TURNITIN_The_effect_of_tempeh_flour

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THE EFFECT OF TEMPEH FLOUR FROM TREE BEAN (*PARKIA TIMORIANA* (DC) MERR.) SEEDS ON LIPID PROFILE IN PROTEIN-ENERGY MALNUTRITION RATS

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ABSTRACT

Children with protein-energy malnutrition (PEM) need a food product that can be easily absorbed by the body because they have impaired nutrient utilization and lipid profile disorder. The provision of fermented products such as tempeh is an alternative approach. Seeds of tree bean (*Parkia timoriana* (DC) Merr.) are a good source of various nutrients needed by children with PEM. This study aimed to prove the effectiveness of the administration of tempeh flour from tree bean (*Parkia timoriana* (DC) Merr.) seeds on the lipid profile in PEM rats. PEM in rats was induced by a zero-protein diet containing granulated sugar (20 g), vegetable oil (1 g), and cornstarch (24 g). The animals were categorized into a normal control group (K+), a PEM group without treatment (K−); a group fed tempeh flour from tree bean (*Parkia timoriana* (DC) Merr.) seeds 1.5 $g.kg^{-1}$ BW (P1); and a group fed tempeh flour from tree bean (*Parkia timoriana* (DC) Merr.) seeds 3 $g.kg^{-1}$ BW (P2). The intervention was administered for 30 days. There was a significant increase in HDL levels ($p = 0.001$) and significant decreases in cholesterol ($p = 0.001$), triglyceride ($p = 0.001$), LDL levels ($p = 0.001$), and atherogenic index of plasma (AIP) ($p = 0.000$) in treatment groups. In conclusion, the administration of tempeh flour from tree bean (*Parkia timoriana* (DC) Merr.) seeds increases HDL levels and decreases cholesterol, triglycerides, and LDL level, and atherogenic index of plasma (AIP) in rats with PEM.

Keywords: atherogenic index of plasma; lipid profile; protein-energy malnutrition; tempeh flour; tree bean

INTRODUCTION

Protein-energy malnutrition (PEM) is defined as malnutrition in which energy and protein intake are under the nutritional adequacy rate **(Adriani and Wirjatmadi, 2016; Data and Information Center Ministry of Health Indonesia, 2015)**. It occurs because of a lack of consumption of foods containing calories and protein and the presence of infectious diseases that affect nutrient utilization **(Adriani and Wirjatmadi, 2016)**.

The highest prevalence of PEM in children under 5 years of age **(Adriani and Wirjatmadi, 2016)**. The prevalence of stunted and wasted children in 2019 in the world was 21.3% and 6.9%, respectively **(UNICEF, 2020)**. Based on Riskesdas 2018, in Indonesia, the prevalence of malnutrition and undernutrition was about 3.9% and 13.8%, respectively **(Center Ministry of Health Indonesia, 2018)**.

Undernutrition and the presence of infectious diseases can aggravate the diseases and worsen nutritional status. Poor nutrition in children leads to slow growth and affects cognitive abilities **(UNICEF, 2020)**. PEM causes hypoalbumin and decreases the immune system **(Hoffer, 2001)**. Also, this condition affects the lipid profile in which children who are malnourished will have high levels of triglycerides, LDL, and cholesterol and low HDL levels **(Alves et al., 2014; Velásquez and Cano, 2012)**.

Children with PEM need food products that are easily absorbed by the body because of impaired utilization of nutrients. Fermented foods contain nutrients that are easily absorbed by the intestines; one of the fermented foods is tempeh **(Masdarini, 2011)**. Tempeh is consumed by Indonesians, as a functional food. It is typically made from soybeans using Rhizopus sp. as its starter **(Cempaka et al., 2018)**.

Tree bean (*Parkia timoriana* (DC) Merr.) is a plant that is still classified as a legume or Leguminosae. Tree bean seeds are high in protein, minerals, essential amino acids, and fatty acids **(Mohan and Janardhanan, 1993)**. Several studies have demonstrated that tree bean seeds have anti-proliferative, antibacterial, and antioxidant functions. **(Angami et al., 2018)**. This study aimed to prove the effectiveness of the administration of tempeh flour from tree bean (*Parkia timoriana* (DC) Merr.) seeds on the lipid profile in rat models of zero-protein-diet-induced PEM.

Figure 1 Tree bean (*Parkia timoriana* (DC) Merr) seeds.

Figure 2 Peeling the black part of tree bean seeds.

Figure 3 Inoculation.

Figure 4 Tempeh from tree bean seeds after fermentation 48 h.

Figure 5 Drying tempeh.

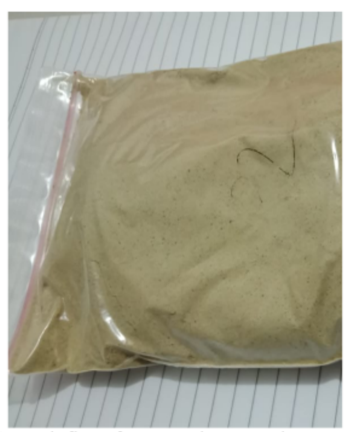

Figure 6 Tempeh flour from tree bean seeds.

Scientific hypothesis

Tempeh flour from tree bean (*Parkia timoriana* (DC) Merr.) seeds increases HDL levels and decreases cholesterol, triglycerides, and LDL level and atherogenic index of plasma (AIP) in rats with PEM.

MATERIAL AND METHODOLOGY

This study is included in the research project Study of the Administration of Tempeh Flour from Tree Bean (*Parkia timoriana* (DC) Merr.) Seeds in Nutritional Status, Hematological Profile, Immune Status, and Metabolic Response on PEM Rats.

Samples

This study used tempeh flour from tree bean seeds as samples. Tree bean seeds (Figure 1) were obtained from suppliers in Klaten, Central Java, Indonesia.

Animals

Male Wistar rats (8 – 12 weeks old, 150 – 200 g, n = 24), were acquired from the Centre for Food and Nutrition of Universitas Gadjah Mada, Yogyakarta, Indonesia.

Laboratory Methods

Albumin and hemoglobin level measurements as criteria of PEM were performed after 14 days of zero-protein diet administration. Measurements of cholesterol, triglyceride, LDL, and HDL levels were performed before the intervention and at the end of the intervention. Blood sampling was done to analyze albumin, hemoglobin, cholesterol, triglyceride, LDL, and HDL through the plexus retroorbital. Examination of triglyceride used Glycerol 3 Phospate Oxidase-Peroxidase Amino Phenazone (GPO-AP) method, meanwhile cholesterol, LDL and HDL used Cholesterol Oxidase-Peroxidase Aminoantipyrine Phenol (CHOD-PAP) with enzymatic spectrophotometric basis. The atherogenic index of plasma (AIP) was calculated from the log (triglyceride/HDL). Blood serum was analyzed in the Centre for Food and Nutrition of Universitas Gadjah Mada, Yogyakarta, Indonesia.

Description of the Experiment

The Processing of Tempeh from Tree Bean Seeds

The seeds were washed and soaked in water for 2 h. Then, the soaking water was removed, and the seeds were washed thoroughly. Tree bean seeds were boiled in water for 2 h. Then, the water was removed and tree bean seeds were drained. Tree bean seeds were peeled (the black part) as seen in Figure 2 and soaked in boiled water for 18 h (the water was changed regularly every 6 h). Tree bean seeds that had been soaked, drained, and boiled for 30 min were then removed and drained again. Tree bean seeds were aerated to dry and then split into two pieces. The tempeh starter was given to tree bean seeds and mixed well. The mixture was placed in plastic bags with a small hole on two opposite sides (Figure 3). It was then left for two nights until mushrooms appeared on the tempeh and began to solidify (Figure 4).

Processing of Tempeh Flour from Tree Bean Seeds

Tempeh from tree bean seeds was sliced using a slicer and then blanched with hot steam for 2 min. Next, tempeh was dried by oven at 60 °C for 6 h (Figure 5), ground with a grinder, and then sieved using a 60 mesh sieve (Figure 6). Tempeh flour was stored in airtight containers. The process was carried out at the Centre for Food and Nutrition of Universitas Gadjah Mada, Yogyakarta, Indonesia.

Animal Treatments

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 (n = 6/group): the normal control group (K+), the PEM group without treatment (K−), the group fed tempeh flour from tree bean (*Parkia timoriana* (DC) Merr.) seeds at 1.5 $g.kg^{-1}$ BW (P1), and the group fed tempeh flour from tree bean (*Parkia timoriana* (DC) Merr.) seeds at 3 $g.kg^{-1}$ BW (P2). All animals, except the K+ group animals, were fed a zero-protein diet for 14 days. This diet contained granulated sugar (20 g), vegetable oil (1 g), and cornstarch (24 g) and was administered orally at 10 g per rat per day. PEM was defined when the rats had albumin <3 $g.dL^{-1}$ and hemoglobin <10 $mg.dL^{-1}$.

Statistical Analysis

Statistical analyses were performed using the IBM SPSS Statistic 22 software. Data are presented as mean ±SD or median. A paired *t*-test and a one-way analysis of variance were used for parametric results; differences between the groups were evaluated using the post-hoc test. Wilcoxon, Kruskal–Wallis, or Mann–Whitney U test was used, as appropriate, for non-parametric results.

RESULTS AND DISCUSSION

The average body weight of the animals significantly increased in all the groups (p <0.05), as seen in Table 1. The P1 group (29(29-31) g) experienced the highest weight gain after the administration of tempeh flour from tree bean seeds compared to the P2 group (27.5(25-28) g). K+ (33(31-35) g) and K− (10(7-12) g) groups that were not given any treatment experienced a significant increase in body weight (p <0.05). Bodyweight gain is due to the nutrient content in the feed. Protein and fat content in tempeh flour from tree bean seeds may increase body weight in rats with PEM.

The results of collaboration research showed that the rats acquired PEM after the administration of the zero-protein diet for 14 days. Rats achieved PEM with albumin (1.21 – 1.33 $g.dL^{-1}$) and hemoglobin (11.86 – 12.13 $g.dL^{-1}$) **(Ulfa, 2020)**. In the previous study, the provision of a zero-protein diet for 2 weeks in rats indicates decreases in albumin and hemoglobin levels **(Anggraeny et al., 2016)**. PEM causes hypoalbuminemia where albumin levels are under a normal value <3.5 $g.dL^{-1}$. Hypoalbuminemia leads to impairment of albumin synthesis and other proteins by the liver **(Murray et al., 2003)**.

A significant difference in triglyceride was found after the administration of tempeh flour from tree bean seeds in the K+ (p = 0.001), K− (p = 0.036), P1 (p = 0.001), and P2 (p = 0.056) groups, as seen in Table 2. Significantly decreased triglyceride levels were found in the P1 and P2 groups, implying that two doses of tempeh flour from tree bean seeds could significantly decrease triglyceride levels in rats with PEM. Based on Table 3, there was a significant decrease in total cholesterol in the K+ (p = 0.028), K− (p = 0.028), P1 (p = 0.028), and P2 (p = 0.028) groups after the administration of tempeh flour from tree bean seeds.

Table 1 The average body weight before and after treatments.

Groups	Bodyweight (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*	

Note: normal control group (K+), PEM group without treatment (K-), tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 1.5 $g.kg^{-1}$ BW group (P1), and tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 3 $g.kg^{-1}$ BW group (P2); *p* = Wilcoxon test, p^1 = Kruskal Wallis test; * = significant (p <0.05)

Table 2 Triglycerides level before and after the administration of tempeh flour from tree bean seeds.

Groups	Triglycerides ($mg.dL^{-1}$)			
	Pre	Post	Δ	*p*
K+	67.8 ±2.089 ^{b,c,d}	69.29 ±2.58^{b,c,d}	1.44 ±0.517^{c}	0.001*
K-	105 ±3,7276^{a}	109 ±3.88^{a,c,d}	3.87 ±3.32^{c}	0.036*
P1	105 ±2,888^{a}	80.82 ±2.46^{a,b,d}	-24.45 ±3.68^{a,b,d}	0.001*
P2	103 ±5,83^{a}	95.61 ±3.059^{a,b,c}	-7.94 ±784^{c}	0.056*
p^1	0.001*	0.001*	0.001*	

Note: normal control group (K+), PEM group without treatment (K-), tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 1.5 $g.kg^{-1}$ BW group (P1), and tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 3 $g.kg^{-1}$ BW group (P2); *p* = Wilcoxon test, p^1 = Kruskal Wallis test; * = significant (p <0.05).

Table 3 Total cholesterol levels before and after the administration of tempeh flour from tree bean seeds.

Groups	Total cholesterol levels ($mg.dL^{-1}$)			
	Pre	Post	Δ	*p*
K+	74.82(71.53-76.64)	76.57(72.8-78.66)^{b,c,d}	1.75(0.97-2.54)^{c,d}	0.028*
K-	1.56(151.82-167.88)	1.59(155.65-168.2) ^{a,c,d}	2.2(0.32-8.01)^{c,d}	0.028*
P1	1.50(146.72-155.47)	93.31(91.21-97.91) ^{a,b,d}	-55.44(-64.26-(-51.73))^{a,b}	0.028*
P2	1.54(146.28-157.66)	1.03(99.58-107.11) ^{a,b,c}	-49.04(-57.24-(-42.53))^{a,b}	0.028*
p^1	0.001*	0.001*	0.001*	

Note: normal control group (K+), PEM group without treatment (K-), tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 1.5 $g.kg^{-1}$ BW group (P1), and tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 3 $g.kg^{-1}$ BW group (P2); *p* = Wilcoxon test, p^1 = Kruskal Wallis test; * = significant (p <0.05).

Table 4 LDL levels before and after treatments tempeh flour from tree bean seeds.

Groups	LDL levels ($mg.dL^{-1}$)			
	Pre	Post	Δ	P
K+	21.11(18.69-23.53)	22.38(20.98-25.17)^{b,c,d}	0.94(0.22-3.69)^{c,d}	0.028*
K-	56.05(54.67-58.13)	57.34(53.85-59.44) ^{a,c,d}	0.59(-0.82 -1.99)^{c,d}	0.172
P1	56.06(53.29-57.44)	27.62(23.78-30.77) ^{a,b,d}	-27.74(-33.66-(-25.98))^{a,b,d}	0.028*
P2	55.02(53.98-60.9)	36.01(33.57-37.76) ^{a,b,c}	-20.05(-25.24-(-16.22))^{a,b,c}	0.028*
p^1	0.004*	0.001*	0.001*	

Note: normal control group (K+), PEM group without treatment (K-), tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 1.5 $g.kg^{-1}$ BW group (P1), and tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 3 $g.kg^{-1}$ BW group (P2); *p* = Wilcoxon test, p^1 = Kruskal Wallis test; * = significant (p <0.05).

Table 5 HDL levels before and after treatments tempeh flour from tree bean seeds.

Groups	HDL levels ($mg.dL^{-1}$)			
	Pre	Post	Δ	*p*
K+	79.25(76.87-81.63)	77.775(72.03-80.46)^{b,c,d}	-1.34(-6.2-(-0.24))^{c,d}	0.028*
K-	46.26(44.22-51.02)	46.36(43.68-48.28) ^{a,c,d}	-0.5(-2.74-1.16)^{c,d}	0.249
P1	45.24(42.86-47.62)	66.285(64.37-70.5) ^{a,b,d}	21.55(20.32-22.88)^{a,b,c}	0.028*
P2	44.9(42.86-47.62)	58.62(55.17-61.3) ^{a,b,c}	12.57(9.85-18.44)^{a,b,d}	0.028*
p^1	0.003*	0.001*	0.001*	

Note: normal control group (K+), PEM group without treatment (K-), tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 1.5 $g.kg^{-1}$ BW group (P1), and tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 3 $g.kg^{-1}$ BW group (P2); *p* = Wilcoxon test, p^1 = Kruskal Wallis test; * = significant (p <0.05).

Table 6 AIP before and after treatments tempeh flour from tree bean seeds.

Groups	AIP ($mg.dL^{-1}$)			
	Pre	**Post**	**Δ**	***p***
K+	-0.07±0.01	-0.048±0.03	0.02(0.01-0.05)	0.048*
K-	0.35±0.01	0.38±0.01	0.03±0.01	0.001*
P1	0.36(0.35-0.41)	0.09±0.02	-0.28±0.01	0.027*
P2	0.36(0.34-0.42)	0.21±0.03	-0.13(-0.25-(-0.11))	0.027*
p^1	0.004*	0.000*	0.000*	

Note: normal control group (K+), PEM group without treatment (K-), tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 1.5 $g.kg^{-1}$ BW group (P1), and tempeh flour from seeds of *Parkia timoriana* (DC) Merr., 3 $g.kg^{-1}$ BW group (P2); p = Wilcoxon test, p^1 = Kruskal Wallis test; * = significant (p <0.05).

The highest decrease in total cholesterol occurred in the P1 group, followed by that in the P2 group. LDL levels in the K+ (p = 0.028), P1 (p = 0.028), and P2 (p = 0.028) groups showed significant differences after the administration of tempeh flour from tree bean seeds. Meanwhile, there was no significant difference in LDL levels in the K− group (p = 0.172) before and after treatment (Table 4). Significantly decreased LDL levels were found in the P1 and P2 groups after treatment. A significant difference was observed in HDL levels after treatment in the K+ (p = 0.028), P1 (p = 0.028), and P2 (p = 0.028) groups. There was no significant difference in the K− (p = 0.249) group. HDL levels in the P1 group were higher than in the P2 group, as seen in Table 5.

Atherogenic index of plasma (AIP) in K+ (p = 0.048), K− (p = 0.001), P1 (p = 0.027) and P2 (p = 0.027) groups showed significant differences after treatments (Table 6). AIP in P1 and P2 groups decreased significantly after the administration of tempeh flour from tree bean seeds. Significantly decreased triglyceride levels and increased HDL levels in the P1 and P2 groups caused AIP in both groups to decrease. AIP is the strongest marker to predict cardiovascular disease (CVD) risk (Khazaál, 2013). Malnutrition worsens the prognosis of heart failure **(Amare, Hamza, and Asefa, 2015)**.

Total cholesterol, triglyceride, and LDL levels increased in rats with PEM; meanwhile, HDL levels decreased compared to the normal control group. Chronic malnutrition causes endocrine changes leading to metabolic profile disorders. Previous studies demonstrated that 98.9% of children have dyslipidemia with low HDL and hypertriglyceridemia **(Veiga et al., 2010)**. A previous study that was conducted on preschool children indicated that malnourished children had lower HDL levels than normal children, and higher triglyceride levels were found in malnourished children **(Velásquez and Cano, 2012)**.

Tree bean seeds in this study were processed into fermented food as tempeh **(Masdarini, 2011)**. Fermentation can increase protein digestibility through the breakdown of complex proteins into a more soluble form **(Nkhata et al., 2018)**. Besides that, tempeh is one of the functional foods that have long been consumed by Indonesians **(Cempaka et al., 2018)**. Tree bean seeds contain rich protein (albumin and globulin), minerals (magnesium, potassium, phosphorus, zinc, iron, and manganese), essential amino acids (phenylalanine, leucine, isoleucine, and tyrosine), and fatty acids such as linoleic acid and oleic acid **(Mohan and Janardhanan, 1993)**. Tree bean seeds contain a thioproline compound that gives them a distinctive odor **(Thangjam and Sahoo, 2012)**. Thioproline is an antioxidant compound and a free radical scavenger. These compounds inhibit the production of nitroso compounds by reducing bacteria and the reaction of nitrogen species **(Kumagai, 2003)**.

In this study, the administration of tempeh flour from tree bean seeds reduced triglycerides, cholesterol, and LDL levels and increased HDL levels in PEM rats. These results are consistent with previous studies, in which, the administration of tempeh flour decreased total cholesterol in high-saturated fat-diet induced rats **(Ridwan, 2015)**. The provision of black soybean tempeh and yellow soybean tempeh decreased LDL cholesterol **(Priastiti and Puruhita, 2013)**. The administration of ethanol extract of tree bean seeds in hyperlipidemia rats could reduce triglycerides, cholesterol, and LDL levels and increase HDL levels **(Dewi, 2018; Fitriani, 2018)**. In a previous study, the administration of a tree bean seed extract decreased high levels of triglycerides and total cholesterol **(Sheikh et al., 2016)**. Flavonoids in tree bean seeds have a hypolipidemic effect that can reduce cholesterol, triglyceride, and LDL levels and increase HDL levels **(Bao et al., 2016; Dubey et al., 2020)**. Flavonoids can inhibit the expression of fatty acid synthase in the liver, which is stimulated by AMPK activity on hepatocyte cells through the kinase B1 pathway **(Pil Hwang et al., 2013)**. Besides, flavonoids reduce fatty acid synthesis in the liver and fat accumulation **(Do et al., 2013)**.

The content of essential amino acids found in tree bean seeds also plays a role in reducing triglyceride, cholesterol, and LDL levels in PEM rats. A previous study explained that the provision of essential amino acid supplements and phytosterols are taken 3 times a day for 4 weeks can reduce triglyceride, cholesterol, and LDL levels in the subject **(Coker et al., 2015)**. The administration of a protein-rich mucuna product in hyperlipidemic rats reduced LDL cholesterol, total cholesterol, and triglyceride levels. Proteins have hypolipidemic activity due to the presence of secondary metabolites, such as phytates and polyphenols. Polyphenols induce metabolic hypolipidemia with their ability to reduce cholesterol acyltransferase and HMG-CoA reductase activity **(Ngatchic et al., 2016)**. The administration of leucine and valine in high-fat-diet rats for 8 weeks can increase HDL cholesterol levels **(Cojocaru et al., 2012)**.

Tree bean seeds contain a thioproline compound that produces a distinctive odor in tree bean seeds. Thioproline contains amino acids and is a natural metabolite that acts as an intracellular sulfhydryl antioxidant and a free radical scavenger and protects cell membranes from damage caused by oxygen derivatives **(Cavallini et al., 1956)**. Thioproline that binds to proteins by hydrogen bonds and van der Waals forces exhibits a strong inhibitory effect. Thioproline is also effective in protecting against oxidative damage in rat brain tissue **(Lyu et al., 2020)**. Antioxidants play a role in decreasing LDL levels and increasing HDL levels through several mechanisms, such as activating the transcription factor NrF2 and increasing antioxidant enzymes, as well as neutralizing oxidative species and inhibiting the activation of the nuclear transcription factor-κB (NF-κB) signaling pathway **(Ademuyiwa et al., 2005; Brunzell et al., 2008; Hegazy et al., 2019; Oršolić and Car, 2014; Oršolić et al., 2019)**. Thioproline in dietary supplements can protect the body from diseases associated with oxidative stress, such as atherosclerosis **(Ham, Jason Chan and Chan, 2020)**.

CONCLUSION

The administration of tempeh flour from tree bean (*Parkia timorina* (DC) Merr.) seeds increases HDL levels and decreases cholesterol, triglycerides, LDL level, and atherogenic index of plasma (AIP) in rats with PEM with the most effective dose is 1.5 $g.kg^{-1}$ BW.

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