



Macronutrient Analysis and *In vivo* Test in the Utilization of Barracuda Fish (*Sphyraena barracuda*) Asap Liquid as an Action to Meet Protein Needs

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The smoking process has undergone significant development and liquid smoke has been widely used for the food industry as an ingredient that gives a distinctive aroma and flavor to food products. The purpose of this study was to determine the macronutrient quality of smoked fish and protein absorption in mice. Quality testing of barracuda smoked fish refers to SNI 2725: 2013. Test types include water content, ash content, protein content, fat content, pH, and Benzo[a]pyrene. *In vivo* analysis was conducted on mice to observe Protein Efficiency Rate (PER), Net Protein Ratio (NPR), Total Protein Digestibility (TPD), Biological Value (BV), and Net Protein Utilization (NPU)

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values. Data were analysed with One-Way ANOVA. The results showed that the use of liquid smoke at a concentration of 5% had a significant effect ($p < 0.05$) on the test of moisture content of 55.2 ± 0.50 , ash content of 4.28 ± 0.02 , protein content of 35.47 ± 0.23 , fat content of 4.55 ± 0.08 , pH 6.77 ± 0.05 and benzo[a]pyrene < 0.02 . The parameter results of TPD 78.21 ± 31.87 , NPU 80.43 ± 3.75 in liquid smoked barracuda fish have higher values than traditional smoked barracuda fish, while PER 3.6 ± 4.06 , NPR 4.06 ± 1.76 and BV 200 ± 4.24 have higher values in liquid smoked barracuda fish compared to traditional smoked barracuda fish. The addition of liquid smoke can to maintain product quality and improve the nutritional quality of protein because protein can be digested and absorbed by the body properly.

Keywords: Barracuda fish; liquid smoke; protein quality; In vivo.

1. INTRODUCTION

One of the fishery products that has high production value is barracuda fish (*Sphyraena barracuda*). The potential production of barracuda fish in Indonesia in 2020 is 1,549,963 tons (Ministry of Marine Affairs and Fisheries/KKP, 2021). This potential is supported by the processing of fishery products which increases every year. Fish processing products are a commodity that is in high demand in Indonesia and the world. FAO predicts that by 2030 the world's demand for fish will reach 172 million tons [1]. The data proves that the global community's preference for fish-based food is increasing. The potential of fisheries production in Indonesia is an important food commodity because it contains nutrients that provide benefits for human health. one of these potentials is found in barracuda fish.

Barracuda fish (*Sphyraena barracuda*) can be an alternative source of protein besides other economically important fish whose presence has begun to be small in the waters. Barracuda fish has the following nutritional content, 71.40% water content, 20.45% protein, 0.25% fat, 6.56% carbohydrates, and 1.34% ash [2]. Utilization of this potential with proper processing needs to be done so that it can be utilized optimally. Smoking is a traditional processing method and produces fish products that have a distinctive aroma and flavor of smoke that consumers like. This processing has several disadvantages, including uncontrolled temperature, non-uniform products and many nutrients that have been damaged by the heating process, the content of carcinogenic compounds in the product, and relatively low selling value. These shortcomings trigger product innovation, one of the innovations made is to utilize liquid smoke in smoked fish processing.

One of the efforts to meet the protein intake needs of the community is to pay attention to the quality and food safety aspects of smoked fish

products. Studies on product quality can be carried out by analysing proximate values (moisture, fat, protein, and ash content), pH value, and benzo[a]pyrene content. Meanwhile, food safety aspects can be studied *In vivo*, namely testing using test animals whose physiological properties are similar to humans. The test animals used are mice (*Mus musculus*). Therefore, to produce smoked barracuda fish products that have high nutritional value and are safe for consumption, research on liquid smoked barracuda fish processing on nutrient absorption *In vivo* needs to be done.

2. MATERIALS AND METHODS

Smoked fish products are made from traditional and semi-modern smoking processes. Fresh barracuda (*Sphyraena barracuda*) was obtained from Rejomulyo market, Semarang City. Liquid smoke was obtained from PT Asap Cair Multiguna, Semarang City. Barracuda fish used in the study were fresh barracuda fish with an average weight of 150 g to 250 g. The fresh barracuda fish used is barracuda fish that meets the quality requirements of fishery product raw materials. The characteristics of barracuda fish used include, among others, having a compact texture, bright match colour, scales that are not easily separated, and have reddish gills. Sample making is divided into 2 types of smoking. The first smoking uses liquid smoke in the process and the second smoking is smoking without liquid smoke. The concentration of liquid smoke used in this study was 5%. The oven process is carried out at a temperature of 80°C and carried out gradually to a temperature of 100°C . The next process after the product is obtained then quality testing is carried out including tests of moisture content, ash content, protein content, fat content, pH, and Benzo[a]pyrene. This test was carried out with 3 replicates and then analysed to determine whether the different treatments given had a significant effect or not.

Phase II research was carried out with observations during the maintenance of mice. mice were divided into 3 groups with each cage containing 5 mice of balb c mice aged 3 months with an average weight of 26 grams. mice were maintained by being given 3 different types of rations for 28 days. during the maintenance process, body weight was measured once every 1 week and the weight of feces and the weight of the remaining feed every day. Feeding is done every morning and evening, the weight of feed given per process is 50 grams. the feed groups given include standard ration feed with complete nutritional composition, liquid smoked barracuda fish feed group and traditional smoked barracuda fish. in liquid smoked barracuda fish and traditional ration composition only protein. Standard ration was used as control feed. Tests conducted in phase II of the study included Protein Efficiency Ratio (PER), Net Protein Ratio (NPR), Total Protein Digestibility (TPD), Net Protein Utilization (NPU), Biological Value (BV) and Total consumption, body weight gain and feces weight.

3. RESULTS AND DISCUSSION

The normality and homogeneity test results at the 95% confidence interval showed that the water content of barracuda smoked fish spread normally. This can be seen in the sig value of 0.118 which shows that the sig value > 0.05. Analysis of variance (ANOVA) to determine the effect of differences in smoking methods. The test results showed that the difference in the smoking process was significantly different Sig (0.00) < 0.05. The test was continued by using the Real Difference Test (BNJ). The highest water content was found in smoked barracuda fish with an average value of 61.6%. The

decrease in water content that occurred was 17.2%. The water content value of smoked barracuda fish using liquid smoke has met SNI 2725, which has is with a maximum value of 60%.

The higher the moisture content in the product the shorter the shelf life, conversely if the moisture content in a product is low then the shelf life of the product is longer. One way to reduce the moisture content in the product is by utilizing high temperatures because with the use of high temperatures, the moisture content contained in the material will easily evaporate. One way to reduce the moisture content in food is by using the smoking method, in this case by utilizing liquid smoke. According to Yuliasri et al. [3], the processing process with smoking is expected to extend the shelf life of the product and reduce the moisture content in the product. The decrease in water content in the product is influenced by temperature and length of processing time.

The normality and homogeneity test results at the 95% confidence interval showed that the ash content of barracuda smoked fish spread normally. This can be seen in the sig value of 0.141 which shows that the sig value > 0.05. Analysis of variance (ANOVA) to determine the effect of differences in smoking methods. The test results showed that the difference in the smoking process was significantly different Sig (0.00) < 0.05. The test was continued by using the Real Difference Test (BNJ). The highest ash content was found in traditional smoked barracuda fish with an average value of 5.97%. The increase in ash content was 5.87%. The ash content of smoked barracuda fish using liquid smoke has fulfilled SNI 2725 with a maximum value of 20%.

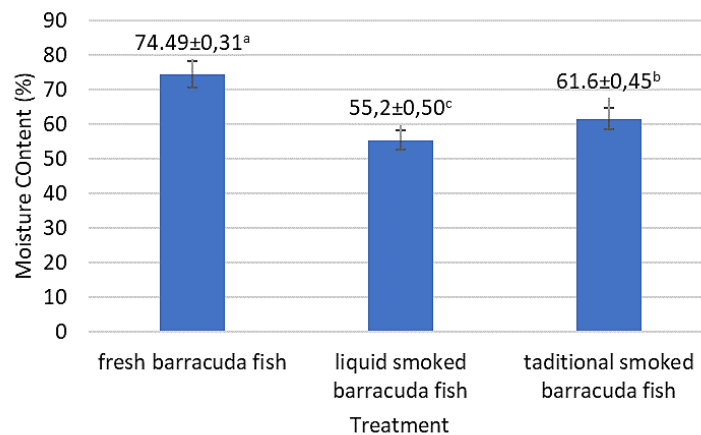


Fig. 1. Moisture content

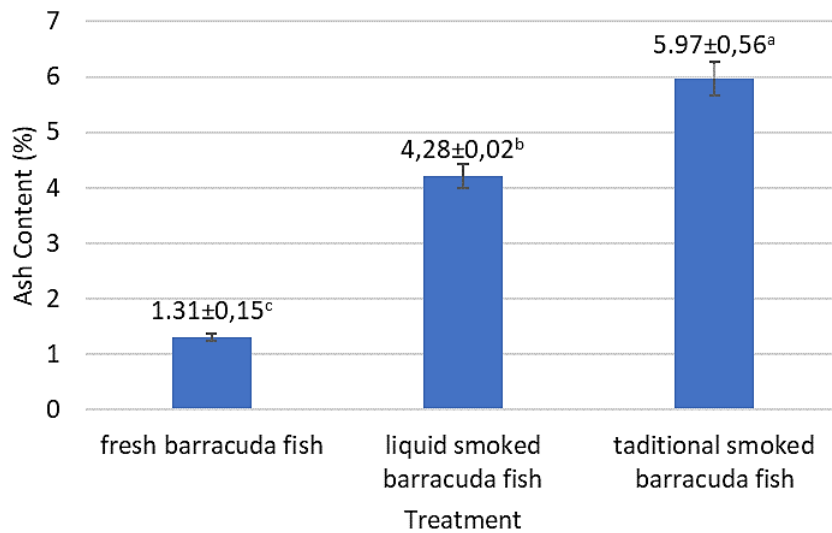


Fig. 2. Ash content

The highest ash content in traditional smoked barracuda fish was 5.97%. The ash content of fresh barracuda fish when compared with traditional smoked barracuda fish and liquid smoke increased. The increase is influenced by various factors during the smoking process. One of the main factors is the salt content contained in the product. This is because in the process of processing fresh barracuda fish that will be processed is soaked in a salt solution. This is in line with the results of research by Swastawati et al. [4], which stated that the ash content of smoked fish increased by about 2.58% from the ash content value of ariid catfish, this may be influenced by the salt content in the product added during soaking before smoking and may also be influenced by chemical processes that cause

changes in the composition of water, protein, and fat.

The normality and homogeneity test results at the 95% confidence interval showed that the protein levels of barracuda smoked fish spread normally. This can be seen in the sig value of 0.2 which shows that the sig value > 0.05. Analysis of variance (ANOVA) to determine the effect of differences in smoking methods. The test results showed that the difference in the smoking process was significantly different Sig (0.00) < 0.05. The test was continued by using the Real Difference Test (BNJ). The highest protein content was found in smoked barracuda fish using liquid smoke with an average value of 35.47%. The increase in protein content that occurred was 74.47%.

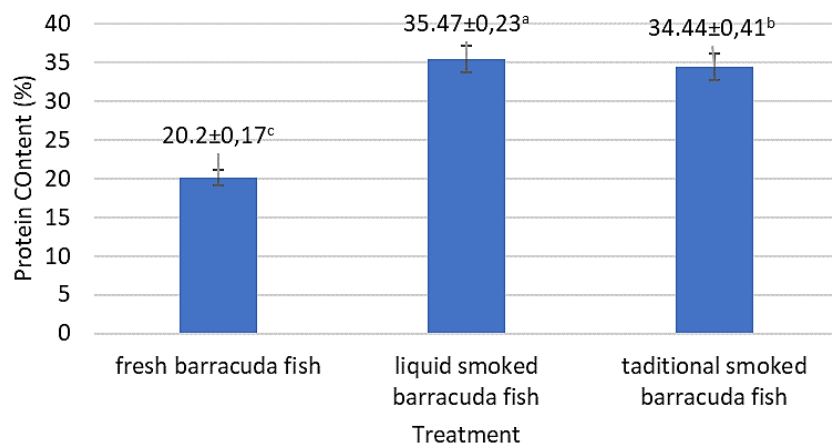


Fig. 3. Protein content

Nitrogen content has a role as a shaper of protein quality. The quality and stability of a food is determined by high protein content. Protein content is inversely proportional to water content, the lower the water content, the higher the protein content of the product. The nugget quality results are better than the standard because it is animal protein, then the protein content of liquid smoked barracuda fish is 1.03% higher than traditional. In line with the research of Swastawati et al. [5], the use of 3% liquid smoke affects the protein content of milkfish ($p < 0.05$), with a value of 15.62%. Liquid smoke has a high osmotic pressure so that it can draw water from fish meat and cause coagulation of proteins. Protein content increases because the content of liquid smoke composition functions as a water binder, so a lot of water loss will increase the percentage of protein content [6].

The normality and homogeneity test results at the 95% confidence interval showed that the fat content of barracuda smoked fish spread normally. This can be seen in the sig value of 0.144 which indicates that the sig value > 0.05 . Analysis of variance (ANOVA) to determine the effect of differences in smoking methods. The test results showed that the difference in the smoking process was significantly different Sig (0.00) < 0.05 . The test was continued by using the Real Difference Test (BNJ). The highest fat content was found in smoked barracuda fish using liquid smoke with an average value of 4.55%.

The process of smoking or processing food ingredients causes some damage to the components of the food ingredients, one of which is the fat content of the food ingredients. Based on the data obtained, it can be seen that the fat content of the material treated with the addition of liquid smoke is able to maintain or have a higher fat content compared to the fat content of traditional aspa fish. Liquid smoke has antioxidant abilities derived from phenol compounds contained in the liquid smoke. In addition, in the processing, process some ingredients that easily evaporate when processed using high temperatures, one of which is the easy dissolution of fat when processed with high temperatures. Fat content is also influenced by the water content contained in the material if the water content in the material is higher, the fat content in the material is also low, and vice versa. According to Hutomo et al. [7], smoking by utilizing liquid smoke is able to maintain the quality of ingredients with a fat content that does not decompose.

The normality and homogeneity test results at the 95% confidence interval showed that the pH of barracuda smoked fish spread normally. This can be seen in the sig value of 0.20 which shows that the sig value > 0.05 . Analysis of variance (ANOVA) to determine the effect of differences in smoking methods. The test results showed that the difference in the smoking process was significantly different Sig (0.00) < 0.05 . The highest pH was found in smoked barracuda fish using liquid smoke with an average value of 7.91%.

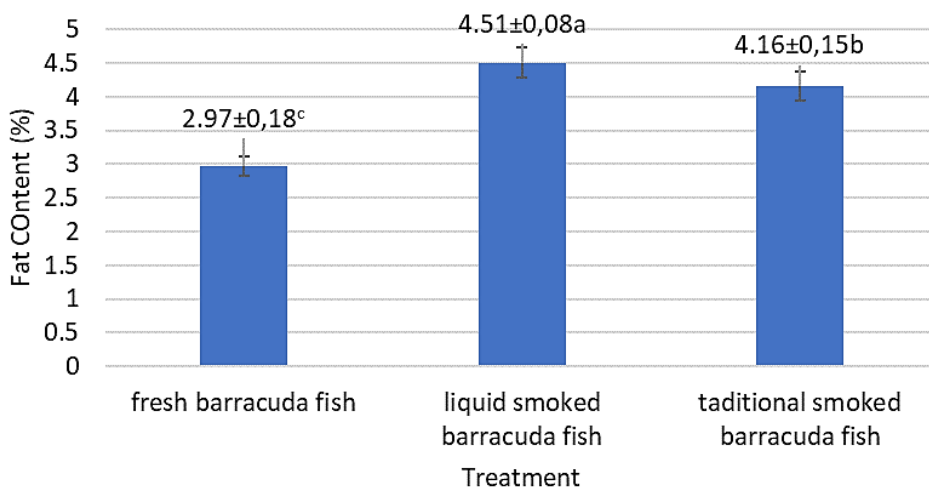


Fig. 4. Fat content

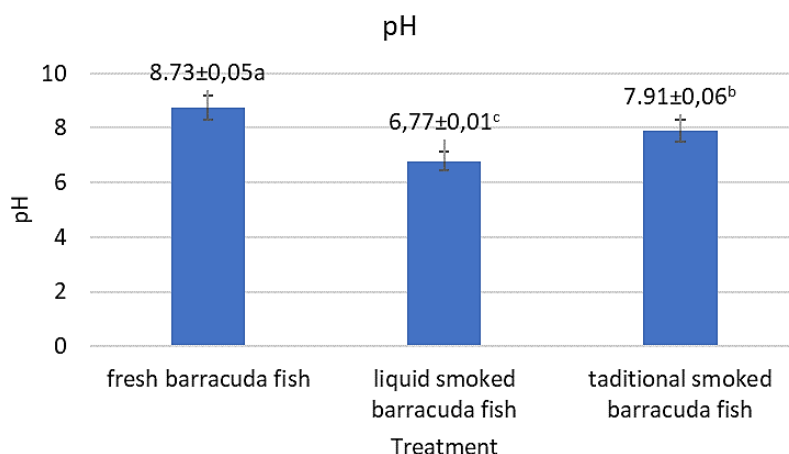


Fig. 5. pH values

The pH value is one of the indicators used to determine the freshness of fish. In the process of fish spoilage, changes in meat pH are very large because they affect the process of autolysis and bacterial attack. The pH results of the fish - smoking process can be seen in the diagram above. The pH of traditional smoked fish is more alkaline than the pH of smoked fish using liquid smoke. The difference between the two is 1.14. According to Fardiaz [8], a good pH for preserved fish is between 2.0-5.5 while a pH between 6.0-8.0 is a good medium for the growth of microorganisms.

Benzo[a]pyrene is one of the Polycyclic Aromatic Hydrocarbon (PAH) compounds. This compound is one of the carcinogenic compounds commonly found in processed products that use high temperatures. Based on the Table 1, shows that benzo[a]pyrene compounds in smoked fish products using liquid smoke have met SNI 2725, which is a maximum of 5 µg/kg. The results of this study indicate that benzo[a]pyrene from traditional smoked fish is quite high. This is in line with the results of research from Swastawati

et al. [9] which states that the value of benzo[a]pyrene in traditional smoked catfish is 8.25 µg/kg.

The percentage results obtained in the total feces of the standard ration compared to liquid smoked barracuda fish were 19.5 g and traditional smoked barracuda fish were 20.1 g, while between liquid and traditional smoked barracuda fish was 0.6 g. The percentage of total feces obtained was influenced by the level of consumption. The total feces obtained is influenced by the level of consumption. Total consumption has a comparison of the difference value between liquid smoked barracuda fish and traditional barracuda fish which is not high at 0.92%. According to Ernaningsih et al. [10] the total consumption and weight of feces on different rations are not significantly different ($P>0.05$). The level of ration consumption is influenced by body size, adult body weight, sex, age, genetic factors, and nation. Feed factors that affect feed consumption are physical properties, nutrient content, and palatability of feed.

Table 1. Benzo[a]pyrene analysis result

No	Treatment	Benzo(a)Pyrene (µg/kg)
1.	Fresh baracuda fish	0,028
2.	Liquid smokes baracuda	<0,02
3.	Traditional barracuda fish	7,12

Table 2. Total consumption of smoked barracuda fish in mice

Group	Total Feces (g)	Total Consumption (g)	Weight (g)
PS	36,8±3,45	158,6±8,2	38±5,4
IBA	17,3±2,87	186,4±2,7	28±4,8
IBT	16,7±3,83	184,7±4,3	27±5,8

Table 3. Parameter values of PER and NPR growth methods

Group	PER (%)	NPR (%)
Standard	3,6±4,06	4,06±1,76
IBA	2,8±0,64	3,81±0,38
IBT	2,1±0,94	2,74±0,91

Table 4. Nitrogen balance method parameter values (BV, NPU, TPD)

Group	BV (%)	NPU (%)	TPD (%)
Standard	148±4,71	78,86±3,15	85,12±0,23
IBA	200±4,24	80,43±3,75	78,21±1,87
IBT	188±2,7	70,87±2,86	72,43±1,76

Feed that contains high protein can be absorbed and retained by the body so that little feces are released, while feed that is not well absorbed by the body is released more feces and higher weight composition. Increased fecal weight occurs along with the high total feed consumed. More active fecal behavior will affect body weight because feces will consume more feed for maintenance. According to Hidayat and Alfien [11], food consumption intake is regulated by the brain, and the hypothalamus plays an important role. The expression of various peptides that regulate appetite and specific circuits of the hypothalamus allow the complex interaction of several nuclei that control energy balance.

The body weight of the mice in the observation is affected by the total appetite of the mice. Changes in body weight that occur can be used as a reference in observing whether the feed given has an effect on the growth of the mice. Based on the data above, it shows that the heaviest body weight is found in mice with standard rations of 38g and the smallest in mice with traditional smoked barracuda fish rations of 27g, which is not significantly different from mice on liquid smoked barracuda fish rations. This is influenced by several factors, one of which is the composition of the ration. Standard rations have a complete nutritional composition such as carbohydrates, fats, proteins, and minerals. Different from the provision of liquid smoke and traditional barracuda fish rations, the composition of nutrients contained in it is only protein. not fulfilled. This is in line with the opinion of Sandana et al. [12], which states that body mass is very sensitive to sudden changes, for example due to increased and decreased appetite or increased and decreased amounts of food consumed, one way to increase appetite can be done with a variety of foods so as to cause an increase in body weight.

Parameter analysis of PER value to measure body weight in mice through the growth of the mice. The PER value of the standard ration is 3.6%, liquid smoked barracuda fish is 2.8%, and traditional barracuda fish is 2.1%. The increasing body weight growth of mice proves that the protein in the feed is efficient, so the PER value obtained is high. The PER value obtained in the standard feed is the difference in comparison with liquid smoked barracuda fish 0.8%. Standard with traditional barracuda fish is 1.5%. This means that smoked barracuda fish have a high efficiency in the growth of mice because of the quality of the barracuda fish itself. The results of research by Rahmi et al. [13], stated that a mixture of banana and soybean base ingredients substituted with sesame seed flour (*Sesame indicum*) with a composition of 67%: 16.5%: 16.5%, with casein (10% protein).

The NPR method was used to determine the maintenance of den results by adding one non-protein group. The NPR value obtained was 4.06% in the group of standard ration mice "A594K", liquid smoked barracuda fish at 3.81% and traditional smoked barracuda fish at 2.74%. The highest NPR value obtained from the results of this study was in the standard ration. The difference between the standard ratio and liquid smoked barracuda fish was 0.25% while liquid smoked barracuda fish and traditional barracuda fish was 1.07%. The comparison between the standard ration and traditional barracuda fish was 1.32%. These results indicate that the availability of protein in smoked barracuda fish is sufficient for growth and maintenance. According to Cheng et al. [14], PER and NPR values support the addition of amino acids in increasing protein utilization.

True Protein Digestibility (TPD) is an indicator of the amount of protein that the body digests from

food. The results obtained in the standard feeding treatment "A594K" obtained a result of 85.12%, liquid smoked barracuda fish feed 78.21%, and traditional barracuda fish 72.43%. The percentage difference between the standard and liquid smoked barracuda fish was 9.2% while the standard with traditional barracuda fish was 14.9%. The value of the standard group and liquid smoked barracuda fish obtained higher results, namely $\geq 70\%$. Protein digested by the body is seen from how much feed is eaten and excreted by rats, namely urine and feces. According to Research by Elango et al. [15], digestibility is the percentage of amino acid intake that has disappeared from the digestive tract and is a standard measure of estimating bioavailability.

Biological Value (BV) is a parameter to evaluate the value of protein absorbed by the body. Feed consumption absorbed and retained by mice measures how much the body uses protein obtained from food for tissue formation. Biological value ≥ 70 of food consumption is able to provide growth. Mice that consume sufficient amounts of feed and energy have good growth and behavior. The BV results obtained in the consumption of standard feed "A594K" were the lowest. The standard ration with liquid smoked barracuda fish is 12% then the standard with traditional smoked barracuda fish is 52%. Based on the results in the table above, the value of feed consumption obtained the greatest results in liquid smoked barracuda fish whose value is $\geq 70\%$, the protein absorbed in the body is used properly. According to Anyalogbu et al. [16], biological value (BV) to measure absorbed food protein (nitrogen) is used by the organism for physiological processes. The BV of rats in the test group above 70% indicates that nitrogen is absorbed by the body or not wasted with urine.

Net Protein Utilization (NPU) is a quality index to determine the amount of protein retained and digested by the body. $NPU \geq 70$ is a value that is said to be sufficient to show high nitrogen derived from protein in food can be retained and digested in the body. The consumption of the standard ration "A594K" compared to the traditional smoked barracuda fish was 9.56%. The comparison of the two rations had the highest difference compared to the standard with liquid smoked barracuda fish at 1.57% and liquid smoked barracuda fish and traditional smoked barracuda fish at 7.99%. Mice that have high NPU values can be seen from the liveliness and consumption of food containing sufficient protein.

This is based on 28 days of observation in mice that consume liquid smoked barracuda fish and feces urine is small, therefore the amount of protein retained and digested by the body is better. Based on Almatsier's [17] research, the NPU value limit is at least 70%. Results $> 70\%$ of nitrogen from food is retained in the body or not wasted with feces and urine.

4. CONCLUSION

The conclusion obtained from the macronutrient analysis research and *In vivo* tests in the utilization of liquid smoked barracuda fish (*Sphyraena barracuda*) as an effort to fulfill protein needs is that the characteristics of smoked and traditional barracuda fish have significant differences ($p < 0.05$) in water content, ash content, protein content, fat content, and pH. The content of benzo[a]pyrene in liquid smoked and traditional barracuda fish meets SNI 2725: 2013. The addition of liquid smoke is able to maintain the nutritional quality of protein compared to the traditional smoking process, but the absorption of protein is most optimal in standard feed because it has a complete nutritional composition. Based on the results obtained, liquid smoked barracuda fish has the potential as a source of protein for the community.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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