www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 18: 913-920 (2018) DOI: 10.4194/1303-2712-v18_7_09

RESEARCH PAPER

Effects of Three Different Dietary Binders on Juvenile Sea Cucumber, *Apostichopus japonicus*

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Received 31 May 2017
Accepted 30 October 2017

Abstract

This study was conducted to evaluate the effects of three different dietary binders on growth performance and water analysis in the culture of juvenile sea cucumber, *Apostichopus japonicus*. The basal diet without binder was used as control (CON), three diets with each of three different binder sources such as guar gum (G_{10}), carrageenan (C_{10}) and xanthan gum (X_{10}) at 10%, and other three diets (G_5C_5, C_5X_5 and X_5G_5) were formulated to contain mixture of two different binders at 5% for each. Juvenile sea cucumber averaging 1.09 ± 0.01 g (mean ± SD) were fed one of the seven experimental diets for 12 weeks. Weight gain and specific growth rate of sea cucumber fed G_{10} and X_5C_5 diets were significantly higher than those of sea cucumber fed the CON diet. Diet cumulative melt away rates for G_{10}, X_{10}, X_5C_5, G_5C_5 and G_5X_5 diets were significantly higher than CON diet. G_{10} diet resulted in a significantly lower chemical oxygen demand comparing to CON diet. Therefore, the present study demonstrated that 10% of dietary guar gum and mixture of 5% xanthan gum and 5% carrageenan could improve growth and water quality in juvenile sea cucumber aquaculture.

Keywords: Apostichopus japonicus, binder, growth performance, water quality.

Introduction

Sea cucumbers with high commercial importance are distributed all around the world, and Japanese sea cucumber, *Apostichopus japonicus,* is one of the most harvested species that appears in Asian markets especially China, Japan and Korea (Sloan, 1985; Yang *et al*., 2005). This species is traditionally used as a food source and has been proven to have antimicrobial, anticoagulant, and anti-inlammatory activities (Nagase *et al*., 1995; Beauregard, Truong, Zhang, Lin, & Beck, 2001; Cho & Bureau, 2001). In addition, cancer prevention, wound healing, aphrodisiac and curative properties are other benefits brought by this invertebrate (Gu *et al*., 2010; Seo & Lee, 2011). Because of these reasons, high world demand for sea cucumber has resulted in severe losses in stocks (Conand, 2004; Uthicke, 2004). In 2016, the South Korean production of sea cucumber was 2,386 metric tons, which valued approximately 26 million dollars (National Statistical Office, 2016). Whereas, feed development of sea cucumber aquaculture for the current state in South Korea has known to be inadequate. Besides, sea cucumber culture depends on imported feeds that mostly come from untrusted sources that have not been tested in South Korean culture environment (Kim *et al*., 2016). According to what was mentioned, it seems necessary for researchers to develop a high quality feed with reasonable price. Binders are feed additives incorporated into aquafeeds in order to increase water-stability, improve firmness and prevent easy breakdown of feed particles (NRC, 2011; Lee *et al*., 2016). Generally, several carbohydrate sources including guar gum, carrageenan and xanthan have been used as binding sources in aquaculture (Storebakken, 1985; Hashim & Saat, 1992; O`mahoney, Mouzakitis, Doyle, & Burnell, 2011). Guar gum is the most common product derived from guar bean (*Cyamopsis tetragonoloba*) that is an annual legume belonging to the Fabaceae family. It has been previously shown that the addition of guar gum to fish feed can result in more stable feces and increasing water quality (Amirkolaie, Leenhouwers, Verreth, & Schrama, 2005; Brinker, 2007). Carrageenan is extracted from red seaweed and has been used widely as a food additive for humans from many years ago (Klose & Glicksman, 1968). Bautista, Millamena & Kanazawa (1989) reported the successful use of a carrageenan microdiet for nutrition of *Penaeus monodon* larvae. This additive also has been previously used as a binder in the microdiets

(Liu *et al*., 2008). Xanthan gum is an extracellular polysaccharide that is resulted from pathogenic bacteria (*Xanthomonas campestris*) of brassicas (Becker, Katzen, Puhler, & Ielpi, 1998). By addition of salts and reduction of temperature a conformation transition occurs in xanthan gum and it turns into a rigid coil (Morris, Rees, Young, Walkinshaw, & Darke, 1977). Xanthan gum has been previously tested as binder in pharmaceutical (Eyo-okon & Hilton, 2003) and aquafeeds (O`Mahoney, Mouzakitis, Doyle, & Burnell, 2011).

In order to reduce nutrient leakage, increase feed efficiency, reduce wastage and improve water stability, proper application of binders seems to be indispensable (Fagbenro & Jauncey, 1995; Hardy, 1989; Huang, 1989). However, current studies on the guar gum, carrageenan, xanthan gum as a binders have been scarce. Therefore, the aim of the present study was to evaluate the effects of different dietary binders such as guar gum, carrageenan, xanthan gum as potential binders on growth performance and water analysis in juvenile sea cucumber *Apostichopus japonicus* culture.

Materials and Methods

Experimental Diets

Formulation and proximate composition of the experimental diets are shown in Table 1 and Table 2, respectively. A basal diet without binder was used as control (CON), three diets with each of three different binder sources such as guar gum (G_{10}), carrageenan (C_{10}) and xanthan gum (X_{10}) at 10%, and other three diets (G_5C_5, C_5X_5 and X_5G_5) were formulated to contain mixture of two different binders at 5% for each. The basal diets were formulated to contain 24% crude protein (Zhu,

Table 1. Composition of the basal diet

Ingredients	
Fish meal[1]	5.00
Pork blood meal[1]	3.10
Dehulled soybean meal[1]	9.00
Squid liver powder[1]	6.00
0 Squid meal[1]	2.65
Corn gluten meal[1]	3.84
Umitorano-powder[2]	3.00
Comb pen shell by-product meal[2]	2.00
Seaweed powder[1]	20.5
Wheat flour	20.5
Fish oil[1]	1.41
Tideland[2]	20.5
Saccharomyces cerevisiae[2]	0.50
Vitamin premix[3]	1.00
Mineral premix[4]	1.00
Moisture (%)	9.05
Crude Protein (%)	24.0
Crude Lipid (%)	5.14
Ash (%)	34.0

[1] Supplied by Suhyup Co., Buan, Korea.
[2] Supplied by Myeongcheon Co., Wando. Korea.
[3] Contains (as mg/kg in diets) : Ascorbic acid, 300; dl-Calcium pantothenate, 150 ; Choline bitatrate, 3000; Inositol, 150; Menadione, 6; Niacin, 150; Pyridoxine . HCl, 15; Riboflavin, 30; Thiamine mononitrate, 15; dl-α-Tocopherol acetate, 201; Retinyl acetate, 6; Biotin, 1.5; Folic acid, 5.4; B_{12}, 0.06.
[4] Contains (as mg/kg in diets): NaCl, 437.4; $MgSO_4 \cdot 7H_2O$. 1379.8; $NaH_2P_4 \cdot 2H_2O$, 877.8; $Ca(H_2PO_4)_2 \cdot 2H_2O$, 1366.7; KH_2PO_4, 2414; $ZnSO_4 \cdot 7H_2O$, 226.4; Fe-Citrate, 299; Ca-lactate, 3004; $MnSO_4$, 0.016; $FeSO_4$, 0.0378; $CuSO_4$, 0.00033; Calcium iodate, 0.0006; MgO, 0.00135; $NaSeO_3$, 0.00025.

Table 2. Proximate composition of seaweed & binders[1]

	Seaweed	Guar gum	Carrageenan	Xanthan gum
Moisture (%)	19.4	10.63	9.94	12.07
Protein (%)	6.7	4.23	0.58	4.45
Lipid (%)	4.1	0.71	0.24	0.33
Ash (%)	25.0	4.73	0.64	5.06

[1] Results reported on the dry matter basis, average of triplicate

Mai, Zhang, Wang, & Xu, 2005) and 5% crude lipid. Fishmeal, blood meal, dehulled soybean meal, squid liver power and corn gluten meal served as the major protein sources in the experimental diets. Fish oil was used as lipid source while umitorano-powder, comb pen shell by-product meal and seaweed powder were the carbohydrate sources. Briefly, after thoroughly mixing the dry ingredients, fish oil and filtered tap water were added to the experimental diets. After drying, the experimental diets were broken up and sieved into 150 µm, and stored at −20 °C in refrigerator until use.

Experimental Fish and Feeding Trial

The feeding trial was carried out at the National Institute of Fisheries Science (NIFS), Pohang, Republic of Korea. Juvenile sea cucumbers were obtained from a commercial hatchery, wando, Republic of Korea, and their health condition was checked until one weeks, upon arrival and one weeks thereafter. Prior to the start of the feeding trial, all sea cucumbers were acclimatized to the experimental status and facilities. At the start of the experiment, 15 juvenile sea cucumbers with an initial average weight of 1.09 ± 0.01 g (mean ± SD) were randomly distributed into each of the 21 plastic rectangular tanks (50 L) supplied with flow-through sea water at 2 L min^{-1}. Each tank was then randomly assigned to one of the three replicates of seven dietary treatments. Sea cucumbers were fed twice daily the experimental diets at the rate of 1~3% wet body weight per day for twelve weeks. Supplemental aeration was provided to maintain the dissolved oxygen (DO) near saturation, and water temperature and pH during the experiment were maintained at 14 ± 0.5 °C using an air conditioner and 8.0 ± 0.5, respectively. A photoperiod of 12h light: 12h dark was used throughout the experimental period.

Sample Collection and Analysis

At the end of the feeding trial, all of sea cucumbers were starved for 24 h, and used weight method (Liao, Ren, He, Jiang, & Han, 2014). The number of sea cucumbers in each tank were checked for the calculation of weight gain (WG), specific growth rate (SGR) and survival. Five sea cucumbers per tank were randomly collected and individually weighed, then dissected to evaluate viscera for the determination of viscerosomatic index (VSI). Five sea cucumbers were randomly selected from each aquarium and frozen at −20 °C for analysis of whole body proximate composition. The proximate composition analyses of the experimental diets and whole-body of fish were performed by the standard methods of AOAC (AOAC, 1995). Samples of diets and fish were dried at 105 °C to determine their moisture contents. The ash content was determined by incineration at 550 °C, crude protein was determined using the Kjeldahl method (N × 6.25) after acid digestion, and crude lipid was measured by ether extraction using the Soxhlet system 1046 (Tacator AB, Hoganas, Sweden) after freeze-drying the samples for 20 h.

Melt Away Rate

A piece of nylon net sheet (mesh size, 1 mm) was made to measure the underwater loss rate. Approximately 2 g of the experimental feed was placed in the nylon net sheet that was submerged in water. To evaluate the loss rate, dry feed was weighted before and after submersion at 0, 1, 2, 4, 8 and 12 hours using an oven.

Water Quality Analysis

About 2 g of feed was put into 1 L seawater to investigate loss rate of feed on time variation (0, 1, 2, 4, 8 and 12 hours) of chemical oxygen demand (COD) measure. COD was determined using a water quality analyzer (HACH, DR/850, USA).

Statistical Analysis

All data were analyzed by one-way ANOVA (Statistics 3.1; Analytical Software, St. Paul, MN, USA) to test for the dietary treatments. When a significant treatment effect was observed, a LSD test was used to compare means. Treatment effects were considered significant at $P<0.05$ level.

Results and Discussion

Several studies have been conducted to develop artificial diets of sea cucumber using different plant materials (Seo, Shin, & Lee, 2011; Wu *et al*., 2015; Yu *et al*., 2015). In the present study, we evaluated the effects of different plant based ingredients as binders in the diet of Japanese sea cucumber. After 12 weeks of feeding trial, the growth performance and survival of sea cucumbers fed different experimental diets are presented in Table 3. WG of sea cucumber fed G_{10} and X_5C_5 diets was significantly higher than those of sea cucumber fed CON diet ($P<0.05$). However, there were no significant differences in WG of sea cucumber fed CON, C_{10}, X_{10} and G_5C_5 diets ($P>0.05$). On the other hand, the sea cucumber fed G_5X_5 diet had the lowest WG ($P<0.05$). SGR indicated the same trend with WG and SGR of sea cucumber fed G_5X_5 diet was significantly lower than those of sea cucumber fed CON, G_{10}, X_{10} and X_5C_5 diets ($P<0.05$). Sea cucumber fed G_{10} and X_5C_5 diets showed the highest SGR, although there was no significant difference among CON, C_{10}, X_{10} and G_5C_5 diets ($P<0.05$). Sea cucumber survival rate varied range from 68.9 to 82% and no significant differences were observed among the dietary treatments. In addition, VSI was not significantly affected by inclusion of different dietary binders. Previous researches demonstrated the effective results of binders such as guar gum, carrageenan, xanthan gum on growth performance of fish (D`Abramo, 2003; Kumar *et al*., 2014; O`mahoney *et al*., 2011; Pearce, Daggett, & Robinson, 2002). Results of our study showed that binders such as guar gum and mixture of carrageenan and xanthan gum were utilized more efficiently than seaweed powder by sea cucumber. Pearce *et al*. (2002) reported that guar gum based diet may be effective in terms of growth comparing with control diet in green sea urchin *Strongylocentrotus droebachiensis*. In addition, Leenhouwers, Adjei-boateng, Verreth, and Schrama (2006) and Brinker and Reiter (2011) demonstrated that guar gum diet did not show adverse effects on growth performance as well as digesta viscosity of fish. Moreover, Slater, Lassudrie, and Jeffs (2011) indicated that best results on digestibility can be achieved by carrageenan-based diet as comparing with other carbohydrate sources in juvenile sea cucumber, *Australostichopus mollis*. In our study, the results for growth performance showed that some binder sources can improve growth performance of juvenile sea cucumber, *A. japonica*.

Whole-body proximate composition of sea cucumber is shown in Table 4. There were no significant differences in whole-body crude protein, crude lipid, moisture and ash content of sea cucumbers fed different experimental diets (P>0.05). Seo *et al*. (2011) also reported that when different plant ingredients are used as carbohydrate sources, whole body proximate composition of sea cucumber is not significantly affected.

Binders play a very important role in the manufacture of aquaculture diets to protect heavy losses from feed disintegration, nutrient leaching, and water contamination (Paolucci, Fabbrocini, Volpe, Varricchio, & Coccia, 2012). Because of these features, many experiments on binders have been carried out for the artificial diets in aquaculture and it was shown that mostly binder-based diets caused improved water stability (Liu *et al*., 2008; Obaldo, Divakaran, & Tacon, 2002; Orire, Sadiku, & Tiamiyu, 2010). Cumulative melt away rate of the experimental diets of sea cucumber for 12 hours is shown in Figure 1. The cumulative melt away rate for G_{10}, X_5C_5, X_{10}, G_5C_5 and G_5X_5 diets showed significantly lower amounts than CON diet during 12 hours (P<0.05). This is while, the cumulative melt away rate of C_{10} diet was not significantly different with CON diet

Table 3. Percent weight gain, specific growth rate and survival for sea cucumber fed the experimental diets (CON, G_{10}, C_{10}, X_{10}, X_5C_5, G_5C_5 and G_5X_5) for 12 weeks[1]

	IW[2] (g)	FW[3] (g)	WG[4] (%)	SGR[5] (%/day)	VSI[6]	Survival (%)
CON	1.11	3.87^{bc}	249^{bc}	1.78^{bc}	12.6	78.9
G_{10}	1.10	4.77^{a}	332^{a}	2.09^{a}	12.6	81.0
C_{10}	1.11	3.44^{bcd}	210^{bcd}	1.62^{bcd}	12.7	72.0
X_{10}	1.09	3.83^{bc}	246^{bc}	1.77^{bc}	12.4	82.0
X_5C_5	1.11	4.51^{a}	311^{a}	2.02^{a}	12.8	74.5
G_5C_5	1.11	3.41^{cd}	207^{cd}	1.60^{cd}	12.5	73.3
G_5X_5	1.12	3.24^{d}	190^{d}	1.52^{d}	13.0	68.9
Pooled SEM[7]	0.00	0.13	11.9	0.05	0.16	2.03

[1] Values are means from triplicate groups of fish where the values in each row with different superscripts are significantly different (P<0.05).
[2] Initial weight (g/fish)
[3] Final weight (g/fish)
[4] Weight gain (%) = (final weight - initial weight) × 100/initial weight
[5] Specific growth rate (%) = 100 × (loge final weight - loge initial weight) / days
[6]VSI: visceral somatic index = visceral weight / body weight × 100
[7] Pooled standard error of means: SD/√n

Table 4. Proximate analysis of whole-body composition for sea cucumber fed the experimental diets (CON, G_{10}, C_{10}, X_{10}, X_5C_5, G_5C_5, G_5X_5) for 12 weeks (% dry matter basis)[1]

	Moisture (%)	Crude protein (%)	Crude lipid (%)	Ash (%)
CON	90.3	42.6	4.3	41.2
G_{10}	91.5	43.0	4.2	40.4
C_{10}	90.2	43.6	4.3	41.4
X_{10}	90.6	42.7	3.7	42.2
X_5C_5	90.6	43.3	4.2	42.2
G_5C_5	90.7	43.4	3.9	42.3
G_5X_5	90.8	43.1	3.6	41.0
Pooled SEM[7]	0.18	0.09	0.30	0.33

[1]Values are means from triplicate groups of fish where the means in each row with a different superscripts are significantly different (P<0.05)
[2]Pooled standard error of means: SD/√n

during 4 hours. However, after 8 hours C_{10} diet showed lower melt away rate than the CON diet. Feed stability in water is considered as retention of physical pellet value with minimal decomposition and nutrient leaching during immersion before utilization. Experimental diets which easily break up into small particles can cause nutrient leakage that could lead to induced water pollution, impaired growth, inefficient feed conversion, and poor survival rate (Obaldo *et al*., 2002). Caltagirone, Francour, and Fernandez (1992) found that 23.9% of guar gum in the feed could improve stability after 48 h immersion in seawater. In addition, Ali, Gropal, Ramana, and Nazer (2005) reported that guar gum and wheat flour-based pellets showed good water stability. Similar studies have been observed in many other fish species (Meyers & Zein-Eldin, 1972; Knauer, Britz, & Hecht, 1993; Brinker, 2007). In agreement with the results of this study, several studies have shown the reduction in water pollution by inclusion of different binders in the diet (Brinker, Koppe, & Rosch, 2005; Cho & Bureau, 2001; Rosas *et al*., 2008).

Variations of water chemical oxygen demand (COD) after for 12 hours is shown in Figure 2. According to the COD results, G_{10} group showed significantly lower value than the CON group during 8 hours ($P<0.05$). X_{10} diet indicated the similar trend with G_{10} diet until 8 hours and X_5C_5 and G_5C_5 diets

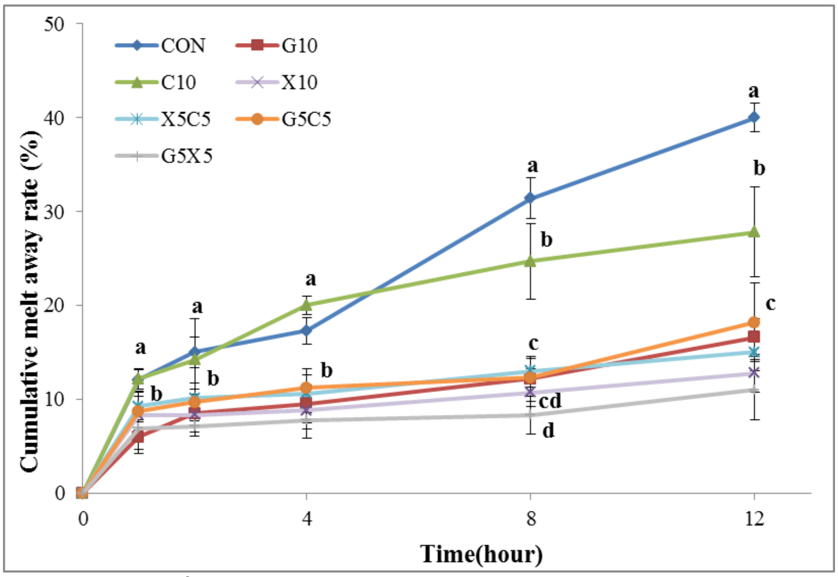

Figure 1. Cumulate melt away rate for experimental diets (CON, G_{10}, C_{10}, X_{10}, X_5C_5, G_5C_5 and G_5X_5) for 12 hrs into the water.

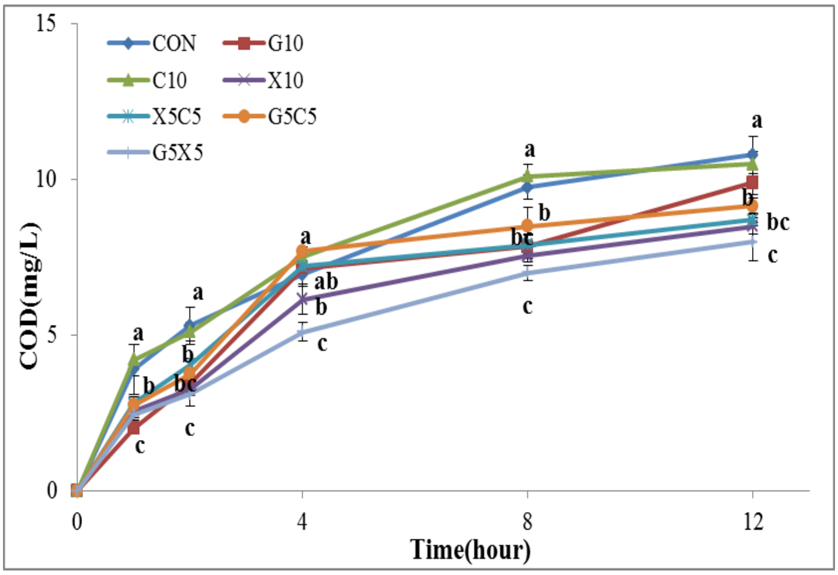

Figure 2. Water COD changes after the addition of diets (CON, G10, C10, X10, X5C5, G5C5 and G5X5) for 12 hrs into the water.

caused no significant differences in COD with CON diet after 1~4 hours. However X_5C_5 diet showed significantly lower value than the CON group after 8 hours (P<0.05). The COD is a value of water quality and defined as the quantity of consumed oxygen in relation to chemically oxidation of inorganic products (Zhao, Jiang, Zhang, Catterall, & John, 2004; Kim *et al*., 2017). However, no studies were found on estimation of the relation between dietary binders in aquafeeds and water COD value.

According to what was mentioned, binder based experimental diets gave better results in regard to growth and water quality compared with CON diet.

Therefore, the present study demonstrated that dietary guar gum for 10% and 5% xanthan gum + 5% carrageenan of diet could improve growth and water quality in juvenile sea cucumber culture. Future research could be dedicated to estimate optimum levels of guar gum in diet of Juvenile Sea cucumber *Apostichopus japonicus*.

Acknowledgments

This research has been financially supported by National Institute of Fisheries Science (20110117217-00) and Feeds and Foods Nutrition Research Center (FFNRC), Pukyong National University, Busan, Rep. of Korea.

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