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ORIGINAL ARTICLE

The Potency of Sea Urchin (*Diadema setosum*) Gonad on Brain Cells of White Rats (*Rattus norvegicus*)

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Abstract

Aim: To investigate the amino and fatty acid contents in the gonad of *Diadema setosum*; body weight, hemoglobin count, number of neuron cells, weight and volume of brain of *Rattus norvegicus*, and lab rats. **Materials and Methods:** The contents of fatty and amino acids in gonad of *D. setosum* were analyzed by gas chromatography and high-performance liquid chromatography methods. The next stage of this research was carried out by administering treatments of 0.5, 1, and 1.5 g/subject/day of sea urchin gonad rations to different groups of rats. A special group of subjects were treated with fish oil was setup as well. **Results and Discussion:** Research on fatty acids content in dried ground sea urchin gonad resulted in the saturated fatty acids variety and content of lauric acids, myristiric acid, palmitic acid, stearic acid, archidic acid, behenic acid, and unsaturated fatty acids variety and content of palmitoleic acid, oleic acid, linoleic acid, linolenic acid, erucic acid, eicosapentaenoic acid, and docosahexaenoic acid. The analysis result of amino acid in dried ground sea urchin gonad showed that the ration contained variety and content of essential amino acids of threonine, methionine, valine, phenylalanine, I-leucine, leucine, lysine, tryptophan; semi-essential amino acid of histidine, arginine, and non-essential amino acids including aspartic acid, glutamic acid, serine, glycine, alanine, and tyrosine. The measurement result of rats showed the most significant increase in body weight with 1 g treatment group and the most significant increase in brain weight with 0.5 g treatment group. The group with 1.5 g treatment showed most amount of hemoglobin (21.67 d/dL) and most percentage of neuron cell count (36.68%). ANOVA statistical analysis of the experiment showed that there is no significant change in body weight, hemoglobin count, brain weight, and brain volume albeit that there is a notable change in the mount of neuron cells. **Conclusion:** It can be concluded that the administration of dried and ground *D. setosum* gonad resulted in the highest increase in body weight of subject rats at 1 g. The administration of 1.5 g dried and ground *D. setosum* gonad gave the most significant amount of neuron cells percentage and hemoglobin in subject rats.

Key words: Amino acids, barin, *Diadema setosum*, fatty acids, hemoglobin, neuron cells

INTRODUCTION

Echinoidea, commonly known as sea urchin, is a taxonomical class of marine animals which are comparable to hedgehog in physical appearance with spines covering its body. The spines are made up of calcium and are varied in length from short, medium, and long. One of the most common encountered species of sea urchin in the coasts of Central Java is *Diadema setosum*. It is often found burrowing in sands or among the rocks in the coast, living in the bottom of shallow waters, seagrass meadows water ecosystem, or reef flats.

Consumable body parts of sea urchins are its eggs and gonads. Gonad, as a gamete producing organ, has high nutritional value. Several previous investigation gave insight on how gonad of sea urchin is one of the most nutritious and valuable consumable marine commodity.[1,2] Gonad of sea urchins is a delicacy in the area of Nusa Tenggara Timur of Indonesia. Gonad of sea urchin contains several amino acids such as valine, threonine,

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glycine, histidine, alanine, glutamic acid, lysine, leucine, I-leucine, methionine, tyrosine, and arginine.[3] Sea urchin gonad is also rich in polyunsaturated fatty acid (PUFA), which was measured as 40% of its total fatty acids content.[4] Arginine and histidine are amino acids which play a key role in growth and development of children, particularly their brain development.

The main factor which influences brain development of human beings is nutrition intake.[5] Nutrition is a primary component including protein with amino acids (essential and non-essential) content, carbohydrate or fat a source of calorie, vitamins, and minerals. The human brain is made up of neurons and neuralgia cells.[6] Neuralgia cells protect and support neuron cells as it carries information to central nervous system. In the nervous system, neuron cells communicate with each other using various chemical substances known as neurotransmitters. Amino acids known play an important role in the synthesis of neurotransmitters for brain and nerve function.[7] In addition, elaborate that long-chained PUFAs (LC-PUFA) are critical during the development stages of human brain, making its intake vital to the development of brain nervous system.[8] Deficiency of fatty acids, including docosahexaenoic acid (DHA), lead to lower neuron processing performance and decrease in brain memory capacity.[9]

Based on the facts above, this research aims to investigate the amino and fatty acid contents in the gonad of *D. setosum* body weight, hemoglobin count, number of neuron cells, weight and volume of the brain of *Rattus norvegicus* lab rats.

MATERIALS AND METHODS

Sample collection and treatment

Sea urchin samples were collected from Panjang Island, Central Java (6° 34' 40.4400'' S, 110° 37' 45.7068'' E). Samples were then dehydrated by indirect sun exposure, and then, were put in wide basin which was covered in fabric and was left in drying area for 3-4 days. Dried samples were made into powder using a blender and were refined by 0.1 mm sieve. Dried ground sea urchin gonad was prepared as a ration for 1-month-old *R. norvegicus* subjects.

The subjects were divided into five treatment groups, and each group consists of three repetitions. The subjects are put into treatment Group I (control), treatment Group II (0.5 g of dried ground sea urchin gonad administered), treatment Group III (1 g of dried ground sea urchin gonad administered), treatment Group IV (1.5 g of dried ground sea urchin gonad administered), and treatment Group V (fish oil administered) with each group given three repetition of treatment. Before the treatment phase, the subject animals were acclimatized by *ad libitum* feeding for 7 consecutive days. Primary ration for the subject animals was private company manufactured B-11S pellets with the following ingredients and contents: Water 13%, protein 21-23%, fat 5%, raw fiber 5%, ash 7%, calcium 0.9%, and phosphor 0.6%. Fish oil used in this research was manufactured by Trilite, with every 1.695 g contains eicosapentaenoic acid (EPA) (0.18 g), DHA (0.12 g), vitamin E (0.02 g), energy (10.7 kcal), protein (0.284 g), carbohydrate (0.152 g), and fat (1.000 g). Sample doses (dried ground *D. setosum* gonad) for each treatment were 0.5 g, 1 g, 1.5 g, and fish oil dose for the positive control group was 0.2 g as seen in Figure 1.[10] The previous report observed that diet of 0.2 g of fish oil is a normal dose, so the administration of such dose was performed on the positive control group. Subject animals were fed with the main ration as much as 0.005 g/subject/day for 7 consecutive days, and then, were treated by their respective groups for 70 days, which is the time in which the mice reach adulthood and their brain tissue development ceases.

Hemoglobin analysis

The blood sample of each subject was collected from sinus orbitalis using microhematocrit. The blood samples were contained in 1.5 ml Eppendorf vials. Hemoglobin count was measured by Sahli haemometer using standard procedures before the measurement, 20 µl of blood sample from each vials was taken to a concave plate with a mixture of 2 ml ethylenediaminetetraacetic acid (EDTA) 10% solution and 2 ml physiological sodium chloride solution.

Body weight analysis

At the end of the treatment phase, body weight of each subject was measured using digital scale. This measurement

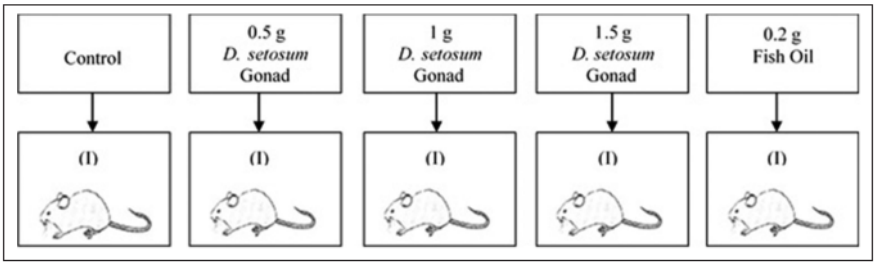

Figure 1: Scheme of sample treatment variations with *Rattus norvegicus* as subjects. Three repeated treatments were performed for each treatment group

allowed comparison of body weight between subjects in the control group and subjects treated with 0.5, 1, 1.5 g of dried ground *D. setosum* gonad, and 0.2 g of fish oil. The body weight of each subject was measured before treatment phase (T_1) and following the treatment phase (T_2). The difference of body weight measurement results before and following the treatment phase are set as the weight gain value in this research.

Brain weight and volume analysis

Brain tissue samples of *R. norvegicus* subjects were collected using head dissection. The weight of the each sample was measured. Each tissue sample was put into measurement glass filled with water. The difference of water volume before and after each brain tissue was added is the brain tissue volume gain value used in this research.

Brain histology analysis

Collected brain organ of subjects was fixated with formalin 10% (10 ml formaldehyde 38%, 90 ml distilled water). The brain organs were then separated into three slices of right, central, and left with each slice measuring in 1 cm × 1 cm wide and 4-5 mm thick. The organs were prepared as samples for histological analysis according to classical histology methodology. Following the preparation, the prepared samples were put into the histological analysis. Histology of the brain organ samples of subjects was carried out to observe and count the number of brain neuron cells of subjects. The observation was carried out using light microscope with ×400 and ×1000 magnification, each in 10 power fields.

Fatty acid analysis

Gas chromatography (GC) was used for analyzed fatty acid compound in measured sample. Gonad sample was prepared by *in situ* transesterification. GC (GC-4A/B, Shimadzu, Kyoto, Japan) was equipped with flame ionization detector and column CP CIL 88 (30 m × i.d. 0.22 µm). 0.2 µL transesterification product was injected to GC. Identification of fatty acid was conducted by injected standard fatty acid. Column heating was initiated at 150°C and linearly increased to 230°C by 5°C increasement per minutes. This chromatography process proceeds under 160 KPa.[11]

Amino acid analysis

The sample was fist prepared according Kjedahl method. High-performance liquid chromatography (HPLC) instrument (LC 20AD, Shimadzu, Kyoto, Japan) was equipped with fluorescence detector and ultra techsphere column. Gradient mobile phase was applied for 40 min. Mobile phase was composed by buffer A (Na-acetate 0.0025M, Na - 0.05% EDTA, 9% methanol, and 1% tetrahydrofuran in 1 L water) and buffer B (95% methanol). Mobile phase was filtered with 0.45 µm before use. Mobile phase system was set at 0% B for min and linearly change to 15% B for 3 min, then 42% B for 10 min, 70% B for 5 min, 100% B for 6 min, and 0% B for 12 min. Flow rate was set to 1 mL/min.

Statistical analysis

To understand significantly of the treatment, we tested all the data using one-way ANOVA. Numeric data were plotted using Origin Software (Origin Lab, version 7.0).

RESULTS

Through this investigation, we conducted several parameters for analysis the rat growth quality, i.e., hemoglobin, body weight, brain weight, brain volume, and number of neuron produced during treatment.

Hemoglobin analysis [Figure 2a] results of the control group subjects and subjects with all treatments for 70 consecutive days show that the subject with 1.5 g of dried ground *D. setosum* gonad treatment exhibits the highest hemoglobin count (14.67 ± 1.33 g/dL) when compared to control group subject (12.00 ± 0.46 g/dL), subject from 0.5 g (13.07 ± 0.67 g/dL), and 1 g (13.23 ± 0.98 g/dL) dried ground *D. setosum* gonad treatment, as well as with that from 0.2 g fish oil treatment (13.60 ± 0.64 g/dL).

Body weight measurement data of *R. norvegicus* subjects for 70 consecutive days on control group [Figure 2b], treatment groups of 0.5 g, 1 g, 1.5 g dried ground *D. setosum* gonad, and 0.2 g of fish oil shows that the subjects receiving treatment of 1 g dried ground *D. setosum* gonad display the most weight gain (146.67 ± 21.42 g) when compared to control subjects (94.00 ± 15.09 g), subjects with 0.2 g treatment of fish oil (144.66 ± 20.51 g) and subjects with 0.5 g (149.00 ± 24.17 g), and 1.5 g treatment (131.00 ± 15.56 g) of dried ground *D. setosum* gonad.

The measurement of brain volume [Figure 2c] of *R. norvegicus* subjects from all groups for 70 days with control treatment, 0.5, 1, and 1.5 g of dried ground *D. setosum* gonad treatment, and 0.2 g of fish oil treatment reveals that subjects with 0.5 g gonad treatment possessed the most brain weight (1.24 ± 0.06 ml) when compared with subjects from control treatment (1.11 ± 0.05 ml), 1 g gonad treatment (1.14 ± 0.003 ml), 1.5 g gonad treatment (1.05 ± 0.11 ml), and from 0.2 g fish oil treatment (1.16 ± 0.02 ml).

The measurement of brain weight [Figure 2d] of *R. norvegicus* subjects from all groups for 70 days revealed with control treatment, 0.5, 1, and 1.5 g of dried ground *D. setosum* gonad treatment, and 0.2 g of fish oil treatment showed that the control group possesses the

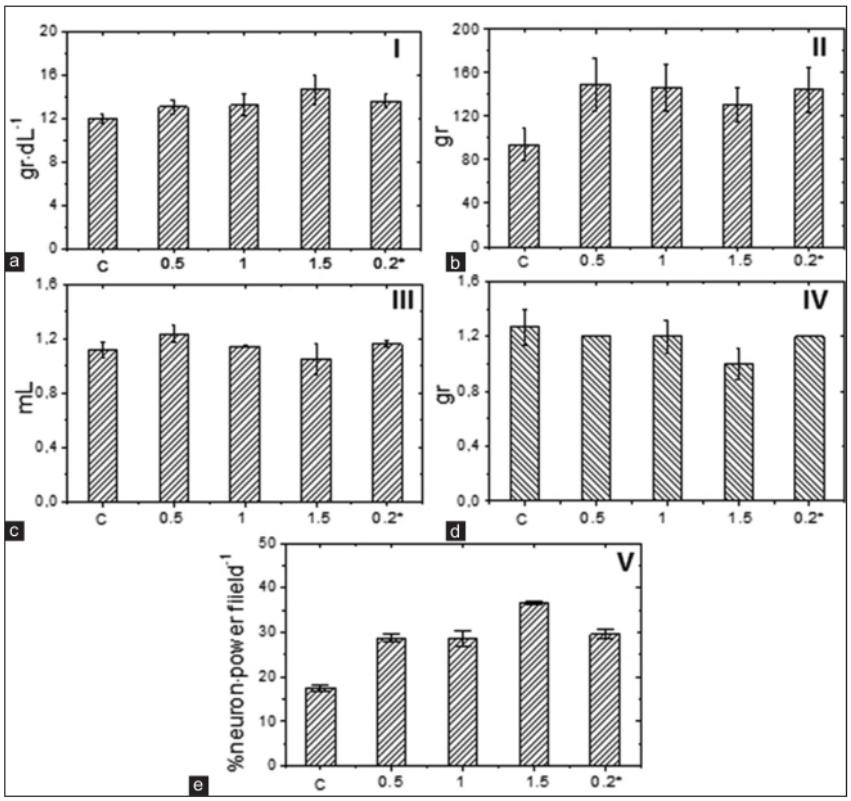

Figure 2: Hemoglobin (a), body weight (b), brain volume (c), brain weight (d), and number of neuron (e) from treated rodent after consumption of 0.5, 1, and 1.5 g sea urchin gonads for 70 days. These treatments were compared to control sample (C) and 0.2 g of fish oil treated rodent (*). All data measurements were conducted in triplicate

highest brain volume (1.27 ± 0.13 g) when compared to that of 0.5 g gonad (1.20 ± 0.00 g), 1 g gonad (1.20 ± 0.11 g), 1.5 g gonad (1.00 ± 0.11 g), and 0.2 g fish oil (1.20 ± 0.00 g) treatment groups.

The unit in which neuron cells count of the brain of *R. norvegicus* subjects is stated is percent per power field [Figure 2e]. The observation and measurement of neuron cells of *R. norvegicus* subjects from control treatment, 0.5, 1, and 1.5 g of dried ground *D. setosum* gonad treatment, and 0.2 g of fish oil treatment for 70 days revealed that brain tissue sample from the subjects with 1.5 g dried ground *D. setosum* gonad treatment possess the most neuron cells (36.68 ± 0.24%) when compared with subjects from control (17.43 ± 0.65%), dried ground *D. setosum* gonad treatment group of 0.5 g (28.73 ± 0.87%), 1 g (28.56 ± 1.70%), and 0.2 g of fish oil treatment group (29.63 ± 1.21%).

Those five parameters results were then tested using one-way ANOVA statistical method to provide better understandable analysis related to significantly between treatment and result. We found among hemoglobin, body weight, brain volume, brain weight, and number of neuron produced $P = 0.37$, 0.31, 0.42, 0.38, and 3.14×10^{-6}, respectively.

Analysis of dried ground *D. setosum* gonad using GC method shows that the highest content of fatty acid is palmitic acid (27.603%) when compared to lauric acid (0.079%), myristic acid (15.549%), stearic acid (6.045%), arachidic acid (1.133%), palmitoleic acid (8.196%), oleic acid (4.315%), linoleic acid (3.947%), linolenic acid (0.592%), behenic acid (17.113%), erucic acid (1.903%), EPA (12.322%), and DHA (1.202%) as shown in Table 1.

Analysis of dried ground *D. setosum* gonad using HPLC method shows that the highest content of amino acid is glutamic acid (15.191%) compared to the content of aspartic acid (11.590%), serine (5.976%), glycine (3.336%), alanine (8.303%), tyrosine (4.952%), histidine (2.455%), threonine (5.479%), arginine (8.544%), methionine (3.3331%), valine (5.877%), phenylalanine (5.638%), I-leucine (5.052%), leucine (9.105%), lysine (4.343%), and tryptophan (0.837%) as displayed in Table 2.

DISCUSSION

Analysis of variation and content of fatty acids in *D. setosum* gonad using GC (GCM) showed that palmitic acid is the

Table 1: GC chromatogram identification table of dried ground *D. setosum* gonad for fatty acid analysis

t_R (min)	Identified fatty acid*	Peak area	% Total fatty acid
5.783	Lauric acid	124	0.079
7.287	Myristic acid	25,110	15.549
9.508	Palmitic acid	66,655	27.603
9.905	Palmitoleic acid	14,423	6.045
11.812	Stearic acid	11,050	1.133
12.252	Oleic acid	21,341	17.113
13.842	Linoleic acid	9243	8.196
14.112	Linolenic acid	2430	4.315
16.645	Behenic acid	26,285	3.947
17.197	Erucic acid	2723	0.592
17.847	EPA	5937	1.903
19.898	Arachidic acid	1864	12.322
21.593	DHA	1343	1.202

*Unknown peaks not shown. EPA: Eicosapentaenoic acid, DHA: Docosahexaenoic acid, GC: Gas chromatography, *D. setosum*: *Diadema setosum*

Table 2: HPLC chromatogram identification table of dried ground *D. setosum* gonad for amino acid analysis

t_R (min)	Identified amino acid*	Peak area	% Total amino acid
1.292	Aspartic acid	41,652,522	11.590
1.991	Glutamic acid	46,488,810	15.191
6.911	Serine	25,940,007	5.976
8.214	Histidine	5,581,040	2.445
9.698	Glycine	6,867,961	3.336
10.220	Threonine	26,160,653	5.479
12.131	Arginine	21,293,835	8.544
12.973	Alanine	39,129,331	8.303
14.403	Tyrosine	12,586,784	4.952
18.021	Methionine	10,790,696	3.331
18.497	Valine	26,835,138	5.877
19.880	Phenylalanine	14,412,130	5.638
21.118	l-leucine	20,518,830	5.052
21.594	Leucine	33,541,975	9.105
23.626	Lysine	5,289,169	4.343
28.255	Tryptophan	3,550,081	0.837

*Unknown peaks not shown. HPLC: High-performance liquid chromatography, *D. setosum*: *Diadema setosum*

dominating content (27.603%) out of total fatty acid content. The content of both saturated and unsaturated fatty acids are found in *D. setosum* gonad sample. Saturated fatty acids are straight-chain fatty acids in terms of their carbon chains.[12] Fatty acids content produced by *D. setosum* gonad in this research were lauric acid (0.079%), myristiric acid (15.549%), palmitic acid (27.603%), stearic acid (6.045%), archidic acid (1.133%), and behenic acid (17.113%).

Unsaturated fatty acids composed by fatty acids molecules with double bond. Unsaturated fatty acids are grouped into monounsaturated fatty acids (MUFAs) and PUFAs. Analysis of unsaturated fatty acids in *D. setosum* gonad samples in this research showed contents of palmitoleic acid (8.196%), oleic acid (4.315%), linoleic acid (3.947%), linolenic acid (0.592%), erucic acid (1.903%), EPA (12.322%), and DHA (1.202%). Oleic acid content in the finding falls into MUFAs category, whereas linoleic, linolenic, EPA, and DHA are grouped as PUFAs.

Essential amino acids are amino acids which play a key role in normal growth and function of body organs and tissue, and cannot be synthesized internally. Among these fatty acids are DHA and EPA which are derivatives of linolenic acid. Essential fatty acids act as a precursor of hormone-like eicosanoid substances, which are prostaglandin, prostacyclin, thromboxane, and leukotriene. These chemical substances are important in regulations of blood pressure, heartbeat, immunity system, in nervous system stimulation, in muscle contraction, and in regeneration functions.

High concentrations of DHA and arachidonic acid (AA) was found as well as approximately 1-2% linoleic acid and alpha-linolenic acid in the central nervous system.[13] Accumulation of DHA and AA in the brain takes place during growth stages. It is also explained that EPA is a precusor to DHA since the increase in EPA intake is linear with more DHA concentration in the brain and retina, as suggested in DHA gain diet. EPA is also known to play a major role in the prevention of cardiovascular disease. It found that brain development during infancy is not optimal without sufficient intake of DHA.[14] DHA is the basic building block in phospholipid, retinal membrane cells, brain cells, breast milk, and sperm.[15]

The essential fatty acids are vital source of nutrition during brain development in infancy stage in that it helps neuron growth process, development of interaction between brain cell synapses, and brain cells differentiation.[9] Infants with sufficient supplies of DHA are shown to develop better mental and psychomotoric development.

Analysis of dried ground *D. setosum* gonad using HPLC method shows that the highest content of amino acid was glutamic acid (15.191%) compared to other amino acids content. Analysis of dried ground *D. setosum* gonad reveals three kinds of amino acid contents; essential, semi-essential, and non-essential. It is explained[7] that essential amino acids cannot be synthesized internally and must be acquired from external source of nutrition. Semi-essential amino acids are synthesized internally, yet its default production amount cannot suffice the need of human body during growth and

development stages. Non-essential amino acids are amino acids that are sufficiently synthesized in human body. The analysis result of amino acids in dried ground sea urchin gonad showed that the ration contained essential amino acids of threonine, methionine, valine, phenylalanine, I-leucine, leucine, lysine, and tryptophan, semi-essential amino acids of histidine, arginine, and non-essential amino acids including aspartic acid, glutamic acid, serine, glycine, alanine, and tyrosine.

Amino acids are vital for human body functions. It is known that glutamic, aspartic, tyrosine, and tryptophan greatly influence the formation of neurotransmitter, and tyrosine and tryptophan also helps in the regulation and control of emotion and intelligence.[16,17] Tyrosine and tryptophan as basic building blocks of norepinephrine and serotonin respectively directly affect intelligence. A neurotransmitter is chemical substance needed in the impulse delivery from one neuron cell to another.

Another study discovers how amino acids are paramount in growth and development stage of human beings. Amino acids are neurotransmitter substances which quickly and precisely work and are directed to the brain. Glutamic, aspartic, and glycine are amino acids which work most effectively as neurotransmitters. Glutamic acid has been known as a neurotransmitter substance which increases excitatory actions in the brain.

The subjects from control group in this research are shown to have the least body weight gain (36.39 g) compared to subjects from other treatment groups. Lack of nutritional intake is attributed as the cause of this fact. Administration of 1 g *D. setosum* gonad rations was shown to contribute to the most body weight gain (89.37 g) compared to ration administration doses of 500 g (84.10 g) and 1500 g (76.8 g), and 0.2 g fish oil diet (87.20 g).

It has been reported that body weight gain is affected by several internal and external factors, such as nutritional intake and hormone.[18] Hormones have been known to directly influence growth. Thyroxin and androgen (testosterone) work to increase the synthesis of protein. Glucocorticoids are a class of hormone which is vital in the metabolism of water, carbohydrate, fat, and protein. Insulin helps process carbohydrate, protein, and fat.

In addition, it believed that high intake of calcium in *R. norvegicus* subjects negatively affects body weight gain of the subjects; the higher the found calcium concentration, the lower the body weight gain of the subjects. Mechanism of calcium in metabolism appears to be that of energy metabolism regulation within intracellular calcium which regulates the metabolism of adiposity fat and triacylglycerol reserve. High intake of calcium causes raises in calcium ion plasma. This increase will depress calcitirol hormone concentration which prevents calcium absorption through membrane vitamin D receptors and causes depression of intracellular calcium. This depression prevents synthase enzyme (main catalyst of lipogenesis) and encourages lipolysis, a process by which triacylglycerol in adipose tissue is converted into fatty acids and glycerol, and send free fatty acid into the blood stream as main fuel to be turned to CO_2. This phenomenon results in depletion of triacylglycerol reserve in adipose tissue, reducing adiposity fat, and in turn keeping the body weight in check.

Hemoglobin count of *R. norvegicus* results subjects from all treatment groups for 70 consecutive days varies across the board. Normal hemoglobin count of the subjects stands at 11-19 g/dL, considering the subjects physiology. *D. setosum* gonad ration administration of 1.5 g gives the most notable hemoglobin count (14.67 g/dL) when compared to control treatment (12.00 g/dL) or other treatments. Fish oil diet of 0.2 g is capable of increasing hemoglobin count (13.60 g/dL) when compared to control treatment (12.00 g/dL) and *D. setosum* gonad ration administration of 0.5 g (13.07 g/dL) and 1 g (13.23 g/dL). *D. setosum* gonad ration treatment with 1 g administration results in higher hemoglobin count gain than that with 0.5 g administration. Hemoglobin count gains from all treatments are shown to be within acceptable limits.

The difference in nutritional content and value in each ration is believed to be the cause of variation in hemoglobin count in all subjects. Milman[19] states that, in conjunction with hemoglobin count, protein affect iron metabolism in that it transports iron in the form of transferring. Iron is known to be a vital part in hemoglobin synthesis. Therefore, hemoglobin count is directly influenced by the content of iron and protein in a ration.

Brain weight measurements of *R. norvegicus* subjects from all groups reveals that subjects with 0.5 g *D. setosum* gonad treatment display the most notable brain weight (1.24 g) whereas subjects with 1.5 g *D. setosum* gonad treatment gains the least brain weight (1.05 g). It is suspected that the use of energy sources such as carbohydrate, fat, and protein as well as other sources of nutrition to synthesize brain tissue, including neuron cell synthesis and oxygen transport, is more significant in subjects with 1.5 g *D. setosum* gonad treatment compared to that of subjects with other treatments.

Brain volume analysis of all *R. norvegicus* subjects following their respective treatment for 70 consecutive days indicates that subjects from control group possess the most brain volume (1.27 ml), whereas those with 1.5 g *D. setosum* gonad treatment possess the least brain volume (1.00 ml).

Brain weight and volume indicate the number of brain cells. Brain cell count in this matter includes neuron cells and glial cells (supporting cells). However, it has been explained that glial cells are not directly responsible for intelligence functions.[20] Therefore, brain weight and volume cannot be directly linked to intelligence level or quality, since it is

possible that glial cells make up the higher portion of the brain than the neuron cells.

Observation in this research indicates that *R. norvegicus* subjects from treatment group of 1.5 g *D. setosum* gonad possessed the highest neuron cells count (36.68%) of all groups. Therefore, subjects from 1.5 g *D. setosum* gonad treatment have the most neuron cells density of all groups. One-way ANOVA test on the treatment of *D. setosum* gonad shows that $P = 0, < 0.05$ accepts H1 which means that effects of the treatment toward neuron cells are found. Results show that control treatment produces the least neuron cells (17.42 ± 1.13), and that fish oil diet, 0.5 g, 1 g, and 1.5 g *D. setosum* gonad treatments results in relatively similar number of neuron cells (28.2 ± 2.27) with 1.5 g *D. setosum* gonad treatment contributing to the most significant number 1500 (36.68 ± 0.43). It is believed that sufficient fat and protein intake in the group with 1.5 g *D. setosum* gonad treatment contributes to this result. It is known that protein is the source of amino acids and fat is the source of fatty acids. Protein and fat are valuable during the growth and development stage of the brain. It has been explained[21] that DHA is an important part of nutrition during infancy and childhood; the brain is highly dependent in LC-PUFAs, which is converted into phospholipids in brain cortex. In addition, DHA is a substantial component of LC-PUFA. These fatty acids are vital to the well-being of the organs of central nervous system. Furthermore, the formation of fatty components in the brain during postnatal period is highly affected by fatty acids composition in nutritional intake.[22]

Linolenic acid deficiency during infancy stage will result in adverse effects and can lead to abnormal defects in growth and development. However, excessive linolenic acid consumption can also lead to body odor, indigestion, prolonged bleeding, and blood clotting.

The number of neuron cells found highly affecting a person's ability to learn. More and denser neuron cells translate into better learning ability.[23] Neuron cells act as highways to deliver impulse in the central nervous system. Density and coverage of neuron cells positively correlates to the number of cells, which means that the higher number of neuron cells, the denser they will be. Therefore, the number of neuron cells in the brain signifies the number of nerve impulses which in turn affects the amount of information delivered to the brain at a time.

CONCLUSION

1. Research on fatty acids content in dried ground *D. setosum* gonad resulted in the saturated fatty acids variety and content of lauric acid (0.079%), myristiric acid (15.549%), palmitic acid (27.603%), stearic acid (6.045%), archidic acid (1.133%), behenic acid (17.113%), and unsaturated fatty acids variety and content of palmitoleic acid (8.196%), oleic acid (4.315%), linoleic acid (3.947%), linolenic acid (0.592%), erucic acid (1.903%), EPA (12.322%), DHA (1.202%). The analysis result of amino acid in dried ground sea urchin gonad showed that the ration contained variety and content of essential amino acids of threonine (5.479%), methionine (3.331%), valine (5.877%), phenylalanine (5.638%), I-leucine (5.052%), leucine (9.105%), lysine (4.343%), tryptophan (0.837%); semi-essential amino acids of histidine (2.445%), arginine (8.544%), and non-essential amino acids including aspartic acid (11.590%), glutamic acid (15.191%), serine (5.976%), glycine (3.336%), alanine (8.303%), tyrosine (4.952%).
2. The most notable body weight gain in *R. norvegicus* subjects (89.37 g) is obtained from those with 1 g of *D. setosum* gonad treatment and the least significant (36.39 g) is of those with control treatment. The highest hemoglobin count of all subjects is found in 1.5 g *D. setosum* gonad treatment group (14.67 g/dl) and the lowest is exhibited by the control treatment group (12.00 g/dl). The highest brain weight of subjects is found in 0.5 g *D. setosum* gonad treatment group (1.24 g) and the lowest brain weight is displayed by those in 1.5 g *D. setosum* gonad treatment group (1.05 g). Brain volume is found to be highest in the control group (1.27 ml) and lowest in 1.5 g *D. setosum* gonad treatment group (1.00 ml). A number of neuron cells in subjects is found to be highest in 1.5 g *D. setosum* gonad treatment group (36.68%) and lowest in the control treatment group (17.43%).
3. One-way ANOVA statistical test revealed that *D. setosum* gonad treatment contributes to the increase in neuron cells.

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