

The effect of giving moringa tempeh flour on hemoglobin levels and body weight of Wistar Rats

Pengaruh pemberian tepung tempe kelor terhadap kadar hemoglobin dan berat badan Tikus Wistar

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Abstract

Background: Tempeh is a fermented product that has earned the nickname "the miracle food" because of its nutritional content, especially amino acids and fatty acids, which are good for health. Moringa leaves, which are also called "the miracle leaves," have complete nutritional content, especially iron, and vitamin C. Vitamin C plays a significant role in the absorption and metabolism of iron

Objectives: Was to increase absorption of iron, which can increase hemoglobin blood and body weight of rats

Methods: There were 4 groups, each consisting of 6 rats. The rats were kept in animal laboratories at room temperature (25–27°C) for 21 days. This research was conducted from 7 to 27 October 2020 in the Pharmacology Laboratory of Medicine, Faculty of Medicine Universitas Muhammadiyah Malang. This study used a Completely Randomized Design, used 24 *Rattus norvegicus* with treatment conditioned with anemia. The four groups were divided into control anemia rats, control non-anemia rats, anemia rats that consumed tempeh, and anemia rats that consumed tempeh with moringa leaves of 4%. The rats body weight was weighed daily and the blood Hb was taken at the end of the treatment. Statistical test used One-way Analysis of Variance ($\alpha = 0,01$).

Results: The results of statistical tests with ANOVA show not significant influence. The results of the hemoglobin and body weight rats had no significant effect.

Conclusion: The value was not significant between groups in the hemoglobin assessment, but the treatment group that was given moringa leaves tempeh was higher than the anemia control group and the pure tempeh group.

Kata kunci

Body weight, hemoglobin, fermented soybean, moringa leaves, functional food

Abstrak

Latar belakang: Tempe merupakan produk fermentasi yang mendapat julukan "makanan ajaib" karena kandungan gizinya, terutama asam amino dan asam lemak, yang baik untuk kesehatan. Daun kelor yang juga disebut "daun ajaib" memiliki kandungan gizi yang lengkap terutama zat besi dan vitamin C. Vitamin C berperan penting dalam penyerapan dan metabolisme zat besi.

Tujuan: Penelitian bertujuan untuk meningkatkan penyerapan zat besi, yang dapat meningkatkan hemoglobin darah dan berat badan tikus

Metode: Terdapat 4 kelompok, masing-masing terdiri dari 6 ekor tikus. Tikus-tikus tersebut dipelihara di laboratorium hewan pada suhu kamar (25-27°C) selama 21 hari. Penelitian ini dilakukan pada tanggal 7 sampai 27 Oktober 2020 di Laboratorium Farmakologi Fakultas Kedokteran Universitas Muhammadiyah Malang. Penelitian ini menggunakan Rancangan Acak Lengkap, menggunakan 24 ekor *Rattus norvegicus* dengan perlakuan yang dikondisikan anemia. Empat kelompok tersebut dibagi menjadi tikus kontrol anemia, tikus kontrol tidak anemia, tikus anemia yang mengkonsumsi tempe, dan tikus anemia yang mengkonsumsi tempe dengan daun kelor 4%. Berat badan tikus ditimbang setiap hari dan diambil kadar Hb darahnya pada akhir perlakuan. Uji statistik yang digunakan adalah Analisis Varian Satu Arah ($\alpha = 0,01$).

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Hasil: Hasil uji statistik dengan ANOVA menunjukkan pengaruh yang tidak signifikan. Hasil kadar hemoglobin dan berat badan tikus tidak berpengaruh signifikan.

Kesimpulan: Nilai yang tidak signifikan antar kelompok pada penilaian hemoglobin, namun kelompok perlakuan yang diberi tempe daun kelor lebih tinggi dibandingkan dengan kelompok kontrol anemia dan kelompok tempe murni.

Kata kunci

Berat badan, hemoglobin, kedelai fermentasi, daun kelor, pangan fungsional

Introduction

Anemia is a condition of the body with a lower hemoglobin (Hb) level than the normal one (11 mg/dL). (Kemenkes RI, 2018) shows a significant rise in the prevalence of anemia in pregnant women. In 2013, 37,1 % of pregnant women experienced anemia. The number increased to 48,9 % in 2017. Anemia is caused by the lack of nutrition essential to form hemoglobin. Anemia in pregnant women is caused by several factors, such as enhancement of the need for iron (Fe) because of fetal growth and lack of nutrition that can help the absorption of iron, like a food full of fiber, high in animal protein, and high in vitamin C. One of the prevention is to increase the consumption of food as a source of iron, protein, and vitamin C. Tempeh is one of the staple foods in Indonesia and is made from soybeans as a protein source.

Tempeh is a fermented food made from soybeans and uses several molds such as *Rhizopus oligosporus*, *Rhizopus oryzae*, *Rhizopus stolonifer* (bread mold), or *Rhizopus arrhizus*. All of these molds are called "tempeh yeast." The molds that grow on the soybean will hydrolyze the complex compounds into simple compounds easily digested by humans. Tempeh is rich in fiber, calcium, vitamin B, and iron (Winarno FG et al., 2017). One hundred grams of tempeh contain 18,3 grams of protein, 10 mg of iron, and 0 mg of vitamin C. Moringa leaves contain 27% of protein and are rich in vitamin A, vitamin C, calcium, iron, fosfor, and zinc. Moringa leaves are also a source of antioxidants such as flavonoids, ascorbic acids, carotenoids, and phenol (Krisnadi, 2015).

According to Abdullah's et al, there is a relationship significant between weight gain and giving cookies with addition of moringa leaf powder. Based on the research results it can be concluded that there is a significant difference in the average increase in body weight under five which means there is an effect of giving cookies with the addition of leaf flour moringa in undernourished toddlers. Sarmana et al found that

the effect of giving tempeh biscuits on change in body weight in toddlers. Moringa leaf extract is effective for increasing hemoglobin levels in young women (Priyas Hastuti & Novita Sari, 2022). Tempeh has a sufficient nutritional value of iron, vitamin B12, and folic acid, so that tempeh has the potential to increase hemoglobin levels in anemia sufferers (Pinasti et al., 2020).

According to the research, adding 2% and 4% of moringa leaves flour produces tempeh with the same hifa, greenish, and normal taste. There is a significant increase between tempeh without the moringa leaves flour and tempeh with 4% moringa leaves flour, with 0,84% of vitamin C (Suprihartini, et. al., 2020). Advanced research is needed to find out the level of hemoglobin and the weight of the rats, if fed the tempeh with moringa leaves flour.

Methods

This research aims to know the effect of adding moringa leaves to tempeh on the hemoglobin level and Wistar Rats' weight. The research is held in the Pharmacology Laboratory of Medicine at Muhammadiyah University Malang. The research uses an experiment.

There are the hemoglobin levels and the rats' weight as dependent variables. The independent variable is tempeh with moringa leaves flour. This research uses Completely Randomized Design (CRD) with 24 experimental rats (*Rattus norvegicus*) divided into four groups. The inclusion criteria for the chosen rats as a sample are they one which is healthy, around 150-200 grams, and 7 weeks old before the intervention. The exclusion criteria are weight loss of more than 10% while intervention and looking sick. The four groups are given intervention in 7 days and three of them are conditioned to have anemia. These are the following stages of intervention:

1. Prepare the pure tempeh flour and 4% moringa leaves flour
2. Ethical trial on the experimental rats

3. Conditioning the rats in the laboratory. There are four groups and each of them consists of 6 rats. The groupings are the normal rats with the standard feed, anemia rats with the standard feed, anemia rats fed with tempeh, and anemia rats fed with 4% moringa leave tempeh. The indication of sodium nitrite to condition anemic rats was given a dose of 25 mg/200 grams of the weight.
4. The dose of the tempeh flour to the rats is given 4 ml/rat/day, with a concentration of 50% solution filtered. The 4 ml of water is taken and administered orally through a probe to the stomach of the rat. The intervention was given for 21 days.

The hemoglobin was measured on the 21st day with a spectrophotometer. The weight change of a rat was measured every week. This research have ethical approval from research ethic committee Nutrition Academy of Karya Husada Kediri 025/EC/AKZI/KH/III/2020.

Results

Hemoglobin

The measurement of the hemoglobin level (g/dl) in the rats was done on the 21st day. The result of the measurement is shown in tabel 1 below.

Tabel 1. Tabel of Hemoglobin (g/dl)

	A	B	C	D
Rat 1	13,90	19,55	15,74	15,82
Rat 2	17,55	16,17	17,21	16,44
Rat 3	16,94	17,24	15,00	16,74
Rat 4	14,74	17,48	14,40	17,63
Rat 5	Dead	16,36	15,32	17,21
Rat 6	Dead	15,28	15,51	16,28
Average	15,78	17,01	15,53	16,69

Note :

A : Anemia group

B : Normal group

C : Tempeh treatment group

D : Tempeh moringa treatment group

The highest average of hemoglobin is in the normal group with standard feed. The lowest average of hemoglobin is in the anemia rats group with tempeh flour feed. The statistical result using ANOVA showed 0,169 ($\alpha > 0,05$), it means there are not any significant differences between the treatments.

Weight

The weight was measured every week. The average measurement of the weight every week is shown in tabel 2 below.

Tabel 2. Rat Weight During Treatment

	A	B	C	D
1 st week	158,83	153,00	162,66	171,00
2 nd week	163,66	163,50	172,00	181,16
3 rd week	184,50	182,66	183,50	192,50

Tabel 3. Rat Weight Gain During Treatment

	A	B	C	D
1 st week	4,83	10,50	9,33	10,17
2 nd week	16,33	19,66	17,08	16,00
3 rd week	25,67	28,67	20,83	21,50
Average/day	1,22	1,37	0,99	1,02

Note :

A : Anemia group

B : Normal group

C : Tempeh treatment group

D : Tempeh moringa treatment group

The average weight gain a day in the positive control group = was 1,22 grams, the negative control group = was 1,36 grams, the anemia groups fed with tempeh = 0,99 grams, and the anemia groups with tempeh moringa flour = 1,02 grams. Statistical tests with ANOVA showed 0,430 ($\alpha > 0,05$), it means there are not any significant differences between the treatments.

Discussion

Haemoglobin

This research assumed that the addition of moringa leaves flour in the fermentation process may increase the level of hemoglobin in the anemia rats. Moringa leaves flour is a source of non-heme iron and vitamin C. The usage of moringa leaves flour in tempeh is aimed to know the effectiveness to increase the rats' hemoglobin levels. Before collecting the data, the rats are conditioned to have anemia. The treatment used natrium nitrit (NaNO). The usage of NaNO will affect the eritrosit's ability to bring the O₂ which will cause anemia (Widyastuti, 2014).

The highest hemoglobin average is in the non-anemia group with standard feed. The composition of the feed is use the BR 1 consisting of cornstarch, bran, soybean meal, fish flour, palm

oil, mineral mix, amino acids lysine, and methionine. One of the factors is that tempeh is a source of vegetable protein, a group of non-heme iron that has a smaller absorption capacity than heme iron from vegetable protein hemoglobin. Although nonheme iron absorption is strongly influenced by dietary factors, dietary components have little effect on heme iron bioavailability. Plant products that are sources of nonheme iron contain many iron inhibitors such as phytic acid, calcium, and polyphenols (Skolmowska & Głąbska, 2019). There is an effect of giving heme iron and nonheme iron on a daily diet on anemia girls' hemoglobin levels (Rohimah & Haryati, 2014)

The graphic of this research shows there is a higher hemoglobin level in the anemia group which is fed by moringa leaves flour tempeh than others. This is also shown that there is a synergy between micronutrients Fe and Vitamin C, as well as bioactive compounds contained in moringa leaves able to increase the level of hemoglobin in experimental rats which were previously given NaNO_2 . Moringa leaves can be consumed as foodstuffs or vegetables which can increase the level of hemoglobin in people with anemia (Tinna, 2018). Anemia caused by a lack of iron is also called nutritional anemia. Iron is working to form the hemoglobin and vitamin C that affect the absorption and redemption of iron in body tissue. (Dodik Briawan, et al., 2009) put forward that the usage of blood boost tablets still is one of anemia prevention. Therefore, the public is encouraged to fulfill their needs by consuming food high in iron and vitamin from animal and plant sources such as moringa, spinach, and kale.

The occurrence of an increase in Hb between the treatment of pure tempeh and moringa leaves tempeh was due to an increase of iron (Fe) levels in moringa leaves flour in tempeh (Suprihartini et al., 2020). There was a significant increase in vitamin C levels between pure tempeh and moringa leaves tempeh, around 0,84% (Suprihartini et al., 2021). Vitamin C is playing a role to increase the absorption of iron, including heme iron and non-heme iron. Iron absorption enhancers are dominated by the effects of ascorbic acid (vitamin C), which overcomes the effects of all dietary inhibitors when included in a diet rich in nonheme iron availability (Thomas Ems., et al., 2023). There was a significant increase of hemoglobin levels in the treatment which was given moringa leaves extract (*Moringa oleifera*)

and also an addition of vitamin C in the anemia rats. Ascorbic acid or vitamin C has been reported to enhance the absorption and bioavailability of ferrous sulfate preparations in rats. Vitamin C reduces and catalyzes iron absorption inhibitors, thus increasing non-heme iron absorption (Hermayanti et al., 2020)

Statistical test result using ANOVA shows there is no significant difference in hemoglobin levels. The same thing is also shown in the research there is also no significant difference in the rats given 0.18 g/head, 0.36 g/head, and 0.72 g/head doses of moringa leaf extract (Retnaningsih & Safitri, 2021). The standard food of rats contains heme iron from fish flour, whereas tempeh has been fermented. The raw soybean still contains anti-nutrients that inhibit iron absorption such as trypsin inhibitor and phytic acid, and moringa leaves also contain phytic acid. Inhibitors of iron absorption include phytate, a compound found in plant-based diets that has a dose-dependent effect on iron absorption. Polyphenols, found in black and herbal teas, coffee, wine, legumes, cereals, fruits and vegetables, have been shown to interfere with iron absorption (Thomas Ems., et al., 2023). The phytic acid is lost during soaking, cooking, and fermentation, phytic acid is reduced by 22% so that the absorption of iron from tempeh can increase compared to soybeans (Winarno, 2017).

Weight

In this research, there is no difference in weight between the groups from the first week until the third week. According to the graphic, there is an increase in weight in all groups of rats. This signifies that the experimental rats have good health and good appetite. Appetite is affected by environmental conditions, health, and the food given. During the research, the cage was cleaned three times a day, changed the husk, was fed, and drink at once. The portion of each time. The portion of feed given is 20 g/rat, and drinks are given ad libitum (always available at any time). The results of this study indicate that the provision of steeping moringa tempeh does not have a significant effect on the body weight of Wistar rats.

The average increase in body weight of Wistar rats during the treatment was highest in the group of non-anemic rats with standard feed containing fish meal and oil. Fats and oils have the highest energy content among others, 9 kcal/gram

(Winarno, 2017). Statistical test result using ANOVA shows there is no significant difference in the rats' weight between the treatment of standard feed and feed with tempeh flour. This was because the tempeh had been fermented. During fermentation, chemical and biochemical changes occur in macro compounds (protein, carbohydrates, and fats). These changes lead to an increase in bioavailability. Proteins are hydrolyzed by proteases into peptides and amino acids. Some insoluble carbohydrates also undergo changes to simple components, fats undergo hydrolysis of fatty acids (Winarno, 2017). Changing nutrients into simpler compounds will increase digestibility. Thus, there was no significant difference between the groups of rats fed a standard diet containing animal protein and rats fed tempeh.

During the fermentation process, the anti-nutritional compounds in soybean are decomposed, including trypsin inhibitors. Trypsin inhibitors are anti-nutritional compounds that inhibit the action of trypsin. Trypsin is an enzyme that plays a role in protein metabolism, which breaks down protein into simpler compounds so that it can be absorbed easily in the small intestine. The effect of the fermentation of tempeh was Increased bioavailability and activity of crude and soluble proteins, minerals, antioxidants, crude fiber and ash levels. Additional vitamin B12 content. Decreased anti-nutrient levels (phytate, trypsin inhibitors, oxalates, oligosaccharides) (Ahnan-Winarno et al., 2021).

Conclusion

The value was not significant between groups in the hemoglobin assessment, but the treatment group that given moringa leaves tempeh was higher than the anemia control group and the pure tempeh group. This indicates that the treatment with moringa tempeh flour was able to prevent a decrease of hemoglobin but still couldn't be in line with the normal group. This study also proved that the provision of moringa tempeh flour had no significant effect on weight between the groups.

It is recommended to determine the addition of high Fe and vitamin C from natural ingredients with good bioavailability to get the right formula.

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References

- Ahnan-Winarno, A. D., Cordeiro, L., Winarno, F. G., Gibbons, J., & Xiao, H. (2021). Tempeh: A Semicentennial Review on its Health Benefits, Fermentation, Safety, Processing, Sustainability, and Affordability. *Comprehensive Reviews in Food Science and Food Safety*, 20(2), 1717–1767. <https://doi.org/10.1111/1541-4337.12710>
- Dodik Briawan; Ari Adriyani; Pusporini. (2009). Determinan Keberhasilan Program Suplementasi Zat Besi pada Siswi Sekolah. *Jurnal Gizi Klinik Indonesia*, 6(2), 78–83.
- Hermayanti, D., Setiawan, W., & Azhar, R. M. (2020). The Effect of Moringa Leaf (*Moringa oleifera* Lam) and Vitamin C Combination Extract in Improving Anemia Indicators Deficiency of White Rice Iron Male Diet Low Rice Iron. *Jurnal Saintika Medika*, 16(2), 104–114.
- Kemendes RI. (2018). *Riset Kesehatan Dasar 2018*. Badan Penelitian dan Pengembangan Kesehatan. https://kesmas.kemkes.go.id/assets/upload/dir_519d41d8cd98f00/files/Hasil-risikesdas-2018_1274.pdf
- Krisnadi, A. D. (2015). *Kelor Super Nutrisi*. Blora: Moringa Indonesia. <http://kelorina.com>
- Pinasti, L., Nugraheni, Z., & Wiboworini, B. (2020). Potensi Tempe Sebagai Pangan Fungsional dalam Meningkatkan Kadar Hemoglobin Remaja Penderita Anemia. *Action: Aceh Nutrition Journal*, 5(1), 19. <https://doi.org/10.30867/action.v5i1.192>
- Priyas Hastuti, A., & Novita Sari, A. (2022). Pengaruh Teh Daun Kelor (*Moringa oleifera* L) Terhadap Peningkatan Kadar Hemoglobin Penderita Anemia. *Avicenna: Journal of Health Research*, 5(1), 27–36. <https://doi.org/10.36419/avicenna.v5i1.590>

- Retnaningsih, R., & Safitri, R. (2021). Role of Moringa Oleifera Leaf Extract in Increasing Hemoglobin Levels in Pregnant Rats With Anemia. *Journal of Health Sciences*, 14(1), 8–13. <https://doi.org/10.33086/jhs.v14i1.1296>
- Rohimah, Y. T., & Haryati, D. S. (2014). Pengaruh Pemberian Zat Besi Hem dan Non Hem pada Diet Harian Terhadap Kadar Hemoglobin Remaja Putri yang Mengalami Anemia. *Jurnal Terpadu Ilmu Kesehatan*, 3(2), 150–154.
- Skolmowska, D., & Głabka, D. (2019). Analysis of heme and non-heme iron intake and iron dietary sources in adolescent menstruating females in a national Polish sample. *Nutrients*, 11(5). <https://doi.org/10.3390/nu11051049>
- Suprihartini, C., Ulilalbab, A., & Budiman, F. A. (2021). Efek Penambahan Tepung Daun Kelor pada Fermentasi Tempe Terhadap Kadar Vitamin C dan N-amino Tempe Kelor (Pelor). *Jurnal ILKES*, 12(1), 369–374. <http://ilkeskh.org/index.php/ilkes/article/view/204>
- Suprihartini, C., Ulilalbab, A., Budiman, F. A., & Puspitasari, U. L. (2020). Zat Besi Tempe Kedelai Daun Kelor, Kadar Air dan Daya Terima Organoleptik Keripik Tempe Daun Kelor. *WEBINAR NASIONAL STKIP PGRI JOMBANG : “Bangkit Dari Pandemi Menuju Hasil Penelitian Dan Pengabdian Yang Berdampak,” September*, 594–604.
- Thomas Ems, Kayla St Lucia, M. R. H. (2023). *Biochemistry, Iron Absorption*. StatPearls Publishing.
- Tinna, I. (2018). *Pengaruh Pemberian Tepung Daun Kelor (Moringa oleifera Leaves) Terhadap Peningkatan Kadar Hemoglobin Eritrosit pada Ibu Hamil Anemia*. Universitas Hasanuddin.
- Widyastuti, D. A. (2014). Blood Profile of Wistar Rats due to Subchronic Condition Caused by Sodium Nitrite. *Jurnal Sain Veteriner*, 31(2), 201–215. <https://doi.org/10.22146/jsv.3810>
- Winarno, F. (2017). *Kimia Pangan dan Gizi*. M-Brio Press.
- Winarno FG, Winarno Wida, & Driando, A. W. (2017). *Tempe: kumpulan fakta menarik berdasarkan penelitian*. PT. Gramedia Pustaka Utama.