TIK-178 Hematological Response Of Gurami Fish (Osphronemus gouramy) To Feeding With Kelakai Leaf Silage (Stenochlaena pallustris)

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HEMATOLOGICAL RESPONSE OF GURAMI FISH (OSPHRONEMUS GOURAMY) TO FEEDING WITH KELAKAI LEAF SILAGE (STENOCHLAENA PALLUSTRIS)

Fauzana Noor Arida*, **Slamat**, **Rini Ririen Kartika**, **Aisiah Siti, Fatmawati**, **Farisie Ahmad Hidayatullah**
Department of Aquaculture, Faculty of Fisheries and Marine Science,
University of Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia
*E-mail: noor.afauzana@ulm.ac.id

ABSTRACT
An alternative to minimizing fish stress is by providing additional feed that is high in protein and antioxidants. Kelakai is an additional feed in kelakai leaf silage for gourami fish. Kelakai has a fairly high protein and antioxidant content so it can support fish health. The research aims to analyze the hematological response of gourami fish fed kelakai leaf silage. Research was carried out at the Wet Laboratory of the Faculty of Fisheries and Marine Affairs, ULM Banjarbaru for maintenance and the Banjarbaru Veterinary Center for hematological tests. Completely Randomized Design (CRD) with 7 treatments and 3 replications. The treatments in the study were K0: Pellet feed + fresh Kelakai, K1: Pellet feed + Kelakai silage with a shelf life of 1 day, K2: Pellet feed + Kelakai silage with a shelf life of 2 days, K3: Pellet feed + Kelakai Silage has a shelf life of 3 days, K4: Pellet feed + Kelakai Silage has a shelf life of 4 days, K5: Pellet feed + Kelakai Silage has a shelf life of 5 days and K6: Pellet feed + Kelakai Silage has a shelf life of 6 days. Evaluation of feeding kelakai silage in improving the hematological response of gourami fish showed an influence on erythrocytes and hematocrit, and no influence on leukocytes and hemaglobin. Increasing the hematocrit and erythrocyte values means improving the health status of the fish, so kelakai leaf silage provides a good hematological response to gourami fish.

KEY WORDS
Gourami fish, silage, kelakai leaves, hematology.

Osphronemus gouramy (gourami) is a fish native to Indonesian waters which has spread throughout the waters of Southeast Asia and China. Gourami is one of 12 commodities to fulfill community nutrition, to increase production and income of the community, gourami fish is widely developed by cultivators, this is because market demand is quite high and maintenance is relatively easy and has high economic value (Ricky, 2008). Fish will grow well if the substances contained in the feed that are digested and absorbed by the fish's body are greater than the amount needed to maintain the body (Setiawati et al, 2013).

Several studies on gourami fish feed have been carried out using several plants such as cassava leaves (Syahrizal, Ghofur and Fakhrurrozi, 2013), sente leaves and water hyacinth leaves (Nofyan, 2005), lamtoro leaves (Siregar, Suharman and Adelina, 2016), kale leaves (Sulhi, Samsudin and Hendra, 2011), taro leaves (Elfrida and Yuspita, 2017), and papaya leaves (Ratna, 2017), whether given directly, as a mixture in artificial feed formulations, or as additional feed given in combine with artificial feed. The additional feed given in this research was kelakai silage.

The Kelakai plant (Stenochlaena pallustris) is a type of fern. Kelakai is generally used by the people of South Kalimantan as a vegetable and has been used for generations as a traditional medicine, which the Dayak people believe is able to treat anemia and is used to increase energy after giving birth (Maharani et al., 2006). Kelakai has a fairly high protein content. The results of Fatmawati and Fauzana's (2016) research stated that young leaves of kelakai contain up to 27.31% protein compared to 26.79% of old kelakai leaves. Kelakai contains high Fe (41.55 ppm), kelakai also contains CU (4.52 ppm), vitamin C (15.41 mg/100g), beta carotene (66.99 ppm), and folic acid 11.30 ppm). Kelakai also contains flavonoids. Flavonoids are a group of phenolic compounds that have two main roles, namely as antioxidants and antibacterials (Irawan et al., 2006). According to empirical studies, kelakai as an antioxidant, the active substances are iron (Fe), calcium, vitamin C and vitamin A. and has properties related to blood (Fahruni. et al,. 2018).

Blood is very useful as a diagnostic tool in determining the health status of fish. One aspect of infection is a change in the blood picture. Blood experiences serious changes, especially when exposed to infectious diseases. Several parameters that can show pathological changes in the blood are hematocrit levels, hemoglobin, red blood cell count and white blood cell count (Bastiawan et al., 2001). Hematology is often also used to detect physiological changes caused by environmental stress and is also related to fish health status. Parameters that are usually used as indices in determining fish health status are total red blood cells, white blood cells, hemoglobin, hematocrit, while to see stress levels usually also measured cortisol and blood glucose levels (Al-Attar 2005). Hemoglobin (Hb) is a red pigment that carries oxygen in red blood cells which is a protein rich in iron. Some indicators of early stress can be seen from blood glucose levels, hemoglobin percentage (Mazeaud and Mazeaud, 1981). A decrease or increase in hematological parameters in the blood indicates that physiological deviations have occurred in the fish.

Several studies related to the hematological response of gourami fish have been carried out by Yunus et al (2020) on rearing media with different salinities, apart from that, Hastuti & Supriyono (2003) also conducted research on the hematological response related to changes in environmental temperature. Other research related to hematological responses and feed is based on research by Prasetio et al (2017) regarding jelawat fish given aloe vera powder in feed which can increase the hematological response in the body and have a good influence on fish health. According to Eviani et al (2019), the hematological response of tilapia fed with gamal leaf extract (Gliricidiasepium) can also increase the fish's hematological response. Research on the hematological response of gourami fish based on the feed given has not provided any information, so this research was carried out based on the additional feed given. An alternative to minimizing fish stress is by providing additional feed that is high in protein and antioxidants. Kelakai is an additional feed for kelakai leaf silage for gourami fish. Kelakai has a fairly high protein and antioxidant content so it can support fish health. The research aims to analyze the hematological response of gourami fish fed kelakai leaf silage.

METHODS OF RESEARCH

Research was carried out at the Wet Laboratory of the Faculty of Fisheries and Marine Science, ULM Banjarbaru for maintenance and the Banjarbaru Veterinary Center for hematological tests.

The research container used 21 black happa measuring 1 m x 1 m x 1 m which were installed hanging in the pond and equipped with a wooden frame and aeration. The test fish used in this study were gourami fry measuring 4-6 cm.

Sampling was carried out every month for 90 days at the beginning, middle and end of the study (3rd month).

This research was carried out using an experimental method using a Completely Randomized Design (CRD) with 7 treatments and 3 replications. The treatments in this study were as follows:

- K0: Pellet feed + fresh Kelakai;
- K1: Pellet feed + Kelakai silage, shelf life 1 day;
- K2: Pellet feed + Kelakai silage with a shelf life of 2 days;
- K3: Pellet feed + Kelakai silage with a shelf life of 3 days;
- K4: Pellet feed + Kelakai silage with a shelf life of 4 days;
- K5: Pellet feed + Kelakai silage with a shelf life of 5 days;
- K6: Pellet feed + Kelakai silage with a shelf life of 6 days.

Hematological studies are important criteria for diagnosis and determining fish health (Lestari, 2001). Hematological examination of gourami fish was carried out at the Banjarbaru Veterinary Center.

RESULTS OF STUDY

The Liliefors Normality Test on the number of leukocytes shows the maximum L value (0.154) < L table 5% (0.188), so the data is distributed normally. The results of the Bartlett Homogeneity Test (Appendix 15) show the results X2 Calculate (9.305982626) < X2 Calculate table 5% (12.59159) and The Analysis of Variance Test (ANOVA) produced calculated F (0.724) < F table 5% (2.85) and F table 1% (4.46), the results showed that additional feeding of kelakai leaf silage had no significant effect on the number of leukocytes. The graph of mean leukocytes for gourami fish shows that the mean leukocytes range 232.63-277.37. The highest mean was in treatment K3 (pellets + kelakai leaf silage 3 days) and the lowest was in treatment K4 (pellets + kelakai leaf silage 4 days) namely 232.63.

Table 1 – Leukocyte data for gourami fish (Osphronemus gouramy) fed additional kelakai leaf silage (Stenochlaena palustris)

Treatment	Leukocytes ($10^3/\mu l$)
K0	285,50 ± 165,05
K1	282,20 ± 162,93
K2	259,40 ± 17,67
K3	277,37 ± 5,12
K4	297,90 ± 171,99
K5	286,65 ± 165,71
K6	294,70 ± 170,15

Source: Primary Data (2021). Mean Leukocytes (Sig value 0.05 and Sig 0.01).

Table 2 – Erythrocyte data of gourami fish (Osphronemus gouramy) fed with additional kelakai leaf silage (Stenochlaena palustris)

Treatment	Erythrocyte ($10^6/\mu l$)
K0	$1,91 \pm 0,34^{ab}$
K1	$2,05 \pm 0,42^{b}$
K2	$1,54 \pm 0,09^{a}$
K3	$1,75 \pm 0,07^{a}$
K4	$2,41 \pm 0,24^{c}$
K5	$1,89 \pm 0,21^{a}$
K6	$2,28 \pm 0,31^{bc}$

Source: Primary Data (2021). Mean Erythrocytes followed by the same superscript letter are not significantly different (Sig value 0.05 and Sig 0.01).

The erythrocyte data was subjected to data analysis in the form of the Liliefors Normality Test (Appendix 17) showing the maximum L value (0.181) < L table 5% (0.188) so the data was declared to be normally distributed. The results of the Bartlett Homogeneity Test (Appendix 18) show the results X2 Calculate (7.953816864) < X2 Calculate table 5% (12.59159) and The Analysis of Variant Analysis (ANOVA) test (Appendix 19) produced calculated F (3.710) > F table 5% (2.85) and < F table 1% (4.46), so these results indicate that additional feeding of kelakai leaf silage has an effect. significant effect on the number of erythrocytes. Further tests using Duncan (Appendix 20) showed that K0 results were not significantly different from K2, K3, K5, K1 and K6. The results for K1 are significantly different from K2, K3, and K5. The results of K4 are significantly different from K0, K1, K2, K3 and K5. The graph of the mean erythrocytes for gourami fish shows that the mean ranges from 1.54 – 2.41. The highest data was in treatment K4 (pellets + kelakai leaf silage 4 days) and the lowest was in treatment K2 (pellets + kelakai leaf silage 2 days) namely 1.54.

The Liliefors Normality Test for the amount of hemoglobin shows the maximum L value (0.166) < L table 5% (0.188), so the data is declared to be normally distributed. The results of the Bartlett Homogeneity Test (Appendix 22) show the results X2 Calculate (25.45648336) <

X2 Calculate table 5% (12.59159) and The Analysis of Variance Test (ANOVA) produced calculated F (1.299) < F table 5% (2.85) and F table 1% (4.46), so the results showed that additional feeding of kelakai leaf silage had no significant effect on the amount of hemoglobin. The graph of the average hemoglobin for gourami fish shows that the average ranges from 5.97 – 10.63. The highest mean was in treatment K4 (pellets + kelakai leaf silage 4 days) and the lowest was in treatment K5 (pellets + kelakai leaf silage 5 days) namely 5.97.

Table 3 – Hemaglobin data for gourami fish (Osphronemus gouramy) fed additional kelakai leaf silage (Stenochlaena palustris)

Treatment	Hemoglobin (gr/dl)
K0	10,17 ± 0,21
K1	9,07 ± 0,38
K2	7,67 ± 0,48
K3	9,23 ± 0,17
K4	10,63 ± 0,67
K5	5,97 ± 1,02
K6	10,30 ± 0,38

Source: Primary Data (2021). Mean Hemoglobin (Sig value 0.05 and Sig 0.01).

Table 4 – Hematocrit data of gourami fish (Osphronemus gouramy) fed with additional kelakai leaf silage (Stenochlaena palustris)

Treatment	Hematocrit (%)
K0	23,87 ± 0,13 ^{a}
K1	25,13± 0,11^{b}
K2	18,03 ± 0,05 ^{a}
K3	21,00 ± 0,03 ^{a}
K4	33,30 ± 0,07 ^{b}
K5	23,77 ± 0,11 ^{ab}
K6	31,07 ± 0,07 ^{b}

Source: Primary Data (2021). Mean Hematocrit followed by the same superscript letter is not significantly different (Sig value 0.05 and Sig 0.01).

The Liliefors Normality Test for hematocrit data shows the maximum L value (0.197) < L table 5% (0.188), so the data is declared to be normally distributed. The results of the Bartlett Homogeneity Test (Appendix 25) show the results X2 Calculate (6.236434466) < X2 Calculate table 5% (12.59159) and The Analysis of Variety Test (ANOVA) produced calculated F (3.397) > F table 5% (2.85) and < F table 1% (4.46), so the results showed that additional feeding of kelakai leaf silage had a significant effect on the hematocrit number. Further tests using Duncan (Appendix 27) showed that K6 was significantly different from K5 but not significantly different from K0, K2 and K4. K5 is not different from K4 and K2 but is significantly different from K0, K3 and K4, while K0, K2 and K3 are not significantly different. The graph of the average hematocrit for gourami fish shows that the average ranges from 18.03 – 33.30. The highest mean was in treatment K4 (pellets + kelakai leaf silage 4 days) and the lowest was in treatment K2 (pellets + kelakai leaf silage 2 days) namely 18.03.

DISCUSSION OF RESULTS

Hematological conditions can describe the health of fish. Hematology is closely related to pathology, especially to obtain an overview of the fish's health condition, whether it is healthy or sick. The circulatory system has many functions, in general its function is as a transportation system, including oxygen, carbon dioxide, food essences, and body metabolism. Blood carries substances from where they are formed to all parts of the body and keeps the body from carrying out its functions properly (Fujaya, 2002). Blood parameters that can indicate a disturbance in the fish's body are the hematocrit value, hemoglobin concentration, number of erythrocytes (red blood cells) and number of leukocytes (white blood cells) (Lagler et al., 1977). According to Salasia et al., 2001; Lukistyowati and Windarti, 2007 and Alamanda et al., 2007) that the use of hematology methods is quite effective for diagnosing fish diseases early, namely by paying attention to parameter values in the blood. Observing the hematological condition of fish that are cultivated as a non-specific defense system can be done to determine their health condition as an initial detection in diagnosing fish diseases, so that treatment and disease prevention efforts can be carried out quickly and precisely.

The aim of observing leukocytes is to determine the white blood cells in the fish's blood. This leukocyte value provides clues about the health of the fish and helps to determine the emergence of abnormalities Fitriana (2019). The gourami leukocyte results in this study had an average of 231.573 – 277.37 x 103/μl. The highest leukocytes were in K3 (pellet feed + kelakai silage with a shelf life of 3 days). The lowest leukocyte count was in K4 (pellet feed + kelakai silage with a shelf life of 4 days).

The leukocyte value of other freshwater fish is in the range above the standard value of 200-300 x 103/μL, namely in research by Titrawani et al., (2011) which states that the total leukocyte of paweh fish is around 125-330 x 103/μL. Bastiawan et al (1995) suggested that the number of leukocytes in fish is influenced by the type or species of fish. Apart from the type of fish, the number of leukocytes is also influenced by physiological factors, namely age and muscle activity. The high total leukocytes in fish are a form of fish adaptation to an environment with temperatures that allow pathogenic microorganisms to reproduce (Titrawani et all, 2011).

The leukocyte values were distributed normally and the ANOVA test showed that the number of leukocytes was not significantly different. The number of leukocytes will increase when fish are infected because they are an active unit in the body's defense system, and leukocytes play a role in fighting infectious diseases (Kimball, 1988). Then the number of leukocytes can also decrease if the body is stressed. Factors that influence the normal number of leukocytes must be controlled during fish maintenance so that the fish are always in healthy condition.

Erythrocytes are closed sacs of plasma membrane that contain a compound, namely hemoglobin. Erythrocytes themselves have the function of transporting oxygen to all cells in the body (Sherwood, 2017). Erythrocytes are the most abundant cells compared to other blood cells. This large content causes blood to be red. The lifespan of erythrocytes is approximately 120 days, so that approximately every day 1% of the total number of erythrocytes die and are replaced by new erythrocytes. (Kiswari, 2014).

In this study, the erythrocytes of gourami fish that were fed additional kelakai leaf silage had a mean ranging from 1.54 – 2.41 x 106/μl. According to Irianto (2005) the number of erythrocytes in teleost fish ranges between (1.05-3.0) x 106 /μL. The highest erythrocytes were in K4 (pellet feed + kelakai silage with a shelf life of 4 days) and the lowest were in K2 (pellet feed and kelakai silage with a shelf life of 2 days). According to Wedemayer and Yasutake (1977), low erythrocytes are an indicator of anemia, while a high number of erythrocytes indicates that the fish is in a state of stress. A decrease in the number of erythrocytes indicates a kidney infection, and a low erythrocyte value indicates that the fish is suffering from anemia, while a high number of erythrocytes (above normal) indicates that the fish is in a state of stress..

The erythrocyte results showed that they were distributed normally and the data were homogeneous. The anova test showed that the erythrocytes were significantly different. Erythrocytes in fish are the most abundant type of blood cell. According to Amlacher (1970), the eating response can also influence blood composition, including the number of erythrocytes, which also influences hematocrit. According to Salasia (2001), the number of erythrocytes for each individual varies depending on age, gender, hormones and environment. According to Fitriana (2019), it affects the number of erythrocytes in fish. Hypoxic conditions during blood collection are very likely to cause an increase in erythrocytes.

Hemoglobin (Hb) is part of blood plasma which has an important function in the circulatory system. Hemoglobin plays an important role in transporting gases, especially oxygen, from the gills which are pumped by the heart to all cells and organs of the body, transporting nutrients into cells, removing metabolic waste and so on. Hemoglobin must be in normal condition. Hemoglobin physically has an important relationship with oxygen. When erythrocytes pass through the lung capillaries, hemoglobin binds oxygen to form oxyhemoglobin. On the other hand, when passing through systemic capillaries, hemoglobin will release oxygen to the tissue and become hemoglobin again (Swenson, 1977 in Dopongtonung, 2008).

The research results showed that the amount of hemaglobin in gourami fish was 5.97 – 10.63 g/dl. According to Salasia et al., (2001) the hemoglobin concentration of freshwater fish ranges from 5.05 – 8.33 g/dl. According to Hastuti and Subandiyono (2011), the size of hemoglobin levels in the blood shows the ability of the blood to transport oxygen from the gills to the tissues. Most teleost fish (hard-boned fish) have the same hemoglobin in erythrocytes as other vetebrates. Stress conditions can affect physiological activities and hemoglobin levels in fish. The physiological state of fish blood varies greatly, depending on environmental conditions such as humidity, temperature and pH (Adelbert, 2008).

Gouramy hemoglobin data in this study showed that the data was normally distributed and homogeneous. The anova test showed that the amount of hemoglobin was significantly different. Hemoglobin levels are in harmony with the number of erythrocytes, the higher the hemoglobin level, the higher the number of erythrocytes. Hemoglobin levels are related to the number of erythrocytes, but do not necessarily correlate with the number of erythrocytes because hemoglobin is the pigment content of red blood cells. Hemoglobin levels did not change significantly, although the number of erythrocytes increased (Lagler et al., 1977).

Hematocrit is the volume of blood cells compared to blood plasma expressed as a percentage. This increase in hematocrit levels is influenced by two factors, namely changes in environmental parameters, especially water temperature, and the physiological state of the fish related to the energy required (Marthen, 2005). Hematocrit is the percentage of erythrocyte volume in fish blood or a comparison between the volume of blood cells and blood plasma. Hematocrit can provide clues about the health of fish and help determine the occurrence of abnormalities (Fitriana (2019).

The hematocrit results of gourami fish fed kelakai leaf silage obtained an average hematocrit of between 18.03 – 33.30%. Fange (1992), who stated that the hematocrit in a number of teslostei fish ranges from 20 – 40%. The best hematocrit value of all these treatments was in treatment K4, namely 33.30%. According to Bastiawan et al., (2001), if fish are infected with disease or their appetite decreases, the hematocrit value will be low. Increasing the hematocrit value means improving the health status of the fish, this is in accordance with the opinion of Wedemeyer and Yasutake (1977), according to which the hematocrit level can be used as an indication of low protein content, vitamin deficiency or fish getting an infection. During the research, the hematocrit level values fluctuated quite a bit. Angka et al., (1990) stated that fish hematocrit varies depending on nutritional factors and the age of the fish.

The results of the normality test show that the hematocrit is normally distributed and the data is homogeneous. The anova test shows that the hematocrit is significantly different. Increasing the hematocrit value means improving the health status of the fish. This is in accordance with the opinion of Wedemeyer and Yasutake (1977), according to which the hematocrit level can be used as an indication to determine low protein content, vitamin deficiency or if the fish has an infection. During the research, the hematocrit level values fluctuated quite a bit. Figures et al. (1990) stated that fish hematocrit varies depending on nutritional factors and the age of the fish.

CONCLUSION

Evaluation of feeding kelakai silage in improving the hematological response of gourami fish showed an influence on erythrocytes and hematocrit, and no influence on leukocytes and hemaglobin. Increasing the hematocrit and erythrocyte values means improving the health status of the fish, so kelakai leaf silage provides a good hematological response to gourami fish.

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