TIK-87 Effects of Vitamin C and Squid Oil Supplementation on Gonad Maturation of Climbing Perch Broodstock (Anabas testudineus Bloch).

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Submission date: 19-Jun-2024 03:10PM (UTC+0700)
Submission ID: 2405224320
File name: TIK-87.pdf (386.27K)
Word count: 4279
Character count: 20923

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Abstract. This study aims to determine the effectiveness of adding vitamin C and squid oil to commercial feed for broodstock fish (*Anabas testudineus* Bloch) on the speed of gonadal maturation and growth. The study was conducted for three months using a completely randomized design with 4 treatments and 3 replications. The test fish were 10 each in each treatment hapa, the treatment of feed A (Commercial feed =CF); B (CF + Vit c 2gr/kg; C (CF+ squid oil 3 ml/kg) and D (CF+ Vitamin C 2 g/kg + Squid oil 3 ml/kg). Feed was given in the morning and evening satiation for 30 days. The results showed that the commercial feed added with a combination of vitamin C 2000mg/kg feed and squid oil as much as 3mg/kg feed (Treatment D) gave the best gonadal maturity level. This is indicated by the average Gonadosomatic Index (GSI) of 4.20% and the average HepatoSomatic Index (HSI) of 3.91%, higher than other treatments. Supported by statistical test results showed very significant differences in somatic gonadal index, hepatosomatic index, egg diameter and absolute fecundity. Meanwhile, growth and condition factors did not show significant differences. There is a tendency that if the dose of vitamin C and squid oil is increased again, it will be able to increase the gonad maturity of the broodstock of climbing perch.

1. Introduction

Papuyu fish or betok fish is one of the local fish species that are commonly found in the inland waters of Kalimantan, especially swamp waters. Although the spiny papuyu fish is very popular with the surrounding community because of its savory and slightly fatty taste [1], so its cultivation business is very attractive. The key to the success of local fish farming is the availability of quality and quantity of seeds. Quality seed is not only determined by genetic factors, but is determined by health factors and the feed given to the parent [2]. From various studies that have been carried out on several marine and freshwater fish, it can be seen that good parental feeding can improve broodstock performance in gonadal development, spawning quality and larval quality [3].

One of the main problems in cultivation-scale seed production is the lack of availability of mature broodstock. Where in the culture container, gonad maturity can be stimulated in various ways, for example by manipulation of environmental factors, diet (nutrient content of feed), and the use of hormones. It is reported that the dominant environmental factors influencing the development of the gonads are temperature and food [4].

Vitamin C is one of the micronutrients needed by brood fish in the reproductive process. This is based on fluctuations in the vitamin C content of the ovaries during the reproductive cycle. The content of vitamin C in the ovaries will increase at the beginning of its development and then decrease in the late phase before ovulation. Generally, fish species do not have the ability to biosynthesize vitamin C. For this reason, fish's vitamin C needs must be supplied from outside.

Fatty acids are one of the factors that greatly affect the reproductive success and survival of hatched larvae. Fish cannot synthesize linoleic and linolenic fatty acids by themselves, so to meet their needs they need to be added to the feed [5].

Based on the above, it is necessary to do research on the addition of important nutrients to feed, one of which is vitamin C and squid oil as a source of fatty acids to trigger gonad maturation of brood fish. The purpose of this study was to determine the effect of adding vitamin C and squid oil to commercial feed given to brood fish (*Anabas testudineus* Bloch) living in tidal swamp waters of Kalimantan, on the speed of the gonadal maturation process.

2. Materials and Methods

2.1. Place and time of research

This research was conducted at the Wet Laboratory of the Faculty of Fisheries, Lambung Mangkurat University, Banjarbaru with a time of about 3 months.

2.2. Research Management

The broodstock used in this study came from the tidal swamp waters of Marabahan. The number of broodstock required is 120 female broodstock, with a stocking density of 10 per cage. The size of the cage used is 1 x 1 x 1 m.

The feed used was commercial feed with 30% protein content which was given satiation. The commercial feed was added with supplements in the form of vitamin C 2000mg/kg feed and squid oil 3mg/kg feed, and as a control was given commercial feed without additional supplements. Feeding is done every day as much as 2 times a day, namely in the morning and evening.

Observations of the fish gonads were carried out at the end of the study by dissecting the fish's body. In addition, supporting parameters were also observed, namely the growth of test fish, condition factors and measurements of water quality including temperature, DO, pH and NH3 will be observed at the beginning and end of the study.

For gonad collection and sexing, fish were sacrificed and dissected carefully. Gonads were isolated and weighed by using a sensitive portable electronic balance (XS Analytical105).

The GSI was determined by the following formula [6] and HSI was calculated by using the formula [7].

2.3. Experimental Treatment and Design

In this study, the treatments used were 4 treatments and 3 replications:
A = Commercial feed (control)
B = Commercial feed + Vitamin C
C = Commercial feed + squid oil
D = Commercial feed + Vitamin C + squid oil
The supplements used were Vitamin C 2000mg/kg feed and squid oil 3mg/kg feed.
This study used a completely randomized design (CRD) with 4 treatments (A, B, C and D) and 3 replications (1,2,3), so it would produce 12 experimental units.

2.4. Observation Parameter

The parameters observed included the main parameters, namely GSI (Gonado Somatic Index), HSI (Hepato Somatic Index), Egg Diameter, and Absolute Fecundity. As for the supporting parameters, namely the Relative Weight Growth Rate, Relative Length Growth Rate, Condition Factors and Water Quality.

3. Results and Discussion

3.1. SGI (Somatic Gonad Index)

The somatic gonadal index value was used to predict when the fish would be ready to spawn.

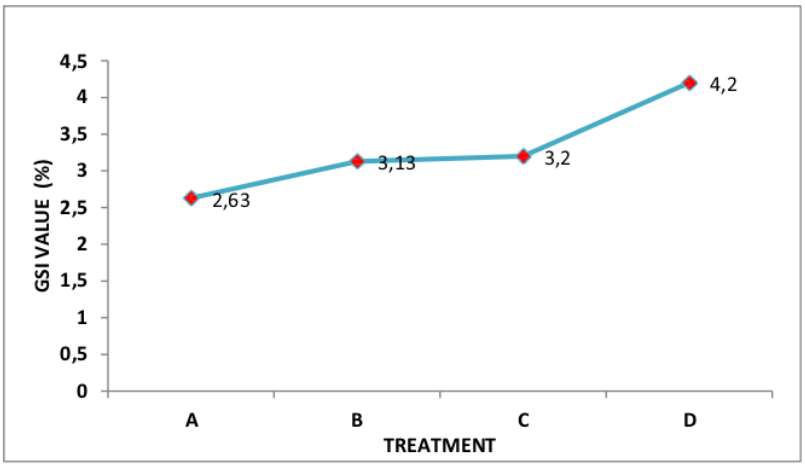

Figure 1. Average Gonadosomatic Somatic Index (GSI) Value of Climbing Perch Broodstock

Based on Figure 1, it is known that the average value of the largest Gonada Somatic Index (GSI) is shown in treatment D of 4.20%, then followed by treatment C of 3.20%, then treatment B of 3.13% and treatment D of 2, 63%. The lack of and excess of vitamin C and squid oil in the feed can affect the success of the gonad maturity level and the process of embryogenesis which causes low fecundity, the degree of fertilization of eggs and the degree of hatching of eggs [5].

If you look at the range of GSI values at GSI stage I (unripe) to GSI stage IV (ripe) which is 0.0189% to 14.9830%, it can be seen that in this study the fish were in GSI stage III (go to ripe/cooking) with the characteristics of the eggs that can be distinguished by eye. Usually, the gonads gain weight rapidly, the testes change from a transparent color to a pinkish color. The GSI values ranged from 2.63% to 4.20%. The weight gain of the gonads will continue until it reaches its maximum weight at GSI IV (ripe).

The increase in GSI value can be caused by oocyte development. During the vitellogenesis process, the yolk granules increase in number and size so that the oocyte volume increases [8]. During this process, most of the metabolic products are focused on the development of the gonads.

3.2. HSI (Hepato Somatic Index)

The Hepato Somatic Index (HSI) is used to describe the energy reserves that exist in the fish body when the fish undergoes the development of gonadal maturity.

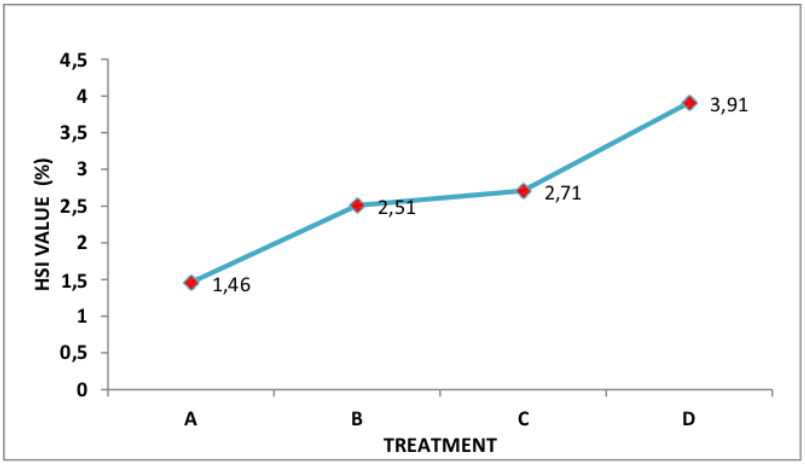

Figure 2. Average Hepato Somatic Index (HIS) of Climbing Perch Broodstock

From Figure 2, it is known that the average value of the largest HIS is shown in treatment D at 3.91%, then followed by treatment C at 2.71%, then treatment B at 2.51% and treatment A at 1.46%. The hepatosomatic index value is related to the somatic gonadal index value. During the development of gonadal maturity, the HSI value and SGI value will continue to increase except when the fish will spawn, the GSI value will reach the maximum range limit, while the HSI value will decrease..

If you look at the range of HSI values at Gonad Maturity Level (GML) I (unripe) to GML IV (ripe) which is 0.6067% to 5.5327%, it can be seen that in this study the fish were in GML III (cooking) with HSI values ranging from 1.46%. up to 3.91%. The increase in liver weight will continue until it reaches its maximum weight at GML IV (ripe). The HSI value in line with the progress of ovarian development. As mentioned [9], the liver is an organ that stores energy reserves and will be dismantled to support the development of oocytes (egg cells) during the vitellogenesis process (egg yolk synthesis process). [10] added that the liver plays an important role in fat metabolism. Fat synthesized in the liver will be partially stored in the form of fat granules.

[9] In the liver, it can be seen changes in energy content, where liver energy levels tend to increase at the beginning of the first to third gonad maturity, then decrease to the final gonadal maturity level (GML V). In the present study on histological analysis of female *Anabas testudineus*, it was observed that vitellogenic oocyte with yolk vesicles increased in number and size. Maturing stage with perinucleolar and previtellogenic oocytes were noticed during July. The mature stage with primary, secondary and tertiary oocytes were found in their early phase and ovary with migratory nucleus at the peripheral region with numerous vacuoles. This indicates that the ovarian development of A. testudineus is group asynchronize in nature. These findings are complemented with the outputs of [11] who worked with *Pangasianodon hypophthalmus*. Similar result was also observed for C. striatus. Presence of different development stages in ovary at a time indicates the asynchronous nature of ovary development in this fish.

In the present study of Vietnamese Climbing Perch, the GSI showed higher values during the June to July; therefore, this period may be regarded as the peak spawning season even though normal breeding season may be started before these months. Highest fecundity was found 46186.14± 2219.15 in July. Based on the histological findings of ovary of the species is considered to have group asynchronous nature which indicates long duration required to manipulate with the brood stock in the hatchery. This condition makes it clear that natural environmental factors influence.

3.3. *Egg Diameter*

[12] state that egg size is indicated by egg diameter. The use of vitamin C and fatty acids can affect the size of the resulting fecundity value has something to do with egg diameter. The size of the egg diameter is closely related to the accumulation of nutrients in the egg itself.

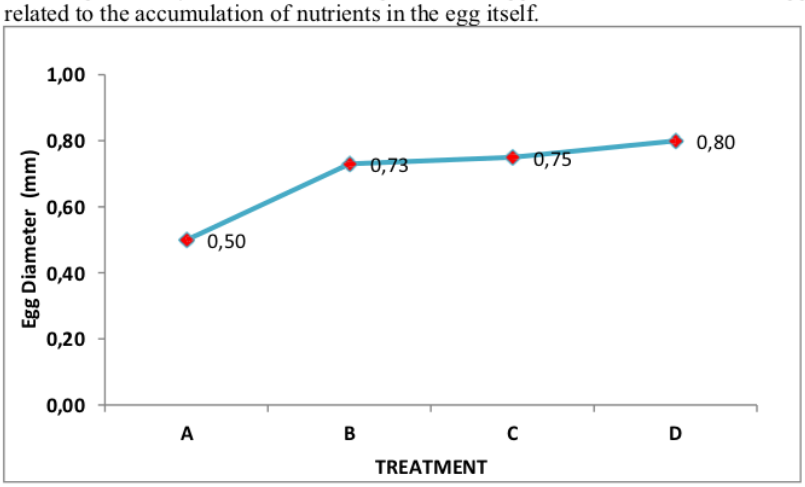

Figure 3. The Average Result of Egg Diameter of Climbing Perch Broodstock

Based on Figure 3, it is known that the average result of the largest egg diameter is shown in treatment D of 0.80 mm, then followed by treatment C of 0.75 mm, then treatment B of 0.73 mm and treatment D of 0.50 mm.

If you look at the size of the egg diameter of female climbing perch that have matured gonads, namely 0.23-1.42 mm, it can be seen that in this study the climbing perch have experienced gonadal maturity but have not yet matured because the size of the egg diameter has not reached the maximum size, which ranges from 0.78 mm to 0.78 mm. 0.88 mm and will continue to increase in size to maximum size until the egg is cooked and ready to be removed.

The presence of fat in the egg can be preserved before being used for the next development process. According to [5] the essential fatty acids contained in eggs affect the early stages of embryogenesis to determine whether the embryo will develop or not.

The more developed the fish gonads, the eggs contained in them are getting bigger in diameter. This is due to the result of the deposition of egg yolk and the formation of oil droplets that proceed gradually in the development of the gonad maturity level [13].

3.4. *Fecundity*

Absolute fecundity is the number of eggs present in fish ovaries.

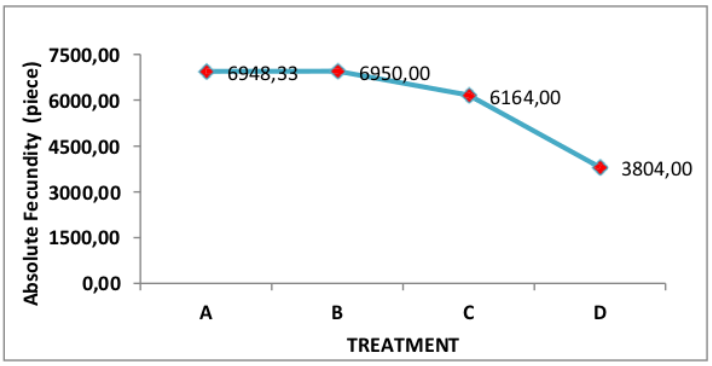

Figure 4. Results of Absolute Fecundity of Climbing Perch Broodstock

Based on Figure 4, it is known that the highest absolute fecundity average results were shown in treatment B of 6950 items, then followed by treatment A of 6948.33 items, then treatment C of 6164 items and treatment D of 3804 items. In contrast to the results of the parameters of GSI, HSI and egg diameter, in absolute fecundity this time, treatment B was the best where the fish kept were fed with additional vitamin C and fatty acids. Fish fecundity is closely related to its environment because the environment can affect the growth of fish length and weight. Changes in fecundity related to food availability. In general, fast-growing individuals have higher fecundity than slow-growing fish of the same size. Fish fecundity is influenced by fish size (length and weight) and fish age, however the greatest fecundity is determined by the amount of feed consumed [14], stated that there is a close relationship between gonad weight and absolute fecundity, this is because 66% of the absolute fecundity of fish is determined by gonad weight while 34% by other factors.

3.5. Relative Weight Growth Rate

The relative weight growth is defined as the percentage of fish body weight growth at each time interval.

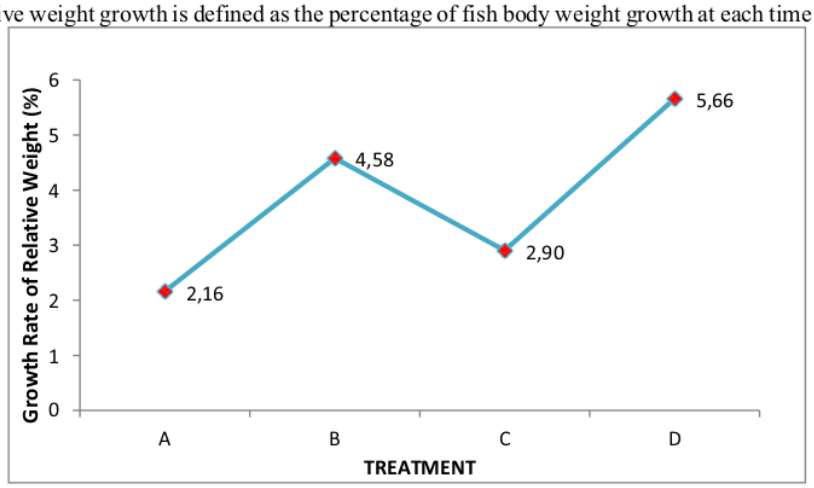

Figure 5. Results of Average Growth Rate of Relative Weight of Climbing Perch Broodstock

Based on Figure 5, it is known that the average result of the largest relative weight growth rate is shown in treatment D of 5.66%, then followed by treatment B of 4.58%, then treatment C of 2.9% and treatment A of 2, 16%.

From the results obtained, it is known that in treatment D it turned out that the climbing perch broodstock experienced the greatest weight growth compared to other treatments, this was because the

addition of vitamin C and squid oil to the feed could meet the nutritional needs of climbing perch broodstock in the weight growth process. So that besides being able to be used for gonad maturation, the existing nutrients can also be used for the weight growth of the brood fish.

In treatments A and B there was also a growth in broodstock weight but the results were not too large, this was because the content of vitamin E in the feed in treatments A and B was mostly used for the gonad maturation process and only a small portion was used for the growth process. Treatment A, treatment B and treatment C experienced somatic growth, which is the normal growth of fish without focusing on gonadal development. [15] reported the role of squid oil as an attractant in feed, where feed with the addition of squid oil gave the fastest response to attract fish compared to fish fed a diet without squid oil. According to [16] attractants are generally produced from free amino acids, and it is reported that the use of a single amino acid often does not provide a stimulus for fish to eat the feed.

3.6. Relative Length Growth Rate

Relative length growth is defined as the percentage of fish body length growth at each time interval

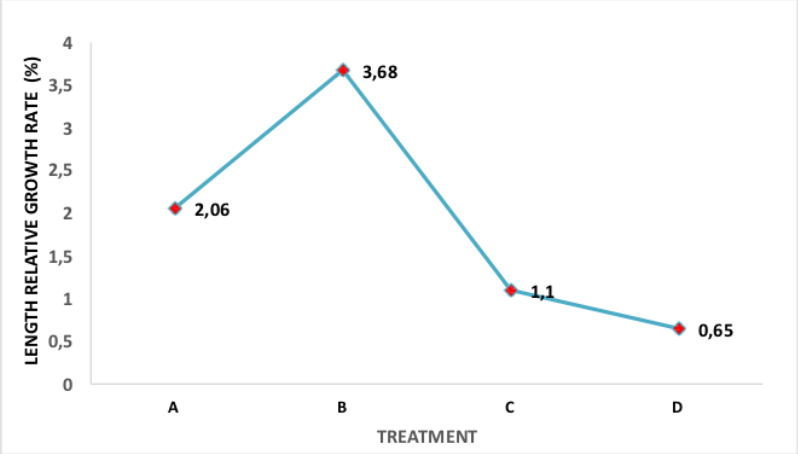

Figure 6. Results of Average Growth Rate of Climbing Perch Broodstock

Based on Figure 6, it is known that the average result of the largest relative length growth rate is shown in treatment B of 3.68%, then followed by treatment A of 2.06%, then treatment C of 1.1% and treatment D of 0.65%. Seeing from the results obtained, the length growth of the broodstock of the four treatments did not differ much. Because the mother fish are reared, so that most of the metabolic products are focused on the development of the gonads, even if there is a small portion for growth, it is for weight growth, not length growth. So the results obtained are not much different. And from the results of this length parameter, it can be seen that the feed given to the treatment was good for somatic growth.

3.7. Condition Factor

Condition factor is a condition that states the quality of fish plumpness, the calculation of which is based on the length and weight of the fish.

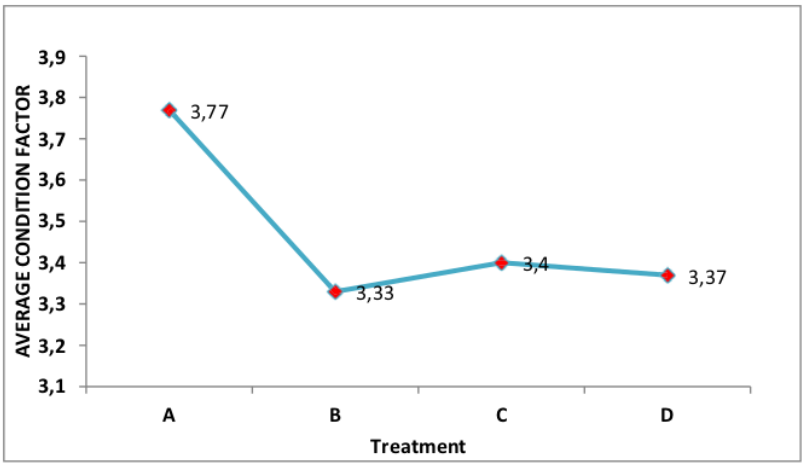

Figure 7. Average Result of Climbing Perch Broodstock Condition Factors.

Based on Figure 7, it is known that the average result of the greatest condition factor is shown in treatment A of 3.77, then followed by treatment D of 3.47, then treatment C of 3.4 and treatment B of 3.33. Parent fish reared with four treatments resulted in weight growth that was faster than the growth in length. This is also followed by the condition factor (K) of brood fish, where the K value ranges from 3.33 to 3.37. According to [17] the K value for fish with less flat bodies ranges from 1 to 3. The relationship between standard length and body weight and the condition of a fish depends on food, age, sex type and gonad maturity [17].

After the data above was tested for normality and homogeneity, it turned out that all data were normal and homogeneous. And after testing the analysis of variance (ANOVA) the results of SGI, HSI, egg diameter and absolute fecundity were very significantly different, and not significantly different from the results of relative weight growth rate, relative length growth rate and condition factors [18].

The results of water quality measurements which include temperature, DO, pH and ammonia (NH3) at the time of the study stated that the water temperature conditions in the study area were still within normal limits and supported the life of the betok fish [19]. This is because all the measured parameter values still meet the standard of living.

The value of water quality parameters, ammonia (NH3) at the time of the study tends to increase, along with the addition of vitamins C and as. Fat added to commercial feeds. Although there is an increase, the value still meets the standard of living so that it does not affect the condition of the fish [20].

4. Conclusion

Feed with a combination of vitamin c and squid oil gave a better increase in SGI and HSI parameters than other treatments.

Acknowledgments

We would like to thank the Aquaculture department Fsheries and Marine Faculty University Of Lambung Mangkurat for Laboratory Analyzed Support of this research. The gratefull expression is also delivered to the lecturer program must research University Of Lambung Mangkurat. for financial support of this research.

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