, where deep-colored animals are well appreciated. Astaxanthin is the most widespread color enhancing pigment used as a food supplement for aquatic organisms. There is no specific commercial food for apple snails, emphasizing the need to search for food alternatives and supplements that improve their performance and attractiveness. The objectives of this study are to measure the synthetic astaxanthin accumulation capacity of *Pomacea diffusa* at different levels of diet supplementation, and the effect on pigment concentration and zootechnical indexes at the end of the culture. It was also aimed to verify the effect of the diet change from fish food to extruded shrimp food on the mollusks' body condition. Apple snails fed with commercial shrimp ration had low feed conversion rates ( $0.81 \pm 0.13$ ), high food efficiency ( $126.7 \pm 18.3\%$ ), and condition factor ( $1.0 \pm 0.14$ ) above the initial average ( $0.90 \pm 0.17$ ), demonstrating that this might be an advantageous alternative food. Dietary supplementation with synthetic astaxanthin did not result in a significant concentration increase of this pigment in muscle tissue of these mollusks ( $P > 0.05$ ).

**Keywords:** Aquaculture. Mollusks. Gastropods. Pigments. Carotenoids.

### Resumo

Popularmente conhecidas como ampulárias, esses moluscos gastrópodes são usualmente cultivados como organismos ornamentais pelo mundo. A coloração desses animais ornamentais é um dos pontos que atrai os seus admiradores e estimula sua aquisição no mercado. O pigmento mais difundido como suplemento para intensificação de cor em organismos aquáticos é a astaxantina. Ainda não existem rações específicas para a ampulária reforçando a necessidade de testar alternativas de alimentos e suplementos que melhorem o desempenho produtivo e a atratividade do animal. Os objetivos desse estudo foram mensurar a capacidade de acumulação de astaxantina sintética em diferentes níveis de inclusão na dieta de *Pomacea diffusa*, sua influência na concentração do pigmento e nos índices zootécnicos ao final do cultivo. E em paralelo verificar a influência da mudança da dieta de ração de peixes para ração de camarão na condição corporal dos moluscos em comparação com o início do trabalho. As ampulárias alimentadas com ração comercial extrusada para camarão marinho tiveram baixos índices de conversão alimentar ( $126,7 \pm 18,3$ ), altos índices de eficiência alimentar ( $126,7 \pm 18,3\%$ ) e fator de condição ( $1,0 \pm 0,14$ ) acima da média inicial ( $0,90 \pm 0,17$ ), o que demonstra que esse alimento pode ser utilizado para seu cultivo. A suplementação dietética de astaxantina sintética não resulta em aumento significativo da concentração desse pigmento na musculatura desse molusco ( $P > 0,05$ ).

**Palavras-chave:** Ampulária. Moluscos. Gastrópodes. Pigmentos. Carotenoides.

### 1 Introduction

Apple snails of the genus *Pomacea* are widely used for use as ornamental species (NG *et al.*, 2016). There are populations of this genus with expressive growth as invasive species introduced in some Asian, European, and American continent countries (HORGAN *et al.*, 2014b; Liu *et al.*, 2019). The most common ampoules in fishkeeping are from the colored lineages of the species *Pomacea diffusa*, which has highlighted aesthetic appeal as an ornamental organism, and for its food habit of consuming periphyton, contributing as an aquarium cleaning organism. Its origin is Amazonian and its creation in aquariums has a wide distribution in the world (LIU *et al.*, 2017).

The shell color patterns and the musculature of these

mollusks attract attention and determine the choice of the specimen to be acquired by buyers and growers of this species in the ornamental aquaculture. The coloring patterns of *Pomacea diffusa* are basically of two types of the main color in the *Pomacea* muscle, being light (albino or yellow) and dark (with the presence of pigment) (YUSA, 2004), with variations, and several color patterns in the shell that are likely a result of the mixture of yellow/orange and violet pigments, as occurs in other mollusks (KOZMINSKY; LEZIN, 2006). The apparent shell color is still influenced by the background color that is given by the animal's muscle, since the shell has a certain degree of transparency, and also by the stripes that may or may not be present (ESTEBENET *et al.*, 2006).

In the musculature of these mollusks, the presence of

chromatophores filled with reddish or orange color can be identified, coming from carotenoid pigments that are acquired by the animal's diet (WADE *et al.*, 2017; COSTA; MIRANDA-FILHO, 2019; ZHAO *et al.*, 2019). As the value and importance of ornamental organisms are generally linked, among other factors, to their color, an increase in pigmentation can increase the commercial attractiveness, and take advantage of the full potential of color expression naturally inherent in mollusks, as is already known for other species (DANIEL *et al.*, 2017).

For aquatic organisms, the main pigment used to increase the red color is astaxanthin. This pigment in artificial form is the most used in the commercial production of aquatic organisms, both ornamental and for food (DANIEL *et al.*, 2017; PATTANAİK *et al.*, 2020).

Astaxanthin has the highest antioxidant activity among natural carotenoids, having a free radical chelation potential higher than that of vitamin E (MIKE, 1991). Additionally, it inhibits mitochondrial lipid peroxidation and contributes to cell membrane stability (GOTO *et al.*, 2001). The ability to transform precursor pigments into astaxanthin varies according to the species of aquatic organism and may be non-existent, or possible from carotenes or some xanthophylls, a process commonly observed in mollusks, crustaceans, and fish (COSTA; MIRANDA-FILHO, 2019).

Astaxanthin is present in *Pomacea canaliculata* tissues in low concentrations concerning other carotenoids (TSUSHIMA *et al.*, 1997). Therefore, it is not known whether the supplemental astaxanthin supply to *P. diffusa* would bring performance and color benefits to these mollusks. Therefore, the pigment accumulation capacity needs to be tested in animals.

The artificial feed provided in tests can influence the mollusks' body condition. As the genus *Pomacea* naturally consumes food with high humidity and/or remains of fish feed, such as secondary fauna in aquariums, these animals can suffer a positive influence if fed with invertebrate rations such as marine shrimp.

The objectives of this study were to measure the accumulation capacity of synthetic astaxanthin at different inclusion levels in the diet of *Pomacea diffusa*, its influence on pigment concentration, and zootechnical indexes at the end of the cultivation. And in parallel to verify the influence of the change from the diet of fish feed to shrimp feed on the mollusks body and productive condition in comparison with the beginning of the work.

## 2 Material and Methods

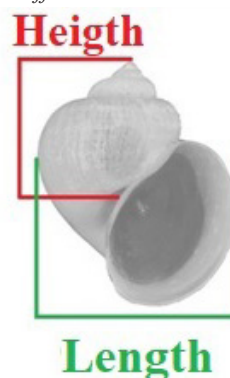
The study was carried out at the Laboratory of Mariculture at LAQUA (Laboratory of Aquaculture at the Veterinary School of UFMG). 180 golden lineage mollusks from the laboratory were used with ornamental fish and raised with fish food containing 35% crude protein (Guabi® Nutrição e

Saúde Animal SA, Sales Oliveira, SP, Brazil) with an average weight of  $0.60 \pm 0, 23$  g divided into six treatments with three repetitions of 10 individuals for each 15-liter tank in a water recirculation system with filtration by floating media by MBBR aeration (Nanoplastic®, São Paulo, SP, Brazil). The treatments were: a control fed once a day with 0.53 g of commercial feed extruded for marine shrimp containing 35% crude protein (Guabi® Nutrição e Saúde Animal SA, Sales Oliveira, SP, Brazil) without the addition of pigments and in the others, synthetic astaxanthin Carophyll Pink 10% (DSM® - Heerlen, Netherlands) was used in the proportions of 25, 50, 100, 200 and 400 mg/kg of feed for 60 consecutive days.

The following variables were measured for the composition of the zootechnical study indexes: the initial weight, at 30 and 60 days, obtained with a precision scale 0.01 g, the length, and height of the shell were measured on the same days as the previous parameter with a digital caliper with a precision of 1 mm as shown in Figure 1, and the quantification of the feed consumed by the animals.

The parameters of water quality, temperature, pH, salinity, and dissolved oxygen (DO) were measured with a multi-parameter COMBO pH and EC probe and OD meter HI 9146 (Hanna Instruments®, Woonsocket, Rhode Island, USA), respectively. The concentrations of Total Ammonia (TA-N), Nitrite (NO<sub>2</sub>-N), were monitored according to the methodology described by UNESCO (1983). For alkalinity, the APHA method (1998) was used.

**Figure 1** - Measurement points in *Pomacea diffusa*



Source: Research data.

The zootechnical indexes were calculated based on the following equations:

Specific growth rate (SGR) - relative to the daily animals' growth, in percentage values. For its calculation, the following expression (1) is used:

$$\text{SGR (\% day)} = \frac{\ln(\text{final weight (g)}) - \ln(\text{initial weight (g)})}{(\text{time (days)}) \times 100}$$

Apparent feed conversion (AFC) - equivalent to the amount of feed needed for the animal to gain 1 kg of live weight (2):

$$\text{AFC} = \frac{(\text{feed consumed (g)})}{(\text{final weight (g)} - \text{initial weight (g)})}$$

Feed efficiency (FE) - constitutes the average weight gain per mollusk in the group, divided by the average feed consumption per individual. It demonstrates the efficiency that the animal had to convert the feed consumed into live weight (3):

$$FE (\%) = (\text{mass gain (g)}) / (\text{amount of feed ingested (g)}) \times 100$$

Weight gain (WG) – it is the animal’s final weight minus the animal’s initial weight. This calculation is obtained by the following formula (4):

$$WG = \text{final weight (g)} - \text{initial weight (g)}$$

Height gain (HG) – it is the animal’s final height minus the initial height. This calculation is obtained by the following formula (5):

$$HG = \text{final height (mm)} - \text{initial height (mm)}$$

Length gain (LG) is the animal’s final height minus the initial height. This calculation is obtained by the following formula (6):

$$LG = \text{final length (mm)} - \text{initial length (mm)}$$

Relative condition factor (Kn) – it indirectly measures the animal’s physiological state concerning stored energy reserves, such as liver glycogen and body fat, practically indicating whether the animal is fat or thin according to the regression curve of the population average (LE CREN, 1951). For its determination, the following formula (7) was used:

$$Kn = \text{observed weight (g)} \div \text{expected weight (g)}$$

The concentration of total carotenoids based on astaxanthin (C-AST) - After slaughtering by crionarcose, samples of the muscles of an animal by treatment were macerated in porcelain gral with a pistil until a fine paste was formed. A portion of one gram of weight was separated in a test tube for extraction of the carotenoids in acetone and then filtered on filter paper, leaving it to be protected from light until measurement. This process was repeated twice more. The extracts were corrected in volume until the solution obtained a 5-mL sample for analysis. The amount of astaxanthin in the gastropod muscles was determined by UV spectrophotometry in the Libra S22 device (Biochrom®, Cambridge, the United Kingdom) based

on the peak of astaxanthin at 480 nm as the main indicator. The value was calculated according to Rodriguez-Amaya (2001) and Rodriguez-Amaya and Kimura (2004), as in expression (8).

$$C\text{-AST} = (\text{ABS} \times \text{DF} \times \text{V}) / (\text{AC} \times \text{CO})$$

Where:

AC = Astaxanthin A1% 1cm = 2100 absorption coefficient

ABS = Absorbance of astaxanthin in the acetone solution at 480 nm

DF = Dilution factor

V = Sample volume in g

CO = optical bucket length = 1 cm

The statistical analysis was performed initially with the Shapiro-Wilk normality test. Parametric variables were analyzed using the Duncan test and non-parametric variables using the Kruskal-Wallis test, both at a significance level of 5%. The statistical program chosen was INFOSTAT version 2017 (open access version).

### 3 Results and Discussion

The water quality parameters measured in the experiment remained unchanged ( $P > 0.05$ ) among the treatments by the Duncan test as the recirculation system kept the same water and the filtration system was efficient to maintain stability in all the treatments. The mean values were: temperature  $31.5 \pm 1.06$  °C, pH  $6.84 \pm 0.42$ , dissolved oxygen  $6.24 \pm 0.56$  mg/L, salinity  $0.38 \pm 0.07$ , alkalinity  $53.33 \pm 4.88$  mg/L  $\text{CaCO}_3$ , ammonia  $0.58 \pm 0.29$  mg/L, nitrite  $0.26 \pm 0.11$  mg/L. The animals’ survival rate was 100% in all the treatments.

The zootechnical indexes did not show significant differences among the treatments ( $P > 0.05$ ). The results are shown in Table 1. These results demonstrate that the supplementation of synthetic astaxanthin in the diet at the tested levels did not alter the mollusks’ development (Figure 2). SANTOS *et al.* (1996) stated that feeding *Pomacea sordida* with bulky foods such as lettuce *Lactuca* sp. and rami *Boehmeria nivea* obtained a 6:1 feed conversion and food acceptance may vary according to the mollusk’ age.

**Table 1** - Zootechnical indices measured in different treatments with the addition of synthetic astaxanthin in the feeding of golden apple snail *Pomacea diffusa*. There were no significant differences among the treatments ( $P > 0.05$ ) by the Duncan test

Diets	Zootechnical index						
	AST mg/kg	SGR (%)	AFC	FE (%)	WG (g)	HG (mm)	LG (mm)
0		$3.31 \pm 0.19$	$0.85 \pm 0.13$	$119.6 \pm 18.4$	$3.83 \pm 0.59$	$4.11 \pm 0.21$	$7.55 \pm 0.97$
25		$3.37 \pm 0.44$	$0.83 \pm 0.14$	$122.0 \pm 19.7$	$3.89 \pm 0.63$	$4.38 \pm 1.37$	$8.21 \pm 1.22$
50		$3.34 \pm 0.16$	$0.85 \pm 0.19$	$121.6 \pm 24.2$	$3.9 \pm 0.77$	$4.81 \pm 0.35$	$6.67 \pm 2.01$
100		$3.44 \pm 0.14$	$0.79 \pm 0.03$	$127.2 \pm 4.4$	$4.07 \pm 0.14$	$4.96 \pm 0.29$	$8.3 \pm 0.44$
200		$3.5 \pm 0.48$	$0.77 \pm 0.17$	$133.2 \pm 27.3$	$4.26 \pm 0.87$	$5.24 \pm 0.77$	$8.38 \pm 1.4$
400		$3.52 \pm 0.13$	$0.74 \pm 0.09$	$136.6 \pm 15.8$	$4.37 \pm 0.51$	$5.4 \pm 0.07$	$8.8 \pm 0.48$

Source: Research data.

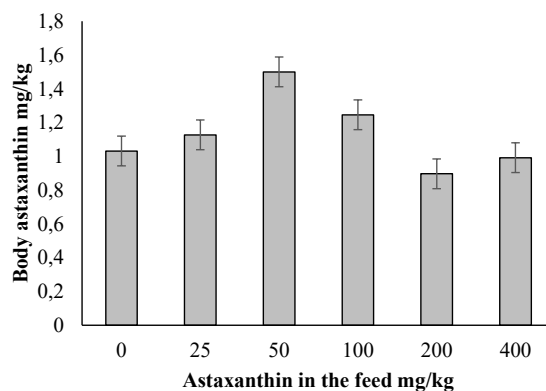
Compared to the present study, Souza Júnior *et al.* (2013) working with *Pomacea lineata* and *P. diffusa* (at the time identified as *Pomacea bridgesii*) fed with shrimp ration (35% crude protein), reported that the animals presented lower and very similar feed conversion rates, therefore, more efficient than bulky diets. In the item Specific growth rate, the present study presented values higher than the work previously mentioned, in which the values were between 1.65 and 1.77% per day. Morrison and Hay (2011) observed a lower percentage of growth of *P. diffusa* about the consumption of *Utricularia* sp. and *Bacopa caroliniana* than other species of the genera *Pomacea*, *Pomacea insularum*, *Pomacea caniculata*, *Pomacea paludosa* and *Pomacea* sp. (at the time identified as *Pomacea haustum*). It was also detected low consumption in general of bulky diets with these and other plants consumed by *P. diffusa* concerning the other species. This may be an indication of a less herbivorous habit in this species, associated to the fact that the good conversion of commercial feed is recommended to use concentrated diets in the creation of this gastropod.

Prabhakaran *et al.* (2019) observed that including at least 0.5% of fish meal in the baits for *Pomacea maculata* increases its attractivity in relation to rice baits. Ramnarine (2004) tested different protein levels in the *Pomacea Urceus* diet and observed that at the level of 20% crude protein (CP) containing the highest amount of animal protein (casein 11.40%, gelatin 2.22%) was better than the control containing 15% CP (casein 6.30%, gelatin 1.26%), which is an indication of the protein demanded by *Pomacea*, which has omnivorous-herbivorous characteristics.

The astaxanthin level in the diets did not significantly change the astaxanthin levels in the mollusks' muscles ( $P > 0.05$ ) (Figure 2). This result has demonstrated that the muscle accumulation capacity of this pigment in these mollusks in the studied life stage is limited and did not change even with supplementation. The pigment levels in the muscle were very low with the supplemented amount, and the pigment may have been directed to other tissues or eliminated in the feces.

Tsushima *et al.* (1997) studying wild *P. caniculata* found  $\beta$ -carotene,  $\beta$ -cryptoxanthin, lutein, and zeaxanthin in the hepatopancreas and muscles, with a greater amount of  $\beta$ -carotene ( $39.1 \pm 1.10\%$ ) in the hepatopancreas and zeaxanthin in the muscle ( $40.8 \pm 3.25\%$ ), which is the main pigment responsible for the yellowish color in albino animals. In gonads ( $61.0 \pm 3.57\%$ ), eggs ( $75.0 \pm 4.56\%$ ) and larvae ( $79.0 \pm 2.46\%$ ) the main pigment was astaxanthin.

**Figure 2** - Body concentration of astaxanthin in *Pomacea diffusa* after 60 days of feeding with food containing different levels of synthetic astaxanthin. There were no significant differences by the Duncan test at 5% probability



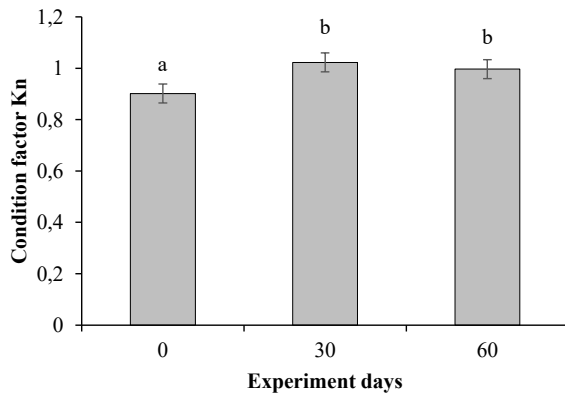
Source: Research data.

*Pomacea* can accumulate these pigments and convert them to astaxanthin only during the reproductive period as a way of protecting eggs and larval forms, as described by Yang *et al.* (2016). Still, in the mentioned study, the greatest accumulation of astaxanthin (when added to the diet) was observed in the *Pomacea*'s hepatopancreas.

In the study of Nurjanah *et al.* (2019), the accumulation of  $313.48 \pm 19.73$  mg/L of astaxanthin was observed in the eggs of *Pomacea caniculata*, which according to the authors is high compared to other species such as chinook salmon (*Oncorhynchus tshawytscha*). According to Dreon *et al.* (2004), the carotenoids of *Pomacea* eggs with proteins form a complex called ovorubin which is responsible for the protection against microsomal oxidation. Due to the properties of these eggs, studies have been directed to their use in fish feeding to enhance color in fancy carp (*Cyprinus carpio*) (BOONYAPAKDEE *et al.*, 2015), parrots as an antioxidant and feather coloring (YANG *et al.*, 2016), also to improve egg quality in Arabic chicken (NUSANTORO *et al.*, 2020).

For the relative condition factor (Kn) expressed in Figure 3, it was verified that the animals of all treatments obtained significant gains with feeding, observing the fact 30 and 60 days after administration of this diet in replacement of the pre-experimental diet made with commercial freshwater fish ration. According to Tamburi and Martin (2016), the condition factor in *Pomacea* reflects the amount of body reserve available in relation to the population and varies according to the availability and quality of the food provided. Thus, it is considered that the food that presented low feed conversion (below 1:1) and the condition factor above the initial value indicate that the food supplied fulfilled its nutritional function efficiently. Therefore, it is also possible to reduce the emission of effluents since the amount of unused food will also be reduced. Such fact for aquariums and aquaculture systems is very interesting, as the animal can use leftover feed from other animals and reduce the load of pollutants in the system, being an alternative species for polyculture systems.

**Figure 3** - Condition factor (Kn) of *Pomacea diffusa* fed with commercial shrimp feed at the beginning (0) with 30 and 60 days of cultivation. Different letters above the data show significant differences ( $P < 0.05$ ) by the Kruskal-Wallis non-parametric test



Source: Research data

In the present work, it can be seen that *P. diffusa* frequently fed on the periphery that grew in the cultivation tank, corroborating Horgan *et al.* (2014a) on Ampullariidae. Thus, it is believed that this habit is one of the factors responsible for low feed conversion and reducing the need for food for its cultivation. The low food interest in plants (MORRISON; HAY 2011) is also a positive point for the species as this prevents it from harming planted aquariums.

This is an indication that this feed satisfactorily meets the needs of these mollusks at this stage of growth concerning that provided previously. This information can help in choosing food for the species cultivation since there are no specific rations available on the market.

#### 4 Conclusion

The marine shrimp diet had a good result in feeding *P. diffusa* in captivity, what can contribute to its commercial cultivation as an ornamental organism and reduce the need for fish food, thus lowering the food conversion and enabling food with reduced cost concerning the ornamental fish ration and avoiding the waste generation. However, dietary supplementation with synthetic astaxanthin does not result in a significant increase in the concentration of this pigment in the *P. diffusa* muscle. Apparently, this mollusk accumulates other pigments in these tissues. Future studies will be able to determine its type and more efficient forms of supplementation.

#### Acknowledgments

Capes for granting scholarships to the students involved. POLI-NUTRI for the supply of pigment for the tests. NANOPLASTIC for the supply of filter media. Kleber C. Miranda-Filho is a research fellow of CNPq (302286/2018-7).

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