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EFFECTIVENESS OF THE ASTAXANTHIN ADDITION IN COMMERCIAL FEED TO OSCAR (*Astronotus ocellatus*) COLOR INTENSITY

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ABSTRACT

The research was functioned to understand the effectiveness of astaxanthin addition with color intensity change and to understand the optimal astaxanthin concentration which is added to commercial feed to increase oscar color intesity. This research was done in Hatchery of Fisheries and Marine Science Faculty of Padjadjaran University in September to October 2018. The research method that has been used was experimental with completely randomize design (CRD) with six treatments and three replications. The treatments of astaxanthin addition are 0 mg/kg, 10 mg/kg, 20 mg/kg, 30 mg/kg, 40 mg/kg, and 50 mg/kg feed, it was based on the amount of feed. The parameter observed was color score as a main data that using Toca Colour Finder (TCF) whereas the weight addition and survival rate as supporting data. The data from color observation was analyzed using Kruskal-Wallis test, if there is a real difference, the Z test will be done. On the other hand, the weight addition was analyzed using ANOVA F test. The F test was conducted to find treatment effect toward parameter, if there was a real difference, the Duncan test will be done. The result conclude that 30 mg/kg astaxanthin addition to the feed is the most effective treatment toward oscar with color intesity score 5.69.

Keywords: Astaxanthin, Color, Commercial Feed, Intensity, Oscar

INTRODUCTION

Ornamental fish is a one of the fisheries comodity which can open business opportunity that is increasing today. Ornamental fish will have optimal high selling value if the color intensity is considered good (Sukarman and Rina 2014). The emminents of ornamental fish is in the color intesity in its body (Rochmawaty 2010). The pigment content inside the feed is one of the factor that effect fish color intensity (Bachtiar 2002).

Increasing ornamental fish color intesity can be done with various ways such as giving enough light from the lamp or the sun and feeding process with additional composition that increase carotene pigment quality, it is a subtance that can give color especially to the fish. The main function of the pigment in a fish naturally is for giving colorful appearance so the fish will be more exquisite. To stimulate color inside the fish body, the color subtance or pigment addition such as astaxanthin in the feed can help stimulate the color of the fish body (Kurniawati 2012).

Oscar that has a complete wide batican feature and bright red color will gain high price and economical value, but as its growth the pigment change still happened that is caused by the decreasing of the amount of pigment cell. This change of the amount is caused by severak factors such as environment (water quality and other factors), light deficiency, the unbalanced of feed compotition and the color subtance component in the feed deficiency during nurture (Satyani and Sugito 1997). Oscar pigment cell quality must be kept up or maintaned, if it isn't done, it will cause the growth of oscar's body color. The faint color will appear and the red bright batican or golden yellow color will not appear. One of the treatment is adding nutrition through additional feed contain carotene.

Carotenoids is essential bioactive compound that is needed by aquaculture biota (fish and *crustacea*) inside the feed nutrition for increasing growth, survival rate, stress endurance and illness endurance (Sudaryono and Endang 2005).

Astaxanthin is a compound that is often use as a pigment source in increasing ornamental fish's color appearence. Astaxanthin also can be used as a suplement, feed, and medicine. It is because astaxanthin has beta-carotene subtance. Astaxanthin is found in skin, shell and exterior structure of mollusca, crustasea, and fish (Oryza 2010).

METHOD

Research Time and Place

The research activity was conducted from September – October 2018 in Hatchery of Fisheries and Marine Science Faculty, University of Padjadjaran.

Equipments and Materials

The equipments that were used in this research as follows 18 aquariums with 45x40x35 cm^3 size, 1 fiber tub with 1 m diameter and 1,5 m height, blower, Dissolved Oxygen (DO) meter, pH meter, termometer, milimeter block, aquarium fish net, hose, digital scale, zip lock plastic, and Toca Colour Finder (TCF). The materials that were used in this research as follows oscar with 10-12 cm with density 5 fish each aquarium, astaxanthin, *Carboxymethyl Cellulose* (CMC) and commercial feed with 1.3-1.7 mm size.

Research Method

The research method is using completely randomize design (CRD) that consists of six treatments and three replications.

The treatments conducted in the process are as follows :

Treatment A : Without Astaxanthin (control)
Treatment B : Astaxanthin addition 10 mg/kg
Treatment C : Astaxanthin addition 20 mg/kg
Treatment D : Astaxanthin addition 30 mg/kg
Treatment E : Astaxanthin addition 40 mg/kg
Treatment F : Astaxanthin addition 50 mg/kg

PROCEDURE

Container Preparation

Container preparation was done with cleaning container such as aquarium and fiber tub at first. They are soaked with Potassium Permanganate (PP) for one day. Clean the container that has soaked with PP solvent then rinsed and dried. After equipments are clean and dry, the container is filled with water. Installation of aeration pump and hose are done after the container filled.

Acclimatization

Acclimatization was conducted within more or less 7 days. When the acclimatization pediod, all tested fish are given same treatment in feeding that is only giving commercial feed in the form of pellet without astaxanthin addition with the percentage

3% of its weight. Feed was given twice a day. The purpose of acclimatization is to give fish an adaptation chance with the environtment which will be used for research and to decide the healthy fish.

Preparation of mixed feed

Feed that was given in the form of pellet with the persentage 3% from fish weight body which will be added with 10 mg, 20 mg, 30 mg, 40 mg, and 50 mg astaxanthin /kg feed each. Contruction of feed was started with mixing astaxanthin and *Carboxymethyl Cellulose* (CMC) as adhesive which is dissolved with water. Then a feed was mixed and stirred with liquid astaxanthin form, then the mixture was put in to the spray bottle and stirred with hands. If there was astaxanthin left in the bottle then it poured to the feed and stirred again, after that dried the feed by letting it expose to air within 5 minutes. If there is a color or smell change in the isolated feed, then we must make a new one.

Feeding

This research happens within 40 days with treatement frequency in feeding twice a day, in the morning and afternoon from 3% of fish body weight. Color intensity measurement was done every 10 days with water changing, the cleaning of wasted feed was done every morning before feeding.

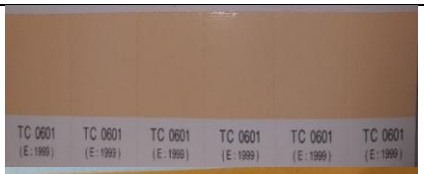
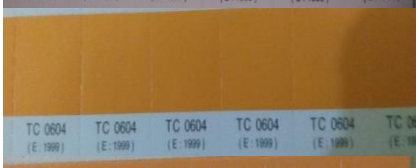
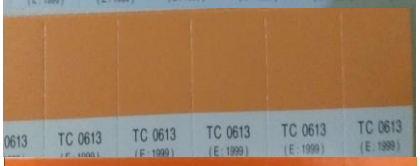
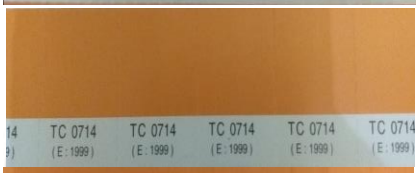
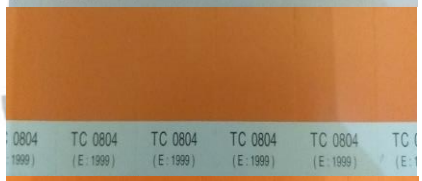
OBSERVATION PARAMETERS

Observation of Oscar Color Intensity

The observation to color changing is done within 40 days and checked every 10 days. The observed parameter is oscar color intensity changing as a main data. Parameter equiment used Toca Colour Finder, that is the equipment to compare color. The scoring is started from the lowest score 1 until the highest score 7 with orange color gradation until dark orange.

The technique to use Toca Colour Finder is focused on two colors that are close to tested fish color body. The color measurement is done by 3-5 persons who aren't having visual impairment such as color blind. The observation is done visually with compare fish color using Toca Colour Finder and analyzed with Kruskal-Wallis test.

Table 1. TCF Color Code

No	TCF Picture	Information Code
1		Score 1 Code TCF 0601
2		Score 2 Code TCF 0604
3		Score 3 Code TCF 0613
4		Score 4 Code TCF 0705
5		Score 5 Code TCF 0714
6		Score 6 Code TCF 0804
7		Score 7 Code TCF 0814

Observation of Oscar Absolute Growth

The absolute growth to fish weight is observed from beginning until the end of the research. The absolute growth is stated as deviation of fish weight which is measured at the end and the beginning of the research. The absolute growth is calculated using Effendie (1979) formula, as follows:

$$W_m = W_t - W_0$$

Note:
Wm : Fish absolute growth weight (g)
Wt : Fish weight time at-t (g)
W_0 : Fish weight time at-0 (g)

Survival Rate

Fish survival rate (SR) is calculated using Effendie (1997) formula, as follows:

$$SR = \frac{\text{Nt}}{\text{No}} X\ 100$$

Note:
SR = Survival rate (%)
No = Number of fish at the beginning
Nt = Number of fish at the end of research

Water Quality

Good water quality is an important factor in improving the color quality and health of ornamental fish. Fish will live healthy and have excellent appearance in an environment with suitable water quality.

Water quality measurement consist of temperature, pH, Dissolved Oxygen (DO) which are checked every 10 days.

DATA ANALYSIS

Color observation data on the fish head and head were analyzed using the Kruskal-Wallis test, if there were significant differences then Test Z. Weight gain observation data were analyzed using F test with a 95% confidence level to determine the effect of treatment on the parameters. If the treatment has a significant effect (F count> F table) then it is continued by Duncan's multiple distance test with a 95% confidence level to find out which treatment has a significantly different effect. Data from observation of SR parameters and water quality were analyzed in a comparative descriptive manner.

RESULT AND DISCUSSION

Oscar Color Intensity Change

Based on the research result that has been done within 40 days, it shows that astaxanthin addition increase oscar color score (Figure 1).

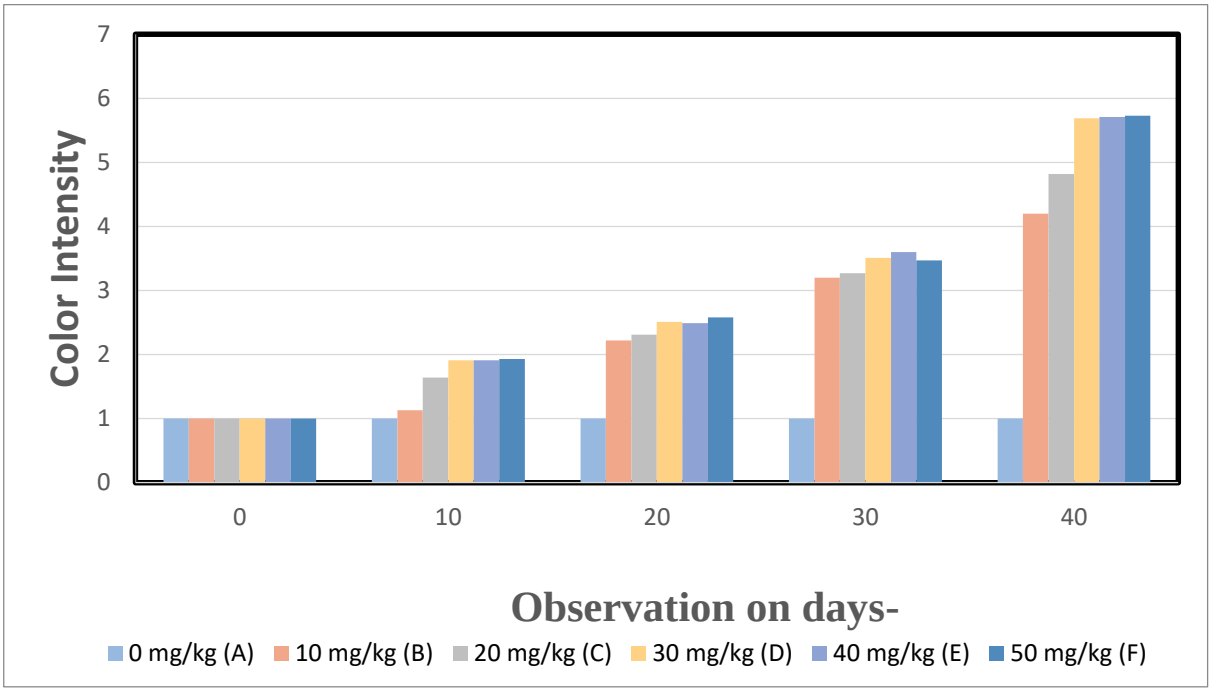


Figure 1. Average of Color Enhancement

On the 10th, 20th, 30th and 40th day of observation showed the color score enchantment to B, C, D, E and F treatments. The treatment A (Control) or without astaxanthin addition showed no color enhancement. It is because commercial feed isn't given carotenoids, so its chromatophores cells don't spread all over the fish's skin and can caused pale to fish skin (Sari et al. 2012).

a b

Figure 2. Color Intensity at Beginning (a) and (b) at The End of the research

Color intensity score keep increasing from day 0 until day 40 within observation. On 40th day, the orange color enhancement happened to every treatments that is given astaxanthin[724]addition (Figure 2). The highest color score is on the treatment F with the average score 5.73.

Table 2. Color Intensity Enhancement Within 40 days

Astaxanthin Addition (mg/kg)	Observation on day-				
	0	10	20	30	40
0 mg/kg (A)	1	1	1	1	1
10 mg/kg (B)	1	1.13	2.22	3.20	4.20
20 mg/kg (C)	1	1.64	2.31	3.27	4.82
30 mg/kg (D)	1	1.91	2.51	3.51	5.69
40 mg/kg (E)	1	1.91	2.49	3.60	5.71
50 mg/kg (F)	1	1.93	2.58	3.47	5.73

Oscar color enhancement keep increasing, it is estimated because oscar still needs carotenoids in the feed to be synthesized become chromatophores cells. The appropriate concentration is given to Oscar also can caused optimal absorption and synthesized carotenoids toward astaxanthin. This also happened to Maesaroh's research (2017), where color intensity enhancement to Oranda goldfish which is given addition to the feed using *Spirulina plantesis* keep increasing until 60th day.

Carotenoids is given with the largest concentration to fish's feed will not give color intensity addition won't be synthesized by fish to grow orange color. The waste from the carotenoids will become feces (McCoy 1999).

Table 3. Average of Color Intensity Score at The End of Oscar Research

Treatments	Color Intensity Score Enhancement
A (Without astaxanthin)	$1^{a}\pm0$
B (Astaxanthin Addition 10 mg/kg)	$4.20^{b}\pm0.40$
C (Astaxanthin Addition 20 mg/kg)	$4.82^{b}\pm0.39$
D (Astaxanthin Addition 30 mg/kg)	$5.69^{c}\pm0.46$
E (Astaxanthin Addition 40 mg/kg)	$5.71^{c}\pm0.47$
F (Astaxanthin Addition 50 mg/kg)	$5.73^{c}\pm0.45$

Note: Number which is followed with letter notation mean there is no significant difference with 95% level of trust.

Based on Kruskal-Wallis' test result showed there is significant difference on the treatment with astaxanthin addition and without astaxanthin addition (Table 3). The result shows the highest color intensity enhancement happened to treatment F with 50 mg/kg astaxanthin and the lowest happened to treatment A without astaxanthin addition.

Treatment without astaxanthin addition doesn't give significant change. It is because the fish body can't synthesize carotenoids without external addition to the feed. As Ikhsan (2012) said that aquatic animal can't synthesize carotenoids inside his body because it must get triggered pigment from outside in the form of feed.

Treatment A has significant difference with treatment B, C, D, E and F, meanwhile treatment B and C has significant difference with treatment D, E, and F .It happen because the difference of fish's absorption ability toward carotenoids. As the research of Jannah (2015) who states that botia for every treatment is having fluctuation change, it is caused by various carotene source in the feed that is given to every treatment and the botia's absorption ability toward carotene subtance in the feed.

Along with astaxanthin addition then the color changing is getting increased because of the additional feed substance will trigger the increasing of color pigment in fish. According to Wayan et al. (2010) stated that adding carotene source or color enchantment will encourage the increasing color or pigment in the fish body, or at least can maintain color pigment that has been appeared in the body. It is supported by Satyani and Sugito (1997), fish color changing depends on the amount of color substance composition in the feed. It needs pigment source concentration that matched and exact, not

exaggerating and not lacking for getting the best color intensity in the fish.

The increasing of color intensity mechanism showed that the brighter color is affected by chromatophores cells that is located in fish's epidermis layer (Wallin 2002). Chromatophores is cell that contains pigment and can change dissemination of pigment cell which is gathered and spreaded in minute or second (Isnaeni 2006). Furthermore, the fish color can also be affected by pigment particle movement in the cell that is controled by nerv system and to chemical substances that is produced, they are epinephrine and acetylcholine. Epinephrine is neurohormone causes pigment in the chromatophores cells gathered in the middle part of the cell. If pigment particle gathered in the middle of the cell, it will cause the fish losing its color. Pigment dissemination in chromatophores is controled by acetylcholine that is taken out by nerv cell. Acetylcholine causes color pigment in chromatophores spreaded, so it makes fish's color brighter (Pardosi et al. 2014).

Growth

Absolute weight observation supported parameter that is done to understand the influence of astaxanthin that is added in comercial feed toward oscar growth. According to Handajani and Widodo (2010), stated that growth is volume and weight addition in certain time. Fish growth is related with protein availability in feed. The average of oscar weight growth in this research gives significant real difference result (Table 4).

Table 4. Oscar Absolute Growth

Treatments	Average (gram)
A (Without astaxanthin addition 0mg/kg)	3.57±0.51
B (Astaxanthin addition 10 mg/kg)	4.40±1.04
C (Astaxanthin addition 20 mg/kg)	4.83±0.06
D (Astaxanthin addition 30 mg/kg)	4.90±0.10
E (Astaxanthin addition 40 mg/kg)	4.40±0.72
F (Astaxanthin addition 50 mg/kg)	4.97±0.67

The highest oscar weight growth during the research happened in treatment F (astaxanthin 50 mg/kg) that is 4.97 gram and the lowest is happened in treatment A (without astaxanthin addition) that is 3.57 gram. Carotene substance addition that is getting higher gives influence toward fish weight growth. It shows that oscar growth isn't obstructed by astaxanthin addition. According to Huda (2013) research result that stated carotene doesn't obstruct koi growth, on the other hand it can increase brighter color in koi. The result also shows that carotene addition in feed can increase feed nutrition and fish's appetite, so it can increase fish weight during the research (Yunisari et al 2014).

Oscar with astaxanthin addition treatment has bigger weight growth than the one without astaxanthin addition. According to Sulawesty (1997) stated feed that is added carotene produces bigger weight growth than the one without carotene addition. Oscar is one of the fish that is considered grows faster and absorbs feed nutrition well. Because of that, oscar can decrease production cost of fish cultivator (Firouzbakhsh and Aliasghari 2009).

According to Mudjiman (2007) to get optimal growth, it is needed a balance of protein, fat, vitamine, carbohidrate and mineral in the feed. Feed quantity that is given will not usually add fish weight, because feed can be used directly by the fish and there is a substance that can't be used or trashed. The optimal growth needs feed quantity that has appropriate quality and quantity that fish needed. Furthermore, the frequency of feeding also has to be concerned, so the feed that is given can be used effienctly by the fish.

Survival Rate

Survival Rate (SR) is comparison between the fish that still live at the end of the research with the total amount at the beginning. Mortality is determinant for cultivation success. The result of oscar SR shows that astaxanthin addition in the comercial feed doesn't give significant influence toward oscar SR level. The average of SR is affected by internal factor included gender, breed, age, reproduction, immunity, and external factor included water quality and fish amount (Herper 1988). The result of SR observation shows in Table 5.

Table 5. Oscar Survival Rate

Treatments	*Survival Rate* (SR)
A (Without astaxanthin addition)	100%
B (Astaxanthin addition 10 mg/kg)	100%
C (Astaxanthin addition 20 mg/kg)	100%
D (Astaxanthin addition 30 mg/kg)	100%
E (Astaxanthin addition 40 mg/kg)	100%
F (Astaxanthin addition 50 mg/kg)	100%

The research has 100% SR that base on Table 5. It is suspected because of carotene substance in astaxanthin, except as a color pigment source it also doesn't affect fish health. Satyani and Sugito (1997) stated not only as a color pigment, but also carotene can protect fish from light and help oxygen metabolism cycle. Carotene substance can also use as a basic material of vitamin A, support thermogulation or body temperature controled, help forming yolk in the reproduction process, and influence fish health (Bachtiar 2002).

Water Quality

Water quality observation is one of the parameter that should be done, because water quality is one of the factor that influence cultivation. Water quality parameter should be observe in the research are temperature, pH and Dissolved Oxygen. This parameter is done in 10 days. Water quality observation result is showed in Table 6.

Table 6. Oscar Water Quality Observation

Parameters	Result	Reference (Baensch & Fischer 2007)
Temperature (ºC)	25	22-30
DO (ppm)	4.0-4.9	> 3
pH	6.9-7.7	7.0-8.1

a. Temperature

The result is concluded from temperature observation during the research in safe limit. According to Baensch and Fischer (2007) optimum temperature for cultivating oscar is 22-23ºC. The average temperature during the research is still around 25ºC. The result of temperature observation during the reasearch is not real difference in every treatment, because temperature treatment is done at the same time and the place is isolated, so the external room condition that often changes doesn't effect the research container.

b. Acidity (pH)

PH analysis result during research conclude the average of all treatments around 6.90-7.70. PH score during research is still in the level of approriate limit. The result at the beginning of the research shows 6.90 score (outside the optimum) and it increases. It is because of high metabolism of the fish during the research. According to Baensch and Fischer (2007) the optimum pH in oscar cultivating is around 7.0-8.1.

c. Dissolved Oxygen (DO)

Dissolved oxygen is the amount of oxygen in the milligram inside one liter of water (ppm). DO observation result is concluded the average in every treatment is around 4.0-4.9 ppm. From average result is concluded that DO is still in the tolerance limit for oscar cultivating. According to Baensch and Fischer (2007) the optimal DO for oscar cultivating is around >3 ppm.

CONCLUSIONS

Based on the research it can be concluded as follows :

1. Astaxanthin addition in commercial feed can effect oscar color enhancement
2. 30mg/kg astaxanthin addition to commercial feed on 40 days can increase significant oscar color with color intensity score 5.69.

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