The Effect of Dietary Carotenoids of Different Forms: Microemulsified and Non-microemulsified on the Growth Performance, Pigmentation and Hematological Parameters in Hybrid Catfish (Clarias Macrocephalus × Clarias Gariepinus)

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Abstract

We tested the feasibility and effect of including dietary carotenoids of different forms, microemulsified (MY) and non-microemulsified (NMY), in diets for hybrid catfish (Clarias macrocephalus × Clarias gariepinus). The results showed that the abdominal skin and back muscle yellowness (b*) of fish fed with 0.7 kg/t MY was higher than the other treatments (1.0 kg/t NMY and control) (p<0.05) with a score of 8.30 and 16.33 respectively. This group also gave the highest total carotenoid deposited in the muscle of 88.27 mg/kg. Carotenoid addition to the control diet at 1.0 kg/t NMY and 0.7 kg/t MY dosage have no adverse effect on the growth, in fact they helped to improve the body weight gain by 82.98 g and 84.17 g (p<0.05) with an FCR improvement of 13 points and 16 points (p<0.05) respectively. There was no adverse effect on the immune system after feeding carotenoid to the catfish and enhancement was observed in certain immune response indices when compared to the control. The findings in the present study were very significant because it supported that microemulsified yellow carotenoids (with smaller particle size of ~0.25 µm) and when used at lower inclusion rate of 30% could achieve better overall performance when compared to the regular size carotenoids and control due to its enhanced bioavailability.

Keywords: Microemulsified; Bioavailability; Carotenoids; Pigmentation; Hybrid catfish; Growth; Hematological parameters

Introduction

In aquaculture, maintenance of the natural skin pigmentation is of great importance from a commercial point of view, as it has a direct impact on consumer acceptance or rejection [1,2] as well as product market price. A variety of natural and synthetic carotenoids are available to enhance coloration in the flesh of salmonid fish [3] and in the skin of others such as European red porgy, Pagrus pagrus [4]. Both synthetically produced pigments, astaxanthin and canthaxanthin, either alone or in combination, have been efficiently used as dietary additives for muscle pigmentation in salmonids [5]. While synthetic carotenoid pigments are commercially available as feed additives, they are expensive and up-take levels are poor, estimated between 5% and 10% [6]. Moreover, there is increasing consumer awareness about safety of synthetic feed additives. This increases the interest in use of natural carotenoids for some fish and shrimp species of economic interest. Carotenoids, which are lipid-soluble pigments, are divided into two groups: (red) capsanthin and (yellow) xanthophylls. The carotenoids are also vital nutrients for healthy growth, metabolism and reproduction [7]. Since fish, like other animals, are not able to synthesize carotenoids [8,9], they have to obtain carotenoids from dietary sources. Fishes can modify alimentary carotenoids and store them in the integument as droplets of microemulsions have the advantage of presenting the carotenoids in a controlled manner with low particle size (in the range 0.1 to 0.4 µm) and when used at lower inclusion rate of 30% could achieve better overall performance when compared to the regular size carotenoids and control due to its enhanced bioavailability.

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Materials and Methods

Production of carotenoid products

The microemulsified yellow (MY) and non-microemulsified yellow (NMY) (marigold flower extract (Tagetes erecta) contains mainly the natural yellow pigments lutein/lutein esters (>80%) and zeaxanthin (~5%) were produced using Kemini Industries (Asia) pilot production facility. Briefly, silica and wheat pollard were first added into a ribbon mixer. MY or NMY concentrate containing the saponified yellow concentrate was then sprayed into the mixer at a rate of 15Hz with constant mixing at 28rpm for 40 minutes until a homogenous mixture was obtained. The targeted total xanthophyll content for both MY and NMY products is at min 20.5 g/kg. Homogeneity of both the MY and NMY products were assessed by analysing 6 independent bags measured by HPLC following the official AOAC (Association of Analytical Communities) method number 970.64 (1990). For MY product, the average analysed total xanthophyll was 20.58 g/kg ± 0.242 while the average total xanthophyllanalysed for NMY product was 20.62 g/kg ± 0.733.

Particle size analysis and transmission electron microscopy observations of carotenoid products

The particle size analysis of the MY and NMY was carried out using a particle size analyzer (HORIBA SZ-100Z). To observe the morphology, the MY was directly deposited onto carbon film supported by copper grids, stained with a 1% aqueous solution of osmium tetroxide (OsO4) and some sodium sulphate anhydrous were added. The mixture was ground and filtered through glass microfiber filters (GF/A, whatman paper) and rinsed with chloroform to increase the boiling point of the mixture. After mixing and phase separation between diethyl ether and water in separatory funnel, the upper layer was taken and placed in a round bottom flask to evaporate in a rotary evaporator at 35°C. The extract was concentrated and dissolved in benzene. Total carotenoids concentration in the muscle was determined spectrophotometrically in benzene using E (1%, 1 cm) = 2500 at 460 nm for yellow carotenoid.

Haematological assay

Blood was analysed with routine methods used in fish haematology [15,16]. In short, blood was collected from the caudal vein with 1 mL nonheparinized disposable syringes fitted with 0.55 × 25 mm disposable needles. Blood samples (approximately 1 mL/fish) were centrifuged at 300 × g, 25°C for 10 min. A volume of 500 μL of the serum was removed and vortexed with 1 mL of ethanol for 30s, then 2 mL of petroleum ether was added, and the mixture was vortexed for 1 min. The petroleum ether was separated by centrifuging at 300 × g, 25°C for 10 min. Red
and white blood cell count was determined with chamber method using Neubauer's haemocytometer; haemoglobin concentration with cyanmethaemoglobin method [17] and haematocrit in capillary tubes of 75 µL volume, which were centrifuged in a microhaematocrit centrifuge and the haematocrit values were read with a reader. The total protein determination followed method [18].

**Statistical analysis**

Significant differences among treatment groups were tested by one-way analysis of variance (ANOVA) and the comparison of any values was made by Duncan’s multiple range tests. A significance level of p<0.05 was used. The statistical analysis was performed using Statgraphics 5.1.

**Results**

**Particle size analysis and transmission electron microscopy observations of carotenoid products**

The MY carotenoids were found to be approximately 0.25 µm in size, as analyzed by electron microscopy and light-scattering diffraction study (Figure 1). This was a dramatic reduction from the approximately 20 m size measured for NMY. The results also suggested that the surfactant and oil phases used in this study not only influenced the formation of protective colloid responsible for establishing colloidal stability against agglomeration but also facilitating the uptake of carotenoids during the intestinal passage.

**Growth performance and feed utilization**

The effects of carotenoid diets on the growth parameters for the fishes throughout the experimental periods are given in Table 2. All fish grew normally, and no specific signs of disease were observed (Figure 2). No mortality occurred throughout the experiment. Feed intake among treatments showed no significant differences (p<0.05), so that total carotenoids intake was proportional to dietary carotenoid concentration. The results showed that carotenoid addition to the control diet at 1.0 kg/t of NMY and 0.7 kg/t of MY helped to improve body weight gain by 82.98g and 84.17g (p<0.05) with an FCR improvement of 13 points and 16 points (p<0.05) respectively.

![Figure 1: Particle size analysis by transmission electron microscopy and light scattering diffraction study of the (a) non-microemulsified (NMY) and (b) microemulsified yellow carotenoids (MY).](image-url)
However, similar growth performance achieved even when dosage used for MY is at 30% lower than that of NMY (p<0.05).

**Colorimetric and total carotenoids analysis**

Colour intensity of hybrid catfish fed with experimental diets throughout the experimental periods is shown in Table 3. Lightness (L) was not affected by carotenoid supplementation (p<0.05), although the white skin of these groups changed slightly from a white hue to a yellow hue. There were, however, significant (p<0.05) differences in yellow (b') among the treatment groups. The group fed the control diet showed a weak redness and yellowness, which differed significantly from values found for group fed the other diets. Yellow tonality was best in fish fed the diet supplemented with 0.7 kg/t MY followed by 1.0 kg/t NMY and the control (Figure 3). The highest numerical values were obtained for the groups fed with diets containing 0.7 kg/t MY (with a score of 8.30 and 16.33 for abdominal skin and back muscle, respectively). The effect of different experimental diets on the total carotenoids in muscle was also determined (Table 3). Total carotenoids measured in fish fed with 0.7 kg/t MY is the highest compared to the other treatments (p<0.05).

**Haematological assay**

Fishes fed on carotenoid diets exhibited increasing RBCs counts for the fishes fed with 1.0 kg/t NMY and 0.7 kg/t MY, respectively (p<0.05; Table 4). The highest counts of WBC’s were obtained for the 1.0 kg/t NMY treatment (1.28 × 10^5 cell/mL, p<0.05). Dietary carotenoids significantly affected the haemoglobin of fishes (7.19 and 7.23 g/dl for 1.0 kg/t NMY and 0.7 kg/t MY, respectively). Haematocrit percentage was higher for carotenoid fed fish compared to the control fish (p<0.05). Similarly, the total protein measured was also higher for the carotenoid fed fish (p<0.05) and the highest value was observed for 0.7 kg/t MY diet.

**Discussion**

We tested the feasibility and effect of including dietary carotenoids of different forms, microemulsified and non-microemulsified, in diets for hybrid catfish (Clarias macrocephalus × Clarias gariepinus). The data in this study showed neither the growth nor the feed conversion efficiency were affected significantly. As a micronutrient, the effects of carotenoids on fish are discreet and are not easily translated into somatic growth. However, its positive role in the intermediary metabolism of fish [19,20] could enhance nutrient utilization and may ultimately result in improved growth. A few studies have reported enhanced growth with carotenoid supplementation [21] whereas others claim reduced feed conversion [22].

In contrast to growth, addition of dietary carotenoid supplements significantly increased total carotenoid deposited in the catfish muscle and enhanced yellowness in skin and muscle colour. Skin lightness was the only color parameter not influenced positively by carotenoid enhancers, which is not surprising considering the generally negative correlation observed between this color variable and skin carotenoid concentration in other species [23,24]. From this study, bioavailability
The effect of dietary carotenoids of different forms: microemulsified and non-microemulsified on the growth performance, pigmentation and hematological parameters in hybrid catfish (Clarias Macrocephalus × Clarias Gariepinus).


The authors would like to thank Dr Orapint, Department of Aquaculture, Faculty of Fisheries, Kasetsart University for conducting the trial and the valuable comments and suggestions.

References

Natural antioxidants, including carotenoids, are important to animal health. They function to remove harmful free radicals produced through normal cellular activity and thus help to maintain the structural integrity of e.g. immune cells. Immuno-enhancement by dietary carotenoid manipulation may complement, if not offer an alternative to, the use of drugs in aquaculture. In fish, an increasing number of studies have demonstrated the role of various dietary carotenoids in immune responses [29,30]. The results of the present study showed that the exposure of hybrid catfish to carotenoids resulted in higher RBC and WBC counts. The haemoglobin values were also increased as compared with fish fed with the control diet. This increase could be due to the presence of lutein and zeaxanthin in the natural yellow pigment, which can help build the immunity capacity. These results showed the improvement of fish health when fed carotenoid-supplemented diets which increased the ability to fight off infections through the reduction of stress levels. The major functions of WBC are to fight infection; defend the body against foreign organisms and in immune response [31]. Feeding dietary carotenoids significantly (p<0.05) increased the levels of haematocrit and total protein content which contradicts the finding reported by [32] where the haematocrit value in treated fish was lower than that of control fish. In all, the haematological results showed some stimulation in the immune response for the carotenoid-fed fish compared to control fish and these effects could be attained with the microemulsified carotenoid when used at a reduced dosage of 30%.

Table 4: Hematological factors of hybrid catfish fed with experimental diets for 12 weeks.

<table>
<thead>
<tr>
<th>Hematological parameter</th>
<th>Experimental diets</th>
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<tr>
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<td>C</td>
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<tr>
<td>Red blood cell (RBC) (x 10^6 cell/ml)</td>
<td>1.59 ± 0.06^a</td>
</tr>
<tr>
<td>White blood cell (WBC) (x 10^5 cell/ml)</td>
<td>1.20 ± 0.08^a</td>
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<tr>
<td>Hemoglobin (Hb) (g/dl)</td>
<td>6.64 ± 0.13^a</td>
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<tr>
<td>Hematocrit (HCT) (%)</td>
<td>36.00 ± 1.00^a</td>
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<tr>
<td>Total protein (mg/dl)</td>
<td>6.18 ± 0.13^a</td>
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Conclusion
Colour intensity, growth and immune resistance are important quality criteria and market value determinants for hybrid catfishes. The use of natural carotenoid may contribute to an enhanced image and quality of hybrid catfish. The findings as reported in this paper clearly demonstrated that the microemulsified yellow carotenoids when supplemented at a 30% reduced dosage can achieved on par growth performance, enhanced pigmentation and has no adverse effect in the immune response of the catfish when compared to the regular carotenoids and control. In summary, microemulsified yellow carotenoids can provide greater cost saving with its better bioavailability due to its characteristic size of ~0.25 μm, making it the carotenoid of choice in catfish aquaculture for control over pigmentation as well as maintaining fish growth and health.

Acknowledgement
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