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Original Article

Profile Hemoglobin and Ferritin of Rattus Wistar with Iron Deficiency Anemia After Consumption of a Snack Bar from Cowpea Flour (*Vigna Unguiculata*) And Haruan Fish (*Ophicephalus Melanopterus*)

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ABSTRACT

Background: In 2019, the prevalence of anemia in children under 5 years of age was 39.8%, while the prevalence of anemia in women of childbearing age was 29.9%, and in pregnant women of childbearing age, the prevalence of anemia ranged from 34.0% to 39.1%. The purpose of this research is to investigate the hemoglobin and ferritin of rattus wistar with iron deficiency anemia after consumption snack bar from cowpea flour and haruan fish. **Methods:** Pre-posttest group design was used in this experiment, consisting of 2 groups. Groups P0 was given commercial feed for 30 days dan group P1 was given snack bar from cowpea flour and haruan fish for 30 days. Before to treatment, all rattus were made into iron deficiency anemia by given commercial feed free iron. **Results:** The results this study increased the high hemoglobin levels ranged from 2.83 to 3.35 g/dl and ferritin levels ranged from 30.61 to 37.45 μg/l in the rattus was given snack bar from cowpea flour and haruan fish in comparison with the rattus was given commercial feed that hemoglobin levels ranged from 0.22 to 0.48 g/dl and ferritin levels ranged from 0.55 to 5.98 μg/l. **Conclusions:** The hemoglobin and ferrin profiles in Wistar rattus increased after receiving a snack bar of cowpea flour and haruan fish.

Key words: Cowpea flour, Haruan fish, Snack bar, Hemoglobin levels, Ferritin levels.

INTRODUCTION

Anemia impairs children's cognitive and motor development and diminishes adults' job capacities, which in turn has an impact on a nation's economy.[1,2] In 2019, the prevalence of anemia in children under 5 years of age was 39.8%, while the prevalence of anemia in women of childbearing age was 29.9%, and in pregnant women of childbearing age, the prevalence of anemia ranged from 34.0% to 39.1%.[3] This condition demonstrates that anemia prevalence in pregnant women falls into the intermediate group, which comprises a 20–40% range. Developmental issues can result from anemia in pregnant women because it can induce early labor, low birth weight kids, and diminished iron reserves in the infants.[4]

Anemia is most often caused by an iron shortage. A diet deficient in iron, a problem with the intestine's ability to absorb iron, or significant blood loss from things like severe monthly flow or internal bleeding can all contribute to iron deficiency.[5-7] Studies on the prevalence of anemia and iron profiles in children and adolescents with low socioeconomic status have shown the prevalence of anemia caused by iron deficiency, with the results indicating that the prevalence of anemia, iron deficiency, and iron depletion is higher in the adolescent group compared to children's.[8] Several other studies have also shown that food fortification with various micronutrients is beneficial; one of the fortified micronutrients is iron, and this study shows the results of effective interventions to increase hemoglobin concentration and reduce the risk of anemia.[9]

Iron consumption is important enough to prevent iron deficiency and iron deficiency anemia. If a person is deficient in iron in his diet, then the iron stores in the body, which are mainly represented by ferritin levels, can be reduced. Conversely, excessive consumption of iron from food or supplements can also lead to increased levels of ferritin in the blood, which can indicate iron overload.[7]

The main problem with the utilization of iron by the body is the low absorption in the intestine. Iron absorption is influenced by two factors, namely absorption of heme iron and non-heme which indicate the presence of two different types of iron in food. Sources of heme in human food are meat, fish and poultry, while non-heme sources are cereals, nuts, vegetables and fruit.[10]

Snack bar is a practical form of distraction, with complete nutritional value and includes food that is durable to store. The basic ingredient is Cowpea flour. Cowpea flour has very low gluten levels, low sodium levels, and high amounts of protein, fat, fiber, high in Fe content and carbohydrate content that is easy to digest.[11] However, cowpea flour itself must require an enhancers factor (protein and vitamin C) to help absorb the Fe in the body. Iron in the food consumed is in the form of ferric (vegetable) and ferrous (animal) bonds. Iron in the form of ferric with the role of gastric juice (HCL) is reduced to a ferrous form which is more easily absorbed by intestinal mucosal cells. The presence of vitamin C can help the reduction process. Iron in the form of ferrous in the mucosal cells is oxidized to ferric, thus the union between ferric and ferrous, which then combines with apoproteins to form protein containing iron, namely ferritin which then through several other processes can enter the blood plasma.[12]

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Increasing the absorption of Iron in beans in this study using cowpea flour, it is necessary to add protein, especially contractile proteins actin and myosin and the amino acids cysteine and histidine, to increase the absorption of Fe in legumes. Making a snack bar with the addition of haruan fish is the development of the material from these distracted food products. This study aims to determine the profile of hemoglobin, dan ferritin in rattus wistar anemia who get snack bars on Cowpea flour and haruan fish.

RESEARCH METHODS

This study uses a pure experimental research design to determine the profile of hemoglobin and ferritin, in rattus wistar anemia who get snack bars on Cowpea flour and haruan fish. The pretest-posttest research design used a control group without random assignment.

The population and sample at this stage is the female *Rattus novergicus strain Wistar* aged 8-10 weeks, with a body weight of 180-200 grams. In this experiment there are 2 kinds of treatments, so the number of experimental animals for each treatment obtained more accurate data required the number of samples or replications at least 3 times for each treatment.

The course of the study first of all experimental animals grouped in 2 groups (random) each group of 5 animals, namely 1) P0 = control group (get commercial / standard feed); 2) Treatment group (get Cowpea flour and haruan fish snack bars). Feed given as much as 20 grams / day, drink provided is water, and the rest of the feed is measured every day. Before being given treatment all animals tried to be anemic as a result of iron deficiency by giving Fe-free feed every day for 8 days. Treatment by providing standard feed in the control group and given a bean snack bar and haruan fish in the treatment group for 30 days. On the 9th day and the 30th day blood drawn through the retro orbital plexus vein to check hemoglobin and ferritin levels. The day before the process of blood drawing is done, experimental animal fasted (only given to drink)

Data collection for of this research are the Hemoglobin Profile (Hb) is the hemoglobin level carried out by sampling the blood of experimental animals and subsequently measured by the *Cyanmethemoglobin* method; Ferritin Profile is Ferritin level which is done by sampling blood plasma of experimental animals and subsequently measured by spectrophotometer.

After the data is collected, it is then re-examined (editing) to ensure the completeness and correctness of the data. The processed data is entered in the frequency distribution table to be analyzed descriptively, while to find out testing the influence between variables will use the t-test. Processing and data analysis using computer programs.

RESULTS

Hemoglobin profile

Hemoglobin levels were measured by taking blood samples from experimental animals and then measuring them using the cyanohemoglobin method. The results of the hemoglobin profile analysis in experimental animals are presented in Table 1.

Table 1 shows hemoglobin levels in experimental animals ranged from normal hemoglobin levels, ie 12.29 - 13.21 g / dL. After all experimental animals were made to experience anemia as a result of iron deficiency by giving Fe-free feed every day for 8 days, the amount of hemoglobin decreased significantly, which ranged from 8.83 - 9.27g/ dL which is the category of anemia. During the treatment period the standard ration giving to the experimental animals group and the Cowpea Flour Bar Snack Bar and Haruan Fish in the treatment group, the increase in hemoglobin level in the control group ranged from 0.22 to 0.48 g / dL, whereas the treatment group occurred increased hemoglobin levels ranged from 2.83 to 3.35 g / dL.

There was a statistically significant difference in the hemoglobin profile of the control group before and after receiving the standard ration (p = 0.001). There was also a significant difference in the hemoglobin profile of the treatment group before and after getting the Cowpea Flour Snack Bar and Haruan Fish (p = 0.000).

Ferritin profile

Based on the results of measuring blood serum in experimental animals using the spectrophotometer method, the ferritin profile was obtained, which is presented in table 2.

Table 2: Ferritin levels during the treatment period of standard ration in the experimental animals group decreased in one experimental animal while in other experimental animals there was an increase in the range of 0.55 - 5.98 5,g/l while the experimental animals that consumed Cowpea Flour Snack Bar and Haruan Fish in the treatment group, there was an increase in ferritin levels ranging from 30.61 - 37.45 µg/l.

There was no statistically significant difference from the ferritin profile of the control group before and after receiving the standard ration (p = 0.107). There was a significant difference from the ferritin profile of the treatment group before and after getting the Cowpea Flour Snack Bar and Haruan Fish (p = 0,000).

DISCUSSION

Hemoglobin profile

Increased hemoglobin levels coincided with an increase in the concentration of Fe content in the control and treatment rations. Based on these averages and analytical tests, the iron content in

Table 1: Profiles of hemoglobin in animals' model.

Code	Hemoglobin Profile (g/dL)			
	Start	Before	After	Enhancement
P0.1	12.70	8.94	9.20	0.22
P0.2	12.88	8.87	9.35	0.48
P0.3	12.66	9.20	9.57	0.37
P0.4	13.17	9.27	9.68	0.41
P0.5	12.84	8.83	9.31	0.48
P1.1	13.01	9.13	12.48	3.35
P1.2	12.29	9.24	12.07	2.83
P1.3	12.55	9.09	12.14	3.05
P1.4	13.21	8.98	12.00	3.02
P1.5	12.51	9.16	12.33	3.17

Note: P0 (Control Group) and P1 (Treatment Group)

Table 2: Ferritin profiles of experimental animals.

Code	Ferritin Profile (µ/l)		
	Before	After	Enhancement
P0.1	42.32	47.33	5.01
P0.2	41.90	40.82	-1.08
P0.3	43.75	49.73	5.98
P0.4	42.40	45.72	3.32
P0.5	41.29	41.84	0.55
P1.1	40.32	70.93	30.61
P1.2	40.54	77.84	37.30
P1.3	39.64	77.09	37.45
P1.4	42.18	76.83	34.65
P1.5	41.11	75.11	34.00

Note: P0 (Control Group) and P1 (Treatment Group) standard meals and snack bars may increase the levels of hemoglobin in experimental animals.

There are several substances that play a role in the formation of hemoglobin, including iron, protein, vitamin B6 which acts as a catalyst in the synthesis of heme in the hemoglobin molecule, vitamin C which affects absorption and release of iron from transferrin into body tissues, and vitamins E which affects the stability of the red blood cell membrane, protein and some other compounds that support the formation of blood and hemoglobin.[12,13] The iron content of food does not indicate its bioavailability because iron absorption depends on several factors, especially the form of iron. The majority of the iron in plants is non-heme iron, and despite the high iron content, iron absorption is minimal as a result of iron-based plant-molecular interactions.[12] The physical state of iron as ferrous and ferric has a significant impact on how much iron is absorbed. Although ferrous iron is more likely to be transported into enterocytes, non-heme iron in food is primarily found in the oxidized or ferric form. While the majority of iron in its ferrous form is still soluble at a neutral pH, iron in its ferric form precipitates in solutions with a pH greater than 3. Therefore, in order for ferrous iron to be absorbed in the less acidic proximal small intestine, it must first be dissolved and chelated in the stomach. Because iron is liberated in the intestinal lumen, chelation happens quickly in other meal ingredients. These chelators can impact iron absorption through iron solubility and act as both enhancers and inhibitors of iron absorption. Therefore, food composition is one of the main factors affecting the absorption of non-heme iron.[10] Animal tissues, such as those of beef, chicken, fish, pork, and lamb, have a positive effect on the absorption of nonheme iron from food. The enhancing effect of animal tissue on non-heme iron absorption was demonstrated in the present study. Haruan fish meat added to the snack bar increased the absorption of non-heme iron by 2.83 to 3.35 g/dL in experimental animal subjects. As a result of the study, it was concluded that fish meat enhances absorption by inactivating the luminal factor that prevents iron absorption. The most likely mechanism for this effect is the formation of luminal transporters that transport iron to the mucous cell membranes.[12] The results of this study showed the same results as several studies that were referred to in a systematic review study.[14]

The increase in hemoglobin levels in the control group was relatively small when compared to the treatment group, this was due to the protein and Fe content in the ration given. The ration used in the control group was the AIN-93 formula with a protein content of 20% where >85% in the form of Casein, and Fe 0.0606 mg,[10] while the Cowpea Flour Snack Bar and Haruan Fish had 10.86% protein content and 3.82 mg Fe content. At the Cowpea Flour Snack Bar and Haruan Fish the main source of protein is the haruan fish which is rich in albumin.[15,16]

The high protein content of the AIN-93 formula which is mainly in the casein form can inhibit iron absorption. Casein can reduce iron absorption. Meanwhile animal protein, including from fish can increase iron absorption.[17]

Ferritin profile

Increased ferritin in experimental animal blood comes from rations consumed, both from standard rations and snack bars. These findings are consistent with research on the effects of soybeans, lentils, and pumpkin seeds on individuals with iron deficient anemia.[18-21] Mice with low ferritin levels will readily absorb iron-rich foods, which causes ferritin levels to rise as iron reserves in the blood grow.[22,23]

Absorption of iron in the body (especially in the form of ferric) can be increased if there are booster factors (enhancers) of iron absorption such as vitamin C and protein. Iron absorption can also be inhibited by the presence of absorption inhibitors (inhibitors) such as tannins, phytic acid, oxalate, and calcium in food. With reduced absorption of iron, the amount of ferritin (iron stored in the body) will also be reduced which will have an impact on decreasing the amount of iron that will be used for the synthesis of hemoglobin so that it can cause anemia.[22,24-29]

The increase in hemoglobin levels in the control group was relatively small when compared to the treatment group, in the treatment group that is given a snack bar with high Iron and protein content could increase ferritin levels in the blood. Increased levels of ferritin in the treatment group were given snack bars of cowpea flour and haruan fish due to a combination of Iron and protein content. After entering the gastrointestinal tract, iron will turn into ferrous form. This ferrous form will then be absorbed. Absorption can occur in the stomach, but the biggest absorption occurs in the upper intestine, then decreases dramatically once it reaches the distal part. The absorption mechanism penetrates mucosal cells using active transport. Ferritin contained in the mucous membrane regulates the amount of iron absorbed. After reaching the plasma, the ferro form is oxidized to ferric and binds to transferrin. The amount of iron absorbed will depend on the composition of the diet which includes heme iron, vitamin C or phytate.[12] Other studies have shown that how much food affects iron absorption is influenced by differences in the chemical composition of foods that can increase or reduce iron absorption.[10,12,24]

CONCLUSION

Cowpea Flour Snack Bar and Haruan Fish can increase hemoglobin levels in experimental animals, ranging from 2.83 to 3.35 g / dl and ferritin levels range from 30.61 - 37.45 µg/l.

SUGGESTION

Overcoming the problem of anemia needs to be given additional foods high in Fe and protein, such as Cowpea Flour Snack Bar and Haruan Fish.

Further clinical research is needed on the effectiveness of snack bars based on high Fe and protein ingredients, accompanied by counseling for dietary changes.

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