

## Gonad quality of sea urchin *Tripneustis gratilla* with different seaweed diets

### Kualitas gonad bulubabi *Tripneustis gratilla* dengan pakan rumput laut yang berbeda

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#### ABSTRACT

Gonad quality of sea urchin *Tripneustis gratilla* is influenced by consumed seaweed type. Therefore, this study was conducted to determine the type of seaweed as potential source of carotenoids and produce a good quality of sea urchin gonad. The study was conducted in two phases, namely first phase was to obtain the content of carotenoids and  $\beta$ -carotene from several species of seaweed; and second phase was to testify the seaweed that produced high quality of sea urchin gonad. This study used completely randomized design with 4 treatments of macroalgae diets, namely *Enhalus* sp., *Sargassum* sp., *Ulva* sp., and *Eucheuma* sp.. Each treatment was repeated three times. Measured parameters were gonad weight, gonad maturity index, gonad color quality, and gonad texture. The study result showed that *Enhalus* contained the highest carotenoid and  $\beta$ -carotene content with 1409.53 and 639.37 mg/kg respectively. The highest gonad weight was 2.8 g; gonad color and texture was good (score 3); and the highest gonad maturity index was 5.4% that obtained from sea urchin fed with *Enhalus*. Therefore, this study concludes that *Enhalus* is potential source of carotenoids for sea urchin *T. gratilla* diet that will produce a good quality of gonad.

Keywords: carotenoid, diet, gonad quality, sea urchin, seaweed.

#### ABSTRAK

Kualitas gonad bulubabi *Tripneustes gratilla* dipengaruhi oleh jenis rumput laut yang dimakan bulu babi tersebut. Oleh karena itu, penelitian dilakukan untuk menganalisis jenis rumput laut yang potensial sebagai sumber karotenoid dan dapat menghasilkan gonad yang berkualitas baik. Penelitian dilakukan dalam dua tahap, yaitu tahap I mengetahui kandungan karotenoid dan  $\beta$ -karotene dari beberapa jenis rumput laut; dan tahap ke II menguji jenis rumput laut yang dapat menghasilkan gonad berkualitas baik. Penelitian ini menggunakan rancangan acak lengkap yang terdiri atas empat perlakuan pakan makroalga yaitu *Enhalus* sp., *Sargassum* sp, *Ulva lactuca*, dan *Eucheuma* sp.. Setiap perlakuan diulang sebanyak tiga kali. Parameter yang diamati adalah bobot gonad, indeks kematangan gonad, kualitas warna, dan tekstur gonad bulubabi. Hasil penelitian menunjukkan *Enhalus* memiliki kandungan karotenoid dan  $\beta$ -karotene tertinggi masing-masing sebesar 1.409,53 dan 639,37 mg/kg. Bobot gonad tertinggi sebesar 2,8 g, warna dan tekstur gonad berkualitas baik (skor 3), serta indeks kematangan gonad (IKG) tertinggi sebesar 5,4% dihasilkan pada pemberian pakan *Enhalus*. Oleh karena itu, dapat disimpulkan bahwa *Enhalus* potensial sebagai sumber karotenoid dalam pakan bulu babi *T.gratilla* dan dapat menghasilkan kualitas gonad yang baik.

Kata kunci: bulu babi, karotenoid, kualitas gonad, pakan, rumput laut

## INTRODUCTION

*Tripneustes gratilla* is one kind of high economical sea urchin found in Kupang Bay, East Nusa Tenggara. Sea urchin gonads are luxurious food in some Asian and Mediterranean, as well as Western countries, such as Barbados and Chile (Rahman *et al.*, 2012; Garama *et al.*, 2012; Elmasry *et al.*, 2013; Elmasry *et al.*, 2015; Rahman *et al.*, 2014; Rahim & Nurhasan, 2016; Cirino *et al.*, 2017; Samuel *et al.*, 2017). Sea urchins are also bioactive ingredient source, namely echinochrome A, polyunsaturated fatty acids (PUFAs),  $\beta$ -carotene, amino acids, and EPA (Chantaro *et al.*, 2008; Chen *et al.*, 2010; Bragadeeswaran *et al.*, 2013; Rahman *et al.*, 2014).

The price of sea urchin gonad in Kupang local market ranges from Rp 10,000 – 15,000 per collective sea urchin (one collective contains 10 sea urchins with 50–60 mm diameter). The price of sea urchin gonad in international market ranges from \$200–450 per kg (Robinson *et al.*, 2002; Rahman *et al.*, 2014). The gonad price depends on the gonad quality from color, flavor, texture, and gametogenesis (Feng *et al.*, 2015). Good gonad color quality varies from the characteristics as yellow/orange color has better quality than brown/black color gonad (Phillips, 2009a; 2009b; 2009c; Garama *et al.*, 2012).

High demand for sea urchin gonad causes this biota being continuously exploited in several countries, such as Japan, Chile, France, Canada, United States, and Colombia (Rahman *et al.*, 2012; 2013; and 2014). The global production of sea urchin capture from 1995 to 2008 showed decreased until 32% (FAO, 2010; Carboni *et al.*, 2012; Rahman *et al.*, 2014). Reduction in sea urchin supply from the nature coupled with high demand, encouraging increased sea urchin culture.

Sea urchin culture is generally directed at the production of qualified gonad based on market demand (Cirino *et al.*, 2017). Gonad quality is influenced by several factors, namely diet type and reproductive cycle (Elmasry *et al.*, 2015; Cirino *et al.*, 2017; Samuel *et al.*, 2017). Gonad quality attracted by the market is reddish yellow color, solid texture, and sweet flavor. Gonad color is determined by Echinenone and  $\beta$ -carotene content (Robinson *et al.*, 2002; Garama *et al.*, 2012). Echinenone in gonad is converted from  $\beta$ -carotene content in macroalgae consumed by sea urchin. Gonad texture quality is influenced

by gonad maturity level. Gonad solid texture is generally produced during premature period. Gonad quality are also determined by the type of diet given and maturity level. Gonads at the final maturation or closed spawning period are usually soft with brownish and creamy color, then producing bitter taste (Tjendanawangi *et al.*, 2014a).

Sea urchin caught from nature has various gonad quality (Garama *et al.*, 2012; Tjendanawangi *et al.*, 2014b). Gonad weight and quality in sea urchin *Evechinus chloroticus* can be modified based on the diet given and 8–12 week maintenance in cage or tank (James & Heath, 2008; Phillips, 2009a; 2009b 2009c; Garama *et al.*, 2012). Therefore, it is necessary to culture sea urchin with similarly good quality gonad and all time availability by determining the type of seaweed given as diet source and gonad maturity level of sea urchin. Based on this condition, this study was performed to examine different seaweed types that are potentially utilized as carotenoids and produce good quality gonad.

## MATERIALS AND METHOD

### First phase study

In the first phase study, ten dominant seaweed types found in sea urchin *T. gratilla* habitat at Kupang Bay, namely *Padina* sp., *Gracilaria* sp., *Halymenia* sp., *Enhalus* sp., *Ulva* sp., *Eucheuma* sp., *Galaxaura* sp., *Halimeda* sp., and *Sargassum* sp. Seaweed types were taken from the water, air dried, then grinded. Grinded seaweed was furtherly analyzed the total content of carotenoids,  $\beta$ -carotene, protein, lipid, carbohydrate, and ash. The total content of carotenoids and  $\beta$ -carotene were measured using spectrophotometer and HPLC (Garama *et al.*, 2012). Seaweed protein content was measured by inhouse method, while lipid, carbohydrates, and ash content were measured by SNI 01-2891-1992 standard method.

### Second phase study

This study used completely randomized design using four diet treatments. Seaweeds utilized as diet treatments were based on the high availability of  $\beta$ -carotene and carotenoids from the first phase, i.e *Enhalus* sp., *Sargassum* sp., *Ulva* sp., and *Eucheuma* sp. Each treatment was repeated three times.

Sea urchins *T. gratilla* with 50–60 mm diameter were maintained in plastic basket measuring 30 × 20 × 10 cm. Nine plastic baskets were hung on

three tanks measuring 2×1×1.5 m under running water system. Each tank contained three baskets and each basket was placed 10 sea urchins *T. gratilla*. Before being given diet treatment, sea urchins were adapted in the tank for one week. Sea urchins were then fasted for one week before diet treatments were given. Sea urchins were fed as much as 150 g every two days for six weeks maintenance.

Three sea urchins were taken to observe the gonadal development on the second, fourth, and sixth week maintenance. Measured parameters were the gonad weight, maturity index, color, and texture. Sea urchins were dissected from aboral to oral section that had no spines segment presented (interambulacral section). The inner organs were slowly removed, except the gonad attached to the shell. Furthermore, attached gonad was cleaned with water, then released from the shell using small thin spoon and placed on the filter paper. Gonad was measured to obtained the weight

data. Furthermore, gonads maturity index was calculated based on:

$$\text{Gonad maturation index} = \frac{\text{Gonad weight}}{\text{Body weight}} \times 100$$

Gonad color and texture assessment were subjectively performed by three panelists. Gonad color was compared with painted color card, then constructed based on the scoring table (Table 1) below.

### Data analysis

Gonad weight and maturity index were analyzed using ANOVA. Gonad color and texture score were analyzed descriptively.

## RESULT

### Carotenoids and $\beta$ -carotene content in seaweeds

The analysis result of total carotenoids and  $\beta$ -carotene content on some seaweeds indicated

Table 1. Gonad color and texture score

Quality	Score	Color	Texture
Very good	4	Light yellow or reddish orange	Solid with soft surface
Good	3	Faded yellow or orange	A bit softened with granules
Fairly good	2	Cream	Softened (easily damaged)
Less good	1	Brown	Fluid

Table 2. Total carotenoids and  $\beta$ -carotene content on some seaweeds

Macroalgae type	Total carotenoids (mg/1000g)	$\beta$ -carotene (mg/1000g)
<i>Enhalus</i>	1409.53	639.37
<i>Sargassum</i>	282.42	246.52
<i>Padina</i>	139.57	118.99
<i>Halimeda</i>	45.50	Nd
<i>Ulva</i>	367.06	Nd
<i>Euchema</i>	146.71	Nd
<i>Halymenia</i>	186.93	Nd
<i>Galaxaura</i>	201.71	Nd

Note : nd (undetected)

Table 3. Proximate analysis result on some seaweeds chosen

No	Macroalgae type	Protein (%)	Lipid (%)	Carbohydrate (%)	Ash (%)
1	<i>Enhalus</i>	12.85	0.56	45.30	31.72
2	<i>Sargassum</i>	10.78	0.84	38.14	40.20
3	<i>Ulva</i>	10.52	0.32	51.96	25.50
4	<i>Halimeda</i>	15.78	0.32	41.60	42.25
5	<i>Padina</i>	8.36	0.55	40.60	48.05
6	<i>E Euchema</i>	16.97	0.55	38.60	43.32

that *Enhalus* (seagrass) contained the highest total carotenoids and  $\beta$ -carotene with 1409.53 and 639.37 mg/kg respectively. The completes data were presented on Table 2.

Based on the total carotenoids and  $\beta$ -carotene content, seaweeds that were potentially utilized as diet source for sea urchin were *Enhalus*, *Ulva*, and *Sargassum*. High carotenoids and  $\beta$ -carotene content in seaweed can be utilized to improve the gonad color of sea urchin. The good quality of sea urchin gonad color with reddish yellow color is determined based on the echinenone content in gonad, which is converted from  $\beta$ -carotene in

the diet (Garama *et al.* 2012; Vizzini *et al.*, 2015). Furthermore, those three seaweeds are preferred by sea urchins.

**Proximate content**

Proximate analysis showed that six seaweeds chosen contained rangely (Table 3) 8.36–16.97 protein, 0.32–0.84% lipid, 38.6–51.96% carbohydrate, and 31.72–48.05% ash.

Low protein and lipid content indicated that seaweed is less potentially utilized as protein and lipid diet source, however potentially utilized as carbohydrate and mineral source. Low protein

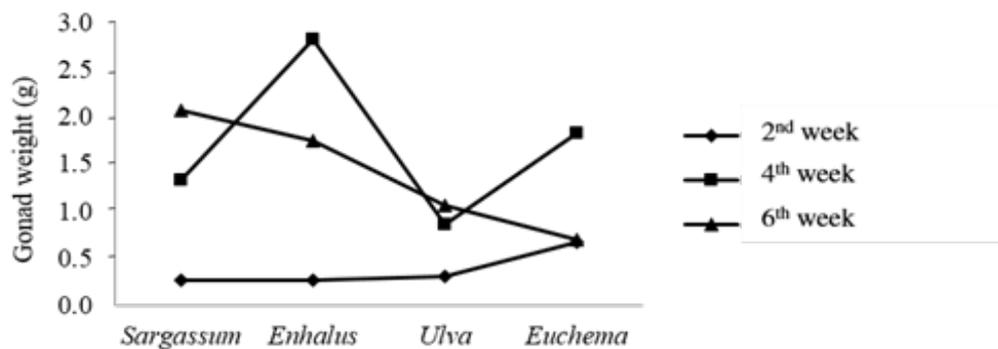


Figure 1. *T. gratila* gonad weight on the second until sixth week after given *Sargassum*, *Enhalus*, *Ulva*, and *Eucheuma*.

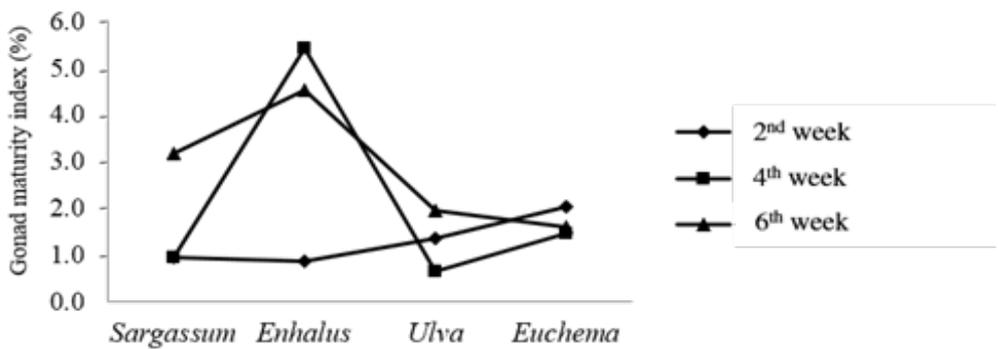


Figure 2. Gonad maturity index of *T. gratila* on the second until sixth week obtained from *Sargassum*, *Enhalus*, *Ulva*, and *Eucheuma* given.

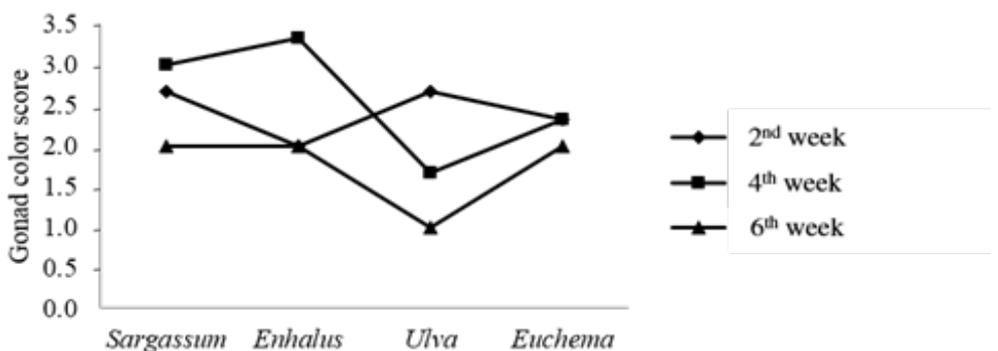


Figure 3. *T. gratila* gonad color on the second until sixth week after given *Sargassum*, *Enhalus*, *Ulva*, and *Eucheuma* diet.

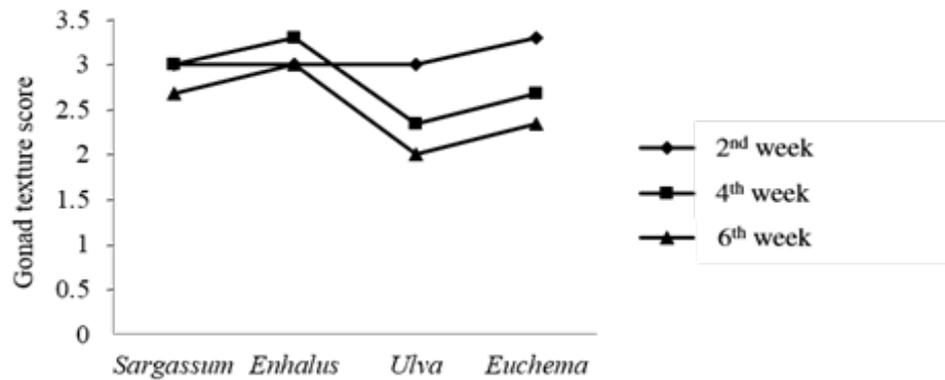


Figure 4. *T. gratila* gonad texture on the second until sixth week after given *Sargassum*, *Enhalus*, *Ulva*, and *Eucheuma* diet.

and lipid content on seaweed means that seaweed diet alone cannot be used as sea urchin diet, therefore it should be combined with other sources to increase the protein content.

### Gonad weight and maturity index

*Sargassum* and *Ulva* diet given produced induced gonad weight from second to sixth week maintenance, while *Enhalus* and *Eucheuma* diet produced induced gonad weight from second to fourth week maintenance, then reduced on sixth week (Figure 1). The highest gonad weight was presented on the fourth week with 2.80 g after given *Enhalus*. The statistical analysis showed that there were insignificant difference ( $P > 0.05$ ) against the gonad weight produced after given some macroalgal type diets.

During six week maintenance, the highest gonad maturity index was obtained from the fourth week with 5.40, then reduced on the sixth week with 4.53 after given *Enhalus* diet (Figure 2). *Sargassum*, *Ulva*, and *Eucheuma* diet produced lower gonad maturity index with  $\leq 3.00$ . The statistical analysis obtained  $P > 0.05$ , indicating insignificant different level of gonad maturity level produced after given some macroalgal types.

### Gonad color and texture

The highest gonad color quality was produced from sea urchin given *Enhalus* and *Sargassum* on the fourth week with good scores (3.3 and 3.0 respectively). The lowest gonad quality was produced on the sixth week with less good score (1) after given *Ulva* (Figure 3).

The highest gonad texture quality was obtained from *Enhalus* and *Sargassum* diet treatment on the fourth week with good scores (3.3 and 3.0 respectively). The lowest quality was obtained on the sixth week with fairly good score (2) from *Ulva* diet treatment (Figure 4).

## DISCUSSION

The gonad weight produced in this study was still very low compared to the gonad weight obtained from nature reaching 12 g (Tjendanawangi, 2014b). Gonad maturity index produced in this study was also lower than obtained from nature with 0.09–15.14 (Tjendanawangi *et al.*, 2010) and 3.22–12.47 (Tjendanawangi & Dahoklory, 2011), as well as lower than Cirino *et al.* (2017), earning 8.70 maturity index after cultured for six weeks. This happened due to one type seaweed given on each treatment diet, whereas the sea urchin is capable of consuming various types of diet. Sartori *et al.* (2015) stated that diet containing maize kernel and some kind of fresh macroalgae produced 6.59 g gonad weight of *Paracentrotus lividus* with twelveth week maintenance. In addition, low gonad weight and maturity index in this study was because of low seaweed protein content ( $< 20\%$ ), which was inadequate to fulfill sea urchin protein requirement as sea urchin requires 20–40% protein (Schlosser *et al.*, 2005; Hammer *et al.*, 2006). Gonad weight and maturity index were influenced by nutrient contents (protein, lipid, energy) of seaweed consumed by sea urchin, besides affected by seaweed digestibility (Sartori *et al.*, 2015) and palatability (Cyrus *et al.*, 2015).

The reddish-yellow color of sea urchin was influenced by Echinenone as the dominant carotenoids in gonad. Echinenone is metabolized from  $\beta$ -carotene in diet, then transported and subsequently deposited in the gonad (Garama *et al.*, 2012). The gonad color produced in this study achieved good quality after being given *Enhalus* diet containing 639.37 mg/kg  $\beta$ -carotene dry weight. This result did not achieve very good quality compared to Robinson *et al.* (2002) on *Strongylocentrotus droebachiensis* who used

*Dunaliella salina* microalgae with 200–250 mg/kg  $\beta$ -carotene dry weight. This indicated that high content of carotenoids in diet did not directly increase the gonad color quality, but it is likely influenced by the diet bioavailability consumed by sea urchin. Robinson *et al.* (2002) expressed the alteration of  $\beta$ -carotene in diet into echinenone in gonad is influenced by macroalgae consumed by sea urchins. Macroalgae as the main diet for sea urchins also contains astaxanthin, cantaxanthin, and fucoxanthin suspected to produce dark color on the sea urchin gonad (Symonds *et al.*, 2009; Garama *et al.*, 2012). The content of astaxanthin and fucoxanthin did not give any influences on sea urchin. Conversely, in fish and crustacean, astaxanthin might increase the gonad maturation rate, spawning, embryonal development, growth performance, and larval survival rate (Daly *et al.*, 2013; Zhang *et al.*, 2013).

The gonad color quality was not only influenced by carotenoids in the diet, but also maturation period and process. Tjendanawangi *et al.* (2010) found that very good *T. gratilla* gonad color quality is generally obtained during the growth phase, i.e. prematuration and early maturation period, while low quality was obtained from the final maturation, spawning, and recovery period. Gonad color variation depends on the diet variation and sea urchin reproductive stage (Rahman *et al.*, 2014).

Gonad texture quality result was in line with gonad color quality. Gonad texture was also influenced by the maturity level. When associated with gonad maturity level, the sea urchin gonad in this study reached the maturity level on the fourth week, then declined on the sixth week. Excellent color and texture gonad quality (score 4) was produced on prematuration period (Tjendanawangi *et al.*, 2010).

## CONCLUSION

Based on this study, it can be concluded that seaweed *Enhalus* diet is capable of producing good gonad color and texture quality of *T. gratilla*, but incapable of producing high gonad weight and maturity index.

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