

Analysis of Macro Nutritional Substances, Branched-Chain Amino Acids, Viscosity Enteral Formula for Hepatic Cirrhosis Patients

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Article history

Posted, Oct 25th, 2021

Reviewed, Jan 13rd, 2022

Received, Mar 17th, 2022

ABSTRACT

Patients with hepatic impairment are particularly at risk of malnutrition because of impaired absorption, hypermetabolism, and poor oral intake. Enteral diet therapy based on pumpkin and soybean flour is one of the local foods functional as an alternative enteral formula for patients with liver cirrhosis because it contains high Branched-Chain Amino Acids (BCAA) and Medium-Chain Triglyceride (MCT). The purpose of this study was to analyze the nutritional value (proximate test), BCAA test (Leucine, Isoleucine, Valine) and Viscosity Test (thickness) and organoleptic test of enteral formulas of patients with liver cirrhosis from pumpkin and soybean flour. This descriptive qualitative research method uses three levels of treatment, comparison of pumpkin with soybean powder. The characteristics analyzed are macronutrients (energy, protein, fat, carbohydrates), BCAA (leucine, isoleucine, valine), viscosity. Statistical analysis with One Way Anova and organoleptic test with a hedonic method. The macronutrient content analysis following the diet's requirements and principles was enteral formula two at a relative concentration (80:20). The statistical test results showed a p-value of 0.000 (<0.05), which means a significant difference in the content of leucine, isoleucine, and valine in the three enteral formulas. Increasing the ratio of pumpkin tends to increase the viscosity of the enteral cirrhosis formula. The enteral formula has characteristics per 1000 ml as follows: 38 grams of protein, 161.1 grams of carbohydrates, 25.2 grams of fat, 1023 kcal of energy, 5743.43 mg of BCAAs, and the results of the organoleptic test selected for the enteral formula 2.

Keywords: BCAA; enteral formula; hepatic cirrhosis; viscosity

ABSTRAK

Pasien dengan gangguan hati sangat beresiko malnutrisi karena terjadi gangguan absorpsi, adanya hipermetabolik, dan asupan oral kurang. Terapi diet formula enteral berbasis labu kuning dan tepung kedelai merupakan salah satu pangan lokal yang bermanfaat sebagai alternatif formula enteral untuk pasien sirosis hepatitis karena mengandung *Branched-Chain Amino Acids (BCAA)* dan *Medium -Chain Triglyceride (MCT)* tinggi. Tujuan penelitian ini adalah untuk menganalisis nilai gizi (uji proksimat), uji BCAA (Leusin, Isoleusin, Valin) dan uji viskositas (kekentalan) dan uji organoleptik formula enteral pasien sirosis hepatitis dari

labu kuning dan tepung kedelai. Metode penelitian ini deskriptif kualitatif menggunakan 3 taraf perlakuan, perbandingan labu kuning dengan bubuk kedelai karakteristik yang dianalisis yaitu zat gizi makro (energi, protein, lemak, karbohidrat), BCAA (leusin, isoleusin, valin), viskositas. Analisis statistik dengan *One Way Anova* dan uji organoleptik metode hedonik. Hasil p analisa kandungan zat gizi makro yang sesuai dengan syarat dan prinsip diet adalah formula enteral 2 pada konsentrasi perbandingan (80:20). Hasil uji statistik menunjukkan *p-value* 0,000 ($<0,05$) yang berarti adanya perbedaan yang nyata kandungan leusin, isoleusin dan valin pada ketiga formula enteral tersebut. Peningkatan perbandingan labu kuning cenderung membuat viskositas formula enteral sirosis hepatitis semakin meningkat. Formula enteral mempunyai karakteristik per 1000 ml sebagai berikut kandungan protein 38 gram, karbohidrat 161,1 gram, lemak 25,2 gram, energi 1023 kkal, BCAA 5743,43 mg, serta hasil uji organoleptik terpilih formula enteral 2.

Kata Kunci: BCAA; formula enteral; sirosis hepatic; viskositas

INTRODUCTION

Liver cirrhosis is the final pathological course of various liver diseases, such as chronic viral hepatitis, alcoholism, autoimmune hepatitis, nonalcoholic steatohepatitis (NASH), biliary cirrhosis. Due to the cirrhosis process, decreased hepatic synthesis function, decreased synthetic hepatic function, reduced ability of the liver to detoxify, and portal hypertension with all its complications (Ndraha, 2015). Cirrhosis is the eighteenth cause of death, with an 800,000 deaths prevalence of 1.3% (Nurdjanah, 2019). According to basic health research (Badan Penelitian dan Pengembangan Kesehatan, 2018), is estimated million people suffer from hepatitis B, and 3 million people suffer from hepatitis C in Indonesia. About 50 per cent of these people have potential liver disease, and 10 per cent progress to cirrhosis of the liver. Meanwhile, one case of liver cirrhosis requires a treatment cost

of around IDR 1 billion and liver cancer treatment of around IDR 5 billion with a minimal cure rate (Kementerian Kesehatan RI, 2017). In the province of South Sumatra, the number of patients with liver cirrhosis in 2012 was recorded at 402 people, consisting of 204 men and 198 women (Lesty Nurainy & Trisnawarman, 2020).

The number of patients with liver cirrhosis in 2017 – 2019 at the Sekayu Regional General Hospital 2017 found 33 people consisting of 21 men and 12 women. In 2018 patients with liver cirrhosis were collected among 32 people consisting of 24 men and eight women, and in 2019 patients with liver cirrhosis opened among 31 people consisting of 23 men and eight women (RSUD Sekayu, 2020). The above incidence shows that cirrhosis is a critical problem; even though eradication efforts have been implemented, this can cause

energy and protein malnutrition problems. In fact, from data in Indonesia, 54-88% of patients with liver cirrhosis have experienced malnutrition. The more severe the cirrhosis, the more influential the malnutrition. Malnutrition in cirrhosis increases morbidity and mortality. The more powerful the malnutrition, the higher the incidence of hepatic encephalopathy, infection, variceal bleeding, and refractory ascites (Lovena, Miro, & Efrida, 2017).

The results of a preliminary survey and interviews with patients hospitalized at Sekayu Hospital regarding the administration of the old liver diet formula using manufactured products showed that 40% did not like drinking milk and other reasons because the price of milk was high. They could not afford to buy it. Dietary requirements in liver disease, according to the European Society for Clinical Nutrition and Metabolism (ESPEN), are given energy of 25-40 kcal/kgBW/day, protein 1-1.5 g/kgBW/day without hepatic encephalopathy or 0.6-0.8 g/kgBW/day with hepatic encephalopathy, carbohydrates 45-65%, and fat 25-30% (Göktürk & Selçuk, 2015). The principles/requirements of the standard Enteral formula are energy content of $\pm 1.0 - 2$ kcal/ml, 1.0-1.5 gram/kg/BW protein,

20-25% fat, and 45-65% carbohydrates (Persatuan Ahli Gizi & Asosiasi Dietisien Indonesia, 2019). Specific enteral formulas related to disease diagnosis have different composition proportions (Rahmadanti, Candra, & Nissa, 2020).

Protein is preferred over vegetable protein and dairy products because it is easier to tolerate and low in Aromatic Amino Acid (AAA) and ammonia than fish and meat protein. Vegetable protein contains fibre that can accelerate the excretion of ammonia through faeces and contains high Branches-Chain Amino Acids (BCAAs) (Silva et al., 2015). In this research, enteral formulas use local food ingredients with high energy content and complete nutrients, are readily available and are sourced from local food ingredients. One of the local food sources of vegetable protein high in BCAA and protein digestibility is soybeans. Soybean (*Glycine max*) can be modified in the form of flour because of the higher protein content of the fresh product, eliminating the unpleasant taste (beany), increasing digestibility and increasing shelf life (Astawan, Wresdiyati, & Ichsan, 2016). Pumpkin (*Cucurbita moshata*) is a fruit that contains beta-carotene. Beta-carotene in the body is converted into vitamin A

which helps maintain health. Besides, beta-carotene is also helpful as an antioxidant. The nutritional content of pumpkin is quite complete at a relatively affordable price, and the availability of pumpkin in Indonesia is abundant (Najiah, 2015). Pumpkin fruit contains active compounds such as saponins, tannins, flavonoids and beta-a-carotene. Beta-carotene in the body will be converted into vitamin A which is helpful for the growth maintenance of body tissues and reduce the risk of cancer and liver disease (Aditya, Ihwan, & Jamaluddin, 2016).

This study uses an enteral formula made from pumpkin. It can be seen that the nutritional content of this hepatoglycurbi formula will be analyzed for its nutritional content and BCAAs. Not only that, but viscosity tests also need to be carried out to show the physical quality of enteral formulas because viscosity is an essential characteristic of liquid foods in food processing. It is done to determine if the enteral product is made within normal limits, not too runny or thick. Based on the background of the problem above, the writer wants to know more about the analysis of macronutrients, BCAAs, and the Viscosity Test of enteral formulas for patients with liver cirrhosis. To examine whether the formula made and researched

is under the standards set for enteral formula products.

METHOD

This research is included in nutrition and food and is a qualitative descriptive study using three levels of treatment, namely the comparison of pumpkin with soybean powder. This research is an experimental laboratory development to analyze physical quality (viscosity), nutritional value / Proximate (energy, carbohydrates, protein, fat, ash, water) and BCAA (Leucine, Isoleucine, Valine) and acceptability test or organoleptic test.

The manufacture of Enteral Hepatoglycurbi Formula and organoleptic tests were carried out at the Sekayu Regional General Hospital, for viscosity tests and proximate analysis carried out at the Chemical and Microbiological Laboratory of Agricultural Products, Department of Agricultural Technology, Faculty of Agriculture, Sriwijaya University (UNSRI), Palembang and analysis of BCAA content (Leucine, isoleucine, valine) was carried out at the Saraswanti Indo Genetech (GIS) manufacture, Bogor. This research was carried out from February to April 2021. The materials used in this study were soybean flour, pumpkin, heptosol milk, chicken eggs, rice flour, canola oil, orange

juice, and refined granulated sugar. The study was conducted to determine the level of treatment by calculating the calculation of the ingredients used based on the dietary requirements of the liver by entering the program in Microsoft Office Excel. Nutritional requirements for liver disease according to the principles & requirements of the standard Enteral

formula are energy content $\pm 1.0 - 2$ kcal/ml, protein 1.0-1.5 gram/kg/BW, fat 20-25%, and carbohydrates 45 – 65% (Persatuan Ahli Gizi & Asosiasi Dietisien Indonesia, 2019). The formulation of the ingredients can see in Table 1 with the ratio of soybean flour and goat's milk flour, namely FE1 (70:30), FE2 (80:20), P3 (90:10).

Table 1. Formulation of Modified Liver Disease Enteral Food

Food Material	Enteral Formulas		
	FE1 (70:30)	FE2 (80:20)	FE3 (90:10)
Pumpkin	105 gr	120 gr	135 gr
Soybean Powder	45 gr	30 gr	15 gr
Rice Flour	15 gr	15 gr	15 gr
Chicken Eggs	25 gr	25 gr	25 gr
Orange Juice	200 ml	200 ml	200 ml
Hepatosol Milk	30 gr	30 gr	30 gr
Sugar	115 gr	115 gr	115 gr
Canola Oil	20 ml	20 ml	20 ml
Water	650 ml	650 ml	650 ml

After the pumpkin was washed and peeled, it was weighed, blanched (10 minutes), and blended. Mix all dry ingredients (rice flour, soy powder, granulated sugar). The dry ingredients mix with the blended pumpkin. Mix the canola oil with the beaten egg. Add half of the water. After boiling, cool, add orange juice and add milk diluted with warm water with the half measure of water, stir, strain, and ready to be tested (viscosity test, proximate test,

BCAA content test (Leucine, Isoleucine, Valine) and test organoleptic).

Viscosity test using a gravimetric method with Ostwald viscometer. Nutrient content includes protein content using the Kjeldahl method, fat content using the Soxhlet method, carbohydrate content using the by difference method. Energy content was obtained by calculating four kcal/g protein + 9 kcal/g fat + 4 kcal/g carbohydrates. After the energy content is known, the

energy density gets by dividing the energy content by the volume test of the range of BCAA (leucine, isoleucine, valine). The organoleptic test includes colour, aroma, taste, and texture parameters with five scales: one = dislike very much, 2 = dislike, 3 = like, 4 = like very much. The Organoleptic assessment was carried out on 17 moderately trained panellists, namely nutritionists at Sekayu Hospital. Data on the values of viscosity, fat content, carbohydrates and energy content which are normally distributed, were analyzed by the One Way ANOVA test followed by

Tukey's post hoc test. The data not customarily distributed (protein, fat, carbohydrate and energy density levels) were analyzed using the Kruskal-Wallis test and the Mann-Whitney further test to determine the significant difference between treatments. The organoleptic test was analyzed using Friedman's non-parametric difference test with Wilcoxon's advanced test. This research has passed the Health Research Ethics Commission of Palembang Health Polytechnic's ethical clearance with No. 736 KEPK/Adm 2/III/2021.

RESULTS AND DISCUSSION

Table 2. The results of the analysis are based on the principles and requirements of the liver disease diet

Enteral Formulas Hepatoglycurbi	Energy (Kal)	Protein (%)	Fat (%)	CH (%)	Selected Formula Based on Nutrition
Diet Terms	1-2	10-16	20-25	45-65	
FE1	1	13,64	23,4	63,0	Selected
FE2	1	15,20	22,9	64,4	Selected
FE3	0,9	18,76	16,0	64,8	Not selected

The three empirical formulas' energy, protein and fat content is only formula three, which does not meet the requirements because the results for less energy, excess protein and fat are less than the dietary requirements for liver disease patients. The high-calorie, high-protein enteral formula has an energy density of 1.0 to 2.0 kcal/ml with an administration

of 200 ml to 250 ml. Enteral formula administration can be done at intervals of 3 to 4 hours so that enteral nutrition can provide energy up to 2000 kcal to prevent gastric retention and regurgitation (Lestari, Rahmawati, Shita, & Eka, 2019).

Protein Content

In the three comparisons, the enteral formula for liver cirrhosis has protein

levels ranging from 13-18%, wherein formula two protein is 15.2% of total calories. It shows that formula two has met the requirements and principles of the liver disease diet, which is 10-16% of the total energy (Persatuan Ahli Gizi & Asosiasi Dietisien Indonesia, 2019). Protein intake is prioritized over vegetable protein and dairy products because it is more easily tolerated, low in Aromatic Amino Acid (AAA) and ammonia, high in Branched-Chain Amino Acids (BCAA) compared to fish and meat protein (Silva et al., 2015).

Fat Content

In the three comparisons, the enteral formula for liver cirrhosis has fat content ranging from 16-23%, whereas formula 2 is 22.9% fat. It shows that formula two has met the requirements and principles of the liver disease diet according to (Persatuan Ahli Gizi & Asosiasi Dietisien Indonesia, 2019), which is 20-25% of the total energy. Sources of MCT fat in the enteral hepatic glycurbi formula were canola oil, soybean flour and hepatosol milk. Vegetable fats, especially soybeans, contain many phospholipids such as lecithin, which can increase the absorption of nutrients into cells through its function as a protective cell membrane (Hamad, Septhea, & Ma'ruf, 2015).

Carbohydrate Content

In the three comparisons, the enteral formula for liver cirrhosis has carbohydrate levels ranging from 63-64%, whereas procedure two carbohydrates are 64.4% total calories. That shows that formula two has met the requirements and principles of the liver disease diet according to Persatuan Ahli Gizi & Asosiasi Dietisien Indonesia (2019), which is 64.4% of the total energy. Carbohydrates are organic compounds formed from carbon, oxygen and hydrogen atoms. Carbohydrates have become the primary energy source for metabolism in humans, where the amount of energy produced by 1 gram of carbohydrates is 4 kcal. Apart from being a source of energy, carbohydrates also function as food reserves and provide a sweet taste in food (Siregar, 2014). Carbohydrate sources for enteral cirrhosis hepatic formula come from sugar, milk and soy flour.

BCAA test (Leucine, Isoleucine and Valine)

BCAA content testing was carried out using the UPLC method to determine the content of leucine, isoleucine, and valine content in each sample. The sample used to carry out the test is 1000 ml of enteral formula liquid. The test results and the comparison of leucine, isoleucine and

valine in the comparison sample of below. pumpkin and soybean flour are presented

Table 3. Results of Analysis of BCAA Content and Viscosity of Enteral Formulas in Patients with Liver Cirrhosis

Enteral Formulas Hepatogly curbi	Leusin (mg/kg)		Mean	Isoleucine (mg/kg)		Mean	Valin (mg/kg)		Mean	P-value	viscosity
	Simplo	Duplo		Simplo	Duplo		Simplo	Duplo			
FE1	2871.57	2887.93	2879.74	1710.21	1722.91	1716.56	1743.57	1754.74	1749.15	0.000	9.65
FE2	2582.76	2595.72	2589.24	1564.53	1571.14	1567.83	1582.94	1590.08	1586.36	0.000	8.75
FE3	1575.14	1572.91	1574.02	1036.43	1037.54	1036.98	1031.56	1026.32	1028.94	0.000	8.7

One-way ANOVA statistical test results p-value of 0.000 (<0.05), which means a significant difference in the content of leucine, isoleucine, and valine in the three enteral formulas. The viscosity value of the enteral formula is directly proportional to various concentrations of soy flour. The higher the concentration of soybean flour, the higher the viscosity value. The viscosity test results on the three enteral cirrhosis hepatitis formulas showed that the highest viscosity value was in FE1, 9.65 millipascal seconds (mPa.s), while the lowest was in the lowest FE3, which was 8.70 millipascal-seconds (mPa.s).

The benefits of branched-chain amino acids (BCAAs) on protein metabolism and the nutritional status of patients with chronic liver disease are well known. In liver cirrhosis conditions, decreased levels of BCCA will inhibit protein synthesis and turnover. A lower serum aromatic amino

acid BCAA ratio is associated with a poorer prognosis in patients with advanced liver disease. The following study aims to determine the long-term effectiveness of oral BCCA supplementation by analyzing the model for end-stage liver disease (MELD) scores and the occurrence of complications in advanced liver disease (Park et al., 2020). BCAAs are essential amino acids for protein synthesis, protein turnover, and regulation of energy metabolism. BCAA consists of leucine, isoleucine, and valine, while AAA consists of tryptophan, phenylalanine, and tyrosine. Ammonia and AAA usually are metabolized or detoxified by the liver. AAA accumulates in patients with advanced liver disease due to impaired hepatocyte function. Patients with cirrhosis have a low BCAA: AAA ratio. BCAA decreased because it was taken up by skeletal muscle cells, as are energy substrate or ammonia degradation. In

contrast, AAA was increased due to the impaired capacity of hepatocytes in deamination (Park et al., 2020).

Organoleptic test on enteral hepatic glycerin formula

Flavour

The results organoleptic test on the taste of the enteral formula showed a difference in the acceptance score for the taste of the enteral formula. The average acceptance of the panellists ranged from 3.16 to 3.36. The results of the Friedman test analysis showed that the p-value was 0.650 (>0.05). So there is no significant difference.

Taste is an assessment factor that determines the results of ingredient formulations assessed through the tongue by measuring sweet, sour, salty, bitter or a combination and picks the level of preference for a product (Ladamay & Yuwono, 2014). The taste of the four enteral formulas ready to brew (F1, F2, F3). It is influenced by the use of ingredients and pumpkin and soybeans composition. Based on the interviews with panellists, panellists prefer the enteral formula that is not too sweet and smells unpleasant. Dislike for food is one of the causes of the panellists' lack of assessment of the taste of enteral formulas.

Viscosity

The results of the organoleptic test on the viscosity of the enteral formula showed that the difference in acceptance scores on the thickness of the enteral formula was not too different. The average acceptance of the panellists ranged from 3.12 to 3.52. The results of the Friedman test analysis showed that the p-value was 0.002 (<0.05). So there is a significant difference in the addition of pumpkin. Many factors affect viscosity, such as temperature, solution concentration, solute molecular weight and pressure. The enteral hepatic glycerin formula (FE1, FE2, FE3) was in a warm state at the organoleptic test. The higher the temperature, the lower the viscosity (Lestari et al., 2019).

Colour

The results of the organoleptic test on the colour of the enteral formula showed that the difference in acceptance scores for the colour of the enteral formula was not. The results of the Friedman test analysis showed that the p-value was 0.005 (<0.05). So there is a significant difference. The characteristics of the enteral hepatoglycurbi formula produced are slightly yellowish-white in line with the increasing ratio of pumpkin. The panellists prefer the colour of the enteral formula, which is whiter but not too yellow. The white colour of the enteral hepatoglycurbi

formula comes from heptosol milk powder, which has a whiter colour characteristic.

Scent

The results of the organoleptic test on the scent of the enteral formula showed that the difference in acceptance scores for the smell of the enteral formula was not too different. The average acceptance of the panellists ranged from 3.16 to 3.36. The results of the Friedman test analysis showed that the p-value was 0.650 (>0.05). So there is no significant difference. The

results of the organoleptic values showed that FE2 had the most favourable scent with the highest score of 3.36 compared to the other two formulas, where the formula FE2 had fewer soybeans than FE1 and gave off an unpleasant aroma, and FE3 had more pumpkin scent. The unpleasant scent of soybeans is produced by the enzymatic oxidation of linoleic acid and linolenic acid by lipoxygenase genes (Lox) to produce hexanol (Ravi, Taheri, Khandekar, & Millas, 2019).

Table 4. Friedman Test Result

Formula	Color	Scent	Viscosity	Taste	Total
FE1	3,32	3,20	3,12	3,20	12,84
FE2	3,76	3,36	3,52	3,36	14,00
FE3	3,56	3,16	3,48	3,16	13,36
p-value	0,005	0,650	0,002	0,650	

The organoleptic test requirements include the presence of samples, panellists and honest response statements. The organoleptic test is closely related to the human senses. The sensory assessment consists of 6 stages: receiving, recognizing, clarifying the nature of the material, recalling and re-elaborating (Melati, 2017). The most preferred formula from the aspect of colour assessment is F2, with an average of 3.76. The most preferred procedure from the element of aroma assessment is F2, with an average of 3.36. The most preferred

formula from the aspect of viscosity assessment is F2, with an average of 3.52. In contrast, the most preferred procedure from the element of taste assessment is F2, with an average of 3.36. The results showed that the panellists preferred formula 2, namely the number of ingredients in the ratio of 120 grams of pumpkin and 30 grams of soybean powder for the right taste.

CONCLUSION

Recommendations for enteral formulas for selected liver cirrhosis patients follow the

liver disease diet (Persagi and AsDI, 2019). FE2 with a ratio of pumpkin and soybean flour of 80:20. BCAA content (leucine, isoleucine, valine) averaged 2589.24 mg, 1567.83 mg, 1586.36 mg, viscosity value 8.75 millipascal-seconds (mPa.s), preferred by 17 panellists, further research is needed regarding the effect of giving enteral formula intervention in patients with liver cirrhosis, and further modification of the formula is needed for this flask to obtain BCAA composition and viscosity according to the standard.

ACKNOWLEDGEMENT

We would like to thank the Chairperson of the Applied Nutrition & Dietetics Undergraduate Study Program, Department of Nutrition, Poltekkes, Palembang, Sriwijaya University Agricultural Laboratory, PT Saraswati Indo Genetech Laboratory, Bogor, and research subjects participated in this research.

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