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Keterangan: Bagian yang direvisi pada Abstrak artikel.

The effect of bromelain enzyme on pineapple core on the relationship between platelet count and hematocrit value in carp (*Cyprinus carpio*) infested with *Argulus japonicus*

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Abstract. Carp (*Cyprinus carpio*) is a freshwater fish commodity that has high economic value and is growing rapidly it is widely cultivated in Indonesia. Based on data from the Maritime and Fisheries, carp production in Indonesia during 2015-2019 increased by 12.09%. Bromelain enzyme gives the best results in hematology, which can affect blood coagulation by increasing the ability of serum fibrinolytic. Purpose of this study was to determine the effect of bromelain enzyme, infestation *Argulus japonicus*, interaction and relationship between platelet count and hematocrit value in carp. Analysis of data used in this study is Analyze of Variance, while to determine the relationship between platelet count and hematocrit value using the Spearman Correlation. Results statistical tests showed that there was no effect ($p>0.05$) between different dosage enzyme bromelain in pineapple core on the platelet count, but there was an effect ($p<0.05$) on hematocrit value. Statistical test results showed that there was no effect ($p>0.05$) between infestations *Argulus japonicus* ~~differentiation~~differentiation platelet count and hematocrit value. Results statistical tests also showed that there was no interaction and relationship between platelet count and hematocrit value in carp that were infested with *Argulus japonicus* and given the enzyme bromelain in pineapple core.

1. Introduction

Carp (*Cyprinus carpio*) is a freshwater fish commodity that has high economic value and is growing rapidly so it is widely cultivated in Indonesia [1]. The advantages of this carp are quite large, easy to breed, fast growth, and resistant to disease [2]. Based on data from the Ministry of Maritime Affairs and Fisheries, carp production in Indonesia during 2015-2019 increased by 12.09%. In 2015-2019, carp production reached 461,546 tons with a target of 558,700 tons, 497,208 tons with a target of 558,700 tons, 320,941 tons with a target of 679,900 tons, 536,349 tons with a target of 558,700 tons, 584,496 tons with a target of 770,700 tons. The target for carp production has not been achieved, mainly due to the spread of various fish diseases that cause productivity levels to decline [3].

The main problem in carp cultivation is disease. This disease causes economic losses because it can inhibit growth, longer maintenance period, high feed conversion, decreased selling value and can cause fish death [4]. Diseases that often attack goldfish are caused by parasites such as *Myxobolus* sp., *White spot disease*, *Argulus* sp., *Lerneae* sp. [5].

The parasite that most often attacks carp is *Argulus japonicus*. This parasite infests the host on the fins, skin, gills and the entire body surface by piercing the host's body using a stylet and sucking the

host's blood using a proboscis. Infestation *Argulus japonicus* causes the host to be injured so that the host experiences bleeding, anemia and increased mucus production [6]. An alternative solution that can be attempted in tackling this parasite is by adding the bromelain enzyme to the feed [7].

Bromelain is an enzyme that can hydrolyze peptide bonds in proteins into amino acid molecules. Bromelain enzyme is a proteolytic enzyme found in pineapple [8]. The highest content of bromelain enzymes is found in pineapple core ranging from 0.100-0.60% [9]. The bromelain enzyme gives the best results in hematology because it can affect blood coagulation by increasing the ability of serum fibrinolytic and inhibiting the synthesis of fibrin, a protein involved in blood clotting [10]. Platelets are blood cells that function in hemostasis. These cells do not have a nucleus and are produced by megakaryocytes in the bone marrow [11]. The hematocrit value is the concentration of erythrocytes in 100 ml of whole blood. The hematocrit value will increase (hemoconcentration) due to an increase in blood cells or a decrease in blood plasma volume, otherwise the hematocrit value will decrease (hemodilution) due to a decrease in blood cellular or an increase in blood plasma levels as in anemia [12]. Therefore, based on the above background, it is necessary to conduct research on the effect of the bromelain enzyme on pineapple core in commercial feeds on the relationship between platelet count and hematocrit value of carp (*Cyprinus carpio*) infested with *Argulus japonicus*.

2. Materials and methods

2.1. Materials

The Tools used in this study were 24 aquariums with a diameter of 15 cm, 2 reservoirs, a scoop, aerator, aeration hose, aeration stone, digital scales, ruler, siphon, pH meter, DO (Dissolved oxygen) meter, Ammonia test kit, label paper, plastic clip, sample pot, knife, blender, refrigerator, oven, centrifuge, petri dish, seser, bucket, scissors, basin, tweezers, tray, beaker glass, syringe, hematology analyzer, edta tube, microscope, and stationery. The experimental fish used in this study were carp (*Cyprinus carpio*) with a body length of 7-9 cm, body weight ranging from 2-3 grams with a total of 48 carp obtained from the Gunung Sari Fish Market, Surabaya and 180 *Argulus japonicus*. The aquarium is filled with a stocking density of 2 g/liter with an initial fish weight of 2 g/head with a total of 48 fish for 24 aquariums. The feed material used in the study was commercial feed which was added with pineapple core in several dosage. The bromelain enzyme production process requires 70% alcohol. The maintenance medium used in this research is PDAM water which is deposited by aeration in a reservoir for one day. This research was carried out from March 2021 to May 2021 located behind the Anatomical Cultivation Laboratory, and the Microbiology Laboratory of the Faculty of Fisheries and Marine Affairs, Universitas Airlangga. Data analysis consisted of univariate and bivariate analysis with Spearman correlation test. The dependent variable is the hematocrit value, while the independent variable is the platelet count consisting of 8 treatments with 3 replications each as follows:

A₀B₀ = 100% commercial feed + Bromelain enzyme 0%

A₀B₁ = 100% commercial feed + Bromelain Enzyme 0% + 5 *Argulus japonicus*

A₀B₂ = 100% commercial feed + 0% Bromelain Enzyme + 10 *Argulus japonicus*

A₀B₃ = 100% commercial feed + Bromelain enzyme 0% + 15 *Argulus japonicus*

A₁B₀ = Feed commercial 100% + Bromelain enzyme 2.25%

A₁B₁ = 100% commercial feed + Bromelain enzyme 2.25% + 5 *Argulus japonicus*

A₁B₂ = 100% commercial feed + Bromelain enzyme 2.25% + 10 *Argulus japonicus*

A₁B₃ = 100% commercial feed + Bromelain 2.25% + 15 *Argulus japonicus*

2.2. Making pineapple core enzyme

First stage of making pineapple core enzyme is preparing tools and materials. The pineapple core that has been prepared is then cleaned, cut into small pieces, blended until smooth, squeezed, and filtered until a clear liquid is obtained from the pineapple core juice. The pineapple core juice is added with 70% alcohol in a 1:4 ratio (extract: alcohol), then the enzyme is left for 1 hour at room temperature, the goal is that the enzyme must settle before entering the refrigerator. Enzymes are included in the refrigerator at a temperature of 10°C. The precipitate obtained centrifuged at 5000 rpm for 30 minutes.

The precipitate formed was then placed in a petri dish and dried in an oven at 40°C. The dried precipitate was then crushed with mortar and pestle to obtain crude bromelain enzyme in powder form, weighed as much as the required dosage of 0% and 2.25%. The enzyme was dissolved using 10 ml of distilled water. The bromelain enzyme was mixed with commercial feed and then given to the test feed using a sprayer. Ready feed was given to the test fish [13].

2.3. Making treatment feed

Feed material used is commercial feed. The commercial feed used as research feed contains protein above 30% because carp requires a minimum of 30% protein [14]. The dosage of commercial feed in carp pellets was 100% in each treatment. The dosage of pineapple core enzymes added to commercial feeds were 0% and 2.25%. Then the two ingredients are mixed thoroughly using a sprayer. The pellets are then aerated and kept out of direct sunlight. Dried pellets are stored in a dry place.

2.4. Aquarium preparation and aquarium maintenance media

Used with a diameter of 15 cm as many as 24 pieces. Before use, the aquarium must be cleaned and sterilized to avoid disease. Cleaning is done by washing the aquarium using water and detergent, then soaking it with 20-30 ppm chlorine for 24 hours [15]. After the chlorine, the aquarium is rinsed thoroughly and dried. Carp rearing media is PDAM water that has been aerated for one day to increase the dissolved oxygen content in the water.

2.5. The culture of *Argulus japonicus*

Aquarium that will be used is cleaned first of dust and dirt attached to it and then dried in the sun. Once ready for use, the stones are put in the aquarium, then filled with water and the installation of aeration and aeration stones is carried out. Sex selection in *Argulus japonicus* can be seen in the shape of the abdominal lobes or the posterior part of the body of *Argulus japonicus*. The female sex can be distinguished by the presence of seminal receptacle (spermathecae) and ovarian pouches that are visible along the midline of the body, whereas in males there is a pair of testes located on the posterior abdomen [16]. The ready aquarium is then filled with *Argulus japonicus* male and female along with carp as hosts and then left until *Argulus japonicus* lays eggs and hatches.

2.6. Fish rearing

Fish used in this study were carp (*Cyprinus carpio*) measuring 7-9 cm. Each aquarium is filled with 2 fish with an initial weight of 3-4 grams of fish. The acclimatization process is carried out first so that the fish can adjust to their new environment. Feed is given with a frequency of three times a day in the morning, afternoon, and evening as much as 5% of the fish biomass [17].

2.7. Implementation of research

The artificial infestation of *Argulus japonicus* was divided into 4, namely without *Argulus japonicus*, mild infestation with 5 *Argulus japonicus*, moderate infestation with 10 *Argulus japonicus*, heavy infestation with 15 *Argulus japonicus*. Artificial infestation was carried out by placing *Argulus japonicus* and carp (*Cyprinus carpio*) into a 500 ml glass beaker containing 400 ml of water for 15-30 minutes. *Argulus japonicus* is fasted for approximately 2 hours to make it stick faster. The fish were reintroduced into the aquarium after *Argulus japonicus* had infested the carp. The maintenance of carp infested with *Argulus japonicus* was carried out for 7 days. The examination of the platelet count and hematocrit value was carried out on the last day of the study, which was the seventh day, to avoid death in the fish to be examined.

2.8. Examination of platelet count and hematocrit value

Blood was taken through the caudalis vein under the spine. The syringe was rinsed using 10% EDTA to prevent blood clots. Taking and storing blood into the tube is done slowly to reduce damage to blood cells. Examination of the platelet count and hematocrit value is carried out using a Hematology

Analyzer by first turning the device on, then writing the sample code to be examined so that a needle appears to suck blood, in one minute the tool will read the results, then the results from the blood sample come out.

2.9. Data analysis

Analysis used in this study is Analyze of Variance (ANOVA) to determine the treatment given, if there are significant results then the calculation is continued with Duncan's multiple distance test while to determine the relationship between platelet count and hematocrit value using univariate data analysis and bivariate. In the bivariate analysis, the relationship between two variables was searched using the Spearman Correlation test [18].

3. Result and discussion

3.1. The average number of platelets and the hematocrit value of carp (*Cyprinus carpio*) influenced by different doses of bromelain enzyme.

The results showed that the average number of platelets ranged from 340.75 cells/mm³-342.33 cells/mm³ and the hematocrit value of carp ranged from 27.650%-32.242%. Test results are presented in Table 1.

Table 1. Average number of platelet and hematocrit values carp (*Cyprinus carpio*) that influenced the bromelain enzyme dosage be difference

Bromelain enzyme	Mean Platelet Count (cells/mm ³) ± Standard Deviation	Mean Hematocrit Value (%) ± Standard Deviation
A0	342,33 ^a ±5,416	27,650 ^a ±3,2081
A1	340,75 ^a ±6,369	32,242 ^b ±1,4706

The different subsets column showed significant differences (p<0,05)

The results of statistical analysis showed that the addition of the pineappleenzyme to the commercial feed of carp (*Cyprinus carpio*) showed that there was no significant difference (p>0.05) in the average amount. goldfish platelets. According to Kelly in 1996 bromelain enzyme selectively degrades thromboxane which functions to inhibit platelet aggregation, can act as an anticoagulant and does not cause hepatotoxicity [19]. The results of statistical analysis showed that the addition of pineapple weevil bromelain enzyme in commercial carp (feed*Cyprinus carpio*) showed that there was a significant difference (p<0.05) in the average value of carp hematocrit. According to Lukistyowati in 2007 the hematocrit value can change depending on the season of feeding temperature and immunostimulants [20].

3.2. The average number of platelets and hematocrit values of carp (*Cyprinus carpio*) affected infestations *Argulus japonicus* by different

The results showed that the average number of platelets ranged from 337.00 cells/mm³-344.83 cells/mm³ and the hematocrit value of carp ranged from 28.950% to 31.583%. The results of the examination are presented in Table 2.

Table 2. The average number of platelets and the hematocrit value of carp (*Cyprinus carpio*) influenced infestations *Argulus japonicus* by different

<i>Argulus japonicus</i>	Mean Platelet Count (cells/mm ³) ± Standard Deviation	Mean Hematocrit Value (%) ± Standar Deviation
B0	337,00 ^a ±7,975	28,950 ^a ±3,4755
B1	344,83 ^a ±5,811	29,683 ^a ±5,4197

B2	342,17 ^a ±2,714	31,583 ^a ±1,9934
B3	342,17 ^a ±3,656	29,567 ^a ±1,6158

The different subsets column showed significant differences ($p < 0,05$)

Statistical analysis results showed that the infestation of *Argulus japonicus* in carp (*Cyprinus carpio*) showed that there was no significant difference ($p > 0.05$) in the average number of carp platelets and the average hematocrit value of carp (*Cyprinus carpio*). According to Christianty *et al* in 2017 the presence of dehydration and bleeding can increase the hematocrit value [21], but in this study there was no increase in the hematocrit value. This is presumably due to the effect of storage time starting from blood collection to the sample testing process using a hematology analyzer. With increasing storage time, the number of hematocrit decreased [22].

3.3. *The average number of platelets and the hematocrit value of carp (Cyprinus carpio) influenced by the interaction between the dosage of bromelain enzyme and different infestations of Argulus japonicus.*

The results showed that the average number of platelets ranged from 332.67 cells/mm³-345.00 cells/mm³ and the hematocrit value of carp ranged from 25.533%-33.833%. Test results are presented in Table 3.

Table 3. Average number of platelet and hematocrit values carp (*Cyprinus carpio*) that affected the interaction between the dosage of enzyme bromelain and infestations *Argulus japonicus* of different

Interaction Beetwen Dosage Bromelain and <i>Argulus japonicus</i> Infestations	Mean Platelet Count (sel/mm ³) ± Standard Deviation	MeanHematocrit Value (%) ± Standard Deviation
A0B0	341,33 ^a ±9,292	26,600 ^a ±3,6661
A0B1	345,00 ^a ±5,196	25,533 ^a ±4,3085
A0B2	342,00 ^a ±3,464	30,300 ^a ±2,1656
A0B3	341,00 ^a ±4,583	28,167 ^a ±0,7767
A1B0	332,67 ^a ±4,041	31,300 ^a ±0,4359
A1B1	344,67 ^a ±7,572	33,833 ^a ±1,7898
A1B2	342,33 ^a ±2,517	32,867 ^a ±0,5508
A1B3	343,33 ^a ±2,887	30,967 ^a ±0,2082

The different subsets column showed significant differences ($p < 0,05$)

Blood consists of liquid plasma and blood cells namely red blood cells, blood cells white, and platelets. Blood plasma is a clear liquid containing dissolved minerals, the result of absorption from digestion of food, waste products of metabolism by tissues, enzymes, antibodies and dissolved gases [23]. Blood plasma contains inorganic salts, proteins, fats, hormones, vitamins, enzymes, and nutrients [24]. From the results of statistical analysis showed that the interaction between the enzyme bromelain in pineapple core with infestation *Argulus japonicus* in carp (*Cyprinus carpio*) showed that there was no significant difference ($p > 0.05$) in the average number of carp platelets and the average value hematocrit carp (*Cyprinus carpio*). The results also showed that the platelet count and hematocrit value in carp were in the normal range, meaning that along with good water quality, non-specific defenses in carp will increase so that they are able to respond to parasitic infestations. According to Almendras (2001) Non-specific defenses are permanent and do not need to be stimulated first, so it often determines that one type of fish is more resistant to pathogens than other types [25]. Non-specific defense consists of the first defense system (skin, scales, mucus) and the second defense system (blood). Irianto (2005) explained that mucus has the ability to inhibit the colonization of

microorganisms on the skin, gills and mucosa [26]. Fish mucus contains *immunoglobulin* natural (IgM) which can destroy invading pathogens [27]. Another important non-specific defense is blood, blood cells consisting of monocytes, lymphocytes, neutrophils that can move to the entry site of foreign antigens through capillary walls and also have lysozyme enzymes [28]. Ellis (1989) explains that the defense system that initially functions is a non-specific defense system, then develops into a specific defense system that functions well. The mechanism of action of the two defense systems support each other through mediators and communicators such as cytokines, interferons, and interleukins [29].

3.4. Data on distribution of platelet count and hematocrit value in carp (*Cyprinus carpio*)

Data on distribution of platelet count in carp (*Cyprinus carpio*) influenced by the interaction between bromelain enzyme dosage and infestations *Argulus japonicus* differentare presented in Table 4.

Tabel 4. Distribution of carp platelet count.

Platelet Count (cells/mm ³)	n (%)
<200.000	0
200.000-500.000	24 (100)
>500.000	0
Total	24 (100)

Description : n=frequency

Based on the results of the study, the overall platelet count ranged from 200,000 cells/mm³ – 500,000 cells/mm³ as much as 100%. The lowest platelet count was 332.67 cells/mm³ and the highest was 345.00 cells/mm³. In this study, the average number of goldfish platelets was 341.54 cells/mm³. This result is still within the normal range as stated by Kuswardani in 2006 normal goldfish platelet levels ranged from 200,000 cells/mm³– 500,000 cells/mm³ [30].

3.5. Data on the distribution of hematocrit values in carp (*Cyprinus carpio*)

Influenced by the interaction between bromelain enzyme dosage and infestations *Argulus japonicus* differentare presented in Table 5.

Tabel 5. Distribution of carp hematocrit value.

Jumlah Hematokrit (%)	n (%)
<21	0
21-33	24 (100)
>33	0
Total	24 (100)

Description : n=frequency

Based on the results of the study, the overall hematocrit value ranged from 21% - 33% as much as 100%. The lowest hematocrit value was 25.533% and the highest was 33.833%. In this study, the average hematocrit value of carp was 29.946%. This result is still within the normal range as stated by Kuswardani in 2006 normal carp hematocrit levels ranged from 21% - 33% [30].

3.6. The relationship between platelet count and hematocrit value

Data from the analysis of the relationship between platelet count and hematocrit value are presented in Table 6.

Table 6. The result of the analysis of the relationship between platelet count and hematocrit value

Variabel	r	p
Platelet Count with Hematocrit Value	-0,149	0,487

Spearman correlation test, a negative correlation was obtained. means that the greater the value of a variable, the value of other variables will be smaller or vice versa. It can be concluded that the lower the platelet count, the higher the hematocrit value. This is in line with the JACC study in 2002 which found that there was a negative correlation between the platelet count and the hematocrit value [31]. In this study, $p = 0.487$, so a significance value of $p > 0.05$ which indicates that there is no significant correlation between the two variables and it can be concluded that there is no relationship between the number of platelets and the hematocrit value in carp (*Cyprinus carpio*) infested with *Argulus japonicus*. and given the enzyme bromelain on the pineapple hump.

3.7. Water quality

Water quality is an important factor in carp culture because water is the main living medium. The range of water quality values during this study can be seen in Table 7. Water quality that meets the requirements can make fish growth and survival good. The results of the analysis of the measured water quality parameters indicate that the rearing medium for freshwater pomfret (*Colossoma macropomum*) during the study was in a suitable environment for growth and development. Maintenance media and the environment are external factors that affect fish growth, including pH, dissolved oxygen, temperature and ammonia [32]. Water quality parameters in the research aquarium are still in optimal standards with a pH range of 7.3-8.6, dissolved oxygen 5.03 mg/L–5.76 mg/L, temperature 28.4°C-30.5°C, and 0 ppm–0.25 ppm ammonia. In general, the parameters physical and chemical of water during maintenance indicate the range that can be tolerated by carp to live.

Table 7. Water quality of carp during rearing.

Parameter	Range
Dissolved Oxygen (mg/L)	5,03-5,76
pH	7,3-8,6
Ammonia (mg/L)	0-0,25
Temperature (°C)	28,4-30,5

4. Conclusion

The results of statistical tests showed that there was no effect ($p > 0.05$) between different doses of the bromelain enzyme in pineapple cobs on the platelet count, but there was an effect ($p < 0.05$) on the hematocrit value. Statistical test results showed that there was no effect ($p > 0.05$) between infestations *Argulus japonicus* differentiation platelet count and hematocrit value. The results of statistical tests also showed that there was no interaction and relationship ($p > 0.05$) between the number of platelets and the hematocrit value in carp (*Cyprinus carpio*) infested with related with *Argulus japonicus* and bromelain core enzyme in pineapple.

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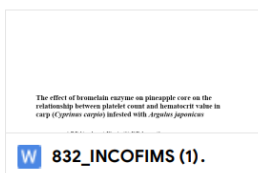
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