

In vivo Iron Availability and Profile Lipid Composition in Anemic Rats Fed on Diets with Black Rice Bran Extract

E. P. Nurlaili, M. Astuti, Y. Marsono, S. Naruki

Abstract—Iron is an essential nutrient with limited bioavailability. Nutritional anemia caused mainly by iron deficiency is the most recognized nutritional problem in both countries as well as affluent societies. Rice (*Oryza sativa* L.) has become the most important cereal crop for the improvement of human health due to the starch, protein, oil, and the majority of micronutrients, particularly in Asian countries. In this study, the iron availability and profile lipid were evaluated for the extracts from Cibeusi varieties (black rices) of ancient rice brans.

Results: The quality of K, B, R, E diets groups shows the same effect on the growth of rats. Hematocrit and MCHC levels of rats fed K, B, R and E diets were not significantly ($P < 0.05$). MCV and MCH levels of rats K, B, R were significantly ($P < 0.05$) with E groups but rats K, B, R were not significantly ($P < 0.05$). The iron content in the serum of rats fed with K, B, R and E diets were not significantly ($P < 0.05$). The highest level of iron in the serum was founded in the B group. The iron content in the liver of rats fed with K, B, R and E diets were not significantly ($P < 0.05$). The highest level of iron in the liver was founded in the R group. HDL cholesterol levels were significantly ($P < 0.05$) between rats of fed B, E with K, R, but K and R were not significantly ($P < 0.05$). LDL cholesterol levels of rats fed K and E significantly ($P < 0.05$) with B and R.

Conclusions: the bran of pigmented rice varieties has, with some exceptions, greater antioxidant and free-radical scavenging activities. The results also show that pigmented rice extracts acted as pro-oxidants in the lipid peroxidation assay, possibly by mechanisms described for the pro-oxidant activities of tocopherol and ascorbic. Pigmented rice bran extracts more effectively increases iron stores and reduces the prevalence of iron deficiency.

Keywords—Anemia, black rice bran extract, iron, profile lipid.

I. INTRODUCTION

IRON deficiency and iron deficiency anemia are prevalent among women of child bearing age as a result of many causes. In Indonesia (Household Health Survey/ SKRT in 2004), this phenomenon has reached epidemic proportions. 40.5% of all children under 5 years old, 50.5% pregnant, 57.1% and 39.5% of all Indonesia women have this serious health condition (10-18 and 19-45 years old). The causes include child birth, heavy menstrual cycles, heavy bleeding from some forms of contraception (IUD), intestinal parasites, ulcers, a high intake of coffee or tea, and a diet lacking in animal proteins, such as beef and other iron rich foods. Iron deficiency, apart from anemia, also results in severe fatigue,

listlessness, poor concentration and susceptibility to infections. True iron deficiency anemia cannot be self diagnosed. It must be determined by a medical exam. Once a positive result has been determined, Iron Replacement Therapy (IRT) should be initiated. The major problem with IRT though, is that most forms of iron prescribed are highly irritating and are a source of dangerous free radicals. An excellent solution to this dilemma is the use of stable, organic iron derived from the Black Rice cultivar of *Oryza sativa*, which is also rich in supportive nutrients, protective antioxidants, and anthocyanidins [1].

Rice is cultivated in over one hundred countries as staple food for more than half the population of the world. Pearled rice, the husk and bran being removed during the rice-milling process, is the most common type of rice consumed because of its high content of starch. Recent evidence indicates that many bioactive compounds do not uniformly exist in a cereal grain but are concentrate in the husk and bran layer [2] so that the consumption of whole grain in regular meals is strongly recommended, to provide desirable health benefits beyond basic nutrition and to reduce the risks of many chronic diseases [3].

The beneficial components of rice bran comprise sterols, δ -oryzanols, tocopherols, tocotrienols and phenolic compounds [4]. Some special phenolic acids, such as ferulic acid, p-coumaric acid and diferulate found in rice bran are not present in significant quantities in fruit and vegetables [5]. The inhibitory activity of ferulic acid on aldose reductase was related to the prevention of diabetic complications [6]. δ -Oryzanol, a mixture of lipophilic phytosterols that are composed of triterpene alcohols or sterols with ferulic acid ester, exhibits antioxidative activity and cholesterol-lowering effect [7]. In addition to δ -oryzanols, tocopherols (T), tocotrienols (T3) and several phenolic compounds in rice bran have potentially beneficial effects, such as antioxidative activity, antimutagenic, and anticancer [8] that play an important role in maintaining health.

Black rice (*Oryza sativa* L. *indica*) is a special cultivar of rice that contains rich anthocyanins in the aleurone layer, has been regarded as a promoting food and widely consumed since ancient times in China and other Eastern Asia countries. Previous studies showed that the supplementation of black rice pigment fraction markedly reduced oxidative stress and improved lipid profile in addition to modulating atherosclerotic lesions in two different animal models [9], [10].

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The form of iron in black rice is naturally chelated to organic compounds, which is the same as the form of iron naturally occurring in most foods, and is one of the safest iron sources available. In addition, black rice organic iron is rich in a wide breadth of naturally occurring antioxidants, such as proanthocyanidins, anthocyanidins, flavonoids, isoflavones, tocotrienols, phytosterols and phytate. These phytochemicals protect arteries from damage, decrease cancer risk, and reduce the overall free radical burden in the body. Isoflavones are thought to possibly help promote stronger bone density, decreasing the risk of osteoporosis and the resulting hip fractures in women; decrease the risk of breast cancer; and reduce symptoms of menopause, such as hot flashes. In addition, these phytochemicals protect skin from UV and chemical damage, helping to slow down the aging process. Proanthocyanidins increase the strength of vascular tissue and help to prevent varicose veins common in women, both after child birth and as one gets older.

The numerous health and nutritional properties of black rice have very important value. Black rice has been given to people traditionally after surgery to promote healing of the surgical wound. Its nutrient make-up as a whole helps to increase red blood cell formation. With over a dozen minerals and at least 17 different amino acids, Black rice has two and a half times more protein than regular rice. This would probably explain why it has been found to be so beneficial in nourishing the yin (body tissue) of older people and those who have weakness.

Black rice has been shown to act as a health tonic for the stomach, liver, and eyes. The positive effect on the eyes may result from its high levels of anthocyanidins, much the same as with bilberry. The dark purple color of black rice is from the anthocyanidins and has value for use as a food coloring agent. Anthocyanidins are known to promoting healthy circulation, and strength in blood vessel walls, helping to prevent bruising.

In Traditional Chinese Medicine, it is thought to nourish the kidney yin, strengthen the spleen system (blood forming function of the body) and warm the liver system (relates to the blood supply and emotional function of the body). According to TCM theory, black rice promotes blood circulation and improves visual acuity (brightens the eyes).

Black rice also contains tocotrienols, relatives of vitamin E known to be more potent antioxidants than vitamin E. Other valuable constituents are ferulic acid, δ -oryzanol, and phytosterols. These compounds help support healthy immune function, and decrease inflammation and cholesterol levels, which are all very important for healthy circulation and longevity [1].

II. MATERIAL AND METHODS

Weanling male Wistar rats (LPPT GadjahMada University Experimental Animals Co), weighing approximately 40 to 45 g were fed either a iron-deficient diet. The composition of the diets was previously described. Deionized distilled water was freely available to all rats. Rats were housed individually in stainless steel cages in a room with a 12-hour light/dark cycle

and maintained at 22°C and 50% to 60% humidity. All rats were weighed weekly.

At the end of the 11-week experimental period, rats were decapitated following an overnight fast. Serum was removed and were taken for Hb, Fe contents, Hematocrit, MCV, MCH, MCHC and TPP levels; lipid composition. Livers were removed and weighed, and liver portions were taken for iron analysis.

A. Samples and Chemicals

One pigmented rice cultivars (Cibeusi) – used as an internal control – were grown and harvested at the Cibeusi, Ciater, Subang West Java, Indonesia. The rice seeds were cleaned and stored at 4°C prior to use. Biochemicals were purchased from Sigma (Louis, MO).

B. Extractions of Rice Bran

The rice seeds were dehulled, degermed, polished in a laboratory mill, and then passed through a 60-mesh sieve, resulting in a uniform fraction of rice bran. The pigments in the hull were extracted by shaking overnight at room temperature with 10 times the sample weight of deionized water (v/v). The solvent was then removed from the extract by rotary evaporation at room temperature.

III. RESEARCH DESIGN

The research is divided into stages:

A. Preparation of Animals

The animals are trying to use are white male Wistar rats by 28 tail, aged 3 weeks, obtained from LPPT University of Gadjah Mada. Determination of the number of animals try referring to Federer's formula, $n =$ number of samples in each group and $k =$ the number of the sample group. The rest of the feed every day weighed. After the adaptation, the animals try to weigh his weight for dose determination of fructose.

B. Making Feed

Before the manufacture of fodder for animals try bran extract preparation, made of rice, black rice bran by way of elected black extracted with 3% acetic acid solution in water deionization (ratio 1: 10) and shaker at a temperature of 40°C, the speed of 125 rpm, for 60 minutes and then kept at a temperature of 4°C for 24 hours. Then do the filtering, in the rotary evaporator for 4 hours at a temperature of 40°C and in the freeze dryer.

C. Maintenance, Animal Surgery and Try Euthanasia

At the beginning of the treatment done analysis on rat blood plasma content of hemoglobin and triglycerides to total, to know the condition of the hypertriglyceridemia and anemia or not. Prior to entering the trial period, all rats has undergone a period of adaptation for 7 days, with the granting of a standard diet (AIN-93G) and drink the water of deionization *ad libitum*. Furthermore all mice conditioned to be anemic given feed with AIN-93G but for a mix of minerals already eliminated the iron substances for 2-5 weeks or mice are anemic until it reached $Hb \leq 6$ g/dL (period of depletion). Mice that had been

anemic is then divided into 4 groups @ 7 tail, and maintained for 5 weeks in individual cages, with the granting of a basal diet without iron and drink the water of deionization *ad libitum* (repletion period). During the period repletion, the need for iron in rat infested Group 4 from the granting of bran, bran extract and extract the residue rice black by force feeding. In mice Group 1, was given the standard diet of AIN 93G (as control). At the beginning, during and end of period repleni, done weighing weight gain, feed intake, and the analysis of the content of hemoglobin total blood mice, performed each week. Of the 28 rats were divided into 4 groups each consisting of 7 tails, which are: Group 1 was given standard food AIN-93G (control = K); Group 2 is given standard food AIN-93G but for a mix of minerals without iron (Ferric citrate), but full of iron on a par with Ferric citrate from bran (B); Group 3 was given standard food AIN-93G but for a mix of minerals without iron (Ferric citrate), but full of iron on a par with Ferric citrate from rice bran extract black deposits (R); Group 4 were given standard food AIN-93G but for a mix of minerals without iron substances (Ferric citrate), but full of iron on a par with Ferric citrate from black rice bran extracts (E). Taking of the blood was done through flexusretroorbital, and later performed surgery and taken his organs. Research carried out at the end of analysis for the parameters of Hematology (hemoglobin content), total Fe (iron) in the blood and lipid composition in the serum.

D. Statistical Analysis

Tests were run in triplicate and the values expressed as means ± SD. Differences in treatment means and correlations of values were analyzed using Minitab PC+ software (version 16.0), significant at P < 0.05

IV. RESULTS

A. Growth and Body Weight Gain

The growth and body weight gain of rats during experiments of repletion are shown in Fig. 1.

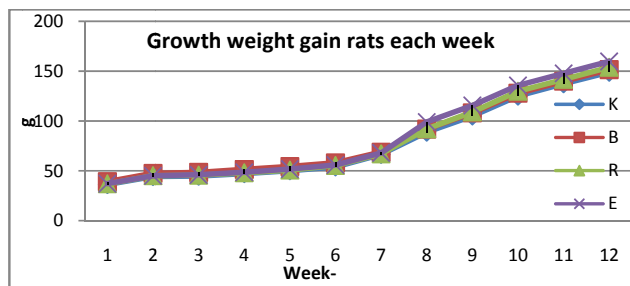


Fig. 1 Growth curve during experiments

As shown in Fig. 1, the growth rate for all the group of rats was not significantly different (P<0.05). This means that though the quality of K, B, R E diets groups shows the same effect on the growth of rats.

Body weight gains of rats during the experiment which is expressed as g per rat, g per g food intake, are shown in Fig. 1

the body weight gain which expressed as g/rat during experiments.

B. Food Efficiency

The efficiency of food, which is calculated as the ratio of weight gain to total food intake times 100 percent, is shown in Fig. 2. The efficiency of K, B, R and E diets was not significantly different (P<0.05).

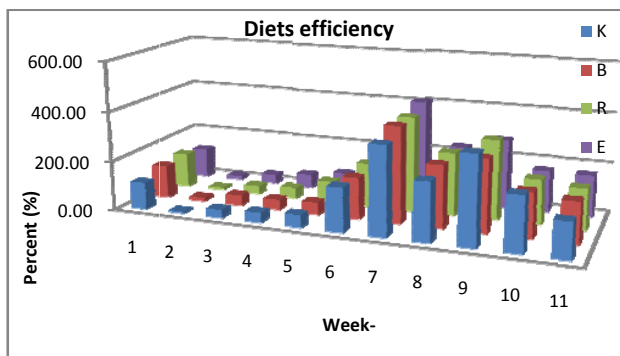


Fig. 2 Food efficiency in rats fed K, B, R, E diets

C. Hematology Parameters

Hematology parameter which were evaluated at the end of the experiments were hemoglobin, hematocrit, MCV, MCH, means capsular hemoglobin concentration (MCHC) and and TPP levels. MCHC was calculated as the ratio of hemoglobin to hematocrit times 100 percent. Hemoglobin levels are shown in Fig. 3; hemoglobin, hematocrit, MCV, MCH, MCHC and TPP levels are illustrated in Fig. 3.

1) Hemoglobin Levels

As shown in Fig. 3, hemoglobin levels at the end of the repletion period of rats fed K, B, R and E diets were g/dL.

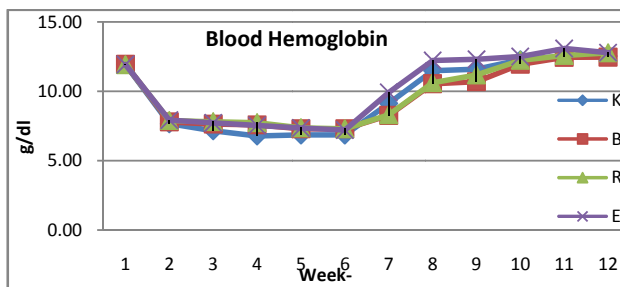


Fig. 3 Hemoglobin levels

2) Hematocrit, MCV, MCH, MCHC and TPP levels

The levels of hematocrit, MCV, MCH, MCHC and TPP levels are shown in Fig. 4. The hematocrit levels of rats fed with K, B, R and E diets were percent (%).

As shown in Fig. 4, hematocrit and MCHC levels at the end of the repletion period of rats fed K, B, R and E diets were not significantly (P<0.05). MCV and MCH levels of rats K, B, R were significantly (P<0.05) with E groups but rats K, B, R were not significantly (P<0.05).

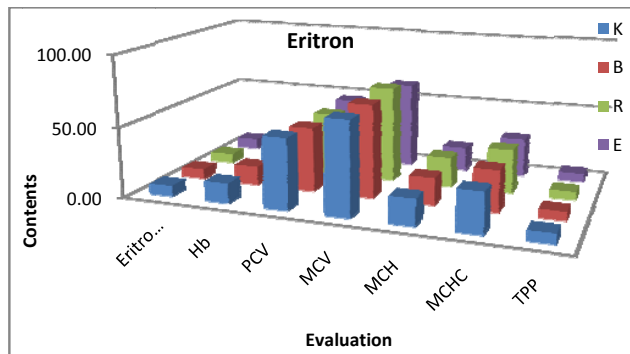


Fig. 4 Hematocrit, MCV, MCH, MCHC and TPP levels

D. Iron in the Serum

The iron content in the serum of rats fed with K, B, R and E diets were not significantly ($P < 0.05$). The highest level of iron in the serum was founded in the B group. The iron content in the serum of rats is shown in Fig. 5.

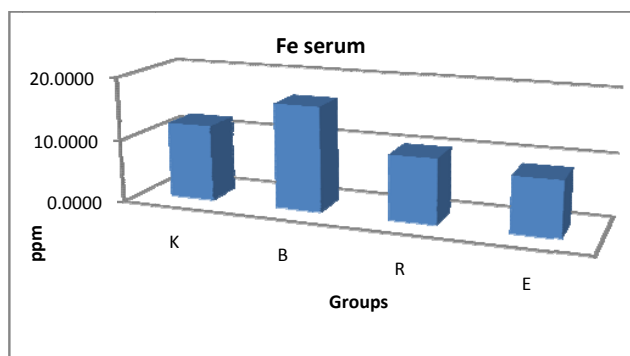


Fig. 5 Iron serum contents

E. Iron in the Organs

The concentration of iron in the liver is expressed in ppm dry weight. As shown in Fig. 6, the iron content in the liver of rats fed with K, B, R and E diets were not significantly ($P < 0.05$). The highest level of iron in the liver was founded in the R group.

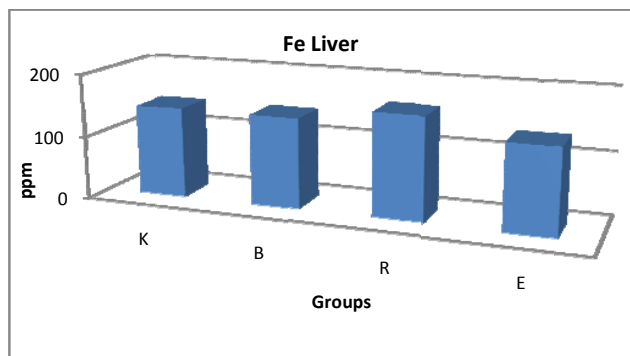


Fig. 6 Iron liver contents

F. Lipid Composition in the Serum

The profiles of lipid in the serum were total cholesterol, triglyceride, HDL cholesterol and LDL cholesterol in rats consuming K, B, R and E diets are listed in Fig. 7.

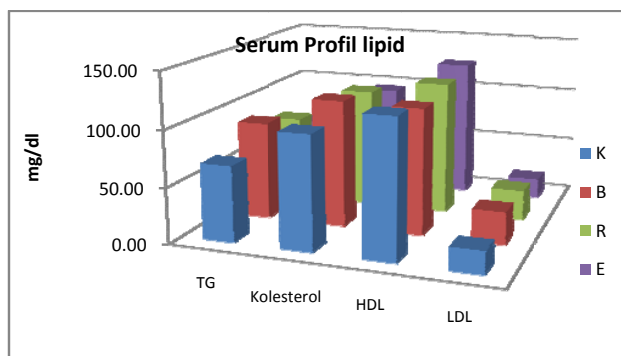


Fig. 7 Lipid composition in the serum

As shown in Fig. 7, TG, total cholesterol, HDL cholesterol, LDL cholesterol levels (mg/dL). Fig. 7 showed that serum LDL increased after therapy and LDL decreased. The highest level of TG, total cholesterol in the serum was founded in the B group. HDL cholesterol levels were significantly ($P < 0.05$) between rats of fed B, E with K, R, but K and R were not significantly ($P < 0.05$). LDL cholesterol levels of rats fed K and E significantly ($P < 0.05$) with B and R.

V. DISCUSSION

Iron is a component of several metalloproteins and plays a crucial role in vital biochemical activities, such as oxygen sensing and transport, electron transfer, and catalysis. Iron is thus indispensable for life. The role of iron in free radical reactions such as lipid peroxidation has been reviewed extensively (4.9-16.8). A variety of studies have demonstrated the ability of iron chelates or complexes to catalyze the formation of reactive oxygen species and stimulate lipid peroxidation [11]. There is evidence suggesting that chelated iron acts as a catalyst for the Fenton reaction, facilitating the conversion of superoxide anion and hydrogen peroxide to hydroxyl radical, a species frequently proposed to initiate lipid peroxidation [11]. Agil and coworkers demonstrated recently that the coinubation of human plasma with ferrous iron and hydrogen peroxide resulted in almost three-fold increase of lipid peroxides, possibly by a Fenton reaction mechanism [12].

Balla and coworkers demonstrated that the combination of physiological concentrations of hydrogen peroxide and hemin induce a rapid per-oxidation of LDL in vitro and that free iron is released from the degraded heme ring [13]. Also, iron released from ferritin has been shown to stimulate the formation of hydroxyl radicals from superoxide radicals and hydrogen peroxide, whereas apoferitin appears to inhibit lipid peroxidation. In iron overload lipid peroxidation has been detected in red blood cells [14].

Upon ingestion of iron, iron is in the iron storage proteins ferritin or associates with the iron transport protein transferrin in the blood stream [14]. Generally, except in states of iron overload, all iron in human serum is bound to proteins. However, when ferrous sulfate is taken, there are high local concentrations of hydrated ferrous ions in the duodenal lumen. The primary oxidation product of hydrated ferrous iron is the hydrated mononuclear $\text{Fe}(\text{OH})_3$, [15]. These two species are those transported most efficiently into the mucosal cell from where they are carried to the blood by transferrin. The net flux of iron from intestinal lumen to the blood may differ greatly whether famous salts or ferric hydroxide type of preparations are supplemented. The ferric hydroxide preparations are also sources of mononuclear $\text{Fe}(\text{OH})_3$, but, due to polynuclear structure, the release occurs at low rate [15].

In this study, the conventional ferrous sulfate supplement increased the susceptibility of the atherogenic lipoproteins to oxidation. Ferrous sulfate is absorbed rapidly in the upper intestine.

VI. CONCLUSION

The main conclusions of this study can be summarized as follows:

- 1) The quality of K, B, R E diets groups shows the same effect on the growth of rats.
- 2) The group is as efficiently utilized by the body as E diets.
- 3) Hematocrit and MCHC levels of rats fed K, B, R and E diets were not significantly ($P < 0.05$). MCV and MCH levels of rats K, B, R were significantly ($P < 0.05$) with E groups but rats K, B, R were not significantly ($P < 0.05$).
- 4) The iron content in the serum of rats fed with K, B, R and E diets were not significantly ($P < 0.05$). The highest level of iron in the serum was founded in the B group.
- 5) The iron content in the liver of rats fed with K, B, R and E diets were not significantly ($P < 0.05$). The highest level of iron in the liver was founded in the R group.
- 6) HDL cholesterol levels were significantly ($P < 0.05$) between rats of fed B, E with K, R, but K and R were not significantly ($P < 0.05$). LDL cholesterol levels of rats fed K and E significantly ($P < 0.05$) with B and R.

In conclusion, the bran of pigmented rice varieties has, with some exceptions, greater antioxidant and free-radical scavenging activities. The results also show that several pigmented rice extracts acted as pro-oxidants in the linoleic peroxidation assay, possibly by mechanisms described for the pro-oxidant activities of α -tocopherol and ascorbic acid [16], [17]. Pigmented rice extracts scavenged superoxide anions more effectively than hydroxyl radicals.

In conclusion, together with our previous iron depletion trial, this study provides evidence for the role of iron in lipid peroxidation in humans. The susceptibility of human lipoproteins to oxidation appeared to be influenced by the presence of different types of iron within the physiological range of iron status. Our findings have important implications regarding the treatment of iron deficiency anemia.

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