

The Potential of Carotenoids and Phytoestrogens Combination on Reproduction Female Fish Parents

ABSTRACT

Aims: This article aims to examine the potential addition of a combination of carotenoids and phytoestrogens in feed to the gonad maturity of various types of fish. The method used in this literature study is to review several research articles on the use of carotenoids and phytoestrogens on fish reproduction and their sources from various plants that can be used as additives to feed. The presence of carotenoids and phytoestrogens in the process of fish reproduction has the potential to improve brood performance. The content of carotenoids which is provitamin A will help in the development of oocytes especially in aiding the accumulation of vitelogenin and retaining fatty acids in oocytes. Meanwhile, the presence of phytoestrogens that have a structure similar to endogenous estrogen, will help the work of estrogen hormones such as estradiol in synthesizing and secreting vitelogenin in the liver. So that the size of oocytes will quickly maximize and have an impact on the rapid maturation of gonads in fish. After maximum oocyte size, phytoestrogens containing isoflavones will work like LH to provide MIH signals that will be received by the surface of the oocyte then forwarded to the cytoplasm to encourage maturation promoting factor (MPF) that causes germinal vesicle breakdown (GVBD) in fish eggs. Sources of producing phytoestrogens and carotenoids are found in plants such as Leguminosae plants that contain lots of isoflavone phytoestrogens and sweet potatoes that contain many carotenoids so that their existence is easily obtained.

Conclusion: The combination of carotenoids and phytoestrogens in feed has the potential to make a considerable contribution to gonadal maturity and reproduction in fish.

Keywords: Phytoestrogens, Fish, Carotenoids, Gonad Maturity

1. INTRODUCTION

The process of gonad maturation in fish is generally related to internal and external aspects in fish. The

internal aspect is the hormones found in fish. Meanwhile, one of the external aspects that affect gonadal maturation is feed. In addition to the

use of hormones that are fairly less economical, the selection of ingredients for good feed quality will support the gonad maturation process, especially in meeting nutritional needs throughout the process of oocyte growth and maturation. The use of quality feed ingredients is certainly more economically affordable for cultivators. The process of growth and maturation of oocytes can be accelerated by the use of quality feed such as high protein content and fat-soluble antioxidant ingredients such as vitamins E, C, and A so that it can trigger the hormone estrogen. Feed that has antioxidants is indispensable for fish throughout reproduction [1].

One of the ingredients that has fat-soluble antioxidant ingredients is carotenoids, besides that these compounds are easy to obtain. Carotenoids are yellow to red fat-soluble pigments [2]. Carotenoids are isoprenoid compounds with a polyene skeleton that has conjugated double bonds [3]. Carotenoids are known as antioxidant compounds that will react with reactive oxygen compounds. Some types of carotenoids are also known to have activity as pro-vitamin A [4]. The structure of carotenoids causes carotenoids to have functions as pigments and antioxidants [5]. Some carotenoids are also known to have activity as provitamin A which can be enzymatically converted to vitamin A in the intestinal mucosa, as is commonly known among others: α -carotene, β -carotene, and β -cryptoxanthin [6].

In addition to the previously mentioned benefits, carotenoids also act as anti-inflammatory, modulate cellular signals, and modulate gene transcription [7].

Carotenoids are compounds that play a role in producing pigments from bright yellow to dark red found in living things, these compounds are widely synthesized by plants, algae, fungi, and bacteria [8]. Based on its structure, carotenoids are divided into 2 main classes, namely carotene and xanthophyll. Carotene (eg: α , β , γ -carotene and lycopene) is an unsaturated hydrocarbon compound and does not contain oxygen. Xanthophylls (e.g. lutein, zeaxantin, bixin, rhodoxanthin) are oxidized carotene derivatives. There are about 750 types of carotenoids that have been found in nature [9].

In the process of vitelogenesis, carotenoids that are fat-soluble are carried by chylomicron plasma or serum albumin through blood plasma to go to the gonads or ovaries. Plasma carotenoids such as xanthophyll can be broken down by ovarian cells and may aid retinol or vitamin A which is thought to be for oocyte formation and development along with vitelogenin or lipoproteins [10,11]. High levels of fatty acids cause the formation of vitelogenin during the process of vitelogenesis in the liver is faster, the faster the process causes the faster vitelogenin is allocated to the ovaries optimally for the formation of eggs. Vitelogenin is produced by the liver and most of the carotenoids in the body are stored

in the liver, so carotenoids are needed by the liver in helping the egg formation process [12]. Antioxidants contained in carotenoids can maintain the presence of fatty acids and accumulate in the yolk, so that the content of fatty acids available is more in the egg and will accelerate the size of oocytes to the maximum which has an impact on the rapid maturity of the gonads. Fish fed with xanthophyll content of 200 ppm were able to produce gonadal weights four times heavier [13].

The pigments produced by carotenoids will also affect the color of fish eggs, β -carotene is a provitamin A compound that can be used in improving the color of egg yolks [14]. There is a positive correlation between the quality of female fish and carotene levels in eggs, the higher the carotenoid levels in eggs the better the quality [15]. Carotene may aid in successful hatching of eggs and juvenile defense against disease and oxidative stress [16]. Each egg containing varying amounts of astaxanthin and β -carotene pigments had a higher fertilization rate than no carotenoids [17].

Increasing the size of oocytes, then gradually the hormones produced by the liver will increase. The hormone is able to continue to produce vitelogenin, then accumulate vitelogenin into the oocyte so that the size of the oocyte increases. One of the estrogen hormones is estradiol which is a stimulant in the biosynthesis of vitelogenin in the

liver. Therefore, it is necessary to have compounds similar to estrogen so that vitelogenin biosynthesis continues to increase, one of these compounds is phytoestrogens.

Fitoestrogen mempunyai struktur yang mirip dengan estrogen endogen, sehingga dapat berikatan dengan reseptor endogen yang berakibat terjadi aktifitas estrogenik. Phytoestrogens have a chemical structure of 2 phenylnaphthalene that resembles the formula of the endogenous estrogen hormone, then phytoestrogens also have an OH group which is one of the requirements for estrogenic activity [18]. Phytoestrogens are widely contained in plants from the leguminosae or fabaceae group, such as red clover, liquorice, bengkoang and soybeans [19]. In addition, plants such as turmeric also contain phytoestrogens that have similarities with estradiol so that they can stimulate the liver to produce vitelogenin [20].

Phytoestrogens have a similar chemical structure to 2 phenylnaphthalene whose chemical formula is the same as endogenous estrogen's chemical formula. There are OH groups in phytoestrogens, estradiol, and diethylstilbestrol, which is one of the requirements for estrogenic activity to occur [18]. Types of phytoestrogens that are often found in soybeans (*Glycine max*) include: Isoflavones consist of Genistein and Daidzein [21].

The stage of vitelogenesis or oocyte development occurs an increase in the production of estradiol-17 β , mediated by the aromatase enzyme in granulosa cells, estradiol-17 β enters the vascular system and stimulates the liver to synthesize and secrete vitelogenin into the blood circulation, then the oocyte membrane binds vitelogenin into the oocyte so that the oocyte grows. This absorption process occurs continuously until the size of the oocyte increases and the number of yolks. So the presence of phytoestrogens will help in increasing estrogen production (estradiol-17 β) causing a buildup of egg yolk, it causes oocytes to grow larger so that it has an impact on maximum egg size, gonadosomatic index value increases.

While oocyte maturation is controlled by LH. LH has stimulated theca cells and produced 17 α -hydroxyprogesterone, then transferred to the basal lamina. LH influences granulosa cells to activate the enzyme 20 β hydroxysteroid dehydrogenase (20 β -HSD), so that the enzyme activity increases and is able to convert 17 α -hydroxyprogesterone to 17 α ,20 β dihydroxyprogesterone (17 α ,20 β -DP), this steroid hormone plays a role in maturation until oocyte ovulation [22,23,24]. In addition, LH also suppresses aromatase activity so that aromatase activity is reduced as a result of a reduction or cessation of estradiol-17 β production, and there is an increase in testosterone production, testosterone will

provide positive feedback to gonadotropins, as a result gonadotropins are more abundant and eventually oocyte maturation and ovulation occur.

The content of isoflavones in phytoestrogens is thought to suppress estradiol 17 β and increase the hormone testosterone. Isoflavones can affect the development of reproductive organs, especially ovaries, by suppressing the synthesis of estrogen hormones. Isoflavones and lignin are inhibitors of 5 α reductase and aromatase [25]. The enzyme 5 α reductase plays a role in the process of testosterone synthesis, while aromatase is an enzyme that plays a role in the formation of estrone from androstenedione. Isoflavones that inhibit aromatase to form estrone will cause the final result of estradiol 17 β to suppress the development of ovarian follicles, besides isoflavones affect estrogen availability by inhibiting 17 β hydroxysteroid dehydrogenase I which also plays a role in estrogen formation. Decreased production of estradiol-17 β and aromatase activity, apparently followed by an increase in 17 α ,20 β -dihydroxy-4-pregnen-3-one (17 α ,20 β -DP) known as MIH (maturation inhibitor hormone). The MIH signal will be received by the surface of the oocyte then forwarded to the cytoplasm to encourage maturation promoting factor (MPF), MPF will play a role in encouraging the migration of the middle egg nucleus to the edge inside the egg so that later the egg nucleus will undergo

fusion shortly before ovulation or GVBD [26].

Isoflavones are one of the phytoestrogen groups. Isoflavone compounds are widely distributed in various parts of the plant, both at the root, leaf stem, and fruit. Isoflavone compounds are secondary metabolite compounds that are widely synthesized by plants. However, unlike other secondary metabolite compounds, these compounds are not synthesized by microorganisms. Thus, microorganisms do not contain this compound. Therefore, plants are the main source of isoflavone compounds in nature. Of several types of plants, higher isoflavone content is found in Leguminoceae plants, especially in soybean plants [27].

The mechanism of action of isoflavones as phytoestrogens is that isoflavones can bind to estrogen receptors as part of hormonal activity, causing a series of reactions that benefit the body. When estrogen levels decrease, there will be a lot of excess estrogen receptors that are not bound, even if the affinity is low, isoflavones can bind to these receptors.

2. SOURCE OF CAROTENOIDS

Sources of carotenoids that can be used as feed ingredients for gonad maturity of fish and have been identified in several plants can be seen in table 1.

Table 1. Types of Carotenoids and Their Sources

Source	Type Carotenoid	Reference
Orange sweet potato	β -karoten	[28]
Chili	β -karoten	[29]
Tomato	Likopen dan β -karoten	[30,31]
<i>Spirulina platensis</i>	β -karoten	[32,33]
Carrot	β -karoten	[34]
<i>Chlorella vulgaris</i>	Lutein	[35]
Moringa Leaves	β -karoten dan Lutein	[36,37]
Duckweed	β -karoten	[38]
Red Dragon Fruit	Carotenoids	[39]
<i>Nymphaea</i> sp.	Carotenoids	[40]
<i>I. aquatica</i>	Carotenoids	[40]
<i>R. maritima</i> ,	Carotenoids	[40]
<i>Najas</i> sp.	Carotenoids	[40]
<i>M. spicatum</i>	Carotenoids	[40]
<i>C. linum</i>	Carotenoids	[40]
<i>S. molesta</i>	Carotenoids	[40]
<i>C. demersum</i>	Carotenoids	[40]
<i>A. pinnata</i>	Carotenoids	[40]
Turmeric	β -karoten	[40]

3. PHYTOESTROGEN SOURCES

Sources of phytoestrogens that have been identified in several plants can be seen in table 2 [41].

Table 2. Sources of Phytoestrogens and Their Content

Source	Content /100 g
Wheat seeds	379380
Soybean	103920
Tofu	27150,1
Sesame oil	8008,1
Rye bread	7540
Soy milk	2957,2
Hummus	993
Garlic	603,6
Bean sprout	495,1
Dried apricots	444,5
Alfafa	441,4
Sunflower seeds	216
Olive oil	180,7
Almond	131,1
Green beans	105,8
Peanut	34,5
Shallot	32
Blue berries	17,5
Corn	9

4. SOURCES OF PHYTOESTROGENS CONTAINING ISOFLAVONES

Sources of phytoestrogens containing isoflavones that can be used as feed ingredients for gonad maturity of fish and have been identified in several plants can be seen in table 3.

Table 3. Sources of Isoflavone Phytoestrogens and Their Content

Source	Reference
Soybean varieties Dena I	[42]
Glycine max (Soybeans)	[43]
Soybean Tempeh	[44]

Extract, Whole Sword Koro Tempeh Extract and Rajang Tempeh	[45]
Soybeans with varieties (Anjasmoro, Argomulyo, and Gema)	[46]
Soy milk and tempeh Tempeh is made from black soybeans (Glycine soja), black koro (Lablab purpureus), and koro kratok (Phaseolus lunatus)	[47]
Peeled and unpeeled soybeans extracted with solvents	[48]
	[49]

5. THE IMPACT OF CAROTENOIDS ON FISH REPRODUCTION

Previous research related to carotenoids that have an impact on fish reproduction is presented in Table 4.

Table 4. Previous research related carotenoids to fish reproduction

Sources of Carotenoids	Result	Reference
<i>Lemna</i> sp. contains β -carotene and xanthophylls	Increased carotenoid content in fish eggs by 21.43 ppm in nilem eggs.	[50]
Carotenoids	Produce egg fecundity and total fatty acids in gonads that	[51]

	are higher than feed without carotenoids in baronang fish.	
Turmeric contains β -carotene	Increases the degree of maturity of the gonads. Improved reproductive performance and increased intensity and spread of orange color in chef goldfish broods	[52]
Moringa leaves contain carotenoids		[53]
Sweet potatoes (Ipomoea batatas) contain β -carotene	Increased body color performance in fish as a reproductive function	[54]

6. CONCLUSION

The combination of carotenoids and phytoestrogens in feed has the potential to make a considerable contribution to accelerating gonad maturity and reproductive performance in fish.

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