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Analysis of Albumin Levels in Cork and Eel Fish Using the Spectrophotometry Method

Análisis de los niveles de albúmina en Pez corcho y anguila mediante el método de espectrofotometría

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SUMMARY

Introduction: Protein albumin in the wound healing process is very important to stimulate the formation of new cell tissue damaged during surgery. One way is by giving Human Serum Albumin (HSA). However, the price for this is expensive. The wound healing process can be stimulated by providing alternative albumin from cork fish and utilizing albumin from eels. The purpose of this study was to compare the albumin levels in cork and eel fish.

Methods: This type of research was a posttest-only control experimental design. The research sample was obtained from the Pacar Keling regional market, Surabaya, totaling 32 samples. The sampling technique used was purposive sampling. To compare the albumin levels between cork fish and eel fish, the data was

analyzed using the t-test independent sample test with an error rate of $\alpha = 0.05$.

Results: The average albumin level in cork fish was 25,7169 % and the average albumin level in eels was 16,9169 %. From the results, both samples (cork and eel fish) have an average yield of albumin levels of 8.8 %. Cork fish had a higher albumin level compared to the albumin level of eels. The independent test results show $\text{sig } 0.0001 < 0.05$. This indicates that there is a difference in the albumin level between cork fish and eels.

Conclusion: The difference in albumin levels in cork and eel fish is caused by weight factors, environmental factors, and food availability factors. An intervention may be provided as an alternative to support the albumin need.

Keywords: Albumin, cork fish, eel.

RESUMEN

Introducción: La proteína albúmina en el proceso de cicatrización de heridas es muy importante para estimular la formación de nuevo tejido celular dañado durante la cirugía. Una forma es administrando albúmina de suero humano (HSA). Sin embargo, el precio de esto es caro. El proceso de cicatrización de heridas se puede estimular proporcionando albúmina alternativa del pez corcho y utilizando la albúmina de las anguilas. El propósito de este estudio fue comparar los niveles de albúmina en corchos y anguilas.

Métodos: Este tipo de investigación fue un diseño experimental de control solo post prueba. La muestra de investigación se obtuvo del mercado regional de Pacar Keling, Surabaya, con un total de 32 muestras.

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La técnica de muestreo utilizada fue el muestreo intencional. Para comparar los niveles de albúmina entre el pez corcho y la anguila, los datos se analizaron utilizando la prueba de muestra independiente t-test con una tasa de error de $\alpha = 0,05$.

Resultados: *El nivel medio de albúmina en corchos fue de 25,7169 % y el nivel medio de albúmina en anguilas fue de 16,9169 %. De los resultados, ambas muestras (corcho y anguila) tienen en su rendimiento medio niveles de albúmina del 8,8 %. El pez corcho tenía un nivel de albúmina más alto en comparación con el nivel de albúmina de las anguilas. Los resultados de las pruebas independientes muestran $\text{sig } 0,0001 < 0,05$. Esto indica que existe una diferencia en el nivel de albúmina entre los corchos y las anguilas.*

Conclusión: *La diferencia en el nivel de albúmina en el corcho y la anguila se debe a factores de peso, factores ambientales y factores de disponibilidad de alimentos. Se puede proporcionar una intervención como alternativa para apoyar la necesidad de albúmina.*

Palabras clave: *albúmina, pez corcho, anguila*

INTRODUCTION

A wound is a damage done to a body part that occurs on the skin in the form of tissue that is cut off, torn, or damaged for some reason. In traumatology, there are several categories of wounds including categories of wounds based on their cause, such as cuts, bruises, stab wounds, abrasions, and torn wounds. Cuts are usually caused by sharp objects such as knives, razors, or scalpels in the surgical process. This is indicated by the edges of the wound being in the form of straight and regular lines (1).

The prevalence of wounds in Indonesia is quite high. Based on the 2013 Basic Health Risks (Riskesdas), the three most common types of injuries experienced by the Indonesian population based on research are cuts/bruises (70.9 %), sprains (27.5 %), and torn wounds (23.2 %) (2). The wound healing process is important because the skin is a single organ that is exposed to the outside world (3,4). When the skin loses its elasticity, the protective, sensory, thermoregulatory, metabolic, and sexual signaling functions cannot function as they should (5).

In the early stages after a major injury, the endocrine and nervous systems react to the injury which then triggers catabolic processes that

then damage the body's tissues to provide the materials needed for immediate repair. Amino acids are required for the synthesis of structural proteins such as collagen and for the synthesis of proteins that play a role in the immune response. Replacement proteins, calories, electrolytes, and fluids are vital components of initial treatment. Protein deficiency not only slows healing but also causes the wound to heal. Albumin is one of the globular proteins that is often applied clinically for nutritional improvement and postoperative wound healing (6-8). The role of albumin in the body is very large, therefore a way is needed to meet the needs of albumin in the body, especially for postoperative patients. Albumin is suitable for stimulating the formation of new tissue cells damaged during surgery. One way is by giving Human Serum Albumin (HSA) (2,9-11). Albumin is produced from human blood, so the price is currently quite expensive (12). An alternative albumin source is needed that is cheaper but has the same clinical aspects such as reduced tensile strength (13).

One of the efforts to minimize the wound treatment cost is to use natural ingredients. Many medicinal plants have an important role in the wound healing process. More than 70 % of pharmaceutical products for wound healing are plant-based products, while 20 % are mineral-based, and the rest are animal-based products (14). The discovery of cork fish albumin extract has been used as an alternative to getting cheaper albumin (12).

Cork fish (*Channa striata*) or cursed fish are common water fish (fresh). They are one type of fish that is widely used by the community for wound healing, especially postoperative wounds, and burns because the main content of cursed fish is protein or albumin, which is quite high. Albumin is the most abundant protein in plasma, totaling around 60 % of the total with a normal value of 3.3-5.5 g/dL. Albumin is also found in the extracellular space, specifically 40 % is found in plasma and 60 % is extracellular (15).

However, cork fish extract products are difficult to obtain because of their availability in the fish. This is due to the high demand for freshwater fish such as cork fish but a lack of good local nurseries. Cork fish is also one of the fish types that are quite rare and expensive. This warrants the need to find alternative types of fish

that are a quality source of protein content that is almost the same as cork fish at a cheaper price.

Eel (*Monopterus albus*) is a type of freshwater fish and a leading fishery commodity. Eel production is quite high. Eel is not a rare item that is hard to find. Currently, eels can be obtained easily in both traditional markets and fish markets in every city (16). The nutritional composition of eels is no less high than other animal protein sources. Evidently, 100 grams of eel meat contains 14 grams of protein, 27 grams of fat, 20 mg of calcium, 1 600 SI (International Units), 2 mg of vitamin A, 2 mg of vitamin C, and 0.1 mg of vitamin B (17).

Eel gel is a semisolid preparation that produces a membrane on the skin surface that functions as a wound cover that can absorb wound exudate. Another preparation is where the membrane also functions as a wound cover and at the same time contains nutrients that help to accelerate wound healing. Extracted eel has been tested for the effectiveness of the eel extract gel and membrane on wound healing using a positive control of circulating preparations and a negative control without drug administration. The eel extract gel and membrane were effective when it came to wound healing compared to the negative control. It had better activity than the circulating preparations. The formulation of the problem in this research is the need for a comparison of the albumin level between cork fish and eel. This study aims to determine the ratio of albumin levels in cork fish and eel respectively (18).

METHODS

The type of research used was a post-test-only control group design to analyze the albumin level found in cork fish and eels. The population and sample of this study were cork fish and eels found in the Pasar Keling area of Surabaya. The criteria for the samples taken were fish of the same size and weight. The number of samples was 32 samples determined using the formula: $(t-1)(r-1) + 15$ with the number of replications or repetitions of 16 times and 2 times of treatment. This meant that the number of samples needed was 32 samples. The sampling technique used in this study was

purposive sampling, i.e. sampling is based on a certain weighing made by the researcher based on the characteristics of the population that have been known previously. The research variables consisted of the independent variables, namely cork fish and eels, and the dependent variable, albumin level. The data analysis method used to analyze the differences in albumin levels in cork fish and eels was the independent t-test.

Inspection Procedure

The examination of the albumin level of cork fish and eels was done using the Kjeldahl method and spectrophotometry. The sample was destroyed to break the nitrogen bonds of the albumin complex form. Nitrogen was liberated by Nessler's reagent complex which formed a yellow-brown colored complex compound. The absorbance was measured at a wavelength of 420 nm with a spectrophotometer. The absorption of complex compounds is directly proportional to the amount of free nitrogen from the albumin which is equivalent to the albumin level.

The tools that need to be prepared during the sample treatment are a Kjeldahl flask, spectrophotometer, Nessler tube, measuring flask, volume pipette, and a Bunsen. The examination reagents consisted of the Nessler reagent, 45 % NaOH solution, concentrated H_2SO_4 , Aquadest, selen reaction catalyst ($CuSO_4$ and K_2SO_4), Rochelle salt solution, and ammonia parent solution.

a. Preparation of cork and eel albumin samples

The albumin samples were prepared using the steam method by washing the cork fish and eel and then putting them into an aluminum basin. They were then steamed until the albumin liquid came out into the basin.

b. Preparation of 100 ppm ammonia mother liquor by dissolving 314.1 mg NH_4Cl with distilled water up to 1 000 mL.

c. Preparation of a series of 100 ppm ammonia solutions by diluting 50 mL of 100 ppm ammonia mother liquor in a 500 mL volumetric flask with distilled water to the mark. 1 mL ~ 0.1 mg NH_4 (10 ppm). The 10 ppm solution was then diluted to make a standard series:

ANALYSIS OF ALBUMIN LEVELS IN CORK AND EEL FISH

0.0 ppm: 100 mL distilled water in a volumetric flask

0.2 ppm: 2.0 mL add 100 mL distilled water in a volumetric flask

0.4 ppm: 4.0 mL add 100 mL of distilled water to a measuring flask

0.6 ppm: 6.0 mL add 100 mL of distilled water to a measuring flask

0.8 ppm: 8.0 mL add 100 mL of distilled water to a measuring flask

1.0 ppm : 10.0 mL add 100 mL distilled water

d. Ammonia solution standard curve

The standard curve was made by stretching the absorbance of the complexed standard solution by preparing 6 Nessler tubes in accordance with the amount of standard solution. Then we took 25 mL of each standard solution concentration and put it in a Nessler tube.

In each tube, 2 drops of Rochelle salt and 1.5 mL of Nessler reagent were added. The color that occurs was immediately checked using a spectrophotometer with a wavelength of 420 nm. Absorbance and concentration graphs were then created. Where there is a standard curve for the absorbance and concentration graphs, when the absorbance and concentration relationship is linear, it must be ensured that the relationship that occurs meets the straight-line equation according to Lambert-Beer.

RESULTS

Based on the examination of the albumin levels using the spectrophotometric method on the samples of cork fish and eels, the results are shown in Table 1.

Table 1
Comparison of the Albumin Levels in Cork Fish and Eels

Sample	Albumin Levels (%)		The difference in albumin levels
	Cork Fish	Eel Fish	
1	26.08	16.91	9.17
2	26.21	16.94	9.27
3	25.80	16.42	9.38
4	24.97	17.93	7.04
5	25.66	15.87	9.79
6	25.08	16.77	8.31
7	25.94	16.89	9.05
8	25.72	17.69	8.03
9	26.22	15.58	10.64
10	25.38	16.29	9.09
11	26.14	17.43	8.71
12	26.73	17.09	9.64
13	25.31	17.22	8.09
14	24.84	17.44	7.40
15	25.68	16.68	9.00
16	25.71	17.52	8.19
Jumlah (Σ)	411.47	270.67	140.8
Rata-rata	25,716875	16,916875	8.8
Sd	0.5103	0.6454	-

Source: BBLK Surabaya, 2018

Based on Table 1, it can be seen that for the albumin levels on average, there is not much of a significant difference in cork fish, with an average of 25.716875 %. The eel obtained an average of 16.916875 %. The average difference in albumin level between the two treatment groups was 8.8 %.

Figure 1 shows that the average albumin level in cork fish is 25.716875 % while the average albumin level in eels is 16.916875 %.

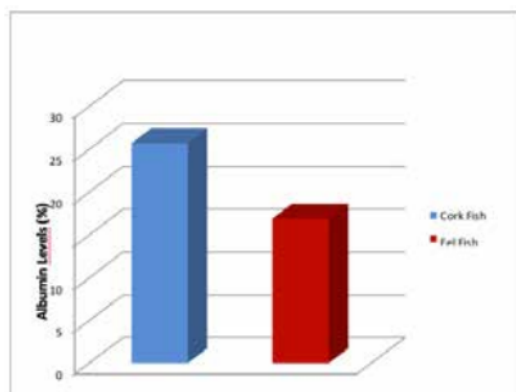


Figure 1. BarChart of Albumin Levels in Cork Fish and Eels.

Based on the results of the study of albumin levels in cork fish and eels, we proceeded with the statistical tests using the SPSS (Statistical Program Social Science) program. The results were tested for normality to determine whether the data was normally distributed using the Kolmogorov-Smirnov and Shapiro-Wilk tests.

From the results of the Kolmogorov-Smirnov and Shapiro-Wilk normality tests above, the results show that the data is normally distributed with a sig value of > 0.05 . The results for cork fish and eel using the Kolmogorov-Smirnov test obtained a significant value of 0.200 where the sig value > 0.05 , namely that the data showed normal distribution. The normality test using the Shapiro-Wilk test for cork fish obtained a significant value of 0.881. For Eel, a significance value of 0.828 was obtained where the sig value was > 0.05 . This means that the data showed a

normal distribution. After that, we proceeded to the paired T-test using the Independent Sample Test.

The paired T-test had an error rate of 0.05 % or sig < 0.05 which indicates that there is a significant difference between the albumin levels in cork fish and eels with sig < 0.05 . This is significant at $P=0.0001$. This means that the alternative hypothesis (H_a) is rejected and (H_o) is accepted, meaning that there are differences in the albumin levels found in cork fish and eels.

DISCUSSION

Analysis of Cork Fish Albumin levels

Based on the results of the study, it can be seen that the average value for albumin level in cork fish as shown in the Table 1 and Figure 1 is 25.7169 %. According to Suprpti, 2008, the cork Fish has a protein content of 25.2 gr. The protein content of cork fish consists of important amino acids, both essential and non-essential. Essential amino acids are amino acids that cannot be synthesized in the body, meaning that they are required as part of our food intake; isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. It can be seen in the table for cork fish content that cork fish contain all of these essential amino acids. Meanwhile, the non-essential amino acid groups in cork fish such as glutamic acid (14.253 %), arginine (8.675 %), and aspartic acid (9.571 %) were also found to be relatively high. The three non-essential amino acids are very important when it comes to wound healing (19).

Analysis of Eel Albumin Levels

Based on the results of the laboratory examination, it can be seen that the average value of eel albumin levels in Table 1 is 16.9169 %. Eel has a protein content of 14.0 g. Eel is a type of fish that is consumed as food and it is a very good prospective source of protein, fat, vitamins, and minerals (2). The main advantage of fish protein compared to other products is the completeness of the amino acid composition and its ease of digestion. Given the large role of nutrition in

health, fish is the right choice for diets in the future. Eel protein is rich in several amino acids that are of quite good quality including leucine, lysine, aspartic acid, and glutamic acid. Leucine and isoleucine are essential amino acids that are indispensable for the growth of children and for maintaining nitrogen balance in adults. Leucine is also useful for the reshuffling and formation of muscle protein.

The average value of albumin levels in eels is sufficient to meet a number of protein needs, therefore it can be considered an alternative food ingredient to meet the protein needs in the wound healing process.

Analysis of the Difference between Cork Fish and Eel

Based on data analysis using the free t-test, the albumin levels in cork fish and eel showed a significant difference of $p < 0.05$. The difference in the acquisition value (edible portion or EP) of the albumin levels in the two fish can be influenced by several factors.

The factors that can affect the size of the acquisition value (edible portion) include the size of the fish in weight and the method of separation between the bone and meat (preparation). The habitat where they live and the method of preparation affect the yield of the meat produced (20). EP is strongly influenced by gender, age, heredity, and food availability (21).

EP can also be influenced by fish growth, especially fish weight gain. The greater the weight of the fish, the higher the EP of the fish (12). The results of this EP calculation as determined by a previous author show that the heavier the fish, the higher the EP. Growth is influenced by internal factors including genetic sex, size, age, eating habits, and other biological factors. External factors include habitat, season, water temperature, type of food, and other environmental factors (22).

The fish condition factors also often have a major influence including the availability of feed and the initial growth of the fish, both of which are dynamic and varied. The average condition of each population varies seasonally and yearly. Within a group of individuals, different

condition factors influence it. Gender and gonadal development also provide variation in the length relationship (23)

However, based on the comparison of the average value, even though the albumin level in the eels was found to be lower than the albumin level in the cork fish, the protein content of the eel can be used as an alternative for the wound healing process (18). The adequacy of the albumin levels in eels for the wound healing process can be seen from the average value of the eel albumin levels which is quite high compared to egg albumin and other fish meat. This review is also based on the completeness and digestibility of the amino acid content contained in the eel. On the other hand, the feasibility of eels as an alternative source of albumin in the wound healing process is based on the availability and price of eels as they are easier to access and cheaper than cork fish. From the description above, the albumin levels possessed by eels can replace the albumin levels in cork fish as part of the wound healing process (24).

CONCLUSION

Based on the results of this study, it can be concluded that the albumin levels in cork and eel fish were found to be significantly different. The average value of the albumin content contained in cork fish is 25.7169 % and the average albumin content contained in eels is 16.9169 %. Eels can therefore be recommended as a source of natural albumin for use in wound healing.

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