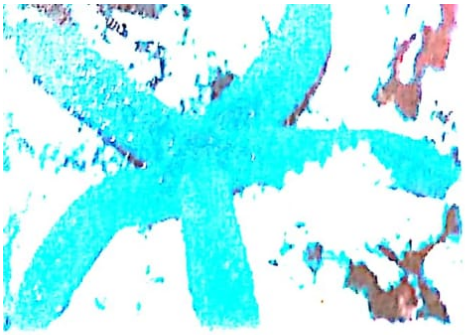

ISFM 2014

The 3rd International Seminar
# of Fisheries and Marine Science

proceeding

"Strengthening Science and Technology
Towards the Development of Blue Economy"

Faculty of Fisheries and Marine Science
University of Riau

ISBN 978-979-792-546-8

October 9-10, 2014
The Premiere Hotel
Pekanbaru, INDONESIA

ISBN 978-979-792-546-8

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ISBN 978-979-792-546-8

Preface

The aquatic ecosystem as a part of earth mega ecosystem is needed to be developed through science and technology that supports the prosperity of a nation. To support this, the International Seminar on Fisheries and Marine Science (ISFM) 2014 held in Pekanbaru took its theme of strengthening the science and technology towards blue economy development. The seminar covered a wide range of disciplines reflecting the vast area in fisheries and marine science, hence the papers received have also been embracing a wide range of topics in research and policy making.

In the seminar, a number of 50 invited papers were presented. A big proportion of these papers were published here in the proceeding. The presenters have been coming from 5 different countries, i.e. Indonesia, Malaysia, Thailand, Nepal, and Germany. Through the seminar, the fruitful discussions were produced via warm and freindly sessions, making a-two day program of seminar passed by thoroughly. The dsicussions touched not only the basic research and findings fishery and marine science, but also revealed the most recent development for the development based on the conservation and economic values of the aquatic resources both inland and of marine systems.

The seminar is entering its 4th anniversary and is expected to be conducted for each year to prosper the academic atmosphere within the Faculty of Fisheries of Univiversity of Riau, Pekanbaru and for the Indonesian and regional fora. It has been considered that the commencement of the seminar has been a very successful for the number of the attendance and the level of anthusiasm. The committee herewith is extending the grateful thankfulness for all the involved personels who were actively contributing for the implementation of the program. We also would warmly thankful for the attendance of the delegations coming from all over Indonesian universities and agencies, both from the state and the private sectors. The special thank was presented to our Minister for Marine Affairs and Fisheries of the Republic of Indonesia, the Honour Mr. Sharif C. Sutardjo, who has officially opened the seminar. His speech has been inspiring for the academicians and officials who attended the seminar. The committee would also like to extend its thankfulness for the Vice Governor of Riau province, Mr. Arsyad Juliandri, who has delivered a supportive message for the development of fishery and marine affairs sector in the province. Finally, we would also send our gratitude to all participants, commitee members, students and the pers communities who all have been considerably helping the commencement of the seminar.

We will be gladly to see you all in the incoming seminars here in Pekanbaru.

Chairman of ISFM 2014

Rahman Karnila

ISBN 978-979-792-546-8

Contents

ISBN 978-979-792-546-8

The Effect of *Caulerpa racemosa* Diet to Cholesterol Level Of Wistar Rats

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ABSTRACT

Caulerpa racemosa has been commonly eaten by people of Jepara coast as vegetables, however there are few reports on the nutritional value.*C. racemosa* seaweed can be categorized as *nutricious food*since its contain fatty acids that is needed by body and others nutrition such as protein, dietary fiber, vitamin and minerals.

The objectives of this research was to analysediet from *C. racemossa* especially on it effect in lowering cholesterol. This research was conducted on August – November 2012.Samples were taken fromthe coastal waters of Jepara, Central Java province.Rats animalsmodel wereobserved individually.The treatments applied on the research were as follow: control; ½ ODH (Optimal Dose of Human); 1 x ODH (ODH=0,09 gr/kg/day of *C.racemosa*) and 2 x ODH (0,18 gr/kg/day of *C. racemosa).*

The dose of seaweed flour of *C racemosa* in the amount of ½ optimal Dose of Human which is 0,045gr/kg/day able to decrease the cholesterol level for 58,13% in 2 weeks. A highly significant effect (P <0,01) on the cholesterol level between treatments. Triglyceride and LDL were significant different (P <0,05) between treatments. Howeverthere was no significant effect (P>0,05) on HDL (High Density Lipoprotein) between treatments.

Key words: *Caulerpa racemossa*, cholesterol, LDL, HDL, rats animal model, trIglycerides.

INTRODUCTION

Caulerpa racemosa or commonly known on the local name as *latoh* is a kind of algae that has been consumed by Jepara coastal (north coastal of central Java) communities. During the season, *C.racemosa* is sold in the traditional markets as fresh vegetables.For some Jepara households, these algae were consumed as supplement food, and even some restaurants in Jepara serve seaweed dishes in their menu.In Maruf *et al*., (2013) analysis showed that carbohydrate was the dominant component and protein was second large amount in *C. racemossa*. This makroalgae was also contained some mineral such as : Na, Ca. Some of amino acid such as L-Threonine and L-Glycine were the dominant amino acids

in the seaweeds whereas L-Asparagine was not detected in sample. From the nutrient and mineral contents, it was concluded that the seaweeds examined in the study may be used as alternate vegetables. Seaweed as vegetable contain of high nutrition for its nature in low calorie, rich in vitamin, protein, mineral,dietary fibre and high antioxidant.The high content of fibreand antioxidant have been known to lower low density lipoprotein (LDL) oxidation hence will decrease the glyserol and cholesterol level in blood plasma. Those kind of lipid are caused in hypercholesterolaemia (Matanjun,2008)

The crude fiber in food will bind and decrease cholesterol inside the body and those were metabolism to the liver, where it is converted into bile acid and subsequentlyexcreted infeces Huang *et al*.,(2010) . Cholesterol level experienced some decrease predicted because of the liver that synthesize the bile acid from the cholesterol and take the cholesterol inside blood is then slowly decreasing (Almatsier, 2002). The high cholesterol and saturated fat diet will cause the increasing intracell cholesterol and its cholesterol will be kept as ester cholesterol. Beside that, this diet will show the decreasing level of LDL and the increasing level of HDL in blood.

Hypercholesterolemia is a condition when the level of cholesterol on blood exceed the normal limit. This condition happened because of the accumulation of saturated fatty acid on the blood vessels. Cholesterol is a molecule that plays important role in cell membran synthetic, steroid hormonal precusor synthetic, cortexs adrenal hormone, bile acid synthetic, and vitamin D. Hypercholesterolemiacaused by the cholesterol level that exceed 239 mg/mL in blood (Cuchel, 1997).

The dietary fiber in food will bind cholesterol inside the body and will be taken out with feces.The high cholesterol dietary and saturated fat caused increasing intracell cholesterol and its cholesterol will be kept as ester cholesterol. Beside that, this diet will show the decreasing level of LDL and the increasing level of HDL in blood.

There was very limited data available on the information related to *C.racemosa*especially on its effect on decreasing the level of cholesterol on wistarrat that has hypercholesterolemia.The aim of the research was to analyze*C.racemosa* asnutricious food on lowering cholesterol level on the blood system. As a edible food that rich of its fiber content, where the fiber content will effect the cholesterol level on rats that has hypercholesterolemia.The animal model diets comprised of four groups: normal diet (control group) as group I,normal diet supplemented with ½ Dosage ODH (II), 1times (III) and 2 times Dosage ODH (Optimal Dose of Human).

MATERIALS AND METHODS

Sample collection

*Caulerpa racemosa*was collected from Jepara‘scoastal water. The fresh seaweed were was hed thoroughly with seawater and wrapped in plastic bags in order to prevent dehydration process. The plastic bags were then wrapped with dry ice and put its on the polysterene cool box. After arriving at Laboratory of Fish Processing Technology Diponegoro University, the samples were again was hed thoroughly with tap water for 3 to 4 times to remove filth or attached epiphytes from algae. All the *C.racemosa*samples were dried using solar dryer for approximately 8 hours until constant weight was obtained. The dried seaweed was then chopped, grounded and powdered to a fine powder using a mill (0,6 mm mesh size sieve) and kept in air tight plastic bag sat room temperature. The fresh samples were analyzed to proximate and fatty acid content and the powder was then feed to rats animal model .

Methods of Quality Parameters

Lipid, protein, ash, crude fiber content and insoluble dietary fiber test were based on AOAC (2005), carbohydrate content test (by difference).Fatty acids in algal were analyzed using Gas Chroma-tography–Mass Spectrometry (GCMS-QP2010 Ultra) of their methyl ester (% of total FAMEs) (Matanjun*et al*.,2008).Determination of total cholesterol level, LDL, HDL and tryglyceride level with CHOD-PAP method (Tranggono,1992). Rats animal model were prepared fromLaboratory of AnimalFisiology of Biology Faculty of Mathematics and Science UNNES.

Animal Model

Preparation of population

Male Wistar (*Rattus norvegicus*) range age of 2 months, weighing 150-200 g, healthy, did not suffer any skin diseases. The animals had free access to water and animalfood standard (a*d libitum)* during acclimatized stage for 7 days and were maintained at a constant temperature of approximately 24°C. The animals, weighed at the start and end of the experiment. Those rats were divided into four groups (n = 6) (WHO, 1975) : (Group I) : Control group, rats were fed with a standarddiet,normal in cholesterol, (Group II) : I/2 ODH (Optimal Dose of Human), (Group III) :1 x ODH (ODH=0,09gr) , (Group IV): 2 x ODH (0,18 gr/kg/day).

Preparation of diet

a. Inducing hypercholesterol in rats

The control group received the standard commercial feed, with 15-19% protein, 8% crude fiber, 3.0% lipids.The other rats group was fed a high cholesterol diet which was quail egg mix, containing 13,1% protein, 11,1% lipids. The diet treatments for lowering cholesterol were consist of *C.racemos*a flour and CMS food grade that were ground and mixed in pellet form. The rat shy percholesterolimia were fed 2ml/day quail egg mixed with standard feed for 21 days.The food standard was made in soluble gel form and binded with CMC in the amount of 2 ml/day. Treatment dose was given until the cholesterol blood level reached to 10-54 mg/dl. After inducing hypercholesterol was finished, the subjet was still fed with standard food and drink in *adlibitum.* The rat reached hypercholesterolia level if the subject cholesterol level was higher than 54 mg/dl (Cuchel *et al.*, 1997).

b. Biochemical analyses.

The rats were fed C*.racemosa* pellet for 14 days, aiming to reduce the cholesterol level. The rats were randomly divided into four following groups: control; ½ ODH (Optimal Dose of Human); 1 x ODH (ODH=0,09gr) ;2 x ODH (0,18 gr/kg/day). Those rats were then killed and blood sampling was undertaken. Total cholesterol, LDL, HDL and triglyceridesserum concentration were determined by using CHODPAP method.

Statistical Analysis

Data on fatty acid profiles were compared between group. Data on lipid profile (total cholesterol, LDL, HDL, triglyceride) were tested using ANOVA and determine whether if there any significant effect were found, analysis was continued using Tukey test.

RESULT AND DISCUSSION

Nutrition Value of *Caulerpa racemosa* Seaweed flour

The nutrition value of *C.racemosa*is presented on Table 1 as follows :

Table 1.Nurition Value of *Caulerpa racemosa* seaweed flour

Analysis	Value (%)
Water	9,12
Protein	13,91
Lipid	0,44
Carbohydrate	53,05
Dietary Fiber	23,29
Crude Fiber	12,53
Ash	7,033
Energy	284,84 Calori/100gr

Based on dry weight (DW) of the current research showed that the protein content in *C. racemossa* obtained from Jepara waters was higher than the value of same species (12.88 ±1.17) as reported by Kumar *et al.,* 2011 who collected the samples from Gujarat India.Based on research by Kumar *et al.*, 2011, it was shown that the energy content of *C.racemossa* was higher than in *C. veravelensis* and *C. scalfoliformis*. This energy comes from proteins and carbohydrates contained in *C. racemossa* and therefore this alga may be consumed by people with obesity problem. The values obtained for protein and lipid contents in the present study were lower to the earlier findings by Rameshkumar *et al*.,(2013), which in the same species that harvested from Gulf of Mannar content 18.3% and 19.1% for protein and lipid respectively.However the present study was agreed to Matanjun (2008) and Venugophal (2010) previous researchs that green algae were low in lipid content range 0.29 to 1,11% (dry weight) basis, they suggested that seaweeds do not contain much lipids however they were rich on PUFA.

Carbohydrate isone of the important components for metabolism and it supplies the energy needed for respiration. The concentration ofcarbohydrate in this study (53.05%) was low compared to the same research of Rameshkumar *et al*.,(2013), where the carbohydrate content was 83.2 %. The difference in chemical composition such as lower content of protein and lipid were probably due to the fact that different source of seaweed,the effects of environmental factors, like temperature, annual cycle, and mineral content of the growth medium and also methods determinations (Van Ginnekene*t al*., 2011). The high fiber content in the seaweed was due to higher polysaccharide content.The total number of crude fibers is the sum of dietary fibers and functional fibers. Crude fiber can be consumed bypeople with obesity or diabetes mellitus.The physico-chemical properties of red alga fibers is similar to that in high fiber commercial foods (Venugophal, 2010).

Fatty acid composition

The fatty acid composition of *Cracemosa* flour is presented on Table 2 bellow :

Table2. Faty Acid's Contentof*C.racemosa* Flour

Type of Faty Acid		Concentration(%)
Saturated Fatty Acid		
Caproic Acid	Hexanoic Acid (C6:0)	0,055
Caprylic Acid	Octanoic Acid (C8:0)	0,014
Capric Acid	Decanoic Acid (C10:0)	0,076
Lauric Acid	Dodecanoic Acid (C12:0)	0,313
Myristic Acid	Tetradecanoic Acid (C14:0)	2,378
Palmitic Acid	Hexadecanoic Acid (C16:0)	60,943
Margaric Acid	Heptadecanoic Acid (C17:0)	0,105
Oleic Acid	9- Octadecenoic Acid (C18:1)	8,334
Stearic Acid	Octadecanoic Acid (C18:0)	0,484
Eicosanoic Acid	Arachidic Acid (C20:0)	0,307
Arachidat Acid	EicosanoatAcid(C20:0)	),452
Behenic Acid	DocosanoicAcid (C22:0)	:,123
Tetracosanoic Acid	Lignoceric Acid	',068
Monounsaturated Fatty Acid		
Palmitoleic Acid	9-Hexadekenoic Acid (C16:1)	3,102
Polyunsaturated Fatty Acid		
Linoleic Acid	9,12-Octadecadienoic (C18:2)	2,241
Linolenic Acid	9,12,15-Octadecatrienoic (C18:3)	1,659
Eicosadienoic Acid	(C20:2)	0,479
Eicosapentaenoic Acid/EPA	5,8,11,14,17 Icosapentaenoic (C20:5)	0,464
Docosahexaenoic Acid/DHA	Docosa-4,7,10,13,16,19 hexaenoic acid (C22:6)	2,647
Unsaturated Fatty Acid		
Gadoleic Acid	9-Eicosenoic (C20:1)	0,755

According to Meyes (1991), palmitic acid is a monosaturated and used as a basic ingredients to form another fatty acid, because it is the longest chain of fatty acid. Madell (1997) reported that people does not like consuming high palmitic acid on their diet since those fatty acid will increase blood lipid content or hyperlipedemic and could increase blood cholesterol. Palmitic acid (C16:0) was measured in at relatively high concentration 60,943% on this present research. This is in a greement with the findings of earlier research by Mantanjun (2008) that green seaweed such as Caulerpa *sp* was rich in C16:0 (palmitic acid) and it higher abundant in amount in comparison to palmitic acid was found in Menhaden fish oil that they only present range between 15,3-25,6% (Stansby, 1990). Whereas the amount of singlechain and double chain unsaturated faty acid in macroalgae is less than single and double chain unsaturated faty acid in Menhaden fish oil.

The important n-3 PUFA α-linolenic acid (C18:3) was on 1,659% while the important n-3 PUFA eicosapentaenoic acid (EPA, C20:5) (which is mainly found in fish) was lower than

DHA (C22:6). (Van Ginnekene*t al*,.2011). In general, the predominant fatty acids were saturated palmitic acid (C16:0; 60.94%), and it was agree with previous research that has been reported by Ambrozova*et al.,* (2012)

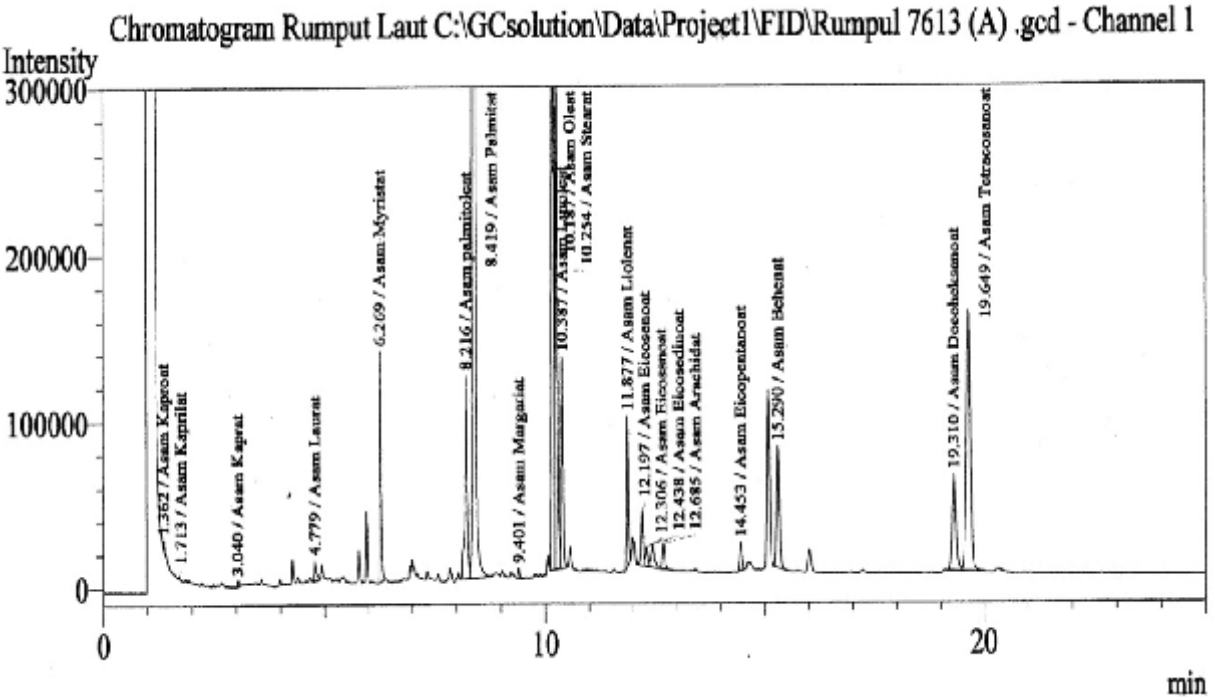

Figure1.**GCMS pattern of fatty acid methyl esters of acyl lipids from the seaweed *C.racemosa.***

*Note the predominance of C16:0 (palmitic acid, Rt = 8,419 min) and C18:1(oleic acid, RT=10.254min)

Profile of Lipid

The cholesterol lowering potentials of the *C.racemosa* were determined by comparing the levels of serum total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglyceride (TG). With regard to total cholesterol there was a significant in crease range of 236,56% to 284,41% when animals were treated high cholesterol treatments induced for 21 days. As well as plasma cholesterol, tryglicerides also increase on the value of 185,94% to 217,21% It can be seen that the consumption of quail egg will increase cholesterol level however decreased HDL blood level. There was a significant different (P<0,01) on cholesterol level between treatments (which was ½, 1 and 2 times ODH Optimal Dose of Human). In the contrary, there was no significant different (P>0,05) on HDL (High Density Lipoprotein) and a significant different (P<0,05) on triglyserida and LDL value. This present study (for the group II) was agree to the finding research by Matanjun (2008) who stated that administration of *C. racemosa* reduced (P<0.05) plasma low-density lipoprotein cholesterol and tryglyceride. It also an significantly increased (P<0.05) on plasma high-density lipoprotein cholesterol. Hence the decreasing on LDL value improved the atherogenic index of rats fed a *C. racemosa* pellet diet. As we known that *C. racemosa* consist of of dietary fiber, omega-3 fatty acids such as eicosapentaenoic acid (C20:5ω3), may probably contributed to the cholesterol-lowering effect.

Data of lipid profile level before and after treatment is presented on Table 3.

Table 3. Lipid Profiles in Rats Fed a High Cholesterol Diet and Treated with Different Concentration of *C.racemosa* pellet (mg/dl).

Lipid Profiles before hypercholesterolimia

Treatment	Cholesterol	Triglycerida	HDL	LDL
K 1	51,1	70,3	39,9	23,97
K II	51,1	64,3	43,7	19,6
K III	50,7	79,7	36,2	27,7
K IV	50,6	80,9	50,2	20,0

Lipid Profiles hypercholesterolemia

Treatment	Cholesterol	Triglycerida	HDL	LDL
K 1	139,5	152,7	26,04	45,96
K II	134,6	138,37	37,47	24,37
K III	144,2	148,2	26,4	57,6
K IV	119,7	151,4	30,2	52,0

Lipid Profiles after lowering Hypercholesterolimia

Treatment	Cholesterol	Triglyserida	HDL	LDL
K 1	67,91	48,16	33,31	41,41
K II	56,35	66,18	29,28	29,57
K III	66,29	39,25	31,94	44,59
K IV	68,18	33,75	37,07	44,33

After14 days of treatment of lowering hypercholesterol using *C racemosa* as a high dietary feeding, all treatments diet groups reduced the serum TC level (range 43,04% to 58%), these values exhibited significant differences from those of the control. In addition, there were no significant differences of LDL level between control and diet treatment. However, *group II* diet was the only seaweed diet that caused a higher depression of LDL level. It seemed that reducing cholesterol using ½ of HOD had a comparatively greater hypocholesterolemic potential than the others.

Good blood lipid profile has the degradation contents of cholesterol, triglyserida and LDL but because of it iwas only for 2 weeks then the degradation was only 43-58%. But there is another opinion which stated that in certain age, a stricht reduction of fat and cholesterol consumption would not decrease hypercholesterol to normal level (Hernawati, 2012).The increasing of food fiber's consumption will not automatically decreasing cholesterol level, because if the absorption of cholesterol is already saturated then the absorption function will not work properly.

CONCLUSIONS AND SUGGESTION

Conclusions.

1. *Caulerpa racemosa* seaweed can be classified as *nutritious food* because it has fatty acids and another nutrient substancese.g protein, lipid and fiber which are needed for the body.

2. The fed of *C racemosa* seaweed pellet in the amount of ½ Optimal Dose of Human which is 0,045 gr/day could reduce cholesterol in rats blood in the amount of 58,13 % in 2 weeks. This result is significant different to rats which fed the same diet for 2 times Optimal Dose of Human (43%).

Suggestion.

1. It is needed to perform an experiment which uses smaller amount of Optimal Dose of Human for longer time experiment.

Acknowledgement

The author sare thankful to the Faculty of Fisheries and Marine Sciences ,University Diponegoro for the financial for this research.

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