

Protein Level and Protein Energy Ratio that Produce the Best Gonad Quality of Sea Urchin *Tripneustes Gratilla*

Agnette T

Department of Fisheries and Marine Resources

University of Nusa Cendana, Kupang

M. Zairin

Department of Aquaculture, Bogor Agricultural University, Bogor

Mokoginta

Department of Water Resource Management, Bogor Agricultural University, Bogor

M. A. Suprayudi

Department of Water Resource Management, Bogor Agricultural University, Bogor

F. Yulianda

Department of Water Resource Management, Bogor Agricultural University, Bogor

Received: December 20, 2013 Accepted: January 3, 2014

doi:10.5296/jbls.v5i1.5076 URL: <http://dx.doi.org/10.5296/jbls.v5i1.5076>

Abstract

The study aims to determine protein level and protein energy ratio that produce the best gonad quality of sea urchin *Tripneustes gratilla*. Sea urchin were fed nine test diets with the combination ratio of protein level (%) and protein energy (kcal GE/g) namely; A (22:9), B (22:11), C (22:13), D (27:9), E (27:11), F (27:13), G (32:9), H (32:11), and I (32 : 13).

Treatment G produced the best gonad quality with gonad weight, gonad protein and gonad colour of 3.49 g, 64.45 % and pale yellow until orange, respectively. Treatment G also showed carotenoid total ranged from 15 to 18 ppm and β -carotene ranged from 5 to 6 ppm. Moreover, this treatment exhibited gonad weight and gonad protein resulting in significant difference ($P < 0.05$) protein level, protein energy ratio and its interaction. However, carotenoid total and β -carotene did not exhibit any difference ($p > 0.05$).

Keywords: Tripneustes gratilla, Protein, Energy, Gonad quality, Carotenoid

1. Introduction

The increasing of gonad price from US \$6 to \$400 kg⁻¹ (Robinson *et al.* 2002; Sphigel *et al.* 2005; Pearce *et al.* 2002), the stable market asking and over fishing in several countries (Hammer *et al.* 2006; Siikavuopio *et al.* 2004, 2006) have supported the development of sea urchin cultivation. The development of sea urchin culture has been purposed to increase production and gonad quality. Production and gonad quality of sea urchin are influenced by the gonad growth level and nutrition quality.

Commonly, species of sea urchin use macroalgae as their food. Nevertheless, macroalgae use as food source has been less optimal due to 1) the limitation of macroalgae species source in nature, 2) variety of algae quality and 3) the difficulty of mass algae storage. Therefore, the development of artificial feed has been carried out to culture several species of sea urchin such as *Pseudocentrotus depressus* (Unuma *et al.*, 1999; Akiyama *et al.*, 2001); *Lytechinus variegatus* (Wasson *et al.*, 1998; Hammer *et al.*, 2004, 2006); *Strongylocentrotus droebachiensis* (Wasson *et al.*, 1998; Hammer *et al.*, 2004, 2006); and *Paracentrotus lividus* (Wasson *et al.*, 1998; Hammer *et al.*, 2004, 2006) but, nutrition need and physical condition of sea urchin differ among species.

The nutrient availability in artificial feed will influence the development and production of sea urchin gonad. Protein content in feed gives the effect on gonad protein content resulting in the increasing of nutritive phagocyte size. The feed energy content is one important factor during gonad development and maturation in reproduction cycle beside protein (Schlosser *et al.*, 2005). Feed with low energy content will cause sea urchin to use a part of protein as energy source for metabolism. It causes the lack of protein need for gonad development and maturation. In turn, feed with high energy content will result in the limitation of protein number eaten. However, the information of feed energy influence particularly protein energy ratio in feed on sea urchin gonad production has still been limited. Since protein has been one rich component in feed of sea urchin culture, it is required to determine the optimal protein level need and protein energy ratio for the maximum growth and gonad production in more efficient protein use.

Tripneustes gratilla is one of dominant sea urchin species which has a potential for being developed in Indonesia particularly in Kupang bay. Nevertheless, study on gonad quality improvement and nutrition need information of sea urchin (*T. gratilla*) culture have still been limited. Therefore, this study aims to determine protein level and protein energy ratio that produce the best gonad quality of *T. gratilla*.

2. Material and Method

2.1 Time and Research Place

Study was carried out from April to September 2009. Sea urchin (*T. Gratilla*) rearing in Department of Fisheries and Marine Laboratory of UNDANA. Preparing of feed materials, feed proximate test and gonad were done in Nutrition and Fish Health Laboratory, Institute of Bogor Aquiculture (IPB). Whilst, carotenoid total and β -carotene of gonad were conducted on postharvest laboratory of Agency for Bogor Agricultural Postharvest Research and Development.

2.2 Test Feed

Materials used for feed formulation were fish flour, soya flour, corn flour, white flour, sargassum flour, fish oil, sargassum extract, agarose, vitamine mixture, mineral mixture, and etoxyquin with ingredients and the result of proximate analysis are listed in table 1 and Table 2.

2.3 Rearing and Data Collection

Adult sea urchin with the body diameter of 50-60 mm were collected from the wild and held in laboratory aquarium. Those sea urchin were acclimatized for two weeks. During the acclimatization process, sea urchin were fed commercial feed at 3 % of body weight once a day. The healthy sea urchin were selected and cultured in aquariums with the sizing of 50 x 50 x 30 cm each containing 10 L filtered seawater at 30 ppt. The density of sea urchin was 20 ind/rearing unit. All rearing units were provided with gentle and continuous aeration. All the rearing units were also completed with thermometer. Sea urchin were then fasted for two weeks thereafter, they were fed with test feed at 3% of body weight every two days satiatedly. Feed residue in the rearing units were removed by siphoning. This study used Completely Randomized Design with 2 factors consisted of 9 treatments and 3 repetitions. The first factor was protein level in which protein level percentage used were 22, 27 and 32%. The second factor was protein energy ratio (C/P) with protein energy level of 9, 11 and 13 (kcalGE/g). The treatment combination ratio between protein level and protein energy was A (22:9), B (22:11), C (22:13), D (27:9), E (27:11), F (27:13), G (32:9), H (32:11), and I (32 : 13). In the end of study, gonad weight, protein level, carotenoid total, β -caroten, and gonad colour of sea urchin was measured. Gonad protein level was determined by using Kjeldahl method as described by Tekeuchi (1988). Meanwhile, carotenoid total and β -caroten of gonad were measured by using spectrophotometer and HPLC as described by Apriyantono *et al.* (1989); Agatsuma *et al.* (2005) and Barclay *et al.* (2006) with some modifications.

Table 1. Formulation percentage of test feed materials (%) with the similar content of vitamin mix, mineral mix, vitamin C, agarose, etoxyquin/BHT, and sargassum extract

Material (%)	Treatment								
	A	B	C	D	E	F	G	H	I
Corn flour	3.00	9.00	15.00	3.00	7.00	10.00	7.00	12.00	8.00
Fish flour	24.00	22.00	22.00	31.00	28.00	27.50	35.00	35.00	35.00

Sargassum flour	5.00	8.00	10.00	6.00	10.00	15.00	7.00	10.00	15.00
Soya flour	15.00	15.00	13.00	17.00	17.00	16.00	21.00	19.00	19.00
White flour	5.00	5.00	5.00	3.00	7.45	7.45	3.00	5.00	1.00
Fish oil	0.50	1.00	3.00	1.00	2.50	6.00	1.00	4.00	12.00
Vitamine Mix	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Mineral Mix	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
vit amine C	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
Agarose	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Etoxyquin/BHT	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Sargassum extract	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Cellulose	38.37	30.87	22.87	29.87	18.92	8.92	16.87	5.87	0.87

Table2. Proximate composition percentage (% of dry weight) of test feed in the third study

Treatment	Proximate composition (%)				
	Ash content	Protein	Fat	Carbohydrate	
				Coarse fibre	BETN
A	16.21	22.21	3.95	25.46	32.17
B	16.02	22.77	5.57	21.76	33.88
C	15.44	22.12	9.85	16.39	36.20
D	17.31	26.73	6.22	18.97	30.77
E	16.42	26.69	8.93	15.71	32.25
F	15.71	26.34	16.02	9.29	32.64
G	16.53	31.82	5.95	19.72	25.98
H	16.96	31.70	12.13	8.96	30.25
I	16.91	31.67	27.25	3.69	20.48

Note: Combination treatment between protein level (%) and protein energy (kcalGE/g) of A (22:9); B (22:11); C (22:13); D (27:9); E (27:11); F (27:13); G (32:9); H (32:11); and I (32:13)

Gonad colour was subjectively tested by three respondents. The colour of gonad was then compared to prepared card colour and continued to be arranged in gonad colour percentage by grouping each gonad in several categories as in category modified by [9] as followed:

1 = very good (light yellow or orange)

2 = good (pale yellow)

3 = mild (yellow-brown, orange – brown, brown red, cream)

4 = bad (dark brown, gray, green)

2.3 Data Analysis

Gonad weight, gonad protein, carotenoid total, and β -caroten data were analyzed by *two-way analysis of variance* (Steel dan Torrie, 1982) using SPSS Version 13.0 software packages. Differences of results were considered statistically significant difference if the *P*

value were ≤ 0.05 . The color of gonad was descriptively analyzed by using Microsoft Excel 2007 programme.

3. Result and Discussion

3.1 Result

3.1.1 Gonad Weight

The highest gonad weight was shown by the treatment of G (32: 9) namely 2.25 g. In contrast, the treatment of A (22:9) gave the lowest gonad weight (0.38 g) (Figure 1). Among the treatment of A, B and C with protein level of 22% and C/P ratio of 9, 11 and 13 kcal GE/g, respectively, the treatment of C (22:13) showed the highest gonad weight. Meanwhile, from the treatments of G, H, I with protein level of 32% and C/P ratio of 9, 11 and 13 kcal, the highest gonad weight was displayed by the treatment of G (32:9). It indicated that the treatment with the low protein level was needed the high C/P ratio. In turn, at the treatment with the high protein level was needed the low C/P ratio. In addition, it could be said that in the G treatment, the optimal feed protein was used to increase gonad weight of sea urchin. Furthermore, protein level and interaction of protein level and protein energy ratio gave the significant effect on the increase of sea urchin gonad weight. However, protein energy ratio itself exhibited no any significant effect ($P > 0.05$) on gonad weight of sea urchin.

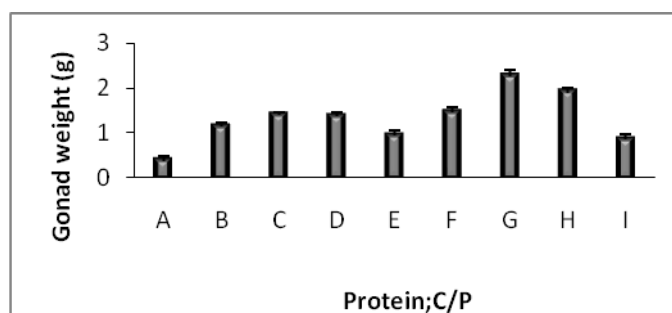


Figure 1. Sea urchin gonad weight (g) at different protein level and protein energy ratio (gonad weight average $\pm$ SD; $n = 3$).

3.1.2 Gonad Protein

The average of gonad protein level in each treatment was range from 48.25 to 64.45 % (Figure 2). Sea urchin (*T. gratilla*) given feed at the treatment G with protein level (P) of 32% and C/P ratio of 11 produced the highest gonad protein (64.45%). It showed that feed protein increasing was able to increase gonad protein content of sea urchin. In this study, protein level and interaction between protein level and protein energy ratio pakan showed a significantly different gonad protein level ($P < 0.05$) in contrast, protein energy ratio did not exhibit any different gonad protein content ($P > 0.05$).

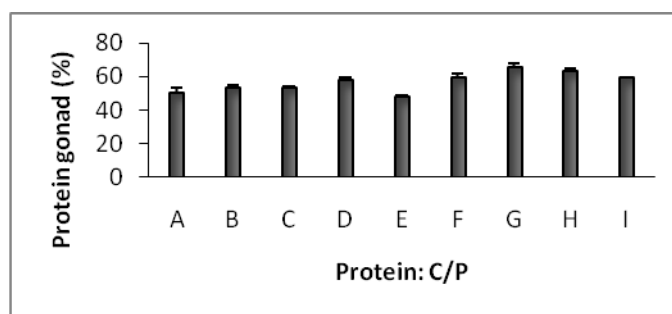


Figure 2. Sea urchin gonad protein at different treatment of protein level and protein energy ratio (protein average $\pm$ SD; $n = 3$).

3.1.3 Carotenoid Total and β -carotene

The highest gonad carotenoid total was found at the B (18.18 ppm) and the lowest gonad carotenoid total was obtained at the treatment F (13.98 ppm). In general, gonad carotenoid total average tended to decrease with protein energy ratio increasing. The lowest gonad carotenoid total was shown by the treatment F (13 kcal GE/g) (Figure 3). The carotenoid analysis displayed that protein level, feed protein energy ratio and interaction between protein level and protein energy ratio gave no significant effect on gonad carotenoid total ($P > 0.05$).

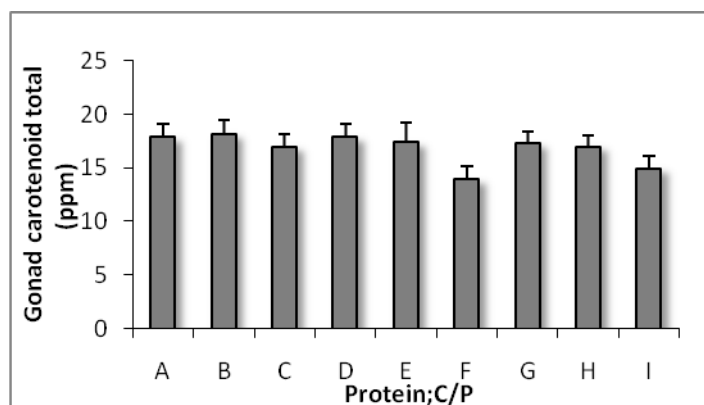


Figure 3. Gonad carotenoid total level of sea urchin at different treatment of protein level and protein energy ratio (Carotenoid total average $\pm$ SD; $n = 3$).

From this study, it could be obtained that gonad carotenoid accumulation was influenced by feed protein energy ratio. The increasing of feed protein energy ratio was suspected to cause the lack of carotenoid taking or absorption binding with protein to gonad due to the more feed fat content as the main energy source of feed.

The highest gonad β -carotene content was also shown by the treatment B (6.26 ppm) and the lowest gonad β -carotene content was obtained at the treatment F (4.78 ppm) (Figure 4). The protein level, protein energy ratio and interaction between protein level and feed protein energy ratio did not give any significant effect on β -carotene of gonad ($P > 0.05$).

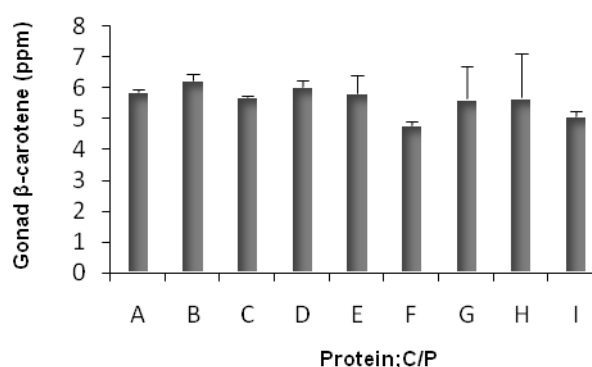


Figure 4. Gonad β-carotene level of sea urchin at different treatment of protein level and protein energy ratio (Carotenoid total average $\pm$ SD; n = 3).

β-carotene content showed the similar pattern to carotenoid total content. Compared to gonad carotenoid total, gonad β-carotene showed a third of carotenoid total and the rest of carotenoid total were converted into the other forms which were suspected to be retinal, retinol and echinenon.

3.1.4 Gonad Colour

Gonad colour obtained was in the second group with gonad colour score average of 2-2.83 indicating a good gonad colour quality. The evaluation of gonad colour (Figure 5) exhibited the similar pattern to carotenoid total and gonad β-carotene.

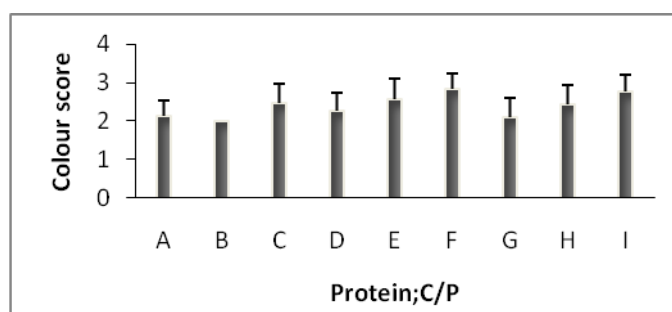


Figure 5. Sea urchin gonad colour evaluation at different treatment of protein level and protein energy ratio (Carotenoid total average $\pm$ SD; n = 3).

The observation of rearing water quality parameters was suitable for the growth of sea urchin during the experiment. Temperature, salinity, pH, and ammonia obtained were ranged from 26 to 28 °C, 29 to 32 ppt, 7.2 to 7.8, and 0.019 to 0.188 mg/l, respectively.

3.2. Discussion

The main component of vitellogenin is protein (lipoprotein). Feed protein content will influence gonad protein content indicated by the increasing of nutritive phagocyte size which has a function as protein storage. Beside protein content, feed energy content and physiology factor of fish give the effect on gonad protein content.

In this study, 32% protein level and 9 kcal GE/g optimal protein energy ratio (C/P) were able to increase gonad weight and gonad protein content of sea urchin (*Tripneustes gratilla*). Investigation by Akiyama *et al.* (2001) on *Paracentrotus depressus* with 15 mm in diameter obtained that sea urchin fed with protein content 20, 30 and 40% had the higher gonad index ($P < 0.05$) than sea urchin fed with protein content 10 and 50% ($P > 0.05$). Meanwhile, Pearce *et al.* (2004) found that adult *Strongylocentrotus droebachiensis* with body diameter of 59.6 ± 4.1 mm fed with feed protein content of 19, 24 and 29% resulted in the similar gonad index and those treatments gave the significantly different gonad index compared to control feed (kelp) with feed protein content of 8.7%. Moreover, *Lytechinus variegatus* with body diameter of 14 mm fed with feed protein content of 9, 15, 21, and 33% for 14 weeks showed the maximum growth and survival rate of sea urchin given feed with protein content of $\geq 21\%$ (Hammer *et al.* 2004). Based on those results, it can be explained that each sea urchin needs a different protein level to grow and produce gonad. The need of protein level is also influenced by the age and size of sea urchin. In general, sea urchin needs protein around 20-40% in its feed (Schlosser *et al.* 2005).

This study also showed the presence of a positive relationship between gonad weight and gonad protein indicated by regression linear namely; gonad protein = $47.25 + 7.3$ of gonad weight with $R^2 = 54.7\%$. It means the higher gonad weight the higher gonad protein level and in turn. Compared to the research done by Tjendanawangi *et al.* (2009), time need to reach maturation and spawn in this study was 2-4 weeks faster. It showed that the effective feed protein would fasten the growth and maturation sea urchin gonad. It was caused by vitelogenesis process in which there was occurred vitelogenin accumulation being the main component from protein to nutritive phagocyte cell causing the increasing of gonad weight and the development and gonad maturation process being faster (Unuma, 1999). Beside protein, the faster growth and gonad were also suspected due to the function of carotenoid content in feed (0.155-0.464 ppm). According to Regunathan and Wesley (2006), carotenoid has the ability to increase shrimp vitelogenesis and directly gave the effect on hormone gene transcription which has an important function in ovary maturation. During vitelogenesis, carotenoids moved from hepatopancreas to vary through haemolymph in which the carotenoid is accumulated in oocyte as the main component of egg yolk protein (lipovitellin) (Torinsen dan Torinsen 1985; Lubzens *et al.* 2003; Regunathan dan Wesley 2006).

The best quality of gonad colour is if gonad has a colour of light yellow or orange and red. Whilst, the good quality of that if it has a colour of pale yellow or orange and the bad quality was shown by gonad with pale colour, cream or brown. Yellow and orange sea urchin gonad were caused by the pigment of carotenoid such as β -carotene and echinenone in which both pigments are the main pigments in sea urchin gonad. Carotenoid total and β -carotene of sea urchin gonad were ranged from 15 to 18 and 5 to 6 ppm, respectively.

The evaluation of gonad colour, carotenoid total and gonad β -carotene tended to decrease at the treatment of C, F and I with feed protein energy ratio increasing of 13 kcal GE/g. It was indicated that the accumulation of gonad carotenoid is influenced by protein energy ratio of feed. The increasing of feed protein energy ratio was suspected to be the causative of carotenoid taking or absorption decreasing binding with protein to gonad. It was caused by the

high carbohydrate or feed fat as the main source of feed. It was also due to a part of intestine absorbed carotenoid converted to be retinol. Retinol was transported in circulatory system and in the plasma it was bound with retinol-binding protein (RBP) synthesized in liver and entered to oocyte. Retinol in retinal form was bound with vitellogenin (VTG) then transported to oocyte via plasma during vitellogenesis (Lubzens *et al.* 2003; Sammar *et al.* 2005). In sea urchin gonad, a big part of carotene are also converted to echinenon through isocriptoxantin (Plank *et al.* 2002; Robinson *et al.* 2002; Shpiguel *et al.* 2005).

4. Conclusion

Protein level and feed protein energy ratio influenced on gonad quality of sea urchin *T. gratilla*. The giving of artificial feed with protein level of 32% and protein energy ratio of 9 kcalGE/g protein resulted in the best production and gonad quality.

Acknowledgement

The authors thanked DIKTI, Indonesia for funding research and Nutrition and Fish Health Laboratory staffs of FPIK, IPB. The authors also thanked to *Balai Besar Penelitian and Pengembangan Pascapanen Pertanian* Bogor staffs.

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