

## ***Pengaruh Probiotik dan Zinc terhadap Kadar Hemoglobin Tikus Malnutrisi***

### **The Effects of Probiotic and Zinc on Hemoglobin Levels in Malnourished Rats**

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#### **ABSTRAK**

**Latar Belakang:** Pemberian suplementasi zat besi untuk mencegah dan mengatasi anemia belum mampu memaksimalkan penurunan angka anemia pada anak. Zat besi yang tidak mencukupi kebutuhan harian, rendahnya penyerapan zat besi, dan kurangnya keanekaragaman makanan yang mengandung zat tersebut menjadi penyebab utama anemia pada anak. Kemampuan absorpsi makro dan mikro-nutrient sangat dipengaruhi oleh struktur histologi usus halus. Perbaikan struktur histologi usus halus dengan pemberian probiotik dan zinc pada kasus malnutrisi diharapkan mampu meningkatkan fungsi absorpsi, sehingga penyerapan nutrisi, terutama zat besi, dapat lebih baik.

**Tujuan:** Penelitian ini bertujuan untuk mengetahui pengaruh probiotik dan zinc terhadap kadar hemoglobin pada tikus malnutrisi.

**Metode:** Penelitian ini menerapkan post test only control group design menggunakan 30 ekor tikus berusia 8 minggu, berat badan 180-200 g, dibagi menjadi 5 kelompok secara acak. Kelompok N diberi diet standar, kelompok M, PZ, P, dan Z diberi diet rendah kalori selama 14 hari untuk membuat kondisi malnutrisi. Perlakuan dimulai pada hari ke-15 sampai 28, kelompok M tetap diberi diet rendah kalori, kelompok PZ, P, dan Z diberi diet standar dan suplementasi. Kelompok PZ diberi kombinasi probiotik dan zinc, kelompok P diberi probiotik, dan kelompok Z diberi zinc. Hari ke-29 seluruh sampel darah diperiksa kadar Hb menggunakan spektrofotometri.

**Hasil:** Terdapat perbedaan rerata kadar hemoglobin yang signifikan antara kelompok malnutrisi dan perlakuan ( $p < 0.0001$ ). Rerata kadar hemoglobin pada kelompok probiotik secara signifikan lebih tinggi dan kelompok kombinasi secara signifikan menunjukkan kadar hemoglobin yang lebih rendah diantara kelompok perlakuan lain.

**Kesimpulan:** pemberian probiotik dan zinc berpengaruh secara signifikan terhadap kadar hemoglobin tikus malnutrisi.

**Kata kunci:** Probiotik, Zinc, Hemoglobin, Malnutrisi

#### **ABSTRACT**

**Backgrounds:** Iron supplementation has not been able to maximize the reduction of anemia in children. Iron consumption that is not sufficient for daily needs and low iron absorption and lack of diversity of food sources of iron are the main causes of anemia in children. The absorption ability of macro and micro-nutrients is strongly influenced by the histological features of the ileum. Improvements in the histological features of the ileum by administering probiotics and zinc in cases of malnutrition are expected to improve absorption function, so that absorption of nutrients, especially iron, can be better

**Objectives:** To assess the effect of probiotics and zinc on hemoglobin levels in malnourished rats.

**Methods:** The experimental study, using 30 rats aged 8 weeks, body weight 180-200 g, divided randomly into 5 groups. Group N was given a standard diet, groups M, PZ, P, and Z were given a low-calorie diet for 14 days to make malnourished. The treatment was started on day 15 to 28, group M was still given a low-calorie diet, groups PZ, P, and Z were given a standard diet and supplementation. PZ group was given a combination of probiotics and zinc, P group was given probiotics, and Z group was given zinc. On the 29th day, all blood samples were checked for Hb levels using spectrophotometry.

**Results:** There was a significant difference in hemoglobin levels between the malnourished group and the treatment group ( $p < 0.001$ ). Hemoglobin levels in probiotic group were significantly higher among the other treatment groups and the combination group indicated significantly lower hemoglobin levels.

**Conclusions:** Probiotic and zinc administration significantly influence hemoglobin levels in malnourished rats.

**Keywords:** Probiotics, Zinc, Hemoglobin, Malnutrition

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## INTRODUCTION

The provision of iron supplementation to prevent and treat anemia has not been able to maximize the reduction of anemia in children. The incidence of anemia is strongly influenced by iron status in the blood, this is closely related to nutritional intake<sup>1</sup>. Anemia in children is caused by inadequate consumption of iron for daily needs and low absorption and lack of diversity in iron sources. The ability to absorb macro and micro-nutrients is strongly influenced by the histological structure of the small intestine<sup>2</sup>. Malnutrition that occurs in children can lead to atrophy of the small intestine which interferes with the absorption of nutrients. The improvement of the histological structure of the small intestine by giving probiotics and zinc in cases of malnutrition is expected to improve the absorption function, so that the absorption of nutrients, especially iron, can be better.<sup>3</sup>

Data in Indonesia shows that the population group aged 1 years suffering from anemia reaches 21.7%. The number of children under five with anemia is quite high, namely 28.1% and shows a decrease after entering school age, adolescents to adults aged  $\pm$  34 years, but the trend is increasing again in higher age groups.<sup>4</sup> Of the total population of people with anemia, the entire incidence of anemia in pre-school children (0-5 years) reaches 47.7% worldwide. Southeast Asia occupies the highest prevalence reaching 65.5%, and in Indonesia itself at 44.5%. Health problems that arise from anemia were serious because anemia can reduce cognitive function and interfere with motor and behavioral development in children. Growth delays, mental development disorders, reduced intelligence can result in decreased work productivity and impaired reproductive function in the future<sup>2</sup>.

Iron Deficiency Anemia (IDA) was the most common anemia found in cases of malnutrition. Improvement of the structure of the small intestine was important to increase the absorption of nutrients, such as iron. Zinc and probiotics were known to reduce the severity of microvilli damage by maintaining the integrity of the intestinal mucosa, increasing immunity, and as an immunomodulator.<sup>3</sup> The integrity of the intestinal mucosa can be maintained if zinc needs are met properly because its function is to regenerate cells and stabilize cell membranes<sup>5</sup>. Administration of zinc was important because it is able to maintain the integrity of the intestinal mucosa and stabilize cell membranes by increasing the expression of zonular occludin (ZO)-1, ZO-2, and claudins at tight junctions.<sup>6</sup> Bacterial fermentation is able to synthesize short chain fatty acids (SCFAs), in the form of polysaccharides which were the main source of nutrition for the intestinal epithelium<sup>7</sup>. Administration of pre- and pro-biotics in malnourished conditions can increase the synthesis and absorption of micronutrients and minerals, such as magnesium, iron, and calcium. This was due to an increase in the production of short chain fatty acids (SCFAs) which causes a decrease in intestinal pH and repairs the epithelium so that it is able to perform its absorption function properly.<sup>8,9</sup> This study aims to assess hemoglobin levels in malnourished rats after administration of zinc and probiotics either in

combination or singly which is expected to improve the microvilli of the small intestine so that nutrients can be well absorbed and can increase hemoglobin levels.

## METHODS

### Place and time of research

The research was conducted in October – November 2019 at the Inter-University Central Laboratory, Center for Food and Nutrition Studies (PSPG) Gajah Mada University, Yogyakarta. Experimental laboratory was the choice of this type of research with a post test only randomized control group design using male white rats of wistar strain (*Rattus norvegicus*) with malnourished conditions. The Ethics Commission of the Sultan Agung Islamic University Faculty of Medicine has given approval to all methods used in this study by letter number 603/VIII/2019/Bioethics Commission.

### Research Flow

Thirty rats with a body weight of around 150-200 grams aged  $\pm$  8 weeks, not sick, active, and no anatomical abnormalities were seen in rats were divided into 5 groups with 6 rats per group (based on WHO rules for research criteria). experimental animal). Group N as a control group that consumed standard feed AD II comfeed as much as 8g/100gBW/day and group M as a malnourished group that received a low-calorie diet during the study. PZ, P, and Z groups were given preconditioning treatment by giving a low-calorie diet for 14 days to make the condition of malnutrition<sup>10</sup>. The low-calorie diet given was feed at a dose of 4g/100gBB/day<sup>10,11</sup>. Fourteen days later, the PZ group was given probiotics and zinc, the P group received probiotics, and the Z group received zinc. The three groups again received standard feed.

### Probiotic Dosage

The probiotics used were composed of *Lactobacillus acidophilus* and *Bifidobacterium longum*. These two probiotics were chosen because they have been widely used before and the products on the market contain these strains<sup>12,13</sup>. The conversion factor based on adult human body weight (70 kg) to mouse body weight (200 g) is 0.018. The daily dose of probiotics for adults is 340 mg/day<sup>14</sup>. Dose calculations were obtained from conversion, which was 6.12 mg/200g/day given with a frequency of once a day orally (sonde) for 14 days.

### Zinc Dosage

Based on the dose of zinc for adults per day of 20 mg/day<sup>15</sup>, then the dose of zinc in this study (conversion factor: 0.018) was 0.36 mg/200g/day given with a frequency of once a day orally (sonde) for 14 days.

### Hemoglobin Check

Examination of hemoglobin levels on the 29th day at the Gajah Mada University laboratory by taking blood through the orbital sinus and examined using spectrophotometry and the unit used g/dL.



**Data analysis**

The results of the normality and homogeneity test showed that the data distribution was normal and the data variance was homogeneous. Data were analyzed by Oneway ANOVA test and then to find out the differences between groups followed by post hoc Tukey's Test. The decision to accept or reject the hypothesis was based on the value of 5% ( $p < 0.05$ ).

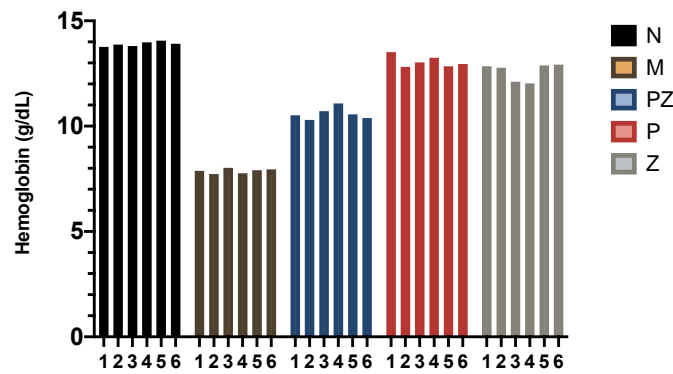
**RESULTS AND DISCUSSION**

The results of the examination of hemoglobin levels using the spectrophotometric method see figure 1, and the average hemoglobin levels see table 1. Analysis of the normality of distribution of data (figure 3) shows that all groups were normally distributed ( $p > 0.05$ ). The results of the homogeneity analysis of data variance showed that the data had a homogeneous variance ( $p > 0.05$ ) (figure 4). From Figure 2, it was known that the highest mean hemoglobin was group N ( $13.90 \pm 0.112$ g/dL) and the lowest in the M group ( $7.88 \pm 0.112$ g/dL). Research that has been conducted at the Integrated Research and Testing Laboratory of Gadjah Mada University states that the normal hemoglobin value

for male Wistar rats aged 8 weeks were  $10.63 \pm 0.26$ g/dL<sup>16</sup>. Based on these values, group M experienced a decrease in hemoglobin levels. The previous theory explained that malnutrition was one of the factors of anemia. The high prevalence of anemia was strongly influenced by intake. A minimal intake of iron was the most common cause of iron deficiency anemia, besides that other nutrients such as folic acid, vitamins C and B12, protein, and zinc were associated with this problem.<sup>17</sup>. The results of giving a low-calorie diet (4g/100gBW/day) during this study were in line with previous studies, which stated that the morphology of the small intestine in experimental animals will experience atrophy in malnutrition conditions. This atrophy of the small intestine will further interfere with the absorption function of the small intestine<sup>18</sup>. Individuals with poor nutritional status experience histological and functional changes in the intestinal mucosa which was the main site for absorption of heme and non-heme iron<sup>19</sup>, seen from group M who received a low-calorie diet from the beginning to the end of the study showed the lowest Hb levels.

**Table 1.** Average Hemoglobin Level

Group	Average	Standard Deviation
N	13.90	0.112
M	7,875	0.112
PZ	10.59	0.279
P	13.07	0.269
Z	12.59	0.408



**Figure 1.** Hemoglobin Levels in each Group of Rats



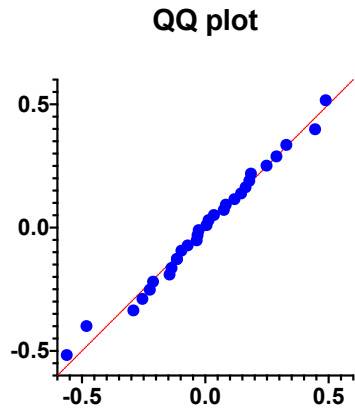


Figure 2. Normality Test Results

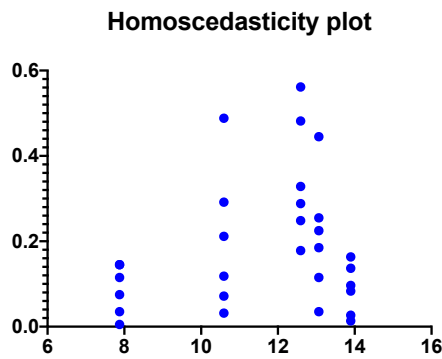


Figure 3. Homogeneity Test Results

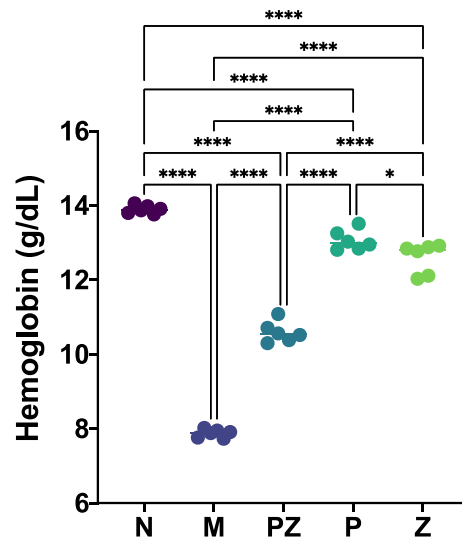


Figure 4. Tukey's Test Post hoc Test Results  
 \*\*\*\*p<0.0001  
 \* p<0.05

Tukey's test	Average Difference	95.00% KP
N - M	6.02	5.579 to 6,465
N - PZ	3,305	2,862 to 3,748
N - P	0.8317	0.3885 to 1.275
N - Z	1.305	0.8619 to 1.748
M - PZ	-2,717	-3,160 to -2,274



M - P	-5,190	-5.633 to -4.747
M - Z	-4,717	-5,160 to -4,274
PZ - P	-2.473	-2,916 to -2,030
PZ - Z	-2,000	-2.443 to -1.557
P - Z	0.4733	0.03021 to 0.9165

In contrast to the normal group given a standard diet, hemoglobin levels were still within normal limits because macro and micronutrient needs were met appropriately. While in the treatment group, the highest mean was group P ( $13.07 \pm 0.269$  g/dL), followed by group Z ( $12.59 \pm 0.408$  g/dL), and the lowest was the PZ group ( $10.59 \pm 0.279$  g/dL). Hemoglobin levels in all treatment groups showed a significant difference compared to group M ( $p < 0.001$ ). The results of the One-way ANOVA test showed that there were differences in each treatment group ( $p < 0.001$ ) and each group was significantly different (figure 2). Giving probiotics in this study was able to increase the average hemoglobin level better than the other groups. This was in line with the benefits of giving probiotics that have been studied previously. Research suggests that the microbiota in the gut can be affected by iron deficiency conditions. Giving probiotics was able to ferment galacto-oligosaccharides which causes a decrease in pH in the intestine so that it can increase iron absorption. In addition, the intestinal epithelium of anemic mice can store iron better after colonization of intestinal bacteria was carried out. These results indicate that *Lactobacillus fermentum* can produce a metabolite in the form of P-hydroxyphenilactic acid, which increases iron absorption through the DMT 1 transporter from enterocytes by reducing Fe<sup>3+</sup> to Fe<sup>2+</sup>.<sup>20</sup>

Hemoglobin levels in the group given zinc alone were better than the combination group ( $p < 0.0001$ ). Research shows that anemia was strongly associated with zinc deficiency, especially in pregnant women and preschool children. Zinc was needed in the process of erythropoiesis to stimulate the formation of red blood cells along with vitamin B12, folic acid, and iron. Giving zinc can increase hemoglobin levels in patients with kidney failure who have undergone hemodialysis<sup>21</sup>. Studies using cell cultures and experimental animals show that the expression of iron transporters and regulatory proteins was regulated by zinc. Zinc induces iron absorption and transcellular transport in small intestinal cells through induction of DMT1 and FPN1 expression<sup>22</sup>. Hemoglobin levels in the probiotic and zinc combination group in this study were not better than the single group ( $p < 0.0001$ ), but were still in the normal range and higher than the malnutrition group ( $p < 0.0001$ ).<sup>16</sup> This study was in line with previous research on the administration of a combination of probiotics and zinc, which was not significantly different from the administration alone<sup>3,23,24</sup>. Hemoglobin levels in baby monkeys aged 2 months and 4 months who were given a single probiotic were higher than the combination group of probiotics and zinc. The low level of iron in the combination group of the study was possible due to the low absorption of iron due to zinc administration, resulting in competition for mucosal transport between zinc, copper, and iron.<sup>23</sup> Research on the interaction of these three elements explains that iron,

copper, and zinc can affect the absorption of each other. When the concentration of copper and zinc increases, the concentration of iron decreases, and vice versa. This competition was related to competition for DMT1 transporters involved in the transport of divalent metals, such as Cu, Zn, Mn, and Pb. The DMT1 transporter was the main Fe<sup>2+</sup> transporter and was active as a Cu<sup>1+</sup> transporter<sup>25</sup>. In addition to the interactions that occur between these metal elements, zinc also interacts with bacteria strains of *Lactobacillus* and *Bifidobacterium*. Some strains of *Lactobacillus* actually use zinc for their growth which was influenced by the activity of these bacteria, so that what was absorbed by the small intestine will be reduced. Anti-nutritional factors such as phytate also affect zinc absorption because phytate will bind zinc strongly so that it cannot be absorbed and was excreted with feces<sup>26</sup>. The potential risks of these interactions were taken into consideration in establishing a fortification or supplementation program<sup>25</sup>.

Each microorganism has different properties, the selection of strains, doses, and ways of administration can have different effects. Aspects of pharmacodynamics, pharmacokinetics, synergism and antagonistic effects when probiotics were combined with zinc still require further research. The results of studies on the combination of probiotics and zinc were still very limited, although their effects have been tested alone<sup>27</sup>. This study limits the method used to only use a post test only control group design, it was hoped that the next research will be more complex in choosing research methods.

## CONCLUSION

Hemoglobin levels in malnourished rats can be improved by giving probiotic and zinc, either alone or in combination. In this study, the combination administration did not give better results of hemoglobin levels than the single group. The literature that discusses the mechanism of the combination of probiotics and zinc was still very limited, so further research was needed. Giving probiotics and zinc can be an alternative in overcoming anemia in cases of malnutrition.

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## Conflict of Interest and Funding Disclosure

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