

**Original****Effectiveness of Fish Protein on Vietnamese Malnourished Type 2 Diabetes Mellitus Patients**

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**ABSTRACT** *Background and purpose* The research was conducted to evaluate the effectiveness of the capsules made from fish protein on malnourished type 2 Diabetes Mellitus (DM) patients in Vietnam. *Methods.* A clinical controlled trial was conducted in 2 groups. Subjects in the intervention group were continuously supplemented with fish protein capsules for 12 weeks, at 9 capsules/day, while those in the control group were not. General information interview, nutritional risk assessment using the Nutritional Risk Screening (NRS) questionnaire, and nutritional status assessment using anthropometric indexes, were assessed at baseline and after 12 weeks of intervention. Serum Albumin and glucose level, as well as lipid profile conditions, and liver and kidney function were further measured before and after intervention. *Results.* 30 subjects per group met the criteria to analyze data. After 12 weeks of using fish protein capsules, the weight and nutritional status of malnourished type 2 DM patients were improved, while tests indicated the liver and kidney function were not significantly changed. *Conclusion.* The fish protein capsules improve the malnourished type 2 DM patient's nutritional status without causing side effects on liver and kidney function and is safe for human use.

**Keywords:** amino acids, non-toxic pufferfish, type 2 Diabetes Mellitus, malnutrition, patients, Vietnamese.

**INTRODUCTION**

Malnutrition (chronic energy deficiency) in adults reduces labor productivity. It increases the severity in patients with chronic diseases (cardiovascular disease, and hypertension...), increasing the rate of infectious complications leads to increased mortality (1–4). In Southeast Asia, malnutrition is estimated to account for 30-50% of adult patients in hospitals. In Vietnam, studies show that at least 1/3 of hospitalized patients are malnourished (5–7) and the rate of malnutrition in hospitalized patients is nearly 60% (8,9). Clinical characteristics of malnutrition in patients usually are unintended weight loss, decreased BMI, decreased muscle mass, and decreased fat mass. The consequences are increased risk of complications, increased severity of the disease, prolonged hospital stay, increased readmission rate, reduced quality of life, increased risk of death, and increased treatment costs (10). Therefore, planning nutritional care during hospitalization and long-term nutrition after discharge for patients is always a necessary issue (11). The Nutrition Risk Screening (NRS) nutritional risk assessment method as recommended by the European Society of Nutrition and Metabolic Diseases in 2002, is a valuable tool for evaluating the effectiveness of nutritional interventions for patients (12).

In the human body, amino acids (aa) play a prominent role, being the building blocks of proteins. Protein is an essential and vital material for the body. Protein is a component of digestive enzymes, hormones, antibodies, and serum. Therefore, when the body is not provided with enough amino acids and proteins, it will affect growth, lead to weight loss, poor digestion, and susceptibility to infections. Studies around the world have shown the effectiveness of amino acid supplementation in improving malnutrition and disease progression in patients (13–15).

Thus, it is important to find many protein and amino acid-rich sources that are available and convenient for the outpatients. Research by the Research Institute for Marine Fisheries (RIMF) have made LW-Protein Capsules from fish meat which are rich in aa and minerals. Duc Giang General Hospital belongs to Hanoi capital, Vietnam, with the number of outpatients is quite large. However, nutritional interventions at this hospital have not yet received much attention. According to Circulars of the Ministry of Health of Vietnam, patients who come for outpatient examination and treatment at the hospital also need to be screened and consulted/intervened nutrition.

Therefore, to improve the nutritional status of patients and support effective disease treatment, the research team conducted a study to evaluate the effectiveness of the fish protein capsules on nutritional status and biochemical indicators in patients with chronic energy deficiency and at risk of malnutrition coming for examination at Duc Giang General Hospital.

## MATERIALS AND METHODS

**1. Location:** Duc Giang General Hospital, Hanoi.

**2. Time:** from July to December 2020.

**3. Design:** A clinical controlled trial was conducted in 12 weeks in 2 groups as following:

- Intervention group include patients continuously supplemented with fish protein capsules;

- Control group include patients who were not provided the capsule.

Both groups kept the same diet, and physical activity and took medications as usual.

### 4. Subjects and sample size

*Sample size:*

The sample size for each group was calculated as 36 patients. Then, for 2 groups, the total estimated study sample size is 72 patients. Subjects in control group was matched with intervention group by age and duration of treatment at the hospital.

*Selection criteria:*

Patients with type 2 DM, aged from 40 years and above, undergoing inpatient or outpatient treatment at Duc Giang General Hospital, with duration < 5 years, and no complications; without lipid disorder, BMI from 17.5 - 18.5 or NRS score  $\geq 3$ , volunteered to participate in the research.

*Exclusion criteria:*

People with gastrointestinal disease or gastrointestinal surgery, cancer, heart disease, liver disease, kidney disease, or acute illness at the time of screening. People who intend to use functional foods/formula milk rich in amino acids/vitamins and minerals during the study period.

### 5. Methods and techniques

#### 5.1. Screening and selecting subjects

All subjects were screened and selected by interviewing General information, nutritional risk assessment using NRS questionnaire, nutritional status assessment using BMI index.

#### 5.2. Baseline survey (T0) and signing informed consent with the subjects

Researchers explain and invite subjects to sign informed consent to participate in the study. Assessing the patient's dietary intake by the 24-hour recall method. Fasting venous blood was collected from the patients to determine serum Albumin, Glucose, lipid profiles, GOT, GPT, and Creatinine concentration.

#### 5.3. Providing fish protein capsules to the subjects and monitoring

The product called LW-Protein Capsules contains fish protein powder > 65%, starch and protein content > 50%, including 16 types of aa. The essential aa composition is described in detail in Table 1.

Patients in the intervention group received the dose of 9 capsules/day and used it throughout 12 weeks. Patients were guided to take 9 capsules in a day, divided into 3 times (at the time of taking breakfast, lunch and dinner). Patients also recorded their own process and illness situation in the daily report diary. After 1 week from the initial time, the researcher contacted the patient and asked about compliance and problems to provide timely instructions. Staffs from the NIN and doctors of Duc Giang General hospital were directly responsible for supervising the study during the 12-weeks of intervention period. Every month, supervisors contacted, met, and reviewed the log book to monitor capsule product intake, and adverse events.

**Table 1. Composition of essential amino acids in LW-Protein Capsules.**

Amino acids	In 1000 mg	In dose of 9 capsules
Isoleucine (mg)	24.98	112.41
Leucine (mg)	36.36	163.62
Lysine (mg)	44.28	199.26
Methionine (mg)	13.94	62.73
Phenylalanine (mg)	20.45	92.03
Threonine (mg)	15.19	68.36
Valine (mg)	30.82	138.67
Histidine (mg)	12.83	57.34

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### 6. Data analysis

Data of subjects met the following criteria were included into analysis:

- Subjects took  $\geq 80\%$  of the prescribed amount of capsules during the intervention period;
- Subjects fully participated in baseline survey and final assessments at the end of study.

All data analysis were done using Excel, STATA, and SPSS 16.0 software with statistical tests.

## RESULTS

After 12 weeks of participating in the study, there were 3 subjects in intervention group and 5 subjects in control group have dropped out of study. Therefore, the analysis was done in 30 pair of subjects who completed the study. Table 2 is the characteristics of the subjects at the beginning of the study (T0).

**Table 2. General characteristics of the subjects at baseline**

Variables	Control group	Intervention group	p*
	(n = 30)	(n = 30)	
mean $\pm$ SD			
Age	68.5 $\pm$ 6.1	68.3 $\pm$ 5.9	
Weight (kg)	45.3 $\pm$ 5.0	45.2 $\pm$ 6.58	
Height (cm)	152.8 $\pm$ 7.5	152.3 $\pm$ 6.9	
BMI (kg/m <sup>2</sup> )	18.98 $\pm$ 2.2	18.95 $\pm$ 2.2	> 0.05
Waist circumference (cm)	73.9 $\pm$ 5.1	74.3 $\pm$ 6.3	
Hip circumference (cm)	87.6 $\pm$ 4.8	87.3 $\pm$ 4.4	
Body fat percentage (%)	22.5 $\pm$ 8.5	22.7 $\pm$ 8.3	

*T-test or Man-Whitney test*

The information shows no difference in age, height, weight, waist circumference, hip circumference, and body fat percentage between the two groups at the time before the study. The results of Table 3 showed that, at baseline, there was no difference in the average concentration of biochemical indices: Albumin, Glucose; indices evaluating blood fat status including Cholesterol, Triglycerides, HDL-C, LDL-C; indices evaluating liver and kidney function: AST, ALT, Creatinine between two groups.

**Table 3. Biochemical indices of the two groups at the beginning of the study**

Variables	Control group	Intervention group	p*
	(n = 30)	(n = 30)	
mean $\pm$ SD			
Albumin (g/L)	39.2 $\pm$ 2.6	39.5 $\pm$ 2.2	
Glucose (mmol/L)	8.1 $\pm$ 3.2	9.7 $\pm$ 3.6	
Cholesterol ( $\mu$ g/L)	4.36 $\pm$ 1.24	4.44 $\pm$ 0.86	
Triglyceride (mmol/L)	1.96 $\pm$ 1.11	1.86 $\pm$ 1.22	
HDL-Cholesterol (mmol/L)	1.07 $\pm$ 0.34	1.18 $\pm$ 0.34	
LDL-Cholesterol (mmol/L)	2.55 $\pm$ 0.88	2.41 $\pm$ 0.76	> 0,05
Creatinin ( $\mu$ mol/l)	89.7 $\pm$ 14.5	98.5 $\pm$ 11.5	
AST (U/L)	24.1 $\pm$ 11.4	28.9 $\pm$ 15.7	
ALT (U/L)	16.5 $\pm$ 15.1	17.1 $\pm$ 14.4	

*T-test or Man-Whitney test*

**Table 4. Changes in anthropometric indexes after 12 weeks of intervention**

Variables	Time	Control group	Intervention group	p*
		(n = 30)	(n = 30)	
		mean ± SD		
Waist circumference (cm)	T0	73.9 ± 5.1	74.3 ± 6.3	> 0.05
	T3	74.1 ± 5.3	74.1 ± 6.5	
	T3 – T0	0.1 ± 1.1	-0.3 ± 1.2	
Hip circumference (cm)	T0	87.6 ± 4.8	87.3 ± 4.8	> 0.05
	T3	86.9 ± 5.3	85.9 ± 3.9	
	T3 – T0	-0.8 ± 3.2	-1.1 ± 3.9	
Body fat percentage	T0	22.5 ± 8.5	22.7 ± 8.3	> 0.05
	T3	22.8 ± 8.6	23.8 ± 7.6	
	T3 – T0	0.3 ± 4.7	1.1 ± 6.3	
BMI (kg/m <sup>2</sup> )	T0	19.0 ± 2.2	18.9 ± 2.2 <sup>a</sup>	> 0.05
	T3	18.9 ± 2.4*	19.9 ± 2.2 <sup>a*</sup>	
	T3 – T0	-0.1 ± 0.2*	1.0 ± 0.6*	
Weight (kg)	T0	45.3 ± 5.0	45.2 ± 6.6 <sup>a</sup>	> 0.05
	T3	45.2 ± 5.2*	46.4 ± 6.5 <sup>a*</sup>	
	T3 – T0	0.2 ± 0.4*	1.1 ± 1.6*	

\*p < 0.05 comparison between 2 groups (Man-Whitney test)

<sup>a</sup>p < 0.05 compare before and after intervention in the same group (paired t-test).

The results showed that after 3 months of supplementing with LW-Protein Capsules, patients had an average weight increase to 46.4 ± 6.5 (kg) and BMI of 19.9 ± 2.2, statistically significant compared to the beginning (average weight is 45.2 ± 6.6 kg and BMI is 18.9 ± 2.2). The average weight of the intervention group increased by 1.1 ± 1.6 (kg). The intervention group's waist and hip circumference indexes mildly decreased, and body fat percentage increased compared to the baseline, but there was no statistical significance in these 3 indexes. For the control group, after 12 weeks, no changes in anthropometric indexes were seen.

Regarding the effects on biochemical indexes in patients participating in the study.

**Table 5. Changes in albumin concentration after 12 weeks of intervention**

Variables	Time	Control group	Intervention group	p*
		(n = 30)	(n = 30)	
		mean ± SD		
Albumin (g/L)	T0	39.2 ± 2.6	39.5 ± 2.2 <sup>a</sup>	>0.05
	T3	40.1 ± 2.7	41.1 ± 2.5 <sup>a</sup>	
	T3 – T0	0.4 ± 0.6	1.5 ± 0.4*	

\*p < 0.05 compared to the control group (Man-Whitney test)

<sup>a</sup>p < 0.05 compare before and after intervention in the same group (paired t-test).

The result demonstrated that the plasma albumin concentration of the intervention group (41.1 ± 2.5 g/L) was statistically significantly higher than the control group (39.5 ± 2.2 g/L) with p<0.05. The increase in average albumin concentration before and after 3 months in the intervention group was 1.5 ± 0.4 (g/L), which was higher than the corresponding difference in the control group (0.4 ± 0.6 g/L) with p < 0.05.

Changes in blood glucose, lipid concentrations, and liver and kidney function before and after the intervention of patients are presented in Table 6.

**Table 6. Changes in biochemical indexes after 12 weeks of intervention.**

Variables	Time	Control group	Intervention group
		(n = 30)	(n = 30)
		mean ± SD	
Glucose (mmol/L)	T0	8.3 ± 3.2	8.7 ± 3.6
	T3	8.4 ± 3.5	8.8 ± 4.1
	T3 – T0	0.1 ± 2.6	0.2 ± 2.4
Cholesterol (mmol/L)	T0	4.36 ± 1.24	4.44 ± 0.86
	T3	4.51 ± 1.14	4.53 ± 0.84
	T3 – T0	0.15 ± 0.85	0.09 ± 0.76
Triglycerid (mmol/L)	T0	1.96 ± 1.11	1.86 ± 1.22
	T3	1.91 ± 1.01	1.69 ± 0.97
	T3 – T0	-0.07 ± 0.68	-0.18 ± 0.87
HDL-C (mmol/L)	T0	1.19 ± 0.34	1.18 ± 0.34 <sup>a</sup>
	T3	1.31 ± 0.36	1.34 ± 0.41 <sup>a</sup>
	T3 – T0	0.11 ± 0.41	0.16 ± 0.20
LDL-C (mmol/L)	T0	2.55 ± 0.88	2.41 ± 0.76
	T3	2.68 ± 0.69	2.57 ± 0.79
	T3 – T0	0.12 ± 0.84	0.15 ± 0.60
Creatine (μmol/L)	T0	78.0 ± 14.1	98.45 ± 9.15
	T3	78.8 ± 14.8	97.33 ± 9.56
	T3 – T0	0.9 ± 5.4	1.12 ± 6.5
GOT (U/L)	T0	24.1 ± 11.4	28.97 ± 8.53
	T3	25.9 ± 9.0	27.93 ± 10.4
	T3 – T0	1.8 ± 4.6	1.04 ± 5.2
GPT (U/L)	T0	17.2 ± 14.6	17.1 ± 9.49
	T3	18.8 ± 9.8 <sup>a</sup>	18.03 ± 17.84
	T3 – T0	1.6 ± 5.3	1.02 ± 4.9

<sup>a</sup>*p* < 0.05 compare before and after intervention in the same group (paired *t*-test).

In the intervention group, HDL-C concentration at the end was statistically significantly higher than before the intervention. The average HDL-C concentration in the intervention group increased by 0.16 ± 0.20 mmol/L after 12 weeks of taking LW-Protein Capsules.

There was no difference in the concentrations of Glucose, Cholesterol, Triglyceride, and LDL-C between the 2 study groups before and after intervention as well as no difference when comparing between 2 time points in the same group.

The concentrations of biochemical index assessing liver and body function in both the control group and the intervention group did not change with statistical significance, proving that the product is effective in protein metabolism and patient health without causing side effects on liver and kidney function.

**Table 7. Dietary intake after 12 weeks of intervention**

Variables	T0 (n=36)		T3 (n=30)	
	Control	Intervention	Control	Intervention
	Median (p25; p75)		Median (p25; p75)	
Energy (kcal)	1007 (873;1417)	1078 (826;1398)	1021 (827; 1234)	1188 (1036; 1398)*
Protein (g)	40.5 (38.2; 52.4)	42.4 (28.0; 61.2)	41.3 (32.4; 48.6)	48.3 (31.7; 60.2)*
Lipids (g)	18.2 (18.9; 351)	19.6 (28.4; 38.9)	20.3 (18.5; 33.4)	27.3 (25.1; 41.0)*
Glucid (g)	176.5 (119.8; 175.3)	178.2 (121.7; 181.2)	174.7 (145.7; 206.3)	193.1 (162.2; 215.5)*
Calcium (mg)	306 (216; 474)	311 (195; 468)	289 (221; 468)	367 (272; 497)*
Iron (mg)	9.2 (5.9; 10.1)	9.1 (6.3; 9.3)	9.2 (6.1; 11.3)	10.1 (6.2; 11.6)*
Zinc (mg)	5.4 (4.4; 8.6)	5.4 (3.9; 7.6)	5.5 (4.5; 9.0)	6.3 (4.2; 8.6)*
Vitamin A (mcg)	60.1 (8.7; 151.2)	53.9 (15.6; 161.2)	60.3 (19.0; 134.3)	64.2 (12.4; 175.2)*
Vitamin C (mg)	90.5 (37.2; 153.5)	89.9 (40.8; 186.3)	91.5 (35.8; 145.1)	189.6 (99.8; 324.6)*
Folate (mcg)	193.3 (128.2; 345.8)	196.2 (116.5; 375.2)	195.8 (125.6; 367.8)	223 (135.2; 338.6)*
Vitamin D (mcg)	0.04 (0.0; 2.24)	0.03 (0.0; 2.21)	0.03 (0.0; 2.16)	0.04 (0.0; 2.37)*

\* $p < 0.01$  using the Wilcoxon rank sum-test to compare between 2 groups

Research results showed that, at the time before intervention, there was no difference in nutrient consumption between the 2 groups. However, after 12 weeks of conducting the study, the total energy consumption, protein, lipid, carbohydrate, and micronutrients are all significantly higher in intervention group compared to the control group. The results showed that supplementing with LW-Protein Capsules after 3 months stimulated the diet of the patients, effectively increasing food consumption.

## DISCUSSION

Research using capsules rich in amino acids made from fish meat on malnourished patients has shown the effectiveness of the nutritional intervention for patients with malnutrition.

The study was designed as a controlled study, evaluating both comparison with control and before - and after-intervention. The study lasted 3 months (12 weeks) in accordance with the minimum recommended duration of use of functional foods. The initial comparison showed that there was no difference between the 2 groups in terms of basic information assessing nutritional status, biochemical indices assessing protein intake, blood sugar status, lipid profile, liver, and kidney function, showing that 2 groups and sample sizes were appropriate to conduct the study (Tables 1, 2, 3). The average BMI of all subjects was about 19.0, in the normal range (because the study used BMI criteria from 17.5 - 18.5 for people 40 - 70 years old, or BMI < 20 for people 71 - 80 years old) but is the lower limit of normal, indicating the patient's malnutrition. After 12 weeks of intervention research, the 2 groups had the above indicators measured again and compared. The results showed an increase in the patient's BMI and weight (Table 4), demonstrating an improvement in the patient's weight. In addition, albumin concentration, representing protein in the patient's body, also increased with statistical significance. Besides, to further understand the nutrient consumption of participants, diets were also analyzed, before and after using the product (Table 7). The results showed that there was a significant improvement in the nutritional diet of patients using the product.

However, the improvement results are not too great, possibly because the time of using the product is not long, and the patient's psychological condition affects eating and absorption. The study also did not evaluate the effectiveness of the LW-Protein Capsules on the improvement of the disease progression. However, the research results have resolved some of the issues mentioned above, showing the safety of the product, and the role of nutritional intervention (essential amino acids) on humans (patients).

In conclusion, after 12 weeks of using LW-Protein Capsules, the weight and nutritional status of diabetic patients being treated at Duc Giang General Hospital were improved, while the liver and kidney function of the

subjects were not increased, proving that the product is effective for the patient's nutritional health without causing side effects on liver and kidney function, and the product is safe for users.

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