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Amino Acid and Fatty Acid of Abalone *Haliotis squamata* Cultured in Different Aquaculture Systems

Magdalena Latuihamallo[a*], Dulmi'ad Iriana[b], Danny Apituley[a]

[a]*Marine Science and Fishery Faculty Pattimura University, Ir. M. Putuhena Street Poka, Ambon 97116, Indonesia*
[b]*Marine Science and Fishery Faculty Padjadjaran University, Dipati Ukur Street 35, Bandung 40016, Indonesia*

Abstract

The aim was to analyze the amino acid and fatty acid of abalone *Haliotis squamata* cultured in different aquaculture systems. The percentage of total amino acid of abalone cultured in laboratory, floating cage, and longline system valued 11.02%, 9.53% and 8.24%. respectively. Abalone contained 14 saturated fatty acids and 16 unsaturated fatty acids. The saturated fatty acids of abalone were dominated by palmitenoic acid (C16:0) and steraenoic acid (C18:0). The high percentage of unsaturated fatty acids in abalone were oleat acid (C18:1n9), linolenic acid (C18:3n3), arachidonoic acid (C20:4n6) and eicosapentaenoic acid/EPA (C20:5n3).

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Keywords: Abalone *Haliotis squamata*, Amino Acid, Fatty Acid.

INTRODUCTION

Abalone is a kind of gastropod which has high economic value and being a profitable income source to fishers in Indonesia caused by its high price and simple processing. The others benefit of this commodity are its high nutrition value and the safety of consuming it, because abalone do not consume the red tide planktons which produced PSP toxins [10, 23].

Abalone *Haliotis squamata* has some comparative benefits compared with other spesies of abalone such as *H. asinina*, like: (a) higher price; (b) better in performance; (c) higher demands [11]. The flesh of abalone has high nutrition with composition of protein (71.99%), lipid (3.2%), crude fiber (5.6%), ash (11.11%), and water (0.6%). While the shell has estetic and economic value which have been used as jewelry and trinkets, button made, and other kinds of handicraft [24].

This research was aimed to analyze the amino acid and fatty acid of abalone *Haliotis squamata* cultured in different aquaculture systems.

MATERIALS AND METHODS

Materials

The tools used in this research *i.e.*: Kjedahl flask, flask, erlenmeyer, lipid beaker, and Soxhlet tube. While the materials are catalyst (K_2SO_4 and $CuSO_4$), concentrated H_2SO_4, H_2O_2,H_3BO_3, indicator solution, $Na_2(SO_4)_3$ (alkali), HCl, chloroform, aquadest, and abalone *Haliotis squamata*.

* Corresponding author
E-mail address: magda_latu@yahoo.co.id

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Sampling Method

Abalone *Haliotis squamata* were cultured in three different type of aquaculture systems, *i.e.*: floating cage system, longline system, and laboratory system. The abalones were fed with some natural foods *i.e.Gracillaria lichenoides, Ulva fasciata,* and combination of both *Gracillaria lichenoides* and *Ulva fasciata.* The abalones were cultured for one year and fed 5 times a day. Then the flesh of abalones were taken to analyze the composition of amino acids and fatty acids. Beside that, the analyzes were also conducted to male and female gonads.

Analysis Method

Protein

Protein is estimated by Micro Kjedahl Method [1]. As much as 0.75 grams of sample put into Kjedahl flask, then added 6.25 grams of K_2SO_4 and 0.6625 grams of $CuSO_4$ as catalyst. 15 ml of concentrated H_2SO_4 and 3 ml of H_2O_2 pour cautiously into the flask and put in acid chamber for 10 minutes.

The next step is destruction process at a temperature of 410 ^oC for 2 hours or acquired the pure solution, then pour 50-75 ml of aquadest after the temperature is normal.

Erlenmeyer contained 25 ml solution, 4% of H_3BO_3 with indicator (bromocherosol green 0.1 % and mathyl red 0.1 % (2:1)) as distiller. Kjedahl flask is affixed to the distillation tool, then add 50 ml of $Na_2(SO_4)_3$ (alkali). The distillation is conducted and the distillat is collected into the erlenmeyer until gain 150 ml of distillat volume (the distillat is green). The distillat is titrated excess HCl 0.2 N until the color changed to grey. Natural form, HCl titration, are made liked the sample steps. The sample testing is done in duplo. The protein is estimated by ((A-B) x normality of HCL x 14.007 x 6.2) / W (g) where: A = ml sample of HCl titration, B = ml form of HCl titration

Lipid

Lipid beaker dried in oven (105 ^oC) is scaled up to constant weight (A). 2 grams of sample (C) covered with filter paper (lipid free) then put into soxhlet tube. 150 ml of chloroform is poured into the lipid beaker. The sample is refluxed for 8 hours. If the solvent is pure-looking, it sign that all lipid has been extracted. Then the solvent in lipid beaker is evaporated to separate the solvent and the lipid. Then the lipid beaker is dried in oven at a temperature of 105 ^oC for 30 minutes, and scaled up to constant weight (B). The lipid is calculated with (B-A)/C [2].

RESULTS AND DISCUSSION

The Proximate of Abalone *Haliotis squamata*

Table 1 shows the proximate analyzes of abalone which cultured in different aquaculture systems. The analyzes show that the protein percentage of abalone and the gonads are 21.66 % for floating cage system, 19.46% for longline system, 28.63% for laboratory system, 33.01% for male gonad, and 13.15% for female gonad. While the lipid percentage are 0.17% for floating cage system, 0.20% for longline system, 0.16% for laboratory system, 2.20% for male gonad, and 4.50% for female gonad. Different type of natural foods show insignificant result to proximate composition, while the aquaculture system seems to be more significant affecting the proximate composition [16].

Overall, it can be assumed that abalone cultured in laboratory system has better percentage and value of proximate and nutrition. It is showed by the higher percentage of protein, lipid, and crude fiber, compared to the abalones cultured in floating cage and longline system. Although it is not too significantly different in lipid percentage. The varies can affect the quality of protein and lipid in abalone, in both the flesh and the gonads.

Amino Acid Profile of Abalone *Haliotis squamata*

Table 2 shows the profile of amino acid of abalone which cultured in different aquaculture systems. The result shows that both the flesh and the gonads of abalone contained the amino acids which consisted of five non essential amino acid *i.e.* aspartic acid, glutamic acid, serine, glycine and alanine, and ten essential amino acid *i.e.*: arginine, histidine, threonine, tyrosine, phenylalanine, methionine, valine, leucine, isoleucine and lysine. By the result, the abalones fed by the three types of natural foods in the aquaculture systems qualified for the resource of animal protein in accordance with the amino acid adequacy determined by FAO/WHO/UNU [2, 6].

Table 2 also shows that different aquaculture systems can affect the quality of protein in abalone. Total percentage of amino acid in abalone cultured in laboratory system is higher than those cultured in floating cage and longline system, which valued 20.42%, 16.84%, and 14.62%, respectively. While the total of amino acid in male abalone is higher than the female, which valued 17.20% and 12.40%.

From the result, it can be assumed that the protein quality of abalone cultured in laboratory system is better than those cultured in floating cage and longline system. Figure 1 shows the amino acids contained in abalone. The percentage of essential amino acid of abalone cultured in laboratory system valued 11.02% which is higher than the abalones cultured in floating cage and longline systems, ranged 8.31-10.16% and 7.60-9.10%, respectively. Arginine and leucine are the highest percentages of essential amino acid found in abalone cultured in laboratory.

Protein is the major constituent of animal's body, and also function as enzymes and hormones. Protein and lipid are the major energy sources for marine animals in early development and that blacklip abalone is no exception in this respect [18]. In tissues of blacklip abalone, arginine, leucine, lysine, threonine, valine and isoleucine were the major amino acids, and taurine was not reported [15]. However, in the present study taurine + proline are found in significant amounts, in the highest quantities, in all the development stages, in the free amino acid pool. Taurine and glycine were reported as the main NEAA in the FAA pool in the muscle of New Zealand abalone, *H. iris* [4]. Taurine has also been observed to be the predominant amino acid in the FAA pool of the muscle of *H. tuberculata* and *H. discus* [19, 13].

Table 1.The Proximate Composition of Abalone *Haliotis squamata*

No.	Proximate Composition	Percentage (%)				
		Floating Cage	Long Line	LAB	Male Gonad	Female Gonad
1	Protein	21.66	19.46	28.63	33.01	13.15
2	Lipid	0.17	0.20	0.16	2.20	4.50
3	Water	67.53	69.07	68.64	55.42	70.20
4	Ash	6.76	7.53	7.35	8.23	7.61
5	Crude Fiber	5.05	4.29	4.40	3.90	2.12

The FEAA, a higher level of arginine, threonine and valine in muscle is documented for *H. discus* [29]. In the muscle of *H. iris*, arginine is the second major amino acid in the FAA pool after taurine followed by hydroxylysine, methionine and threonine [4]. In muscle and viscera of juvenile *H. diversicolor*, taurine, arginine, glycine, glutamic acid and alanine are found to be dominant in the FAA pool, and an increase in taste active amino acids (arginine, glycine, glutamic acid and alanine) in the autumn and winter months are also recorded [14].

The same amino acids dominate the FAA pool in muscle and viscera of *H. diversicolor*, and account for about 81 to 94 % of the FAA pool in muscle and viscera [5]. In general, the observations on the FAA pool in muscle and viscera of *H. diversicolor* are comparable to the present findings on the different development stages of blacklip abalone. Alanine, glycine and arginine, which are normally contained at a higher level in tissues of marine mollusks, are proven to be crucial in energy metabolism by maintaining glycolysis through the formation of opines

under hypoxic conditions [12]. Arginine is also an important endogenous energy compound as a phosphogen (phosphoarginine) in abalone [27].

Table 2. Amino Acid Profileof Abalone *Haliotis squamata*

No.	Amino Acid	Percentage (%)				
		Floating Cage	Long Line	LAB	Male Gonad	Female Gonad
1	Aspartic Acid	1.94	1.68	2.29	1.82	1.27
2	Glutamic Acid	3.15	2.69	3.75	2.32	1.71
3	Serine	0.99	0.86	1.15	1.03	0.77
4	Glycine	1.44	1.20	1.48	1.02	1.00
5	Alanine	1.99	1.81	2.35	2.64	1.39
	TNEAA	**7.31**	**6.38**	**9.4**	**7.81**	**6.14**
6	Histidine	0.24	0.22	0.31	0.38	0.24
7	Threonine	0.72	0.63	0.85	1.06	0.58
8	Arginine	1.69	1.48	2.16	1.50	1.68
9	Tyrosine	0.57	0.48	0.68	0.77	0.48
10	Methionine	0.32	0.29	0.47	0.38	0.26
11	Valine	0.68	0.59	0.83	0.96	0.53
12	Phenylalanine	0.63	0.47	0.71	0.77	0.50
13	Isoleucine	0.58	0.51	0.74	0.79	0.45
14	Leucine	1.29	1.11	1.57	1.30	0.87
15	Lysine	0.60	0.57	0.95	1.48	0.67
	TEAA	**9.53**	**8.24**	**11.02**	**9.39**	**6.26**
	TAA	**16.84**	**14.62**	**20.42**	**17.20**	**12.40**

TNEAA = Total of Non Essential Amino Acid
TEAA = Total of Essential Amino Acid
TAA = Total of Amino acid

Fatty Acid Profile of Abalone *Haliotissquamata*

Table 3 shows the profile of fatty acid of abalone which cultured in different aquaculture systems. Both the flesh and the gonads contain 14 saturated fatty acids and 16 unsaturated fatty acids. Similar to other fishery products, the saturated fatty acid of abalone, both the flesh and the gonads, are dominated by palmitenoic acid (C16:0) and stearenoic acid (C18:0). While the unsaturated fatty acid in abalone which has high percentage consisted of oleat acid (C18:1n9), linolenic acid (C18:3n3), arachidonoic acid (C20:4n6) and eicosapentaenoic Acid/EPA (C20:5n3).

By the result, just liked the other fishery products, it can be assumed that lipid of abalone has better value in nutrition, qualitatively, compared to the lipid of ruminant. Fatty Acid omega 3 and omega 6 liked linolenic acid (C18:3n3), eicosapentaenoic acid/EPA (C20:5n3) and docosahexaenoic acid/DHA show the high quality of lipid in abalone, in both the flesh and the gonads.

Figure 2 shows that different culture systems affected the quality of lipid of abalone. By the figure, it can be assumed that fatty acids, both the saturated or unsaturated, in floating cage and longline system has higher percentages compared to result of laboratory system.

One of the lipid function is to co-operate with protein to mold the membrane structure of body which has the physiological function as protector to organ it gild from external factors, *e.g.*, extreme temperature alterations which can affect the organism. It can be assumed that the temperature varieties in floating cage and longline systems may cause the higher percentage of lipid in abalone, particularly the lipid structural.

Table 3. Fatty Acid Profile of Abalone (*Haliotissquamata*)

No.	Fatty Acid	Percentage (%)				
		Floating Cage	Long Line	LAB	Male Gonad	Female Gonad
	Saturated Fatty Acid					
1	C8	nd	nd	0.10	Nd	0.05
2	C10	nd	nd	nd	Nd	0.03
3	C12	nd	nd	nd	0.10	0.23
4	C13	nd	nd	nd	0.04	nd
5	C14	0.39	0.39	0.59	3.07	4.53
6	C15	0.2	0.14	0.22	0.65	0.40
7	C16	5.3	4.99	5.67	12.73	18.44
8	C17	0.67	0.43	0.61	1.05	0.60
9	C18	2.22	1.91	2.16	2.57	2.93
10	C20	0.12	0.13	0.09	0.06	0.07
11	C21	nd	0.017	0.03	0.05	0.05
12	C22	0.51	0.55	0.34	0.13	0.07
13	C23	0.23	0.03	0.22	0.05	0.04
14	C24	0.16	0.48	0.12	Nd	nd
	Unsaturated Fatty Acid					
15	C14:1	nd	nd	0.02	0.02	0.10
16	C16:1	0.15	0.13	0.31	0.44	1.46
17	C17:1	0.04	0.2	0.07	0.10	0.14
18	C18:1n9t	0.14	0.03	0.12	0.21	0.20
19	C18:1n9c	3.59	2.83	4.02	6.51	10.95
20	C18:2n9t	nd	nd	0.04	0.23	13
21	C18:2n6c	0.18	0.11	0.24	0.46	0.34
22	C18:3n6	nd	nd	nd	0.06	0.03
23	C18:3n3	0.29	0.24	0.42	1.51	0.89
24	C20:2	nd	0.06	0.08	0.11	0.09
25	C20:3n6	0.5	0.01	0.09	0.63	0.28
26	C20:3n3	nd	0.01	0.03	0.07	0.07
27	C20:4n6	1.74	1.03	1.69	11.91	5.76
28	C20:5n3	0.92	1.22	0.54	2.09	0.84
29	C22:1n9	nd	nd	nd	0.04	0.03
30	C22:6n3	nd	0.02	nd	0.14	0.12

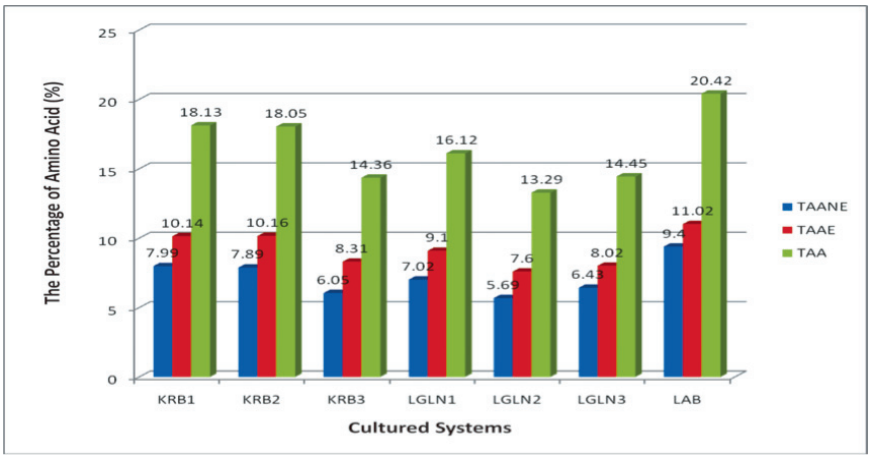
Note: nd = no detected

The fatty acid contents of wild and cultured Australian adult blacklip abalone, *Haliotis rubra*, are analysed by gas liquid chromatography. Wild abalone contains significantly higher levels of total n-3 polyunsaturated fatty acids (PUFA), eicosapentaenoic acid (20:5n-3), docosapentaenoic acid (22:5n-3) and alpha-linolenic acid (18:3n-3) than cultured abalone ($P<0.05$). The predominant n-3 PUFA is docosapentaenoic acid in wild abalone, while in cultured abalone a high level of eicosapentaenoic acid is found. The concentration of docosahexaenoic acid (22:6n-3) is low in both wild and cultured abalone, and cultured abalone has a significantly higher percentage composition of this fatty acid than wild abalone ($P<0.01$). Significantly higher levels of arachidonic acid (20:4n-6), 22:2n-6, 22:4n-6 and total n-6 PUFA are also found in wild abalone than in cultured animals ($P<0.05$). The ratio of n-3 PUFA to n-6 PUFA is the same in wild and cultured abalone. Manipulation of nutrient sources of cultured abalone may influence their lipid composition. Consumption of either wild or cultured abalone will contribute to dietary n-3 PUFA intake, with benefits to human health [24].

Cultured abalone also represent a source of LC n-3 PUFA and good source of DPA or consumers [8, 20, 30, 25, 26, 3]. The LC n-3 PUFA and total lipid contents of marine animals vary depending on various biological and environmental factors, such as diet of the animals, water temperature the reproductive cycle, and the latitude at which they were harvested [8, 17, 26].

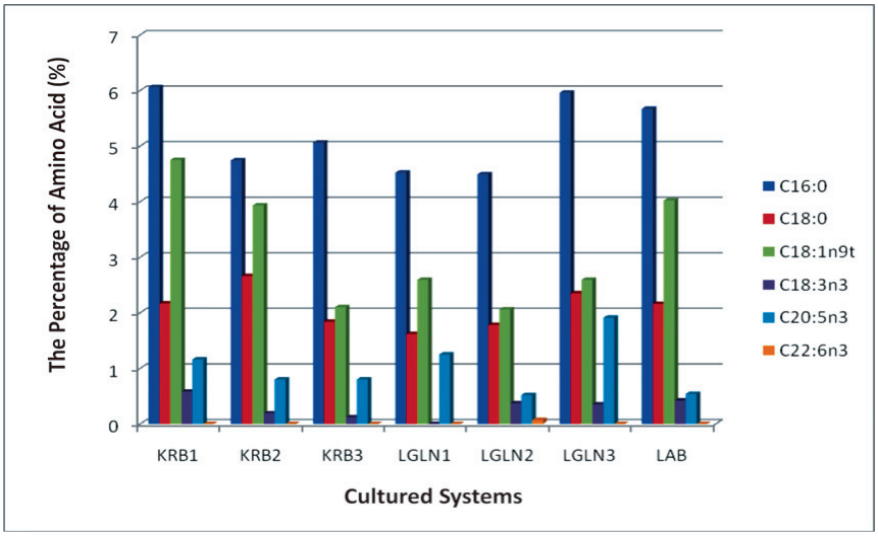
Studies on mollusks have also found that lipid content in these animals vary depending on the nutritional value of the food supply and the environmental influences on the metabolic activities [21, 22]. Available information suggests that *H. discus hannai* [31] have a capacity to synthesize EPA, and DHA from alpha-linolenic acid (18:3n-3, ALA) and ARA from linoleic acid (18:2n-6, LA) through elongation and desaturation. However, this study was based on the feeding trial only.

Similar studies also reported that *H. laveigata*, *H. fulgens* and *H. asinina*Linne have limited capacity to synthesize LC n-3 PUFA from ALA [7, 9, 23]. The same results were recorded in the study of *H.discus hannai* with its capacity to synthesize ALA to LC n-3 PUFA [28]. It appears likely that abalone is able to biosynthesize LC-PUFA to a certain extent, by elongation and desaturation of shortchain fatty acids.



Note :KBR = Floating Cage; LGLN = Long Line

Figure 1. Graphic of Amino Acid Analyzes of Abalone *Haliotis squamata* Cultured in Different Aquaculture Systems



Note :KBR = Floating Cage; LGLN = Long Line

Figure 2. Graphic of Fatty Acid Analyzes of Abalone *Haliotis squamata* Cultured in Different Aquaculture Systems

CONCLUSION

1. The quality of protein of abalone cultured in laboratory is better than those cultured in net floating cage and longline, caused by the higher value of essential amino acid it had.
2. Linolenat acid, EPA, and DHA found in the flesh of abalone are fatty acid omega 3 and omega 6 which very important for brain growth, shows that both male and female abalone are potential and have good quality of nutrient.

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