



Importance of Carotenoids and Phytoestrogens in Gonad Maturity of Various Types of Fish

Iskandar ^a, Muhamad Erpan Saputra ^{a*} and Zuzy Anna ^a

^a *Faculty of Fisheries and Marine Sciences, Universitas Padjadjaran, Jl. Raya Bandung Sumedang KM.21, Jatinangor, Sumedang Regency, West Java 45363, Indonesia.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript

Article Information

DOI: 10.9734/AJFAR/2023/v24i1623

Open Peer Review History:

This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: <https://www.sdiarticle5.com/review-history/103170>

Mini-review Article

Received: 26/05/2023

Accepted: 15/07/2023

Published: 20/07/2023

ABSTRACT

Aims: The purpose of this article is to examine the potential for adding combinations of carotenoids and phytoestrogen in their feed to the maturity of gonads of different kinds of fish. The method of writing used is the library study, with the journal search stages, journal selection, journal analysis, and journal synthesis. Based on the review of several related journals. The presence of carotenoids and of estrogen in the reproductive process of fish is highly profitable. The carotenoid compound, which is a provitamin a, will help in the development of oocytes primarily with the accumulation of vitellogenin and maintaining fatty acids in the oocytes. Meanwhile, the presence of phytoestrogen that have structures like endogenous estradiol will assist in synthesizing and producing vitellogenin in the liver. So the size of oocytes would accelerate and impact the rapid ripening of the fish. After a maximum Estrogen measure, a full-flesh-based phytoestrogen will act like a hoflavon to give a MIH signal to be received by the surface of oocyte then passes to the cytoplasm to encourage maturation promoting factor (MPF) that causes germinal vesicle breakdown (GVBD) to the fish's egg. The presence of easily obtained carotenoids and phytoestrogen provided potential additives.

*Corresponding author: Email: erpansaputra@gmail.com;

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 gonadal maturation in fish is generally related to internal and external aspects of fish. The internal aspect is the hormone found in fish. While one of the external aspects that affect gonad maturation is feed. In addition to the use of hormones which are considered less economical, the selection of ingredients for good quality feed will support the gonadal maturation process, especially in meeting nutritional needs during the growth and oocyte maturation process. Utilization of quality feed ingredients is certainly more affordable economically by farmers. The process of growth and maturation of oocytes can be accelerated by using quality feed such as high protein content and fat-soluble antioxidant ingredients such as vitamins E and A which can trigger the hormone estrogen. Gonad maturation in brood stock can be stimulated in various ways, namely by improving environmental factors, nutrition, and giving hormones [1]. Feed that has antioxidants is needed by fish throughout reproduction [2]. The induction of the hormone estrogen from the outside can help trigger the formation of vitellogenin during the vitellogenesis process. The phytoestrogen content in plants has similarities with estradiol so that it can stimulate the liver to produce vitellogenin [3]. However, scientific information on the results of research on the use of a combination of carotenoids and phytoestrogens specifically on the subject of fish is still very scarce. Based on this, the purpose of writing this article is to conduct an in-depth scientific study on the use of carotenoids and phytoestrogens as an alternative to reproductive problems in fish.

2. MATERIALS AND METHODS

The method used in this research is literary research. Literary studies, also known as literary review, literary review, theoretical basis, literary review, or theoretical review, is an approach to research based on written works. Literature research refers to research that uses only written sources, including published and unpublished research results. Several stages are carried out as follows:

1. Journal search. First of all, a search was carried out on previous journals about the factors that influence gonad maturity in fish. Searches were made on scientific journal databases such as ScienceDirect, Google Scholar, and ProQuest. The keywords used were "the effect of carotenoids on gonadal maturity" and "potential of phytoestrogens on fish reproduction".

2. Journal selection. From the search results, journal selection is based on certain criteria such as relevance, credibility, and accuracy of information. The selected journals should be relevant to the research topic and recognized by the scientific community.

3. Journal analysis. Once the journals have been selected, the articles are analyzed. This analysis includes identification of the factors that influence the feeding habits of fish and an explanation of the mechanisms involved in this process.

4. Journal synthesis; From the analysis, information from relevant journals is synthesized. The information gathered will be used as a basis for preparing the trial

3. RESULTS AND DISCUSSION

3.1 Potential of Carotenoids in Fish Reproduction

One of the ingredients that has fat-soluble antioxidant ingredients is carotenoids, besides that these compounds are easy to obtain. Carotenoids are fat-soluble pigments that are yellow to red in color [4]. Carotenoids are isoprenoid compounds with a polyene framework that has conjugated double bonds [5]. Carotenoids are known as antioxidant compounds which will react with reactive oxygen compounds. Several types of carotenoids are also known to have activity as pro-vitamin A [6]. The carotenoid structure causes carotenoids to function as pigments and antioxidants [7]. Several carotenoids are also known to have activity as provitamin A which can be converted enzymatically to vitamin A in the intestinal mucosa, as is well known, among others: α -carotene, β -carotene, and β -cryptoxanthin [8]. In addition to the benefits previously mentioned,

carotenoids also act as anti-inflammatories, modulate cellular signals, and modulate gene transcription [9].

Carotenoids are compounds that play a role in producing pigments from bright yellow to dark red that are found in living things, these compounds are synthesized by plants, algae, fungi, and bacteria [10]. Based on the structure of carotenoids can be divided into 2 main classes, namely carotenes and xanthophylls. Carotenes (eg: α , β , γ -carotene and lycopene) are unsaturated hydrocarbon compounds and do not contain oxygen. Xanthophylls (eg: lutein, zeaxanthin, bixin, rhodoxanthin) are oxidized carotene derivatives. There are about 750 types of carotenoids that have been found in nature [11].

In the process of vitellogenesis, fat-soluble carotenoids are carried by plasma chylomicrons or serum albumin through the blood plasma to go to the gonads or ovaries. Plasma carotenoids such as xanthophyll can be broken down by ovarian cells and can help retinol or vitamin A which is thought to be for the formation and development of oocytes along with vitellogenin or lipoprotein [12,13]. High levels of fatty acids cause the formation of vitellogenin during the vitellogenesis process in the liver to be faster, the faster the process causes the faster the vitellogenin is optimally allocated to the ovary for the formation of egg cells. Vitellogenin is produced by the liver and most of the carotenoids in the body are stored in the liver, so

that carotenoids are needed by the liver to help the egg formation process [14]. The antioxidants contained in carotenoids can maintain the presence of fatty acids and accumulate in the yolk, so that the available fatty acid content is more available in the egg and will accelerate the size of the oocyte to be maximized which has an impact on the rapid maturity of the gonads. Fish that were given feed containing xanthophyll of 200 ppm were able to produce gonad weight four times heavier [15].

Pigments produced by carotenoids will also affect the color of fish eggs, β -carotene is a provitamin A compound that can be used to increase the color of egg yolks [16]. There is a positive correlation between the quality of female fish and the levels of carotene in the eggs, the higher the levels of carotenoids in the eggs, the better the quality [17]. Carotene may aid in egg hatching success and juvenile defense against disease and oxidative stress [18]. Each egg containing various amounts of astaxanthin and β -carotene pigment has a higher fertilization rate than without carotenoid administration [19].

3.2 Sources of Carotenoids

Sources of carotenoids that can be used as feed ingredients for maturing fish gonads and their presence has 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	[30]
Chili	β -karoten	[31]
Tomato	Likopen dan β -karoten	[32,33]
<i>Spirulina platensis</i>	β -karoten	[34,35]
Carrot	β -karoten	[36]
<i>Chlorella vulgaris</i>	Lutein	[37]
Moringa Leaves	β -karoten dan Lutein	[38,39]
Duckweed	β -karoten	[40]
Red Dragon Fruit	Carotenoids	[41]
<i>Nymphaea</i> sp.	Carotenoids	[42]
<i>I. aquatica</i>	Carotenoids	[42]
<i>R. maritima</i> ,	Carotenoids	[42]
<i>Najas</i> sp.	Carotenoids	[42]
<i>M. spicatum</i>	Carotenoids	[42]
<i>C. linum</i>	Carotenoids	[42]
<i>S. molesta</i>	Carotenoids	[42]
<i>C. demersum</i>	Carotenoids	[42]
<i>A. pinnata</i>	Carotenoids	[42]
Turmeric	β -karoten	[42]

Table 2. Previous studies related to carotenoids on fish reproduction

Sources of Carotenoids	Result	Reference
<i>Lemna sp.</i> contains β -carotene and xanthophylls	Increased carotenoid content in fish eggs by 21.43 ppm in Nile eggs.	[52]
Carotenoids	Produce egg fecundity and total fatty acids in gonads that are higher than feed without carotenoids in baronang fish.	[53]
Turmeric contains β -carotene	Increases the degree of maturity of the gonads.	[54]
Moringa leaves contain carotenoids	Improved reproductive performance and increased intensity and spread of orange color in chaf goldfish broods	[55]
Sweet potatoes (<i>Ipomoea batatas</i>) contain β -carotene	Increased body color performance in fish as a reproductive function	[56]

3.3 Impact of Carotenoids on Fish Reproduction

Previous research related to carotenoids which have an impact on the reproduction of tesaji fish is in Table 2.

3.4 Phytoestrogen Potential in Fish Reproduction

As the size of the oocyte increases, the hormone produced by the liver will gradually increase. This hormone is able to continue to produce vitellogenin, then accumulate vitellogenin into the oocyte so that the size of the oocyte increases. One of the estrogen hormones is estradiol which is a stimulant in vitellogenin biosynthesis in the liver. Therefore, it is necessary to have compounds that are like estrogen so that the biosynthesis of vitellogenin continues to increase, one of these compounds is phytoestrogens.

Phytoestrogens have a structure similar to endogenous estrogens, so they can bind to endogenous receptors resulting in estrogenic activity. Phytoestrogens have a chemical structure of 2 phenyl naphthalene that resembles the endogenous estrogen hormone building formula, then phytoestrogens also have an OH group which is one of the requirements for estrogenic activity to occur [20]. Phytoestrogens are abundant in plants that come from the Leguminosae or Fabaceae group, such as red clover, liquorice, bengkoang and soybean [21]. In addition, plants such as turmeric also contain phytoestrogens which have similarities with estradiol so that they can stimulate the liver to produce vitellogenin [22].

Phytoestrogens have a chemical structure like 2 phenyl naphthalene whose structural formula is

the same as that of endogenous estrogens. There is an OH group in Phytoestrogens, estradiol, and diethylstilbestrol, which is one of the requirements for estrogenic activity to occur [20]. The types of phytoestrogens that are often found in soybeans (*Glycine max*) include: Isoflavones consisting of Genistein and Daidzein [23].

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

Meanwhile, 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 dihydroxy progesterone (17 α ,20 β -DP), this steroid hormone plays a role in the maturation until oocyte ovulation occurs. [24,25,26]. In addition, LH also suppresses aromatase activity so that aromatase activity decreases resulting in a reduction or cessation of estradiol-17 β production, and an increase in testosterone production.

The content of isoflavones in phytoestrogens is thought to suppress estradiol 17β and increase the hormone testosterone. Isoflavones can affect the development of reproductive organs, especially the ovaries by suppressing the synthesis of the hormone estrogen. Isoflavones and lignin are inhibitors for 5α reductase and aromatase [27]. The 5α reductase enzyme 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 prevent the formation of the final product in the form of 17β estradiol thereby suppressing the development of ovarian follicles, besides that isoflavones affect the availability of estrogen by inhibiting 17β hydroxysteroid dehydrogenase I which also plays a role in the formation of estrogen. The decrease in estradiol- 17β production and aromatase activity was apparently followed by an increase in $17\alpha,20\beta$ -dihydroxy-4-pregnen-3-one ($17\alpha,20\beta$ -DP) which is known as MIH (maturation inhibitor hormone). The MIH signal will be received by the surface of the oocyte and then forwarded to the cytoplasm to encourage the maturation promoting factor (MPF). MPF will play a role in encouraging the migration of the egg cell nucleus in the middle to the edges in the egg so that later the egg cell nucleus will undergo fusion just before ovulation or GVBD [28].

Isoflavones are a group of phytoestrogens. Isoflavones are widely distributed in various parts of the plant, both in the roots, stems, leaves and fruit. Isoflavones are secondary metabolites that are widely synthesized by plants. However, unlike other secondary metabolites, these compounds are not synthesized by microorganisms. Thus, microorganisms do not contain these compounds. Therefore, plants are the main source of isoflavones in nature. From several types of plants, the higher isoflavone content is found in Leguminosae plants, especially in soybean plants [29].

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 an excess of unbound estrogen receptors, although the affinity is low, isoflavones can bind to these receptors.

3.5 Phytoestrogen Sources

Phytoestrogen sources that have been identified in several plants can be seen in Table 3 [43].

Table 3. Sources of phytoestrogens and their ingredients

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

3.6 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 4.

Table 4. Sources of isoflavone phytoestrogens and their content

Source	Reference
Soybean varieties Dena I	[44]
Glycine max (Soybeans)	[45]
Soybean Tempeh Extract, Whole	[46]
Sword Koro Tempeh Extract and Rajang	
Tempeh	[47]
Soybeans with varieties (Anjasmoro, Argomulyo, and Gema)	[48]
Soy milk and tempeh	[49]
Tempeh is made from black soybeans (Glycine soja), black koro (Lablab purpureus), and koro kratok (Phaseolus lunatus)	[50]
Peeled and unpeeled soybeans extracted with solvents	[51]

6. CONCLUSION

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

ACKNOWLEDGEMENTS

Praise be to God Almighty for all the abundance of His grace and guidance so that the author can complete this writing. In the preparation of this article, the author has tried his best and of course with the help of various parties. For that, the author would like to thank: all researchers who have provided information contained in this writing.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Dewantoro E. Gonad performance of catfish (*Barbonymus schwanenfeldii*) after periodic injection of HCG hormone. Indonesian Aquatics [Journal]. 2015;6(1): 244-55.
2. Izquierdo MS, Fernández-Palacios H, Tacon AGJ. Effect of broodstock nutrition on reproductive performance of fish. Aquaculture. 2001;197(1-4):25-42.
3. Ibrahim Y, Saputra F, Yusnita D, Karim AUT. Evaluation of the growth and development of gonads of Serukan *Osteochilus* sp fed with turmeric flour. J Aquacult. 2018;2(2):1-6.
4. Rodrigues E, Mariutti LRB, Mercadante AZ. Scavenging capacity of marine carotenoids against reactive oxygen and nitrogen species in a membrane mimicking system. Mar Drugs. 2012;10(8):1784-98.
5. Rodriguez-Concepcion M, Avalos J, Bonet MI, Boronat A, Gomez-Gomez L, Hornero-Mendez D et al. Aj, Olmedilla-Alonso B., Palou A. A Global Perspective On Carotenoids: Metabolism, Biotechnology, And Benefits For Nutrition And Health. Prog Lipid Res. 2018;04.004;2018:7062-93.
6. Sartori AG de O, Silva MV. Main food sources of carotenoids, according to the purpose and degree of processing, for beneficiaries of the "Bolsa Família" in Brazil. Food Sci Technol, Campinas. 2014; 34(2):408-15.
7. Young AJ, Lowe GL. Carotenoids—antioxidant properties. Antioxidants (Basel). 2018;7(2):1-4.
8. Harrison EH. Mechanisms involved in the intestinal absorption of dietary vitamin A and provitamin A carotenoids. Biochim Biophys Acta. 2012;1821(1):70-7.
9. Murillo AG, Fernandez ML. Potential of dietary non-provitamin A carotenoids in the prevention and treatment of diabetic microvascular complications. Adv Nutr. 2018:714-24.
10. Misawa N. Pathway engineering of plants toward astaxanthin production. Plant Biotechnol. 2009;26(1):93-9.
11. Merdekawati W, Karwur FF, Susanto AB. Carotenoids in algae: Studies on the biosynthesis, distribution and functions of carotenoids. Bioma. 2017;13(1):23-32.
12. Lubzens E, Young G, Bobe J, Cerdà J. Oogenesis in teleosts: how fish eggs are formed. Gen Comp Endocrinol. 2010; 165(3):367-89.
13. Tizkar B, Soudagar M, Bahmani M, Hosseini SA, Chamani M, Seidavi A, et al. Effects of dietary astaxanthin and β carotene on gonadosomatic and hepatosomatic indices, gonad and liver composition in goldfish *Carassius auratus* (Linnaeus, 1758) Broodstocks. Lat Am J Aquat Res. 2016;44(2):363-70.
14. Azrimaidaliza. Vitamin A, immunity, and its relation to infectious diseases. J Public Health. 2007;2:9-96.
15. James R, Sampath K, Thangarathinam R, Vasudevan L. Effect of dietary Spirulina Level on growth, fertility, coloration and leucocyte count in red swordtail, *Xiphophorus helleri*. Isr J Aquacult Bamidgeh. 2006;2; 58:97-104.
16. Bidura IGNG, Partama IBG, Utami IAP, Candrawati DPMA, Puspani E, Suasta IM, et al. Effect of Moringa oleifera leaf powder in diets on laying hen performance, β -carotene, cholesterol, and minerals contents in egg yolk. IOP Conf S Mater Sci Eng. 2020;823(1).
17. Azka A, Nurjanah AM. Jacob. Profile of fatty acids, amino acids, total carotenoids, and α -tocopherols of flyingfish eggs. JPHPI. 2015;18(3).

18. Letcher RJ, Heath JW, Heath DD. Why are salmon eggs red? egg carotenoids and early life survival of Chinook salmon (*Oncorhynchus tshawytscha*). Tyndale S.T. *Evol Ecol Res.* 2008;10:1187-99.
19. Tizkar B, Soudagar M, Bahmani M, Hosseini SA, Chamani M. The effect of dietary astaxanthin and β -caroten on the reproductive performance and egg quality of female goldfish (*Carrastus auratus*). *Caspian J Environ Sci*; 2013.
20. Biben. Fitoestrogen: efficacy for the reproductive, nonreproductive system and the safety of its use. Prosiding, seminar ilmiah nasional. Bandung: Universitas Padjadjaran; 2012.
21. Lukitaningsih E. Phytoestrogens: safe natural compounds as a substitute for estrogen hormones in women. Herbal research information media. Fakultas farmasi. Yogyakarta: Universitas Gadjah Mada; 2012.
22. Ibrahim Y, Saputra F, Yusnita D, Karim AUT. Evaluation of the growth and development of gonads of Serukan *Osteochilus sp* fed with turmeric flour. *J Aquacult.* 2018;2(2):1-6.
23. Suparman E. Phytoestrogens/HRT: pros and cons. Scientific journals. Manado: Universitas Sam Ratulagi; 2006.
24. Nagahama Y. Gonadotropin action on gametogenesis and steroidogenesis in teleost gonads. *Zool Sci.* 1987;4: 209-22.
25. Nagahama Y. Regulasi of oocyte growth and maturation in fish. *Dev Biol.* 1995;30:103-45.
26. Nagahama Y. Endocrine regulation of gametogenesis in fish. *Int J Dev Biol.* 1994;38(2):217-29. PMID 7981031.
27. Whitten PL, Patisaul HB. Cross species and interassay comparisons of phytoestrogen action. *Environ Health Perspect.* 2001;109;Suppl 1:5-20.
28. Basuki F. Optimization of oocyte maturation and ovulation in goldfish (*Carassius auratus*) through the use of aromatase inhibitors [thesis]. Institut Bogor Agriculture (IPB). Bogor; 2007.
29. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. *J Nutr Sci.* 2016;5:e47. Available: <https://doi.org/10.1017/jns.2016.41>
30. Ginting E. Carotenoid extraction of orange-fleshed sweet potato and its application as a natural food colorant. *jtip.* 2013;24(1):81-8.
31. Octaviani T, Guntarti A, Susanti H. Determination of β -carotene levels in several types of chilies (genus *Capsicum*) by visible Spectro-photometric method. *Pharmaciana.* 2014;4:101-9.
32. Hassan. Lycopene content of tomato (*Lycopersicum esculentum* L.) on heating time and temperature. *Chemistry Journal.* 2015;16:28-35.
33. Herrero M, Cifuentes A, Ibañez E. Sub and supercritical fluid extraction of functional ingredients from different natural sources: plants, food-by-products, algae, and microalgae: a review. *Food Chem.* 2006;98(1):136-48.
34. Dey S, Rathod VK. Ultrasound assisted extraction of β -carotene from *Spirulina platensis*. *Ultrason Sonochem.* 2013; 20(1):271-6.
35. Roohinejad S, Everett DW, Oey I. Effect of pulsed electric field processing on carotenoid extractability of carrot purée. *Int J Food Sci Technol.* 2014;49(9):2120-7.
36. Luengo E, Martínez JM, Bordetas A, Álvarez I, Raso J. The influence of the treatment of medium temperature on lutein extraction assisted by pulsed electric fields from *Chlorella vulgaris*. *Innov Food Sci Emerg Technol.* 2015;29:15-22.
37. Joshi P, Mehta D. Effect of dehydration on the nutritive value of drumstick leaves. *J Metab Syst Biol.* 2010;1:5-9.
38. Zhang M, Hettiarachchy SN, Horax R, Kannan A, Praisody MDA, Muhundan A et al. Phytochemicals, antioxidants and antimicrobial activity of *Hibiscus sabdariffa*, *Centella asiatica*, *Moringa oleifera* and *Murraya koenigii* leaves. *J Med Plants Res.* 2011;5(30):6672-80. Available: <https://doi.org/10.5897/JMPR11.621>
39. Hillman WS, Culley DD, Jr. The Uses of duckweed: the rapid growth, nutritional value, and high biomass productivity of these floating plants suggest their use in water treatment, as feed crops, and in energy efficient farming. *Am Sci.* 1978;66(4):442-51.

40. Kalidupa N, Kurnia A, Nur I. Study of the utilization of red dragon fruit peel flour (*Hylocereus polyhizus*) in feed for the coloring of koi carp (*Cyprinus carpio* L.). *Aquat Media*. 2018;3(1):590-7.
41. Ariyanti H, Apriliana E. The effect of phytoestrogens on menopause symptoms. *majority*. 2016;5(5).
42. Kurniawan M, Izzati M, Nurchayati Y. Chlorophyll, carotenoid, and vitamin C content in several aquatic plant species. *Anatomy and physiology bulletin*. XVIII. 2010;1.
43. Daud A, Sulistyarti H, Retnowati R, Ginting E. High-performance liquid chromatography (hplc) method for determining isoflavones content in shade-tolerant soybean with i. *IOP Conf S Mater Sci Eng*. 2019;546(3).
Available: <https://doi.org/10.1088/1757-899X/546/3/032004>
44. Li B, Tian L, Zhang J, Huang L, Han F, Yan S, et al. Construction of a high-density genetic map based on large-scale markers developed by specific length amplified fragment sequencing (SLAF-seq) and its application to QTL analysis for isoflavone content in *Glycine max*. *BMC Genomics*. 2014;15(1):1086.
Available: <https://doi.org/10.1186/1471-2164-15-1086>
45. Nemitz MC, Yatsu FKJ, Bidone J, Koester LS, Bassani VL, Garcia v. C, et al. A versatile, stability-indicating and high-throughput ultra-fast liquid chromatography method for the determination of isoflavone aglycones in soybeans, topical formulations, and permeation assays. 2015;134. *Talent*;:183-93.
Available: <https://doi.org/10.1016/j.talanta.2014.10.062>
46. Istiani Y, Handajani S, Pangastuti A. Characterization of isoflavone bioactive compounds and antioxidant activity test of ethanol extract of tempeh made from koro sword (*Canavalia ensiformis*). *Biopharmaceuticals*. 2015;13(2):50-8.
47. Setiawati A, Yuliani SH, Istyastono EP, Gani MR, Veronica EF, Putri DCA, et al. Quick and simple quantitative analysis of tempeh isoflavones using the thin-layer chromatography method densitometry. *J Sci Community Pharm*. 2014;11(1):13-7.
48. Sulistyowati E, Martono S, Riyanto S, Lukitaningsih. Analysis of daidzein and genistein on soybean (*Glycine max* L. Merrill) Varieties Anjasmoro, Argomulyo, and Dena 2 Using the HPLC Method. *Indonesian Pharmaceutical Media*. 2018;13(1):1299-304.
49. Fawwaz M, Natalisnawati A, Baits M. Isoflavone aglycone levels in soy milk extract and Tempe determination of isoflavone Aglicone in extract of soymilk and Tempe. *Ind J Agro-Ind Technol Manag*. 2017;6(3):152-158.
Available: <https://doi.org/10.21776/ub.industria.2017.006.03.6>
50. Sulistiani HR, Handayani S, Pangastuti A. Characterization of isoflavone bioactive compounds and test of antioxidant activity from the ethanol extract of tempeh made from black soybean (*Glycine soja*), black koro (*Lablab purpureus*), and koro kratok (*Phaseolus lunatus*). *Biopharmaceuticals*. 2014;12(2):62-72.
51. Djide MN, Permana D, Ismail. Soybean Isoflavones Extraction and Determination of Their Concentration by Ultra Fast Liquid Chromatography (UFLC) Soybean Isoflavones Extraction and Analysis Their Concentration by Ultra Fast Liquid Chromatography. *Sartini. Sci J*. 2014;3(2):130-4.
Available: <http://ojs.unm.ac.id/index.php/sainsmat>
52. Subhan U, Mawar R, Andriani Y. Effect of Lemna Administration on Nilem Fish Egg Carotenoids (*Osteochilus hasselti*). *BIOTICS*. 2018;16(2).
53. Laining A, Usman U, Ika T. Fecundity, fatty acid profile, and carotenoid content in baronang fish, *Siganus guttatus*, fed two types of maturation feed. *Aquaculture Technology Innovation Forum*; 2015.
54. Farida GS, Hasan H. Addition of turmeric flour and oodev in feed to induce gonad maturation of biawan fish (*Helostoma temminckii*). *Ruaya [journal]*. 2018;6(2):70-80.
55. Pratiwi YE. Reproductive performance of broodstock chef (*Carassius auratus*) given a combination of oodev and Moringa Leaf Flour (*Moringa oleifera*) mixed feed [thesis]. Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Bogor Agricultural University; 2019.

56. Yaeni T, Suminto YT. Utilization of sweet potato extract (*Ipomoea Batatas* var *Ayumurasaki*) in feed for body color performance, growth and survival of rainbow fish (*Melanotaenia praecox*) utilization. *J Aquacult Manag Technol.* 2017;6(3): 293-302.

© 2023 Iskandar et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:

<https://www.sdiarticle5.com/review-history/103170>