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Profile of Macro-Micro Mineral and Carotenoids in *Pomacea Canaliculata*

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Abstract
Golden apple snail (*Pomacea canaliculata*) can lay 15-20 groups of eggs that each group is about 500 eggs. The purpose studi were to determine the content of macro-micro mineral and carotenoids in *P. canaliculata* from Dramaga Aquatic, Bogor. The study that was done is a measurement of morphometric size and weight in golden apple snail eggs group, analysis of proximate, mineral, and total carotenoids. One group of golden apple snail eggs has length average of 3.7±0.7 cm; width (2.2±0.3) cm; height (1.2±0.3) cm, and weight (4.4±1.4) grams. Results proximate analysis showed that 75.55±3.20% moisture, 13.81±3.37% ash, 3.32±0.22% protein, 0.19±3,37% fat, and 7.12±0,11% carbohydrate. Macro mineral content of golden apple snail eggs from the highest to the lowest were calcium (17925.18±116.64 ppm), natrium (402.92±4.55 ppm), calium (252.02±12.06 ppm), phosphorus (197.28±0.33 ppm), and magnesium (112.29±0.36 ppm). Micro mineral content of golden apple snail eggs showed that copper (10.16±0.33 ppm), iron (7.83±0.14 ppm), and zinc (5.28±0.05 ppm), Carotenoids in golden apple snail eggs was 313.48±19.73 ppm. This is higher than the carotenoids in carrots of hybrid various (60.21±0.66 to 79.47±0.42 ppm), flying fish eggs (245.37 ppm) and *chinook* salmon egg (*Oncorhynchus tshawytscha*) (17.9 ppm).

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Keywords

Carotenoid;
Eggs;
Golden Apple Snail;
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Introduction

Pomacea canaliculata is easy to find in rice fields area or at the edge of the stagnant waters in Indonesia. Golden apple snail can cause damage up to 10-40% of the total rice field areas in Indonesia, such as in Java, Sumatra, Borneo, NTB, and Bali. Pests from the class of molluscs is potentially a major pest of rice plants as they rapidly proliferate

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and attack the young plants. Golden apple snail are able to produce eggs during its life as much as 15-20 groups, that is each group numbered approximately 500 grains, the hatching percentage of more than 85% (Budiyono, 2006).

Golden apple snail eggs in Indonesia has been used as crackers and juice (healthy beverages) that allegedly due to the high content of minerals (calcium) and has been used as a fertilizer, plant growth regulator (growth stimulating substances) allegedly because of the high organic carotenoids in golden apple snail eggs. Scientific studies on the mineral *P. canaliculata* have not been done in Indonesia. A scientific study of golden apple snail eggs outside Indonesia was done Cheesman (1958) who found a carotene-glycoprotein complex in the golden apple snail eggs (*P. canaliculata*), without being bound fatty esters, called ovorubin. Abdullah *et al.* (2017) found pigmen in golden apple snail eggs. The study of the golden apple snail eggs (*P. canaliculata*) then continued on biochemical analysis perivitelline during embryogenesis form lipoproteins, lipids, carbohydrates, carotenoids Astaxanthin, and antioxidants. Perivitellin is the main nutrient supply during embryogenesis in the form of yellow granular proteins (Heras and Pollero, 2002). Proximate analysis, analysis of macro-micro minerals, and total carotenoids have not done so in this research. The purpose study were to determine the mineral content of macro-micro and total carotenoids *P. canaliculata*).

Materials and Methods

Materials and Equipment

The main material used in this study was eggs of g (*P. canaliculata*) obtained from the fishing area in Situ Gede, Bogor Barat, Bogor City and Cultivation Experiment Pool, Department of Aquaculture, Faculty of Fisheries and Marine Science, Bogor Agricultural University with coordinat 6.556182°Lu and 1067.264125°BT. Materials for proximate analysis using distilled water, kjeltab selenium species, a solution of concentrated sulfuric acid H_2SO_4 (Merck), NaOH (Merck-KGA), 0.1 HCl solution (Sigma Aldrich), containing 2% H_3BO_4 bromocresol indicator green-methyl red (1: 2) pink, fat-free cotton, and solvent fat (n-hexane). The materials used for the analysis of minerals (Ca, K, Mg, Zn, Fe) is HNO3 (Sigma Aldrich), HClO4 (Merck-KGA), H_2SO_4 and HCl. The materials used for the analysis of mineral phosphorus (P) was $FeSO_4.7H_2O$ and ammonium molybdate 10%. The materials used for the analysis of total carotenoids were β-carotene standard, acetone-hexane solution of 3: 7.9% acetone solution in hexane, hexane solution, fat-free cotton, anhydrous sodium sulfate (Na_2SO_4) powder, sea sand, and activated alumina.

The proximate analysis consists of the analysis of water content using tools oven (Yamato DV-41), fat content using a Soxhlet method (SIBATA SB-6), protein content using distillation with term control (La bentech HMIC- F100), and ash content using the furnace (Yamato FM 38- My71e). Analysis of minerals (Ca, K, Na, Mg, Fe, Cu, Zn) was done using an Erlenmeyer flask, hotplate, flask, atomic absorption spectrophotometer (AAS) (Shimadzu AA-7000). Phosphorus (P) was analysed using a UV-VIS spectrophotometer-200-RS. Analysis of carotenoids was done by ultrasonic (power sonic 405) and UV-VIS-6500.

Sample Preparation

The study begins with the collection of data such as size and weight morphometric golden apple snail eggs, then analyzed proximate, analysis, of total carotenoids and minerals. Data of morphometric size, weight, contents proximate, mineral, and total carotenoids performed on fresh condition. Golden snail eggs were prepared by cleaning thedirt that is patched, then crushed until homogeneous.

Proximate Analysis

Several grams of fresh samples were analyzed for its nutrients through the proximate test according to AOAC (2005). Mineral analysis was done for the composition of macro and micro minerals contained in eggs. Samples to be assayed mineral wet ashing by the method of Reitz *et al.* (1960). The wet ashing process was carried out with a sample weighed as much as 20 grams, then put in a 150 mL Erlenmeyer flask. A total of 25 mL of HNO_3 (Sigma Aldrich) was added to the Erlenmeyer flask and allowed to stand for 1 hour. Erlenmeyer placed on a hotplate for ± 600oC for 6 hours and was added 2 mL of concentrated H_2SO_4 (Merck) , $HClO_4$ (Merck-KGA) and HNO_3 (Merck) mixture of 15 drops, 10 mL of deionized water, and 3.0 mL of concentrated HCl. The sample solution was then diluted to 100 mL in a flask. A number of the standard stock solution of each mineral was diluted using deionized water until the concentration was within the working range of the desired metal and the addition of 0.05 mL $Cl_3La._7H_2O$ (Merck) and 5 mL of deionized water. Standard solution, blank, and sample flowed into the AAS for minerals Ca, K, Na, Mg, Fe, Cu, Zn. The third solution was measured absorbance or high peaks of standard, blank, and sample at a wavelength and the corresponding parameters for each mineral with a spectrophotometer.

Mineral Analysis

Phosphorus Mineral Analysis using the Method pf Taussky and Shorr (1953)

A 10% solution of ammonium molybdate was prepared by dissolving 10 g of ammonium molybdate in deionized water with final volume 100 mL (10%). The solution was then added 28 mL of H_2SO_4, and diluted with deionized water to 100 mL (solution A). The next stage is to make the solution B, a total of 10 mL of solution A was added with 60 mL of deionized water and 5 g $FeSO_4$. $7H_2O$, then diluted with deionized water to 100 mL. Samples of wet ashing cuvette was inserted into the tube and then added with 2 mL of solution B. The intensity of the color was measured using a spectrophotometer with a wavelength of 660 nm for the mineral phosphorus.

Caretenoid Analysis

Analysis of total carotenoids using the method of Apriyanto *et al.* (1989). A solution of a homogeneous golden apple snail eggs in 1 gram incorporated into Erlenmeyer flask, then 100 mL of acetone-hexane was added to bring the ratio to 3:7. The mixture was shaken using orbital shaker for 30 minutes, then allowed to stand overnight with black plastic cover at room temperature. Samples were taken homogenization 30 minutes. The sample solution was centrifuged to separate the emulsion acetone, hexane, and water. The aqueous phase was then removed and the acetone-hexane extract was filtered on a chromatography column containing fat-free cotton, anhydrous sodium sulfate (Na_2SO_4) powder, and activated alumina. The filtrate obtained is called the sample extract.

Separation of carotene with a chromatography column. Sea sand was put in the column to a height of 0.5 cm. The activated aluminum oxide suspended in n-hexane entered. The suspension was poured slowly into the column and the column pat so that the particles uniformly distributed throughout the column, it is done to a high aluminum oxide layer 8-9 cm. Column was always wetted with hexane. Concentrate carotene was quantitatively transferred into the column with the aid of a pipette. The container of concentrate was washed with hexane, and the laundry was inserted into the column. Elution was carried out with n-hexane until all carotene yellow-orange out of the column. Elution was terminated when the eluent from the column no longer colored. The absorbance of the test solution was measured with a spectrophotometer at a wavelength of 452 nm

Results and Discussion

Nutrient Content

One group of golden apple snail eggs in this study had an average of length of (3.7 ± 0.7) cm; width of (2.2 ± 0.3) cm; height of (1.2 ± 0.3) cm; and weight of (4.4 ± 1.4) grams. The chemical composition of golden apple snail eggs is presented in Table 1. The results of the analysis of water content in the golden snail eggs in the amount of 75.55 ± 3.20%. The water content of golden apple snail eggs did not show significant changes in its development although exposed to air for about 15 days. According to Dreon *et al.* 206, this is probably because saccharides of ovorubin with high amounts galaktogen in perivitelline so efficiently prevent loss of water by maintaining an adequate environment for the embryo.

The results of the analysis of the ash contains in the *P. canaliculata* eggs in the amount of 13.81 ± 3.37%. High ash contains in golden apple snail eggs indicates higher mineral content in the golden apple snail eggs. This is caused when the biological material is burned, all organic compounds will be broken; most of the carbon turns into carbon dioxide (CO_2), hydrogen into water vapor and nitrogen into nitrogen vapor (N_2). Most of the minerals will be left behind in the form of ash is in the form of simple inorganic compounds, as well as the merger will occur between individuals or with oxygen to form inorganic salts (Arifin, 2008). Golden apple snail eggs shell is harder than the protective layer of catfish eggs, sea urchin eggs, and como tuna eggs. This led to the ash content of golden apple snail eggs higher than the golden apple snail meat, como tuna eggs, sea urchin eggs, and catfish eggs (Nurjanah[a] *et al.* 2012)

The results of the analysis of protein content in golden apple snail eggs was in the amount of (3.32 ± 0.22%). Age, season, and food intake affects protein content in a biological material. 300-kDa thermostable oligomers Ovorubin which is the dominant protein in the fluid perivitellin (PV) that surrounds the embryo *P. canaliculata.* Ovorubin has an essential role to the development of the embryo, protease inhibition, photoprotection, and storage of food. Ovorubin very high to stability in the pH range is quite large and can resist pepsin. Lipoproteins of the golden apple snail eggs serve to provide energy and nutrients for the developing embryo (Dreon *et al.*, 2008). Garin *et al.* (1996) showed that the lipoprotein fractions (PV1, 2, and 3) was detected for the first time in gastropod eggs with a protein content of 57.0; 7.5; and 35.5%. Tuna are carnivorous and catfish are omnivorous animals, so they intake protein more than snails and sea urchins. This enables golden apple snails eggs protein content lower than tuna eggs and catfish eggs (Nurjanah[b] *et al.* 2012).

Fat content analysis in golden apple snail eggs in the amount of 0.19 ± 0.00%. Species, food, habitat, size, and degree of maturity of gonads affect the fat content in a biological material. Garin *et al.* (1996) have observed regarding lipoproteins in golden snail eggs. Three lipoprotein fractions in the golden apple snail eggs observed that the lipid content of PV1, PV2, PV3 using microchromatography. Complex glyco-protein-carotene (PV1) have characteristics very high density lipoprotein (VHDL) which has a 0.33% fat, composed of free sterols and phospholipids. PV2 particles is also a VHDL (very high-density lipoprotein) 400 Kd with 3.75% fat. The main fat in the form of free sterols and phospholipids, and also has triasilgliserida as energy providers, as well as free fatty acids. Particles PV3 is HDL (high-density lipoprotein). PV3 fraction consists of at least three lipoproteins. Analysis PV3 fat fraction was split into two subfractions"h" and "p". Fraction "h" made up 5.16% of fat, in the form of free sterols, phospholipids, and free fatty acids. Fraction "p" made up 9.5% of fat which is 30% of the total fat golden snail eggs. This fraction has a high contains of carotene pigments, in addition to free fatty acids, hydrocarbons, sterols esterification and triglycerides (Heras and Pollero, 2002). Dreon *et al.* (2004b), palmitic acid (16:0) is the major fatty acids in eggs snails, followed by stearic acid (18:0), oleic acid (18:1n-9), and eikosamonoenat acid (20:1n-7). The fatty acids represent more than 60% of fatty acids in egg carotenoids ovorubin snails.

The results of the analysis of carbohydrate content on golden apple snail eggs in the amount of 7.12 ± 0.11%. Research Dreon *et al.* (2004a) showed that the total carbohydrate in the eggs accounted for 17.8% snails from ovorubin and 2.5% (w / w) of PV2. Analysis by size exclusion chromatography showed that the number of O-linked oligosaccharides was higher than N-linked species (59% of total carbohydrates ovorubin and 67% w / w of carbohydrate PV2). Polysaccharides in PV golden apple snail eggs consisting of galactose and fucose unit (Heras and Pollero, 2002).

Table 1: Chemical composition of some aquatic raw materials (%)

Chemical composition	Golden snail eggs	meat golden snail	Tuna eggs como[2])	Sea urchin eggs[3])	Cat fish eggs)
Air change moisture	75.55±3.20	77.60	73.03±0.71	12.03±1.26	70.85-73.46
Abu change ash	13.81±3.37	3.20	1.79±0.25	2.25±0.24	0.48-3.98
Protein	3.32±0.22	12.20	18.16±0.91	12.03±1.26	54.12-59.48
Lemak change fat	0.19±0.00	0.40	4.26±0.05	3.05±0.50	38.09-41.90
Karbohidrat change carbohydrate	7.12±0.11	6.60	2.76±0.21	2.80±2.41	-

source: 1) Pambudi (2011);
2) Intarasirisawat *et al.* (2011);
3) Mol *et al.* (2008);
4) Yulfiperius *et al.* (2003)

Mineral Content

Mineral content presented in Tables 2 and 3. The content of calcium in the golden apple snail eggs are 17925.18 ± 116.64 ppm. Calcium content of golden apple snail eggs was found to be bigger than the calcium mullet eggs and meat snails. High calcium content in golden apple snail eggs because of the egg snails. Catalan *et al.* (2002) suggested that the shell/golden apple snail egg capsules containing calcium carbonate in the protein matrix and mucopolysaccharides acid. Calcium is in the golden snail eggs formation of the albumen gland complex and capsule gland. Mineral absorption capacity is influenced by several factors, the driving factor is the pH of the acid and the inhibiting factor are the alkaline pH conditions, the presence of fiber and pitat acid (Yanuar *et al.*, 2009).

magnesium contains in the golden snail eggs is 112.29 ± 0.36 ppm. Total of carbohydrate was determined which showed that the N-acetylglucosamine on ovorubin is the second major monosaccharide golden snail eggs. Magnesium is a cofactor for many enzymes involved in glucose metabolism in particular the use of high-energy phosphate bonds. This causes magnesium golden apple snail eggs higher than mullet eggs and como tuna eggs. The mineral content of a material is also influenced by environmental conditions and age (Yenny and Suastika, 2011).

Table 2: Mineral composition of golden snail eggs and belanak fish eggs in wet basis (ppm)

Mineral	Golden snail eggs	Belanak fish eggs[1)]
Macro mineral		
Ca	17925.18 ± 116.64	11.56 ± 0.00
Na	402.92 ± 4.55	-
K	252.02 ± 12.06	-
P	197.28 ± 0.33	-
Mg	112.29 ± 0.36	17.34 ± 0.06
Micro mineral		
Cu	10.16 ± 0.33	tt
Zn	5.28 ± 0.05	11.35 ± 0.04
Fe	7.83 ± 0.14	0.87 ± 0.12

Source : 1) Olgunoglu dan Olgunoglu (2011)

Table 3: Mineral composition of some aquatic raw materials (ppm)

Type of mineral	Golden snail eggs	Tuna eggs *como*[1)]	Meat golden snail[2)]
Macro mineral			
Ca	73313.60 ± 477.06	-	7593.81
Na	1647.93 ± 18.62	768.25 ± 12.14	620.84
K	1030.75 ± 49.34	2194.34 ± 51.35	824.84
P	806.86 ± 1.36	1456.63 ± 14.86	1454.32
Mg	459.28 ± 1.48	486.33 ± 5.45	238.05
Micro mineral			
Cu	41.55 ± 1.37	34.35 ± 0.45	tt
Fe	32.01 ± 0.56	122.17 ± 0.88	44.16
Zn	21.6 ± 0.22	-	20.57

Source : 1) Intarasirisawat *et al*. (2011); 2)Pambudi (2011) sodium *P. canaliculata* contains is 402.92 ± 4.55 ppm. Changes in blood pressure is influenced by sodium imbalance. More than 90% of the osmotic pressure in the extracellular fluid is determined by a salt containing sodium, especially in the form of sodium chloride (NaCl) and sodium bicarbonate ($NaHCO_3$). Osmotic pressure changes in extracellular fluid sodium concentration describe changes (Yaswir and Ferawati, 2012). Golden apple snail eggs are protected by a shell so that the osmotic pressure of the extracellular fluid and sodium concentration of golden apple snail eggs are quite stable.

The content of potassium in the golden apple snail eggs are 252.02 ± 12.06 ppm. Age and physiological state affect the potassium content in a material (Yaswir and Ferawati, 2012). Environmental conditions also affect potassium levels in a material (Manalu, 2007). The content of phosphorus in the golden apple snail eggs, is 163.929 ppm. Food affects the content of phosphorus in biological creatures (Zainudin, 2010). The marine food contains more phosphorus to phosphorus golden apple snail eggs lower than como tuna fish eggs.

The zinc content in the egg snails is 5.28 ± 0.05 ppm, higher than the zinc content in the egg mullet, and meat snails. Zinc is involved linking amino groups with active genes. The level of zinc absorption in the intestine is influenced by several factors, there are factors that help and hinder the absorption. Substances that are endogenously produced and digested as well as low molecular weight, such as methionine, histidine, cysteine, citrate, picolinate, prostaglandin E2, reduced glutathione, and other small ligands assist zinc absorption in the intestine (Sunar, 1999). Inhibiting inorganic and organic zinc absorption in the intestine, among others, cadmium, copper, phosphate, calcium, iron (non-heme), mionositol heksaphospate (phytate), dietary fiber components including hemicellulose and lignin, and oxalate. Environment influences the zinc content in a biological material (Shindu, 2005). The iron content in eggs snails is 7.83 ± 0.14 ppm. Transferrin is a protein that transports iron carrier plasma and extracellular fluid to meet the body's needs. Transferrin receptor is a glycoprotein located on the cell membrane, acts bind the transferrin-iron complex (Ani, 2011). Golden snail eggs containing glycoproteins with enough amount that binds to carotene. This causes the iron content of eggs snails higher than mullet eggs. The content of copper in the golden snail eggs, which is 9.75 ppm. Environment affects the copper content in a biological material (Shindu, 2005). Nurjanah et al (2015) have osbserved mineral analyzed preferably using deionized water than using distilled water because there is still the possibility of some mineral content in distilled water.

Total Carotenoids Golden Apple Snail Eggs

Total carotenoids golden snail eggs are 313.48 ± 19.73 ppm. Results of research Rakcejeva *et al.* (2012) showed that levels of total carotenoids in carrots of various types of hybrids ranged from 60.21 ± 0.66 to 79.47 ± 0.42 ppm. According to Azka *et al.* (2015) Total carotenoid flaying fish eggs at 245.37 ppm and 37.92 ppm. Results of research Garner *et al.* (2010) indicates the total content of carotenoids in eggs *chinook* salmon (*Oncorhynchus tshawytscha*) of 17.9 ppm. Environmental factors, temperature, season, dietary intake, level of maturity affects carotenoid content in a material. The carotene content is high on golden snail eggs expected to be used as fish feed (beautify skin color), cosmetic ingredients, or health supplements.

Carotene can help in the success of hatching eggs and juvenile defense against disease and oxidative stress. There is a positive correlation between the quality of the females with the carotene content in eggs, the higher the carotenoid content in eggs, the better the quality. Female parent body tissues and carotenoid concentrations in eggs reflects dietary intake of carotenoids by a female parent (Garner *et al.*, 2010).

The main constituent is nitrogen golden snail eggs in jelly around the eggs a red glycoprotein with prosthetic makeup carotenoids. This protein has a high stability against denaturation of the protein. One egg snails weighs 20 mg and 0.23 mg protein has an embryo and 1.5 nmol astaxanthin. Golden snail eggs carotenoid content of about 72 nmol/gram. Astaxanthin is a major carotene in golden snail eggs. Astaxanthin is a powerful antioxidant. Astaxanthin on golden snail eggs consists of a free form (40%), monoester form (24%), and the form of diester (35%) esterified with fatty acids 16: 0.

Conclusion

Golden snail eggs contain high levels of minerals, macro-micro and total carotenoids were high. High calcium content in eggs snails for their shells on a golden snail eggs. eggs snails of Macro minerals from the highest to the lowest, are calcium, sodium, potassium, phosphorus, and magnesium. Micro minerals in eggs snails contains from the highest to the lowest, namely copper, iron, and zinc. Total carotenoids golden snail eggs are higher than carrots of various hybrid eggs *chinook* salmon (*Oncorhynchus tshawytscha*).

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Conflict of Interest

The author(s) declare no conflict of interest.

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