JPPIPA 11(2) (2025)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

The Effect of Different *Guanidino Acetic Acid* (GAA) Levels and Protein Source on Blood Profile of Tilapia Fish (*Oreochromis niloticus*)

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Received: May 08, 2024
Revised: December 07, 2024
Accepted: February 25, 2025
Published: February 28, 2025

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DOI: 10.29303/jppipa.v11i2.7608

Abstract: Tilapia (*Oreochromis niloticus*) is a freshwater fish that has high economic value and potential to be cultivated. The problem that often arises in tilapia cultivation is the lack of optimal use of the feed given. The purpose of this research was to find out blood profile of tilapia during culture period. The method used was a completely randomized design (CRD) with five treatments and every treatment with four replications. This study used 240 Tilapia with average weight of 15 g. The stocking density of the aquaria is 15 fish. Test feed in this study is the form of dry pellets consisting combination of GAA levels, protein content (25% dry matter bases), and source of protein (fish meal and non-fishmeal). During the cultured period of 70 days, fish fed by formula diet of 3% biomass per day. Sampling for blood profile tested of erythrocytes, leukocytes, and hematocrit is carried out after the research. The water quality of temperature, pH, and DO were daily monitoring. The combination treatment of guanidino acetic acid (GAA) levels with different protein sources (fishmeal and non-fishmeal) had a significant effect on total erythrocyte of tilapia, but did not have significant differences between treatments on total leukosyte and hematocrit. Treatment with 1.2 g/kg GAA, fish meal protein source was the best treatment in this research.

Keywords: Blood profile; *Guanidino Acetic Acid* (GAA); Protein source; Tilapia

Introduction

Tilapia (*Oreochromis niloticus*) is a freshwater fish that has high economic value and potential to be cultivated. The increasing production of tilapia from year to year to meet export market demand indicates the high prospect of tilapia cultivation (Rohma et al., 2013). According to KKP (2022), the total fisheries production in 2022 is 5.89 million tons. This value consists of capture fisheries of 1.90 million tons and aquaculture of 2.72 tons. FAO data (2022), shows that in 2021 the value of tilapia production reached 1.12 million tons or 31.94% of the total production of freshwater fish farming in Indonesia. Tilapia is widely cultivated because it is easy to breed, and easily adapts to the environment, so the distribution of tilapia in nature is very wide, both in the tropics and in temperate regions (Niode et al., 2017). The factor that plays an important role and determines the success of aquaculture activities is feed. Feed is one of the important aspects that must be considered in aquaculture because feed is a source of energy to support growth. Feed is also one of the main factors to produce maximum production (Yang et al., 2021). Good feed has a large nutritional content, can be obtained and processed easily, fish are easy to digest, has an economical price in the sense that it does not compete

How to Cite:
Afifah, F. O., Mahariawan, I. M. D., Kusuma, W. E., Hidayat, B. R., Yuniarti2, A., & Hariati, A. M. (2025). The Effect of Different Guanidino Acetic Acid (GAA) Levels and Protein Source on Blood Profile of Tilapia Fish (Oreochromis niloticus). *Jurnal Penelitian Pendidikan IPA*, *11*(2), 138–144. https://doi.org/10.29303/jppipa.v11i2.7608

with human needs, and is not toxic to fish. Peng et al. (2023), argue that the lack of optimal utilization of feed for growth is one of several factors inhibiting the success of tilapia aquaculture. Therefore, the addition of guanidino acetic acid (GAA) to artificial feed can be useful for increasing the growth and health status of tilapia (Dilger et al., 2013).

The problem that often arises in tilapia aquaculture is sub-optimal utilization of the feed given for the growth and health status of fish, especially the blood profile of tilapia. *Guanidino acetic acid* (GAA) is a natural precursor of creatine that can increase energy metabolism in the body. Creatine can usually be found in processed animal ingredients, processed animal ingredients are found in meat meal, bone meal, poultry by-product meal, and fish meal. Creatine found in these types of flour is very small and fluctuates (Gonzalez-Esquerra et al., 2006). GAA is formed from non-essential amino acids arginine and glycine that react with the enzyme L-Arginine: glycine amidinotransferase (AGAT) in the kidney (Zhang et al., 2014). GAA then goes to the liver through the circulatory system (Dilger et al., 2013). GAA then goes through a chemical process (methylated) by S-adenosyl methionine (SAM) which is then converted into creatine.

The addition of GAA used in feed is mostly done in feed manufacturing, compared to adding creatine directly to the feed. This is because GAA contains molecules that are quite stable, and GAA is resistant to temperatures when making feed up to 1900C and GAA has a more affordable price than creatine (Tossenberger et al., 2016). GAA plays a role in cellular energy metabolism because it is the sole precursor of creatine in animals and humans. *Guanidino acetic acid* (GAA) according to Li et al. (2020), has been used as a feed additive. Feed additives are used to improve feed intake, growth performance, feed efficiency, antioxidant status, and meat quality in monogastric animals.

Blood profile can be used as one of the growth parameters that are closely related to the health status of fish (Fajriyani et al., 2017). Blood profile includes examination of hemoglobin, hematocrit, and total erythrocytes and leukocytes. Hemoglobin in fish blood functions as a carrier of oxygen, nutrients, and hormones that will be flowed to all parts of the fish body. A decrease in total erythrocytes in the fish body can result in a lack of oxygen in the fish body so that it can interfere with metabolism and reduce the immune system (Lestari et al., 2024). Erythrocytes are important for fish because of their role in binding oxygen needed by the fish body. Fish blood composition is an important diagnostic factor, so changes in blood picture are widely used. Lack of erythrocytes can cause anemia and fish appear lethargic and appetite decreases, erythrocytes also contain hemoglobin (Sumantri et al., 2020). Hematocrit value has a relationship with hemoglobin and total erythrocytes and leukocytes (Lestari et al., 2024). Factors that affect the number of erythrocytes and leukocytes are the condition and health of the fish body. Sick fish will produce many leukocytes to phagocytize bacteria and synthesize antibodies. Stress in fish can also cause changes in blood glucose which will reduce fish health (Ariyanti et al., 2022). So that fish growth can be disrupted. Low hemoglobin levels cause the metabolic rate in fish to decrease and the energy produced decreases. Low hemoglobin can also indicate that the feed given to fish has low protein (Widyantoro et al., 2014).

Observation of blood profile in this study was conducted to determine the stress response in tilapia feed with the addition of GAA. Stress response in animals can be seen from changes in cortisol hormone levels, blood glucose, hemoglobin, total erythrocytes, leukocytes, and hematocrit. When fish experience stress, there will be changes in the number of erythrocytes, hematocrit values, and hemoglobin levels. While the number of leukocytes tends to increase. Stress is a survival response in fish to the cause of stress or commonly referred to as stressors. Many factors can be a source of stress both from external factors in the form of the environment (temperature, pH, light, DO, and feed) and biotic factors such as microorganism infections (Royan et al., 2014). The addition of GAA to artificial feed at the right level is expected to improve the growth and health status of fish, especially the blood profile of tilapia (*O. niloticus*).

Method

This research was conducted from May to November 2023, at the Aquaculture Laboratory and Fish Disease and Health Laboratory, Faculty of Fisheries and Marine Science, Universitas Brawijaya.

The method used was a completely randomized design (CRD) with five treatments and every treatment with four replications. This study used 240 Tilapia with average weight of 15 g. The stocking density of the aquaria is 15 fish. Test feed in this study is the form of dry pellets consisting combination of *guanidino acetic acid* (GAA) levels, protein content (25% dry matter bases), and source of protein (fish meal and non-fishmeal). The pellet size is adjusted to the fish mouth opening of 3 mm. Composition of the ingredients in test feed is shown in Table 1.

The procedure of this research went through three stages, namely, preparation, manufacture of feed, implementation, blood profile sampling and testing. The process of making feed includes preparation of raw

materials, grinding of coarse raw materials using a grinding machine to a flour texture, balancing raw materials, mixing, molding dough using a molding machine, and ovens with a temperature of 600C for 24 hours. After making the pellets, a proximate analysis is carried out to determine the nutrients contained in the feed that has been made. During the cultured period of 70 days, fish fed by formula diet of 3% biomass per day. Sampling for blood profile tested of erythrocytes, leukocytes, and hematocrit is carried out after the research. The water quality of temperature, pH, and DO were daily monitoring.

Table 1. Composition of the Ingredients in Test Feed

Material	K (g)	A (g)	B (g)	C (g)	D (g)
Fish meal 60%	0.0	30.0	30.0	0.0	0.0
Rice Bran	200.0	200.0	200.0	200.0	200.0
Soybean Meal	341.2	333.7	333.9	340.3	340.6
DDGS	50.0	50.0	50.0	50.0	50.0
CPO	11.0	11.4	11.5	11.2	11.3
Poulty by Product Meal	60.0	30.0	30.0	60.0	60.0
Pollard	100.0	100.0	100.0	100.0	100.0
Cassava	95.0	95.0	95.0	95.0	95.0
Corn Yellow	136.3	143.4	141.9	136.4	135.4
Vitamin Premix	0.5	0.5	0.5	0.5	0.5
Mineral Premix	1.0	1.0	1.0	1.0	1.0
CMC	5.0	5.0	5.0	5.0	5.0
GAA	0.0	0.6	1.2	0.6	1.2
Total	1.000	1.000	1.000	1.000	1.000

The treatments in this research are described as follows:

K : Control treatment

A : Treatment with 0.6 g/kg GAA, fish meal protein source (FM)

B : Treatment with 1.2 g/kg GAA, fish meal protein source (FM)

C : Treatment with 0.6 g/kg, non-fishmeal protein source (NFM)

D : Treatment with 1.2 g/kg, non-fishmeal protein source (NFM)

Blood samples of test fish were taken after a maintenance period of 70 days. Blood samples were taken using a 1 ml syringe that contained 10% EDTA as anti-coagulant used to avoid blood clots during blood collection and storage. Blood was taken from the *linea lateralis* of tilapia. Blood samples that have been taken are put in *eppendorf* then analyze for total erythrocytes, leukocytes, and hematocrit.

Total erythrocyte measurements were counted on 5 small boxes of the hematocytometer. Total erythrocytes can be calculated using the formula from Blaxhall et al. (1973), as follow:

$$N = n\ x\ 104\ cells/mm^3 \quad (1)$$

Description:

N : Total red blood cells in 1 mm^3

n : Total red blood cells in 5 counting rooms (counted)

104 : Dilution factor (1:20)

Total leukocytes measurement is counted as many as 4 boxes or fields. Total leukocytes can be calculated based on the formula proposed by Blaxhall et al. (1973), as follows:

$$N = n\ x\ 50\ cells/mm^3 \quad (2)$$

Description:

N : Total white blood cells in 1 mm^3

n : Total white blood cells in 4 counting rooms (counted)

50 : Dilution factor (1:20)

The hematocrit level was measured by comparing the volume of red blood cell solids with the hematocrit scale, which was determined by the following calculation (Anderson, 1993):

$$Hematocrit = \frac{Length\ of\ Erythrocyte\ Settled\ Volume}{Lenght\ of\ Total\ Blood\ Volume}\ x100\% \quad (3)$$

Result and Discussion

Blood profile can be used to see the physiological response in fish. Observation of blood profiles in this study was conducted to determine the stress response in tilapia fed with the addition of guanidino acetic acid (GAA). The results of erythrocyte, leukocyte, and hematocrit counts after the rearing period are presented in Table 2.

Table 2. Composition of the Ingredients in Test Feed

Treatment	Blood Profile		
	Erythrocytes (x10^6 cells/mm^3)	Leukocytes (x10^3 cells/mm^3)	Hematocrit (%)
K (0 g/kg GAA)	1.6^a	23.1^a	28.4^a
A (0.6 g/kg GAA, FM)	1.8^{ab}	22.1^a	29.5^a
B (1.2 g/kg GAA, FM)	2.2^d	21.4^a	30.4^a
C (0.6 g/kg GAA, NFM)	2.0^c	21.8^a	29.6^a
D (1.2 g/kg GAA, NFM)	2.0^c	21.8^a	29.5^a

Erythrocyte

The combination treatment of guanidino acetic acid (GAA) levels with different protein sources (fishmeal and non-fishmeal) had a significant effect on total erythrocyte of tilapia ($P<0.05$). The results of the measurement of total erythrocytes after treatment for 70 days are presented in Figure 1.

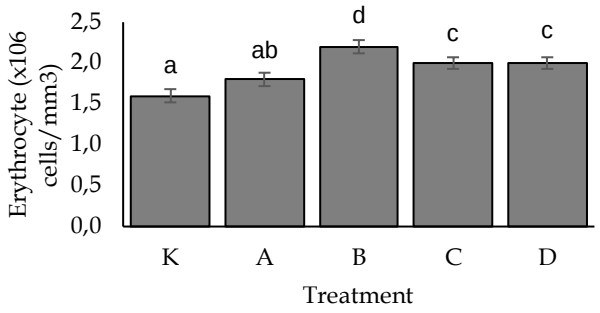

Figure 1. Total erythrocytes of tilapia

The result showed that the average value of total erythrocytes had different results in each treatment. The highest result was aimed at treatment B (1.2 g/kg GAA, FM) with total erythrocyte count of $2.2x10^6$ cells/mm^3. The lowest result was obrained by the control treatment (K) with total erythrocyte count of $1.6x10^6$ cells/mm^3. The calculation value obtained in this study is still within the normal limits of tilapia erythrocyte count. The normal erythrocyte count in tilapia ranges from 20,000 - 3,000,000 cells/mm^3 (Hartika et al., 2014). A high number of erythrocytes exceeding the normal limit indicates that the fish is under stress. Stress can affect the performance of growth and health of fish in the form of blood cell disorders, one of which is erythrocytes (Bangsa et al., 2015).

Erythrocytes are one aspect of blood profile parameters. Erythrocytes or red blood cells are one of the blood components that have the function of transporting hemoglobin throughout the body and spreading the oxygen and nutrients it contains (Z. Li et al., 2021). Erythrocytes are produced from the spinal cord and have a function to examine how the blood reacts to pathogens that attack fish (Kuhn et al., 2017). Ciftci et al. (2015), stated that there was an effect on the supplementation of GAA to feed at 0.6 g/kg and 1.2 g/kg, namely an increase in the number of erythrocytes in tilapia. Barbalato et al. (2022), mentioned that the main physiological role of red blood cells, or erythrocytes, is to transport oxygen and carbon dioxide gases from the lungs to the tissues and to maintain systemic acid/base balance. In addition, red blood cells are equipped with an antioxidant system, which essentially contributes to their function and integrity. Damage to red blood cell integrity, defined as hemolysis, has been shown to contribute significantly to severe pathologies, including endothelial dysfunction (Purwanti et al., 2014).

Leukocyte

The results showed that the addition of different guanidino acetic acid (GAA) levels as follows 0.6 g/kg and 1.2 g/kg with fishmeal and non-fishmeal protein source had different result in each treatment. The results of the measurement of total leukocytes after treatment for 70 days are presented in Figure 2.

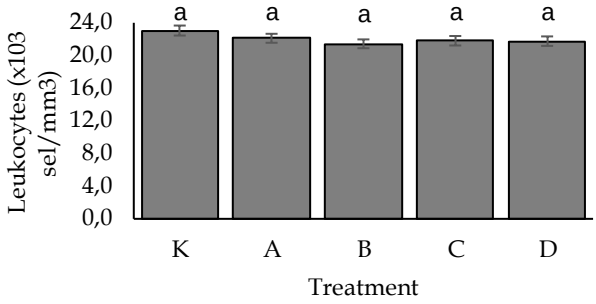

Figure 2. Total leukocytes of tilapia

The combination treatment of GAA levels with different protein sources (fishmeal and non-fishmeal) did not have significant differences between treatments on total leukocytes of tilapia ($P>0.05$). The result showed that the average value of total leukocytes had different results in each treatment. The highest result was aimed at control treatment (K) with total erythrocyte count of $23.1x10^3$ cells/mm^3. The lowest result was obtained by the treatment B (1.2 g/kg GAA, FM) with total leukocytes count of $21.2x10^3$ cells/mm^3. The leukocyte count value obtained in this study is still within the normal limits of tilapia leukocyte count. The normal leukocyte count in tilapia ranges from 20,000 - 150,000 cells/mm^3 (Fauzan et al., 2017). Leukocyte counts that are still within normal numbers indicate that the hematopoiesis process is still occurring in tilapia.

Leukocytes will increase when fish are infected as a form of immune response against microorganisms. Changes in aquatic environmental conditions, changes in water quality and lack of feed can cause a decrease in the number of leukocytes in fish, causing a decrease in antibody production, decreased body resistance, and susceptibility to disease (Afrianto et al., 2015). Leukocytes play an important role in the cellular and humoral defense of the organism against foreign substances (Tugiyanti et al., 2018). Leukocytes move freely and are functional cells in the innate immune system. Factors that affect the number of leukocytes is the condition and health of the fish body. Leukocytes help rid the body of foreign substances. Sick fish will produce many leukocytes to phagocytize bacteria and synthesize antibodies (Firman et al., 2022). The number of leukocytes in tilapia can also be influenced by type or species, age, and muscle activity (Salasia et al., 2001).

Hematocrit

The results showed that the addition of different guanidino acetic acid (GAA) levels as follows 0.6 g/kg

and 1.2 g/kg with fishmeal and non-fishmeal protein source had different result in each treatment. The combination treatment of GAA levels with different protein sources (fishmeal and non-fishmeal) did not have significant differences between treatments on total hematocrit of tilapia (P>0.05). The results of the measurement of total leukocytes after treatment for 70 days are presented in Figure 3.

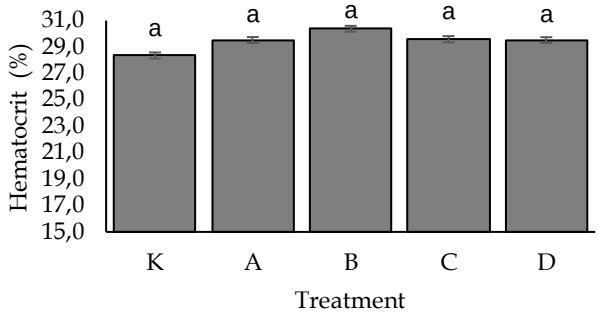

Figure 3. Total hematocrit of tilapia

The result showed that the average value of total hematocrit had different results in each treatment. The highest result was aimed at treatment B (1.2 g/kg GAA, FM) with a calculated hematocrit value of 30.4%. The lowest result was obtained by the control treatment (K) with hematocrit value is 28.4%. The values obtained in this study, both the highest and lowest values, are still included in the normal levels of tilapia hematocrit values. Normal hematocrit values in tilapia range from 27.3% - 37.8% (Firman et al., 2022).

Tilapia blood hematocrit values can be abnormal due to age, sex, environment, oxygen deficiency conditions, and lack of nutrients in fish feed. Lack of nutrients in the feed given to fish can cause fish to experience stress and then disease and decreased appetite. This can affect growth performance in tilapia. Calculation of hematocrit value reflects oxygen carrying capacity in the blood. Low hematocrit values can be caused by gill damage or defective osmoregulation, while high values indicate increased oxygen demand or acute environmental stress (Fauzan et al., 2017). If the fish is exposed to stress factors, the fish's appetite will decrease and the blood hematocrit value will decrease. Fish can experience microcystic anemia, causing the number and size of red blood cells to decrease, resulting in a low hematocrit value. In addition to stress factors, hematocrit value can also be influenced by gender, body size, and spawning period (Royan et al., 2014).

Conclusion

The combination treatment of guanidino acetic acid (GAA) levels with different protein sources (fishmeal and non-fishmeal) had a significant effect on total erythrocyte of tilapia, but did not have significant differences between treatments on total leukosyte and hematocrit. Treatment with 1.2 g/kg GAA, fish meal protein source was the best treatment in this research.

Acknowledgments

The author would like to thank to Rifqy Yuga, Novi Juniar Ananda, and Shilfanny Juniar for helping the authors in carrying out the research.

Author Contributions

FO. Afifah, IMD. Mahariawan, WE. Kusuma, BR. Hidayat, A. Yuniarti, and AM. Hariati conceived of the presented idea. FO. Afifah developed the theory and performed the computations. A. Yuniarti, IMD. Mahariawan, and AM. Hariati verified the analytical methods. All authors discussed the results and contributed to the final manuscript.

Funding

This research was funded by Numega/Nutrivet Research Grant.

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