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Research Article
Influence of Fortified Tempe with Iron and Vitamin A to Increase Hemoglobin Level of Rats with Iron Deficiency Anemia

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Abstract

Objective: The study was conducted to assess the influence of fortified tempe with iron and vitamin A to increase hemoglobin level. **Methodology:** An experimental study of randomized pre test, post test and control group design was conducted on 30 Sprague-Dawley rats with iron deficiency anemia. Samples were divided into 6 groups randomly and then the rats were treated for 6 weeks. The treatments were as follows: (1) Standard feed (SF), (2) SF+TWF, (3) SF+TFe230, (4) SF+TFe271, (5) SF+TFe271+VA15 and (6) SF+TFe271+VA50, which TWF was tempe without fortification, TFe230 and TFe271 was tempe fortified with iron 230 and 271 ppm, VA15 was 15 ppm vitamin A and VA50 was 50 ppm vitamin A. Iron used was ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and vitamin A used was retinyl acetate. **Results:** Average hemoglobin level at baseline was 12.0±0.3 g dL^{-1}. After a period of depletion, it decreased to 5.7±0.2 g dL^{-1}. Average hemoglobin levels after treatment in the control rats (rats fed SF) was 11.6±0.2 g dL^{-1}, rats that was given tempe fortified with iron had not reached the average level of 12.0 g dL^{-1} while rats that were given tempe fortified with iron+vitamin A SF+TFe271+VA15 and SF+TFe271+VA50 the average of hemoglobin levels were, respectively 12.2±0.1 and 12.4±0.2 g dL^{-1}. ANCOVA test results showed that tempe fortified with iron and vitamin A significantly ($p = 0.001$) improved the level of hemoglobin (Hb). The highest average hemoglobin level was in the treatment group iron 271 ppm+vitamin A of 50 ppm. **Conclusion:** Iron and vitamin A fortification in tempe with ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) 271 ppm and retinyl acetate 50 ppm may increase hemoglobin level in iron deficiency anemia rats.

Key words: Tempe fortification, iron, vitamin A, hemoglobin level

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Anemia is one of the most common nutritional problems in the world, including in Indonesia. The findings of several studies in Indonesia showed that anemia prevalence of teenagers remained high (26.1- 42.6%). WHO[1] stated that one region is considered to have moderate level of public health problem if its anemia prevalence is between 20.0-39.9%.

Iron deficiency or anemia may influence infant's to adult's psychomotor development and cognitive performance[1-4]. Iron deficiency or anemia may also influence physical work performance[5]. The severity levels of patients with iron deficiency anemia upon the infectious disease increase due to the influence of immune system[6]. Severe menstruation blood loss is also one of the important factors of iron deficiency anemia[7].

Due to public health programs, iron supplementation is one of the efforts to control iron deficiency anemia of the targeted group. The other alternative is fortification in food consumed by many people in Indonesia. The results of study upon female teenagers of junior high school in Semarang, Indonesia showed that 37.8% consumed tempe daily, 46.7% consumed 2-3 tempe per week and only 1.1% did not consume tempe[8]. The result of another study upon teenagers of senior high school in Tegal, Central Java, Indonesia showed that 54.4% students always consume tempe ($\geq$7 times per week); 33.3% frequently consume tempe (4-6 times per week) and 12.2% rarely consumed tempe (1-3 times per week), respectively. Amount of 64.7% of students consumed high quantity of tempe ($\geq$150 g day^{-1}) while 37.3% of students consumed low quantity of tempe (<150 g day^{-1})[9].

In this study, fortification was conducted with the addition of iron and vitamin A due to various studies that indicate the presence of roles of vitamin A in hematopoietic. The relationship between vitamin A deficiency and anemia have been studied for many years. In Indonesia, the results showed that baby-mother with retinol serum of <0.7 μ mol L^{-1} have 2.4 times risk upon iron deficiency anemia[10]. Other study showed that children who consumed soup fortified with iron and vitamin C could increase the iron serum and saturated transferrin level better when the levels of serum retinol was >40 mg dL^{-1} than <20 mg dL^{-1}. Thus, it can be concluded that the status of vitamin A influence the deposited iron mobilization[11,12]. According to WHO[1], anemia can be diagnosed by measuring hemoglobin levels in blood. Thus, the level of hemoglobin was eventually measured in this study.

MATERIALS AND METHODS

Study design: This study was conducted with randomized pre test, post test and control group design. A total of 30 Sprague Dawley rats underwent depletion period for 2 weeks with standard fed (AIN-93G) of free-Fe to made them iron deficiency anemia. Samples were divided into 6 groups randomly and then the rats were treated for 6 weeks. The treatments were as follows: (1) Standard Feed (SF), (2) SF+TWF, (3) SF+ TFe230, (4) SF+TFe271, (5) SF+TFe271+VA15 and (6) SF+TFe271+VA50, which TWF was tempe without fortification, TFe230 and TFe271 was tempe fortified with iron 230 and 271 ppm, VA15 was 15 ppm vitamin A and VA50 was 50 ppm vitamin A. The dependent variable was the level of hemoglobin (Hb). The independent variable was the intervention of fortified soybean tempe with iron and vitamins A and the measured confounding variables were the status of protein (albumin level), Cu (levels of Cu), Zn (levels of Zn) and status of infection.

Tempe fortification preparation: The soybean used in this study was the local variety soybean from Grobogan, Central Java, Indonesia. Iron in form of ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and vitamin A in the form of retinyl acetate were purchased from Kimia Farma Indonesia. Iron level, vitamin A level and production of tempe refers to Tawali[13] and Astuti *et al.*[14]. Briefly, soybean was washed, boiled for 30 min, soaked for 22 h, peeled and washed. After steamed for 40 min, tempe then cooled down in room temperature. After tempe cooled down, yeast, iron and vitamin A were added, stirred between the process and then wrapped. Tempe was incubated in room temperature (31°C) for 30-34 h.

Animal: Sprague Dawley (SD) rats aged 4 weeks were weighed (100-175 g) then adapted for 7 days with standard feed AIN-93G[15]. Amount of 36.5% casein protein available in standard feed was substituted with fortified tempe flour. The body weight was measured weekly. At 7 am everyday, rats were fed with 10 g pellet. Residual feed was weighed every day. After adapted for 7 days, the rats were fed with AIN-93G free-iron for depletion period of 14 days. After depletion period, hemoglobin level was measured until hemoglobin level was $\leq$6.0 g dL^{-1} [16]. Blood sample was taken through the orbital sinus. The intervention with standard feed supplemented with the fortified tempe were lasted for 42 days according to the treatment for each group. The EDTA blood were taken for the measurement of hemoglobin and blood

serum for the measurement of protein status (albumin), Cu and Zn levels as well as infection status. During the study, none of the rats were dead. This study was conducted in the Center of Food and Nutrition Study (PAU) of Gadjah Mada University, Yogyakarta. This study was approved by Comittee of Health Research Ethics, Medical Faculty of Diponegoro University with reference No. 334/EC/FK/RSDK/2012.

The hemoglobin level was measured with cyanmethemoglobin methods[17] using a spectrophotometer HACH DR/2000 made in USA at a wavelength of 546 nm with C/F program and factor of 36.8. Albumin serum was measured using Bromocresol Green method[18]. The status of infection was with C Reactive Protein (CRP). Cuprum (Cu) is determined with Atomic Absorption Spectrophotometer (AAS) brands Perkin Elmer 3110 models made in USA at a wavelength of 324.8 nm. Zinc (Zn) with AAS was determined at a wavelength of 213.9 nm.

The collected data were analyzed with univariate, bivariate and multivariate analyses (analyze the difference of hemoglobin level between groups by controlling the confounding variable). Univariate analysis was used to describe each measured variable and were expressed as Mean±SD. The ANOVA and Kruskal Wallis test were used to analyze the difference between group for body weight before and after treatment, weight gain after treatment, the status of protein (albumin level), Cu, Zn and infection. Before bivariate analysis, the normality test of data was conducted using Kolmogorov Smirnov test[19]. The analysis of covariance (ANCOVA) was used to analyze the difference of hemoglobin level between groups by controlling the confounding variable. The differences were considered significant at $p<0.05$.

RESULTS

Weight: At the beginning of the study, the rat weight ranges from 112-175 g each, with an average of weight 140.5±15.2 g each. After the adaptation and depletion period, the average body weight of rats with iron deficiency anemia was 158.7±15.4 g and there was no significant difference in the average weight before treatment ($p = 0.315$). After treatment, there was significant difference in weight gain due to the treatment ($p = 0.000$). The rat feed were given in the form of pellets. About 10 g day^{-1} of pellets were given to rats. The remaining feed was weighed at each morning to calculate the eaten feed. The eaten feed for 6 weeks (42 days) after intervention ranging from 297-337 g. The average feed eaten for 6 weeks and per day were 320.3±8.3 and 7.6±0.2 g, respectively. The average weights of each treatment were presented in Table 1.

Levels of hemoglobin: The average level of hemoglobin at baseline and depletion period was 12.0±0.3 and 5.7±0.2 g dL^{-1}, respectively. There was no significant difference in the average levels of hemoglobin before intervention due to the given treatment. The average level of hemoglobin after intervention in the controlled rats (SF rats) were 11.6±0.2 g dL^{-1}; rats fed with the fortified soybean tempe with iron has not reached 12.0 g dL^{-1}, while rats fed with fortified soybean tempe with the combination of iron and vitamin A (SF+TFe271+VA15 and SF+TFe271+VA50 treatments) has, respectively reached 12.2±0.1 and 12.4±0.2 g dL^{-1}. ANCOVA test results showed that the intervention of the fortified soybean tempe with the combination of iron and vitamin A may significantly increase the levels of hemoglobin ($p = 0.001$). The highest average level of hemoglobin was in the treatment of iron of 271 ppm plus vitamin A of 50 ppm. The influence of combination between iron and vitamin A significantly increases the levels of hemoglobin. Trend of hemoglobin levels was based on treatment and measuring time presented in Fig. 1. Before intervention, all rats were in iron-deficiency anemia and after intervention, rats in treatments with SF and SF+TWF were still in anemic stage. Whereas, rats in treatment with SF+TFe230 and SF+TFe271, 60.0% of which suffer from anemia. Whereas, rats in treatment with combination of iron and vitamin A (SF+TFe271+VA15 and SF+TFe271+VA50 were found with no anemia after the treatment).

Table 1: Average and gain weight of rats

	Average±SD		
Treatments	Weight before intervention	Weight after intervention	Weight gain after intervention
SF	156.6±11.4	193.4±8.1	36.8±4.3[a]
SF+TWF	167.6±9.8	203.4±10.1	35.8±1.3[a]
SF+TFe230	161.8±15.0	208.4±15.0	46.6±6.3[b]
SF+TFe271	152.2±21.8	199.8±22.1	47.6±3.8[b]
SF+TFe271+VA15	165.6±16.6	218.0±16.8	52.4±2.1[b]
SF+TFe271+VA50	148.6±12.6	203.2±12.1	54.6±1.7[b]
p-value	0.315	0.202	0.000**

**Significant difference in ANOVA test/Kruskal-Wallis test, [a,b]Same letter(s) in column shows that there is no significant difference in posterior test (each treatment is compared with controlled/standard feed)

Table 2: Average levels of Hb, albumin, Cu and Zn

Treatments	Mean±SD Hb levels before treatment	Hb levels after treatment	Levels of albumin (g dL^{-1})	Levels of Cu (μg mL^{-1})	Levels of Zn (μg mL^{-1})
SF	5.7±0.2	11.6±0.2[a]	6.5±0.1[a]	1.1±0.7	1.2±0.3
SF+TWF	5.9±0.1	11.4±0.4[a]	6.2±0.1[b]	1.3±1.1	0.9±0.2
SF+TFe230	5.6±0.3	11.9±0.3[b]	6.5±0.1[a]	1.4±0.4	1.3±0.5
SF+TFe271	5.8±0.2	11.7±0.3[a]	6.3±0.1[a]	0.7±0.2	1.8±0.9
SF+TFe271+VA15	5.7±0.2	12.2±0.1[b]	6.4±0.1[a]	0.7±0.2	1.3±0.1
SF+TFe271+VA50	5.7±0.2	12.4±0.2[c]	7.3±0.1[c]	0.6±0.3	1.4±0.7
p-value	0.398	0.001**	0.000**	0.091	0.229

**Significant difference in ANOVA test/Kruskal-Wallis test and Hb level after treatment with ANCOVA test, covariate: Hb pre, albumin level, Cu and Zn level, [a-c]Same letter(s) in column shows that there is no significant difference in the posterior test (each treatment is compared with the controlled/standard feed)

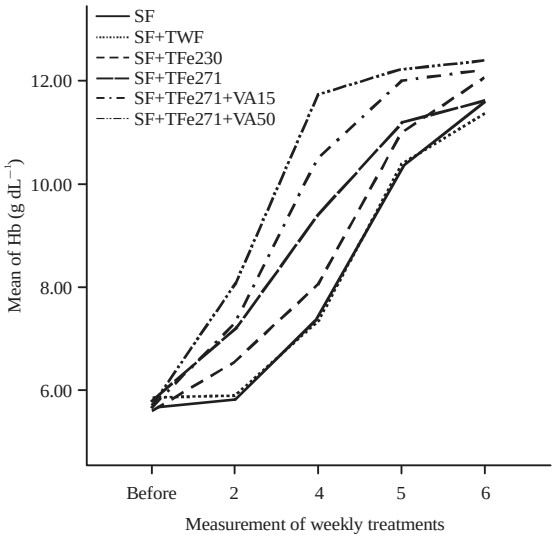

Fig. 1: Trends of haemoglobin levels based on treatment and time measurement

Levels of CRP, albumin, Cu and Zn: The rats on the condition iron deficiency anemia shows that 27 of 30 rats (90.0%) had positive infection status while after treatment decreased into 12 rats (40.0%) with positive infection status. The status of protein which was measured using the level of albumin ranges from 6.0-7.5 g dL^{-1} with the average of 6.5±0.4 g dL^{-1}. The average level of albumin of each treatment was presented in Table 2. The highest average level of albumin in the treatment of SF+TFe271+VA50 was 7.3±0.1 g dL^{-1}. The level of copper in serum (Cu level) ranges from 0.4-3.1 mg mL^{-1} with an average of 1.0±0.6 mg mL^{-1}. The results of statistical test showed that there was no significant difference upon the level of Cu between the treatment groups. The level of Zinc in serum (Zn levels) ranged from 0.6-3.2 mg mL^{-1} with an average of 1.3 mg mL^{-1}. The average level of Zn in each treatment was presented in Table 2. The results of statistical test showed that there was no significant difference upon the average level of Zn, between the treatment groups.

DISCUSSION

The level of hemoglobin (Hb) in the baseline was ranging from 11.4-12.8 g dL^{-1} while after the depletion period; the rats were in iron deficiency anemia with hemoglobin levels ranging from 5.2-6.0 g dL^{-1}. The result of this study was similar with earlier findings of Kamei *et al.*[20] who reported that the rats which were fed an iron-deficient diet, 3 ppm iron, *ad libitum* for 16 days resulted in anemic rats, hemoglobin level was 6.1±0.2 g dL^{-1} and a control diet with standard feed, 48 ppm iron for 16 days, the hemoglobin level was 14.4±0.4 g dL^{-1} with the initial hemoglobin level before fed with standard feed was 11 g dL^{-1}. The results of this study showed that the influence of fortified soybean with iron significantly increases Hb level. Iron is an essential micro mineral in the formation of hemoglobin level. Hemoglobin is composed of four polypeptide chains of globins that each of which contains heme molecules[21,22]. There are various proteins with important roles in cellular physiology that require iron to operate their functions. In mitochondria, the electron transport chains, ferrochelatase enzyme and hemoglobin are needed for bioenergetics and oxygen transport[23,24].

The status of infection was also measured in this study as variable used to control in multivariate analysis. First, the rats were situated with iron-deficiency anemia that 80.0% of the rats have positive infection status. The measurement of this infection status in this study uses C Reactive Protein (CRP), that the appearance of CRP measurement principle in individual serum responding to various conditions of tissue inflammatory and necrosis was not found that the cause of condition decreases. The CRP is the acute protein phase with various functions to protect body, such as stimulating the immune system, promoting wound healing and changing free iron in the circulation to prevent bacteria to use it for their growth[22]. In the depletion period, the decrease of iron consumption may result in iron metabolism disturbance and changes of iron metabolism contributing to anemia prevalence that C Reactive Protein (CRP) increases.

In iron deficiency anemia condition, the average level of hemoglobin was 5.7±0.2 g dL^{-1}. This situation can be explained that the regulation of iron is in normal iron metabolism condition under the control of Iron Regulatory Protein (IRP), binding mRNA sequence and protecting it from damage. In iron deficiency, IRP binds mRNA and increases transferrin receptor protein expression and pressed ferritin synthesis that the use and absorption of iron increase[25]. In the repletion period, when iron intake is adequate and intracellular ferritin synthesis increases, the iron is eventually deposited[25] which resulted hemoglobin level increases.

The status of protein in this study was used to measure the level of albumin. The albumin level of given feed plus fortified soybean tempe (iron and vitamin A) at higher level ($p = 0.000$). Then this result associated with the weight of rats given feed+higher fortified soybean tempe with iron and vitamin A and also shown a greater weight gain. This indicates that the absorption of nutrients such as carbohydrates, fats and proteins is better when the vitamins and minerals consumption are adequate.

Rats in the treatment of fortified tempe with iron of 271 ppm and vitamins A of 50 ppm has the highest level of albumin of 7.3±0.1 g dL^{-1}. It shows that the increase of vitamin A consumption influences the transport of retinol in liver. According to Gropper *et al.*[22] the retinol transport in liver requires two particular proteins: Retinol Binding Protein (RBP) and transthyretin (TTR) as thyroxine-binding globulin. Both proteins are synthesized by parenchyma cells of liver. Thus, higher retinol in the liver may increase RBP and TTR synthesis. The RBP synthesis depends on status of protein, retinol and zinc. Regardless to retinol which is mobilized from liver as transport to the other tissues, retinoic acid is produced in small quantities by individual cells and generally bound with albumin as transport in blood[22]. Retinoic acid can also modify the cell surfaces by increasing glycoprotein synthesis at the gene level or by increasing glycoprotein on the cell surfaces to induce adhesion cell[26]. The changes at glycoprotein glycans can greatly influence cells or tissues differentiation through its influence on cell recognition, adhesion and aggregation[22].

The results of this study showed that combination of iron and vitamin A may increase hemoglobin level. The relationship between iron status and vitamin A has been widely studied. The influence is due to the presence of vitamin A roles in hematopoietic[27,22]. There are several mechanisms that may explain the influence of vitamin A deficiency upon the status of anemia; the decrease of iron mobilization from iron deposit to bone marrow[12,22], lower resistance to infection which may increase the status of anemia due to infection[12,28], the influence of iron absorption or metabolism and direct modulation or stimulation of erythropoiesis[28,12,22].

In this study, although tempe fortified with the combination of iron and vitamin A may significantly increase the hemoglobin levels but its influence was not significant to the levels of Cu and Zn (respectively, $p = 0.320$ and $p = 0.217$). Thus, it can be stated that either iron fortification level of 230 ppm or 271 ppm did not significantly reduce the level of Cu. The fortification levels of 230 ppm and 271 ppm is considered based on the recommended iron fortification. The WHO[1] Guidelines suggest that no more than 3 mg of fortificant iron be added to a 50 g serving portion of a solid food or 250 mL of beverage, contributing a maximum of 22% of daily iron needs from a diet with high biological availability[29]. Iron used in this study is the ferrous sulfate heptahydrate of 271 ppm, which contains iron element 54.4 mg kg^{-1} tempe. That is the tempe 1 piece (50 g) contained 2.7 mg of iron supplements, still below the limit set by the WHO. Another study on infants with formula consumption supplemented with iron of 10.2 mg L^{-1}, Cu absorption of 13.4% and with iron of 2.5 mg L^{-1}, Cu absorption of 27.5% show significant differences. However, the absorption of Zn is not significantly different[30]. All the nutrients in the body interact with others, including iron, zinc, Cu and vitamin A. However, this study shows that the fortified soybean with different levels of iron and vitamin A did not influence the level of zinc (Zn).

CONCLUSION

In conclusion, iron and vitamin A fortification in tempe with ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) 271 ppm and retinyl acetate 50 ppm may increase hemoglobin level in iron deficiency anemia rats. The level of iron and vitamin A did not influence the level of Cu and zinc.

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REFERENCES

1. WHO., 2001. Iron Deficiency Anaemia: Assessment, Prevention and Control. A Guide for Programme Managers. World Health Organization, Geneva.
2. Soemantri, A.G., 1989. Preliminary findings on iron supplementation and learning achievement of rural Indonesian children. Am. J. Clin. Nutr., 50: 698-701.

3. Lozoff, B., E. Jimenez and A.W. Wolf, 1991. Long-term developmental outcome of infants with iron deficiency. N. Engl. J. Med., 325: 687-694.
4. McLean, E., M. Cogswell, I. Egli, D. Wojdyla and B. de Benoist, 2007. Worldwide Prevalence of Anemia in Preschool Aged Children, Pregnant Women and Non-Pregnant Women of Reproductive Age. In: Nutritional Anemia, Kraemer, K. and M.B. Zimmerman (Eds.)., Sight and Life Press, Basel, Switzerland, pp: 1-12.
5. Husaini, M.A., D. Karyadi and H. Gunadi, 2001. Evaluation of Nutritional Anaemia Intervention Among Anemic Female Workers on a Tea Plantation. World Health Organization. Iron deficiency Anaemia. Assessment. Prevention and control. A guide for programme managers. WHO/NHD/01.3. Genewa.
6. Ekiz, C., L. Agaoglu, Z. Karakas, N. Gurel and I. Yalcin, 2005. The effect of iron deficiency anemia on the function of the immune system. Hematol. J., 5: 579-583.
7. Brabin, L. and B.J. Brabin, 1992. The cost of successful adolescent growth and development in girls in relation to iron and vitamin A status. Am. J. Clin. Nutr., 55: 955-958.
8. Astuti, R. and E. Handarsari, 2009. Usia menarche, indeks masa tubuh, frekuensi konsumsi dan status sosial ekonomi orang tua pada siswi SLTP di pinggir kota dan pusat kota, kota semarang. Semarang: Fakultas Kesehatan Masyarakat Universitas Muhammadiyah Semarang, Indonesia.
9. Alawi, F., 2003. Faktor-faktor yang berhubungan dengan konsumsi tempe pada remaja di SMUN 1 slawi dan SMUN 1 tegal. Skripsi, Jurusan Gizi Masyarakat dan Sumberdaya Keluarga, Fakultas Pertanian, Institut Pertanian Bogor, Bogor.
10. Dijkhuizen, M.A., F.T. Wieringa, C.E. West and M. Muherdiyantiningsih, 2001. Concurrent micronutrient deficiencies in lactating mothers and their infants in Indonesia. Am. J. Clin. Nutr., 73: 786-791.
11. Van Stuijvenberg, M.E., M. Kruger, C.J. Badenhorst, E.P. Mansvelt and J.A. Laubscher, 1997. Response to an iron fortification programme in relation to vitamin A status in 6-12-year-old school children. Int. J. Food Sci. Nutr., 48: 41-49.
12. Zimmermann, M.B., 2007. Interactions between Iron and Vitamin A, Riboflavin, Copper and Zinc in the Etiology Anemia. In: Nutritional anemia, Kraemer, K. and M.B. Zimmerman (Eds.)., Sight and Life Press, Basel, Switzerland, pp: 199-213.
13. Tawali, A.B., 2000. Fortifikasi zat besi pada ragi tempe dan dan analisis ketersediaan (availability) zat besi pada tempe yang dihasilkan (Suatu kajian fortifikasi mikronutrien pada makanan tradisional). Seminar Makanan Tradisional, Pusat Kajian Makanan Tradisional (PKMT) Universitas Brawijaya, Malang.
14. Astuti, R., A. Syamsianah and S. Aminah, 2012. Tempe fortifikasi untuk penanggulangan anemia gizi besi pada Remaja. Laporan Hibah Bersaing Tahun I. Universitas Muhammadiyah Semarang, Indonesia.
15. Reeves, P.G., F.H. Nielsen and G.C. Fahey Jr., 1993. AIN-93 purified diets for laboratory rodents: Final report of the American institute of nutrition *ad hoc* writing committee on the reformulation of the AIN-76A rodent diet. J. Nutr., 123: 1939-1951.
16. Naruki, S., M. Astuti, Y. Marsono and S. Raharjo, 2010. Sifat prooksidatif fortifikan nafeedta, dengan kecap kedelai manis sebagai makanan pembawa, dalam system biologis (Tikus). Majalah Ilmiah Agritech., 30: 244-249.
17. WHO. and CDC., 2007. Assessing the iron status of population. Including literature reviews, Report of a joint World Health Organization/ Centers for Disease Control and Prevention Technical Consultation on the assessment of iron status at the population level, Geneva, Switzerland, 6-8 April 2004.
18. Hill, P.G., 1985. The measurement of albumin in serum and plasma. Ann. Clin. Biochem., 22: 565-578.
19. Sheskin, D., 2004. Handbook of Parametric and Nonparametric Statistical Procedures. 3rd Ed., Chapman and Hall /CRC, Boca Raton, ISBN: 9781584884408, Pages: 1193.
20. Kamei, A., Y. Watanabe, T. Ishijima, M. Uehara and S. Arai *et al.*, 2010. Dietary iron-deficient anemia induces a variety of metabolic changes and even apoptosis in rat liver: A DNA microarray study. Physiol. Genom., 42: 149-156.
21. Hoffbrand, V.A. and A. Mehta, 2008. At a Glance Hematologi. Erlangga, Jakarta.
22. Gropper, S.S., J.L. Smith and J.L. Groff, 2009. Advanced Nutrition and Human Metabolism. 5th Edn., Cengange Learning, Wadsworth, USA.
23. Hatefi, Y., 1985. The mitochondrial electron transport and oxidative phosphorylation system. Annu. Rev. Biochem., 54: 1015-1069.
24. Ajioka, R.S., J.D. Phillips and J.P. Kushner, 2006. Biosynthesis of heme in mammals. Biochim. Biophys. Acta (BBA)-Mol. Cell Res., 1763: 723-736.
25. Hesketh, J.E., M.H. Hesketh and G. Bermano, 1998. Regulatory signals in messenger RNA: Determinants of nutrient-gene interaction and metabolic compartmentation. Br. J. Nutr., 80: 307-321.
26. Olson, J.A., 1993. Atwater lecture: The irresistible fascination of carotenoids and vitamin A. Am. J. Clin. Nutr., 57: 833-839.
27. Fishman, S.M., P. Christian and K.P. West, 2000. The role of vitamins in the prevention and control of anaemia. Public Health Nutr., 3: 125-150.
28. Semba, R.D. and M.W. Bloem, 2002. The anemia of vitamin A deficiency: Epidemiology and pathogenesis. Eur. J. Clin. Nutr., 56: 271-281.
29. WHO., 2006. Guidelines on Food Fortification with Micronutrients. World Health Organization and Agriculture Organization of the United Nations, Geneva, Switzerland.
30. Haschke, F., E.E. Ziegler, B.B. Edwards and S.J. Fomon, 1986. Effect of iron fortification of infant formula on trace mineral absorption. J. Pediatr. Gastroenterol. Nutr., 5: 768-773.