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Effect of Sago worm flour (*Rhynchhorus feirugineus*) on Albumin and Haemoglobin in Protein Energy Malnutrition (PEM) Wistar rats

Aleda Florince Oyay[1]*, Muchlis Achsan.Udji Sufro[2], Gemala Anjani[3]

[1]Master Student of Nutrition, Faculty of Medicine, Diponegoro University, Jalan Prof Sudharto Semarang 50275, Indonesia
[2]Department of Interna, Faculty of Medicine, Diponegoro University, Jalan Prof Sudharto Semarang 50275, , Indonesia
[3]Department of Nutrition, Faculty of Medicine, Diponegoro University, Jalan Prof Sudharto Semarang 50275, Indonesia
**Correspondence*: florincealeda@gmail.com

ABSTRAK

Latar Belakang: *Kurang energi protein (KEP) merupakan salah satu penyakit gangguan gizi yang penting di Indonesia. Dampak kekurangan protein yang parah umumnya terjadi pada bayi dan balita. Dalam keadaan KEP terdapat perubahan nilai Hemoglobin dan albumin dalam darah. Ulat sagu dikenal sebagai makanan yang kaya akan protein, sehingga diharapkan dapat memperbaiki status albumin dan Hemoglobin anak dengan KEP.*
Tujuan: *Mengetahui pengaruh pemberian tepung ulat sagu terhadap kadar albumin dan Hemoglobin (Hb) tikus wistar KEP*
Metode: *Penelitian true experimental dengan Pre post test control group design. Menggunakan 24 ekor tikus wistar kemudian dibagi menjadi 4 kelompok: K- (tikus normal); K+(tikus KEP); P1(tikus KEP dengan intervensi tepung ulat sagu 0,36g/100gBB); dan P2(tikus KEP dengan intervensi tepung ulat sagu 1,36g/100gBB). Intervensi dilakukan selama 28 hari. Analisis statistik menggunakan metode Shaphiro Wilk, One Way Anova.*
Hasil: *Terdapat perbedaan yang signifikan (p>0,05) pada berat badan, kadar albumin, dan Hemoglobin pada intervensi pemberian tepung ulat sagu pada tikus wistar KEP.*
Kesimpulan: *Tepung ulat sagu meningkatkan berat badan, kadar albumin, dan kadar Hb tikus wistar KEP.*

KATA KUNCI*: Albumin; Hemoglobin; Kurang energi protein (KEP); tepung ulat sagu*

ABSTRACT

Background: *Protein energy malnutrition (PEM) is one of the most important nutrition disorder in Indonesia. The impact of a severe protein deficiency generally occurs in infants and toddlers. In the PEM there is a change in the albumin and Hemoglobin (Hb) levels. Sago caterpillar flour contain high protein, so it can improve the nutritional status of albumin and Hemoglobin levels in children with PEM.*
Objectives: *To observe the effect of sago caterpillar flour on albumin and Hemoglobin (Hb) levels on PEM Wistar rats*
Methods: *True experimental pre-post control group design. 24 Wistar rats were divided into 4 groups: K-(normal rats); K+(PEM rats); P1(PEM rats with intervention of 0.36g/100gBW sago caterpillar flour); P2(PEM rats with intervention of 1.36g/100gBW sago caterpillar flour). Statistical analysis using Shapiro Wilk method and One Way ANOVA.*
Results: *There were significant differences (p.0.05) in body weight, albumin levels, and Hemoglobin in the intervention of sago caterpillar flour in PEM Wistar Rats.*
Conclusions: *Sago caterpillar flour increased body weight, albumin levels, and Hemoglobin levels in PEM Wistar Rats*

KEYWORDS*: Albumin; Hemoglobin; Protein energy malnutrition (PEM); Sago worm flour*

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INTRODUCTION

Protein Energy Malnutrition (PEM) form of malnutrition caused by a lack of energy and protein in various proportions. PEM divided into three categories: kwashiorkor, marasmus, and marasmus kwashiorkor (1). The prevalence of PEM mostly found in children under five years and pregnant-lactating women. In children under 5 years, the most common cases of PEM are marasmus and kwashiorkor (2). PEM often founding in developing countries, one is Indonesia. Data from Basic Health Research of Indonesia (RISKERDAS) in 2018 show, 13.8% of children with malnutrition and 3.9% of children with severe malnutrition (3). The distribution of PEM prevalence in Indonesia covers 19 provinces from a total of 34 provinces. Province of Papua and West Papua included in the PEM distribution area (4,5).

The impact of severe protein deficiency is related to changes in Hemoglobin and albumin levels. The albumin levels selected as an indicator for determining of PEM because it is the most abundant manifestation of protein plasma in the blood (6). Function of serum albumin is to maintain plasma osmotic pressure and carry the various types of substance and hormones to the organs (7,8,9). In the condition of PEM, one of the clinical symptoms that can be observed is anemia (10). It is related because erythroprotein plays a role in stimulating the production of red blood cells (11).

Various alternatives for handling incidence of PEM have been carried out. One of them is the assistance for family food security by utilization of local food materials. Sago worm is one of the local food ingredients that can be found in eastern of Indonesia (mainly in Papua and West Papua) and has health benefits effect (12). Papua Sago worm contain high protein (10.39gr/100gr) and has good digestibility (92%) (13). All types of essential amino acid are found in Papua Sago worms (14). The highest amino acid in Papua Sago worm are phenylalanine and lysine. Study show, intervention of sago worm flour decrease parasitemia index and increase the Hemoglobin levels of mice induced with malaria (15). Studies related sago worm still very limited, so this study aim to observe the effect of sago worm flour on albumin and Hemoglobin levels in PEM model rats.

MATERIALS AND METHODS

True experimental pre-post control group design with 24 male rats (*Rattus norvegicus*) aged 4 weeks with 100 gr weight. Subject divided into 4 group: K- (normal rats); K+(PEM rats model); P1(PEM rats model with intervention of 0.36gr/100grBB sago worm flour); and P2(PEM rats model with intervention of 1.36gr/100grBB sago worm flour). Dose calculation of sago worms based on lysin content and conversion of Laurence and Bacharach table (16,17). Protein Energy Malnutrition (PEM) rat model was made by feeding with low protein (0%) for 14 days. Rats was PEM if the albumin levels was <3 g/dL and Hemoglobin levels was Hb<10 g/dL, hair loss, dull in color, and the cornea is cloudy. The rats were given standard feed of comfeed AD II 10gr/100grBB/day and *ad libitum* water (18). Intervention of sago worm flour given by 28 days with syringe. Sago worm flour made by bake at 100^{0}C then mashed and sieved with an 80 mesh sieve. (19). Blood samples for observe albumin and Hemoglobin levels were obtained from *plexus retroorbitalis*. Albumin and Hemoglobin levels were analyzed with DiaSys kit. The manufacture of sago worm flour, animal maintenance, treatment, sampling, and analysis of albumin and Hemoglobin levels were carried out at the Central Laboratory of Food and Nutrition Studies, Gadjah Mada University, Yogyakarta.The ethical clearance for the study obtained from Dr. Kariadi Central Hospital Semarang with number No.111/EC/H/FK-UNDIP/XI/2020. Statistical analysis using *Shaphiro Wilk* then continued with *One Way Anova*. Data with abnormal distribution were analyzed using *Kruskal-Wallis*.

RESULTS AND DISCUSSIONS

Body Weight

Body weight is an anthropometric indicator that used to assess nutritional status. Body weight greatly influenced by food and nutrition because it will affect the body condition (20). Adequate of food and nutrition intake will increasing the body weight. The increasing of rat body weight affected by the feed intake and consumption.

Table 1. Rats Body Weight Before and After Intervention

Groups	Pre Mean ± SD	Post Mean ± SD	Δ	P-*Value*
K-	122.29 ± 1.49	115.14 ± 2.34	-7.15 ± 0.85	0.007
K+	111.86 ± 1.34	120.71 ± 2.05	8.85 ± 0.71	0.008
P1	112.14 ± 1.67	130.57 ± 1.98	18.43 ± 0.31	0.000
P2	112.43 ± 1.90	138.00 ± 2.58	25.57 ± 0.68	0.026

P: Paired t-test

Table 1 show the result of normality test for rat body weight using *Saphiro wilk test* (p>0.05) has normal distribution. The *Paired t-test* show the different of rat body weight in the all groups. P2 group (PEM rat model with intervention of 1.36g/100gBB sago worm flour) show the increasing of body weight on protein energy malnutrition rats model compared with K+ group and P2 group. (25.57 gr).

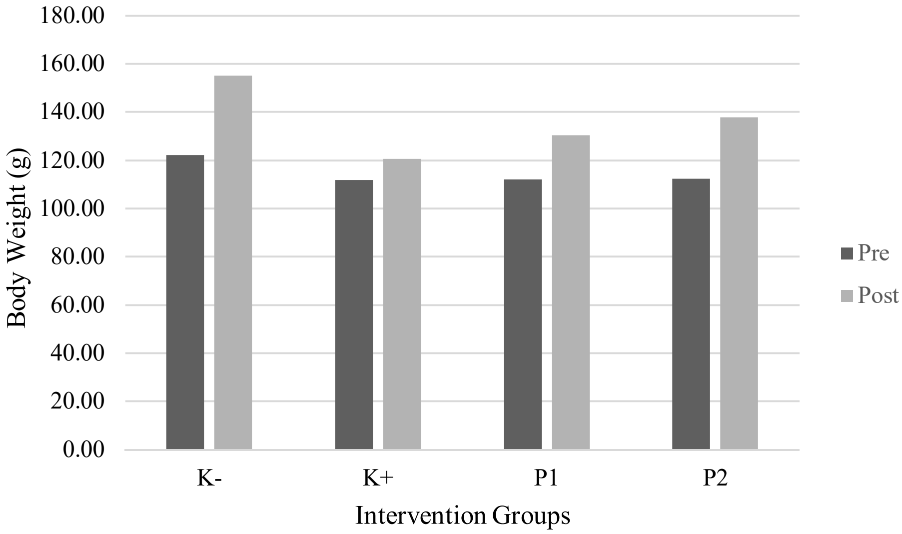

Figure 1. Body Weight Comparation

Intervention with sago worm flour show the increasing of rat body weight. The increasing of body weight is the result of a growth process at the cellular level which in affect to all body components. In human, the largest organ growth occurs in long bones, skeletal muscles, digestive system, respiratory system, blood circulation and blood volume. Child have early growth and development of the brain, eyes, and ears. New born baby has 25% weight of adult human brain. At 2 years, brain develops up to 75% from adult brain and at 10 years, the brain has 95% of the adult brain. Lymphoid tissue has maximum growth before adulthood and decreases with age. Reproduction organs develop slowly in preadolescence and significancy increasing in adolescence. (21,22,23).

Nutrition has important role in growth and development. Macronutrient like carbohydrate, fat, and proteins are necessary in the process of growth and development. Carbohydrate are the main nutrients for supplying energy to body for carry out the activities. Carbohydrates needed in every life cycle. In toddler, carbohydrates needed due to the high level of children's activity and energy for brain development (24). Fat is an energy storage in the body. Fat contains essential fatty acid which have role in regulating nervous system, strengthening cardiovascular system, build up the immune system, and help the body for absorb nutrients (25). Fat also play a role for transporting and dissolving vitamins in the body which will affect the toddler growth function (26). Protein is a macronutrient for building the tissue system. Body need the protein for growth, building body structure like muscles, skin, and bones, and tissue regeneration. Protein has the most important value because it is related to life processes. All living cells related to proteins. Protein play a role for transporting nutrients from digestive tract through the intestine walls to the blood circulation (25).

Albumin Levels

Albumin is a water soluble protein that synthesized in the liver and plays a role for maintaining blood colloid osmotic pressure (6). Albumin has a large molecular weight and shape, so it cannot penetrate the vessels walls or capillaries and will help to maintain fluid vascular system (9). Albumin is the most abundant protein plasma in the human body, 55-60% of the measured protein serum (27). Albumin plasma levels has correlated with protein storage in the body. Decreasing of plasma albumin levels indicates protein deficiency in the body. Albumin levels measurements used to indicate nutritional and health status for detecting the protein energy malnutrition (PEM) (28). Intervention with sago worm flour show the increasing of rat albumin levels. Normal albumin levels in male Wistar rat is 3.0-5.1 g/dL (29). Intervention of sago worm flour in PEM rat model proven to increase albumin levels. High protein intake shows a tendency towards better nutrition improvement in PEM rat model.

Table 2. Rats Albumin Levels Before and After Intervention

Groups	Pre (g/dl)	Post (g/dl)	Δ	P-*Value**
K-	4.70 ± 0.08	4.59 ± 0.05	-0.11 ± 0.03	0.014
K+	1.13 ± 0.07	1.07 ± 0.06	-0.06± 0.01	0.022
P1	1.20 ± 0.07	3.86 ± 0.05	2.66 ± 0.02	0.975
P2	1.19 ± 0.09	4.19 ± 0.11	3.00 ± 0.02	0.001

P: Paired t-test

Table 2 show the albumin levels of rats with sago worm flour intervention. Intervention with sago worm flour show the increasing of rat albumin levels. The result of the normality test for albumin levels in rats using *Shapiro wilk test* show normal distribution (p>0.05). Further test with *Paired t-test* show the P2 groups (PEM rat model with intervention of 1.36g/100gBB sago worm flour) has the most significant increasing of albumin levels (p=0.001). Increasing of albumin levels in P2 groups is 3.00 g/dL. Intervention of high dose sago worm flour show an increase in albumin levels of PEM rat model.

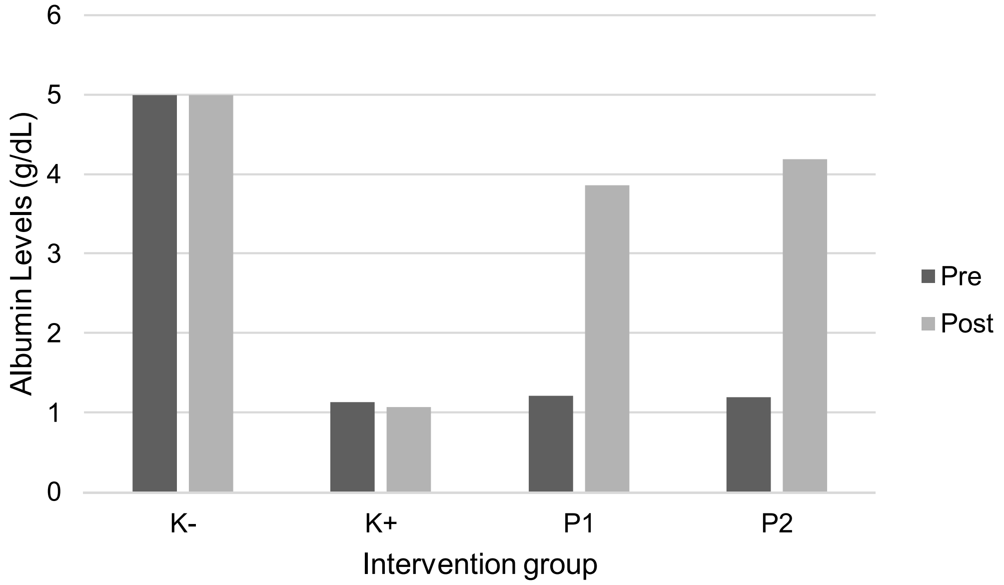

Figure 2. Albumin Levels Comparation

In Protein Energy Malnutrition (PEM) there is a degradation of blood albumin levels. Albumin serum levels are used as an indicator of severe protein deficiency. PEM lead to hypoalbuminemia. Albumin serum is the largest component in protein serum and can be a marker for determining personal nutrition status. The albumin serum concentration affected by various factor like infection, trauma, hydration status, hepatic function and renal disfunction (11). PEM condition will affect the albumin serum levels directly or indirectly. On directly pathway, decreasing of protein synthesis will decrease protein levels. Inadequate protein intake will aggravate the condition. Protein amount in liver, gastrointestinal system, kidney, and heart will decrease too. This can ve exacerbated by changes of the intestinal villi which cause protein absorption reduced (11). Inadaquate protein intake will slow down the synthesis of albumin mRNA and decreasing the albumin serum levels. Indirectly decreasing albumin serum levels commonly happened on stunting children. In this condition there is an increasing of oxidative stress and decreasing of immune system. Oxidative stress will lead the damaged cell in the body, including hepatic cells or hepatocytes. Damage of hepatocytes will inhibits the production of albumin that synthesized by liver cells (7).

Insects are an alternatives source of protein that has ecological advantages in PEM (30). The consumption of insect has been carried out by African, Asian, and European communities to resolve PEM (31,32). Insect cultivation has begun to become a government program in Africana and Asian as a source of animal protein. Intervention with sago worm flour show significancy effect for increasing albumin serum levels in PEM rat model. This influenced by protein intake improvement. Papua sago worm provides good benefits as a source of protein in consumption patterns of Papuans (33,34). Nutritional analysis of sago worm flour shown high content of protein and antioxidant (15). Nutritional content of sago worm flour is almost equivalent to standard protein (Casein). It means that sago worm flour can used to support growth and maintenance of body tissue. In 100gr sago worm flour contains 10.39gr protein with bioavailability of 92%. Mono unsaturated fatty acid (MUFA) contains 8.18gr (dominated by 45% oleic acid), poly unsaturated fatty acid (PUFA) contains 79gr (dominated by linoleate acid), 43.25 μg/gr of α-tokoferol, 24–88% magnesium and 29–34% zinc (14).

Hemoglobin Levels

Hemoglobin is main component of erythrocytes and a protein that contains a lot of iron. Hemoglobin plays an important role in oxygen transport from lungs to all of body tissues (35). Examination of Hemoglobin levels is done to observe the anemia status and PEM status that leading the decreasing of nutrient status.

Table 3. Rats Hemoglobin Levels Before and After Intervention

Groups	Pre Mean ± SD	Post Mean ± SD	Δ	P-*Value**
K-	14.32 ± 0.26	14.11 ± 0.25	-0.21 ± -0.001	<0.001
K+	8.53 ± 0.36	8.31 ± 0.35	-0.22 ± -0.001	<0.001
P1	8.86 ± 0.21	11.97 ± 0.45	3.11 ± 0.24	<0.001
P2	8.92 ± 0.18	13.18 ± 0.11	4.26 ± -0.07	<0.001

P : One Way Anova

Table 3 show the increasing of Hemoglobin levels of PEM rat model with sago worm flour intervention. P2 groups (PEM rat model with intervention of 1.36g/100gBB sago worm flour) has the highest increasing of Hemoglobin levels (4.27 g/dL). The result of the normality test for Hemoglobin levels in rats using *Shapiro wilk test* show normal distribution (p>0.05). ANOVA test show the significantly different between intervention groups after sago worm flour intervention compared with control groups (0.00 from p<0.05).

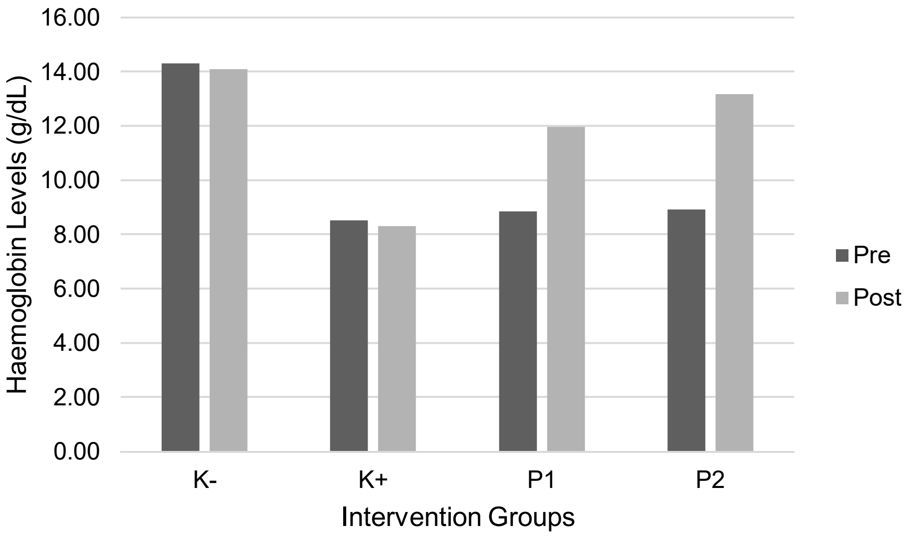

Figure 3. Hemoglobin Levels Comparation

Hemoglobin synthesis affected by availability of iron and protein adequacy. Albumin levels in blood circulation correlate with Hemoglobin levels. Decreasing of albumin levels in blood circulation will lead the decrease of Hemoglobin levels. It is correlate because protein is one of important elements that needed in Hemoglobin synthesis. When the albumin levels in body was low. the Hemoglobin synthesis will defected and lead the decreasing of Hemoglobin levels in body. Protein in the form of glycine amino acid and succinyl-coA are needed to convert protoporphyrin to heme by interacting with iron and ferrocelatase enzyme. Globin synthesis need biotin amino acid. folic acid. vitamin B6. and vitamin B12. To forming Hemoglobin. 4 heme molecules that binding with 1 globin molecule are needed (36). Iron and protein deficiency will lead the disruption of Hemoglobin synthesis resulting anemia and ultimately occurs PEM.

Apart material for building blocks of heme and globin. protein is needed as iron transporter. Iron that has been absorbed by intestinal mucosa will transported in blood circulation with binding protein. Binding of iron and protein called transferrin. Transferrin is stored in liver, spleen, and spinal cord for formation of red blood cells (eritropoiesis). When protein intake was inadequate, the Hemoglobin synthesis will disrupted. In the cells, iron interact with electron transport protein chains that play role in the final steps of energy metabolism. Proteins remove hydrogen and electron form from energy-producing nutrient to bind oxygen and form to water and produce ATP. Most of the iron storage are in Hemoglobin and myoglobin in muscles. Protein in form of transferrin can carrying iron. In the inadequate protein intake, iron cannot be carried and lead to disruption of Hemoglobin formation. This will affect the iron absorption and transportation. (37).

CONCLUSIONS AND RECOMMENDATIONS

Intervention with 1.36gr/100grBW sago worm flour increasing the body weight. albumin levels. and Hemoglobin levels in PEM rat model significantly. From the study. sago worm flour is expected to be applied directly to improve the PEM condition in toddler. Further research on the dosage of sago worm flour still needs to be explored to complement the research.

REFERENCES

1. Soeters P. Bozzetti F. Cynober L. Forbes A. Shenkin A. Sobotka L. Defining malnutrition: A plea to rethink. Clin Nutr [Internet]. 2017;36(3):896–901. Available from: http://dx.doi.org/10.1016/j.clnu.2016.09.032
2. Munthali T. Jacobs C. Sitali L. Dambe R. Michelo C. Mortality and morbidity patterns in under-five children with severe acute malnutrition (SAM) in Zambia: A five-year retrospective review of hospital-based records (2009-2013). Arch Public Heal. 2015;73(1):1–9.
3. Iztapalapa M G. MC.Infant Malnutrition. 2014. 527–41
4. Riskesdas K. Hasil Utama Riset Kesehata Dasar (RISKESDAS). J Phys A Math Theor [Internet]. 2018;44(8):1–200. Available from: http://arxiv.org/abs/1011.1669%0Ahttp://dx.doi.org/10.1088/1751-8113/44/8/085201%0Ahttp://stacks.iop.org/1751-8121/44/i=8/a=085201?key=crossref.abc74c979a75846b3de48a5587bf708f
5. Akbar J. Akbar M. Irianto D. Measurement and Development of Humanware and Technoware Competencies in Order to Meet Pintle Chain Product Requirements in Bandung Manufacture Polytechnic. IOP Conf Ser Mater Sci Eng. 2016;114(1):2015–9.
6. Rahman MZ. Begum BA. Serum total protein. albumin and A/G ratio in different grades of protein energy malnutrition. Mymensingh Med J. 2005;14(1):38–40.
7. Adriani. Merryana BW. Gizi dan Kesehatan Balita. jakarta: Jakarta: KENCANA; 2014.
8. Adriani. M.. Wirjatmadi B. Peranan Gizi Dalam Siklus Kehidupan. Kencana Pustaka; 2012.
9. Sekine S. Terada S. Aoyama T. Medium-chain triacylglycerol suppresses the decrease of plasma albumin level through the insulin-Akt-mTOR pathway in the livers of malnourished rats. J Nutr Sci Vitaminol (Tokyo). 2013;59(2):123–8.
10. Elisabeth K. Changing profile of Undernutrition (E-SAM). Indian Pediatrics. 2012.
11. Wagnew F. Dejenu G. Eshetie S. Alebel A. Worku W. Abajobir AA. Treatment cure rate and its predictors among children with severe acute malnutrition in northwest Ethiopia: A retrospective record review. PLoS One. 2019;14(2):1–13.
12. Ariani A. Anjani G. Sofro MAU. Djamiatun K. Tepung ulat sagu (Rhyinchophorus ferrugineus) imunomodulator Nitric Oxide (NO) sirkulasi mencit terapi antimalaria standar. J Gizi Indones (The Indones J Nutr. 2018;6(2):131–8.
13. Security KF. Ringkasan Studi Pangan dan Tata Guna Lahan (FOLU). 2019.
14. Köhler R. Irias-Mata A. Ramandey E. Purwestri R. Biesalski HK. Nutrient composition of the Indonesian sago grub (Rhynchophorus bilineatus). Int J Trop Insect Sci. 2020;677–86.

15. Manggaprow L. (Rhynchophorus ferrugineus) Terhadap Indeks Parasitemia Dan Kadar Hemoglobin Mencit Strain Swiss Yang Terinfeksi Malaria The Effect Of Sago Worm (Rhynchophorus Ferrugineus) Flour On Parasitemia Index And Hemoglobin Level Of Swiss Strain Mice Magister . 2018.
16. Nirmala IR. Trees. Suwarni. Pramono MS. Sago worms as a nutritious traditional and alternative food for rural children in Southeast Sulawesi. Indonesia. Asia Pac J Clin Nutr. 2017;26(May):S40–9.
17. Raja R Pillai. Rajavel Elango. Ronald O Ball. Anura V Kurpad. and Paul B Pencharz. Lysine Requirements of Moderately Undernourished School-Aged Indian Children Are Reduced by Treatment for Intestinal Parasites as Measured by the Indicator Amino Acid Oxidation Technique. J Nutr. 2014;(Nutrient Requirements and Optimal Nutrition).
18. Anggraeny. O. Dianovita. C. Putri. EN Minarty Sastrina RSD. Korelasi Pemberian Diet Rendah Protein Terhadap Status Protein. Imunitas. Hemoglobin. dan Nafsu Makan Tikus Wistar Jantan. Indones J Hum Nutr. 2016;3(2):105–22.
19. Astawan M. Wresdiyati T. Pengaruh Konsumsi Tempe Kedelai Grobogan Terhadap Profil Profil Serum . Hematologi Dan Antioksidan Tikus [Effect of Grobogan Soybean Tempe Consumption on Rats ' Serum Profiles . Hematology . and Antioxidant]. 2019;(January).
20. Rokhe. J. E. 1979. Prioritas Pediatri di Negara Berkembang. Yayasan Estetika Medika. Penerbit Buku-buku Ilmiah Kedokteran. Yogyakarta.
21. Meadow. R dan Newll. S. Lecture Notes Pediatrica. Jakarta: Erlangga. 2002.
22. Cameron. N. Human Growth and Development. California: Academic Press. 2002.
23. Tanuwijaya. S. Konsep Umum Tumbuh dan Kembang. Jakarta: EGC.2003.
24. Panel. E. & Nda. A. Scientific Opinion on nutrient requirements and dietary intakes of infants and young children in the European Union. EFSA J. 2013 ;11. 1–103.
25. Susetyowati. Ilmu Gizi Teori dan Aplikas. EGC; 2017.
26. Adriani. Merryana. and Bambang Wirjatmadi. “Peranan gizi dalam siklus kehidupan.” Jakarta: Kencana prenada media group 2 (2012): 245-278.
27. Soeters PB. Wolfe RR. Shenkin A. Hypoalbuminemia: Pathogenesis and Clinical Significance. J Parenter Enter Nutr. 2019;43(2):181–93.
28. Sja’bani. Mochammad. 1998. ‘Arti Klinis Pemeriksaan Albumin Serum sebagai Pertanda Progres Malnutisi dengan Metode Brom Cresol Green (BCG) dan Elektroforesis pada Penderita Hemodialisis Rutin’. Berkala Ilmu Kedokteran 30 (4) : 181-187.
29. Sutedjo. SKM. 2007. Mengenal Penyakit Melalui Hasil Pemeriksaan Laboratorium. Amara Books. Yogyakarta.
30. Lindgren E. Harris F. Dangour AD. Gasparatos A. Hiramatsu M. Javadi F. et al. Sustainable food systems—a health perspective. Sustain Sci. 2018;13(6):1505–17.
31. Raheem D. Carrascosa C. Oluwole OB. Nieuwland M. Saraiva A. Millán R. et al. Traditional consumption of and rearing edible insects in Africa. Asia and Europe. Crit Rev Food Sci Nutr. 2019;59(14):2169–88.
32. Caparros Megido R. Sablon L. Geuens M. Brostaux Y. Alabi T. Blecker C. et al. Edible insects acceptance by belgian consumers: Promising attitude for entomophagy development. J Sens Stud. 2014;29(1):14–20.
33. Bustaman S edrus i. Ternak. pengkajian ulat sagu sebagai budidaya pakan. 2007.
34. Tubert-Brohman I. Sherman W. Repasky M. Beuming T. Improved docking of polypeptides with glide. J Chem Inf Model. 2013;53(7):1689–99.
35. Meliana. Dina. 2004. ‘Studi Banding Beberapa Metode Pengukuran Kadar Hemoglobin’. Skripsi. Fakultas Kedokteran-UNS. Surakarta.
36. Susilo. Joko.. dan Hamam Hadi. 2002. ‘Hubungan Asupan Zat Besi dan Inhibitornya sebagai Prediktor Kadar Hemoglobin Ibu Hamil di Kabupaten Bantul Propinsi DIY’. Berita Kedokteran Masyarakat 18 (1) : 1-8.
37. Sunita. Almatsier. Prinsip Dasar Ilmu Gizi. Jakarta: Gramedia Pustaka Utama. 2010. 51-75.