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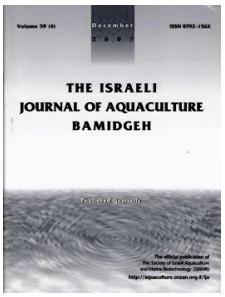

Biochemical Components of Different Colored Strains of Cultured Japanese Scallop (*Mizuhopecten yessoensis*) Under Different Cultivation Systems

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Key words: *Mizuhopecten yessoensis*; total crude protein; total fat; ash; amino acids; fatty acids; mineral elements

Abstract

In this study, the water and total fat content, total crude protein, ash, fatty acids, amino acids and mineral elements of scallop adductor muscle were used to understand the biochemical components of different colored strain of Japanese scallop, *Mizuhopecten yessoensis,* cultured using different cultivation methods. Common scallops had slightly higher moisture, and total protein content, significantly higher total fat content, and significantly lower ash content than ivory white scallops when cultivated under both suspended and bottom culture conditions. For scallops of both colors, suspended culture individuals had slightly higher moisture, total protein content, significantly lower total fat and ash content, compared to bottom culture conditions. Most amino acids were more abundant in scallops from the bottom culture group than in scallops from the suspended culture group. The ivory white scallops contained slightly higher amounts of total amino acids, essential amino acids, and flavor-imparting amino acids, than the common scallops under a given culture method. In the suspended culture group, common scallops had higher content of unsaturated fatty acids, mono-unsaturated fatty acids, polyunsaturated fatty acids, lower contents of eicosapentaenoic acid and docosahexaenoic acid, compared with ivory white scallops. In the bottom culture group, common scallops contained more unsaturated fatty acids, polyunsaturated fatty acids, eicosapentaenoic acid and docosahexaenoic acid compared with ivory white scallops. In common scallops, levels of mineral elements (apart from Fe and Mg) were higher in suspended culture than in bottom culture. Levels of mineral elements in ivory white scallops in suspended culture were higher than or the same as those in bottom culture with the exception of K and Mn. In conclusion, different cultivation methods and shell color of *M. yessoensis* affected biochemical composition, amino acid content, fatty acid content, and mineral element content of the scallops. The research results may provide useful information for selective breeding, culture methods, deep processing, and comprehensive utilization of *M. yessoensis*.

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Introduction

The Japanese scallop *Mizuhopecten yessoensis*, is a cold-water shellfish that lives in 0-23^{0}C water and is distributed widely along the coastline of the northern islands of Japan, the northern part of the Korean Peninsula, and the Sakhalin and Kuril Islands. It was introduced to China from Japan in the 1980s. As *M. yessoensis* is larger in size and commands a higher market price than the native scallop *Chlamys farreri*, and the introduced bay scallop *Argopecten irradians*, *M. yessoensis* aquaculture has expanded rapidly and has become an important economic aquaculture species along the northern coastline of China under two cultivation methods, suspended culture and bottom culture (Chen et al., 2010).

Because the scallop *M. yessoensis* is a filter-feeding bivalve that feeds on particles suspended in the water column, it is possible that different cultivation methods (i.e., suspended culture and bottom culture) affect growth and biochemical components of this species. Different strains of *M. yessoensis* may exhibit different growth and nutritional characteristics, for example, our research team produced an ivory white strain of *M. yessoensis* through long-term selective breeding (Fig.1 B). Its obvious characteristics are that both the left and right side shells are white, whereas in common *M. yessoensis*, the left shell is brown and the right shell is creamy-yellow (Fig.1 A). Compared with common *M. yessoensis*, the ivory white strain shows better performance in suspended culture in terms of growth, survival rate, and resistance to environmental stresses (Chang et al., 2007; Ding et al., 2011).

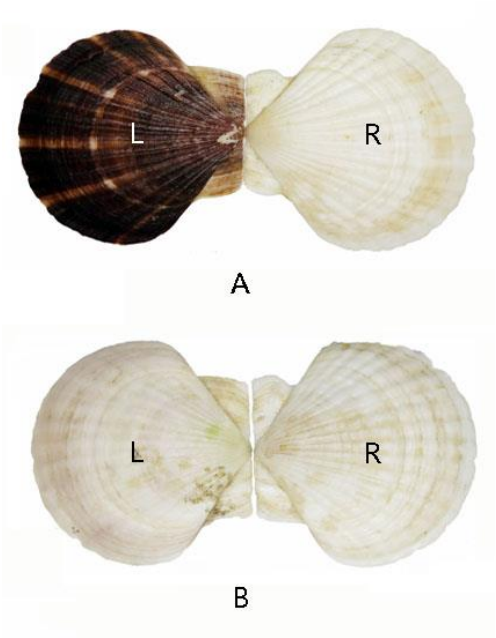

Fig. 1. The characteristics of common scallop and ivory white scallop.
A: common scallop. B: ivory white scallop. L: left shell. R: right shell

Studies of *M. yessoensis* have focused mainly on ecological habits (Silina, 1994; 1996), seed production (Chang et al., 2001; Yu et al., 2006; Li et al., 2010), aquaculture (Zhang et al., 2008; Liu et al., 2013), semen cryopreservation (Yang et al., 2008), induction of polyploidy (Wang et al., 2009), gynogenesis (Yang et al., 2006), genetic diversity (Chang et al., 2007), and population genetic structure (Meng et al., 2010; He et al., 2012). However, the effects of cultivation methods and shell color on the nutrient composition of *M. yessoensis* are poorly understood. The purpose of this study was to evaluate the differences in biochemical content between ivory white and common strains of *M. yessoensis* cultured using different cultivation methods. Our research results may provide some useful information for selective breeding, culture method, deep processing, and comprehensive utilization of *M. yessoensis*.

Materials and Methods

Two-year-old scallops were used in this study. The ivory white strain was the second generation of *M. yessoensis* bred by our research team in March 2009. The ivory white and common scallops were cultured on Zhangzi Island, Liaoning Province China, using two different cultivation methods (suspended culture and bottom culture). In April 2011, we selected 20 ivory white scallops from the suspended culture and 20 from bottom culture (shell length 87.49±6.04mm, shell width 24.43± 2.66 mm, shell height 86.58±6.46mm, wet weight 85.00±10.74 g). We also selected 20 common

scallops from suspended culture and 20 from bottom culture (shell length 84.32±8.73 mm, shell width 22.86±2.06 mm, shell height 84.15±8.07mm, wet weight 84.25±7.60 g). There were no significant differences ($P > 0.05$) between the four groups in shell length, shell width, shell height and wet weight. The adductor muscle of each specimen was collected to measure water content, total fat, total crude protein, ash, fatty acids, amino acids, and trace elements.

The atmospheric pressure drying method (Chaijan et al., 2010) was used to measure the water content. The protein content was determined according to the Kjeldahl method modified by Liu et al. (1999). Total fat content was determined by extraction according to Bligh and Dyer (1959). Ash content was determined according to Jensen (2013). The water-free sample was then combusted at 500^{0}C for 12 h to determine ash content gravimetrically.

To determine the total amino acid profile, adductor muscle proteins were hydrolyzed with 6 N hydrochloric acid (with 0.1% phenol) in a hydrolysis tube which was filled, under vacuum, with nitrogen at 110^{0}C for 22 h. Hydrolysis was performed under inert and anaerobic conditions to prevent the oxidative degradation of amino acids. The reaction mixture was diluted with deionised water to a volume of 50 ml and filtered. The filtered liquid (1 ml) was dried in a vacuum desiccator (40^{0}C to 50^{0}C liquid) and diluted with 2 ml distilled water. After the liquid was dried, the sample was dissolved in 1 ml of buffer solution (pH 2.2). The amino acids were separated by ion exchange liquid chromatography in an automatic analyzer (Biochrom 30, Amersham Biosciences) and identified by comparing their retention times with those of specific standards (Sigma).

Lipids were transmethylated to fatty acid methyl esters (FAMEs) for analysis by gas chromatography according to Metcalfe et al. (1966). An HP5890 (FID detector) and an SPTM-2380 column (30 m × 0.25 mm × 0.20 μm) were used in the experiment. The separation was performed using nitrogen as the carrier gas. The initial column temperature was set at 150^{0}C for 1 min, and then increased to 200^{0}C and 250^{0}C at increasing rates of 15 and 2^{0}C/min, respectively. The injector was maintained at 270^{0}C with an injection volume of 1 μl under a splitless mode. The FID detector temperature was set at 270^{0}C. FAMEs were identified by comparison with the retention times of the authentic standards (Sigma Co., USA). An internal standard (19:0 fatty acid; Sigma Co., USA) was added to quantify the absolute amount of fatty acids.

The content of minerals was digested by nitric acid and perchloric acid and determined using wet nitration and flame atomic absorption spectrophotometry (TAS-986, Beijing's PERSEE General Instrument Co., LTD, China) according to Xin et al. (2007). The muscle was dried at 120^{0}C and weighed 0.25 g was placed in the digestion jar with the mixed acids (HNO3: HClO4= 5:1) at 140^{0}C for 2.5 hours. Deionized water and acid-washed glassware were used in this study.

Differences in biochemical composition between the different shell color and cultivation methods were analyzed by one-way ANOVA on SPSS 13.0. Normality and homogeneity of variances were tested by Kolmogorov-Smirnov and Bartlett tests, respectively. Nonparametric ANOVA equivalent (Kruskal-Wallis test) and post-hoc Games-Howell tests were performed when data did not meet the criteria of ANOVA. The post-hoc Duncan test was used to determine significant differences between groups.

Results

Common scallops had slightly higher moisture, total fat, total protein content ($P > 0.05$), and slightly lower ash content ($P > 0.05$) than ivory white scallops when cultivated under suspended culture conditions (Table 1). Under bottom culture conditions, common scallops also had slightly higher moisture and total protein content, but slightly lower total fat and ash content than ivory white scallops ($P > 0.05$). For scallops of both colors, moisture and total protein content from individuals cultivated via suspended culture were slightly higher than those of scallops cultivated under bottom culture conditions ($P > 0.05$), whereas values for total fat and ash content was significantly lower in scallops from suspended culture compared to bottom culture conditions ($P < 0.01$).

Table1. Biochemical composition of ivory white and common *M. yessoensis* (g/100g fresh)

Essential nutrients	*Suspended culture*		*Bottom culture*	
	common	*'Ivory white'*	*common*	*'Ivory white'*
moisture	78.63±5.31	78.52±5.09	78.33±5.19	78.22±3.86
total protein	18.91±1.50	18.82±2.27	18.49±1.82	18.39±1.86
total fat	0.18±0.06^a	0.13±0.04^a	0.4±0.08^b	0.41±0.04^b
ash	1.25±0.10^a	1.38±0.12^a	1.7±0.05^b	1.88±0.20^b

Values with different letter superscripts in rows are significantly different ($P<0.05$).

Table 2 shows the amino acid composition (which includes almost all types of amino acids) of the scallops. Glutamic acid (Glu) was the most abundant among the amino acids detected, accounting for 16-17% of the total. The levels of glycine (Gly) and aspartic acid (Asp) were also very high, whereas methionine (Met) and tyrosine (Tyr) were lowest. Total amino acids (TAA) were slightly more abundant in scallops cultured under bottom culture than in scallops from suspended culture ($P > 0.05$). With the exception of threonine (Thr), lysine (Lys), alanine (Ala), and Tyr, all amino acids were more abundant in scallops from the bottom culture group than in scallops from the suspended culture group, but the differences were not significant ($P > 0.05$).

Within a given culture method, the ivory white scallops contained slightly higher amounts of total amino acids, essential amino acids, and flavor-imparting amino acids, than the common scallops ($P > 0.05$). In suspended culture, the content of each essential amino acid except Tyr and Met was higher in ivory white scallops than in common specimens ($P > 0.05$). In bottom culture, the content of each essential amino acid except arginine (Arg), Met, and phenylalanine (Phe) was higher in ivory white scallops than in common scallops ($P > 0.05$).

Table2. Amino acid content of ivory white and common *M. yessoensis* (g/l00g fresh)

Amino acid	*Suspended culture*		*Bottom culture*	
	common	*'Ivory white'*	*common*	*'Ivory white'*
Asp[2]	1.21±0.08	1.28±0.13	1.26±0.13	1.31±0.21
Ala[2]	0.72±0.05^{ab}	0.78±0.06^{a}	0.67±0.05^{b}	0.71±0.02^{ab}
Glu[2]	1.93±0.31	2.01±0.24	1.94±0.27	2.03±0.13
Gly[2]	1.47±0.05^{a}	1.51±0.22^{a}	1.77±0.13^{b}	1.8±0.07^{b}
Ser	0.48±0.07	0.49±0.04	0.50±0.06	0.52±0.01
Pro	0.33±0.03	0.35±0.09	0.36±0.04	0.37±0.06
Tyr	0.22±0.04^{a}	0.19±0.03^{ab}	0.17±0.02^{ab}	0.15±0.02^{b}
His[1]	0.27±0.02	0.27±0.01	0.29±0.02	0.27±0.02
Arg[1]	0.59±0.02^{a}	0.64±0.03^{a}	1.12±0.19^{b}	1.05±0.12^{b}
Thr[1]	0.70±0.08^{b}	0.72±0.05^{b}	0.57±0.02^{a}	0.65±0.03^{ab}
Val[1]	0.49±0.07	0.51±0.04	0.51±0.03	0.52±0.05
Met[1]	0.23±0.04	0.22±0.03	0.28±0.04	0.24±0.03
Lys[1]	1.05±0.09	1.10±0.07	1.00±0.13	1.05±0.08
Ile[1]	0.54±0.08	0.56±0.07	0.57±0.09	0.59±0.1
Leu[1]	0.92±0.07	0.95±0.15	0.95±0.15	0.96±0.14
Phe[1]	0.42±0.03	0.43±0.01	0.48±0.06	0.47±0.07
TAA	11.57±0.28^{a}	12.02±0.49^{ab}	12.45±0.82^{ab}	12.68±0.27^{b}
EAA	5.20±0.19^{a}	5.42±0.25^{ab}	5.77±0.49^{ab}	5.8±0.24^{b}
NEAA	6.36±0.29^{a}	6.6±0.27^{ab}	6.68±0.33^{ab}	6.88±0.12^{b}
FAA	5.32±0.33^{a}	5.58±0.27^{ab}	5.64±0.3^{ab}	5.84±0.04^{b}
E/T(%)	0.45±0.02	0.45±0.01	0.46±0.01	0.46±0.01
E/N(%)	0.82±0.06	0.82±0.03	0.86±0.03	0.84±0.04
D/T(%)	0.46±0.03	0.46±0.01	0.45±0.01	0.46±0.01

Note: EAA:essential amino acids, NEAA: nonessential amino acids, FAA: flavor-imparting amino acids, TAA: total amino acids, E / N: essential amino acids / nonessential amino acids, E / T: essential amino acids / total amino acids, D / T: delicious amino acids / total amino acids, 1: EAA, 2: DAA.
Values with different letter superscripts in rows are significantly different ($P<0.05$).

Cultivation method and shell color had some effect on the fatty acid composition of scallops (Table 3). In the suspended culture group, common scallops had higher contents of unsaturated fatty acids (UFA), mono-unsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), and lower contents of eicosapentaenoic acid and docosahexaenoic acid (EPA+DHA) compared with ivory white scallops, but the difference was not significant ($P > 0.05$). In the bottom culture group, common scallops contained slightly more UFA, PUFA, and EPA+DHA compared with ivory white scallops, whereas the MUFA content was slightly lower than that in ivory white scallops ($P > 0.05$).

Table3. Fatty acid content of ivory white and common *M. yessoensis* (g/l00g dry)

Fat acid	*Suspended culture*		*Bottom culture*	
	common	*'Ivory white'*	*common*	*'Ivory white'*
14:00	1.299±0.103^{a}	1.475±0.119^{ab}	1.635±0.119^{b}	1.566±0.14^{b}
15:00		0.621±0.046	0.703±0.100	0.686±0.088
16:00	14.900±2.224	16.367±3.087	16.455±3.065	16.262±3.541
17:00	0.783±0.168	0.806±0.061	0.763±0.166	0.800±0.048
18:00	7.456±0.590	7.157±1.376	7.875±1.226	8.148±1.194
16:01	1.729±0.181^{bc}	1.329±0.238^{ab}	1.091±0.152^{a}	2.037±0.262^{c}
17:01	2.727±0.368	2.535±0.232		
18:01			5.477±0.785	6.056±1.539
18:1n-7		2.185±0.175		
18:1n-9	5.018±0.938	2.519±0.354		
20:01	3.718±0.207	3.439±0.759	3.245±0.299	3.011±0.227
16:02	0.978±0.067^{a}	0.910±0.013^{b}		
16:03	0.602±0.038			
18:2n-6	0.793±0.037^{a}	0.724±0.070^{a}	1.159±0.068^{b}	1.625±0.205^{c}
18:4n-3	2.481±0.299^{a}	2.516±0.254^{a}	0.939±0.175^{b}	0.919±0.143^{b}
20:02			1.685±0.248	1.585±0.127
20:2n-6	0.632±0.089	0.692±0.045		
20:4n-6	3.765±0.372^{a}	3.651±0.304^{a}	2.698±0.399^{b}	2.771±0.236^{b}
20:5n-3(EPA)	18.961±2.957	20.628±2.958	22.619±3.385	21.264±2.356
21:5n-3	0.602±0.038			
22:3n-6			0.783±0.044	0.751±0.083
22:5n-6	1.009±0.037^{b}	1.043±0.052^{b}	0.742±0.110^{a}	
22:5n-3	0.739±0.054	0.715±0.035	0.731±0.115	0.71±0.031
22:6n-3(DHA)	31.808±3.201	30.687±2.418	31.399±2.442	31.828±2.542
UFA(%)	75.56±2.271	73.575±1.940	72.569±7.074	72.557±1.654
MUFA(%)	13.192±1.688^{a}	12.007±1.738^{ab}	9.813±1.046^{b}	11.104±1.141^{ab}
PUFA(%)	62.369±0.838	61.568±0.551	62.756±6.139	61.452±2.320
EPA+DHA(%)	50.769±1.149	51.315±0.642	54.019±5.790	53.092±2.302

Note: EPA: eicosapentaenoic acid, DHA: docosahexaenoic acid, UFA: unsaturated fatty acids, MUFA: mono-unsaturated fatty acids, PUFA: polyunsaturated fatty acids, EPA+DHA: eicosapentaenoic acid and docosahexaenoic acid.
Values with different letter superscripts in rows are significantly different (P<0.05).

For common scallops, the content of UFA and MUFA in the suspended culture group was slightly higher than that in the bottom culture group, but the content of PUFA and EPA+DHA was slightly lower than that in the bottom culture group (P > 0.05). For ivory white scallops, UFA, MUFA, and PUFAs were more abundant in the suspended culture group than in the bottom culture group, and the content of EPA+DHA was lower than that in the bottom culture group, but the difference was not significant (P > 0.05).

Mineral elements present in ivory white and common *M. yessoensis* under different culture conditions are listed in table 4. For common scallops, the content of the mineral elements (except Fe and Mg) was higher in suspended culture than in bottom culture. The content of the mineral elements in ivory white scallops in suspended culture was higher than, or the same as, that in bottom culture, with the exception of K and Mn. In the suspended culture group, the mineral elements were more abundant in common scallops than in ivory white scallops. Except for K, the same was true for the bottom culture group.

Table 4. Mineral elements content of ivory white and common *M. yessoensis* (mg/kg)

Mineral elements	*Suspended culture*		*Bottom culture*	
	common	*'Ivory white'*	*common*	*'Ivory white'*
Ca	379.00±24.98^{a}	175.33±19.4^{b}	114.00±11.36^{c}	91.00±10.15^{c}
K	5,200.00±323.87^{a}	4,700.33±244.08^{b}	4,600.33±224.44^{bc}	4,900.33±170.19^{abc}
Se	0.29±0.02	0.26±0.05	0.27±0.03	0.23±0.03
Zn	16.03±1.53	14.00±1.67	15.00±2.12	14.00±1.05
Fe	4.10±0.53^{a}	2.20±0.17^{b}	13.00±1.92^{c}	2.02±0.44b
Mg	567.00±58.95	551.33±28.01	572.00±44.4	467.33±35.3^{a}
Mn	0.88±0.03^{c}	0.39±0.05^{a}	0.59±0.08^{b}	0.48±0.08^{ab}

Values with different letter superscripts in rows are significantly different (P<0.05).

Discussion

High protein and low fat content have become symbolic of the ideal food. Results of this study showed that *M. yessoensis* was rich in protein and low in fat content. In addition, scallops cultivated in suspended culture had higher protein content and lower fat content than those in the bottom culture group, which may be because the suspended culture environment had a richer food supply. The gonad index of scallops cultivated in suspended culture was significantly higher than those of scallops in the bottom culture group (Uddina et al., 2007). The carbohydrate content and gonad index of *Crassostrea gigas* cultured in the shallow layer were significantly higher than those of individuals cultivated in the deep layer (Thao et al., 2005). In addition to the food source, we believe that different breeding environments also can affect the biochemical content of scallops. In our study, the water temperature in the bottom culture experiment was lower than that in the suspended culture, and after long-term growth and adaption, the bottom cultured individuals had higher fat content.

Content and composition of amino acids, especially the eight essential amino acids (EAA), determine protein quality. Glu is not only a flavor-imparting amino acid, but it is also important in brain tissue biochemical metabolism, as it participates in the synthesis of a variety of physiologically active substances. Lys can prevent cell degeneration, adjust the function of mammary glands, corpus luteum, and ovaries, increase appetite, promote offspring growth, improve absorption of calcium, and accelerate bone growth (Zhang et al., 2006). Additionally, Lys is a very important amino acid in human milk, thus we speculate that *M. yessoensis* may be a good food to eat during lactation. Many young mammals require Arg for growth, and it also plays many biochemical and therapeutic roles in the human body. Intake of Arg helped reduce the loss of nitrogen and promote wound healing in subjects who had experienced trauma (Barbul et al., 1987). Our results show that *M. yessoensis* is a source of high quality protein, as it contains nearly every type of amino acid and has an essential amino acid (EAA) content of 45-46%, and a flavor-imparting amino acid content of 45-47%.

We found that the total amino acid (TAA), essential amino acid (EAA), and flavor-imparting amino acid (FAA) contents of scallops cultivated in bottom culture were slightly higher than those of scallops cultivated in suspended culture. We speculate that this difference is related to the different culture environments. Bottom cultured scallops had better growth conditions, better water quality, and no limitations caused by the presence of an aquaculture cage. However, further research is needed to address this issue.

Some researchers have reported that UFAs can reduce blood fat content and blood pressure, inhibit platelet aggregation, improve immunity, and significantly reduce the incidence of cardiovascular disease (Bao, 2006; Zhang et al., 2006; Sun et al., 2008). EPA and DHA can enhance memory, prevent Alzheimer's disease and cancer, protect eyesight, reduce deposition of fat in blood vessel walls, and improve the toughness of blood vessels (Chi et al., 2007). Therefore, we suggest that consumption of *M. yessoensis* is helpful for maintaining human health, as we detected the presence of 24 types of fatty acids in this scallop; more specifically, UFA content was 72.5-75.6% of the total, and the values for MUFA, PUFA, and EPA+DHA were 9.8-13.2%, 61.5-62.8%, and 50.8-54%, respectively. In addition, the UFA content of ivory white *M. yessoensis* in the suspended culture group was higher than that of scallops in the bottom culture group.

The opposite trend was found for EPA+DHA content. Differences in food quantity and availability and aquaculture environment may explain this difference. Deep sea scallops had higher 24-methylene cholesterol and DHA content than scallops from shallower environments, which ensures the membrane liquidity needed to survive in the low water temperatures of the deep sea (Napolitano et al., 1992).

Mineral elements play a very important physiological role in the human body. They help maintain the acid-base balance, osmotic pressure, water and electrolyte balance, neuromuscular excitability, and normal heart function of the body. As mineral elements cannot be synthesized by the body, they must be acquired from food (Ye et al., 2004). We found that the mineral element content of common scallops was significantly higher than that in ivory white scallops in both the suspended and bottom culture groups, especially for Ca, Fe, and Mn. We conjecture that the mineral element content is associated with shell color, as the shell color of common scallops was dark, which may result from high Fe and Mn content.

In conclusion, different cultivation methods and shell color of *M. yessoensis* affected biochemical composition, amino acid content, fatty acid content, and mineral element content of the scallops. Our results support work done by both our research team and others on selective breeding to improve the quality of scallops in culture. Our results may provide information on culture methods (bottom versus suspended) which may be useful in aquaculture.

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