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Effects of Kedawung Seed Tempeh Flour (Parkia Roxburghii G. Don) on Albumin Levels and Hemoglobin Levels in Protein Energy Malnutrition (PEM) Rats

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A B S T R A C T

Protein energy malnutrition (PEM) is a condition of malnutrition where food intake does not provide adequate amounts of energy and protein for growth and development. Clinical signs of PEM include hypoalbuminemia and anemia. Fermentation of kedawung (Parkia Roxburghii G. Don) seeds is an effective process that shows an increase in nutrients and reduces anti-nutrients. This study was aimed to investigate the effect of kedawung seed tempeh flour (Parkia Roxburghii G. Don) on albumin levels and hemoglobin levels in PEM rats. True-experimental randomized pre-post with control group design was used. PEM in rats was induced by a low protein diet. A total of 24 Wistar rats were categorized into normal control group (K-), protein energy malnutrition control group without treatment (K+), kedawung seed tempeh flour (Parkia Roxburghii G. Don) 1,5 gr/100g BW/d group (P1), kedawung seed tempeh flour (Parkia Roxburghii G. Don) 3,0 gr/100g BW/d group (P2) for 28 days. Albumin levels were measured using ELISA, hemoglobin levels with a hematology analyzer. Albumin levels showed a significant difference between groups (p=0.000) and hemoglobin levels showed a significant difference between groups (p=0.001). Kedawung seed tempeh flour (Parkia Roxburghii G. Don) 1,5 gr/100g BW/d for 14 days increased albumin and hemoglobin levels in PEM rats.

Kata kunci:

Tepung Tempe biji kedawung *(Parkia Roxburghii G. Don)*
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A B S T R A K

KEP merupakan kondisi gizi kurang dimana asupan makanan tidak memberikan jumlah energi dan protein yang adekuat untuk pertumbuhan dan pemeliharaan tubuh. Tanda klinis KEP meliputi hipoalbuminemiadan anemia. Fermentasi biji kedawung (Parkia Roxburghii G. Don) merupakan proses efektif yang menunjukkan peningkatan zat gizi dan mengurangi zat antigizi. Penelitian ini bertujuan untuk mengetahui pengaruh pemberian tepung tempe biji kedawung (Parkia Roxburghii G. Don) terhadap kadar albumin dan kadar hemoglobin (Hb) tikus KEP. Penelitian ini menggunakan desain true-experimental randomized pre-post-test with control group design. Kondisi KEP pada tikus di induksi dengan diet rendah protein. Tikus wistar sebanyak 24 ekor dikelompokkan ke dalam kelompok kontrol normal (K-), kelompok kontrol KEP tanpa perlakuan (K+), kelompok tepung tempe biji kedawung (Parkia Roxburghii G. Don) 1,5 gr/100g BB/hari (P1), dan kelompok tepung tempe biji kedawung (Parkia Roxburghii G. Don) 3gr/100g BB/hari (P2) selama 28 hari. Kadar albumin diukur menggunakan ELISA dan kadar hemoglobin dengan hematology analyzer. Kadar albumin menunjukkan adanya perbedaan signifikan antar kelompok (p=0,000) dan kadar hemoglobin menunjukkan adanya perbedaan signifikan antar kelompok (p= 0,001). Tepung tempe biji kedawung (Parkia Roxburghii G. Don) 1,5 gr/100g BB/hari selama 14 hari meningkatkan kadar albumin dan kadar hemoglobin pada tikus KEP.

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INTRODUCTION

One of the nutritional imbalances is the condition of Protein Energy Malnutrition (PEM). PEM is a condition of malnutrition where food intake does not provide adequate amounts of energy and protein for growth and development (P. Soeters et al., 2016). Based on data from Riskesdas 2018, the prevalence of malnutrition in Indonesia in 2018 reached 13.8% and malnutrition was 3.9%.Clinical signs of PEM include hypoalbuminemia, decreased immunity, anemia, and impaired metabolic responses (Lehman & Ballow, 2020).

Serum albumin is the largest component of serum protein and is a marker for nutritional status. PEM can cause hypoalbuminemia. Albumin works to maintain plasma oncotic pressure and provides various types of substances and hormones to the body's organs(Keller, 2019; Seiji Sekine, Shin Terada, 2013; P. B. Soeters, Wolfe, & Shenkin, 2019). PEM and anemia in children are related. Anemia in childhood can occur as a result of micronutrient deficiency or lead to malnutrition due to poor synthesis of macronutrients, especially protein (Akalu, 2020; Guerrant, Oriá, Moore, Oriá, & Lima, 2008; Wagnew et al., 2019).

Children with PEM experience malabsorption of nutrients, therefore it is recommended to give food products with easily absorbed nutritional components, one of which is fermented food (Kvissberg et al., 2015). Fermented foods contain molecules that are easily absorbed because they have undergone molecular breakdown into simple molecules, one of the fermented foods in Indonesia that are well known is tempeh. Tempeh has been known as a functional food that is generally made from soybean seeds or some other ingredients (Michaelsen, K. F., Hoppe, C., Roos, N., Kaestel, P., Stougaard, M., Lauritzen, L., Mølgaard, C., Girma, T., & Friis, 2009; Vital, Rayane J, Priscila Z Bassinello , Quédma A Cruz , Rosângela N Carvalho , Júlia C M de Paiva, 2018). It is typically made from soybeans using Rhizopus sp. as its starter (Ahnan-Winarno, Cordeiro, Winarno, Gibbons, & Xiao, 2021).

Kedawung seeds (*Parkia roxburghii G. Don*) contain high enough protein and phytochemical compounds such as amino acids, fatty acids, phytosterols, antioxidants, and bioactive compounds Thioproline or Thiazolidine-4-carboxylic Acid (TCA). Previous studies of kedawung seeds(*Parkia roxburghii G.Don*) showed antibacterial, antidiabetic, antiproliferative properties (Angami et al., 2017). Fermentation of kedawung seeds (*Parkia roxburghii G. Don*) is an effective process that shows an increase in protein, fat, amino acids, fatty acids and reduces anti-nutritional substances (Sathya & Siddhuraju, 2015).

In this study, the flouring process of kedawung seed tempeh (*Parkia roxburghii G.Don*) was carried out using the oven drying method. The flouring process is useful for increasing the storage period of a material due to the drying process which causes a reduction in the water content in the material, thereby inhibiting the growth of microorganisms (Mohammed, Edna, & Siraj, 2020). The application of kedawung on food products such as tempeh has not been carried out. The aim of this study is to determine and investigate the effect of kedawung seed tempeh flour *(Parkia Roxburghii G. Don)* on albumin levels and hemoglobin levels in protein energy malnutrition.

METHOD

This study was included in the research project Study of The Administration of Kedawung seed tempeh flour *(Parkia Roxburghii G. Don)* in Nutritional Status, Hematological Profile, Immune Status and Metabolic Response on PEM ratssupported by funding received from the Faculty of Medicine, Universitas Diponegoro 2020. Research begins with the processing of kedawung tempeh and kedawung seed tempeh flour continued with nutrition analysis at the Integrated Laboratory of Diponegoro University Semarang, and intervention research on experimental animals at the PSPG PAU UGM Yogyakarta Animal Laboratory for 28 days with acclimatization process to blood collection and analysis. Research on experimental animals has been approved by The Ethical Committee of Medical Research the Faculty of Medicine, Universitas Diponegoro (No. 22/EC/H/FK-UNDIP/IV/2020.), Indonesia.

The Processing of Kedawung Seed Tempeh *(Parkia roxburghii G. Don)*

The sample used was kedawung seeds (*Parkia roxburghii G. Don*) obtained from suppliers in Sukoharjo, Central Java and the plant classification was determined at the Biology Laboratory of Diponegoro University. The ingredients for making kedawung seed tempeh are kedawung seeds and "Raprima" brand tempeh yeast. Kedawung tempeh is processed by washing the kedawung seeds, soaked in water for 2h then the soaking water was removed, the seeds were washed. Next the seeds were boiled in water for 2h then drain well the water peeledling the seed coat, then soaked seeds again in water for 18h (the water was changed regularly every 6h. The last step to giving yeast, and put the seeds into the perforated plastic. After 48 hours the mushrooms on tempeh begin to solidify and reach the point of maximum fermentation.

The Processing of Kedawung seed Tempeh Flour *(Parkia roxburghii G. Don)*

Tempeh from kedawung seeds was sliced using a slicerandthen blanched with hot steam for 2 min. Then tempeh was dried by the oven with a temperature of 50°C for 6h, milling using a continuous feeding grinding machine brand "Fomac", and sifting with a 60 mesh sieve,[7] at the PSPG PAU UGM Yogyakarta Animal Laboratory.

Experimental Animals

This was an experimental study with a randomized pre-post-test design with a control group offour weeks-old male Wistar rats (Rattus novergicus), each weighing 90 – 120 g, healthy condition and activewere acquired from the PSPG PAU UGM Yogyakarta Animal Laboratory. Exclusion criteria rats have wounds and bleeding. Mice who got sick and died during the study will be dropped out. The animals were individually housed and provided a standard feed of Comfeed II at 15 g per rat per day and water ad libitum. Rats were randomly divided into four groups (6 mice per group): normal/healthy control (K-), protein energy malnutrition without treatment (K+), the group fed kedawung seed tempeh flour *(Parkia Roxburghii G. Don)* 1.5 gr/100g BW/d (P1), the group fed kedawung seed tempeh flour *(Parkia Roxburghii G. Don)* 3gr /100g BW/d (P2). All animals, except the K+ group animals, were fed a low protein diet for 14 days.This diet contained granulated sugar (67%), vegetable oil (17%),and cornstarch (15%) and was administered orally at 10 gper ratper day(Olivia Anggraeny, Chardina Dianovita, Ekanti Nurina Putri, Minarty Sastrina, 2016).

Albumin measurements after 14 days of low protein diet administration and after intervention. Albumin levels were analyzed *Bromocerol Green* (BCG) methods. Hemoglobin measurements before and after intervention with a *hematology analyzer.* Protein Energy Malnutrition was defined when the rats had albumin levels <3g/dl and hemoglobin levels <13 g/dl.

Statistical analyses were performed using the IBM SPSS Statistic 20 software. Data are presented as mean ±SD or median. Shapiro-Wilk test to determine the normality of data distribution, paired t-test and one-way ANOVA were used for parametric results, followed by Post-hoc Bonferroni was performed to analyze the difference in the effect of the four groups. Wilcoxon and Kruskal-Walli′s test was used for nonparametric results.

RESULTS AND DISCUSSION

The average body weight of the rats significantly increased in all the groups (p<0,05). In table 1, the highest weight gain after intervention occurred in the P1 group (29(29-31) g). Wilcoxon test results showed a significant difference between body weight before and after administration of kedawung seed tempeh flour *(Parkia roxburghii G. Don)* in groups K- (p=0.026), K+ (p=0.027), P1 (p=0.020) and P2 (p=0.026). The test was continued with the Kruskal Wallis test which showed that there was a difference in the average body weight gain of rats between groups (p=0.000). There is protein and fat content in kedawung seed tempeh flour *(Parkia roxburghii G. Don)* helps increase the body weight of rats, after weight loss in protein energy malnutrition conditions.

Table 1.
The Average Body Weight Before and After Intervention

Group	Body Weight (g)			
	Pre	Post	Δ	*p*
K-	124 (120-128)	156 (154-161)	33 (31-35)	0,026*
K+	99 (97-104)	109 (107-113)	10 (7-12)	0,027*
P1	100 (99-103)	130 (128-132)	29 (29-31)	0,020*
P2	102,5 (98-105)	129 (126-132)	27.5 (25-28)	0,026*
p^1	0,002	0,000	0,000	

Notes: p= Wilcoxon test, p^1= Kruskal Wallis test; * = significant (p <0.05)

Table 2.
Albumin Levels Before and After Kedawung Seed Tempeh Flour *(Parkia roxburghii G.Don)* Intervention

Group	Albumin Levels (g/dl)			
	Pre	Post	Δ	*P*
K-	4,84 ± 0,37	4,61 ± 0,25	-0,23 ± 0,14	0,012*
K+	1,29 ± 0,24	1,26 ± 0,24	-0,03± 0,17	0,009*
P1	1,33 ± 0,08	4,28 ± 0,13	2,95 ± 0,19	0,001*
P2	1,21 ± 0,08	3,95 ± 0,05	2,74 ± 0,10	0,001*
P^1	0,001	0,001	0,001	

Notes: p= Paired t-test, p^1= One way Anova test;* = significant (p <0.05)

Table 3.
Hemoglobin Levels Before and After Kedawung Seed Tempeh Flour *(Parkia roxburghii G. Don)* Intervention

Group	Hemoglobin Levels (g/dl)			
	Pre	Post	Δ	P
K-	14,9 (13,40 – 16,20)	15,75 (14,80 – 16,70)	0,95 (−0,20–2,50)	0,104
K+	12,10 (11,40 – 13,00)	10,10 (8,60 – 11,30)	– 2,25 ((−3,20)– (−1,20))	0,027*
P1	12,05 (10,40 –13,20)	14,75 (12,70 – 17,10)	2,40 (2,20 – 3,90)	0,027*
P2	12,30 (9,50 –12,80)	12,90 (12,30 – 13,70)	1,10 ((−0,30) – 3,60)	0,116
P^1	0,005	0,001	0,001	

*Notes; p= Wilcoxon test, p^1= Kruskal Wallis test; * = significant (p <0.05)*

Serum albumin levels in rats ranged from 3.0-5 g/dl. Table 2 shows groupsP1 and P2 after treatment each had an average normal albumin level (4.28 ± 0.13 g/dl) and (3.95 ± 0.05 g/dl). The K- group had the highest albumin level (4.61 ± 0.25 g/dl) than the P1 and P2 groups, but after the intervention the average albumin level in the P1 group was closer to the K- group. Paired t-test results showed a significant difference in albumin levels before and after treatment in groups K- (p = 0.012), K+ (p=0.009), P1 (p=0.001) and P2 (p=0.001). Analysis data continued with One-way Anova test showed that there was a significant difference after giving kedawung seed tempeh flour *(Parkia roxburghii G. Don)* (p = 0.001).

In table 3, after the intervention periods, groups P1 and P2 each had hemoglobin levels (14.75(12.70 – 17.10 g/dl) and (12.90 (12.30–13.70 g/dl). K- had higher hemoglobin levels (15.75(14.80 – 16.70) g/dl) compared to groups P1 and P2, but hemoglobin levels in the P1 group were closer to the K- groups. The results of the Wilcoxon test showed a significant difference between hemoglobin levels before and after administration of kedawung seed tempeh flour *(Parkia roxburghii G. Don)* in the K+ group (p=0.027) and P1 group (p=0.027). However, there was no significant difference in hemoglobin levels before and after the intervention in the K- group (p=0,104) and P2 group (p=0,116). The test was continued with the Kruskal Wallis test which showed differences in hemoglobin levels between groups (p=0.001).

The weight gain of rats after treatment in the K-group was higher than in all groups, because the K-group rats were given standard feed. The standard feed provided contains complete macronutrients including carbohydrates, protein, fat, fiber, and vitamins. The K- group from the beginning when the K+, P1 and P2 groups were given a low-protein diet had a higher body weight than the three groups, so that the intervention group also gave more weight gain.

Nutrient intake is one of the factors for increasing body weight. The composition of feed affects the optimization of digestibility and absorption of nutrients in the body (Khasanah, Ariani, Angwar, & Nuraeni, 2015). There is a fairly high protein, fat, amino acids content in kedawung seed tempeh flour *(Parkia roxburghii G. Don)* so that it helps increase the body weight of rats, after experiencing weight loss in PEM conditions. Protein plays an important role in the growth and maintenance of body tissues, as well as a source of energy for humans and animals, so it is possible to improve the nutritional status of rats with increased body weight (Triawanti, Yunanto, Sanyoto, & Nur′Amin, 2018). Essential amino acids play a major role in protein synthesis in the body related to the growth and development of children (Semba et al., 2016).

The K+ group fed a low protein diet (0%) had the lowest albumin levels, thus proving that a low protein diet (0%) for 2 weeks decreased albumin levels. This is in line with the before studywhich showed that feeding low-protein diets for 14 days in rats reduced albumin levels (Utariani, Rahardjo, & Perdanakusuma, 2020). Studies in PEM children also show that the mean albumin levels are significantly lower than in well-nourished children (Gupta & Gupta, 2020). Low serum albumin levels are a common finding in PEM, and are usually used as a parameter to assess a child's nutritional status (Kumar & Singh, 2013).

The decrease in albumin levels in the K+ group was due to a lack of protein intake that lasted for 2 weeks. Protein is synthesized in the liver (Warrier, Dole, Warder, & Suskind, 1990), and protein synthesis is modulated by dietary factors, such as amino acids and protein intake (Anna E. Thalacker-Mercer, Craig A. Johnson, Kevin E. Yarasheski & Campbell, 2007; Caso et al., 2007; Feo, Haymond, Medicina, Feo, & Horber, 1992; Moore et al., 2009). Reducing protein consumption slows the synthesis of albumin mRNA which causes low albumin levels (Nicholson, Wolmarans, & Park, 2000), so the decrease in protein synthesis is related to albumin synthesis.

In groups P1 and P2 there was an increase in albumin levels after the rats hypoalbuminemia. Elevated serum albumin levels are closely related to protein and amino acids, which increase muscle protein synthesis leading to protein balance. Thus the availability of amino acids greatly affects protein synthesis, because amino acids modulate cellular processes leading to protein synthesis through the initiation of translation. Amino acids can alter the redox state of serum albumin leading to increased levels of reduced serum albumin (Ricardo et al., 2015; Verbruggen et al., 2011; Wada, Takeda, & Kuwahata, 2018). There is protein and amino acids content in kedawung seed tempeh flour*(Parkia roxburghii G. Don)* helps increase albumin levels in protein energy malnutrition rats. Studies on PEM children under five with soybean tempeh juice have shown an increase in albumin levels (Saputra, 2014). The results of this study also showed that the administration of kedawung seed tempeh flour *(Parkia roxburghii G. Don)* was able to increase albumin levels when compared to the K+ group who were not given kedawung seed tempeh flour *(Parkia roxburghii G. Don).*

The K+ group fed a low protein diet (0%) had the lowest hemoglobin content, thus proving that a low protein diet (0%) for 2 weeks could reduce hemoglobin levels. This is in line with the before study which showed that feeding low in protein to rats reduced hemoglobin levels(Ling et al., 2004). The decrease in hemoglobin levels in the K+ group was due to the lack of protein intake in rats. Protein plays an important role in the transportation of iron in the body. Decreased protein intake results in inhibited iron transport so iron deficiency will occur and experience a deficiency in hemoglobin levels (Victor W. Rodwell; Robert K. Murray, 2015).

The P1 treatment group experienced an increase in hemoglobin levels after administration of kedawung seed tempeh flour *(Parkia roxburghii G. Don),* although the P2 group was not significant. Hemoglobin is a complex protein compound, therefore the availability of Fe and protein plays a role in the synthesis process (Linder, 2009). There is protein and Fe content in kedawung seed tempeh flour*(Parkia roxburghii G.Don)* helps increase hemoglobin levels in protein energy malnutrrition rats.The protein content of Kedawung seed tempeh flour is 32.4% and the iron content of Kedawung seed tempeh flour is 9.98%. In human studies, supplementation of soy tempeh in infants aged 12-18 months when eating for 6 months can increase hemoglobin, serum iron, and ferritin (Wulandari Sidharta & Susanto, 2017). The results of this study also showed that the administration of kedawung seed tempeh flour *(Parkia roxburghii G. Don)* was able to increase hemoglobin levels when compared to the K+ group who were not given kedawung seed tempeh flour *(Parkia roxburghii G. Don)*. The effect of kedawung seed tempeh flour *(Parkia roxburghii G. Don)* on increasing body weight, albumin levels, and hemoglobin levels indicates that kedawung seed tempeh flour *(Parkia roxburghii G. Don)* can be used as a functional food to resolve malnutrition problems in children.

CONCLUSIONS AND SUGGESTIONS

The administration of kedawung seed tempeh flour *(Parkia Roxburghii G. Don)* increases albumin levels and hemoglobin levels in rats with PEM with the most effective dose being 1.5 g/100 gr BW/d. It is recommended that further research carry out organoleptic and acceptability tests on the application of kedawung seed tempeh *flour (Parkia Roxburghii G. Don)* in food processing for protein energy malnutrition children.

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ETHICAL CONSIDERATIONS

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Conflict of Interest Statement

There are no conflicts of interest.

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