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# Comparison of astaxanthin and oxidized β - carotene in red tilapia diets: Effects on growth, pigmentation, immunity, and antioxidative functions

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**Abstract:** The study explored the effect of astaxanthin (AX) and oxidized β-carotene (OX) on the growth performance, skin coloration, and hematological parameters of red tilapia (Oreochromis spp.). The tilapia were fed one of three treatments: (a) commercial control diet, (b) commerical control diet supplemented with astaxanthin, and (c) commercial control diet supplemented with oxidized β-carotene. The experminet was based on a completely randomized design (CRD). Each treatments had three replicates. Red tilapia with an initial mean weight at 42.34 ± 1.326 grams per individual were distributed 8 fish per replicate: in total 72 fish. The feed was fed at a rate of 1.5% of body weight three times a day. Results indicated that fish fed the OX diet exhibited numerically superior growth performance compared to those fed the AX diet and control diets. Furthermore, fish fed the OX diet had significantly enhanced skin carotenoid pigmentation ($p<0.05$). Hematological analysis revealed significantly higher levels of superoxide dismutase (SOD) and lysozyme activity in fish fed the OX diet compared to the other diet groups ($p<0.05$). Therefore, it can be concluded that the fish fed the OX diet tended to outperform the fish fed AX diet and control diet and exhibited significantly better coloration and antioxidant status in this study.

***Keywords:*** *Astaxanthin, Oxidized β--carotene, Red Tilapia.*

## 1. Introduction

Red tilapia (*Oreochromis spp.*) has become a significant component of aquaculture in Thailand, contributing to Thailand's economy and food security. In 2024, tilapia farming in Thailand is projected to cover an area of 525,207 rai, with an estimated production volume of 258,160 metric tons. The average yield per rai is expected to be 492 kilograms. These figures indicate a significant growth in tilapia production, reflecting advancements in farming practices and increase productivity per unit area [1]. Red tilapia is favored due to its rapid growth rate, high value, adaptability to various farming conditions, and its appealing color and taste [2, 3, 4, 5]. Ways to improve the fish's performance, to reduce the fish's stress and to promote fish's gut health are of interest to the fish industry. Production of healthy fish relies in part on homeostasis in the gut. Carotenoid products are well-known antioxidant additives used in the tilapia diets, especially astaxanthin (AX), a xanthophyll carotenoid. AX exerts a crucial role in upregulating antioxidant capacity, stress resistance, and immunity in fish [6]. AX can be produced naturally and/or synthetically. Natural sources of astaxanthin include algae, yeast, salmon, trout, krill, shrimp and crayfish. Synthetic astaxanthin is widely used in feed preparation and animal feeding [7]. In this study, the astaxanthin was sourced from *Paracoccus carotinifaciens,* a gram-negative bacteria capable of producing astaxanthin [8]. Recently, a carotenoid related feed additive product called oxidized β-carotene (OX) has been extensively studied in the monogastric animal feed but only a few studies have been undertaken aquaculture. The full oxidation of β-carotene yields a complex product called oxidized β-carotene (OX). After oxidation, the main antioxidant nutritional benefits of Vitamin A and/or β-carotene have completely diminished but these are replaced by an innate immune


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modulating protection and anti-inflammatory effect [9]. Initially, the innate immune system is primed to face microbial challenges. Subsequently, the anti-inflammatory effects are attributed to the downregulation of gene expression associated with various inflammatory responses. Both benefits are derived from the antioxidant properties of carotenoid. However, to date, there is no research in aquaculture, red tilapia in particular, determining the possibility that whether oxidized β-carotene benefits have carry-over benefits on fish pigment and antioxidative capacity. Therefore, the present study was conducted to clarify the effects of AX and OX growth performance, skin pigment, hematological and anti-oxidative parameters in red tilapia.

## 2. Material and Methods

*2.1. Experimental design*

The experiment was designed using a completely randomized design (CRD) into three treatments and three replicates per treatment (nine replicates in total). There were eight fish in each replicate, in total 72 fish in this experiment. The fish tank served as a replicate. The experiment was conducted at the Nutrition and Aquafeed Laboratory, Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand, under the guide for the use of animals of Kasetsart University, Bangkok, Thailand. Three dietary treatments included (1) a commercial control diet (C) ; (2) a commercial control diet supplemented with astaxanthin (AX) at 100 ppm and (3) a commercial control diet supplemented with oxidized β-carotene (OX) at 100 ppm.

*2.2. Experimental Condition*

Red tilapia (*Oreochromis spp.*) were obtained from a commercial farm in Ayutthaya province, Thailand. They were stocked and acclimated in 3000 L fiber tank and fed with commercial diet (control diet) for one week before grading the same size for the experiment. The initial average weight of red tilapias was 42.34 ± 1.326 grams per individual. The red tilapia were then stocked in tanks at a density of eight fish per tank (53 fish/$m^3$). Each tank had a volume of 200 liters with water filled to 150-160 liters. The trial was carried out in a shaded outdoor environment for the six-week trial with the temperature ranging between 26-29 °C. During the feeding period, the water quality was monitored and controlled to remain within the optimum condition for fish culture; pH 6.5-7.5 and dissolved oxygen of (DO) level of more than 4 mg/L, alkalinity of 70-120 mg/L and hardness of 80-150 mg/L. The feeding scheme of the experimental diets was three times a day at a rate of 1.5% of fish body weight per day.

*2.3. Dietary Treatments Preparation*

In this study, a commercial tilapia feed was used as the basal diet to prepare AX and OX dietary treatments. All treatments started with 2,000 g of the commercial fish diet then coated evenly with 2% w/v carboxymethyl cellulose solution containing 0.3 g of AX. The coated AX feed was mixed thoroughly and aired dry for 3 hours at 27-30 °C. The diet was kept in the room temperature until used. OX treatment was prepared in the same manner as AX with 0.3 g of OX used in the coating solution. All three diets were analysed for proximate analysis.

*2.4. Data Collection*

*2.4.1. Growth Performance*

Fish and three dietary treatments were weight every two weeks for the calculation of weight gain (g/ind.), average daily weight gain (ADG; g/ind./day), specific growth rate (SGR); %/day), feed conversion ratio (FCR).

*2.5. Hematological and Immune Measurement*

After the six-week trial, blood samples (one mL for whole blood content and two mL of serum content) were collected by randomly selecting two fish from each replicate per treatment. The fish were removed from their tanks and anesthetized with clove oil (100 ppm). Whole blood was withdrawn via the caudal vein, and an EDTA solution was used as an anticoagulant agent. The hematocrit was

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determined by using microhematocrit heparinized capillary tubes, a microhematocrit centrifuge, and a reader, expressed as the percentage of blood cell volume [10]. The hemoglobin was measured using the Drabkin and Austin method [11]. The number of erythrocytes/ leukocytes was counted with a Neubauer cell counting chamber or hemocytometer per one $mm^3$ of blood.

Serum samples, two mL, were collected without adding anticoagulant and centrifuged at 3000 rpm for 15 minutes. The supernatant from the serum was collected and stored at -20 °C. The supernatant was used to assess serum protein and immunoglobulin M contents by using the method of Lowry et al [12]. Serum lysozyme levels was determined with enzymatic assay of lysozyme products. The spectrophotometric determination (A450, light path = one cm) was based on the lysis reaction from lysozyme activity. The unit definition was as follows: one unit of lysozyme activity was the activity required to produce a ΔA450 of 0.001 per minute at pH 6.24 and 25 °C using a suspension of *Micrococcus lysodeikticus* (Sigma-Aldrich, St. Louis, MO, USA) as the substrate in a 2.6 mL reaction mixture. Serum superoxide dismutase (SOD) activity was quantified using the Sigma-Aldrich SOD Determination Kit which utilized WST-1, a water-soluble tetrazolium salt that generates a formazan dye upon reduction by superoxide anions. The reduction rate is linearly correlated with xanthine oxidase (XO) activity and is inhibited by SOD. Thus, the IC50, representing 50% inhibition of SOD and SOD-like materials, was measured using a colorimetric method. Additionally, the absorbance of WST-1 formazan at 440 nm is proportional to the amount of superoxide anions, the SOD activity was determined by measuring the increase in color intensity at 440 nm. The levels of total glutathione (GSSG+GSH) were measured using a commercial kit (Sigma-Aldrich, Switzerland) in the sera of the biological samples [13].

### *2.6. Fish Skin Color Measurement*

Six fish were randomly selected from each treatment group, and their skin color was visually scored using the Salmofan™ color chart.

### *2.7. Carotenoid Measurement*

Total carotenoid analysis was performed with a colorimetric method. The fish scales were removed, and skin was separated from the flesh, ensuring no flesh remained attached. The fish skin was then chopped into small pieces. One gram of chopped fish skin sample was weighed, and 15 mL of a solution of hexane, acetone, ethanol, and toluene in a ratio of 10:7:6:7 was added. The mixture was sonicated for 15 minutes under cool and dark conditions. A portion of the solution was extracted, and its absorbance was measured at a wavelength of 470 nm. The total carotenoid was calculated.

### *2.8. Statistical Analysis*

All data were subjected to one-way ANOVA (analysis of variance) using statistical software (IBM, SPSS Inc.). Differences between the means were evaluated using the Tukey HSD test for variance. Overall significance was defined at a level = 0.05, and the results were presented as mean ± SD (standard deviation). Alphabetical notation was used to identify significant treatment differences at $p < 0.05$ [14].

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## 3. Results

**Table 1.**
The proximate analysis of three dietary treatments.

| Proximate Parameters | Control | T1 (AX) | T2 (OX) |
|---|---|---|---|
| Moisture, % | 9.97 | 10.27 | 9.83 |
| Ash, % | 13.70 | 13.67 | 13.81 |
| Protein, % | 31.45 | 30.82 | 31.50 |
| Lipid, % | 4.77 | 4.75 | 4.79 |
| Fiber, % | 4.32 | 4.47 | 4.56 |
| Calcium, % | 1.59 | 1.54 | 1.51 |
| Phosphorus, % | 0.76 | 0.74 | 0.72 |

**Table 2.**
The effects of astaxanthin (AX) and oxidized β-carotene (OX) on growth performance in red tilapia for 6 weeks raising period[1]

| Growth Parameters | Control | T1 (AX) | T2 (OX) |
|---|---|---|---|
| Final weight (g/fish) | 63.00 ± 2.612 | 66.27 ± 3.490 | 67.01 ± 2.764 |
| Weight gain (g/fish) | 20.61 ± 2.510 | 22.99 ± 3.638 | 25.66 ± 3.725 |
| ADG (g/fish) | 0.49 ± 0.059 | 0.55 ± 0.083 | 0.61 ± 0.085 |
| SGR (%/day) | 0.95 ± 0.107 | 1.01 ± 0.138 | 1.15 ± 0.155 |
| FCR | 1.50 ± 0.196 | 1.45 ± 0.164 | 1.26 ± 0.194 |

**Note:** [1]: Data were expressed as mean±SD and values in the same row were not significant ($p>0.05$).

**Table 3.**
The effects of astaxanthin (AX) and oxidized β-carotene (OX) on hematological and anti-oxidative parameters in red tilapia[1,2]

| Hematological Parameters | Control | T1 (AX) | T2 (OX) |
|---|---|---|---|
| RBC ($10^4$ cell/mL) | 1.50 ± 0.055$^b$ | 0.43 ± 0.010$^c$ | 6.40 ± 0.299$^a$ |
| WBC ($10^3$ cell/ mL) | 0.94 ± 0.006$^a$ | 0.70 ± 0.122$^b$ | 0.88 ± 0.046$^{ab}$ |
| Ht (%) | 31.00 ± 0.000$^a$ | 21.33 ± 1.155$^c$ | 26.33 ± 1.528$^b$ |
| Hb (g/dL) | 5.47 ± 0.058$^a$ | 3.87 ± 0.153$^c$ | 4.63 ± 0.252$^b$ |
| Serum protein (mg/dL) | 8.55 ± 0.567$^a$ | 7.27 ± 0.299$^b$ | 8.50 ± 0.505$^a$ |
| IgM (g/L) | 0.53 ± 0.045 | 0.42 ± 0.056 | 0.53 ± 0.033 |
| Lysozyme activity (U/mL) | 820 ± 126.2$^b$ | 1117 ± 109.7$^a$ | 1263 ± 110.2$^a$ |
| SOD (U/mL) | 2.90 ± 0.755$^b$ | 6.17 ± 0.493$^b$ | 20.67 ± 2.517$^a$ |
| Glutathione (nMGSH/mL) | 30.20 ± 0.200 | 30.43 ± 0.208 | 30.47 ± 0.115 |

**Note:** [1]: Data were expressed as mean±SD and means in the same row with different letters ($^{a,b,c}$) were significantly different from each other ($p<0.05$).
[2]: RBC=red blood cell; WBC=white blood cell; Ht=hematocrit; Hb=hemoglobin; IgM=immunoglobulin M

**Table 4.**
The effects of astaxanthin (AX) and oxidized β-carotene (OX) on carotenoid in feed and in red tilapia skin.

| Carotenoid Parameters | Control | T1 (AX) | T2 (OX) |
|---|---|---|---|
| Feed (ug/g) | 149.427 ± 11.529$^b$ | 222.31 ± 8.703$^a$ | 153.47 ± 10.700$^b$ |
| Fish skin (ug/g) | 245.05 ± 6.689$^c$ | 419.90 ± 12.089$^b$ | 632.70 ± 47.610$^a$ |
| Avg. SalmoFan™ | 21.50 ± 1.049$^b$ | 25.33 ± 2.422$^a$ | 27.17 ± 2.137$^a$ |

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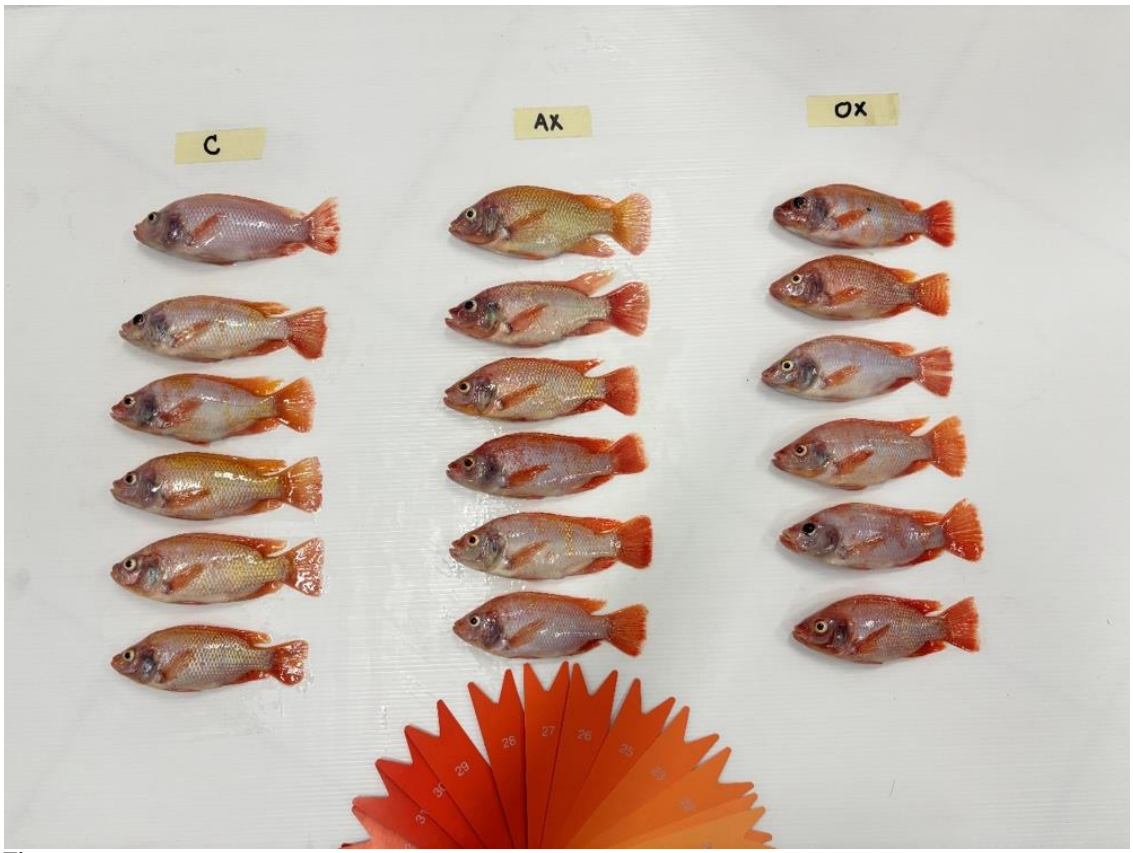


**Figure 1.**
Red tilapia skin color scoring from SalmoFan™ from individual fish fed with three dietary treatments for six weeks.
**Note:** Three dietary treatments include (1) a commercial control diet (C), (2) a commercial control diet supplemented with Astaxanthin (AX), and (3) a commercial control diet supplemented with oxidized β-carotene (OX).

The proximate composition of all three treatments (Table 1) were similar within the range of 30.82-31.45% for protein, 1.51-1.59% for calcium, and 0.72-0.76% for phosphorus. The average initial weight of fish among the three treatments was at 42.39 ± 1.326 per individual. There were large numerical differences (NS) in growth rate and feed efficiency (Table 2) with the OX diet performing very well compared with the AX, and particularly the control diets. Notably, the trend in weight gain improvement in fish fed the OX diet showed the highest at 25% (25.66 ± 3.725 vs 20.61 ± 2.510) compared to fish fed the control diet.

From the hematological results in Table 3, there were significant ($p< 0.05$) differences in RBC, WBC, Ht, Hb and serum protein. Fish fed the OX diet had the highest RBC count per mL compared to those fed the control and AX diets. In contrast, WBC, Ht, Hb and serum protein was highest in fish fed the C diet, followed by fish fed the OX diet. Fish fed the OX diet had the highest SOD and lysozyme concentration followed by fish fed the AX diet and control diet. There was no significant difference in IgM and glutathione parameters.

Fish skin carotenoid levels, at 632.70 ± 47.610 ug/g, and the average Salmofan™ scores, 27.17 ± 2.137, were significantly higher in the fish fed the OX diet compared with other diet treatments (Table 4). In contrast, the carotenoid concentration in fish feed supplemented with OX treatment and the control feed were comparable. Figure 1 illustrated the SalmoFan™ skin color scoring of six individual fish at six weeks of age after feeding the dietary treatments. Fish fed the OX and the AX diets showed brighter and more vibrant pigmentation while fish fed the C diet appeared noticeably muted and paler.

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## 4. Discussion

Differences in growth performance were substantial numerically but not significant. Many factors such as the number of replicates, weather condition, environmental temperature affect the growth performance of all three treatments, adding to variation which could only be countered by better environmental control and use of more replicates. However, at least, these preliminary growth performance results showed that the AX and the OX fed diets tended to be superior to the control. Dietary astaxanthin significantly increased the growth and reduced the FCR of Nile Tilapia [15] and increased weight gain in red tilapia [16] in previous works. Additionally, the preliminary results in this study showed that fish fed the OX diet tended to further improve the growth performance of fish compared to fish fed the AX diet. The enhancement in growth performance of fish fed the OX diet could be due to inhibition of free radicals due to the oxidative stress which corresponds with the observed significant increase in SOD ($p<0.05$) and lysozyme levels ($p<0.05$). However, there was no significant difference in glutathione and IgM. With pathogenic infection, IgM and lysozyme are the proteins involved in the defensive mechanisms from the immune system. Lysozyme is an important defensive molecule of fish innate immune system [17, 18]. This immune defensive process also produces free radicals and leads to the oxidative stress. SOD and glutathione were the critical enzymes in antioxidant defensive mechanism. The development of a pathogenic and/or oxidative stress defensive mechanism was through enzymatic and non-enzymatic processes in the innate immune system. The main benefit of oxidized β-carotene was priming the innate immune system [9]. Therefore, fish fed the OX diet had a better innate immune system to support better fish growth performance.

Priming its innate immune response and mitigating the inflammatory response occurred without depleting stored energy [9]. Animals often use their stored energy to support growth and its gut which would result in weight loss. The more vibrant coloration of the red tilapia fed the OX diet in this study was hypothesized to reflect a healthy gut and thus more efficient absorption of pigments, not from differences in carotenoid pigmentation concentration in the feed as these were the same in the C and OX diets. On the other hand, fish fed the AX diet likely derived their vibrant red coloration directly from the carotenoid pigment added to the feed. The total carotenoid in AX feed diet was the highest compared to C and OX diets. Previous studies indicated that astaxanthin supplementation can lead to a more vibrant and red coloration, a desirable trait in the aquaculture industry [19]. Dietary supplementation with astaxanthin at 400 mg/kg markedly increased carotenoid levels in the skin, fins, and gills, leading to improved body coloration [16, 20]. Further studies are suggested to explore optimum dosage, the mechanism underlying the impact of OX, and broader applications of OX in the aquaculture industry etc.

## 5. Conclusion

This study shows that oxidized β-carotene surprisingly outperformed astaxanthin in improving skin pigmentation of red tilapia (*Oreochromis* spp.) and at the same time resulted in numerical differences increments in gain and feed efficiency, which if proven in subsequent work would be of great interest to the industry. The mechanism of action of oxidized β-carotene seems to be through reduction of oxidative stress and support of the innate immune system, allowing for more efficient absorption and deposition of the pigments present in the control diet.

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