Advances in Social Science, Education and Humanities Research, volume 535
Proceedings of the 1st Paris Van Java International Seminar on Health, Economics, Social Science and Humanities (PVJ-ISHESSH 2020)

Role of Tempeh Formula as a Source of Vitamin B_{12} and Its Implementation for Vegetarian Diet

1st Susianto
Graduate School of Public Health
STIKes Kuningan
Indonesia
susiantostikku@gmail.com

***Abstract*—Tempeh is a traditional and nutritious food of Indonesia which contains vitamin B_{12} and vegetarians have higher risk of vitamin B_{12} deficiency. The objective was to investigate the effect of tempeh consumption on the level of serum vitamin B_{12} and homocysteine in vegetarian. Methods: Experimental study with randomized control and double blind. The total samples were 118 vegetarians from Pekanbaru in 2010 consisted of 42 vegetarians with vitamin B_{12} deficiency treated by vitamin B_{12} fortification. The 76 normal vegetarians aged above 17 years old were randomly assigned to consume 100g tempeh formula (5.9 µg vitamin B_{12}) daily (n=38) or placebo (n=38). Vitamin B12 in tempeh formula and serum were analyzed by spectrophotometer before and after 3 months intervention, and after retention for 3 months without intervention. Results: Prevalence of vitamin B_{12} deficiency in lacto-ovo-vegetarian was 35.6%. Delta of serum vitamin B_{12} after retention for 3 months without intervention was +3.5 pmol/L in tempeh group (p = 0.804) and -19.2 pmol/L in placebo group (p = 0.038). There was a significant correlation between serum vitamin B_{12} and homocysteine. Conclusion: Tempeh could maintain normal level of serum vitamin B_{12} after retention for 3 months without intervention in vegetarian.**

Keywords—Tempeh Formula, Vitamin B12, Vegetarian Diet

I. INTRODUCTION

Vitamin B_{12} is an essential micronutrient that plays a role in thes formation of cells such as red blood cells [3]. Declining level of vitamin B_{12} due to chronic low intake over a period of time or due to mal-absorption of vitamin B_{12} by intestine will have a negative balance which can cause problem in DNA synthesis as seen obviously in red blood cell division and nerve cells as well. This can lead to megaloblastic anemia and nerve damage [13]. Naturally, vitamin B_{12} (cobalamin) only comes from the synthesis of bacteria or microorganisms that grow in soil or water, feces, intestine of ruminant animal and human. Natural sources of vitamin B_{12} is limited to animal-based foods [14] so that people who adopt a vegetarian diet are at risk of vitamin B_{12} deficiency [5]. A vegetarian diet consists of plant-based foods with or without eggs and milk [8].

Vegetarian diet has been linked with a normal lipid profile, higher antioxidant, phytochemical and fiber [12]. Thus, studies believe that vegetarians could potentially prevent heart disease, stroke, obesity, hypertension, cancer, diabetes and osteoporosis [1] [7] [11] Herrmann et al in 2003 reported that there were 52% of adult vegans, 26% of lacto-ovo-vegetarians and 1% of non-vegetarian have vitamin B_{12} deficiency in Germany and Netherlands [6]. Prevalence of vitamin B_{12} deficiency in elderly over fifty years is estimated at 3 percent to 40 percent [2].

Tempeh is a non-salted fermented soybeans inoculated with Rhizopus oligosporus mold after boiling, soaking and wrapped incubated at 30^0 – 37^0 C for 24 - 36 hours. The average level of vitamin B_{12} in tempeh is about 5µg/100g [10]. Vitamin B_{12} in tempeh is produced by Klebsiella pneumoniae and Citrobacter freundii bacteria during the soaking and fermentation process. Klebsiella pneumoniae and Citrobacter freundii are not pathogenic and harmful to health [4].

II. METHODOLOGY

This was an experimental study, community trial with randomized control, double-blind, obtaining primary data to determine the effect of consumption of tempeh formula as a source of vitamin B_{12} on level of serum vitamin B_{12} and serum homocysteine in the intervention group compared with the control group who were given a placebo (without tempeh). This study was conducted in the city of Pekanbaru for 6 months from March to September 2010. The population was the whole vegetarians in Pekanbaru. The sample was vegetarians aged 17 years old in Pekanbaru that had been a member of Indonesia Vegetarian Society (IVS) Pekanbaru branch, selected by random sampling based on IVS members list. The inclusion criteria was applied for those vegetarians who were members of IVS Pekanbaru branch, both men and women age 17 years old, agreed to be interviewed about the individual characteristics, diet and willing to consume tempeh formula (100g/day) for 3 months, to follow blood test for 3 times at the initial intervention in March 2010, the end of the intervention in June 2010 and 3 months retention after the intervention in September 2010. The exclusion criteria was applied for those vegetarians who were vitamin B_{12} deficiency (serum vitamin B_{12} level less than 156 pmol/L),

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smokers, alcohol drinkers and kidney diseases. The sample size in this study was calculated by hypothesis testing for two population means [9].

$Sp^2 = [(n_1 - 1)\ S_1^2 + (n_2 - 1)\ S_2^2] / [(n_1 - 1) + (n_2 - 1)]$

$$n = \frac{2\sigma^2}{} \frac{\{Z_{1-\alpha/2} + Z_{1-\beta}\}^2}{(\mu_1 - \mu_2)^2}$$

Total number of samples required for this study was 64 people consisting of 32 people for the intervention (tempeh) group and 32 people for the control (placebo) group.

Value S_p^2 or σ2, μ1, μ2 obtained from the intervention study done by Winkels et al in Netherland, using vitamin B_{12} fortification in bread [15]. The total sample was 118 lacto-ovo-vegetarians consisted of 42 subjects with vitamin B_{12} deficiency treated by fortification of vitamin B_{12}. Cut-off point used in this study for vitamin B_{12} deficiency was below 156 pmol/L and hyperhomocysteinemia was above 12 μmol/L [6]. Men and women aged above 17 years old were randomly assigned in this 3 months double-blind, placebo-controlled trial to consume tempeh formula with average content of 5.9 μg vitamin B_{12} daily (n=38) or placebo (n=38). Vitamin B_{12} in tempeh formula was analyzed by uv-vis spectrophotometer using microbial assay method. Serum vitamin B_{12} was measured by Electrochemiluminescent immunoassay method, serum homocysteine was measured by Microparticle enzyme immunoassay method. Serum vitamin B_{12} and serum homocysteine were analyzed before and after 3 month intervention, then retention for 3 months without intervention.

A. Procedures and Materials of Intervention

The intervention material provided in this study was tempeh formula called "VEGAN 1" made by the Nutrition Research and Development Center in Bogor, West Java with ingredients of tempeh, oatmeal, sugar, cocoa powder and emulsifier. The "Placebo" contained oatmeal, sugar, cocoa powder and emulsifier. Each subject of the intervention group (tempeh group) consumed 100 gram of "VEGAN 1" every day with average content of 5.9 μg vitamin B_{12} and each subject of the control group (placebo group) consumed 100 gram of "Placebo" every day for 3 months from March 20th, 2010 until June 19th, 2010, and then retention for 3 months without intervention until September 26th, 2010.

B. Therapy for the B12 Deficiency Group

The "B_{12} fortification" was made by the Nutrition Research and Development Center in Bogor, West Java and contained 50 μg vitamin B_{12} from tablet, oatmeal, sugar, cocoa powder, emulsifier. According to the ethical review of research, therapy must be given to the vitamin B_{12} deficiency group. Each subject of vitamin B_{12} deficiency group (B_{12} fortification group) consumed 100 gram "B_{12} fortification" every day for 3 months from March 20th, 2010 until June 19th, 2010, and then retention for 3 months without intervention until September 26th, 2010.

C. Determination of Vitamin B12 in "VEGAN 1", Serum Vitamin B12, Serum Homocysteine

Vitamin B_{12} in "VEGAN 1" was determined by using microbial assay which measures the growth of the bacteria Lactobacillus leichmannii [10]. The analysis was conducted at the Laboratory of Microbiology, Nutrition Research and Development Center in Bogor, West Java. The collection and preparation of blood samples from the subjects were done by a health care analyst from the clinical laboratory and diagnostic center "Pramita Utama" in Jakarta with ISO and GLP certification and assisted by two health care analysts from the University of Riau in Pekanbaru. Serum vitamin B_{12} was analyzed by Electrochemiluminescent (ECLIA) Immunoassay using Modular E-170 (Roche), whereas serum homocysteine were analyzed by Microparticle Enzyme Immunoassay (MEIA) using AxSYM Plus (Abbott).

III. OUTCOMES AND DISCUSSIONS

Determination of vitamin B_{12} was performed on 12 samples of "VEGAN 1" from 4 batches of production. The average level of vitamin B_{12} was 5.9 ± 0.7 μg/100g (Table 1). Six of 38 subjects were dropped out in tempeh group with n=32 and four of 38 subjects were dropped out in placebo group with n=34 (Table 2).

Mann-whitney test showed no significant difference ($p > 0.05$) in level of serum vitamin B_{12} and serum homocysteine before and after intervention between placebo and tempeh group (Table 3). Delta of serum vitamin B_{12} after intervention was -3.5 pmol/L in tempeh group and -3.0 pmol/L in placebo group. Independent-T test also showed no significant difference ($p > 0.05$) in level of delta of serum vitamin B_{12} (p=0.954) and delta of serum homocysteine (p=0.722) between placebo and tempeh group (Table 4).

Spearman test showed a significant correlation ($p < 0.05$) between level of serum vitamin B_{12} and serum homocysteine before intervention with a correlation coefficient (r) of -0.418 and after intervention with a correlation coefficient of -0.326. It indicated that a declining level of serum vitamin B_{12} was followed by an increasing level of serum homocysteine (Table 5).

TABLE I. THE AVERAGE LEVEL OF VITAMIN B_{12} IN 12 SAMPLES OF TEMPEH FORMULA "VEGAN 1"

No	Sample Date	Vitamin B_{12} (μg/100g)		Mean B_{12} (μg/100g)
		1 ml	3 ml	
1	24th March 2010	8.563	7.921	7.2
		5.779	6.516	
2	29th March 2010	6.661	6.885	6.3
		5.228	6.377	
3	1st April 2010	6.604	7.027	6.1
		5.448	5.512	
4	8th April 2010	6.261	6.631	6.1
		4.908	6.728	

5	13th April 2010	7.116	7.011	6.3
		5.838	5.212	
6	20th April 2010	7.17	6.447	6.5
		6.355	6.095	
7	26th April 2010	5.323	4.29	4.6
		4.742	3.966	
8	28th April 2010	5.686	4.308	5.4
		6.41	5.179	
9	3rd May 2010	5.334	4.609	5.6
		6.721	5.882	
10	5th May 2010	6.517	5.341	5.7
		5.701	5.117	
11	10th May 2010	5.548	6.201	5.4
		4.073	5.948	
12	12th May 2010	5.928	6.333	5.6
		Not Detected	4.437	

TABLE II. CHARACTERISTICS OF SUBJECTS IN TEMPEH AND PLACEBO GROUPS

Characteristics	Groups			
	Tempeh (n=32)		Placebo (n=34)	
	n	%	n	%
Sex				
Male	13	40.6	21	61.8
Female	19	59.4	13	38.2
Age				
17 – 29 years	18	56.3	16	47.1
30 – 49 years	14	43.8	13	38.2
50 – 64 years	0	0	3	8.8
≥ 65 years	0	0	2	5.9
Type of vegetarian				
Lacto-ovo-vegetarian	32	100	34	100
Length of vegetarian diet				
< 4 years	8	25	11	32.4
4 – 10 years	14	43.7	11	32.4
> 10 years	10	31.3	12	35.2

Note: Tempeh (n=32) with 6 of 38 subjects was dropped out. Placebo (n=34) with 4 of 38 subjects was dropped out.

TABLE III. LEVELS OF SERUM VITAMIN B12 AND SERUM HOMOCYSTEINE BEFORE AND AFTER INTERVENTION IN TEMPEH AND PLACEBO GROUPS

Variables	Groups						p value
	Tempeh			Placebo			
	n	Mean	SD	n	Mean	SD	
B_{12} (before) pmol/L	32	211.1	53.3	34	249.9	106.1	0.223
B_{12} (after) pmol/L	32	207.6	56.7	34	111	111	0.240
Homocysteine (before) µmol/L	32	15.7	6.5	34	6	6	0.847
Homocysteine (after) µmol/L	32	16.2	5.6	34	16.3	6.1	0.807

Mann-whitney: $p > 0.05$, SD = Standard Deviation

TABLE IV. LEVELS OF SERUM VITAMIN B12 AND SERUM HOMOCYSTEINE BEFORE AND AFTER INTERVENTION, MEAN OF Δ B12 AND Δ HOMOCYSTEINE IN TEMPEH AND PLACEBO GROUPS

Groups	B_{12} (before) pmol/L	B_{12} (after) pmol/L	Mean Δ B_{12} pmol/L	p value
Tempeh	211.1	207.6	-3.5	0,954
Placebo	249.9	246.9	-3	
	Homocysteine (before) µmol/L	Homocysteine (after) µmol/L	Mean Δ Homocysteine µmol/L	p value
Tempeh	15.7	16.2	0.5	0.772
Placebo	16	16.3	0.3	

Independent-T: $p > 0.05$

TABLE V. CORRELATION BETWEEN THE LEVELS OF SERUM VITAMIN B12 AND SERUM HOMOCYSTEINE

Variables	n	Median	p Value	r
Vitamin B_{12} (before) pmol/L	66	201.3	0.001*	-0.418
Homocysteine (before) µmol/L	66	13.4		
Vitamin B_{12} (after) pmol/L	66	200.4	0.008*	-0.326
Homocysteine (after) µmol/L	66	14.5		

*) Spearman: $p < 0.0$ r = correlation coefficient

Analysis of ROC curve got a significant correlation ($p < 0.05$) between the incidence of vitamin B_{12} deficiency and length of lacto-ovo-vegetarian diet (Table 6). The cut-off point of vitamin B_{12} deficiency ranged from 4.5 to 9.5 years after becoming a lacto-ovo-vegetarian (Table 7).

TABLE VI. RECEIVER OPERATOR CHARACTERISTIC (ROC) CURVE

Area	SE	p value	95% CI
0.683	0.051	0.001*	0.583 - 0.783

*) ROC Curve: $p < 0.05$ SE = Standard Error

TABLE VII. SENSITIVITY AND SPECIFICITY FOR DETERMINING THE CUT-OFF POINT OF VITAMIN B12 DEFICIENCY

Length of vegetarian (years)	Sensitivity	Specificity
> 0.00	1.000	0.000
1.50	0.976	0.061
2.50	0.976	0.212
3.50	0.976	0.288
4.50	0.952	0.348
5.50	0.857	0.409
6.50	0.810	0.439
7.50	0.738	0.470
8.50	0.714	0.561
9.50	0.714	0.606
10.50	0.595	0.667
11.50	0.548	0.682
12.50	0.476	0.727
13.50	0.357	0.773
14.50	0.357	0.788
15.50	0.333	0.818
16.50	0.286	0.864
17.50	0.262	0.879

Delta of serum vitamin B_{12} after retention for 3 months without intervention was +3.5 pmol/L in tempeh group (p=0.804) and -19.2 pmol/L in placebo group (p=0.038). Statistical test showed no significant difference ($p > 0.05$) in delta of serum vitamin B_{12} after retention for 3 months without intervention in tempeh group, but there were a significant difference ($p < 0.05$) in delta of serum vitamin B_{12} after retention for 3 months without intervention in placebo group (Table 8).

TABLE VIII. LEVELS OF SERUM VITAMIN B12 AFTER INTERVENTION, 3 MONTHS AFTER INTERVENTION WAS STOPPED AND MEAN OF Δ B12 IN TEMPEH AND PLACEBO GROUPS

Groups	B_{12} (after) pmol/L	B_{12} serum (3 months after intervention was stopped) pmol/L	Mean Δ B_{12} pmol/L	p value
Tempeh (n=32)	207.6	215.6	3.5	0.804*
Placebo (n=34)	246.9	224.5	-19.2	0.038**

*) Dependent-T : $p > 0.05$
**) Wilcoxon : $p < 0.05$

Wilcoxon test proved no significant difference ($p > 0.05$) between the intake of dietary vitamin B_{12} from foods at the end of intervention and 3 months retention after the intervention was stopped in tempeh group and placebo respectively. The increasing level of serum vitamin B_{12} in tempeh group and the declining level of serum vitamin B_{12} in placebo were not because of the differences in dietary vitamin B_{12} intakes from foods (Table 9).

TABLE IX. DIETARY VITAMIN B12 INTAKES FROM FOODS AFTER INTERVENTION AND 3 MONTHS AFTER INTERVENTION WAS STOPPED IN TEMPEH AND PLACEBO GROUPS

Groups	B_{12} intakes from foods (after intervention) Median (µg)	B_{12} intakes from foods (3 months after intervention was stopped Median (µg)	p value
Tempeh (n=32)	0.15	0.22	0.476
Placebo (n=34)	0.30	0.36	0.809

Wilcoxon : $p > 0.05$

Dependent-T test showed a significant increase in serum vitamin B_{12} level in B_{12} fortification group ($p < 0.05$) after intervention (mean of Δ B_{12} = 159.9 ± 64.3 pmol/L). In the other hand, Wilcoxon test showed a significant decrease in serum homocysteine level in B_{12} fortification group ($p < 0.05$) after intervention (median of Δ homocysteine = -5.5 µmol/L. The results of analysis by Dependent-T test showed a significant decrease in serum vitamin B_{12} level in B_{12} fortification group ($p < 0.05$) at 3 months retention after the intervention was stopped (mean of Δ B_{12} = -149.3 ± 63.1 pmol/L) (Table 10).

TABLE X. LEVELS OF SERUM VITAMIN B12 AND SERUM HOMOCYSTEINE BEFORE AND AFTER INTERVENTION, 3 MONTHS AFTER INTERVENTION WAS STOPPED AND MEAN OF Δ B12 IN B12 FORTIFICATION GROUP

Groups	B_{12} (before)	B_{12} (after)	Mean Δ B_{12}	p value
B_{12} fortification pmol/L	124.6	284.5	159.9	0.001*
	B_{12} (after)	B_{12} (3 months after intervention was stopped)	Mean Δ B_{12}	p value
	284.5	140.7	-149.3	0.001*
B_{12} fortification µmol/L	Homocysteine (before) Median	Homocysteine (after) Median	Δ Homocysteine. Median	p value
	20.1	15.1	-5.5	0.001**

*) Dependent-T : $p < 0.05$
**) Wilcoxon : $p < 0.05$

IV. CONCLUSION

Tempeh formula "VEGAN 1" was able to maintain normal level of serum vitamin B_{12} after retention for 3 months without intervention in healthy lacto-ovo-vegetarian.

CONFLICT OF INTEREST

Author has declared no conflict of interest.

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