

PJN

ISSN 1680-5194

PAKISTAN JOURNAL OF
NUTRITION

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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Effect of Sodium Iron EDTA Fortification in Tempe in Serum Iron and Ferritin Level of Anemic Female Wistar Rats

Toto Sudargo¹, Rio Jati Kusuma¹, Tonny Arjuna¹, Rasita Amelia Hasnawati¹,
Dianandha Septiana Rubi¹ and Abdul Rohman²

¹Department of Health and Nutrition, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia

²Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract: In Indonesia, the prevalence of iron deficiency anemia is still high. Iron fortification in food using sodium ferric EDTA (NaFeEDTA) potentially reduce the incidence of iron deficiency anemia. Iron deficient anemia was induced in female Wistar rats by feeding low iron (Fe) diet and was randomly divided into ten groups where one group was fed normal diet which serves as control group. Every treatment group will receive FeSO₄, tempe and iron-fortified tempe (temFe) with iron dosage of 6, 12 and 24 ppm respectively for 17 days. Blood was drawn for serum iron (SI) and serum ferritin (SF) measurement. After treatment, temFe 24 ppm group has the highest SI and the highest SI changed compared with other treatment groups although no significant different ($p > 0.05$) was observed between temFe 24 ppm group and FeSO₄ 24 ppm group for SI (139.36 ± 1.58 vs 134.10 ± 2.73 $\mu\text{g/dL}$) and SI changed (89.40 ± 2.78 vs 84.47 ± 2.41 $\mu\text{g/dL}$). SF was increased in all treatment diet with the highest was found in FeSO₄ 24 ppm group (73.25 ± 3.16 ng/mL). However, SF level wasn't significantly different between the FeSO₄ 24 ppm, tempe 24 ppm and temFe 24 ppm and group ($p > 0.05$). Iron fortification in tempe with NaFeEDTA will restore both SI and SF in iron deficiency anemia.

Key words: Iron deficiency anemia, Tempe, Sodium iron EDTA

INTRODUCTION

Anemia is defined as a condition in which the blood hemoglobin concentration was below the normal value although there is still a debate over the cut off of hemoglobin value (Beutler and Waalen, 2006). It is caused by several factors such as iron deficiency, vitamin deficiencies such as folate and vitamin B12, infection, chronic disease and inflammation, blood loss or haemolysis and hemoglobinopathies and affecting 1.6 billion people worldwide or around quarter of the world's population (Benoist *et al.*, 2008; Lutter, 2008; McLean *et al.*, 2009; Milman, 2011). Although folate and vitamin B12 deficiencies have caused anemia in certain groups of population, iron deficiency was the major and the most common cause of anemia, accounting for 75-80% total burden of anemia worldwide (WHO, 2001; Metz, 2008; Milman, 2011).

Iron deficiency anemia occurred as the final stage of iron storage depletion which caused a reduction in hemoglobin production, thus lowering its concentration in the blood (Baker and Greer, 2010; De-Regil *et al.*, 2011). The imbalance in iron homeostasis caused by any or combined factors; inadequate intake of iron, poor iron absorption and utilization, increased physiological requirement during growth and pregnancy and chronic blood losses due to chronic disease, parasitic infection such as hookworm infection, inflammation or

menstruation is the underlying cause of iron deficiency anemia (Bothwell, 2000; Moy, 2006; Balarajan *et al.*, 2011; Shaw and Friedman, 2011). Moreover, iron depletion can occur even before anemia detected and affecting almost 2.5 times population than iron deficiency anemia itself (WHO, 2001).

Improving iron status in iron deficiency and iron deficiency anemia is important to prevent the detrimental health effect especially in children and women including increased risk of death in pregnant and labor women, low birth weight and child death, reduced attention and cognitive development in children and also loss productivity (Lozoff *et al.*, 2000; Haas and Brownline, 2001; Angulo-Kinzler *et al.*, 2002; de Benoist *et al.*, 2008). Iron supplementation and food fortification are two strategies for reducing iron deficiency in population. Although both of the methods are cost-effective to reduce iron deficiency in population, food fortification appears to be cost effective and affordable especially in the low and middle income country with limited health facilities or storage setting (Mora, 2002; Baltussen *et al.*, 2004; Le *et al.*, 2006).

Choosing the right iron salt for food fortification is essential as iron can oxidize nutrient in food, thereby changing the physical properties of food such as color and taste (Hurrell, 2002). Moreover, the presence of inhibitor from food; phytic acid, phenolic compound and

some protein, is another problem regarding to iron fortification in food (Hurrell *et al.*, 1992; Lynch *et al.*, 1994a; Reddy *et al.*, 1996; Ishikawa *et al.*, 2007; Yun *et al.*, 2011; Abizari *et al.*, 2012a). Sodium iron EDTA (NaFeEDTA) is iron salt that has been recommended by WHO and FAO as iron fortificant as it doesn't cause oxidation in food (Hurrell, 2002; Allen *et al.*, 2006). In addition, food fortification using NaFeEDTA is effective to improve iron status in population, which among them came from food with high phytate content (Chen *et al.*, 2005; Thuy *et al.*, 2005; Andang'o *et al.*, 2007; Abizari *et al.*, 2012b; Macharia-Mutie *et al.*, 2012).

However, although epidemiological data have potentially shown the advantage of NaFeEDTA salt as iron fortificant in food, little is known regarding the benefit of this salt to improve iron status when added in tempe. Our result shows that iron fortification in tempe using NaFeEDTA improve serum ferritin and serum iron in anemic rats.

MATERIALS AND METHODS

Materials: Soybean for tempe was bought from local market in Yogyakarta. NaFeEDTA was acquired from Akzo Nobel Chemical with product name of Ferrazone (Amhem, Netherland). Plasma iron kit was acquired from DiaSys (Germany) and rat ferritin kit was acquired from ICL (Portland, USA).

Preparation of tempe and tempe fortification: Tempe fortification was produced as previously described in Sudargo *et al.* (2013). Briefly, soybean was washed, soaked for 1 day and boiled for 30 min. After cooled in room temperature, Ferrazone was added with dose of 28 mg/kg soybean, 56 mg/kg soybean and 112 mg/kg soybean prior to addition of tempe yeast. The mixture was stirred and incubated at 32°C for 16-32 h. Tempe was stored in refrigerator at 4°C prior to experiment.

Animals: Fifty female wistar rats aged two months were obtained from Pusat Antar Universitas (PAU),

Yogyakarta, Indonesia. Rats were caged individually in stainless steel cage in animal room (22-25°C room temperature and 12 h daylight cycle). Rats were fed AIN-93 standard diet *ad libitum* for 3 days prior to iron depletion state. For iron depletion state, 45 rats were fed iron-free diet for 10 days respectively and 5 were served as control groups. The composition of the iron-free diet was reported elsewhere (Martino *et al.*, 2011). After 10 days of iron-free diet, rats were divided into 9 groups: FeSO₄ with iron dose of 6, 12 and 24 ppm, tempe only with iron dose of 6, 12 and 24 ppm and fortified tempe or temFe with iron dose of 6, 12 and 24 ppm in the diet. The composition of diet was described in Sudargo *et al.* (2013). Rats were treated for 17 days and blood was taken for serum ferritin and serum iron analysis. Body weight was measured every day during iron depletion and iron repletion state.

Serum ferritin and serum iron measurement: Blood was drawn from sinus orbitalis and centrifuged immediately to get the serum. Serum was stored at -20°C prior to analysis of serum ferritin and serum iron. Serum ferritin was assayed using ELISA method using a kit from ICL (Portland, USA) according to manufacturer protocol. Serum iron was measured using a kit from DiaSys (Germany) according to manufacturer protocol.

Statistical analysis: All statistical analysis were performed at GraphPad Prism 6 for Windows, GraphPad Software La Jolla, California USA. One way ANOVA was applied to analyze the difference between groups for serum ferritin and serum iron. The alpha of statistical significance was set at p<0.05.

RESULTS

Body weight: There is no different body weight of rats in FeSO₄, tempe only and tempe fortified group during the study. The body weight both of control group and treatment groups tend to increase. Furthermore, in group that receiving tempe fortified or temFe 24 ppm

Table 1: Body weight of rats fed the experimental diet

Group	Week 1	Week 2	Week 3	Week 4
Control	133.34±5.52 ^a	139.8±6.09 ^a	144.69±6.24 ^a	153.43±6.17 ^a
FeSO₄				
6 ppm	155.57±14.79 ^{a,b}	162.17±15.16 ^{a,b}	168.46±15.16 ^{a,b}	175.13±15.05 ^{a,b}
12 ppm	146.94±21.53 ^{a,b}	154.17±21.76 ^{a,b}	160.26±21.99 ^{a,b}	166.37±21.82 ^{a,b}
24 ppm	148.14±9.74 ^{a,b}	154.80±9.73 ^{a,b}	160.89±9.45 ^{a,b}	167.47±9.58 ^{a,b}
Tempe				
6 ppm	160.57±16.74 ^{a,b}	167.20±16.38 ^{a,b}	172.86±15.87 ^b	179.27±15.85 ^{a,b}
12 ppm	143.23±13.35 ^{a,b}	149.66±12.95 ^{a,b}	155.40±12.89 ^{a,b}	161.87±12.84 ^{a,b}
24 ppm	140.51±9.63 ^{a,b}	147.54±9.42 ^{a,b}	153.97±8.86 ^{a,b}	160.53±8.78 ^{a,b}
TemFe				
6 ppm	149.26±10.74 ^{a,b}	155.54±10.58 ^{a,b}	161.57±10.66 ^{a,b}	168.3±10.61 ^{a,b}
12 ppm	149.69±12.18 ^{a,b}	156.89±12.18 ^{a,b}	162.89±12.04 ^{a,b}	169.50±11.83 ^{a,b}
24 ppm	161.69±10.08 ^b	168.80±9.88 ^b	175.11±9.93 ^b	182.03±9.92 ^b
p-value	0.0395	0.0356	0.026	0.037

Data are presented as mean±standard deviation

^{ab}Different values represent p<0.05

Table 2: Serum iron and ferritin level in rats fed experimental diet

Variable	Control	FeSO ₄				Tempe				TemFe				p-value	
		6 ppm	12 ppm	24 ppm	6 ppm	12 ppm	24 ppm	6 ppm	12 ppm	24 ppm	6 ppm	12 ppm	24 ppm		
Serum Iron															
Depletion	142.25±1.54 ^a	49.48±1.69 ^b	52.13±1.54 ^{bc}	49.64±0.97 ^b	49.64±0.93 ^b	52.21±0.94 ^{bc}	53.01±1.88 ^{bc}	50.44±0.92 ^{bc}	52.85±1.16 ^c	49.96±1.52 ^c	52.85±1.16 ^c	50.44±0.92 ^{bc}	52.85±1.16 ^c	49.96±1.52 ^c	<0.001
Repletion	141.43±1.78 ^a	64.07±1.36 ^b	93.15±2.48 ^b	134.10±2.73 ^b	60.24±2.41 ^b	64.07±1.53 ^b	73.55±1.53 ^b	62.15±1.95 ^b	119.05±1.98 ^b	139.36±1.58 ^b	119.05±1.98 ^b	62.15±1.95 ^b	119.05±1.98 ^b	139.36±1.58 ^b	<0.001
Change	-0.82±2.79 ^a	14.59±1.62 ^b	41.02±3.32 ^b	84.47±2.41 ^b	10.6±1.96 ^b	11.85±1.70 ^b	20.53±2.81 ^c	11.71±2.30 ^b	66.19±2.06 ^c	89.40±2.78 ^c	66.19±2.06 ^c	11.71±2.30 ^b	66.19±2.06 ^c	89.40±2.78 ^c	<0.001
Ferritin	45.09±4.36 ^a	39.63±1.13 ^b	63.75±3.58 ^b	73.25±3.16 ^{bc}	55.89±2.15 ^c	65.22±1.82 ^c	67.46±3.08 ^{cd}	47.79±0.82 ^c	50.85±3.26 ^{cd}	64.26±2.97 ^{cd}	50.85±3.26 ^{cd}	47.79±0.82 ^c	50.85±3.26 ^{cd}	64.26±2.97 ^{cd}	<0.001

^{a,b,c}Different values represent p<0.05

Data were presented as mean±standard deviation (n = 6)

the body weight was found significantly different compared control group (Table 1).

Effect of NaFeEDTA fortified tempe on serum iron and ferritin: After 10 days administration of iron free diet, serum iron was significantly lower in all treated groups compared with control (p<0.01). After 17 days of treatment, serum iron was increased in all treatment group with the highest increment was found in iron fortified tempe or temFe 24 ppm, although it wasn't statistically different with FeSO₄ 24 ppm group (Table 2). Furthermore, there is no statistical difference among fortified tempe or temFe 24 ppm group with control group (p>0.05). This result indicating that iron fortification in tempe using NaFeEDTA is effective on alleviating serum iron level in iron deficiency anemia condition.

For serum ferritin, we found that all treatment groups have significantly high level of ferritin compared with the control (p<0.01). Interestingly, serum ferritin level was not statistically different between FeSO₄ 24 ppm, tempe 24 ppm and temFe 24 ppm group (Table 2). This result demonstrating that tempe alone is effective in increasing serum ferritin level in iron deficiency anemia condition, but not the serum iron level.

DISCUSSION

In this study we demonstrated that NaFeEDTA is an effective salt for iron fortification in tempe; a traditional fermented soy food from Indonesia. Iron fortification in soy-based food is challenging not only due to the high phytate content, but also some others iron chelator compound such as soy protein fraction conglycinin and proanthocyanidin which can inhibit iron absorption (Lynch *et al.*, 1994b; Yun *et al.*, 2011). It is also reported that reducing the phytate content in soy protein will not ameliorate iron absorption in the body (Davidsson *et al.*, 2001; Davidsson *et al.*, 2004). However, our result suggest that NaFeEDTA can overcome this problem as indicated by restoration of serum iron and elevation of serum ferritin level in iron deficiency anemia condition. NaFeEDTA is an iron chelator which has high binding affinity with iron compared with other ligands such as phytic acid and phenolic compound (Layrisse *et al.*, 2000). Although the iron was bound with the EDTA, however, iron can be dissociated from EDTA within the lumen of gastrointestinal tract prior to or during intestinal absorption (Zhu *et al.*, 2006). In addition, NaFeEDTA is able to increase iron absorption by 2-4 fold, even in the presence of phytate (Hurrell *et al.*, 2000; Allen *et al.*, 2006). Thus, NaFeEDTA is well suited for fortifying food with high iron-inhibitory components such as soy product as reported from field studies as well as in our study. Fidler *et al.* (2003) reported that iron absorption from NaFeEDTA-fortified soy sauce relatively higher compared FeSO₄-fortified soy sauce (6.1 vs 5.6%).

Chen *et al.* (2005) also reported that subject receiving NaFeEDTA-fortified soy sauce had significantly higher hemoglobin and plasma ferritin level compared subject did not receive NaFeEDTA-fortified soy sauce.

However, although NaFeEDTA-fortified tempe or temFe is effective in improving serum iron anemia, our result also indicated that tempe alone could improve serum iron level although the effect is not as strong as FeSO₄ or when fortified with NaFeEDTA. Interestingly, we also found that tempe alone can increase the serum ferritin level compared with the control group. This condition can be explained as iron in tempe is used to increase iron storage, particularly in the liver, instead of being used to maintain iron status in the body (Kasaoka *et al.*, 1997). Moreover, the presence of antioxidant substance in tempe will also help increasing the iron storage in the liver as iron from iron salts is used to maintain the activity of antioxidant enzyme in the liver when given in the iron deficiency condition (Kasaoka *et al.*, 1997; Nout and Kiers, 2005; Altun *et al.*, 2014; Aycicek *et al.*, 2014).

Conclusion: In conclusion, NaFeEDTA-fortified tempe is effective to improve serum iron and ferritin level in iron deficient anemia rats. Further study is needed to address the mechanism and efficacy of NaFeEDTA-fortified tempe in preventing and alleviating iron deficiency in the population.

Our study showed that iron fortification using NaFeEDTA in tempe can increase the serum iron and ferritin in anemic female rats.

ACKNOWLEDGEMENT

This research supported by Lembaga Penelitian dan Pengabdian Masyarakat Universitas Gadjah Mada (LPPM-UGM): Kegiatan Penelitian Unggulan Perguruan Tinggi (Penelitian Kerjasama Institusi).

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