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Erythrocyte Formation Rate in Wistar Anemia Induced 2,4-Dinitrophenylhydrazine through Intake Maize Biofortified Iron

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Abstract

BACKGROUND: Biofortification through *Pseudomonas putida* IFO 14796 intervention has succeeded to improve iron content in maize grain up to 18.79%.

AIM: This study was carried out to assess the effect intake of maize biofortified iron (MBI) on the red blood cell (RBC) formation rate in Wistar rat anemia induced 2,4 dinitrophenylhydrazine.

METHODS: Randomized complete design was carried out to assess the effect of MBI on the erythrocyte level with four levels of MBI treatment (R1=10%; R2=12%; R3=14%, and R4=16% of rat body weight [BW]) and one of control (Ro=10% maize non-biofortified iron), and five replications, respectively. Erythrocyte level was measured using the Counting Neubauer Chamber Method after Wistar anemia induced 2,4-dinitrophenylhydrazine (DPNH) after intake MBI for 7 days. Data were analyzed by ANOVA and Fisher's least significant difference.

RESULTS: There was an influence intake of MBI level on the improving rate the erythrocyte formation in Wistar anemia ($p < 0.05$). Treatment R1 improved to 0.058±0.034% significantly different with R0 (0.006±0.017%), but non-significantly with R2 and R4 at $p < 0.05$. Linear regression showed the equation, $y=-0.002x+0.07$; $R^2=0.69$.

CONCLUSION: Intake MBI more than 10% BW negatively effect to RBC formation rate of Wistar rat's anemia.

Introduction

Malnutrition and iron-deficiency anemia the main issue in various countries in the world and become a factor to increase the mortality rate. The main type of malnutrition from micronutrient was an iron, Zn, and Vitamin A deficiency [1]. Iron deficiency in the body can be caused by low iron, and Vitamin C intake, low iron absorption from foods high in phytate or phenol compounds, blood loss, parasitic infections, even manifestations of chronic kidney diseases, malignancies, and autoimmune disorders [2], [3], [4], [5]. Deficiency iron supply from food can decrease in hemoglobin, hematocrit, and erythrocytes formation [6], [7].

Strategies to reduce the burden of the iron deficiency can be done by modification food, provide Fe supplement or fortification, and food biofortified iron. While biofortification can be done through transgenic, conventional plant breeding, and agronomic interventions through micronutrient fertilizer application [8]. Biofortification through *Pseudomonas putida* IFO 14796 intervention has succeeded to improve iron content in maize grain up to 18.79% [9].

Consumption of pearl millet biofortified iron (*Pennisetum glaucum*) from transgenic has been increased absorption, and effectively overcome the iron deficiency in people [10]. However, some arguments show that food products from transgenics have serious effects on the genetic changes in consumer. In addition, iron accumulation in the brain plays a central role for inducing oxidative stress in neurodegeneration [6], [11]. This study was carried out to assess the effect intake of maize biofortified iron (MBI) contained 10.117 mg/kg on erythrocyte formation in Wistar rat anemia.

Materials and Methods

Location and time

The experiment was undertaken for 21 days from December 17, 2019, to January 05, 2020, in

the center for an integrated laboratory of Dayanu Ikhsanuddin University, Baubau City, Southeast Sulawesi-Indonesia.

Experimental materials

Female Wistar was accessed from Bandung, Indonesia (Certificate Veterine: No. 524.3/3873-Dispangtan/2019). Female Wistar rats anemia resulted from induced by injection 40 mg/kg 2,4 dinitrophenylhydrazine for 4–7 days. MBI contained 10.117 mg/kg was produced from the previous project through *P. putida* IFO 14796 intervention.

Design experiment

The research was carried out with the randomized complete design, consisting of four treatment groups and one control group. The treatment group contained experiment units R1 (10%), R2 (12%), R3 (14%), R4 (16%), and R0 (10%) of body weight (BW). MBI was an independent variable, and erythrocyte level for the dependent variable. The sample size was 25 female Wistar anemia (*Rattus novergicus*) randomly placed into five groups. Each group consisted of five animals that were placed separately in a 30 cm × 25 cm × 30 cm cage. The treatment group of Wistar anemia was supplied food from MBI 3 times a day as well as a control group by maize non-biofortified iron, and water drinking by *ad libitum* [12]. In addition, Wistar anemia was treated well in a room with good ventilation, normal sun exposure through the window, temperature 27–30°C, and low humidity. Blood was taken from the lateral vein of rat's tail based on standard operational procedure (SOP) *Intravenous Injection in the Rat* [13] for measuring the total erythrocyte of using Counting Neubauer Chamber Method [14].

Data analysis

The effect of intake of the MBI for the erythrocyte formation rate in Wistar anemia was analyzed using statistical ANOVA one-way and Fisher's least significant difference (LSD) at $p < 0.05$.

Results

The mean erythrocyte of Wistar rat anemia was 5.34–6.55 × 10^6 μL^{-1} in range, whereas the number of erythrocytes has increased to be 5.74–8.74 × 10^6 μL^{-1} in range after intake MBI for 7 consecutive days. The highest erythrocyte formation rate occurred in the treatment R1 (0.058±0.034) and the lowest in the R3 (−0.008±0.011%) (Table 1).

Table 1: Average of erythrocyte formation rate of Wistar rats after intake maize biofortified iron (% d^{-1})

Treatment	Number of erythrocyte ($\times 10^6/\mu L$)	($\times 10^6/\mu L$)	Means rate ± SD (% d^{-1})
R0	5.50 ± 0.44	5.78 ± 0.74	0.006 ± 0.017[b]
R1	5.34 ± 0.31	8.74 ± 2.72	0.058 ± 0.034[a]
R2	5.51 ± 0.39	6.65 ± 0.78	0.030 ± 0.022[a]
R3	5.41 ± 0.44	5.03 ± 0.16	−0.008 ± 0.011[b]
R4	6.55 ± 1.42	5.74 ± 0.79	0.010 ± 0.032[a]

The same alphabet was non-significantly different at $p<0.05$

The analysis of variance showed a significant effect of MBI intake on the erythrocytes formation rate in Wistar rats anemia ($p < 0.05$). Likewise, with Fisher's LSD test showed a different means of erythrocyte formation rate in R1, R2, R4, against R0 (control) at $p < 0.05$ (Table 1). The erythrocyte formation rate in Wistar rats anemia tends to decrease with increase the level of MBI intake (%), while based on the linear regression analysis obtained the equation: $Y=-0.018x+0.067$, $R^2=0.688$ (Figure 1).Thus, MBI intake was negatively correlated to the erythrocyte formation rate in Wistar rats anemia. The formation erythrocyte of red blood cells tends to decrease with increasing MBI intake more the 10%.

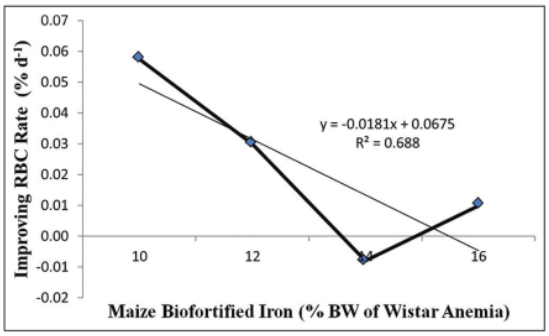

Figure 1: Erythrocyte formation rate with intake maize biofortified iron level

Discussion

The erythrocyte formation tends to decrease with increased intake of MBI. Erythrocyte formation rate was a variety from (−0.008±0.011%) to (0.058±0.034%) per day in range. According to Monette *et al*. [15], the formation of erythrocytes in Wistar rats has been undertaken up to 6.5 h. However, increasing erythropoiesis stimulation in the lymph up to 5–6 times and 2–3 in the bone marrow if there was acute bleeding or hypoxia. During bleeding, lymphatics increase erythropoiesis from 10% to 40% in normal conditions [16]. However, erythropoiesis can only take place perfectly if nutritional supply enough for the production of globins and heme such as amino acids, iron, Vitamins B12, B6, and folic acids (component B2), nickel (Ni) and cobalt (Co), and erythropoietin hormone [17].

Maize grain contains protein stored in the lumen of the endoplasmic reticulum, rich in beta, and gamma-zein [18], and contains about 1756 kind of proteins [19], leucine, lysine, tryptophan, methionine, isoleucine, valine, phenylalanine, glutamic acid, serine, alanine, tyrosine, and proline [20], [21], while the fat content of maize grain was 3.21–7.71% in the range [22]. The low erythrocyte formation in Wistar anemia with increase intake MBI level may be related to high inhibitor iron absorption in the maize grain.

Phytate, polyphenols, calcium, ascorbic acid, and tissue were able to inhibit the absorption of iron [23]. Maize grain contained high phytate compounds in fresh corn up to 1.71 g/kg and dry corn (7.15–7.60 g/kg). However, heating will be able to reduce the levels of phytate acid in fresh corn by 18.1–46.7% and dry corn (11.5–52.6%). This study treated dried maize grain with soaked in water for 24 h and then heated in the presto cooker at 100°C for 10 min. This was intended to help reduce phytic acid levels in dried maize grain. However, the erythrocyte formation rate tends to decrease with MBI level. The weakness of this study is the absence of observations of Vitamin B, folic acid, and others important factor supporting the erythrocyte formation.

Conclusion

The maximum level of MBI for erythrocyte formation in Wistar rats anemia was 10% of BW. Forward research is important to confirm the human equivalence iron dose (HEID) from MBI for children and adult in anemia.

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