The Implementation of Natural Pigments of Pumpkin Meal to enhance the Color Quality of Koi Fish (*Cyprinus carpio*)

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Abstract
Skin color is one of the quality indicators in ornamental fish, especially in koi fish. The objective of research is to determine the exact amount of pumpkin meal as a source of carotenoids in artificial feed and its effect on the color quality of koi fish. This research used koi fish strain kohaku seeds size 7-9 cm, carried out in Main Center for Freshwater Aquaculture Development (BBPBAT) Sukabumi. The method implemented was experimental method using Complete Randomized Design with four treatments and four replications. The treatments were on 10, 20 and 30% pumpkin meal and control (without pumpkin extract), respectively.

Parameters measured were the color brightness and survival rate. Observation of the color brightness was done visually with the approach to color on Toca Color Finder. The color brightness data was analyzed descriptively and chroma score was analyzed using Kruskal-Wallis test to see if there was any difference between the treatments followed by Z test at 5%. Survival rate was analyzed by the F-test and the difference between treatments followed by Duncan's multiple-range test at 5%. The results showed that the addition of 20% pumpkin meal gave the best color brightness with color index TC 0906 as much as 75% with chroma scores 50.525. The addition of pumpkin meal in diet has no effect on the survival rate.

Keywords: Pumpkin Meal, Koi, *Cyprinus carpio*, Color Quality, Survival Rate.

Introduction

Koi fish (*Cyprinus carpio*) is an ornamental fish that is included in the relatives of goldfish, one of types freshwater ornamental fish that many in demand. Important criteria in determining the quality of koi ornamental fish includes body shape, fin shape, body size, color pattern and color quality[1]. Ornamental fish production in 2014 reached 1.19 billion head. The most dominant type of ornamental fish is koi fish (26.8%) due to the high selling price of koi fish, easy to cultivate and relatively easy to reach market that attract the interest of ornamental fish farmers[2].

Intensive ornamental fish maintenance in clear water or aquariums causing fish does not always get natural food, so it should be fed artificial feed containing carotenoids[3]. This causes the color of ornamental fish generally to fade when preserved in the aquarium[4].

The color and pigmentation of ornamental fish are influenced by the absorption and deposit of carotenoids in the body[5]. Carotenoids are the main pigment in ornamental fish skin, but fish are not able to synthesize carotenoids[1]. Carotenoid-rich feeding is an efficient way to enhance the pigmentation process in ornamental fish[6].

To increase the selling value and meet the market needs, it is necessary to engineer the feed by adding carotenoid. One of the innovations in feed formulation is to find an alternative source of carotenoid pigments from readily available and affordable materials[7]. Sources of carotenoids can be derived from foodstuffs, one of which is the pumpkin (*Cucurbita moschata*). Pumpkin is one of the agricultural commodities having many advantages over other commodities, including the source of carotenoids, pectin, mineral salts, vitamins and other bioactive substances such as phenolic compounds[8].

Data from the Central Bureau of Statistics in 2003 showed the average yield of pumpkin in Indonesia ranged from 20 to 21 tons per hectare, but the availability of the abundant pumpkin has not been widely used, the processing of pumpkin is still limited as an alternative food for the community to be processed into food products and has not been widely used as an additive in feed.

Seen from the limited scope of pumpkin processing, high carotene content and complete nutrition, pumpkin can be used as an additional ingredient in feed which aims to increase the color brightness of koi fish, therefore it is necessary to do research on the engineering of feed fish with artificial feeding with the addition of pumpkin meal in a way to improve the color quality of koi fish.

Material and Methods

Materials and Tools: The tools used in this research were aquarium size 60×40×40 cm^3, aeration equipment, Toca Color Finder, digital scales, millimeter block, pelletizer, oven, pH meter, thermometer and winkler bottle. The material used in this research included koi fish strain kohaku seeds seedlings in BBPBAT Sukabumi size 7-9 cm with weight ± 6 g, 60 days old as much as 240 head, commercial feed with protein content between 31-33% and pumpkin meal.

Methods: This research was conducted at Main Center for Freshwater Aquaculture Development, Sukabumi. The research was conducted from December 2016 to February 2017. The method used in this research was an experimental method using complete randomized design with four treatments and four replications. The treatment provided is as follows:

Treatment A: Addition of 0% pumpkin meal on commercial feed (Control).

Treatment B: Addition of 10% pumpkin meal on commercial feed.

Treatment C: Addition of 20% pumpkin meal on commercial feed.

Treatment D: Addition of 30% pumpkin meal on commercial feed.

The research was conducted for 40 days with the feeding treatment in the form of feed with different levels of pumpkin. Feed is given as much as 5% of fish biomass with frequency three times a day at 08.00, 12.00 and 16.00.

Primary data measured in this research was color quality for 40 days performed visually using standard values obtained from Toca Color Finder[9]. Supporting data observed included survival rate and water quality.

Determination of the effect of pumpkin meal to the color brightness was done by counting the number of fish in the treatment population as much as 30% of the total density of fish in the Toca Color Finder in accordance with the color of the fish. The determination of the effect of the feeding process addition to the best color brightness refers to the red color of koi fish kohaku strain (Figure 1) which is the 1st champion of koi fish in kohaku Grand Champion - All Japan Koi Show 2016 which corresponds to the color index of TC-0906 (figure 2).

Figure 1: Kohaku Red Color Reference[10]

Figure 2: Color Index on Toca Color Finder no. 0906

The number of fishes in each replication that has the skin color according to the TC color index is calculated and pigmented against the total population of the group with the calculation referring to the prevalence formula[11] modified to the number of β-carotene-induced fish:

$$\sum = \frac{C}{Ns} \; x \; 100\%$$

where Σ = Percentage of number of fishes that produce a certain color, C = Number of fishes that produce a certain color and Ns = Number of fish samples in color observation

The color brightness data was analyzed descriptively and chroma score was analyzed using Kruskal-Wallis test to check if there was any difference between the treatments followed by Z test at 5%. Survival rate was analyzed by the F-test and the difference between treatments followed by Duncan's multiple-range test at 5%[12].

Results and Discussion

Color Quality: The color quality of koi fish can be assessed through the brightness level of the skin color. The brightness color level of koi fish seeds as much as 180 heads from 240 heads kept for 40 days showed that the pumpkin meal in commercial feed could affect the color brightness of koi fish seeds. The best color standard of koi fish of the kohaku used in this research referred to the color of koi fish Grand Champion at All Japan Koi Show 2016 with color index of TC. 0906 (figure 2). Koi fish seeds were not given pumpkin meal (treatment A) on average produce an orange and yellow-brown color indicated by color index of TC.0806 and TC. 0606 and only produced color with TC color index, 0906 as much as 10%.

In addition, koi fish seeds fed with 10% pumpkin meal addition on commercial feed (Treatment B) produced a red color with a TC color index. 0906 reaches 60%. Likewise, with the treatment of 30% pumpkin meal addition on commercial feed (Treatment D) reached the same percentage. Koi fish of -kohaku seeds produced highest color with TC color index. 0906 was achieved in 20% pumpkin meal addition on commercial feed (Treatment C) as much as 75% according to the color of koi fish Grand Champion at the All Japan Koi Show 2016. Koi fish did not reach the TC color index. 0906 produced color with TC index. 0806 and TC. 0606.

Determining the best treatment with the highest color brightness, score was analyzed by Kruskal-Wallis non-parametric statistical test on the skin color or on the final observation. The scale was the score that occurred at the end of the research. Based on the results of Kruskal-Wallis test in table 1, treatment B (10% pumpkin meal addition commercial feed), C (20% pumpkin meal addition on commercial feed) and D (30% pumpkin meal addition on commercial feed) are significantly different from treatment A (0% pumpkin meal addition on commercial feed) whereas treatments B, C and D were not significantly different.

The result of Kruskal-Wallis test provided an illustration that the addition of carotene to the feed gave positive result to the increase of red color of koi fish. Treatments B, C and D were the best treatment dose because the results were significantly different from treatment A, but when viewed on the basis of the highest value, treatment C with the addition of 20% pumpkin meal was the best treatment. Result of analysis of β-carotene level which has been done in research before, it was known that the content of β-carotene in pumpkin meal was as much as 8.5 µg / mL[13].

With the addition of carotene in the body of the fish, it could increase the absorption of pigment cells so that the color quality of fish would increase, compared with the feeding without carotene. The color changes of koi fish seeds at the beginning and end of the research and the color difference between treatments can be seen in table 2.

Pumpkin meal as a natural source of carotenoids added in artificial feed will be absorbed into the body of koi fish. Carotenoids have various forms of compounds, one of which is carotene. Carotene binding to proteins is called carotenoprotein. These compounds when experiencing a process of heating will split into protein and carotene which can produce a red color[14]. The addition of color enhancer sources in the feed will encourage increased color pigment in the fish body, or at least will be able to maintain the color pigment in the body during maintenance[15].

However, the percentage difference in the treatment of B and D with C treatment might be due to different absorption rates of the color pigment species and the number of carotenoid sources given which might also be caused by genetic, water quality and fish stress levels. In treatment D, it could be concluded that the result was less than optimal because it decreased from the result of treatment C, which exceeded the limits of the ability of koi fish to absorb and accumulate the source of carotene. Suspected on treatment D, excessive carotene source was allocated for fish metabolism. In addition, treatment A gave the lowest result due to the absence of carotenoid content in the feed that could be absorbed by fish in color pigmentation.

Physiologically the fish will change the pigment obtained from its food resulting in color variations. Physiological color changes are the color changes caused by movement activity of pigment or chromatophores[16]. The absorption of fish to the pigment sources is influenced by the amount or dose of pigment, the chemical structure of a given type of pigment and chromatophores cells contained in the fish[17]. Pigmentation in fish is also affected by hormones and central nervous system[18]. The pituitary gland produces melanin dispersing hormone (MDH) that affects color fading and melanin aggregating hormone (MAH) which affects the appearance of color.

Hormones have a limited ability to work providing excessive sources of pigment which could decrease the work of hormones. As the case with treatment D with the addition of 30% pumpkin meal in the feed had a chroma score of 44.45 which decreases from the treatment C with the addition of 20% pumpkin meal with a chroma score of 50.525. This showed that in treatment C, the fishes are able to absorb the pigments resources optimally and in treatment D, providing a source of pigment exceeds the limits of the koi fish in absorption resulting in lower hormone action. Excessive source of pigment, at some point, will not provide a better color change, might even decrease the color score, so the koi fish responded with the fading of the color on the fish skin.

Based on the results of this research, it could be said that the addition of pumpkin meal into the feed as a source of carotene could give an increase in the red color of koi fish of kohaku strain. Sequentially starting from carotenoid absorption in the fish body, carotenoids form provitamin A which was then converted by fish organ (liver) into vitamin A. The vitamin A in the dermis layer began to form a color pigment. Along with the absorption of lycopene in the body, lycopene increased the pigmentation effect on color cells and protects the cell layer from UV rays to keep cells from damage[19]. This is in accord with the research which stated that the addition of pumpkin meal by 20% of the total artificial feed provided an enhanced color quality koi fish kohaku strain[13].

Other research with the addition of carotenoid content in feeds such as marigold meal and carrot meal also provided an increase in the color quality of ornamental fish. In addition, based on research, results showed a difference in the color quality of koi fish between treatments with 5% Spirulina meal compared to the treatment without Spirulina[20]. Koi fish in the treatment with Spirulina had a dominant color that was denser and brighter with a smooth base color, clear and clean, while on treatment without Spirulina involves less bright colors and less smooth base color. This indicated that the fish were able to absorb carotenoid content in the feed derived from Spirulina meal resulting in a brighter color intensity.

Survival Rate: The survival rate of koi fish strain kohaku at the end of research conducted for 40 days, the highest result was achieved by treatment B (10% pumpkin meal addition on commercial feed) that was 98%±3.33 and decreased by the same value on control (treatment A) and treatment D (30% pumpkin meal addition to commercial feed) that was 95%±6.38, while the lowest SR in treatment C (20% pumpkin meal addition to commercial feed) was 93%±9.43. The survival rate of koi fish in each treatment is shown in figure 3.

The results of statistical analysis using F-test of addition pumpkin meal up to 30% did not affect the survival of koi fish strain kohaku at 5% level. Referring to SNI.01-6494.1[21], the survival rate of goldfish production is 90%; this condition indicated that the survival rate of the koi fish seeds during the research is standardized by the SNI, since the

lowest survival value achieved in the treatment C still above 90%. In addition to functioning as a color pigment, carotene plays a role in protecting fish against light and is believed to assist in the metabolism of the oxygen cycle[22]. Carotene also naturally acts as a basic ingredient of vitamin A. In addition, it serves as an antioxidant that plays a role in reproductive regulation, cell differentiation and enhancement of the immune system[23]. So, these things become factors of high survival rate of koi fish seeds.

The survival rate is influenced by several factors such as age, environment (water quality), food and pest. The high survival rate of koi fish seeds, other than determined by the addition of pumpkin meal to 30% on commercial feed with it, is also supported by optimal water quality. The water quality of maintenance media (table 3) is within the normal range in accord with SNI 7775[24] and SNI 7734[25] for the life of koi fish seeds so that the fish can survive.

Table 1
The Color Brightness of Koi Fish Based on The Treatment with The Addition of Pumpkin Meal

Treatments	Color Brightness
A (Addition of 0% Pumpkin meal)	21.6[a]
B (Addition of 10% Pumpkin meal)	45.425[b]
C (Addition of 20% Pumpkin meal)	50.525[b]
D (Addition of 30% Pumpkin meal)	44.45[b]

Description: The data followed by the same letter notation means no significant difference at the 5% level in the Z test

Table 2
Color Changes of Koi Fish According to Index of Toca Color Finder

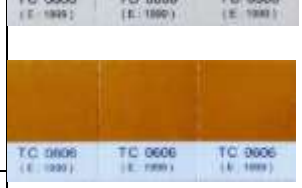
Treatment	Beginning	End	TC Index Color
A			0606 
B			0906 0806 
C			0906 0806 
D			0806 0606 0906

Table 3
Water Quality Measurements in Media

Parameter	Unit	Range In Maintenance Media
Temperature	°C	23.7-27.2
pH	-	6.58-7.54
DO	mg/L	3.16-5.81

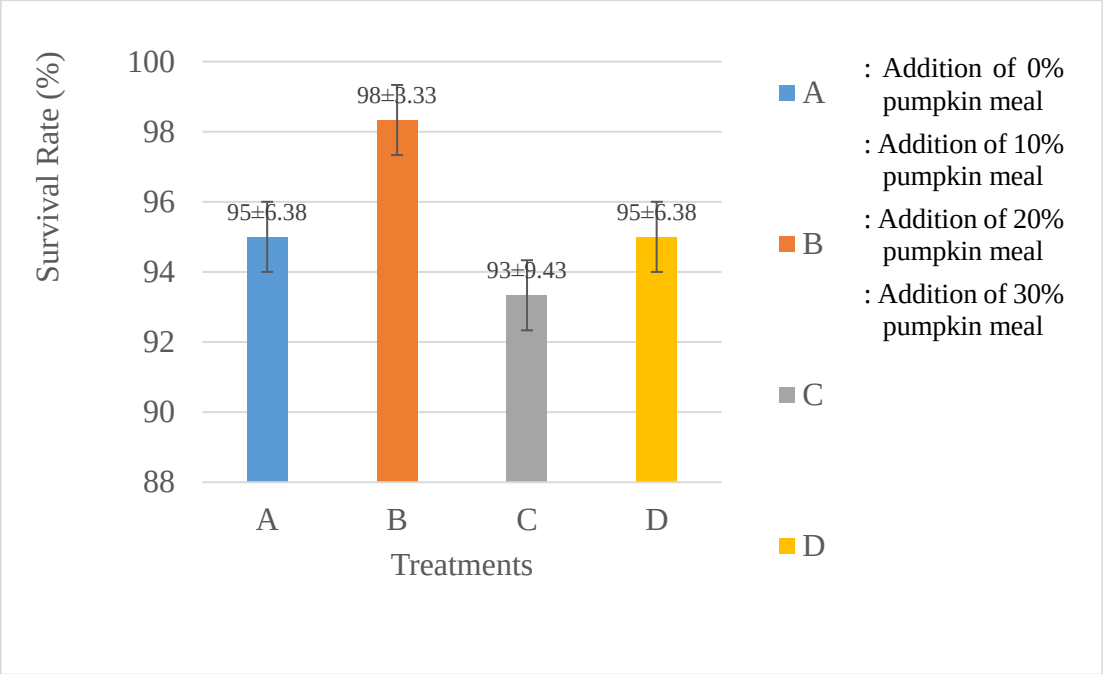


Figure 3: Survival Rate of Koi Fish Seeds

Conclusion

Based on the results of this research it could be concluded that the addition of 20% pumpkin meal in the feed provided the best color brightness with color index TC 0906 as much as 75% with a score of 50.525 which could increase the economic value of koi fish strain kohaku. The addition of pumpkin meal in the feed did not affect the survival rate of koi fish.

Acknowledgement

We would like to express our gratitude to Aquaculture Department, Fishery and Marine Science Faculty, Padjadjaran University who had allowed us to conduct this research. We also would like to thank the Main Center for Freshwater Aquaculture Development (BBPBAT) Sukabumi that had been so kind in providing a place and some materials for this research.

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