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Short Communication

Tempeh extract fortified with iron and synbiotic as a strategy against anemia

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Abstract

Iron fortification is one of potential way to overcome anemia. Probiotics can maintain digestive tract health while prebiotics can improve the absorption of minerals. Tempeh is rich in nutrients required for hemoglobin synthesis, such as protein, vitamin B12, vitamin C, zinc, iron, and copper. The objective of this study was to evaluate the efficacy of tempeh extract fortified with iron and synbiotic in increasing the hemoglobin concentration (Hb). Fermented synbiotic tempeh extract was made using *Lactobacillus plantarum* Dad13 and fructo-oligosaccharides. A total of 24 male and female Wistar rats underwent the iron depletion phase to induce anemia, then divided into 3 groups, given: 1) Fermented synbiotic tempeh extract with 50 ppm Fe/ $FeSO_4$ (Fe), 2) Fermented synbiotic tempeh extract (St), and 3) not receive any interventions (Co). The rats were fed with interventional diet AIN-93 free Fe during intervention. Body weight and Hb were measured before and after intervention for 17 days. There was a significant increase in body weight on Fe and St groups after intervention respectively, 31 g and 32 g, as well as for Hb, from 6.41 ± 0.14 to 11.48 ± 0.31 and 6.47 ± 0.23 to 11.03 ± 0.35 mg/dl ($p < 0.05$). Group without intervention (Co) did not show significant increase both in body weight and hemoglobin. This can be a good opportunity to combat anemia using Indonesian local food, tempeh which fortified with iron and synbiotic.

Introduction

Anemia is one of the major public health problems experienced by more than 600 million human in the world. Mostly, anemia was found in the developing countries than developed countries (Arisman, 2004). In Indonesia, anemia is one of the major malnutrition problem beside Protein-Energy Deficiency (PED), Vitamin A Deficiency (VAD), and Iodium-Deficiency Impairment (IDI). Iron-Deficiency Anemia (IDA) is the most difficult anemia to overcome in Indonesia. Based on Indonesian Basic Health Research (2007), prevalence of anemia in Indonesia is 19.7% on adult woman and 12.1% on adult man. Effect of IDA is really wide, such as alteration of epithelial tissue, impaired growth in children, decreased achievement and low cognitive ability of children at school and low productivity on workers (WHO, 2004).

Strategy to overcome IDA consists of 4 strategies, includes nutritional education, Fe supplementation for nutritional at risk group, food fortification and indirect reduction program, such as routine worm infection examination and giving anti-malaria drugs on the endemic malaria region. Micronutrient fortification of food often conducted by fortified staple food or highly consumed food such as rice or salt (Helmyati *et al*., 2014). Food fortification had not many roles yet to overcome IDA because fortified food is not provided enough. Fortification is more cost-effective way against anemia than supplementation (Baltussen *et al*., 2004).

Iron is an essential trace element for microorganism. In order to obtain iron from the very low iron environment, microorganisms have developed sophisticated mechanisms such as siderophores system. This system helps decreasing iron availability for the host. Iron fortification made excess iron available to microorganisms, thus enhancing their pathogenicity. It is important to look for a new innovative antimicrobial agent to minimize the negative effect of iron fortification (Jurado, 1997). The intestines of healthy animals are heavily colonized by a large and various microorganisms. The indigenous microbiota, as this population is often termed, may give advantages to their host such as provision of nutritional factors, promotion of

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digestion, modulation of immune system, and as a barrier against colonization by potentially pathogen bacteria (Tompkins *et al*., 2000). Variety of food source and its intake tend to impact the balance of gut microbiota (Helmyati *et al*., 2015)

Recent studies suggest that Fe is also absorbed from the proximal colon, which absorption being enhanced by the presence of easily fermentable carbohydrates such as inulin (Scholz-Ahrens *et al*., 2007). Inulin and other prebiotic carbohydrates stimulate the growth of bacteria that produce short chain fatty acid (SCFA) such as propionic acid, which enhances mineral uptake. The absorption of Fe and other divalent cations is enhanced by SCFA in the proximal colon, which are produced by bacterial fermentation in the colon. Non-digestible disaccharides increased caecal SCFA pools and prevented IDA in gastroectomised rats (Shiga *et al*., 2006).

Tempeh is a traditional fermented soy product from Indonesia. It is made by a natural culturing and controlled fermentation process. Nutritionally, tempeh represents food high in proteins and also contains many other important nutrients such as vitamin B12, protein, vitamin C, iron, zinc and copper (Mien *et al*., 2008). These are nutrients required for hemoglobin synthesis. The present study was conducted to evaluate the efficacy of tempeh extract fortified with iron and synbiotic in increasing the hemoglobin concentration (Hb). It is important to optimize the health benefit of local food in overcoming nutritional problems.

Materials and Methods

Study design

This study was conducted at Microbiology Laboratory and Nutrition Laboratory, Universitas Gadjah Mada, precisely in June - August 2014. The study was approved by Medical and Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada (No: KE/FK/542/EC). The interventions were divided into 3 periods: adaptation (6 days), depletion phase/iron-deficiency anemia induction (14-17 days) and intervention (17 days). Rats were fed with AIN-93 free Fe during IDA induction and intervention.

Subjects

In total, three-weeks-old of 24 Wistar rats, consist of 12 male and 12 female rats, were used. They were obtained from Integrated Research and Testing Laboratory, Universitas Gadjah Mada. Rats were individually housed in stainless-steel cages under controlled conditions (24°C, 12-h light/dark cycle) and had access to food and water ad libitum.

Formulation

Tempeh was made using traditional method. There were four steps in the tempeh making process, namely soaking, boiling, inoculating with tempeh starter, and incubating at dark-room temperatured for optimum product. Tempeh was fermented with *Rhizopus* sp. mold, especially *Rhizopus oligosporus, R. oryzae, R. arhizus, R. stolonifer* and *R. microspores*. The procedure of making tempeh extract was as follows: 1) Add 200 ml of hot water of 100 g tempeh, 2) Blend tempeh for 10 minutes, 3) Heating, 4) Straining, 5) Pour them into sterile bottles, 6) Pasteurization for 10 minutes at 80^{o}C (Susanti, 1992).

Fermented tempeh extract making procedure was as follows: 1) Inoculation of *L. plantarum* Dad13 at 37°C for 18 hours in MRS broth media (Merck, Merck and Co.), to be used as a starter, 2) Inoculation of *L. plantarum* Dad13 in skim milk, 3) Dissolved the tempeh extract, 10% sucrose (Gulaku, Sugar Group Companies), FOS (Orafti), skim milk (Lactona, PT. Mirota KSM), 50 ppm $FeSO_4$ or 50 ppm NaFeEDTA, 4) Sterilization at 115^{o}C for 10 minutes, 5) Fermentation with 1% starter at 37^{o}C for 24 hours.

Intervention

In adaptation period, rats were fed with standard diet (10 g) and water ad libitum for 6 days. In depletion phase/iron-deficiency anemia induction, the rats were fed AIN-93 free Fe (10 g) for 14 days or until they were anemic (hemoglobin concentrations < 7 g/dl). Anemic rats then received intervention based on the group and water. The rats were randomly divided into 3 groups: 1) given fermented synbiotic tempeh extract with 50 ppm Fe/$FeSO_4$ (Fe); 2) given fermented synbiotic tempeh extract (St); and 3) without any intervention (Co). Intervention was given by force-feeding. During iron-deficiency anemia induction and intervention, rats were fed AIN-93 free Fe (10 g each).

Measurement

Hemoglobin concentrations data was obtained by analyzing blood samples using photometric method by using diagnostic system (diasys) Embit kits (Diasys, Germany). Analysis adjusted with protocol printed in the kit. Blood was taken from the sinus orbitalis. Rats' body weights data was obtained using digital analytic scales. Hemoglobin concentration was measured before and after 17 days of interventions. The body weights were measured once in 3 days.

Table 1. Rats' body weights (g) before and after interventions

	Groups		
	Fe (g)	St (g)	Co (g)
Before intervention	85 ± 10[a]	82 ± 15[a]	70 ± 16[a]
After intervention	116 ± 7[a]	114 ± 13[a]	84 ± 14[b]

Fe: synbiotic fermented milk fortified with $FeSO_4$
St: synbiotic fermented milk without fortification
Co: without intervention
[a,b]mean scores in rows with the same superscript letters are not significantly different

Statistical analysis

Hemoglobin concentration was expressed as g/dl. Differences between groups were analyzed by one-way analysis of variance (ANOVA) and were considered statistically significant at $p < 0.05$.

Results and Discussion

Rats becomes anemic on 14th days of depletion phase was included in intervention phase that took place for 2 weeks. Rats' body weight before intervention between groups was similar (p>0.05) and significantly increased after interventions with Fe and St (p>0.05). The AIN-93 consumed by rats was similar between three groups (p>0.05), with average of 8 mg/day. The body weights before and after interventions was seen in Table 1.

Anemia was caused by many factors, low iron intake and absorption and physiologic condition such as growth period and pregnancy (WHO, 2006). Deficiency of others nutrients, such as vitamin A, B12, C and folic acid, can also lead to anemia (WHO, 2001). Iron supplementation and fortification are potential ways against anemia. Iron supplementation has some issues, like high metallic-taste and low compliances. In the other hand, fortification is considered as a cost effective way to improve the micronutrient deficiency (Le *et al.*, 2006).

Interventions were done for 17 days. There were similar hemoglobin concentrations at baseline between groups (p>0.05). The increased hemoglobin after interventions in Fe and St were statistically significant (p<0.05) but Co group did not show increase in Hb (Table 2). Tempeh is rich in nutrients which required for hemoglobin synthesis, such as protein, vitamin B12, vitamin C, folic acid, zinc, iron, and copper. In 100 g tempeh, there are 20.8 g protein; 4 mg iron (TKPI, 2008); and 1.7 mg vitamin B12 (Sapuan *et al.*, 1996). Protein is needed for globin synthesis while iron enters the protophorpyrin that result in heme (Shils *et al.*, 1994).

Table 2. The differences in hemoglobin concentrations between groups at before and after interventions

	Fe (mg/dl)	St (mg/dl)	Co (mg/dl)
Before intervention	6.41 ± 0.14[a]	6.47 ± 0.23[a]	6.40 ± 0.29[a]
After intervention	11.48 ± 0.31[a]	11.03 ± 0.35[a]	6.28 ± 0.27[b]

Fe: synbiotic fermented milk fortified with $FeSO_4$
St: synbiotic fermented milk without fortification
Co: without intervention
[a,b]mean scores in rows with the same superscript letters are not significantly different.

Some indigenous probiotics has been isolated from Indonesian local food. One of them is *Lactobacillus plantarum* Dad13 which is isolated from "dadih", the Indonesian traditional fermented food made from buffalo's milk. This bacteria could stimulate the humoral immune system (Kusumawati, 2006) and extracellular anti-microbial activity on Salmonella in vitro which could be able to assimilate and deconjugate bile salt (Ngatirah, 2000) and also potential as hipocholesterolemic agents (Lestari, 2003). The other study revealed that *L. plantarum* 299v was able to increase absorption of non-heme iron (Bering *et al.*, 2006).

Prebiotics can support the growth of probiotics by providing selective substrates for its growth (Gibson *et al.*, 2004). Prebiotics fermentation by lactic acid bacteria can decrease pH (Rowland *et al*, 1997) so that could increase mineral absorption (Rastall, 2010). The prebiotic fermentation by probiotic may produce SCFA (short chain fatty acid) that has many therapeutic effects for human (Bharucha, 2008).

Conclusions

Fermented synbiotic tempeh extract with or without iron fortification can increase body growth and hemoglobin concentrations in anemic rats. Considering the negative effect of iron fortification on gut microbiota balance, it may better to use fermented synbiotic tempeh extract than fortified one. It needs further research.

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