天然と飼育したムラサキウニ生殖巣の遊離アミノ酸組成

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Free Amino Acid Compositions of the Gonad of the Wild and Cultured Sea Urchins *Anthocidaris crassispina*

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Abstract: Sea urchins *Anthocidaris crassispina* caught in subtidal barrens were fed *Ulva pertusa*, wakame, mixture of *Sargassum piluliferum* and *Sargassum patens*, or *Trachurus japonicus* for two months, and the gonadsomatic index and free amino acid composition of the gonads were compared to those of wild sea urchins. The gonadsomatic indexes of cultured sea urchins fed wakame or *U. pertusa* were similar to those of wild sea urchins. The free amino acid composition of sea urchins fed *U. pertusa* was similar to that of wild sea urchins. These findings suggest the potential effectiveness of an aquaculture system utilizing these algae.

Key words: *Anthocidaris crassispina*; Diet; Free amino acids; Aquaculture system

The commercial value of sea urchins of the species *Anthocidaris crassispina* inhabiting the subtidal barrens is poor because the gonadal volume is low. The development of aquaculture systems for these sea urchins could increase their commercial value. In an effort to establish the aquaculture system, we previously examined the dietary effects of algae available in the coastal area of Nagasaki on the gonadal growth and free amino acid composition of the sea urchin (Osako et al. 2006). Our study showed that feeding with wakame (*Undaria pinnatifida*) for only two months increased the gonadsomatic index (GSI) more effectively than that with *Ulva pertusa*, amanori *Porphyra* spp., or fish meat (horse mackerel; *Trachurus japonicus*) for the same period. Also, levels of bitter-tasting free amino acids were lower in the gonads of sea urchins fed *U. pertusa* than in the gonads of sea urchins fed other diets. The results were similar to previous findings (Hoshikawa et al. 1998; Nabata et al. 1999).

As described above, several researchers have studied the use of algae or fish meat as a diet for sea urchins in an aquaculture system. However, there are no reports comparing the gonad free amino acid composition of cultured sea urchins with that of wild sea urchins. Therefore, we investigated the differences between cultured and wild sea urchins, paying special attention to the free amino acid composition of the gonad, which is one of the most important taste components.

Materials and Methods

The sea urchins used for the rearing experiment were collected at a subtidal barren (depth, 0－3 m) in Nagasaki City, Japan on April 20, 2002. There were no recognizable algae in the subtidal barren. The wild samples used in this study were sea urchins collected in a *Sargassum* area and an *Ecklonia* area near 32°34'N, 129°45'E, and in a *Sargassum* area near 32°34'N,

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129°46'E (depth, 0－5 m) between June 22 and 23, 2002 (hereafter, they are referred to as samples caught at areas 1, 2, and 3, respectively). The seaweed in both *Sargassum* areas was mainly *Sargassum macrocarpum* and *Sargassum piluliferum*. The principal seaweed in the *Ecklonia* area was *Ecklonia kurome*. *U. pertusa*, boiled and salted wakame, whole horse mackerel meat, and mixture of *S. piluliferum* and *Sargassum patens* (hereafter, referred to as *Sargassum* spp.) were chosen as diets for the sea urchins, since they are easily obtained in the Nagasaki district. *U. pertusa* and *Sargassum* spp. were collected in the coastal area (32°48'N, 129°46'E). Wakame, cultured in coastal water (32°37'N, 130°15'E), was purchased from local seafood merchants. Horse mackerel was processed as reported previously (Osako et al. 2006). The cultured sea urchins were fed each diet in a square pipe during the period from 24th April to 10th June, 2002; significant gonadal development has been reported during these months of the year (Yamasaki and Kiyomoto 1993). Fifty sea urchins were fed in a polyvinyl chloride square pipe (100 cm on a side; 30 cm in height) with nylon net (0.5 × 0.5 mm) on the bottom. The pipe was put on a container filled with seawater and showered with filtered seawater continuously at the rate of 500 *ml*/min. One feeding pipe was used for each diet. About 200 g of diet was fed at each pipe every 3 days, and leftover diet was then removed. The temperature of the seawater was not controlled; it was between 14 and 22℃ during the experimental periods. In each diet group, 7 to 10 sea urchins were randomly selected, their body wet weights and gonad wet weights were measured, and GSI (gonad weight × 100/body weight) was calculated. Trichloroacetic acid extracts from each sea urchin gonad were prepared by the procedure of Konosu et al (1974), and the free amino acid composition was analyzed using an automatic amino acid analyzer (ALC 1000; Shimadzu, Kyoto, Japan). Averages were compared based on one-way ANOVA followed by Scheffé's F *post hoc* test (Statview, SAS Institute, USA). Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

The body weights of reared and wild sea urchins were 46.0 to 53.7 g and 47.3 to 59.6 g, respectively. The GSI values of sea urchins fed wakame (9.8%) were higher than those of sea urchins fed other diets (5.7 to 9.5%), although the values did not differ statistically from those of sea urchins fed *U. pertusa* (9.5%). In the present study, there were no significant differences in GSI between wakame-fed and *U. pertusa*-fed sea urchins. However, in the other study, the values for sea urchins fed wakame were markedly higher than those for sea urchins fed *U. pertusa* (Osako et al. 2006). Further investigations are needed to compare the effects of *U. pertusa* and wakame on GSI values. The GSI values of wild sea urchins were 5.8 to 9.9%, and the values of those caught at area 2 were the highest (9.9%). The value for wild sea urchins caught at area 2 did not differ significantly from those for cultured sea urchins fed wakame or *U. pertusa*. Thus, cultivation for only 2 months using wakame or *U. pertusa* makes it possible to add value to the lean sea urchins inhabiting subtidal barrens. The cultured sea urchins were originally collected at subtidal barrens, where their GSI values had been 5.1 ± 1.4% (Osako et al. 2006). The free amino acid compositions of the gonads of the sea urchins are shown in Table 1. The levels of the *umami* amino acids, aspartic acid and glutamic acid did not differ substantially between cultured and wild sea urchins. Levels of sweet-tasting amino acids other than glycine, such as threonine, serine, proline and alanine also did not differ between cultured and wild sea urchins. Moreover, the levels of *umami*-tasting and sweet-tasting amino acids did not differ with diet or sampling area of wild sea urchins. On the other hand, the glycine content of cultured sea urchins (1159－1387 mg/100 g) was markedly higher than that of wild sea urchins (813－999 mg/100 g). In general, the levels of bitter-tasting amino acids - valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine and arginine in

Table 1. Free amino acid composition of gonad of *Anthocidaris crassispina* (mg ／ 100 g) [*1,2]

	Cultured				Wild		
	Diet				Sampling area		
	Ulva pertusa (*n* = 10) [*3]	Wakame (*n* = 8)	*Sagassum* spp. [*4] (*n* = 7)	Fish meat (*n* = 7)	Area 1 [*5] (*n* = 10)	Area 2 [*6] (*n* = 5)	Area 3 [*7] (*n* = 9)
B. W. (g) [*8]	53.7 ± 6.9	46.6 ± 2.6	51.5 ± 8.0	46.0 ± 3.7	47.3 ± 5.8	59.6 ± 12.6	57.2 ± 10.2
GSI (%) [*9]	9.5 ± 2.4^{a,b}	9.8 ± 2.1^{a}	7.5 ± 0.8^{b,c}	5.7 ± 0.9^{c}	5.8 ± 2.2^{c}	9.9 ± 3.4^{a,b}	5.8 ± 2.4^{c}
Taurine	43 ± 26^{a}	31 ± 13^{a}	46 ± 9^{a}	40 ± 29^{a}	49 ± 15^{a}	26 ± 9^{a}	42 ± 6^{a}
Aspartic acid	11 ± 7^{c}	10 ± 5^{c}	29 ± 9^{a}	11 ± 4^{c}	21 ± 11^{a,b}	16 ± 5^{b,c}	14 ± 14^{b,c}
Threonine	108 ± 52^{a,b,c}	115 ± 38^{a,b}	110 ± 53^{a,b,c}	133 ± 56^{a}	67 ± 38^{c,d}	73 ± 32^{b,d}	49 ± 26^{d}
Serine	63 ± 13^{a}	51 ± 7^{a,b}	53 ± 26^{a,b}	25 ± 13^{d}	34 ± 11^{c,d}	48 ± 18^{a,b,c}	47 ± 16^{b,c}
Glutamic acid	98 ± 45^{b}	117 ± 43^{a,b}	148 ± 32^{a}	114 ± 54^{a,b}	143 ± 34^{a}	112 ± 29^{a,b}	110 ± 29^{a,b}
Proline	46 ± 36^{a}	55 ± 39^{a}	48 ± 23^{a}	37 ± 22^{a}	62 ± 29^{a}	39 ± 28^{a}	45 ± 39^{a}
Glycine	1387 ± 127^{a}	1409 ± 204^{a}	1214 ± 289^{a,b}	1159 ± 158^{a,b}	937 ± 375^{b,c}	999 ± 244^{b,c}	813 ± 333^{c}
Alanine	231 ± 92^{a}	261 ± 100^{a}	308 ± 104^{a}	225 ± 63^{a}	348 ± 214^{a}	283 ± 129^{a}	275 ± 210^{a}
Cystine	3 ± 4^{b}	15 ± 3^{a}	9 ± 6^{a,b}	18 ± 7^{a}	9 ± 13^{a,b}	10 ± 6^{a,b}	11 ± 15^{a,b}
Valine	30 ± 23^{c}	60 ± 38^{b}	40 ± 23^{b,c}	111 ± 59^{a}	22 ± 5^{c}	19 ± 4^{c}	15 ± 7^{c}
Methionine	3 ± 4^{c}	5 ± 4^{b,c}	8 ± 7^{a,b}	11 ± 10^{a}	2 ± 2^{c}	6 ± 1^{b,c}	2 ± 2^{c}
Isoleucine	9 ± 7^{c}	28 ± 25^{b}	20 ± 17^{b,c}	64 ± 36^{a}	7 ± 4^{c}	7 ± 2^{b,c}	7 ± 2^{c}
Leucine	14 ± 10^{c}	41 ± 37^{b}	35 ± 31^{b,c}	76 ± 46^{a}	13 ± 7^{c}	16 ± 7^{b,c}	14 ± 3^{c}
Tyrosine	21 ± 23^{c}	56 ± 54^{a,b}	34 ± 30^{b,c}	79 ± 60^{a}	6 ± 6^{c}	13 ± 2^{c}	7 ± 4^{c}
Phenylalanine	21 ± 11^{b}	30 ± 11^{a,b}	38 ± 17^{a}	42 ± 21^{a}	19 ± 5^{b}	23 ± 4^{b}	24 ± 3^{b}
Histidine	27 ± 16^{b}	33 ± 26^{b}	34 ± 25^{b}	90 ± 49^{a}	13 ± 6^{b}	15 ± 6^{b}	14 ± 4^{b}
Lysine	152 ± 36^{b}	158 ± 50^{b}	160 ± 32^{b}	247 ± 83^{a}	85 ± 55^{c}	83 ± 28^{c}	86 ± 27^{c}
NH_3	7 ± 2	5 ± 1	10 ± 2	7 ± 2	5 ± 1	7 ± 2	10 ± 5
Arginine	258 ± 63^{a,b}	219 ± 83^{a,b,c}	186 ± 71^{b,c}	288 ± 109^{a}	174 ± 99^{c}	179 ± 47^{b,c}	175 ± 52^{c}
Total	2532 ± 268^{a}	2702 ± 184^{a}	2528 ± 317^{a}	2777 ± 348^{a}	2017 ± 682^{b}	1973 ± 412^{b}	1760 ± 516^{b}

[*1] Data are shown as mean ± standard deviation.
[*2] Different superscripts in GSI and each free amino acid indicate statistical differences ($p < 0.05$).
[*3] number of specimens.
[*4] Sargassum spp. indicates mixture of *S. piluliferum* and *S. patens*.
[*5-7] Areas 1 and 2 indicate Sargassum area and Ecklonia area near 32°34'N, 129°45'E, respectively. Area 3 indicates *Sargassum* area near 32°34'N, 129°46'E.
[*8] "B. W." denotes body weight.
[*9] GSI = gonad weight × 100 ／ body weight.

cultured sea urchins were higher than those in wild sea urchins, except for sea urchins fed *U. pertusa*, in which the amounts of bitter-tasting amino acids, except for arginine, were relatively low. The levels of bitter-tasting amino acids in sea urchins fed *U. pertusa* were generally similar to those in wild sea urchins, except for lysine and arginine. The comparatively lower levels of bitter-tasting amino acids observed in sea urchins fed *U. pertusa* may be caused by lower composition ratios of bitter-tasting amino acids in the algae, as reported previously (Osako et al. 2006). The relatively low levels of bitter amino acids in the wild sea urchins may suggest that they feed on prey that contains relatively low levels of bitter amino acids.

From the above, we conclude that the GSI values of sea urchins inhabiting subtidal barrens could be increased to those of wild sea urchins inhabiting areas dominated by *Sargassum* or *Ecklonia* by feeding with wakame for only two months. Also, our results indicate that feeding with algae for the same period can induce a free amino acid composition in sea urchins that is similar to that of wild sea urchins inhabiting *Sargassum* or *Ecklonia* areas.

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天然と飼育したムラサキウニ生殖巣の遊離アミノ酸組成

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磯焼け帯に棲息するムラサキウニにアナアオサ，ワカメ，マメダワラおよびマアジを用いて2カ月間飼育し，生殖腺指数（GSI）および生殖腺中の遊離アミノ酸組成を，ホンダワラ類やクロメが繁殖している海域の天然ムラサキウニのそれらと比較した。

ワカメあるいはアナアオサを与えることにより，ムラサキウニのGSIは天然のものに最も近づいた。一方，アナアオサで飼育したウニ生殖巣の遊離アミノ酸組成は天然のものに最も近かった。以上の結果より海藻を用いた養殖システムの可能性が示唆された。