Short Communication

Effects of the addition of a marigold extract to diets fed to channel catfish (*Ictalurus punctatus*) on growth parameters

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The expansion of aquaculture in the last 10 years has made it an important source of protein worldwide. However, increased production, and the culture intensification it carries, results in higher risk of infectious disease due to poor water quality and high stocking densities. Disease has several negative effects on fish, which include reduced feed efficiency, impaired growth and death, representing a direct loss of investment in feed, labour and other inputs.

Disease in fish has been traditionally opposed with chemotherapeutants; however, the number of chemotherapeutants available for use in aquaculture has become more restricted as new drugs become available (Alderman, 2002), due to the antibiotic resistance they may induce when used in fish (Belem-Costa and Cyrino, 2006). This has resulted in the search of non-toxic alternatives to ensure fish health during culture, such as plant extracts to be used as appetite stimulators and growth promoters, antimicrobial, anti-parasitic, antioxidant agents, and immunostimulants in fish (Syahidah *et al*., 2015). Furthermore, animal extracts have also been successfully used as fungicides, like those obtained from the sea cucumber, *Holothuria leucospilota* (Farjami *et al*., 2014).

Natural carotenoids have traditionally been used as dietary colouring agents; however, carotenoids have been found to carry out essential

roles in nearly all biological systems, as well as having antioxidant (Brambilla *et al*., 2009) and analgesic properties (Bashir and Gilani, 2008).

Some carotenoids elevate humoral innate factors such as complement and lysozyme activity, as well as non-specific cellular factors like phagocytes and nonspecific cytotoxicity (Amar *et al*., 2001), enhancing the innate immunity of fish (Amar *et al*., 2004), and maintaining fish health and disease resistance (Amar *et al*., 2012).

Marigold (*Tagetes erecta* L.) is a plant native to Mexico whose flowers accumulate carotenoids (Del Villar-Martinez *et al*., 2007). Natural carotenoids extracted from marigold have been used in aquaculture to help in the survival of fish juvenile stages by strengthening their immunological system (Amar *et al*., 2004), thus reducing mortality rates (Amar *et al*., 2012).

The effect of carotenoids in fish health may exert a positive effect on fish growth indices; however, these effects on growth parameters have not been studied in channel catfish (*Ictalurus punctatus*), which is the most cultured fish species in north-eastern Mexico, and one of the most cultured on the southern United States. Consequently, the aim of this study was to investigate if there were significant differences in growth among channel catfish juveniles when fed diets containing various carotenoid doses from a natural source.

In this study, twelve 50-L tanks filled up to a 46 L volume with circulating and aerated well water were utilized. Four hundred healthy juvenile channel catfish (*I. punctatus*) with an average initial size range of 6.06±0.06 to 6.13±0.17cm, and an average initial weight range of 4.32±0.33 to 4.53±0.24 g were used. Fish were acclimated for two weeks to the experimental conditions before the beginning of the trial (Rábago-Castro *et al*., 2006). Tanks were divided into four groups (one control and three experimental groups). Three tanks (replicates) belonging to each group were randomly distributed in the bioassay room to avoid anticipatory stress. Twenty-six fish were randomly allocated to each tank; water flow was kept at 3.33 ml/sec, and was increased as the tank biomass increased (Rábago-Castro *et al*., 2006). Fish were fed *ad libitum* without waste twice a day (0900 and 1700) for 13 weeks (Sanchez *et al*., 1996). Feed consumption was monitored and registered for each tank.

The first group of fish were fed with a basal diet (control) consisting of a commercial catfish feed (Purina®) with a proximal analysis of 35% protein, humidity 12% maximum (max), fat 8% minimum (min), raw fibre 4% max, ashes 10% max, calcium 0.6% min, and phosphorus 1% min); while the three experimental groups (ME50, ME100, ME200) were fed with the basal diet supplemented with 50, 100 and 200 mg/kg of an ethanol extracted marigold extract (*T. erecta*, containing 70% lutein and 30% astaxanthin), respectively. The marigold extract was fixed to the

treatment feed by aspersion (Ong and Tee, 1992).

The trial was run for 13 weeks. All fish were anesthetized with benzocaine (30 µg/L), measured (fork length to the nearest mm) and weighed in an analytical balance at the beginning and end of the trial, and in weeks 3, 7 and 10. Fish growth was measured according to Sanchez *et al*. (1996), and included mean weight (Mw, g); specific growth rate (SGR, %/d) calculated as SGR= [(Log_e MwT-log_e Mwt)/(T-t)]×100, were (log_e)= natural logarithm, (MwT) = final weight of the interval of interest, (Mwt)=initial mean weight of the interest interval; (T-t)=number of days during for which growth was evaluated; condition index (K), calculated as K= 100× (weight/length^3); feed conversion index (FCI), calculated as FCI= (ΣWFI/N)/(MwT-Mwt), where ΣWFI is the sum of feed consumed during the interval of interest, MwT=final mean weight for an interval of interest, Mwt= initial mean weight for an interval of interest, and N=number of fish; feed consumption (FC: expressed as % of body weight) was calculated as ((ΣWFI ×100) /(Mwt×N) /wk), where wk is the time interval given in weeks, and ΣWFI, Mwt, and N are defined as in the equation for FCI. At the end of the trial, fish from all treatment groups were sampled and euthanized with an overdose of benzocaine; the level of catfish fresh muscle pigmentation was visually assessed immediately post slaughter by using Roche Salmo Fan TM (Hoffman-La Roche, Basel, Switzerland) in a light cabinet (Johnston *et al*., 2000).

For each treatment, growth curves for catfish were fitted based on mean weight over time using nonlinear least squares and the quasi-Newton algorithm. Afterwards, analysis of residual sum of squares (ARSS) was performed to evaluate differences in the growth curves between treatments (Chen *et al*., 1992).

SGR (%/d), feed consumption (% of body weight) and FCI were calculated for each tank at intervals of 0-3, 3-7, 7-10, 10-13 and 0-13 weeks, whereas K was calculated at 0, 3, 7, 10 and 13 weeks. Afterwards, differences between the four treatments (control, ME50, ME100, ME200) were evaluated with a one-way ANOVA (Zar, 1999).

Water quality parameters were within the range reported for this species during the trial. No significant differences (p>0.05) were found in the mean weight of fish at the beginning of the trial among the control (4.42±0.33 g) and experimental ME50 (4.32±0.33 g), ME100 (4.38±0.11 g) and ME200 (4.53±0.25 g) groups. Furthermore, no significant differences (p>0.05) were detected in the weight growth curves of control and experimental groups during the first ten weeks. At the end of the experiment (week 13) a weight difference was observed between control fish (35.42±1.39 g) and fish fed with the marigold extract, ME50 (40.99±2.12 g), ME100 (37.54±4.47 g), ME200 (42.92±5.98 g) (Fig. 1). The mean weight of fish in ME200 was 7.5 g

(21%) greater than the final weight of control fish; however, no significant differences were detected (p>0.05).

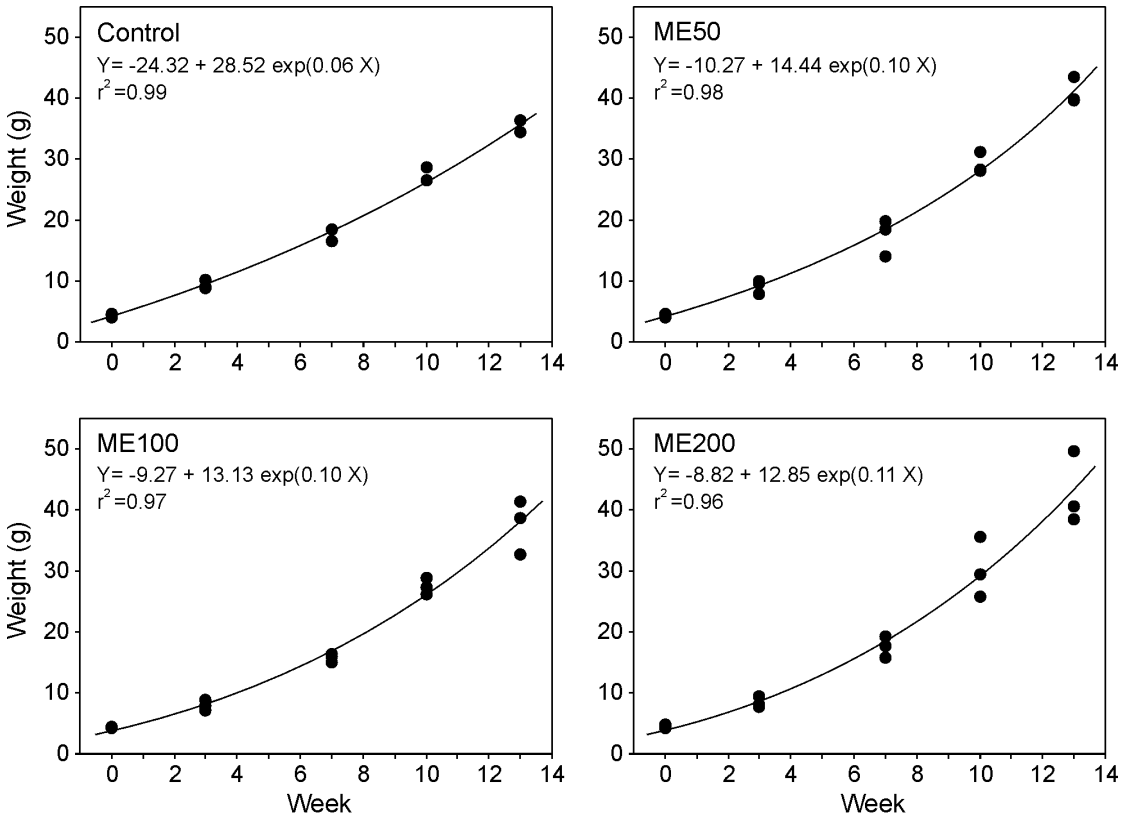

Figure 1: Growth in mean weight for channel catfish fed a basal diet (control) and three experimental diets supplemented with marigold extract at a rate of 50 mg/kg (ME50), 100 mg/kg (ME100) and 200 mg/km (ME200). Exponential equations of the form $Y = a + b\exp^{cX}$ were fitted in all cases.

On the other hand, growth curves for weight exhibited significant differences between treatments (Fig. 1). Catfish fed with 200-mg/kg marigold extract supplementary diet (ME200) showed significantly the fastest growth in weight, followed by those fed the ME50 diet. Significantly lowest growth was observed in fish fed the control diet, which did not differ from the ME100 treatment.

No significant differences (p>0.05) in SGR, K, feed consumption (% of body weight), and FCI were detected.

Results from growth and feed utilization data indicate that the marigold extract had no influence on feed consumption and weight in juvenile channel catfish, concurrent with other studies, which have found no effects of carotenoids on fish growth (Gomes *et al*., 2002; Amar *et al*., 2012), and where diet supplementation did not enhance growth (Buyukcapar *et al*., 2007). While no significant differences in weight were observed, fish fed with the marigold-supplemented diets grew faster than controls, resulting in an overall higher

SGR over control fish; however, the differences were not significant. This is similar to what has been observed in other studies in different species (Amar *et al*., 2004; Diler *et al*., 2005). In the same way, condition index (K), feed consumption or FCI were not affected by the addition of the marigold extract to the fish feed at the end of growth trial. These results are similar to those observed in salmonids (Amar *et al*., 2001), tilapias (Boonyaratpalin and Unprasert, 1989), or red porgy (Chatzifotis *et al*., 2011). In fact, recent studies suggest that although marigold supplemented in the diet has the least significant impact on growth and body composition in *Barilius bendelisis* (Jha *et al*., 2012). Marigold extracts have a good antioxidant potential (Bashir and Gilani, 2008), however, the addition of carotenoids seems to have no effect on the growth rate or survival in an ornamental dwarf cichlid (Harpaz and Padowicz, 2007). Many of these results are similar to what has been observed in other fish; namely that they grow well, but where no differences in weight gain, feed efficiency, or survival are observed among fish fed various carotenoids (Li *et al*., 2007).

No differences in muscle pigmentation were observed between experimental and control fish. Flesh pigmentation is highly valued in fish such as salmon, trout, and ornamental fish; while in others, such as channel catfish it is highly undesirable (Li *et al*., 2007), reducing the acceptability and marketability of catfish fillets (Liu *et al*., 2012). Marigold extract is rich in lutein, which imparts a yellowish coloration to fish muscle (Li *et al*., 2007); however, in this study, no differences were detected in muscle pigmentation between control and experimental fish fed the marigold-supplemented diet. There are studies that report the yellow colour becomes visible after 11 weeks in fish fed pigment-enhanced diets (Li *et al*., 2011), so this is probably the reason why no differences in muscle pigmentation were detected between the different fish. In summary, the addition of a marigold extracts to the diet of catfish elevated but did not significantly enhance channel catfish weight when compared to control fish. The results clearly indicate that, in terms of weight gain in channel catfish, marigold extract does not have a significant effect on fish growth.

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