Journal of Animal and Veterinary Advances 4 (7): 654-658, 2005

Effect of Various Sources of Carotenoids on Survival and Growth of Goldfish (*Carassius auratus*) Larvae and Juveniles

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Abstract: Two separate trials were performed to evaluate the effects of adding various sources of carotenoids on survival and growth of goldfish (*Carassius auratus*) larvae and juveniles. In the first trial (Trial A), larvae (initial body weight: 3.4±0.7 mg) were fed four microparticulate diets (MP) from first feeding to day 28. Diets were formulated to contain a constant level of pigments (45mg kg^{-1}) from different sources of carotenoids (*Chlorella vulgaris*, *Spirulina platensis* and synthetic pigment astaxantin). All treatments were tested in triplicate as well as a fasted control group. In the second growth trial (Trial B), a fifth diet, containing *Haematococus pluvialis* biomass (45 mg kg^{-1}; diet Hp), was also tested. Diets were fed to homogenous groups of 45 juvenile *C. auratus* (1.7±0.1g), in triplicate. Water temperature was maintained at 25±1°C during the 12 weeks long trial. High survival rates were recorded in both studies. Furthermore, there were no significant differences among treatments, for either survival or growth. Our results demonstrate that survival and growth of goldfish larvae and juvenile was not affected by the inclusion of 45 mg carotenoids kg^{-1} of diet.

Key words: Fish larvae, pigments, carotenoids, growth, survival, *Carassius auratus*

INTRODUCTION

Life in the sea and fresh water begins with microalgae, which are a diverse group yielding an almost unlimited range of chemicals: carotenoids, phycobilins, fatty acids, polysaccharides, vitamins, sterols and other biologically active compounds. Besides pigments, microalgae also contain other essential nutrients which determine the quality, survival, growth and resistance to disease of cultured species. Many compounds, such as betaine, inosine 5-monophosphate and amino acids have been shown to stimulate feeding in fish[1-4] and are natural constituents of phytoplankton. Carotenoids, lipid soluble pigments, may be carried through the food chain from primary producers, namely microalgae, because animals cannot synthesize them *de novo*[5]. Carotenoids are the most widespread pigments in nature, as they occur in bacteria, yeast, mold, algae, green plants and in many animals. A positive metabolic role of carotenoids in the nutrition of larval fish and survival of young fry was discussed by Shahidi *et al.*[6] and Lazo *et al.*[7]. Numerous reports show that carotenogenic microalgae appear as suitable sources of carotenoids in fish feeds[8-15] for pigmentation purposes but less work is involved in evaluating the potential benefits of dietary carotenoids for growth and survival of aquatic species.

The aim of this work was to determine the effect of supplementation of diets with carotenoids (natural and synthetic) on growth and survival rates of ornamental goldfish (*Carassius auratus*) larvae and juveniles.

MATERIAL AND METHODS

Fish, diets, microalgae, facilities and feeding protocol: Two separate growth trials were performed to evaluate the effects of adding various sources of carotenoids in survival and growth of *C. auratus*. In the first trial (Trial A), larvae of goldfish (red Oranda variety) were obtained from broodstock in our laboratory by forced spawning induced following intraperitoneal injections of a crude extract of carp hypophysis[16]. Larvae (mean initial length: 6.4±0.8 mm) were randomly distributed in 15 plastic tanks (5L; 200 larvae T^{-1}) in a closed water recirculated system[17]. Four microparticulate diets (MP) were formulated to contain a constant level of pigments (45 mg kg^{-1}) from different sources of carotenoids. Larvae

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Table 1: Composition of the experimental diets (dry weight)

	Diet (g kg^{-1} dry weight)			
Ingredients	C	As	Sp	Cv
[1]Microparticulate diet	1000.0	999.40	992.5	988.8
[2]Synthetic asthaxanthin	0.0	0.56	0.0	0.0
[3]*Spirulina platensis*	0.0	0.00	7.5	0.0
[4]*Chlorella vulgaris*	0.0	0.00	0.0	11.2
*Proximate analysis				
Dry mater (%)	91.6	91.40	91.8	92.0
Crude protein (%DM)	52.1	52.20	52.0	51.8
Lipid (%DM)	7.6	7.70	7.5	7.4
Energy (kJ g^{-1} DM)	19.6	19.30	19.2	19.0
Ash (%DM)	15.7	15.90	15.9	16.0

[1]Microparticulate diet for cyprinid larvae based on yeast and fish protein hydrolysate, [2]Pigments: 8% DM, [3]Pigments: 0.6% DM; Protein: 25% DM, [4]Pigments: 0,4% DM; Protein: 65% DM
*Values are means of two determinations

were subjected to one of the following dietary regimes from first feeding (day 2 post-hatch) to day 28: (1) an MP containing algae *Chlorella vulgaris* biomass (diet Cv), (2) an MP containing *Spirulina platensis* biomass (diet Sp), (3) an MP containing synthetic pigment astaxantin (diet As) and (4) the microparticulate diet without carotenoid supplementation (diet C). The proximate composition of experimental diets is presented in Table 1. The MP control (diet C), based in yeast and fish protein hydrolysate, was routinely used in previous studies with ornamental cyprinid larvae in our experimental fish farm[16]. An automatic feed dispenser[18] was used to feed the experimental diets with particles between 100 to 200 μm during the 1st week, between 200 to 400 μm during the 2nd week and between 400 to 630 μm during the 3rd and 4th weeks. All treatments were carried out in duplicate as well as a fasted control group. Photoperiod was set on a 12:12 h light/dark cycle throughout the study. The trial lasted 4 weeks and water temperature was kept at 25±1°C with a flow rate of 0.4 L min^{-1} tank^{-1} during the 1st week and of 0.8 L min.$^{-1}$ from the 2nd week onwards. Dissolved oxygen was kept above 6.0 mg L^{-1} and total ammonia-nitrogen and nitrite-nitrogen levels were monitored twice weekly using photometric methods[19]. Tanks were cleaned daily and dead animals removed and recorded. At days 7th, 14th, 21st and 28th samples of 10 larvae were taken from each tank, anaesthetized and the total length and weight recorded. At the end of the trial the larvae from each tank were fasted for one day, counted and total biomass was weighed.

The second trial (Trial B), was performed with juvenile goldfish (1.5 month old) raised in UTAD experimental fish farm. Triplicate homogenous groups of 45 fish (initial mean body weight: 1.7±0.1g) were randomly distributed in 15 rectangular plastic tanks (100L capacity) with a flow rate of 0.8 L min^{-1}. Water temperature was maintained at 25±1°C and artificial photoperiod (12L: 12D) was used during the 12 weeks long trial. Five isonitrogenous diets were formulated to contain a constant level of pigments (45 mg kg^{-1}) from different sources of carotenoids: biomass from algae *Chlorella vulgaris* (diet Cv), *Spirulina platensis* (diet Sp), *Haematococus pluvialis* (diet Hp), synthetic pigment astaxantin (diet As) and a control diet without added pigments (diet C). The microalgal biomass and synthetic pigments were finely ground (<800 μm) and mixed with a basal diet of commercial trout feed (Protein: 52% DM; Lipids: 17% DM) for 15 min and pelleted in a California Pellet Miller (CPM) to produce compressed pellets of 1 mm size and stored in airtight containers prior to use. Fish were fed by hand, twice a day (09.00 and 18.00 h), to apparent satiety and daily feed intake was recorded.

The microalgae used in these diets, *C. vulgaris*, *H. pluviallis* and cyanobacteria *S. maxima* were cultivated in raceways ponds, polyethylene bags and alveolar photobioreactors, respectively, using appropriate mediums[20,15].

Experiments were conducted according to the European Council Directive 86/609/EEC regarding the protection of animals used for experimental and other scientific purposes.

Analytical methods: Chemical composition analysis of the diets was made using the following procedures: dry matter, by drying at 105°C for 24 h; ash, by combustion at 550°C for 12 h; crude protein (micro-Kjeldahl; N×6.25); fat by dichloromethane extraction (Soxhlet) and gross energy in an adiabatic bomb calorimeter (IKA).

Statistical analysis: Data are presented as means±standard deviation. Data from each trial was analysed independently. Data on survival and weight of larvae were transformed (angular and logarithmic, respectively). To test differences between dietary treatments, data were subjected to one-way analysis of variance and, when appropriate, means were compared with the Newman-Keuls multiple range test. Statistical significance was tested at a 0.05 probability level. All statistical tests were performed using the Statgraphics (7.0) statistical package.

RESULTS

At the end of the larvae trial high survival rates were observed in all treatments (Table 2) with the exception of the unfed control group with a total mortality recorded at day 16th. There were no significant differences among

Table 2: Mean values of survival, total length and weight of larvae at the end of the trial (day 28) (Trial A)

	Diet			
Parameters*	C	As	Sp	Cv
Survival (%)	98.9±00.1[a]	97.5±00.1[a]	98.6±00.1[a]	97.2±00.2[a]
Mean initial weight (mg)	3.4±00.7	3.4±00.7	3.4±00.7	3.4±00.7
D_{07}	17.0±04.6	17.6±04.6	18.3±05.1	17.6±05.2
D_{14}	47.7±11.5	39.4±05.7	39.3±07.2	41.5±09.4
D_{21}	126.9±30.2	99.5±30.8	109.5±032.1	113.5±34.1
D_{28}	245.1±22.0[a]	223.0±04.7[a]	221.2±6.2[a]	227.3±10.0[a]
Mean initial length (mm)	6.4±00.8	6.4±00.8	6.4±00.8	6.4±00.8
Mean final length (mm)	22.6±01.2[a]	20.6±00.7[a]	21.8±00.3[a]	22.1±00.3[a]
Biomass[1] (mg)	242.4[a]	217.4[a]	218.1[a]	220.9[a]

*Mean±standard error (n=30). In the same line, values with different superscript letter are significantly different ($p<0.05$)
[1]Theoretical final biomass WxS: mean final weight x survival

Table 3: Growth performance and diet utilization by juvenile goldfish [1] (Trial B)

	Diet				
Parameters	C	Sp	Hp	Cv	As
Mean initial weight (g)	1.6±0.10	1.7±0.1	1.7±0.1	1.7±0.1	1.7±0.10
Mean final weight (g)	4.3±0.03	4.4±0.03	4.4±0.1	4.5±0.1	4.3±0.10
[2]Weight gain (%)	173.6±10.3	161.3±7.3	159.4±10.9	167.4±11.6	152.9±5.90
[3]Specific growth rate (%)	1.2±0.04	1.1±0.03	1.1±0.05	1.2±0.05	1.1±0.03
[4]Feed conversion ratio	2.1±0.04	2.1±0.04	2.1±0.06	2.0±0.1	2.1±0.10
[5]Feed efficiency (%)	48.3±0.01	48.6±0.01	47.4±0.01	50.1±0.02	47.2±0.01
Mortality (%)	2.2	0	0	2.2	0

[1]Mean±standard error. In the same line, values with different superscript letters are significantly different ($p<0.05$), [2]Weight gain (%): Final body weight-initial body weight)/ initial body weight 100, [3]Specific growth rate (%): Ln final weight-Ln initial weight 100/time (days), [4]Feed conversion ratio: Feed intake DM/ weight gain, [5] Feed efficiency (%): 1/ feed conversion ratio

treatments ($p>0.05$) in respect of total length and total weight of larvae, although a slight increase in both parameters was noticed in treatment C. The product weight plus survival (WxS) was calculated as a global criterion that allows the comparison of the final biomass of the different larvae groups. The best product value was recorded in the larvae fed diet C but this value was not significantly higher ($p>0.05$) than those of the other larvae groups.

At the end of the trial high survival rates were observed in all treatments. There were no significant differences among treatments as regards mean final weight and weight gain ($p>0.05$). The values for specific growth and feed conversion rates were homogeneous and no significantly differences ($p>0.05$) were observed between treatments (Table 3: Trial B).

DISCUSSION

Astaxanthin (3,3'-dihydroxy-4,4'-diketo-β,β-carotene) and canthaxanthin (4,4'-diketo-β,β-carotene) are widely used as dietary supplements in diets for salmonids as a method for inducing the typical pink colour of their flesh[21-23]. Given the high costs of synthetic pigments, efforts have been deployed to evaluate the potential of some natural compounds, like the pigments obtained from the red yeast *Phaffia rhodozyma*[24], the marine bacteria *Agrobacterium aurantiacum*[25], the green algae *Haematococcus pluvialis*[26,27], *Chlorella zofingiensis*[28] and *Chlorella vulgaris*[29] as dietary carotenoid sources.

Besides the effectiveness of carotenoids in pigmentation of fish skin a positive effect in the nutrition of larval fish and survival rates of young fry was discussed[6,29]. The addition of alga to the rearing tanks of marine fish larvae (green water culture) has been shown to enhance growth and survival as well as the quality of the fry[30,7]. One of the beneficial effects attributed to adding algae is an increase in ingestion rates of food by marine fish larvae[31]. In addition, the presence of algae in rearing tanks of European sea bass larvae has been shown to increase digestive enzyme secretion[32]. The carotenoids level of 45 mg kg^{-1} diet used in this work for survival and growth purposes, was due to the highest pigmentation attained with this concentration[15,33].

The results obtained with goldfish larvae are in accordance with previous trials at UTAD experimental fish farm[16] and the larvae grew within the range normally found in the literature data for this specie (245 mg; 28 days). The high nutritional value of the microparticulate diet C for rearing this particular goldfish larvae variety seems to be one of the major observations in this study.

Despite the long duration of the second trial (Trial B), the results evidenced not sound effect of dietary carotenoids upon the growth, or the survival, of juvenile goldfish. In fact the fish grew within the range normally found in the literature data and neither growth nor feed efficiency was significantly affected by inclusion of carotenoids in dietary treatments. No mortality was associated to the experimental treatments and it is worth mentioning that the rearing conditions, namely water parameters and UV-sterilization, seem to be very effective in the rearing of the fish. The inclusion of 45 mg carotenoids in the diet, besides this effectiveness on skin pigmentation (visual observation) and proved in other works, was not sufficient to induce any differences in growth and survival of larvae and juvenile goldfish, independently of natural or synthetic source.

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