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A REVIEW ON POTENTIAL OF VARIOUS SOURCES OF NATURAL PIGMENT FOR ORNAMENTAL FISH

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ABSTRACT

For ornamental fish, the quality criteria are particularly concerned with their physical attractiveness. Because the aesthetic value or ornament is particularly significant for the sorts of fish in this group, the term decorative fish is very fitting. The bright and lustrous body color of ornamental fish is one of the characteristics that determines their economic value. The pigment concentration in fish feed is a key aspect in bringing out fish colors so that they are bright and lustrous. Based on the components in them, a variety of natural materials have a lot of potential to be used as a source of natural colors. Based on multiple research and scientific references, these natural pigment sources have the ability to improve the color quality of various fish.

INTRODUCTION

Ornamental fish is one of the fishery commodities that bring in quite a large amount of foreign exchange for the country. The diversity of ornamental fish in Indonesia as a tropical environment, both fresh and marine, cannot be denied, and it offers significant economic opportunities. In contrast to consumption fish, which measures production in terms of weight and size of fish, the quality parameters considered for ornamental fish are more focused on their physical appearance. The term ornamental fish is very appropriate for ornamental fish because the aesthetic value or ornament is very important for the types of fish of this group. One of the factors that determine the economic value of ornamental fish is the bright and shiny body color.

An important factor in bringing out fish colors so that they are bright and shiny is the pigment content in fish feed. Pigments are non-nutritive compounds that stimulate the appearance of colors, such as yellow, orange and red in fish. The pigments include carotenoids, xanthophiles and pterins. Each has various derivatives that will produce different colors. For example, yellow colors are produced by beta-carotene, lutein, zeaxanthin and taraxanthin, while orange and red colors are produced by astaxanthin and canthaxanthin. The presence of pigment is very important for fish, because animals in general cannot synthesize pigment for their own body, but are able to store it. Sources of pigments that form natural color for fish are only carotenoids from natural feed, the rest of the pigments must be added to the feed.

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COLOR ON FISH

The color of the fish body is due to the presence of pigment cells or chromatophores found in the dermis on the scales or under the skin. The cells that give fish their color are chromatophores and iridocytes. These pigment cells can be improved and maintained in quality by adding sources of carotenoids to fish feed because carotenoids are the main source of color pigment formation in fish. Cytoplasmic contained in chromatophores called pigment granules which are the source of color. These cells are classified into five basic color categories, namely erythrophores which produce red and orange colors, xanthophores which produce yellow colors, melanophores which produce black colors, leukophores which produce white colors, and iridophores which can reflect light reflections. Fish can only synthesize black and white pigments. Red, orange and yellow colors cannot be synthesized by the fish body, so the formation of color in ornamental fish is highly dependent on the amount of carotenoids present in the feed [1].

Pigmentation in fish color is controlled by the nervous system and two chemicals produced by nerves, namely: epinephrine (adrenaline) is a neurohormone released by organisms when surprised or scared, causing pigment granules to gather in the center of the cell and cause the animal to lose color. The second compound is acetylcholine which is a chemical that is released by nerve cells into the muscles, causing melanin to spread and causing the organism's body color to darken. Pigment granules that spread throughout the cell or collect at a point cause discoloration in fish. If the pigment grains clump together at a point, the resulting color as a whole looks pale, whereas if the pigment grains are spread out, the color will be clearly visible.

According to [2] carotenoids are the main pigments in ornamental fish. The color and pigmentation of ornamental fish are influenced by the absorption and accumulation of carotenoids. The absorption of carotenoids in tissue cells affects the chromatophores in the epidermal layer of fish. The chromatophores found in fish skin allow it to change its color. The structure of the fish skin can be seen in Figure 1 below.

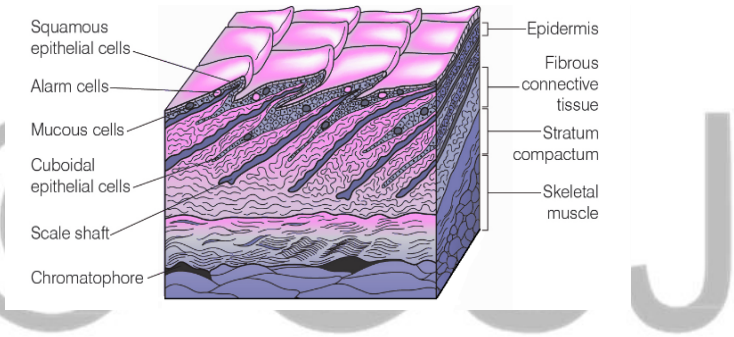

Figure 1. Fish skin structure
Source: [3]

Cromatophore basic colors are divided into five groups, namely red and orange (Eritrophora), yellow (Xantophora), black (Melanophora), white (Leukophora), and reflection sheen (Iridophora). The black and brown pigments formed by melanophores are derived from the amino acid tyrosine and phenol. Red, yellow, and orange pigments are abundant in vitamin A sources, while the green color comes from a combination of basic colors from Chromatopora. In addition to pigments, the color of fish is influenced by water quality, age, maturity of the color display in fish, for example, black pigment tends to be evenly distributed in water as the fish gets older because the number of pigment cells cannot meet the body surface area of larger fish [4].

NATURAL PIGMENT SOURCES

The use of synthetic pigments is a commonly used application in the feed industry. The aspect of ease of use, which is very practical and produces an 'instant' color effect with high color intensity on fish, is the main consideration in its selection. On the other hand, the use of synthetic dyes, apart from being expensive, will have a negative impact on fish and the aquatic environment. Actually, there are many sources of natural pigments that can be used as an alternative to pigment in fish feed. The next section of this paper will describe several natural sources that can be used as pigments.

Red Spinach

Red spinach (*Amaranthus tricolor* L.) belongs to the type of pulled spinach or commonly called "bayam sekul" in Indonesia, with small and soft leaves. Red spinach leaves have a lot of content, such as high enough protein, amino acids, steroids, fatty acids, iron, calcium, and carotene. Red spinach has a high fiber content so consuming it is very good for digestion and is also a source of natural carotenoid dyes [5].

This plant is one of the potential plants as a source of natural dyes, easy to obtain and more environmentally friendly [5]. Red spinach has anthocyanin pigments that are not found in green spinach. Anthocyanins are purplish red pigments that play a major role as antioxidants. Antioxidants are needed by the body to prevent the oxidation of free radicals that can cause various diseases [6].

Red spinach (*Amaranthus tricolor* L.) is a vegetable plant that belongs to the Amaranthaceae family (spinach type plant group). Amaranthaceae is a short-lived plant that is spread in tropical and sub-tropical areas. Amaranthus tricolor comes from tropical America and is called tricolor because its leaves have three colors, namely green, red and purplish. However, some of these species only have red or green colors [5]. The classification of red spinach plants according to [7] is described below:

Kingdom : Plantae (Plants)
Subkingdom : Tracheobionta (vascular plants)
Super Division : Spermatophyta (Produce seeds)
Division : Magnoliophyta
Class : Magnoliopsida
Sub Class : Hamamelidae
Order : Caryphyllales
Family : Amaranthaceae
Genus : Amaranthus
Species : *Amaranthus tricolor* L.

Red spinach will grow well when planted in soil with an acidity (soil pH) of around 6 - 7 and a temperature range of 20 - 32^{o}C [8]. This plant can live in the highlands and lowlands and grows throughout the year. This red spinach is planted in a short time, a maximum of 25 days [7]. The shape of the red spinach plant can be seen in Figure 2.

Figure 2. Red Spinach (*Amaranthus tricolor* L.)

The content of vitamin A in red spinach is 5800 mg. Vitamin A is in the form of beta-carotene which in the body is converted into retinol (vitamin A). In 100 g of red spinach contains 80 mg of vitamin C [8]. Red spinach contains carotenoids that are potential as a source of natural dyes [8].

Carotenoids are fat-soluble pigments or commonly referred to as lipochromes. Currently, more than 800 types of carotenoids have been found in nature and produce yellow, orange, red pigments that can be identified by their color [9]. Sources of carotenoids can be found in animals, plants, and microorganisms [10]. Types of carotenoids that can be used in fish and shrimp feed are beta-carotene, zeaxanthin, isozezanthin, and chantaxanthin. The pigmentation ability of a material is not only determined by the high content of carotenoids but is also determined by the type of carotenoids contained in it [11].

The carotenoid content in red spinach is the lutein compound (as the main component), zeaxanthin, violastin, neoxanthin, and beta-carotene [12]. Based on research by [13] one of the most common types of carotenoids is beta-carotene. In laboratory tests conducted on red spinach, it was found that red spinach contains 15.9 mg/L of carotenoids.

[14] reported that red spinach extract in the form of beta-carotene can increase the color of goldfish with a dose of carotenoids 1500 mg/kg feed. Based on the research conducted by [15] regarding the analysis of beta-carotene content in fresh and boiled red spinach leaves, it was found that the beta-carotene content in fresh red spinach leaves was 14.6 mg/kg and for boiled red spinach leaves was 8.50 mg/kg. The nutritional content of red spinach plants can be seen in Table 1.

Table 1. Red spinach nutritional content (in 100 g)

Component	Value
Energy	51 kcal
Protein	4.6 g
Fat	0.6 g
Carbohydrates	10 g

Calcium	368 mg
Phosphorus	111 mg
Iron	2.2 mg
Vitamin A	5800 mg
Vitamin B1	0.08 mg
Vitamin C	80 mg
Water	86 g
Edible portion	71%

Source: [16]

The content of vitamin A in red spinach is quite high in the form of beta-carotene. Beta-carotene is a compound with antioxidant activity that can counteract free radicals [17]. In the body, beta-carotene will be converted into retinol (vitamin A) [6]. Red spinach in addition to containing the above nutrients, also contains anthocyanin pigments. Anthocyanins are purplish red pigments that give red spinach its red color and act as antioxidants [6]. Anthocyanin levels in red spinach with a total dissolved solids of 5.8 °Brix anthocyanin content of 18.94 mg/ml [18].

Spirulina

Spirulina platensis is a blue green algae that has a protein content of 68% of its dry weight [19]. Protein consists of amino acids such as methionine, cysteine, and lysine. *Spirulina platensis* is rich in gamma-linolenic (GLA), and also contains alpha-linolenic acid (ALA), linolenic acid (LA), stearidonic acid (SDA), eicosapentaeonic (EPA), docosahexaenoic acid (DHA), and arachidonic acid (AA). The vitamins contained in it are vitamins B_1, B_2, B_3, B_6, B_9, B_{12}, a source of antioxidants such as vitamin C, vitamin D and vitamin E, as well as a source of potassium, calcium, chromium, copper, iron, magnesium, manganese, phosphorus, selenium, sodium and zinc. Spirulina contains antioxidants selenium, vitamin E, the enzyme SOD (Superoxidase Dismutase) which can reduce the risk of damage caused by free radicals. Consumption of adequate amounts of antioxidants can also reduce degenerative diseases, such as cardiovascular, cancer, atherosclerosis, and osteoporosis in humans [20]. [21] stated that it was successful in extracting the pigment phycocyanin, one of the pigments from *Spirulina platensis* which is a natural dye (Figure 3).

Figure 3. *Spirulina platensis* flour

The classification of *Spirulina platensis* according to [22] is as follows:

Division : Cyanophyta
Class : Chyanophycae
Order : Oscillatoriales
Family : Oscillatoriaceae
Genus : Spirulina
Species : *Spirulina platensis*

Spirulina has a higher biopigment phycocyanin content than other plants. Phycocyanin has health benefits, including inhibiting tumor growth. Each milligram of phycocyanin (blue pigment) costs between US$6-17. *Spirulina platensis* has antimicrobial activity against *Klebsiella pneumonia, Shigella shigae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Proteus vulgaris*, and *Salmonella typhii*. Phycocyanin from Spirulina can also reduce the viability of EACC (Ehrlich Arcites Carcinoma Cell) [23].

In nature, *Spirulina platensis* lives in an environment that has a high content of carbonate-bicarbonate compounds. *Spirulina platensis* can grow well in fresh or marine waters with a pH range between 8-11. The blue-green color of *Spirulina platensis* is formed due to the presence of phycocyanin and phycobiliprotein pigments. Both pigments are proteins containing a tetrapyrrole group which causes *Spirulina platensis* to have a turquoise color [19].

In contrast to *Chlorella vulgaris* which is a type of chlorophytic microalgae or green algae. In general, chlorophytes or green algae have biopigments used for photosynthesis, namely chlorophyll, in addition to the presence of carotenoid biopigments (carotene and xantrophil). Green algae is dominated by green color because it comes from the pigments chlorophyll a and chlorophyll b. Besides acting as a biopigment, chlorophyll is also known to have potential as an antioxidant. Antioxidants are compounds that at low concentrations are able to prevent or slow down oxidation reactions caused by free radicals. The total carotene contained in Chlorella per 10 g is 0.37% [24].

Carrot

Carrot is a plant that is not native to Indonesia, but derived from abroad with a temperate (sub-tropical) climate, grown in an environment with cold and humid air temperatures. Carrots have short stems that are barely visible. Carrot tubers are reddish yellow in color due to their high carotene content. For the growth and production of tubers, the optimal air temperature is between 15.6-21.1^{o}C. If the air temperature is too high (hot), it often causes the tubers to be small (abnormal) also pale and dull in color. If the air temperature is too low (very cold), then the tubers that are formed will become small in lengths. The short length of the roots (tubers) is influenced by innate factors and by external factors such as the lack of softness of the plant, and soil water amount. If the carrot plant has excess water, it will cause the plant to be susceptible to disease or pests and if the plant lacks water, it will be difficult for the tuber formation process. Watering can be done once or twice a day depending on the conditions [25].

According to [25] carrot plants in the nomenclature and systematic (taxonomy), carrot plants are classified as follows:

Division : Spermatophyta
Sub division : Angiosperms
Class : Dycotyledonae
Order : Umbelliferales
Family : Umbelliferae / Apiaceae / Ammiaceae
Genus : Daucus
Species : *Daucus carota* L.

Figure 4. Carrot tuber/roots

Carrot plants (*Daucus carrota* L.) contain many nutrients needed by the body, especially as a source of vitamin A. Carrot tubers contain a lot of vitamin A due to the high content of carotene or a chemical compound that forms vitamin A. Carotenoids that have vitamin A activity include alpha-carotene, beta-carotene, gamma-carotene, and beta-crytoxanthin. The orange color of carrots indicates a high content of beta-carotene. The more orange the carrot is, the higher the beta-carotene content. The levels of beta-carotene contained in carrots are more than kale, caisim, and spinach. Carrots contain a lot of carotenoids in the phloem than in the xylem. High levels of beta-carotene in carrots can be used as a mixture of natural fish feed and can beautify the color of ornamental fish.

Figure 5. Carrot flour

Carrot flour (Figure 5) is a preserved product that can be used as an alternative to extend shelf life, facilitate storage and transportation to expand marketing reach, besides carrots in the form of flour are easily processed into various products. Carrot flour can be used as a fish feed additive mixed in artificial feeds to produce good color quality in fish. This is because carrots contain carotene compounds (Pro-vitamin A) and can be converted into vitamin A. The content of carotene compounds in carrots is beta-carotene which produces a reddish yellow color in the carrots. The content of beta-carotene possessed by carrot vegetables can increase the yellow, red and orange colors in the test fish as well as spirulina [26].

Table 2. Carrot flour nutritional content

Component	Value (%)
Protein	7.70
Fat	1.15
Crude fiber	24.35
Ash	-
Moisture Content	6.70
Beta-Carotene	51.50 (µg/gr)

Source: [27]

Beta-carotene as other carotenoids in nature are mostly fat-soluble hydrocarbons and bind to compounds whose structure resembles fat. In general, carotenoids have physical and chemical properties, namely fat soluble, soluble in: chloroform, dyes, carbon disulfide and petroleum ether, poorly soluble in alcohol, sensitive to oxidation, stable to heat in oxygen-free air except for some stereo isometric changes [28]. In plants, beta-carotene is biosynthesized by geranyl phosphate. Carotene is a terpene group that is biochemically composed of 8 isoprene groups. As a hydrocarbon compound that does not have an oxygen group, carotene is soluble in fat and insoluble in water.

APPLICATION OF NATURAL PIGMENTS IN ORNAMENTAL FISH FEED

Several studies have shown that the use of natural pigments in fish feed can increase the brightness of fish colors. Marigold flower and red sweet potato extract were noted to be able to improve the red color quality in koi fish because they contain high carotene. In fact, the addition of Spirulina algae in the feed in addition to producing a brighter color, is able to make koi fish scales shinier and increase the red color of ornamental lobsters because they are able to deposit more astaxanthin pigment. The ability of natural pigment sources to produce color is influenced by several factors, including the pigment content in natural ingredients, the level of use in feed, the duration of administration and the ability of fish to absorb the given pigment source.

The use of natural ingredients as a source of pigment is safer for fish, environmentally friendly and cheaper than synthetic pigments. However, the application of pigment materials sourced from natural ingredients is still faced with several obstacles, related to the quality of the pigments produced. Fluctuations in the quality of natural pigments have a high range because their content is influenced by several factors, such as the intensity of sunlight during maintenance or the quality of the soil or water where the material is grown. This will make it difficult to measure the pigment content and optimal dosage for its use in feed. The use of natural pigments from various plants and algae in feed has been shown to improve the color quality of ornamental fish. Some of the results of research on the use of natural pigment sources in various fish are presented in Table 3.

Table 3. Several research on natural pigment application

Research Conclusion	Author
The addition of 6% red spinach powder to Clown Loach (*Chromobotia macracanthus* Bleeker) feed is the best treatment with an increased color brightness value of 5.63	[29]
The addition of 12% Butterfly pea (*Clitoria ternatea* L.) leaves meal (BPLM) to commercial feed was able to enhance the color quality on the tail of the Swordtail fish, i.e average color 5 and a value of color 87.3.	[30]
Giving pellets of 5% will produce carotenoids of nilem fish eggs 27.24% and administration of 40% lemna produce carotenoids of 21.43 ppm	[31]
	[32]

The Marigold petal meal could be used as a pigmenting source at a concentration of 15 g / 100 g feed of dry weight for Red Swordtail, *Xiphophorus helleri* The addition of 9% *S. platensis* flour gave the best color increase in Oranda goldfish	[33]

Conclusion

Various natural materials have great potential to be used as a source of natural pigments based on the content of the ingredients in them. This is proven by the ability of these natural pigment sources to improve the color quality of various fish based on several research and scientific references.

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