

# The Effects of Rumah Panggung Environment, Social Culture, and Behavior on Malaria Incidence in Kori Village, Indonesia

*by Irwan Budiono*

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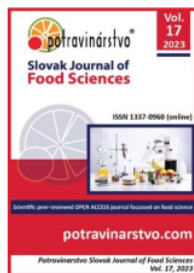
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## The efficacy of Bloso fish (*Glossogobius giuris* sp.) in improving hemoglobin, hematocrit, platelet, and albumin levels of Wistar rats with hypoalbuminemia

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### ABSTRACT

Tuberculosis (TB) is an infectious disease worldwide that causes death. Common clinical manifestations of patients with TB include anemia, hypoalbuminemia, and malnutrition. Most patients with TB are infected with coccus bacteria, such as *Staphylococcus aureus*, that commonly attack the respiratory tract. However, the consumption of heme protein sources could improve the nutritional status of patients with TB. Fish comprise one of the most widely consumed sources of heme. The bloso fish (*Glossogobius giuris* sp.), considered a fish without economic value is a new alternative source of heme protein. This study aimed to develop supplements using bloso fish (*Glossogobius giuris* sp.). This study used an experimental pretest-post-test control group design. Seven male Wistar rats were used as the negative control group. Twenty-eight male Wistar rats were administered *S. aureus*, fed a protein-deficient diet, and divided into the positive control group, the K1 group, which received up to 675 mg/200 g of bloso fish flour, the K2 group, which received up to 67.5 mg/200 g of bloso fish oil, and the K3 group, which received up to 675 mg/200 g of bloso fish flour from oil extraction dregs. Treatment was administered for 28 days. The hemoglobin (Hb), hematocrit (Ht), platelet, and albumin levels in blood serum from the retroorbital vein were measured. Data were processed using a paired t-test and one-way analysis of variance. The results showed differences in Hb, Ht, platelet, and albumin levels were observed before and after treatment. Additionally, differences in Hb, Ht, platelet, and albumin levels were observed in the groups that received bloso fish flour and bloso fish oil. Bloso fish flour and bloso fish oil increased the Hb, Ht, platelet, and albumin levels of rats with hypoalbuminemia.

**Keywords:** hemoglobin, hypo albumin, bloso fish, tuberculosis, fish oil

### INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* that affects the lungs. TB is the second leading cause of death worldwide, and it was declared a global public health emergency by the World Health Organization in 1993. As many as 8 billion new cases of TB are diagnosed each year, resulting in as many as 2 million deaths. In 2017, 10.4 million new cases of TB were diagnosed, resulting in 1.7 million deaths. *Mycobacterium tuberculosis* exposure causes various conditions, such as infection resistance, latent infection without active disease, active pulmonary disease and active extra-pulmonary disease. The TB incidence is higher in low and middle-income countries. Research has shown that as many as 91% of the sputum of patients with TB contains Gram-positive coccus bacteria from the *Staphylococcus* sp. and *Streptococcus* sp. Genera [1].

In Indonesia, the rate of TB is high, and there is an increasing trend of severe TB. The 2017 World Health Organization report indicated that the number of new TB cases in Indonesia is approximately 1,020,000 per year

(399 cases/100,000 population), resulting in 100,000 deaths per year (41 cases/100,000 people). Furthermore, the estimated prevalence of human immunodeficiency virus among patients with TB is 6.2%. Additionally, the number of drug-resistant TB cases has been estimated to be 10,000; these cases comprise 1.9% of drug-resistant TB cases associated with new TB cases and 12% of drug-resistant TB cases associated with TB with repeated treatment [2]. Based on the 2018 Basic Health Research, the magnitude of the incidence of pulmonary TB has decreased; however, at 321 cases per 100,000 population, the incidence of pulmonary TB is still much higher than the national target. Furthermore, this number is too high according to the 2019 strategic plan, which indicates a target prevalence of pulmonary TB of 245 cases/ 100,000 population [3].

TB is closely associated with malnutrition and anemia [4]. Moreover, anemia exacerbates TB and malnutrition. Hemoglobin, hematocrit, platelet, and albumin levels can reflect the nutritional status of patients with TB. Hemoglobin is a routine blood parameter and an illustration of the nutritional status related to the body's iron status. Hemoglobin can bind to iron, which is directly associated with anemia. Albumin is the main plasma protein component, which can reversibly bind with normal levels of 3.5 g/dL to 5.5 g/dL. The main function of albumin is maintaining osmotic pressure in the body; its half-life is 14 to 21 days [5]. Previous studies have shown that serum albumin levels decrease significantly in patients with TB and nutritional problems. Nutritional factors such as low food intake, anorexia, and increased catabolism, including acute phase protein reactions, also affect hypoalbuminemia in patients with TB [6].

It is possible that increased albumin levels in patients with TB who experience hypoalbuminemia can increase the antimicrobial effect of anti-TB drugs and inhibit the production of inflammatory cytokines. Anti-TB drugs include rifampicin and isoniazid, which can bind strongly to albumin in pulmonary patients with TB, thereby improving their clinical condition [7]. Improvements in food consumption, especially protein, can help improve hypoalbuminemia in patients with TB. Fish is one source of heme protein; therefore, the bloso fish (*Glossogobius giuris* sp.) has the potential to be developed into a formula that could improve the nutritional status of patients. Individuals living in the coastal areas of the island of Java believe that consuming bloso fish benefits children and pregnant women. The bloso fish (*Glossogobius giuris* sp.) is an endemic species in Indonesia [8] that lives in brackish waters, is often considered a pest, and has low economic value.

Therefore, developing an appropriate form of processed bloso fish is necessary to help improve the hemoglobin, hematocrit, platelet, and albumin levels of rats with hypoalbuminemia and TB.

### Scientific Hypothesis

Bloso fish flour and bloso fish oil can improve the hemoglobin, hematocrit, platelet, and albumin levels of rats with hypoalbuminemia.

## MATERIAL AND METHODOLOGY

### Samples

Blood samples obtained from 35 Wistar rats.

### Animals, Plants and Biological Materials

This study used male Wistar rats. The inclusion criteria included: Wistar strain; male sex; age 8 to 12 weeks; the weight of 160 to 250 g; no anatomical abnormalities; and healthy and active during the adaptation period. Exclusion criteria included pain or inactivity during the adaptation period, extreme weight loss before treatment, and diarrhoea. The drop-out criterion was death during the treatment period. Thirty-five rats were randomly selected and divided into five groups of seven. A total of thirty-five experimental animals were required.

### Instruments

Hematology Analyzer, Sysmex KX-21, Kobe, Japan.

### Laboratory Methods

The Automated hematology analyzer (AHA) was used to measure hemoglobin, hematocrit, platelet, and albumin levels [9].

### Description of the Experiment

**Sample preparation:** Whole blood was obtained from each Wistar rat and stored with an anticoagulant to measure the hemoglobin, hematocrit, platelets, and albumin.

**Number of samples analyzed:** Blood samples from thirty-five Wistar rats were collected from the retroorbital vein (before and after intervention)

**Number of repeated analyses:** In duplicate.

**Number of experiment replication:** In duplicate.

**Design of the experiment:** The experimental animals were treated at the Center for Food and Nutrition Studies Laboratory of the Universitas Gajah Mada (Yogyakarta, Indonesia). Nutrient testing was performed in the Center for Food and Nutrition Studies Chemical Laboratory of the Universitas Gajah Mada (Yogyakarta,

Indonesia). The Nutrition Laboratory of Universitas Negeri Semarang (Semarang, Indonesia) and the Food Engineering Laboratory of Soegijapranata Catholic University (Semarang, Indonesia) were used to produce bloso fish flour. Hypoalbuminemia in this study used 1% *Staphylococcus aureus* to induce infection. The isolated culture from Center for Food and Nutrition Studies of the Universitas Gajah Mada, Yogyakarta, Indonesia.

A randomized, controlled, pretest-posttest design was used for this study. Wistar rats were randomly divided into a negative control group (K-group), a positive control group (K+ group), and the following three treatment groups: the K1 group, which received 675 mg/200 g of bloso fish flour; the K2 group, which received 67.5 mg/200 g of bloso fish oil; and the K3 group, which received 675 mg/200 g of bloso fish flour from oil extraction dregs. During this study, three servings of animal protein per day, equivalent to 150 g of animal food per day, were administered [10]. The wet bloso fish were floured using a spray dryer. This drying process involved low-temperature evaporation at 40 °C for 8 to 12 hours. The dried bloso fish subjected to the spray dryer shrank by 25%. Bloso fish oil was obtained using a drying method involving a cabinet dryer. Pressing was performed until the bloso fish oil yield reached 10% of the dry weight. The residue from the pressing was referred to as oil extraction dregs. Treatment was administered using a gastric tube once daily during the morning for 28 days. The duration of administration was influenced by the half-life of albumin, which is 14 to 21 days. The negative control group received an AIN93M feed. The positive control and treatment groups received AIN93M modified feed and were injected with 1% *Staphylococcus aureus* to induce infection. Modifying the AIN93M feed was performed to induce protein deficiency, which involved removing the casein component. As a result, the feed contained only corn flour, sucrose, corn oil, alpha cell (non-nutritive bulk), and mineral mix. Rat feed was produced as pellets, similar to rat feed and consumed orally. The rats received 20 g of feed each day.

### 1 Statistical Analysis

The mean  $\pm$  standard deviation (SD) was calculated for each group of seven rats. As all data were normally distributed, the significance of differences before and after treatments was determined using the paired t-test. The significance of differences between the groups was assessed by one-way analysis of variance (ANOVA), calculated by the SPSS version 20 program, with a significance level of  $p < 0.05$  by the Tukey HSD Test.

## RESULTS AND DISCUSSION

The conditions of protein deficiency and cell resistance to pathogenic bacteria allowed cells to form cellular defences by mediating T cells and macrophages [11]. Nutritional components, such as carbohydrates, proteins, fats, and vitamins, can activate T cells and macrophages. Fat can regulate the immune response against pathogenic bacteria [12]. Fatty acids can increase the ability of macrophages to kill bacteria through phagocytosis [13]. Eicosapentaenoic acid and polyunsaturated fatty acid (PUFA) can increase mycobacterial growth by reducing tumour necrosis factor- $\alpha$  secretion in macrophages. The fish oil used during this study was a source of n-3 PUFA. The anti-inflammatory content of n-3 PUFA can be used as therapy for chronic inflammatory diseases, such as TB. The n-3 PUFA can repair interferon- $\gamma$  and stimulate the immune response. The activation of n-3 PUFA to kill bacteria occurs through chemotaxis, antigen presentation, adhesion molecule expression, and the major histocompatibility complex. Fats, especially n-3 PUFA, can influence the maturation process of phagolysosomes and endosomal membrane lipid composition during a critical phase of mycobacterial clearance [14]. The n-3 PUFA can also integrate with cell membranes, including effector cells in the immune system, thereby affecting functional changes that can be referred to as resistance to disease [15].

The bloso fish is a source of heme iron. Bloso fish flour, bloso fish oil, and the dregs of bloso fish oil extraction increased hemoglobin levels during treatment under TB conditions (Table 1). Heme iron is easily absorbed during metabolism [16]. The conditions of our study were in agreement with those of studies of experimental animals with inflammatory anemia conditions that showed that iron mobilization and increased iron absorption could be mediated by erythroferrone suppression. The key regulator of iron homeostasis in humans is hepcidin which acts as ferroportin (FPN-1). Ferroportin is a membrane protein that has a role as the leading exporter of iron in mammalian cells. This ferroportin will have a different position in mammalian cells, such as macrophages which play a role in iron recycling, duodenum as an organ tasked with absorbing iron and hepatocytes which play a role in iron storage. Hepcidin will bind to ferroportin which causes internalization and is followed by cellular degradation which ultimately blocks the release of iron by enterocytes and reticuloendothelial cells to the bloodstream, which can ultimately maintain iron homeostasis. Increased extracellular and iron stores or inflammatory stimulation can trigger hepcidin expression. Hepcidin expression can be inhibited through hypoxia and erythropoiesis [17]. Hepcidin-modulated erythropoiesis to improve anemia. Patients with active TB who lack iron sources can experience anemia and hypoxia and require systemic signalling to enterocytes through the hepcidin-ferroportin axis induced by inflammation and regulation through intestinal hypoxia-inducible factor-2 $\alpha$  [18].

Anemia is common in patients with TB, with an incidence of up to 88%. Furthermore, anemia in patients with TB is caused by chronic inflammation [19]. Research conducted in Rio de Janeiro, Brazil, even showed that the proportion of patients with anemia due to chronic disease reached 75.9%. This proportioned figure is higher than iron deficiency anemia which only reaches 2.4%. This study also shows that the incidence of anaemia can be corrected without iron supplementation after TB therapy, either through food or medicine, [20]. Studies have shown a decrease in serum concentrations of C-reactive protein; and acute phase reactants; and an increase in the production of pro-inflammatory cytokines, such as interleukin-6, tumor necrosis factor- $\alpha$ , and interferon- $\gamma$ , which contribute to the occurrence of anemia by reducing erythropoietin products. Erythropoiesis disorders begin with suppression of the bone marrow response to erythropoietin, thus affecting iron metabolism [21]. TB is a chronic inflammatory condition that begins with increased hepcidin levels.

Furthermore, TB can trigger hepcidin in patients with anemia. Hepcidin levels are affected by iron deficiency, hypoxia, and erythropoiesis. Anemia is related to low hemoglobin levels or red blood cell concentrations caused by major hematological findings with chronic diseases [22]. Table 1 shows that Bloso fish can increase hemoglobin levels. Bloso fish is one of the animal proteins which is rich in iron. Incidents of infection trigger competition between hosts and pathogens for iron reserves. Dysregulation of iron is associated with infectious diseases, including TB. Iron is a micronutrient required by *Mycobacterium tuberculosis* to survive in the host. The ability of *Mycobacterium tuberculosis* to use available iron can change the host's response to infection. During infection, *Mycobacterium tuberculosis* can convert excess Fe into Fe reserves which are then converted into siderophores and used to support their growth and multiplication [16]. Therefore, additional food sources of protein are needed to compensate for the use of iron by *Mycobacterium tuberculosis*.

Anemia affects the quality of life and increases the morbidity and mortality of patients with TB. The cell-mediated immune response and bactericidal capacity of leukocytes of patients with anemia are also observed with suppressed conditions [23]. Research using a cross-sectional and case-control design showed that the TB incidence of patients with anemia was 3.56 times higher than that of patients without anemia. This is possible because, among patients with anemia, immuno-compromised patients have an imbalance of nutrients. Phagocytic macrophage activity is an important immunological response that controls TB infection through granuloma formation [24]. Granuloma formation is characterized by a collection of immune cells and mycobacterium walls that limit the replication and spread of tubercle bacilli. Anemia conditions, especially iron deficiency anemia, can interfere with the immune response mediated by T cells, thereby causing disturbances in the function of polymorphonuclear neutrophils and the intracellular bactericidal activity of immunological cells. Iron deficiency can change the balance between Th1 and Th2 cytokines, ultimately triggering the Th2-dominant response associated with TB [25].

Platelets are effector cells that have a role in the process of homeostasis and the initiation of wound healing. Furthermore, they are important in chronic infection and inflammation [26]. Additionally, they are one of the important markers of pulmonary cavitation. Platelets appear to support the host response to extracellular infection; however, with intracellular infections, they contribute to immune evasion [27]. Platelets can convert monocytes to an M2-like macrophage phenotype, thus creating a permissive environment for mycobacterial growth. Platelets can also induce foamy multinuclear epithelioids and macrophages with immunosuppressive capacities in vitro. Platelets contribute to TB pathology by increasing tissue damage by induction of matrix metalloproteinases in infected monocytes [28]. A study conducted by Fox- et al. showed that patients with TB had increased activated platelet markers and platelet-related factors that could be reduced through antibiotic therapy. Changes in platelet count, especially during TB infection, will be associated with death and severity of infection. Acute phase reactants and proinflammatory cytokines will affect megakaryocytes, reducing platelet size and platelet production from bone marrow in patients infected with *Mycobacterium tuberculosis* [29]. The provision of bloso fish in flour or oil can reduce the number of platelets (Table 1). Therefore, bloso fish have a role in reducing the inflammatory process and tissue damage. Platelets may mediate immunological mechanisms by forming immune cells and releasing chemokines and growth factors. Platelets can mediate the formation of granulomas by producing chemokines that involve innate cell responses such as neutrophils, monocytes, and macrophages in TB patients. Platelets can cause monocyte induction through collagenase activity, which will then differentiate from monocytes to become multinucleated giant cells. Research shows that the secretion of proinflammatory cytokines in TB patients will be activated directly by platelets after discovering *Mycobacterium tuberculosis* through receptors such as toll-like receptor-2 (TLR-2) and TLR-4. TLR-2 in the monocytes of TB patients will increase in the same direction as interleukin-1-beta (IL-1 $\beta$ ), IL-6 and IP-10. Proinflammatory cytokines can trigger the production of platelets by triggering megakaryocytopoiesis. In vitro studies involving murine animal models indicate that vascular endothelial growth factor (VEGF-A) can trigger platelet production by accelerating megakaryocyte maturation when interacting with the VEGFR1 receptor via a paracrine or autocrine mechanism. Platelets and monocytes can produce this receptor [30].



Figure 1 Picture bloso fish (*Glossogobius giuris* sp.).

Bloso fish is a local fish in Indonesia and is well known with buto cina. Bloso fish are included in demersal fish, having a cigar-shaped body, round or slightly flattened, head pointed and depressed, and the muzzle is wider than their length. The general colour is brown above and silver at the bottom. Bloso fish spread in Indonesia until the West Pacific region.

Table 1 Hemoglobin, hematocrit, platelet, and albumin levels of Wistar rats.

Parameters	Groups				
	K <sup>-a</sup>	K <sup>+b</sup>	K1 <sup>c</sup>	K2 <sup>d</sup>	K3 <sup>e</sup>
<b>Hb (g/dl)</b>					
Pretest	15.8 ±0.4	11.7±0.1	11.6 ±0.3	11.4 ±0.1	11.5 ±0.2
Posttest	15.6 ±0.3	11.6±0.1	14.1 ±0.4	14.9 ±0.2	14.1 ±0.2
Δ	-0.2 ±0.1 <sup>c,d,e</sup>	-0.1 ±0.1 <sup>c,d,e</sup>	2.5 ±0.5 <sup>a,b,d</sup>	3.5 ±0.3 <sup>a,b,c,e</sup>	2.6 ±0.3 <sup>a,b,d</sup>
<i>p</i>	0.001*	0.001*	0.001*	0.001*	0.001*
<b>Ht (%)</b>					
Pretest	48.7 ±0.3	35.2 ±0.5	35.1 ±0.6	34.8 ±0.2	34.9 ±0.4
Posttest	48.6 ±0.3 <sup>7</sup>	35.0 ±0.5	46.2 ±0.4	47.8 ±0.5	42.3 ±0.9
Δ	-0.1 ±0.0 <sup>c,d,e</sup>	-0.1 ±0.1 <sup>c,d,e</sup>	11.1 ±0.6 <sup>a,b,c,d,e</sup>	12.9 ±0.6 <sup>a,b,c,d,e</sup>	7.5 ±0.9 <sup>a,b,c,d,e</sup>
<i>p</i>	0.006*	0.041*	0.001*	0.001*	0.001*
<b>Platelets (10<sup>3</sup>/μL)</b>					
Pretest	166.0 ±4.2	787.6 ±3.1	784.4 ±5.3	787.0 ±5.2	784.4 ±3.3
Posttest	163.9 ±4.3	789.6 ±3.4	190.0 ±4.9	175.7 ±5.6	192.3 ±5.1
Δ	-2.1 ±0.7 <sup>c,d,e</sup>	2.0 ±0.6 <sup>c,d,e</sup>	-594.4 ±8.8 <sup>a,b,d</sup>	-611.3 ±7.5 <sup>a,b,c,d,e</sup>	-592.1 ±6.8 <sup>a,b,d</sup>
<i>p</i>	0.001*	0.001*	0.001*	0.001*	0.001*
<b>Albumin (g/dl)</b>					
Pretest	5.9 ±0.2	1.1 ±0.3	0.9 ±0.1	1.0 ±0.1	0.8 ±0.1
Posttest	5.9 ±0.2 <sup>7</sup>	1.0 ±0.2	5.0 ±0.1	5.7 ±0.1	4.1 ±0.1
Δ	-0.0 ±0.0 <sup>c,d,e</sup>	-0.1 ±0.1 <sup>c,d,e</sup>	4.1 ±0.1 <sup>a,b,c,d,e</sup>	4.7 ±0.1 <sup>a,b,c,d,e</sup>	3.3 ±0.1 <sup>a,b,c,d,e</sup>
<i>p</i>	0.001*	0.001*	0.001*	0.001*	0.001*

Note: <sup>a</sup>Sampling was performed 14 days after the induction of hypoalbuminemia and 28 days after the beginning of treatment. K<sup>-</sup>: negative control group (normal); K<sup>+</sup>: positive control group with hypoalbuminemia; K1: hypoalbuminemia group; treated with 675 mg/200 g of bloso fish flour; K2: hypoalbuminemia group, treated with 67.5 mg/200 g of fish oil; K3: hypoalbuminemia group, treated with 675 mg/200 g of the remaining oil extracted from bloso fish that was processed into fish flour. Values represent the mean ± standard deviation of the observation mode for seven rats in each group. Statistical analysis: \**p*; paired t- test; (significant difference at

( $p < 0.05$ ). One-way analysis of variance; when significant, post hoc testing (least significant difference) was performed for intergroup comparisons. <sup>a</sup>Statistically significant difference ( $p < 0.05$ ) when compared with K- values; <sup>b</sup>Statistically significant difference ( $p < 0.05$ ) when compared with K+ values; <sup>c</sup>Statistically significant difference ( $p < 0.05$ ) when compared with K1 values; <sup>d</sup>Statistically significant difference ( $p < 0.05$ ) when compared with K2 values; <sup>e</sup>Statistically significant difference ( $p < 0.05$ ) when compared with K3 values.

Food and nutrition management can reduce TB's incidence and mortality rates [31]. Research conducted by Matos et al. indicated that low serum albumin levels at hospital admission are a strong risk factor for death. Furthermore, a study performed in Korea a relationship between malnutrition and death attributable to TB for hospitalized patients [32]. TB can cause malnutrition due to decreased food intake and increased use of energy and nutrients in the body. Nutritional support is needed for patient survival and the body's functional abilities. Nutritional support provided to patients should be a single component considering the amount of nutrients and composition. A study of HIV-infected TB patients who were given energy and protein supplementation in the form of biscuits showed an improvement in hand-grip strength but not in body weight or body composition [33]. Hand-grip strength is a measure of work capacity in TB patients. Measurement of hand-grip strength also shows the ability to improve food intake and TB care carried out.

The condition of malnutrition in TB patients is characterized by insufficient intake of protein and total calories. A study conducted in China showed that there was a condition of protein-calorie malnutrition in TB patients. This condition can reduce the effectiveness of components that play an important role in cell immunity. Malnutrition makes a negative contribution to TB care. Malnutrition can reduce protein level in the patient's body, slowing down the lesions' healing process. Adequate protein content not only provides benefits in repairing lesions during TB treatment but is able to increase the amount of TB drug carrier protein so as to increase the concentration of anti-tuberculosis drugs in the blood and assist in sputum conversion [34].

The condition of TB is capable of losing weight so that the patient is in a state of undernutrition. This undernutrition condition can be caused by decreased food intake or factors related to TB such as cachexia, due to metabolic dysfunction, poor absorption, fever, and anorexia. Metabolic changes due to TB can trigger a condition called anabolic block. This anabolic block allows the intake of protein sources to act as a source of energy but not as an ingredient in the anabolic process. Research shows that supplementation with macronutrients affects weight gain in TB patients so that they can improve their quality of life, including reducing the risk of hypercatabolism due to febrile illness [35].

Serum albumin is an important marker of the nutritional status of a patient with TB [13]. The serum albumin level with TB can be used as a marker of liver function during the therapy process, is inexpensive, and used as a monitoring tool to determine the success of TB therapy [36]. The albumin serum concentration of the experimental animals injected with *S. aureus* was decreased compared to that of the control group. Albumin levels increased after the administration of bloso fish, as either flour or oil (Table 1). Hypoalbuminemia is a risk factor for death for elderly individuals with TB [37]. Hypoalbuminemia can change the total and relative numbers of T-lymphocytes and various immune system cells, ultimately helping the host fight against *Mycobacterium tuberculosis*. Low serum albumin levels are generally associated with an increased risk of inflammation because of the increased load of tubercle bacilli at pulmonary sites in patients with TB [38]. The administration of bloso fish preparations may be an alternative method of reducing the bacterial load and inhibiting bacterial replication [39].

## CONCLUSION

Hemoglobin, hematocrit, platelets, and albumin are markers used to detect TB. Patients with TB have a variety of haematological manifestations with features of anemia, hypoalbuminemia, and thrombocytosis. Therapy using bloso fish flour and bloso fish oil improved hemoglobin, hematocrit, platelets, and albumin levels. The weakness of this study is that it does not examine proinflammatory cytokines or the components responsible for inducing the inflammatory process. Examination using ferritin and serum transferrin should be advised to determine anemia activation of tuberculosis patients. Future research requires processed forms made from bloso fish flour or bloso fish oil to better understand its effects on TB patients who are experiencing malnutrition.

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The authors declare that they have no conflict of interest.

#### **Ethical Statement:**

The use of animals in this research was approved by the Ethics Committee of Health Universitas Negeri Semarang following the legislation of the Declaration of Helsinki (1964) and CIOMS WHO (2016) (Project identification code: 138/KEPK/EC/2022, Date of approval: March, 24, 2022, Name of the Ethics Committee: Ethics Committee of Health, Universitas Negeri Semarang).

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