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Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

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An Aqueous Extract of Black Rice Bran from the Cibeusi Variety Prevents Anemia and Hypertriglyceridemia in Rats

Enny Purwati Nurlaili[1], Mary Astuti[2], Yustinus Marsono[2] and Sri Naruki[2]
[1]Faculty of Agricultural Technology, Universitas 17 Agustus 1945 Semarang, Jawa Tengah, Indonesia
[2]Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract: Rice is a leading staple food in Southeast Asia and it is typically milled before consumption. Black rice found in Indonesia is classified as *Oryza sativa* L. The color in the grain is caused by anthocyanin pigments that give the hulled rice a dark purple color. We found that extracts from black rice bran have high levels of iron and anthocyanins. The objective of this study was to evaluate the potential of an aqueous extract of black rice bran to prevent anemia and hypertriglyceridemia *in vivo*. Anemia and hypertriglyceridemia were induced in twenty-eight three-week old male albino Wistar rats (*Rattus norvegicus*); the rats were divided into a control (C) group or 3 treatment groups of bran (B), bran extract (E), or extraction residue (R). The results showed that differences among the groups based on feed consumption, Hemoglobin Regeneration Efficiency (HRE) and the erythrocyte morphology of rats were not significant. Black rice bran aqueous extract prevented anemia and hypertriglyceridemia by increasing the hemoglobin Hb level from 7.21 to 12.96 g/dl and by reducing triglycerides from 179.29 to 56.55 mg/dl.

Key words: Anemia, black rice var. Cibeusi, bran aqueous extract, hypertriglyceridemia

INTRODUCTION

Nutrition deficiency is currently a major issue in Indonesia and is mostly caused by iron deficiency anemia (IDA) (Almatsier, 2002), for which food with a low bioavailability of iron is the main cause. Iron deficiency (ID) and iron deficiency anemia (IDA) are highly prevalent among young women and children in South and Southeast Asia (WHO, 2011).

In poor socioeconomic populations in Indonesia, the prevalence of IDA may be as high as 26.4-28.1% in school-aged children and infants (Anonymous, 2013). IDA as indicated by low blood hemoglobin (<12 μg/L), low ferritin (<10-12 μg/l), low transferrin saturation (below 15%), low serum iron content (below 50 μg/dl), high erythrocyte protoporphyrin (above 2.5 μg/g hemoglobin) and a mean corpuscular hemoglobin concentration (MCHC) below 31% (Allen *et al*., 2006). IDA impairs cognitive performance, infant and child growth, immune status and the capacity for work (WHO, 2011). Even mild-to-moderate ID without anemia may lower the capacity for work and resistance to fatigue (Haas and Brownlie, 2001; Brownlie *et al*., 2004) and impair cognition (Stoltzfus *et al*., 2001).

Trace mineral iron is an essential nutrient for humans. Unfortunately, dietary iron deficiency is the most common and widespread nutritional disorder in the world. Iron is usually divided into two types: heme and nonheme. Heme is absorbed as the stable porphyrin complex. Plant iron in food is present in the nonheme form and has more diverse properties than iron in animals. The chemical form of nonheme iron in food is closely associated with iron bioavailability. Compared with heme, the chemical form of small molecular weight nonheme iron compounds is easily altered by other dietary components. For example, phytic acid is present in cereals, rice, legumes and lentils and polyphenols, such as tannic and chlorogenic acids, are found in tea, coffee, red wine, vegetables and herbs. These dietary constituents are capable of capturing iron from the nonheme iron in plant foods and forming insoluble compounds in the intestinal lumen, resulting in the inhibition of iron absorption (Theil, 2004).

Iron deficiency anemia can lead to increased triglyceride levels and promote hypertriglyceridemia, in which chylomicrons and triglycerides are significantly increased (higher than 1000 mg/dL or even above 10,000 mg/dL) due to a mutation either in the lipoprotein lipase (LPL) *gene* critical for chylomicron and very low density lipoprotein (VLDL) metabolism or in apolipoprotein (apo) C-II, a co-factor of the gene. Fields and Lewis (1999) noted that iron deficiency may potentially disrupt essential fatty acid (EFA) metabolism due to the reduction of the activity of the enzyme stearoyl-CoA-desaturase (SCD), for which iron is a co-factor. Moreover, anemia-induced hypertriglyceridemia involves an increased VLDL mechanism, which is caused by a reduction in the synthesis of carnitine, which is critical for the transport of fatty acids into mitochondria where they are oxidized. Such a disruption can lead to a metabolic shift to glyceride synthesis, which results excessive triglycerides in the serum and tissue.

Corresponding Author: Enny Purwati Nurlaili, Faculty of Agricultural Technology, Universitas 17 Agustus 1945 Semarang, Jawa Tengah, Indonesia

To solve these problems, the amount and quality of dietary iron must be improved by means such as iron fortification, the consumption of vitamin C-containing foods that facilitate iron absorption, or other widely consumed alternative foods such as rice, which is a staple food for half of the world's population. A nutritional survey by Gregorio *et al*. (1999) reported that approximately 50% of the iron intake in the Philippines came from rice. Black rice is a far less popular variety than white rice. Indonesia has a local rice variety containing specific genes that regulate the aleuron color, endosperm and the endosperm starch composition. Anthocyanin is highly produced in the aleuron and endosperm of black rice, so the grain has a dark purple color that is almost black.

A high iron content in black rice (106.97 µg/g) has been previously reported (Kaneda *et al*., 2005); black rice iron content is much higher than in white and red rice (39.40 and 53.20 µg/g, respectively) (Meng *et al*., 2005). Because iron is a critical need for red blood cell formation, it is more feasible to introduce black rice into diet than to use iron-fortified rice.

Nevertheless, despite its high iron content of 109.02 µg/g db (Kaneda *et al*., 2007) and its anthocyanin content of 10.70 mg/g (Kong and Lee, 2010), black rice bran remaining from the dehulling process has not been widely used for food due to the presence of components that inhibit absorption, such as phytic acid. Extraction has become an alternative to enhance the functional effect of the iron and anthocyanin of black rice, mainly because iron bioavailability from plant sources is reportedly low, approximately 10% and specifically, is approximately only 1% for rice. Hence, iron and anthocyanin bioavailability is expected to be higher in a black rice bran extract. Black rice bran extract has a highly potential for use to prevent anemia and hypertriglyceridemia because the iron can be more easily absorbed, can bind to hemoglobin and can function as a stearoyl-CoA-desaturase (SCD) co-factor.

Black rice reportedly contains 5.55 mg/g anthocyanin (Ono *et al*., 2003). As a flavonoid, the anthocyanin activity is determined by hydroxylation and the presence of a sugar moiety and mainly inactivates hydroxyl and peroxyl radicals, forms a complex with metal ions such as iron and inhibits metal initiation during lipid oxidation. Anthocyanins are also the most effective scavenger of reactive oxygen species, as well as oxidized lipid and is a platelet aggregation inhibitor (Ghiselli *et al*., 1998).

MATERIALS AND METHODS

Design, experimental period and location: An *in vivo* experiment was performed using male Wistar rats and a randomized post-test-only control group design. The *in vivo* experiment was conducted in January-April 2014, in several laboratories at the Faculty of Agricultural Technology of Universitas Gadjah Mada and at the Food and Nutrition of Inter-university of Universitas Gadjah Mada Yogyakarta. All protocols were approved by the Pre-Clinical Ethical Clearance Commission on Health Research Ethics Faculty of Public Health (Diponegoro University, Semarang, Indonesia No. 19.1/EC/FKM/2014).

Number and sampling of experimental animals: Twenty-eight male albino Wistar rats (*Rattus norvegicus*) aged ±3 weeks old and weighing 33-46 gram were randomly divided into 4 groups, with one normal control group (C) fed a standard feed, AIN-93M, containing anorganic iron as ferric citrate. The three treatment groups consisted of anemic and hypertriglyceridemic rats. All treatment groups were fed with AIN-93M containing ferric citrate-equivalent iron. The first treatment group (B) was supplemented with black rice bran, the second group (R) with a bran extract residue and the third group (E) with black rice bran extract.

Treatments were administered after 7 days each of pre- and post-adaptation periods. During the iron depletion period in the first stage of the experiment, twenty-eight 3-week-old male albino Wistar rats (*Rattus norvegicus*) were maintained for 5 weeks in individual cages, fed a basal diet without iron and were provided with deionized drinking water *ad libitum*. The rats became anemic as a result.

Materials and instruments: Black rice var. Cibeusi from Ciater was the principal material. Rats were also fed AIN-93G and AIN-93M standard feeds whose mineral composition was modified using AIN 76 (Table 1). Analyses were performed using a hemoglobin measurement kit, triglyceride measurement kit and deionized water.

Instruments used during the experiments were animal welfare maintenance equipment, including individual cages (45 x 35.5 x 14.5 cm), water bottles, an analytical balance, aluminum bowls and latex gloves. For biological measurements, disposable suits, laboratory glassware, an analytical balance, oven, a desiccator, a Soxhlet extractor, a water bath shaker, a muffle furnace, a UV-VIS spectrometer, a vortex mixer, a 4°C centrifuge, a rotary evaporator, a freeze dryer and an AAS (Atomic Absorption Spectrophotometer) were used. The feed composition is shown in Table 1.

Extraction of rice bran: The rice seeds were dehulled, degermed and polished in a laboratory mill and then passed through a 60-mesh sieve, which resulted in a uniform fraction of the 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.

Research stages: During the initial stage, the hemoglobin and total triglycerides in the blood plasma were measured to determine any pre-experiment abnormalities. After 7 days of adaptation, anemia was induced in all rats by

feeding them for 2-5 weeks with the AIN-93G feed that did not contain iron. Anemia was defined as Hb ≤6 g/dL (depletion period). In the repletion period, the anemic rats were randomly divided into 4 groups (n = 7) and maintained for 5 weeks in individual cages; the rats were given a basal diet (AIN-93M) without iron and deionized drinking water *ad libitum*. Iron repletion was accomplished with ferric citrate in the control, whereas the treated groups were orally administered black rice bran, the extraction residue and the bran aqueous extract.

Data processing and analysis: A completely randomized experimental design was used to collect data, which was then analyzed using Tukey's analysis with the Minitab 17 software to determine the significant differences ($\alpha = 0.05$).

RESULTS AND DISCUSSION

Rat feed consumption: AIN 93G and AIN 93M standard feed without Fe was given *ad libitum* during the 1-week adaptation and 10-week experimental periods. Organic Fe as well as bran, extract and bran extraction residue containing iron and anthocyanin were orally administered to rats in the different groups (Fig. 1). During the adaptation and experimental periods, the rats were fed with demineralized standard feed and drinking water *ad libitum*. Figure 1 shows that all treated groups had a similar feed consumption pattern and an analysis of variance ($p \leq 0.05$) confirmed that the feed consumption differences among the groups was not significant. At the seventh week, the feed consumption in all of the groups was slightly decreased, probably because of the oral administration. There was a significant increase in subsequent weeks. It has also been reported in the literature that feed consumption might be slightly decreased during an experiment due to treatment-induced stress, such as that caused by daily oral administration or blood sampling during the initial and mid parts of the experiment.

Hemoglobin regeneration efficiency (HRE): Iron bioavailability was indicated by the dietary iron absorbed and utilized by the body for physiological functions. Iron bioavailability *in vivo* can be determined using the hemoglobin regeneration efficiency (HRE) and an iron balance (Hernandez *et al*., 2003). The results are presented in Fig. 2. The HRE of the black rice bran extract treated group (E) was similar to that of the C group, indicating that black rice bran extract contained potentially bioavailable iron. However, a statistical analysis ($p \leq 0.05$) showed that HRE among groups was not significantly different, indicating that the results were determined by the diet and the treatment period.

Experimental hemoglobin (Hb) and triglyceride (TG) levels

Hemoglobin (Hb) level: The Hb level during the experiment was observed to measure pre- and post-anemia and the treatment effects in the 4 groups. During the initial period, the Hb level in all groups was approximately 11.93 g/dl. The Hb analyses of weeks 6 were conducted on anemic rats characterized by Hb levels between 6.85 to 7.30 g/dl. After treatment, the average Hb levels were 13.97 g/dl (C), 13.31 g/dl (B), 14.11 g/dl (R) and 12.96 g/dl (E), as shown in Figure 3. The Hb level was increased in all of the groups and the highest level was found in R, followed by C, B and E.

A statistical analysis of the data from week 6 showed a significant difference for the E group, compared to groups C, B and R ($p \leq 0.05$). At week 8, the C, B, R and E groups showed a significant difference ($p \leq 0.05$). The data at week 10 showed that among the C, B and R groups, B, R and E were not significantly different, whereas C and E were significantly different ($p \leq 0.05$). At 12 weeks, there were no significant differences among the groups. Changes in the Hb levels during the experiment are shown Fig. 4 and changes in the Hb levels during the initial stage, induction and intervention are shown in Fig. 5.

The correlation between the Hb level of the C group and the bran (B), black rice bran extraction residue (R) and extract (E) groups during the experiment are shown in Table 2. Changes in the Hb level cannot be distinguished from other blood profiles. The table shows that the Hb percentage changes were significantly correlated with the changes in TG. A statistical analysis using Pearson's correlation sig ($p \leq 0.01$) indicated that the Hb levels of the C, B, R and E groups were very strongly correlated.

Triglyceride (TG) level: The TG levels and the post-treatment changes in the 4 groups were observed to assess the effect of anemia in the rats. During the initial stage, the TG level was approximately 69 mg/dl for all groups. From weeks 6, the anemic rats also had hypertriglyceridemia, as indicated by a high TG level of approximately 166.02 to 179.29 mg/dl. After treatment, the TG level was reduced to 67.79 mg/dl (C), 88.00 mg/dl (B), 75.76 mg/dl (R) and 56.55 mg/dl (E) or lower in all treatment groups (Fig. 6). The lowest level was in the E group, followed by the control, R and B groups. A statistical analysis using ANOVA showed that the TG level at week 6 showed no significant differences among the C, B, R and E groups ($p \leq 0.05$). At week 8, no significant differences were found between groups C and E or B and R, but groups C and E were significantly different from groups B and R ($p \leq 0.05$). The data at week 10 showed that groups C and E were not significantly different, but groups B and R were significantly different ($p \leq 0.05$). At week 12, there were no significant differences among the groups. The triglyceride level changes during the experiment are shown in Fig. 7 and changes during the

Table 1: Composition of feed per 1000 g of modified mineral mix

Composition	C	B	R	E
Casein	200	200	200	200
L-cysteine	3	3	3	3
Corn starch	529.5	529.5	529.5	529.5
Sucrose	100	100	100	100
Cellulose	50	50	50	50
Soybean oil	70	70	70	70
Mineral mix AIN-76	35	35*	35*	35*
Vitamin mix AIN-93M	10	10	10	10
Colin bitartrate	2.5	2.5	2.5	2.5

Source: Yamagishi *et al.* (2000)
AIN-93M (Reeves *et al.*, 1993), modified with mineral mix AIN-76 (*except Ferric citrate)
C: Normal control group fed using standard feed AIN-93M containing anorganic iron as ferric citrate
B: Fed with AIN-93M and bran containing ferric citrate-equivalent iron
R: Fed with AIN-93M and bran extraction residue containing ferric citrate-equivalent iron
E: Fed with AIN-93M and black rice bran extract containing ferric citrate-equivalent iron

Table 2: Correlation of the level of hemoglobin (Hb) among the control (C), bran (B), black rice bran extract residue (R), black rice bran extract (E) groups

	Control	Bran	Residue	Extract
Control				
Pearson correlation	1	0.982**	0.981**	0.985**
Sig. (2-tailed)		0.000	0.000	0.000
N	12	12	12	12
Bran				
Pearson correlation	0.982**	1	0.997**	0.955**
Sig. (2-tailed)	0.000		0.000	0.000
N	12	12	12	12
Residue				
Pearson correlation	0.981**	0.997**	1	0.947**
Sig. (2-tailed)	0.000	0.000		0.000
N	12	12	12	12
Extract				
Pearson correlation	0.985**	0.955**	0.947**	1
Sig. (2-tailed)	0.000	0.000	0.000	
N	12	12	12	12

**Correlation is significant at the 0.01 level (2-tailed)

Table 3: Correlation of the level of triglyceride (TG) among the control (C), bran (B), black rice bran extract residue (R), black rice bran extract (E) groups

	Control	Bran	Residue	Extract
Control				
Pearson correlation	1	0.960**	0.991**	0.970**
Sig. (2-tailed)		0.000	0.000	0.000
N	12	12	12	12
Bran				
Pearson correlation	0.960**	1	0.934**	0.996**
Sig. (2-tailed)	0.000		0.000	0.000
N	12	12	12	12
Residue				
Pearson correlation	0.991**	0.934**	1	0.956**
Sig. (2-tailed)	0.000	0.000		0.000
N	12	12	12	12
Extract				
Pearson correlation	0.970**	0.996**	0.956**	1
Sig. (2-tailed)	0.000	0.000	0.000	
N	12	12	12	12

**Correlation is significant at the 0.01 level (2-tailed)

initial, induction and intervention stages are shown in Fig. 8. The changes in the TG level were also correlated with other blood profiles. Table 3 shows that changes in the TG percentage were significantly correlated with reductions in

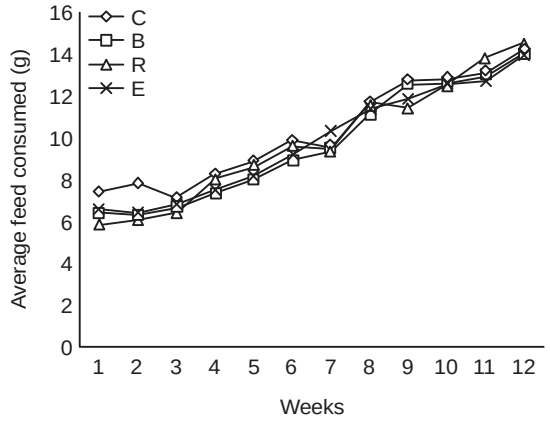

Fig. 1: Average feed consumption ratio (FCR) during the study in the control (C), bran (B), black rice bran extract residue (R) and black rice bran extract (E) groups

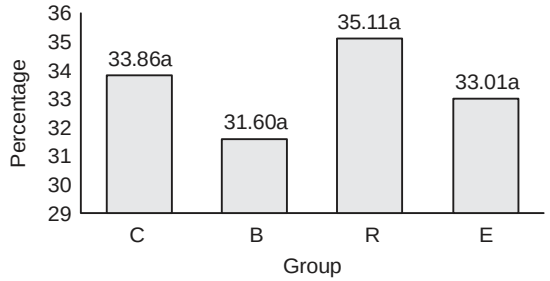

Fig. 2: Average percentage of HRE rats in the control (C), bran (B), black rice bran extract residue (R) and black rice bran extract (E) groups

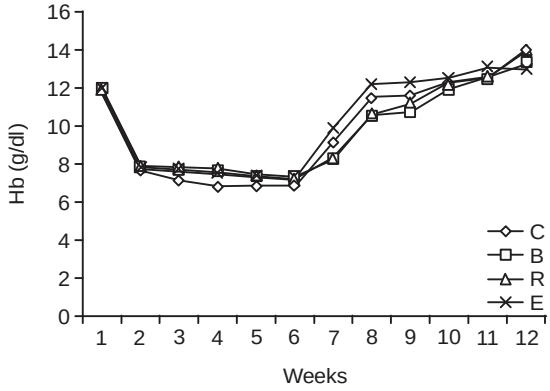

Fig. 3: Hemoglobin levels (Hb) over time in the control (C), bran (B), black rice bran extract residue (R) and black rice bran extract (E) groups

the Hb percentage. A statistical analysis using Pearson's correlation sig. ($p \leq 0.01$) indicated that the TG levels of groups C, B, R and E had a very strong correlation between samples.

Fields and Lewis (1999) noted that an iron deficiency could change essential fatty acid (EFA) metabolism due to a reduction in the activity of the enzyme stearoyl-CoA-desaturase (SCD) because the function of iron as a co-factor is disrupted. Several EFA derivatives such as arachidonic acid (C20:4 ω-6), eicosapentaenoic acid (EPA) (C20:5 ω-3) and docosahexaenoic acid (DHA) (C22:6 ω-3) derived from linoleic acid. EPA and DHA can compensate for arachidonic acid, which can cause inflammation and induce thrombosis and arthritis from the accumulation of metabolites. They also allegedly lower the hepatic production of triglycerides and apolipoprotein-β, which are the primary lipid and protein components of VLDL.

The primary anthocyanin components of black rice are cyanidin-3-glucoside and peonidin-3-glucoside. Natural anthocyanin is present in the glycoside form (i.e., bound to a sugar molecule). Several studies have mentioned that consumed anthocyanin is excreted in the urine attached to a sugar, which is an indication of a low rate of absorption. Anthocyanin is absorbed via several mechanisms, such as direct absorption by mucosal epithelial cells in its glycoside form and via hydrolysis that breaks the sugar-bond transforming it to an aglycone. Unabsorbed anthocyanin is transferred to the colon where it is fermented by the microbiota to produce phenolic compounds.

Black rice bran extract can potentially prevent anemia and hypertriglyceridemia because the iron contained in the extract can be absorbed, bind to hemoglobin and serve as a co-factor of stearoyl-CoA-desaturase (SCD). Moreover, anemia-induced hypertriglyceridemia involves the stimulation of the VLDL mechanism, which is caused by the reduced synthesis of carnitine, which is critical for the transport of fatty acids into the mitochondria where they are oxidized. Such disruption can lead to a metabolism shift to glyceride synthesis that results in excessive triglycerides in the serum and tissues. Hypertriglyceridemia caused by VLDL accumulation also increases enzyme activity.

In addition to its effects on the iron content, the flavonoid anthocyanin is also able to prevent hypertriglyceridemia. Its activities, mainly inactivating hydroxyl and peroxyl radicals, forming complexes with metal ions (e.g., iron) and inhibiting metal initiation during lipid oxidation, are determined by its hydroxylation state and the presence of the sugar moiety. Anthocyanin is also the most effective scavenger of reactive oxygen species, as well as oxidized lipid and is a platelet aggregation inhibitor. According to Mpiana *et al.* (2010), anthocyanin extract from *Justicia second* stabilized the red blood cell membrane and inhibited hemoglobin polymerization. Tedesco *et al.* (2001) reported that red wine anthocyanin prevented oxidative stress in human red blood cells. Kim *et al.* (2013) reported that iron loading is associated with altered lipid metabolism, but underlying mechanisms remain unknown. And explains the relationship between iron status and lipid

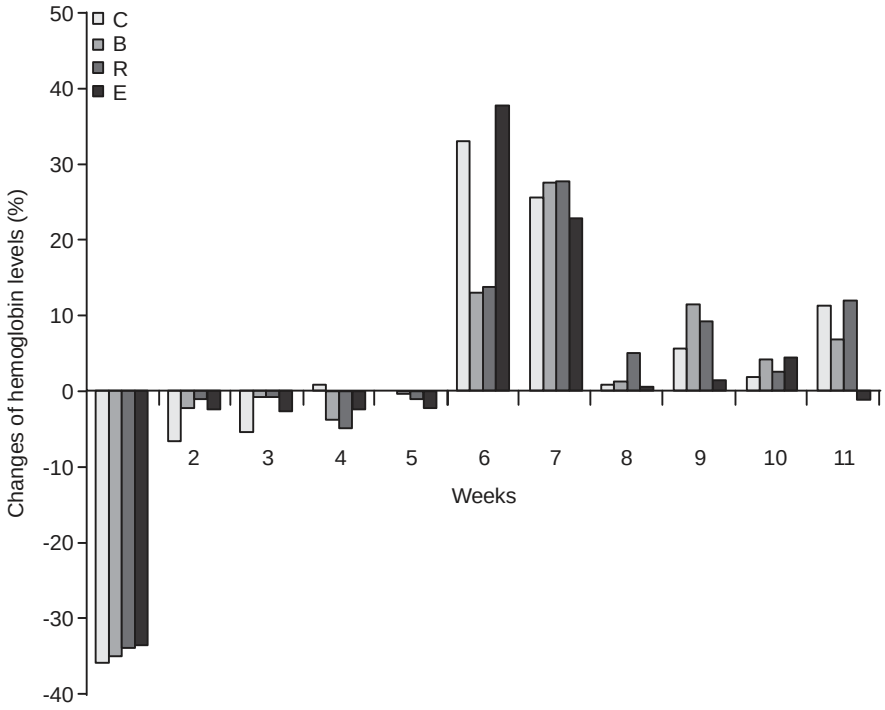

Fig. 4: Percent changes over time in the hemoglobin levels in the control (C), bran (B), black rice bran extract residue (R) and black rice bran extract (E) groups

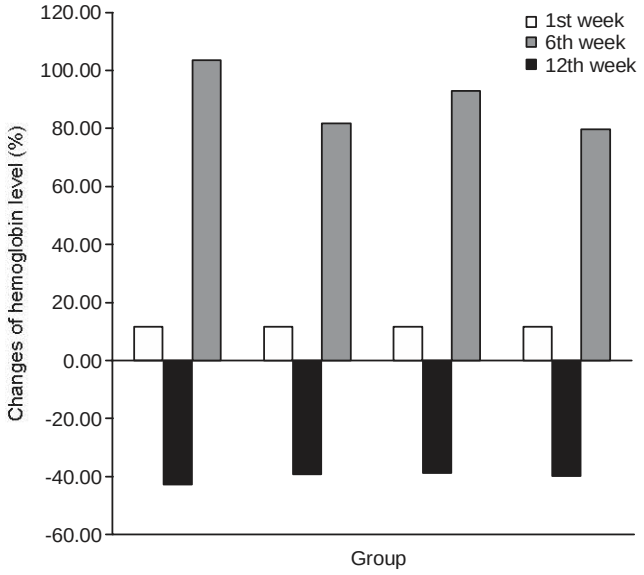

Fig. 5: Percent changes in the levels of hemoglobin at the beginning, induction and intervention periods in the control (C), bran (B), black rice bran extract residue (R) and black rice bran extract (E) groups

metabolism and provides mechanistic support for interventions that reduce serum iron levels in individuals at risk for hypertriglyceridemia. Choi *et al.* (2001) reported that severe iron deficiency anemia in girls is attended by decreased concentrations of serum total cholesterol and triglyceride and that these reduced serum lipid levels return to normal following iron supplementation.

Microscopic blood erythrocyte: Iron deficiency anemia (IDA) occurs due to low iron availability for erythropoiesis.

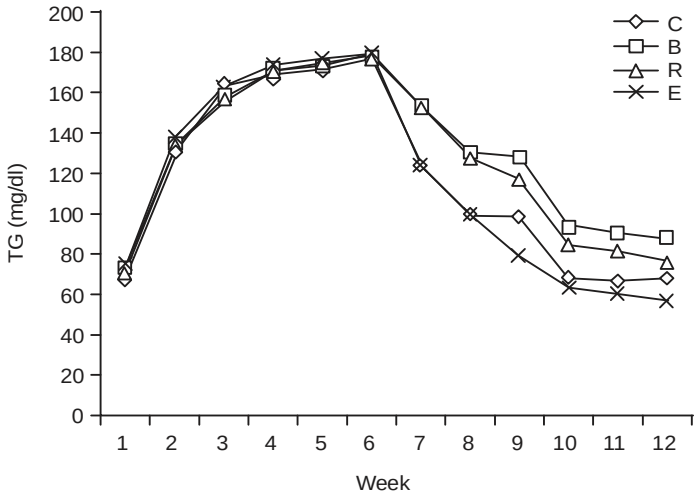

Fig. 6: Levels of triglycerides (TG) in the control (C), bran (B), black rice bran extract residue (R) and black rice bran extract (E) groups

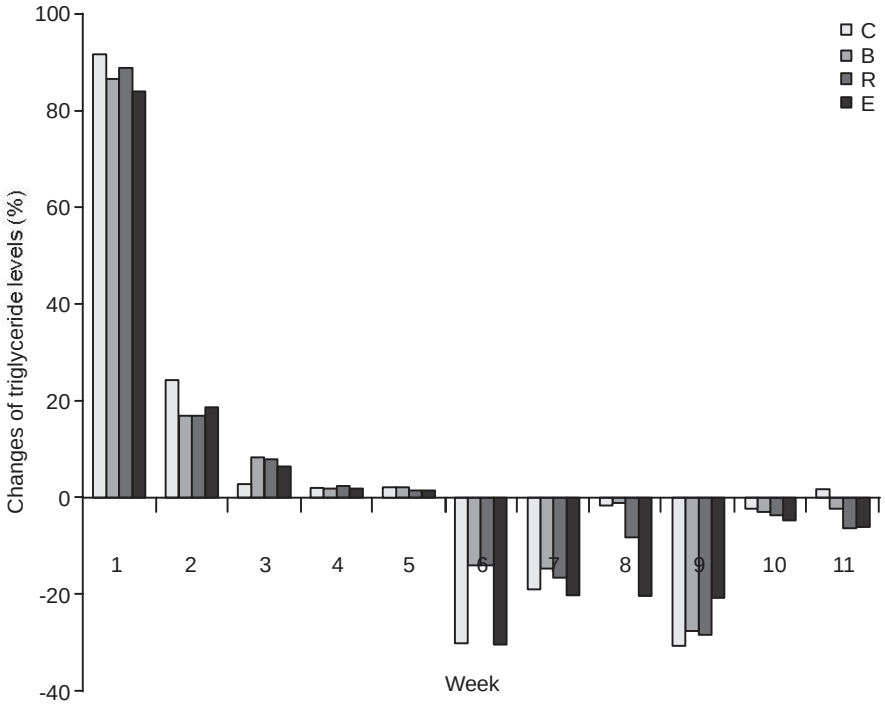

Fig. 7: Percent changes in the levels of triglycerides in the control (C), bran (B), black rice bran extract residue (R) and black rice bran extract (E) groups

A depleted store of iron leads to low hemoglobin formation. Other effects are a reduction in erythrocyte size below normal (microcytic) or an Hb content below normal (hypochromic), which result in a lower capacity of the blood to transport oxygen into the cells and tissues (Provan, 1999).

Our analyses were conducted using a peripheral blood morphology (PBM) method by dripping blood onto a slide using a capillary tube or an applicator. The blood droplet was smeared using another slide with a flat edge. After drying, it was stained using Wright or Giemsa for 15 min, washed and dried again. The assessment was conducted microscopically with 100-fold magnification. The results indicated anisocytosis and the presence of microcytes, hypochromicity and poikilocytosis were indicated in the IDA samples. Many pencil cells and target cells were found (Wu *et al*., 2002). During the initial stage of IDA, the peripheral blood morphology of erythrocytes in particular

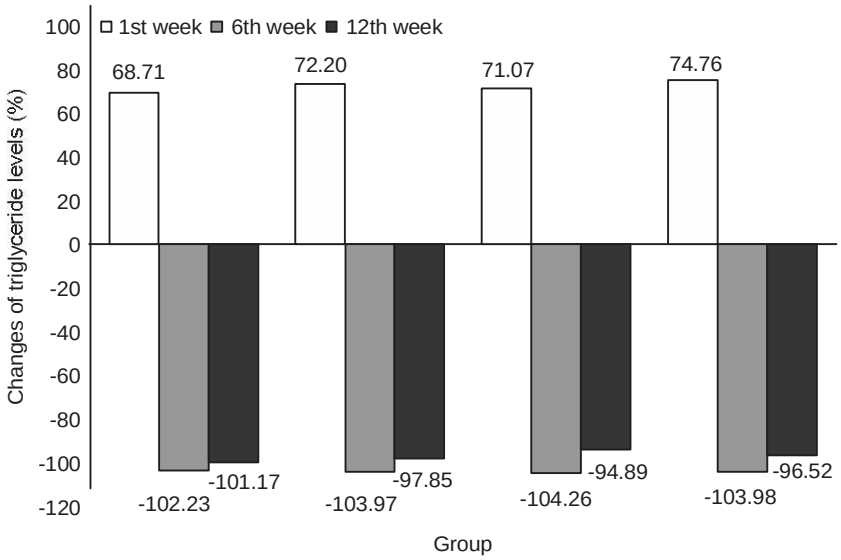

Fig. 8: Percent changes in the levels of triglycerides in the early, induction and intervention periods in the control (C), bran (B), black rice bran extract residue (R) and black rice bran extract (E) groups

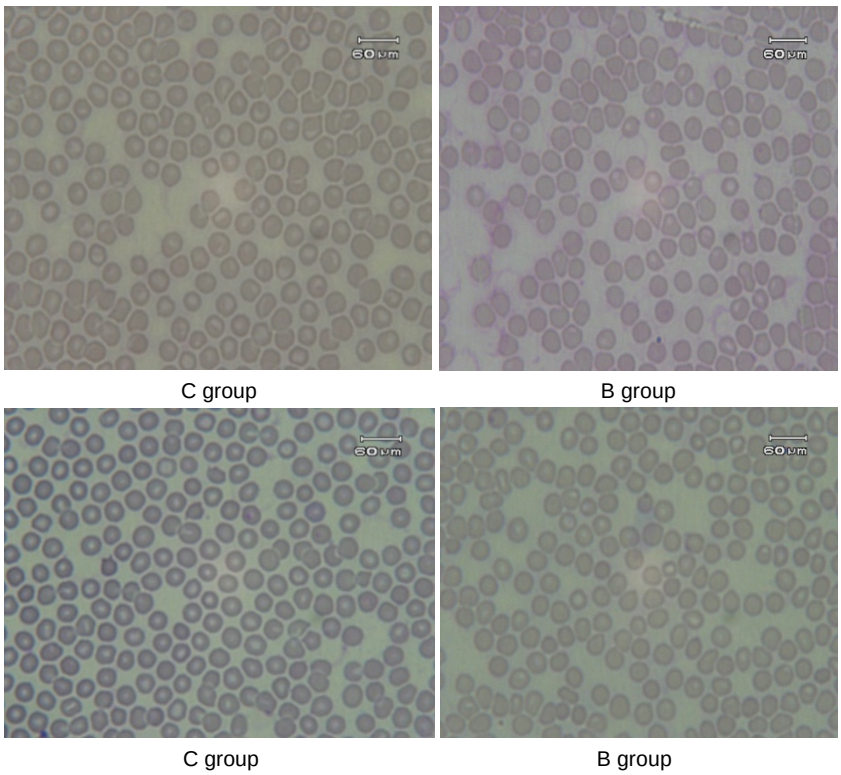

Fig. 9: Microscopic blood erythrocytes at the end of the study in the control (C), bran (B), black rice bran extract residue (R) and black rice bran extract (E) groups

were normocytic and normochromic. Observation of the blood smear preparation was conducted in the final stage of the experiment to assess blood erythrocyte images and are shown in Fig. 9. The images show that the erythrocyte morphology of rats in the C, B, R and E groups was normally biconcave. An ANOVA ($p \leq 0.05$) indicated no significant differences between control and the treated groups, which indicated that the numbers and morphology of the erythrocytes began to return to normal. The erythrocytes of rats fed black rice bran extract had a better

blood smear form, without crenation (acanthocytes) and with a higher amount of normal erythrocytes.

Conclusions: Black rice bran extract prevented anemia and hypertriglyceridemia. An aqueous extract of black rice bran prevented anemia and hypertriglyceridemia as indicated by an increase in the Hb level from 7.21 g/dl to 12.96 and a reduction in triglycerides from 179.29 to 56.55 mg/dl.

The consumption of black rice bran extract has recently become increasingly popular because of its higher iron and antioxidant content (anthocyanin) compared to white rice.

REFERENCES

Allen, L., B.D. Benoist, O. Dary and R. Hurrell, (ed.), 2006. Guidelines on food fortification with micronutrients. World Health Organization (WHO) and Food and Agricultural Organization of the United Nations.

Almatsier, S., 2002. Prinsip Dasar Ilmu Gizi. Gramedia Pustaka Utama. Jakarta.

Anonymous, 2013. Basic Health Research. Indonesia Ministry of Health. Jakarta. Indonesia.

Brownlie, T.T., V. Utermohlen, P.S. Hinton and J.D. Haas, 2004. Tissue iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women. Am. J. Clin. Nutr., 79: 437-443.

Choi, J.W., S.K. Kim and S.H. Pai, 2001. Changes in Serum Lipid Concentrations during Iron Depletion and after Iron Supplementation. Ann. Clin. Laboratory Sci., 31: 2.

Fields, M. and C.G. Lewis, 1999. Cholesterol-lowering nature of unsaturated fat in rats may be due to its inability to increase hepatic iron. Metab., 48: 200-204.

Ghiselli, A., M. Nardini, A. Baldi and C. Scaccini, 1998. Antioxidant activity of different phenolic fractions separated from an italian red wine. J. Agric. Food Chem., 46: 361-367.

Gregorio, G.B., D. Senadhira, T. Hunt and R.D. Graham, 1999. Improving iron and zinc value of rice for human nutrients. Agric. Dev., 23: 68-87.

Haas, J.D. and T.T. Brownlie, 2001. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. J. Nutr., 131: 676S- 88S; discussion 688S-90S.

Hernandez, M., V. Sousa., A. Moreno., S. Villapando and M. Lopez-Alarco, 2003. Iron bioavailability and utilization in rats are lower from lime-treated corn flour than from wheat flour when they are fortified with different sources of iron. The J. Nutr., 133: 154-159.

Kaneda, I., H. Yasui, Y. Adachi, J. Takada and H. Sakurai, 2005. Determination of trace element concentrations in ancient rice (red and black rices) and a Present-day Rice (Koshihikari): Relationship Among the Trace Element Concentrations, Species, Harvest Site and Rice Parts. Biomed. Res. Trace Elements, 16: 241-249.

Kaneda, I., F. Kubo and Sakurai, 2007. Nutrition: Relationship between trace metal concentration and antioxidative activity of ancient rice bran (red and black rice) and a present-day rice bran (Koshihikari). J. Trace Element Med. Biol., 21: 45-51.

Kim, J., X. Jia, P.D. Buckett, S. Liu, Chih-Hao Lee and M. Wessling-Resnick, 2013. Iron loading impairs lipoprotein lipase activity and promotes hypertriglyceridemia. FASEB J., 27: 1657-1663.

Kong, S. and J. Lee, 2010. Short communication: Antioxidants in milling fractions of black rice cultivars. Food Chem., 120: 278-281.

Meng, F., Y. Wei and X. Yang, 2005. Iron content and bioavailability in rice. J. Trace Element Med. Biol., 18: 333-338.

Mpiana, P.T., K.N.N. Ngbolua, M.T. Bogota, T.K. Kasonga, E.K. Abu, D.S.T. Tshibangu and V. Modogo, 2010. *In vitro* Effects of anthocyanin extracts from justicia second vahl on the solubility of hemoglobin S and membrane stability of sickle erythrocytes. Blood Transfus, 8: 248-254.

Ono, K., N. Sugihara, Y. Hirose and K. Katagiri, 2003. An Examination of optimal extraction solvents for anthocyanin pigments from black rice produced in gifu.

Provan, D., 1999. Mechanism and management of iron deficiency anemia. Br. J. Haematol., 105: 19-26.

Reeves, P.G., F.H. Neilsn and G.C. Fahey Jr, 1993. Purified Diet for Laboratory Rodents. J. Nutr., 123: 1939-1951.

Stoltzfus, R.J., J.D. Kvalsvig and H.M. Chwaya, 2001. Effects of iron supplementation and anthelmintic treatment on motor and language development of preschool children in Zanzibar: double blind, placebo controlled study. BMJ., 323: 1389 - 1393.

Tedesco, I., G.L. Russo, F. Nazzaro, M. Russo and R. Palumbo, 2001. Antioxidant effect of red wine anthocyanins in normal and catalase-inactive human erythrocytes. J. Nutr. Biochem., 12: 505-511.

Theil, E.C., 2004. Iron, ferritin and nutrition, Annu. Rev. Nutr., 24: 327-343.

World Health Organization, 2011. Iron deficiency anemia: assessment prevention and control a guide for program managers. NHD01.3 ed. Geneva: WHO.

Wu, A.C., L. Lesperance and H. Bernstein, 2002. Screening for iron deficiency. Pediatrics in the review. 23: 171-178.

Yamagishi, H., H. Okazaki, M. Shimizu, T. Izawa and Komabayashi, 2000. Relationship among serum triacylglycerol, fat pad weight and lipolysis in iron-deficient rats. J. Nutr. Biochem., 11: 455-460.