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Improving Body Weight of Female Wistar Rats Anemia by Using Iron Biofortified Maize

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Abstract
This research aimed to evaluate the effect of iron biofortified maize (IBM) on improving the body weight of Wistar anemia. The randomized complete design was carried out with four IBM levels covered R-1=10%; R-2= 12%; R-3=14%, and R-4=16% of body weight. The body weight was measured after IBM intake for 7 days. Data analyzed by ANOVA, Fisher's LSD, and Linear regression. There was an influence IBM on the improvement of the body weight of Wistar anemia. The R-3 improved up to 0.0109% d^{-1} significantly different from others at $p<0.05$. The body weight tends to increase with the IBM level following the equation Y=0.005x-0.0096; R^2 = 0.79. The maximum safe level of IBM for the body weight of anemic Wistar rat was 14%.

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Iron Biofortified Maize; Maximum Safe Level; Wistar Rat Anemia.

Introduction

Malnutrition and chronic diseases are a global issue, especially in developing countries and it contributes to mortality rates. Malnutrition has become an inhibition factor in the achievement of Millennium Development Goals 2015. The high prevalence of anemia caused by iron deficiency has become pregnant women, infants, and children under 2 years of age, pre school children, school-age children, adolescents, and non-pregnant women.[1] In Indonesia, malnutrition has been a public health issue with a wasting prevalence of 13% and stunting 36%, and anemia in the moderate category.[2] Low nutrient intake to be a significant determinant

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factor as a cause of micronutrient deficiencies, including iron deficiency.[3]

Biofortification of micronutrient in rice (*Oryza sativa* L.), maize, and wheat was believed to be a sustainable strategy to overcome iron and zinc deficiency in humans.[4] Maize grainhas been the staple food of the world community after rice and wheat, including in Mexico, Sahara-Africa, and Indonesia.[5-7] Maize biofortified iron has not been widely applied, and the interventions needed in the future to improve its quality and overcome micronutrient deficiencies.[8] Previously studied show that biofortification through *Pseudomonas putida* IFO 14796 intervention has improved iron content in maize grain up to 18.79%,[9] and intake 10% of the iron biofortified maize (IBM) was able to improve the erythrocyte formation in Wistar rats anemia.[10]

Therefore, to observe the effect of maize biofortified iron on the improvement of the body weigh also needs to be observed how it affects body weight. Wistar rats (Rattus norvegicus) were widely used in laboratory studies as an animal model. They are feasible enough to mimic all aspects of human abnormalities, develop all the main signs of metabolic syndrome, obesity, diabetes, dyslipidemia, hypertension, liver disorders, and kidney dysfunction as well as micronutrient deficiencies.[11] This research aimed to assess the effect of IBM on the bodyweight of Wistar rat anemia.

Materials and Methods

Location and Time: The experiment was undertaken for 21 days from December 17th, 2019 to January 5th, 2020 in the center for an integrated laboratory of Dayanu Ikhsanuddin University, Baubau City, Southeast Sulawesi-Indonesia.

Experiment Materials

Female Wistarwas accessed from Bandung, Indonesia (Certificate Veterine: No. 524.3/3873-Dispangtan/2019). Female Wistar rats anemia 150-200 g induced by injection 40 mg kg^{-1} 2.4 dinitrophenyl hydrazine (DPNH) for 4-7 days resulted in anemia (Hb<12 mg dL^{-1}). Iron biofortified Maize (IBM) 10.117 mg kg^{-1} was produced from the previous project through an experiment using P. putida IFO 14796.

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%), and R4(16%) and Ro (10%) body weight (BW). Iron biofortified maize (IBM) as an independent variable and formation bodyweight as the dependent variable. The sample size was 25 female Wistar anemia (*Rattus novergicus*) randomly placedinto 5 groups. Each group consisted of 5 animals were placed separately in a 30 x 25 x 30 cm cage. The treatment group of Wistar anemia was supplied food from iron biofortified maize (IBM) 3 times a day as well as a control group by iron non-biofortified maize (INBM), and water drinking by addlabitunc[2] Also, Wistar anemia was treated well in a room with good ventilation, normal sun exposure through the window, temperature 27-30°C, and low humidity. Bodyweight of Wistar rats was measured at the beginning and end of the study using the SF-400A "Electronic Compact Scale" digital balance. While improving body weightrate (GR) was calculated using the formula.[13]

GR= (Wt-Wo) / t x 100%

Data Analysis

The effect of intake of the iron biofortified maize (MBI) for the bodyweight formation rate of Wistar anemia was analyzed using statistical ANOVA-ONE WAY and Fisher's LSD (Least Significance Difference) at $p<0.05$.

Ethical Matters

The investigation was approved and that informed consent was obtained from the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University, Protocol number UH19010037.

Results

The mean body weight of female Wistar rat anemia was highest in R-3 (204.50 ± 3.96 g), and the lowest in R-2 (189.40 ± 8.56 g). Variation of MBI intake resulted in a different response to Wistar rat anemia. The IBMlevel in R-3 (14%) can increase bodyweightup to 17 g for 7 days and improving rate in 0.0109% per day (2.04 gd^{-1}), higher than R-o (control), and others. Nevertheless, the body weight in R-1(10%) and R-2 (12%) tends to decrease (Table 1).

There was an effect of the IBM on improving bodyweight of Wistar anemia ($p<0.03$). While based on Fisher's LSD (Least Significant Different) show that body weigh in R-3 significantly different with $p=0.0109<0.05$ (Table 1). However, the body weight tends to increase with MBI level (Figure 2):

Table 1: Average ofbody weight in female Wistar anemia (% d^{-1})

Treatments	BW ±SD (Pretest)	BW±SD (Posttest)	Δ	Rate (% d^{-1})
Ro	188.80±15.53	187.00±8.34	-1.8	-0.0010
R1	194.50±9.75	190.50±6.25	-4	-0.0028
R2	193.40±8.76	189.40±8.56	-4	-0.0029
R3	187.20±7.12	204.20±3.96	17	0.0109*
R4	188.40±17.01	193.00±22.17	4.6	0.0106

Table 2: Distribution of the different effect of IBM to the body weight of Wistarrat anemia

IBM Level (%)		p-value
R0	R1	0.687
R0	R2	0.669
R0	R3	0.001*
R0	R4	0.222
R1	R2	1.000
R1	R3	0.001*
R1	R4	0.126
R2	R3	0.001*
R2	R4	0.106
R3	R4	0.024*

Note: * means significant different at p<0.05

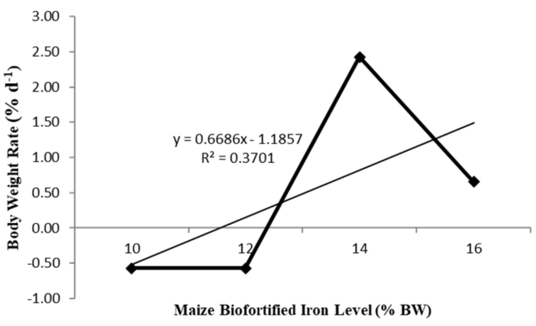

Fig.1: The Correlation Intake Iron Biofortified Maize Level With Improving Body Weight Rate of Wistar Anemia

IBM level in R-3 for Wistar rat anemia has a significant effect on improving the bodyweight ($p= 0.001$) (Table 2) and tends to increase with the IBM level following the equation $Y=0.005x-0.0096$; $R^2 = 0.79$, where Y is body weight, and x is IBM level (Figure 1).

Female Wistar anemia (Hb <12 mg / dL) has been given a single intake of iron biofortified maize (IBM) can survive up to 95%. The body weight tends to increase (0.01% d^{-1}) with the IBM level.Therefore, IBM has the potential to be a single source of energy to maintain the body weight of female Wistar rat anemia. Improving the body weight of rats was 2.04 g per day lower than the normal growth of rat 5 grams per day.[14] Monotonous diet from cereal has a vulnerability in micronutrient deficiency, but the product of bio-fortification positively impact to improve the nutrition in human.[15]

Maize grains contained carbohydrate (79.15-80.64 g / 100 g), 1756 kinds of proteins, and the fat (3.21-7.71%).[16-18] Also, the maize grain contained some amino acid such as leucine, lysine, tryptophan, methionine, isoleucine, valine, phenylalanine, glutamic acid, serine, alanine, tyrosine, and proline,[16, 19, 20] and mineral of Fe, Zn, Mg, Cu, pro-vitamin A, vitamin E and antioxidants in fresh corn.[18] However, vitamin A degradation occurs quickly around 45% in the first 3 months, even more than 85% can be lost if the storage conditions are not supportive harvest.[21,22,23] Therefore, the optimal values of nutrient content and sensory acceptance of corn are relatively higher (55.0-68.5%) compared to peanuts (27.5-35.0%), and malt barley (4.0-10.0%).[24] Besides, maize also contains several of phytochemical compounds in the form of phenolic, flavonoid, anthocyanin. Even corn has higher carotene, tocopherol, and oils than rice and wheat.[5-7] Corn has a fiber positive effect on metabolic processes and weight control of the bodyweight.[25, 26] Whilstphenolic, oligosaccharides, and flavonoids are not easily digested so relatively quite a lot reach the large intestine (10.24-64.4%).[27, 28] Thus, corn consumption was considered to have significant to improve the bodyweight and even to prevent chronic diseases such as cardiovascular disease, obesity, diabetes, and cancer.[29]

Biofortification in maize with cultivars that efficiently mobilize, extract and move iron into edible plant parts, increasing iron bioavailability in staple foods is one of the solutions expected to combat and prevent community iron deficiency, especially in areas with limited resources.[30, 31] The basis for calculating nutritional adequacy is bodyweight, basal metabolic energy (BMR), activities, additional needs for growth, additional energy for food digestion, and factors for body composition, age, and sex. The standard for nutritional adequacy of the diet in Indonesia 2012 to covering energy; protein; fats (including n-3 and n-6), carbohydrates, water, vitamins A, D, E, K, thiamine, riboflavin, niacin, pyridoxine, folic acid, vitamin B12, pantothenic acid, biotin, choline, and vitamin C; also includes minerals such as iron, calcium, phosphorus, magnesium, sodium, potassium, iodine, zinc, copper, chromium, selenium, manganese, fluorine. fiber, choline and biotin, nitrogen and essential amino acids, fatty acids, amino acids, cholesterol, and bio-active substances in food.[32] The challenge ahead is how to bio-fortified staple foods in maize grain to increase the amount of quantity and quality micronutrients and vitamins that can meet individual metabolic needs.

The low rate of weight gain in Wistar rat anemia fed with maize bio-fortified iron illustrates that this single food does not meet the body's metabolic needs. Likewise in humans, a monotonous diet from cereals was susceptible to micronutrient deficiencies, although the maize bio-fortified products have a positive impact to improve the nutritional health in human.[15] The weakness of this studied has not measured the levels of each nutrient component integrated into the maize bio-fortified iron products. While the perfection of the body's metabolism requires many nutrients derived from diets.[33] However, the iron biofortified maize expected to increase the content of vitamins and essential micronutrients in food simultaneously.

Conclusion

Iron biofortified maize has influenced to improve bodyweight of female Wistar ratanemia.Improving the bodyweight of Wistar anemia tends to increase with IBM level. IBM 14% was a maximum safe level for the growth rate of Wistar rat anemia but relatively lower than the normal growth.

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Conflict of Interests

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Author Contribution

All authors contributed to establishing the topic of the research and design experiment. Saifuddin Sirajuddin focused on nutrition for iron deficiency anemia; M. Natsir Djide focused on the bacterial function in biofortification, and Anwar Mallongi focused on statistical analysis.

References

1. WHO. Guidelines on food fortification with micronutrients: World Health Organization and Food and Agricultural Organization of the United Nations; (2006).
2. Barkley JK, Kendrick KL, Codling K, Muslimatun S, Pachon H. "Anemia prevalence over time in Indonesia: estimates from1997, 2000, and 2008 Indonesia Family Life Surveys". *Asia Pac J Clin Nutr*; 24:452-5. (2015).
3. Thomas D, Chandra J, Sharma S, Jain A, Pemde HK. "Determinants of Nutritional Anemia in Adolescents". *Indian Pediatr*; 52:867-9. (2015).
4. Moreno-Moyano LT, Bonneau JP, Sanchez-Palacios JT, Tohme J, Johnson AA. "Association of Increased Grain Iron and Zinc Concentrations with Agro-morphological Traits of Biofortified Rice". *Front Plant Sci;* 7:1463. (2016).
5. Subhash C, Yijiang M, Yirong Z, Jianbing Y, Jiansheng L. "Comparison of Nutritional Traits Variability in Selected Eighty-Seven Inbreds from Chinese Maize (Zea mays L.) Germplasm". *J Agric Food Chem*; 56:6506–11. (2008).
6. Hoisington D. "Opportunities for nutritionally enhanced maize and wheat varieties to combat protein and micronutrient malnutrition". *Food Nutr Bull*; 23:376-7. (2002).
7. Van Hung P. "Phenolic Compounds of Cereals and Their Antioxidant Capacity". *Crit Rev Food Sci Nutr*; 56:25-35. (2016).
8. Klaus Kraemer, Zimmermann MB. "Nutritional Anemia". SIGHT AND LIFE / DSM Nutritional Products Ltd. (2007).
9. Jumadi, Sirajuddin S, Djide MN, Mallongi A. "Root Inoculation with *Pseudomonas putida* IFO 14796 for Improving Iron Contents in Maize Grain". *Journal of Food Resource Science*; 8:1-5. (2019).
10. Jumadi M, Sirajuddin S, Djide MN, Mallongi4 A. "Erythrocyte Formation Rate in Wistar Anemia Induced 2,4-Dinitrophenylhydrazine through Intake Maize Biofortified Iron". *Open Access Macedonian Journal of Medical Sciences*; 8:468-71. (2020).
11. Sunil KP, Lindsay B. "RodentModels forMetabolic Syndrome Research". *Journal of Biomedicine and Biotechnology.* (2011).
12. Toma, Victory NC, Kabir A. "The effect of aqueous leaf extract of fluted pumpkin on some hematological parameters and liver enzymes in 2,4-dinitrophenylhydrazine-induced anemic rats". *African Journal of Biochemistry Research;* 9:95-8. (2015).
13. Dhur A, Galan P, Hercberg S. "Effect of decreased food consumption during iron deficiency upon growth rate and iron status indicators in the rat". *Ann Nutr Metab*; 34:280-7. (1990).
14. IPB. Tikus Putih (*Rattus norvegicus*). Available from: http://www.foxitsoftware.com.
15. De Moura FF, Palmer AC, Finkelstein JL, Haas JD, Murray-Kolb LE, Wenger MJ, *et al*. "Are biofortified staple food crops improving vitamin A and iron status in women and children? New evidence from efficacy trials". Adv Nutr; 5:568-70. (2014).
16. Josiane SA, Bienvenu AV, Wilfried PS, Adolphe A, Djima A, Joachin G, *et al*. "Nutritional Properties Assessment of Endogenous and Improved Varieties of Maize (Zea mays L.) Grown in Southern Benin". *Pak J Biol Sci*; 20:267-77. (2017).
17. Wang G, Wang G, Wang J, Du Y, Yao D, Shuai B, *et al*. "Comprehensive proteomic analysis of developing protein bodies in maize (Zea mays) endosperm provides novel insights into its biogenesis". *J Exp Bot*; 67:6323-35. (2016).
18. Ikram U, Muhammad A, Arifa F. "Chemical and Nutritional Properties of Some Maize (Zea mays L) Varieties Grown in NWPF, Pakistan". *Pakistan Jurnal of Nutrition*; 9:1113-7. (2010).
19. Mariscal-Landin G, Reis de Souza TC, Ramirez Rodriguez E. "Metabolizable energy, nitrogen balance, and ileal digestibility of amino acids in quality protein maize for pigs". *J Anim Sci Biotechnol*; 5:26. (2014).
20. Li Y, Wang Y, Wei M, Li X, Fu J. "QTL identification of grain protein concentration and its genetic correlation with starch

concentration and grain weight using two populations in maize (Zea mays L.)". *J Genet*; 88:61-7. (2009).
21. Hemery YM, Laillou A, Fontan L, Jallier V, Moench-Pfanner R, Berger J, *et al*. "Storage conditions and packaging greatly affects the stability of fortified wheat flour: Influence on vitamin A, iron, zinc, and oxidation". *Food Chem*; 240:43-50. (2018).
22. Xie L, Yu Y, Mao J, Liu H, Hu JG, Li T, *et al*. "Evaluation of Biosynthesis, Accumulation and Antioxidant Activity of Vitamin E in Sweet Corn (Zea mays L.) during Kernel Development". *Int J Mol Sci;* 18. (2017).
23. Jang Y, Park NY, Rostgaard-Hansen AL, Huang J, Jiang Q. "Vitamin E metabolite 13'-carboxychromanols inhibit pro-inflammatory enzymes, induce apoptosis and autophagy in human cancer cells by modulating sphingolipids and suppress colon tumor development in mice". *Free Radic Biol Med*; 95:190-9. (2016).
24. Fikiru O, Bultosa G, Fikreyesus Forsido S, Temesgen M. "Nutritional quality and sensory acceptability of complimentary food blended from maize (Zea mays), roasted pea (Pisum sativum), and malted barley (Hordium vulgare)". *Food Sci Nutr*; 5:173-81. (2017).
25. Casas MI, Duarte S, Doseff AI, Grotewold E. "Flavone-rich maize: an opportunity to improve the nutritional value of an important commodity crop". *Front Plant Sci*; 5:440. (2014).
26. Konings E, Schoffelen PF, Stegen J, Blaak EE. "Effect of polydextrose and soluble maize fiber on energy metabolism, metabolic profile and appetite control in overweight men and women". *Br J Nutr*; 111:111-21. (2014).
27. Luzardo-Ocampo I, Campos-Vega R, Gaytan-Martinez M, Preciado-Ortiz R, Mendoza S, Loarca-Pina G. "Bioaccessibility and antioxidant activity of free phenolic compounds and oligosaccharides from corn (Zea mays L.) and common bean (Phaseolus vulgaris L.) chips during in vitro gastrointestinal digestion and simulated colonic fermentation". *Food Res Int*; 100:304-11. (2017).
28. Hernandez M, Ventura J, Castro C, Boone V, Rojas R, Ascacio-Valdes J, *et al*. "UPLC-ESI-QTOF-MS(2)-Based Identification and Antioxidant Activity Assessment of Phenolic Compounds from Red Corn Cob (Zea mays L.)". Molecules; 23. (2018).
29. Petroni K, Pilu R, Tonelli C. "Anthocyanins in corn: a wealth of genes for human health". Planta; 240:901-11. (2014).
30. Sperotto RA, Ricachenevsky FK, Waldow Vde A, Fett JP. "Iron biofortification in rice: it's a long way to the top". *Plant Sci*; 190:24-39. (2012).
31. Finkelstein JL, Haas JD, Mehta S. "Iron-biofortified staple food crops for improving iron status: a review of the current evidence". *Curr Opin Biotechnol;* 44:138-45. (2017).
32. Kartono D, Hardinsyah, Jahari AB, Sulaeman A, Astuti M, Soekatri M, *et al*. "Angka Kecukupan Gizi (Akg)Yang Dianjurkan Bagi Orang Indonesia". Widyakarya Nasional Pangan dan Gizi (WNPG)X 2012, Gedung LIPI, Jakarta 20-21. (2012).
33. Guamuch M, Dary\ O, Rambelson Z, Cruz Vdl, Villalpando S, Tom C, *et al*. "Model for estimating nutrient addition contents to staple foods fortified simultaneously: Mexico and Kampala data". *Ann NY Acad Sci;* 1312:76–90. (2014).