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Development of effectively formulated diets for juvenile abalone (*Haliotis* spp.)

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요 약

국내 전복 총양식생산양은 2000년에는 20톤이었으나 2018년에는 20,053톤으로 증가하였으며, 소비자들의 높은 수요로 인하여 이러한 경향은 계속될 것으로 예상된다. 이러한 전복의 중요성 때문에 전복용 배합사료 개발을 위한 다양한 연구가 수행된 바 있다. 국내 전복양식의 경우 전복의 사육 및 수질 관리의 용이성으로 인하여 미역이나 다시마와 같은 자연산 해조류를 먹이로 공급하는 것을 선호하고 있다. 그러나 이들 해조류는 주요 영양소(단백질, 지질 등) 함량이 전복의 성장에 필요로 하는 영양소 요구량에 비하여 낮기 때문에, 전복의 성장 둔화를 초래한다. 따라서 전복의 안정적인 양식생산을 위해서는 배합사료의 사용이 필수적이며, 경제적이고 효율적인 배합사료의 개발을 위해서는 단백질, 지질과 탄수화물 등의 영양소 균형이 중요하다. 일반적으로 전복은 어류나 갑각류와는 달리 에너지원으로서 탄수화물을 잘 이용하는 것으로 알려져 있으나, 전복용 배합사료내 탄수화물원의 종류나 탄수화물에 대한 지질의 비율 등에 대한 연구는 여전히 미비한

실정이다. 뿐만 아니라 전복용 배합사료내 다량으로 함유되는 어분과 해조류의 가격 상승이 지속되고 있기 때문에 경제적이고 효율적인 배합사료 개발을 위해서는 이들 어분과 해조류를 대체할 수 있는 새로운 사료원의 개발이 필수적이다. 따라서 본 연구에서는 경제적이고 효율적인 전복용 배합사료 개발을 위하여 배합사료내 탄수화물원의 종류와 탄수화물과 지질의 적정 비율에 따른 전복의 성장과 체조성에 미치는 효과를 평가하고, 상업용 배합사료내 어분과 해조류를 각각 발효대두박과 생미강으로 대체시 전복의 성장에 미치는 영향을 조사하였다.

1. 배합사료내 탄수화물과 지질 비율에 따른 까막전복 치패의 성장과 체조성에 미치는 효과

본 연구는 배합사료내 탄수화물(carbohydrate, C)과 지질(lipid, L) 비율에 따른 까막전복의 성장과 체조성에 미치는 효과를 조사하였다. 총 1,260 마리의 까막전복 치패를 18 개의 유수식 수조에 각각 70 마리씩 수용하였다. 총 6 종류의 탄수화물과 지질 비율이 다른 실험사료[49:1(C49:L1), 48:2(C48:L2), 47:3(C47:L3), 45:5(C45:L5), 43:7(C43:L7), 41:9(C41:L9)]를 준비하였으며, 모든 실험구는 3 반복구를 두었다. 실험사료는 16 주간 매일 1 일 1 회 충분히 공급하여 주었다. 실험사료의 수중안정성 평가는 실험사료를 해수에 담근 후 12 시간, 24 시간과 48 시간 경과시 측정하였다. 수중안정성 평가 결과, 사료내 조단백질, 조지질과 회분함량이 시간경과에 따라 유의적으로 감소하였다. 16 주간의 사육실험 종료시 전복의 생존율은 C48:L2 사료 공급구가

C49:L1 과 C41:L9 사료 공급구보다 높게 나타났으며, 체중 증가와 일일성장률은 C49:L1, C48:L2 와 C47:L3 사료 공급구가 C45:L5, C43:L7 와 C41:L9 사료 공급구보다 높게 나타났다. 16 주간의 사육실험 종료시 전복 가식부의 조지질 함량은 실험사료내 조지질 함량을 잘 반영하였다. 이상의 결과를 고려할 때, 전복용 배합사료내 탄수화물과 지질의 적정 비율은 48:2 또는 47:3 으로 판단되며, 까막전복용 배합사료내 3% 이상의 지질 함량은 전복의 성장을 저해하는 것으로 판단된다.

2. 배합사료내 탄수화물원에 따른 까막전복 치패의 성장 및 체조성에 미치는 영향

본 연구는 배합사료내 탄수화물원 종류에 따른 까막전복의 성장 및 체조성에 미치는 영향을 조사하였다. 총 1,680마리의 까막전복 치패를 24개의 유수식 수조에 각각 70마리씩 수용하였다. 총 7종류의 다른 탄수화물원[dextrin (DT), glucose (GC), corn starch (CS), α-cellulose (CL), maltose (MT), sucrose (SC), wheat flour (WF)]으로 구성된 실험사료를 준비하였으며, 배합사료의 효과를 검증하기 위하여 자연산먹이인 미역 공급구를 두었으며, 모든 실험구는 3반복구를 두었다. 16주간 매일 1일 1회 충분한 양의 사료를 공급하여 주었다. 16주간의 사육실험 종료시 전복의 생존율은 실험사료에 따른 효과가 나타나지 않았지만, 체중 증가와 일일성장률은 CL 사료 공급구가 다른 모든 사료 공급구보다 유의적으로 높게 나타났다. 전복의 각장, 각고와 가식부 무게는 CL 사료 공급구가 가장 높게 나타났으며, WF, DT, SC, CS, GC, MT 및 미역 공급구 순으로 나타났다. 모든 배합사료를 공급한 전복의 체중 증가와

일일성장률, 가식부의 조단백질 및 조지질 함량은 미역 공급구보다 유의적으로 높게 나타났다. 이상의 결과를 고려할 때, CL 은 전복의 성장을 향상시키는 가장 우수한 탄수화물원으로 판단된다.

3. 상업용 배합사료내 어분과 해조류를 대두박과 생미강으로 대체시 참전복 치패의 성장에 미치는 효과

본 연구는 상업용 배합사료내 어분(fish meal, FM)과 해조류(macroalgae, MA)를 각각 발효대두박과 생미강으로 대체시 참전복의 성장에 미치는 영향을 조사하였다. 1개의 6톤 raceway를 3구역으로 균등하게 나누어 사옹하였으며, 총 4개의 raceway를 사용하였다. 총 21,600마리의 참전복 치패를 무작위로 선별하여 12 개의 구역에 수용하였다(1개 구역당 1,800마리 수용). 총 4종류의 실험사료를 준비하였으며(Std, FM50, FM50＋MA50, FM50＋MA100), 대조구(Std)사료는 어분 14%, 발효대두박 25%, 콘글루텐밀 3.4%, 새우분 3%, 소맥분 20%, 덱스트린 5%와 해조류 25%를 함유하여서 시판용 전복사료와 동일하게 제조하였다. FM50사료는 어분 50%를 발효대두박으로 대체하였고, FM50+MA50사료는 어분과 해조류를 각각 50%씩 발효대두박과 생미강으로 대체하였으며, FM50+MA100사료는 어분 50%와 해조류 100%를 발효대두박과 생미강으로 각각 대체하였다. 또한 이들 배합사료의 효과를 평가하기 위하여 자연산 먹이인 미역과 다시마 공급구를 두었으며, 모든 실험구는 2반복구를 두었다. 실험사료는 1일 1회 매일 충분하게 공급하였으며, 사육기간은 총 16주간이었다. 사육실험 종료시 생존한 전복 50마리를 각 수조에서 무작위로 추출하여 성장 효과를 비교하였다. 모든 배합사료를

공급한 전복의 생존율은 자연산 먹이(미역과 다시마)를 공급한 전복의 생존율보다 유의적으로 높게 나타났다. Std사료를 공급한 전복의 체중 증가와 일일성장률은 다른 모든 실험구보다 유의적으로 높게 나타났다. 이상의 결과를 고려할 때 전복양식현장에서 FM을 발효대두박으로 대체시 그 효과는 제한적일 것으로 판단되지만, 상업용 배합사료내 해조류 50%와 100%를 생미강으로 각각 대체한 결과를 고려하면 해조류 50% 대체시 나머지 50% 해조류는 생미강으로 대체 가능한 것으로 판단된다.

Chapter 1.

General Introduction

Abalone (*Haliotis* spp.) is the most commercially important marine aquaculture species globally, particularly in East Asian countries, such as Korea, Japan, and China. In 2000, annual aquaculture production of abalone in Korea and globally were 20 and 480 metric tons, respectively, but reached 16,027 and 168,333 metric tons, respectively, in 2017 (FAO, 2019). This upward trend is expected to continue, with increasing human consumption of abalone and expansion of global abalone cultures. Therefore, many feeding trials have been reported to investigate the dietary nutrient requirements of abalone (Uki et al., 1985a; Uki et al., 1986a, 1986b; Mai et al., 1995a, 1995b; Lee et al., 1998a; Zhang et al., 2009) and alternative sources for fish meal (FM) and macroalgae (MA), which are the most expensive components in abalone diet (Lee et al., 1999; Cho, 2010; Kim et al., 2016; Myung et al., 2016; Jang et al., 2018; Choi et al., 2018; Lee et al., 2018; Ansary et al., 2019) have been considered. Additives have also been developed to improve growth and/or immune responses of abalone (Xue et al., 2008; Lange et al., 2014; Duong et al., 2016; Zhao et al., 2018; Currie et al., 2019) and color changes of abalone shells (Ju et al., 2016; Hoang et al., 2019). The optimum dietary protein and lipid requirements of abalone are reportedly to be 25–35% and 3–7%, respectively (Mai et al., 1995a, 1995b; Fleming et al., 1996; Bautista-Teruel et al.,

2003).

Abalone farmers are likely to feed abalone on MA, such as *Undaria pinnatifida* Harvey or *Saccharina japonica* Areschoug in Korea, which are grown and harvested in the wild during the winter season. In addition, nutrient contents of these MA, such as protein (amino acids) and lipid (fatty acids) in these MA do not satisfy the requirements of abalone (Uki et al., 1986a; Mai et al., 1995a, 1995b). Consequently, feeding abalone on MA may result in a poor growth rate and an increased production cost compared with those in abalone receiving nutrition-balanced diets (Kim et al., 1998; Lee, 1998; Cho et al., 2006; Naidoo et al., 2006; Cho et al., 2008; Gracia-Esquivel and Felbeck, 2009; Dang et al., 2011; Kim et al., 2016; Myung et al., 2016).

Since abalone are marine gastropods and slow eaters, water stability of the feed is another factor to affect performance of abalone. Their natural diet comprises 40–50% carbohydrates, and various abalone enzymes can hydrolyze complex carbohydrates (Fleming et al., 1996). Fish and crustaceans do not utilize carbohydrates as efficiently as abalone, but both carbohydrate and lipid are important nonprotein energy sources for fish and are incorporated into fish diets to maximize the use of dietary protein for growth, as indicated by the “protein-sparing effect” (Dias et al., 1998; Helland and Grisdale-Helland, 1998; Stone et al., 2003). Abalone have been reported to utilize carbohydrate better than lipid as an energy source (Britz et al., 1994; Monje and Viana, 1998; Thongrod et al., 2003). Accordingly, Thongrod et al. (2003) showed that donkey’s ear abalone (*H. asinina*)

achieved higher weight gains on a diet with no fish oil than that fed diets containing 4% increments of fish oil (4–16%) with decreasing starch contents (29.1–13.1%) for 28 weeks. These investigators concluded that high levels of dietary lipid negatively affect abalone growth, whereas high levels of carbohydrate support growth. Given the reported intolerance of abalone to high levels of dietary lipid, these 4% increments in dietary fish oil supplementation may have been too wide to determine optimal dietary carbohydrate and lipid levels for abalone growth. Hence, determination of the optimal ratio of carbohydrate to lipid is an important factor in feed formulation to improve growth performance of abalone.

The effects of various carbohydrate sources on growth performance of aquatic animals remain controversial. Olive flounder (*Paralichthys olivaceus* Temminck & Schlegel) achieved better weight gain when fed diets containing 15% maltose and 15–25% dextrin than with diets containing 15% cellulose and 5% dextrin (Lee et al., 2003). Similarly, Rahman et al. (2016) showed that digestibility in olive flounder was better with dietary potato starch and dextrin than with wheat flour, corn starch, Na alginate, and carboxymethyl cellulose. Lee and Lee (2004) also reported that starry flounder (*P. stellatus* Pallas) efficiently utilize dextrin and α-potato starch. Another study showed that blunt snout bream (*Megalobrama amblycephala* Yih) responded better when fed dextrin (Ren et al., 2015). Enes et al. (2010), however, observed no effects of dietary maltose, dextrin, starch, or glucose on weight gain of gilthead sea bream (*Sparus aurata* Linnaeus). Tan et al. (2006) reported that gibel carp (*Carassius auratus gibelio* Bloch)

performed better on cellulose, on the other hand, did on dextrin for Chinese longsnout catfish (*Leiocassis longirostris* Günther) when different sources (glucose, dextrin, soluble starch and α-cellulose) of carbohydrate were included. Carbohydrate should be also included in abalone diets at levels that optimize their use of dietary protein for growth. However, the effects of various dietary carbohydrate sources on growth performance of abalone have not been well studied yet.

Feed is the largest production cost for aquaculture operations, in which compromises are often made between nutrition-balanced and cost-effective feeds (Neori and Nobre, 2012). As FM and MA are the largest components and the most expensive feed ingredients in formulating abalone feed, their alternative sources need to be developed (Uki et al., 1985a, 1985b, 1986a; Lee et al., 1998a; Cho et al., 2008; Cho, 2010). Several by-products have been considered as alternatives for FM (Lee et al., 2004; Jung et al., 2016; Choi et al., 2018) and MA (Kim et al., 2016; Jang et al., 2018; Lee et al., 2018; Ansary et al., 2019). Kim et al. (2016) revealed that MA (*S. japonica*) could be completely (100%) replaced with an agricultural by-product, rice bran in abalone (*H. discus*) feed in the 16-week feeding trial when 20% MA was included. Hence, this is required to investigate in the extruded pellet (EP) in commercial scale farm before practical applications.

Therefore, the optimal level of carbohydrate to lipid ratio in the diet for juvenile abalone (*H. discus*) was determined in the first study. In the second study, the proper carbohydrate source to maximize growth performance of

juvenile abalone (*H. discus*) was also investigated. Finally, substitution effect of fermented soybean meal and rice bran for FM and MA in EP on growth performance of juvenile abalone (*H. discus hannai*) in commercial scale farm conditions.

Chapter 2.

Effect of dietary carbohydrate-to-lipid ratio on growth and carcass composition of juvenile abalone (*Haliotis discus*, Reeve 1846)

Abstract

The optimal dietary carbohydrate (C) : lipid (L) ratio on growth and carcass composition of juvenile abalone, *H. discus*, was determined. A total of 1,260 juveniles were randomly distributed into 18 containers. Six experimental diets containing different ratios of C to L (49:1, 48:2, 47:3, 45:5, 43:7, and 41:9) were prepared and referred to as the C49:L1, C48:L2, C47:L3, C45:L5, C43:L7, and C41:L9 diets, respectively. Water stability of the experimental diets was measured 12, 24, and 48 h after seawater immersion. Crude protein, crude lipid and ash content of the experimental diets decreased with time. Survival of abalone fed the C48:L2 diet was higher than that of abalone fed the C49:L1 and C41:L9 diets. Weight gain and specific growth rate (SGR) of abalone fed the C49:L1, C48:L2, and C47:L3 diets were higher than that of abalone fed the C45:L5, C43:L7, and C41:L9 diets. Crude lipid content of the soft body of abalone was directly reflected from dietary crude lipid content. In conclusion, the optimal dietary C : L ratio was estimated to be 48:2 and 47:3 based on survival, weight gain, and SGR of juvenile abalone, respectively. Greater than 3% lipid content in the diet deteriorated performance of this species of abalone.

1. Introduction

Annual aquaculture production of abalone, *Haliotis* spp., in Korea reached 20,053 ton in 2018 (KOSIS, 2019) and will continue to increase in the future due to its high demand for human consumption. Abalone (*H. discus*) is the predominant species cultured in Korea and is endemic to the waters off Japan and Eastern Asia, including South Korea's Jeju Island (Han, 1998).

The optimal dietary protein and lipid requirements for abalone were reported to be 25–35% and 3–7%, respectively (Mai et al., 1996; Bautista-Teruel et al., 2003). In addition, optimal dietary protein and energy ratio was reported to be 418 mg protein/kJ (40.5% protein and 6.8% lipid) for early (initial weight of 0.2 g) green abalone (*H. fulgens*) (Gómez-Montes et al., 2003). However, no published study on the optimal dietary carbohydrate requirement for abalone is available yet.

Abalone are slow-feeding marine gastropods. Their natural diet consists of macroalgae, which is composed of 40–50% carbohydrate, and they have various digestive enzymes capable of hydrolyzing complex carbohydrate (Fleming et al., 1996). Mai et al. (1995a) reported that two species of abalone (*H. tuberculata* and *H. discus hannai*) have great potential to utilize carbohydrate for energy and perhaps for other nutritional purposes. Britz et al. (1994) suggested that the gross maintenance energy metabolism of abalone is carbohydrate-based, as in many other gastropods, given that seaweeds have a low fat content and high storage carbohydrate content.

Both carbohydrate and lipid are important nonprotein energy sources for fish and are incorporated in fish diets to maximize use of dietary protein for growth known as the "protein-sparing effect" (Dias et al., 1998; Helland and Grisdale-Helland, 1998; Stone et al., 2003). Despite the fact that predatory fish utilize dietary lipid better than carbohydrate as a nonprotein energy source (Shimeno et al., 1996; Hemre et al., 2002), and excessive carbohydrate in the diet of predatory fish may depress the growth performance, impair some physiological functions, and cause skeletal malformations (Hutchins et al., 1998; Tan et al., 2007; Ren et al., 2011), abalone have been reported to utilize carbohydrate better than lipid as an energy source (Britz et al., 1994; Monje and Viana, 1998; Thongrod et al., 2003). This was also expected by the fact that abalone have high levels of the digestive enzymes amylase, cellulase, and alginase, but low levels of lipases (Emerson, 1967; Gómez-Pinchetti and García-Reina, 1993; Garcia-Esquivel and Felbeck, 2006). Thongrod et al. (2003) showed that donkey's ear abalone (*H. asinina*) fed a diet with no supplementation of fish oil achieved higher weight gain compared to abalone fed by one of all other diets containing 4% increments of fish oil (4–16%) with a decrease of starch (29.1–13.1%) for 28 week. They concluded that high levels of dietary lipid negatively affected growth of abalone, whereas high levels of carbohydrate supported growth. Given the reported intolerance to high levels of dietary lipid by abalone, however, the 4% treatment increments of dietary fish oil used in their study may have been too wide to determine optimal carbohydrate and lipid levels or the optimal carbohydrate : lipid ratio in

diets for growth performance of abalone. Mai et al. (1995a) reported different results of optimal lipid levels of 3.11–7.09% in a 25% protein diet for both abalone, *H. tuberculata* and *H. discus hannai*. Greater resolution of optimal dietary lipid inclusion levels in the low range (< 5%) is essential in ensuring optimal growth of abalone.

Determination of the optimal ratio of carbohydrate to lipid is an important factor in feed formulation to improve growth performance of abalone. Fleming et al. (1996) reported that carbohydrate and lipid levels in commercial abalone diets ranged from 32 to 60% (average: 46.6%) and from 1.5 to 5.3% (average: 3.7%), respectively. The best weight gain was obtained in donkey's ear abalone fed a 37.6% protein diet with 1.3% lipid level (Thongrod et al., 2003).

In this chapter, therefore, the optimal dietary carbohydrate : lipid ratio for growth and body composition of juvenile abalone, *H. discus*, was determined.

2. Materials and methods

2.1 Preparation of abalone and rearing conditions

Juvenile abalone were purchased from a private hatchery (Jeil Abalone Hatchery, Jeju, Korea) and transferred to an abalone farm (Ocean and Fisheries Research Institute, Jeju Special Self-Governing Province, Jeju, Korea). Prior to initiation of the 16-week feeding trial, the abalone were acclimated to the experimental conditions for 2 week and fed on dry *Undaria* spp. once daily at a ratio of 2–3% of total biomass. A total of 1,260 juveniles (mean ± SE: 1.68 ± 0.001 g) were randomly distributed (70 juveniles/container) into eighteen 70-L rectangular plastic screened containers (120 × 36 × 30 cm), and nine containers were randomly placed into each of two 9-ton concrete flow-through raceway tanks provided with a flow rate of 48.2 L/min/tank through a pipeline. Water flowed from the top of each container through a pipeline. Sand-filtered seawater, at 14.7–17.2℃ (mean ± SD: 16.6 ± 0.52℃) measured at 15:00 h, was supplied throughout the feeding trial. Aeration was supplied in each raceway and the photoperiod was determined by natural conditions. Abalone were fed the experimental diets once a day at 17:00 h at a quantity to ensure satiation (2.0–3.5% of their biomass) with a small amount remaining. Dead abalone were removed daily and the bottoms of the containers were cleaned daily. At the end of the 16-week feeding trial, abalone were harvested, and the group from each container was collectively weighed.

2.2 Preparation of the experimental diets

Six experimental (isonitrogenous) diets containing different ratios of carbohydrate to lipid (49:1, 48:2, 47:3, 45:5, 43:7, and 41:9) were prepared and referred to as the C49:L1, C48:L2, C47:L3, C45:L5, C43:L7, and C41:L9 diets, respectively (Table 1). Fish meal, corn gluten meal, and casein were used as the protein sources in the experimental diets. Dextrin, squid liver, and soybean oils were used as the carbohydrate and lipid sources in the experimental diets. As dextrin content decreased in the experimental diets, squid liver and soybean oils increased. Protein levels in the experimental diets were satisfied for dietary requirement (35%) of abalone (Mai et al., 1995b). Each diet was assigned randomly to triplicate tanks of abalone.

Following the addition of sodium alginate (22%) to each experimental diet, the ingredients were mechanically mixed, and water was added at a ratio of 1:1. A paste was created using an electronic mixer and then shaped into 0.15-cm thick sheets to be hand-cut into 1cm^2 flakes. The flakes were dipped in an aqueous solution of 5% $CaCl_2$ for 1min, and the excess solution was drained. They were then dried for 2 d and stored at −20℃ until use.

Table 1 Ingredients and chemical composition of the experimental diets (%, DM basis)

	Experimental diets					
	C49:L1	C48:L2	C47:L3	C45:L5	C43:L7	C41:L9
Ingredient (%)						
Fish meal	10	10	10	10	10	10
Corn gluten meal	5	5	5	5	5	5
Casein	20	20	20	20	20	20
Dextrin	22	21	20	18	16	14
U. pinnatifida	15	15	15	15	15	15
Squid liver oil		0.5	1	2	3	4
Soybean oil		0.5	1	2	3	4
Sodium alginate	22	22	22	22	22	22
Mineral premix[1]	4	4	4	4	4	4
Vitamin premix[2]	2	2	2	2	2	2
Nutrients (%, dry matter)						
Dry matter	86.8	84.2	86.9	86.3	86.2	87.9
Crude protein	37.8	37.3	37.7	38.0	37.2	37.1
Crude lipid	0.9	1.6	2.7	5.3	6.7	9.8
Carbohydrate[3]	48.8	48.2	47.0	44.0	42.4	39.6
Ash	12.5	12.9	12.6	12.7	13.7	13.5
Estimated energy (E) (kJ/g diet)[4]	14.87	14.95	15.24	15.76	15.89	16.57
P : E (mg/kJ)	447	439	435	424	412	394

[1]Mineral premix contained the following ingredients (g/kg mix): NaCl, 10;

$MgSO_4 \cdot 7H_2O$, 150; $NaH_2PO_4 \cdot 2H_2O$, 250; KH_2PO_4, 320; $CaH_4(PO_4)_2 \cdot H_2O$, 200; Ferric citrate, 25; $ZnSO_4 \cdot 7H_2O$, 4; Ca-lactate, 38.5; CuCl, 0.3; $AlCl_3 \cdot 6H_2O$, 0.15; KIO_3, 0.03; $Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2; $CoCl_2 \cdot 6H_2O$, 0.1.

[2]Vitamin premix contained the following amount which were diluted in cellulose (g/kg mix): L-ascorbic acid, 200; α-tocopheryl acetate, 20; thiamin hydrochloride, 5; riboflavin, 8; pyridoxine, 2; niacin, 40; Ca-d-pantothenate, 12; myo-inositol, 200; d-biotin, 0.4; folic acid, 1.5; p-amino benzoic acid, 20; K_3, 4; A, 1.5; D 3, 0.003; choline chloride, 200; cyanocobalamin, 0.003.

[3]Carbohydrate was calculated by the difference (100 – [% crude protein + % crude lipid + % ash]).

[4]Estimated energy (E) (kJ/g diet) was calculated based on 16.7kJ/g for protein and carbohydrate, and 37.6kJ/g for lipid (Garling and Wilson, 1976).

2.3 Measurement of water stability of the experimental diets

The six experimental diets were placed in separate 70-L plastic rectangular containers without abalone in triplicate. These containers were then placed within a 5-ton concrete flow-through indoor raceway tank at a flow rate of 45.6 L/min and subsampled at 12, 24, and 48 h to evaluate leaching of dry matter in the experimental diets to determine their water stability. Water stability of nutrients in the diets was expressed as the percentage of final dry content to initial dry content for each nutrient based on Mai et al. (1995a).

2.4 Analytical procedures for diets and carcasses

Thirty abalone at the start and 20 abalone from each container at the termination of the feeding trial were sampled and frozen at −40℃ for chemical analysis. Prior to examination, all samples were thawed slightly, and the shell was separated from the soft body tissue. Shell length, shell width, and shell height were measured to a precision of 1.0 mm using a digital caliper (Mitutoyo Corporation, Kawasaki, Japan), and the ratio of soft body weight to total body weight (the soft body weight+the excised shell's weight) was calculated to determine a condition index. Specific growth rate (SGR, % body weight gain/d) was calculated as follows: SGR = ([$\ln(W_f)$ – $\ln(W_i)$]/d of feeding) × 100, where $\ln(W_f)$ is the final mean weight and $\ln(W_i)$ is the initial mean weight (Britz, 1996). The soft body tissue of 10 abalone from each container was then homogenized and used for proximate analysis. Crude protein content was determined using the Kjeldahl method

(Auto Kjeldahl System, Buchi B-324/435/412, Switzerland); crude lipid content was determined using an ether-extraction method; moisture content was determined by oven drying at 105℃ for 24 h; and ash content was determined using a muffle furnace at 550℃ for 4 h. All methods were aligned with AOAC (1990) practices.

2.5 Statistical analysis

Differences between treatments were tested for significance using one-way ANOVA and Duncan's multiple range test (Duncan, 1955) in SAS 9.3 (SAS Institute, Cary, NC, USA). Water stability of the experimental diets was tested by ANOVA with repeated measurement designs (Cody and Smith, 1991). All percentage data were arcsine-transformed prior to statistical analysis.

3. Results

3.1 Water stability of the experimental diets

Crude protein content of the experimental diets decreased with time ($P < 0.0001$) and significant ($P < 0.0001$) interaction (experimental diets × time) was observed (Fig. 1). The highest proportion of crude protein content retained was observed in the C49:L1 diet 48 h after sea water immersion, followed by the C48:L2, C43:L7, C45:L5, C41:L9, and C47:L3 diets.

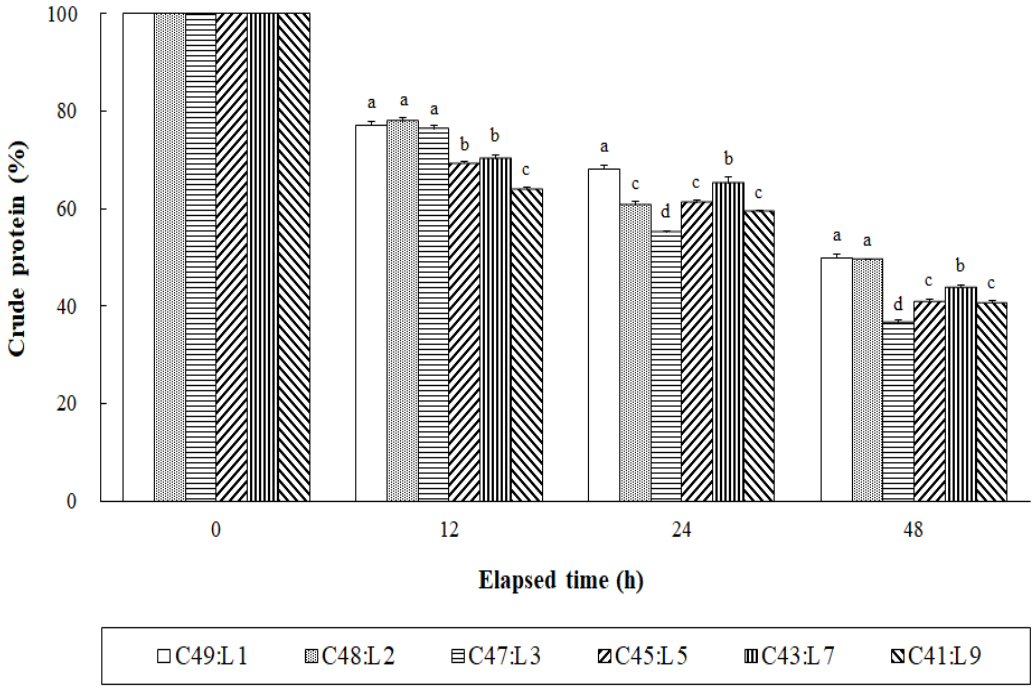

Fig. 1. Changes in retention rate (%) of crude protein content in the experimental diets at 12, 24, and 48 h after sea water immersion (means of triplicate ± SE). (ANOVA with repeated design: time [$P < 0.0001$] and their interaction [experimental diets × time] [$P < 0.0001$]). Different letters in each time point indicates difference between experimental diets with in each time point.

Crude lipid content of the experimental diets decreased with time ($P < 0.0001$) and significant ($P < 0.0001$) interaction (experimental diets × time) was observed (Fig. 2). Crude lipid content retained tended to decrease with dietary lipid content, except for the C47:L3 diet. The highest proportion of crude lipid content was observed in the C41:L9 diet and lowest for the C49:L1 diet 48 h after seawater immersion.

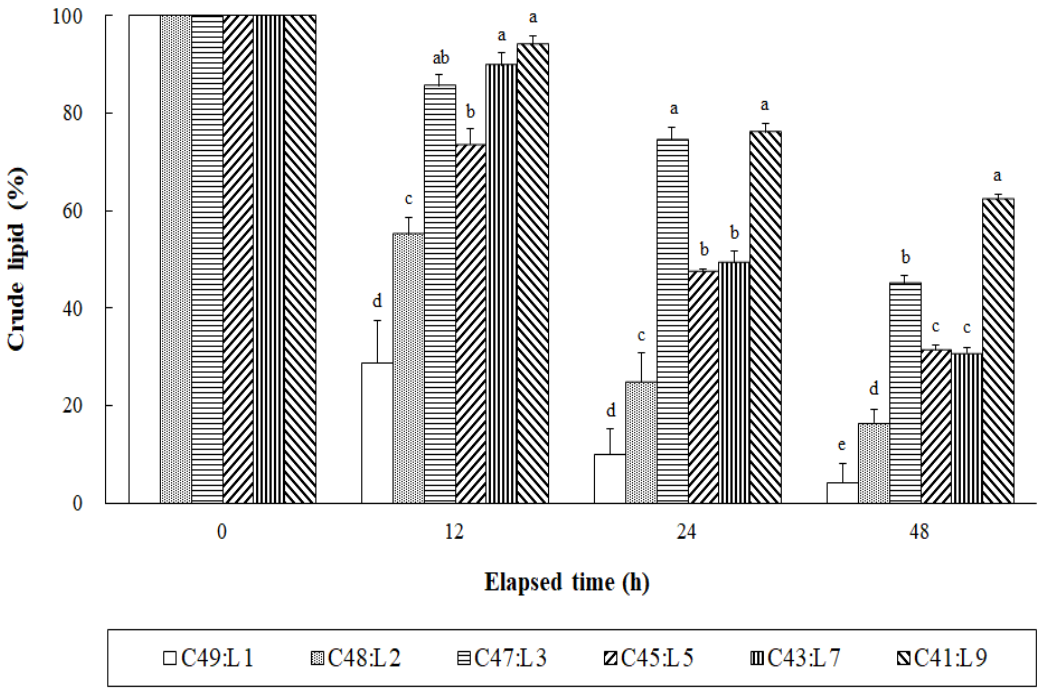

Fig. 2. Changes in retention rate (%) of crude lipid content in the experimental diets at 12, 24, and 48 h after seawater immersion (means of triplicate ± SE). (ANOVA with repeated design: time [$P < 0.0001$] and their interaction [experimental diets × time] [$P < 0.0001$]). Different letters in each time point indicates difference between experimental diets with in each time point.

Ash content of the experimental diets decreased with time ($P < 0.0001$) and significant ($P < 0.0001$) interaction (experimental diets × time) was observed (Fig. 3). The highest proportion of ash content retained was observed in the C45:L5 diet 48 h after seawater immersion, and followed by the C43:L7, C41:L9, C49:L1, C47:L3, and C48:L2 diets.

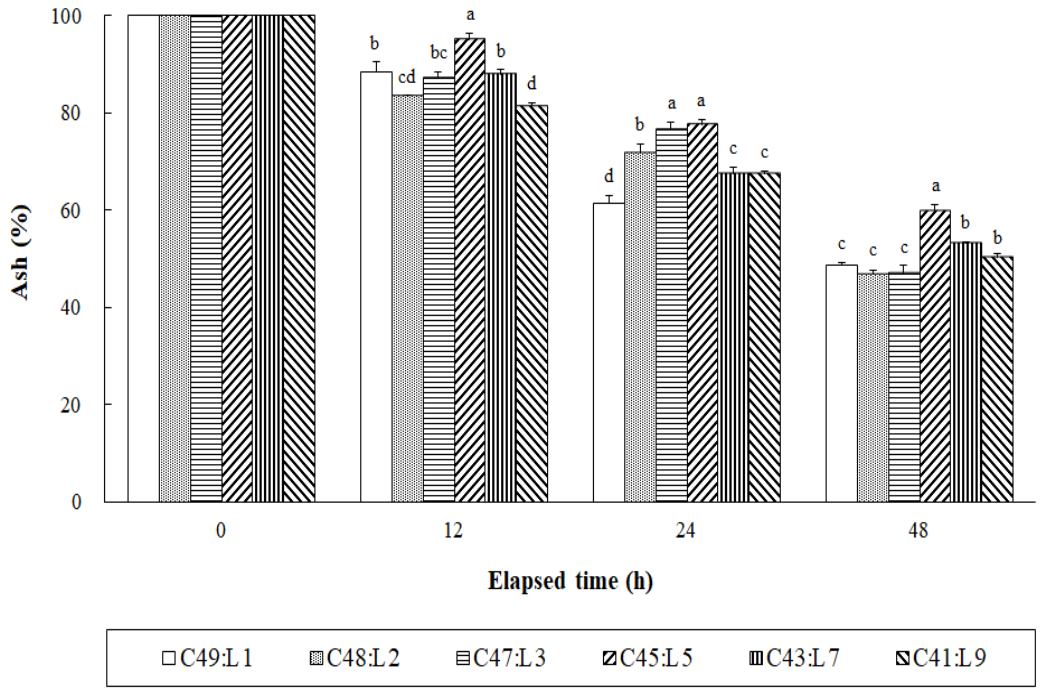

Fig. 3. Changes in retention rate (%) of ash content in the experimental diets at 12, 24, and 48 h after seawater immersion (means of triplicate ± SE). (ANOVA with repeated design: time [$P < 0.0001$] and their interaction [experimental diets × time] [$P < 0.0001$]). Different letters in each time point indicates difference between experimental diets with in each time point.

3.2 Growth performance of abalone

Survival of abalone fed the C48:L2 diet was significantly ($P < 0.05$) higher than that of abalone fed the C49:L1 and C41:L9 diets, but not significantly ($P > 0.05$) different from that of abalone fed the C47:L3, C45:L5, and C43:L7 diets (Table 2).

Weight gain and SGR of abalone fed the C49:L1, C48:L2, and C47:L3 diets were significantly ($P < 0.05$) greater than those of abalone fed the C45:L5, C43:L7, and C41:L9 diets. Weight gain and SGR of abalone fed the C45:L5 and C43:L7 diets were also significantly ($P < 0.05$) greater than those of abalone fed the C41:L9 diet.

Shell length of abalone fed the C48:L2 and C47:L3 diets was significantly ($P < 0.05$) longer than that of abalone fed the other diets (Table 3). The shortest shell length was obtained in abalone fed the C41:L9 diet. Shell width of abalone fed the C47:L3 diet was significantly ($P < 0.05$) wider than that of abalone fed the C49:L1, C45:L5, C43:L7, and C41:L9 diets, but not significantly ($P > 0.05$) different from that of abalone fed the C48:L2 diet. Shell height of abalone fed the C49:L1, C48:L2, and C47:L3 diets was significantly ($P < 0.05$) higher than that of abalone fed the C45:L5, C43:L7, and C41:L9 diets. Soft body weight of abalone fed the C47:L3 diet was significantly ($P < 0.05$) heavier than that of abalone fed the C49:L1, C45:L5, C43:L7, and C41:L9 diets, but not significantly ($P > 0.05$) different from that of abalone fed the C48:L2 diet. However, no significant difference in the ratio of soft body weight to total weight of abalone was found among the dietary ratio of carbohydrate to lipid.

Table 2 Survival (%), weight gain (g/abalone), and specific growth rate (SGR) of juvenile abalone fed the experimental diets containing the various ratio of carbohydrate (C) to lipid (L) for 16 week

Experimental diets	Initial weight (g/abalone)	Final weight (g/abalone)	Survival (%)	Weight gain (g/abalone)	SGR[1]
C49:L1	1.67 ± 0.003	4.77 ± 0.036	94.3 ± 1.43^{bc}	3.10 ± 0.032^{a}	0.91 ± 0.005^{a}
C48:L2	1.67 ± 0.000	4.81 ± 0.040	98.1 ± 0.95^{a}	3.13 ± 0.040^{a}	0.92 ± 0.007^{a}
C47:L3	1.67 ± 0.004	4.84 ± 0.066	95.2 ± 0.48^{abc}	3.17 ± 0.061^{a}	0.92 ± 0.010^{a}
C45:L5	1.67 ± 0.003	4.41 ± 0.056	97.1 ± 1.43^{ab}	2.74 ± 0.059^{b}	0.84 ± 0.012^{b}
C43:L7	1.67 ± 0.003	4.33 ± 0.099	95.7 ± 0.82^{abc}	2.65 ± 0.099^{b}	0.82 ± 0.020^{b}
C41:L9	1.67 ± 0.003	4.07 ± 0.052	91.0 ± 0.48^{c}	2.40 ± 0.049^{c}	0.77 ± 0.010^{c}

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

[1]SGR (%/day) = ([ln(W_f) − ln(W_i)/day of feeding] × 100, where ln(W_f) is the natural log of the final mean weight of abalone and ln(W_i) is the natural log of the initial mean weight of abalone.

Table 3 Shell length (mm), shell width (mm), shell height (mm), soft body weight (g), and the ratio of soft body weight ot total weight of abalone fed the experimental diets containing the various ratio of carbohydrate (C) to lipid (L) for 16 week

Experimental diets	Shell length (mm)	Shell width (mm)	Shell height (mm)	Soft body weight (g)	Soft body weight/total weight
C49:L1	35.4 ± 0.06^{b}	24.8 ± 0.02^{b}	7.5 ± 0.10^{a}	3.4 ± 0.10^{b}	0.63 ± 0.015^{a}
C48:L2	35.8 ± 0.06^{a}	24.9 ± 0.08^{ab}	7.4 ± 0.05^{a}	3.5 ± 0.05^{ab}	0.63 ± 0.011^{a}
C47:L3	36.0 ± 0.05^{a}	25.0 ± 0.14^{a}	7.5 ± 0.05^{a}	3.6 ± 0.04^{a}	0.64 ± 0.013^{a}
C45:L5	34.9 ± 0.08^{c}	24.1 ± 0.04^{c}	7.2 ± 0.02^{b}	3.1 ± 0.06^{c}	0.63 ± 0.005^{a}
C43:L7	34.7 ± 0.05^{c}	24.0 ± 0.06^{c}	7.1 ± 0.02^{b}	2.9 ± 0.02^{d}	0.63 ± 0.008^{a}
C41:L9	34.2 ± 0.07^{d}	23.7 ± 0.11^{d}	6.6 ± 0.02^{c}	2.6 ± 0.02^{e}	0.62 ± 0.006^{a}

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different (P > 0.05).

3.3 Proximate composition of the soft body of abalone

Moisture and ash content of the soft body of abalone was not significantly ($P > 0.05$) affected by dietary ratio of carbohydrate to lipid (Table 4). Crude protein content of abalone fed the C45:L5 diet was significantly ($P < 0.05$) higher than that of abalone fed the other diets. Crude lipid content of abalone fed the C41:L9 diet was significantly ($P < 0.05$) higher than that of abalone fed the other diets, followed by the C43:L7, C45:L5, C47:L3, C48:L2, and C49:L1 diets.

Table 4 Chemical composition (%, wet weight basis) of the soft body of abalone fed the experimental diets containing various ratio of carbohydrate (C) to lipid (L) for 16 week

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
C49:L1	77.5 ± 0.05^{a}	17.3 ± 0.02^{b}	1.0 ± 0.02^{e}	2.1 ± 0.05^{a}
C48:L2	77.4 ± 0.08^{a}	17.1 ± 0.05^{c}	1.0 ± 0.02^{de}	2.0 ± 0.03^{a}
C47:L3	77.4 ± 0.10^{a}	17.2 ± 0.03^{d}	1.1 ± 0.03^{d}	2.0 ± 0.04^{a}
C45:L5	77.4 ± 0.05^{a}	17.4 ± 0.01^{a}	1.3 ± 0.05^{c}	2.0 ± 0.04^{a}
C43:L7	77.4 ± 0.04^{a}	16.9 ± 0.02^{e}	1.5 ± 0.05^{b}	2.0 ± 0.05^{a}
C41:L9	77.4 ± 0.05^{a}	16.8 ± 0.01^{e}	2.7 ± 0.02^{a}	2.1 ± 0.03^{a}

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

4. Discussion

Weight gain and SGR (ranging from 0.77 to 0.92%/day) of abalone in this chapter were comparable to those [ranging from 0.28 to 0.34 (Jung et al., 2016), from 0.45 to 0.46 (Kim et al., 2016), from 0.31 to 0.37 (Myung et al., 2016), and from 0.78 to 0.93%/d (Lee et al., 2017), respectively] in the same species of abalone fed the formulated diet with similar experimental conditions in other studies. The highest survival and weight gain (SGR) were obtained in abalone fed the C48:L2 and C47:L3 diets, respectively, indicating that the optimal dietary carbohydrate : lipid ratio was estimated to be in the region of 48:2 and 47:3 for performance of juvenile abalone. The fastest weight gain of abalone fed the C47:L3 diet corresponded with the largest values for other biological criteria (shell length, shell width, shell height, soft body weight, and the ratio of soft body weight to total weight of abalone) measured in this chapter, agreeing with other studies (Bautista-Teruel et al., 2003; Cho, 2010; Myung et al., 2016; Lee et al., 2017). Similarly, Thongrod et al. (2003) showed that donkey's ear abalone grew the best on the 37.6% protein diet containing 52.1% carbohydrate and 1.3% lipid when abalone were fed with 36–38% protein diets containing different ratios of carbohydrate (35.6–52.1%) using starch as the main carbohydrate source and lipid (1.3–19.0%) using fish oil as the main lipid source for 28 week and concluded that weight gain decreased with the increased lipid and decreased carbohydrate content in the diets, resulting from low feed intake probably because of high caloric value

of lipid. The natural diet of abalone consisting of MA is also composed of 40–50% carbohydrate (Fleming et al., 1996).

Montano-Vargas et al. (2005) reported that carbohydrate seems to be the more preferred energy source than lipid for pink abalone (*H. corrugate*) and lipid levels should be minimized to the extent that essential fatty acids requirements are met. Because high-dietary lipid negatively affects performance of abalone as a result of low feed consumption (Mai et al., 1995a; Britz and Hecht, 1997; Gómez-Montes et al., 2003; Thongrod et al., 2003), low lipid content (<5%) is commonly recommended for commercial abalone feed (Fleming et al., 1996; Bansemer et al., 2014). To lower feed cost, however, carbohydrate has to preferably satisfy the energy requirements of abalone (Dunstan, 2010; Bansemer et al., 2014).

Crude protein and lipid and ash content retained slowly decreased with time throughout 48 h observation, except for the crude lipid content in the C49:L1 and C48:L2 diets in this chapter. High variation in crude lipid content in both diets was probably due to their low lipid content. Water stability (leaching-out loss) of nutrients (crude protein and lipid and ash) in the experimental diets were comparable to other similar formulated diets (Lee et al., 2016, 2017). Because water stability of nutrient (crude protein and lipid and ash) content in the experimental diet in this chapter was relatively high, and given that the diets were supplied to abalone daily, leaching is unlikely to have negatively affected performance of abalone.

Because herbivorous and omnivorous fish utilize carbohydrate more efficiently as nonprotein energy source than carnivorous fish (Hemre et al.,

2002; Stone, 2003), their optimal dietary carbohydrate : lipid ratio is much higher. To compare the optimal dietary carbohydrate : lipid ratio for performance of abalone with fish, carbohydrate : lipid ratios were calculated from previously reported carbohydrate values (100 – [% crude protein + % crude lipid + % ash]). Li et al. (2012) also reported that high-dietary carbohydrate (<carbohydrate : lipid ratio of 9.0) and too-low-dietary lipid caused metabolic stress of herbivorous blunt snout bream (*M. amblycephala*) and led to elevated liver oxidation rates, impaired liver function, depressed immunity, and reduced resistance to *Aeromonas hydrophila* infection when diets containing various carbohydrate : lipid ratios (4.0, 5.1, 6.7, 9.0, 15.6, and 33.8) were fed to fingerling fish for 10 week. Furthermore, the excessive carbohydrate in the diet of carnivorous fish also depressed the growth performance and impaired some physiological functions (Hutchins et al., 1998; Hemre et al., 2002; Ren et al., 2011; Li et al., 2012).

Unlike fish, abalone are known to utilize carbohydrate rather than lipid as a nonprotein energy source (Britz et al., 1994; Hemre et al., 2002; Thongrod et al., 2003), supported by the fact that abalone have high levels of digestive enzymes amylase, cellulase, and alginase, but low levels of lipase (Emerson, 1967; Gómez-Pinchetti and García-Reina, 1993; Garcia-Esquivel and Felbeck, 2006). Again, Mai et al. (1995a) reported that *H. tuberculata* and *H. discus hannai* have great potential for utilizing carbohydrate well for energy. Based on growth performance of abalone, the optimal dietary carbohydrate : lipid ratio (47:3) seems to be 15.7 in this chapter, which is much higher than that of all other fish reported in Table 5.

Crude lipid content of the soft body of abalone was proportionally reflected from the dietary lipid content in the first study, agreeing with other studies (Mai et al., 1995a, 1995b; Thongrod et al., 2003; Cho et al., 2008; Garcia-Esquivel and Felbeck 2009; Cho 2010; Myung et al., 2016).

In conclusion, the optimal dietary carbohydrate : lipid ratio was estimated to be 48:2 and 47:3 based on survival and weight gain (SGR) of juvenile abalone, respectively. Higher than 3% lipid content in diet deteriorated performance of this species of abalone.

Table 5 Optimal carbohydrate (C), lipid (L), and carbohydrate to lipid ratio in various protein (P) diet for fish

Fish species (scientific name)	P (%)	C (%)	L (%)	C : L	References
Herbivorous fish					
Grass carp (*Ctenopharyngodon idella*)	38.9	47.2	0.2	11.2	Gao et al. (2010)
Blunt snout bream (*M. amblycephala)*	32.5	52.7	5.8	9.0	Li et al. (2012)
African catfish (*Clarias gariepinus*)	40.2	37.1	13.5	2.7	Ali and Jauncey (2004)
Yellow carfish (*Pelteobagrus fulvidraco*)	42.9–43.1	39.6–43.7	6.2–9.9	4.0–7.1	Wang et al. (2014)
Walking catfish (*C. batrachus*)	40.0	44.8	8.1	5.6	Erfanullah and Jafri (1998)
Carnivorous fish					
Giant grouper (*Epinephelus lanceolatus*)	49.2	30.4	15.1	2.0	Li et al. (2016)
Rockfish (*Sebastes schlegeli*)	51.4	26.4	12.7	2.1	Lee and Kim (2009)
Chinese longsnout catfish (*L. longirostris*)	44.0	28.8	11.8	2.4	Tan et al. (2007)
Beluga (*Huso huso*)	34.9	40.2	16.3	2.5	Mohseni et al. (2011)
Large yellow croaker (*Lamichthys crocea*)	39.5	38.7	12.2	3.2	Zhou et al. (2016)

Chapter 3.

Effects of dietary carbohydrate sources on growth and body composition of juvenile abalone (*Haliotis discus*, Reeve 1846)

Abstract

A 16-week feeding trial was conducted to assess the effects of different types of dietary carbohydrate on growth and body composition of juvenile abalone (*H. discus*). A total of 1,680 abalone were randomly distributed among 24 containers (70 per container) and fed one of eight diets, including seven experimental diets containing different types of carbohydrates, including dextrin (DT), glucose (GC), corn starch (CS), α-cellulose (CL), maltose (MT), sucrose (SC), and wheat flour (WF), and *U. pinnatifida* to compare effect of experimental diets. Survival was not significantly affected by diet. Weight gain and specific growth rate were greatest in abalone fed on the CL diet. Shell length, width, height, and soft body weight were greatest in abalone fed the CL diet, followed by the WF, DT, SC, CS, GC, and MT diets and *U. pinnatifida*. Weight gain, specific growth rate, crude protein, and crude lipid contents of the soft body of abalone fed on the experimental diets were greater than those fed on *U. pinnatifida*. In conclusion, CL is the most effective carbohydrate source for improving growth of this species of juvenile abalone and practically applicable in formulating abalone feed.

1. Introduction

Annual aquaculture production of abalone (*Haliotis.* spp.) in Korea in 2000 was approximately 20 tons and reached 20,053 tons in 2018 (KOSIS, 2019). This upward trend is expected to continue, given the high demand for abalone for human consumption. Several studies involving feeding trials have been conducted to investigate dietary nutrient requirements of abalone (Uki et al., 1985a; Uki et al., 1986a, 1986b; Mai et al., 1995a, 1995b; Lee et al., 1998a; Zhang et al., 2009). The development of alternatives for fish meal and macroalgae, which are the most expensive components in abalone diets (Lee et al., 1999; Cho, 2010; Kim et al., 2016); dietary inclusion of macro- and microalgae (Lee et al., 1998b; Lee et al., 2000); and improved feeding regimes (Cho et al., 2011) have also been investigated. The optimum dietary protein and lipid requirements for abalone were reported to be 25–35% and 3–7%, respectively (Mai et al., 1995a, 1995b; Fleming et al., 1996; Bautista-Teruel et al., 2003).

Abalone are marine gastropods and slow eaters. Their natural diet consists of 40–50% carbohydrates, and they have various enzymes capable of hydrolyzing complex carbohydrates (Fleming et al., 1996). Mai et al. (1995a) found that two species of abalone, *H. tuberculata* and *H. discus hannai*, have a great capacity to use carbohydrates for energy and perhaps for other nutritional purposes. Britz et al. (1994) demonstrated that the gross maintenance energy metabolism of abalone is carbohydrate based, like many other gastropods (Emerson, 1967), given that seaweeds have low fat content

and high storage carbohydrate content. Previous studies have indicated that differences in the complexity of carbohydrates influence their digestion and utilization by aquatic animals (Wilson, 1994; Cuzon et al., 2000; Lee et al., 2003; Stone et al., 2003; Tan et al., 2006).

Carbohydrates are thus an important nonprotein energy source for abalone, in contrast to fish and crustaceans, which do not use them as effectively. Abalone have high levels of the digestive enzymes protease, amylase, cellulase, and alginase, but low levels of lipases (Emerson, 1967; Gomez-Pinchetti and Garcia-Reina, 1993; Britz et al., 1994; Garcia-Esquivel and Felbeck, 2006). The donkey's ear abalone (*H. asinine*) utilizes high levels of carbohydrates more effectively than lipids to support its growth (Thongrod et al., 2003).

Carbohydrates should, therefore, be included in abalone diets at levels that optimize their use of dietary protein for growth. Research on the effects of dietary carbohydrate sources on the growth and body composition of *H. discus* is, however, very limited. This species is endemic to the waters off Japan and eastern Asia, including the South Korean Jeju Island (Han, 1998).

In this chapter, the effects of various dietary carbohydrate sources on the growth and body composition of juveniles of *H. discus* were investigated.

2. Materials and methods

2.1 Preparation of abalone and rearing conditions of abalone

Juvenile abalone were purchased from a private hatchery (Deagun Fisheries, Jeju, Korea) and transferred to an abalone farm (Ocean and Fisheries Research Institute, Jeju Special Self-Governing Province, Jeju, Korea). Prior to initiation of the feeding trial, the abalone were acclimated to the experimental conditions for 4 week and fed on dry *Undaria* spp. once daily at a ratio of 2–3% of total biomass. A total of 1,680 juveniles (mean ± SE: 1.68 ± 0.001 g) were randomly distributed into twenty-four 70-L rectangular plastic containers (120 × 36 cm; 70 individuals per container), and 12 containers were randomly placed into each of two 9-ton concrete flow-through tanks with a flow rate of 48.2 L/min/tank. Sand-filtered seawater, at 14.7–17.2℃ (mean ± SD: 16.6℃ ± 0.52℃), was supplied throughout the feeding trial. Aeration was supplied in each raceway and the animals were subjected to a natural photoperiod. Abalone were fed, for 16 week, with the experimental diets once a day at 17:00 h at a quantity to ensure satiation, with a small amount (about 2–3.5% of their biomass) leftover. Dead individuals were removed daily and the bottoms of the containers were cleaned daily. At the end of the 16-week feeding trial, abalone were harvested and the group from each container was collectively weighed.

2.2. Preparation of the experimental diets

Eight diets, including seven experimental ones and the dry *U. pinnatifida*, were prepared in triplicate (Table 6). Each experimental diet included a different carbohydrate source (12%), together with fish meal (28%), corn gluten meal (10%), soybean meal (8%), squid liver oil (0.5%), and soybean oil (0.5%), which served as sources of protein and lipid. The different carbohydrate sources were dextrin (DT), glucose (GC), corn starch (CR), α -cellulose (CL), maltose (MT), sucrose (SC), and wheat flour (WF). Protein and lipid levels in the experimental diets were based on published dietary requirements for abalone (Mai et al., 1995a, 1995b). The dry *U. pinnatifida* was also included as a control.

After the addition of sodium alginate (22%) to each experimental diet, the ingredients were mechanically mixed, and water was added at a ratio of 1:1. A paste was created using an electronic mixer and then shaped into 0.15-cm thick sheets to be hand-cut into 1-cm^2 flakes. The flakes were dipped in an aqueous solution of 5% $CaCl_2$ for 1 min, and the excess solution was drained naturally. They were then dried naturally for 2 days and stored at –20℃ until use.

Table 6 Feed formulation of the experimental diets (%, DM basis)

	Experimental diets							
	DT[1]	GC[2]	CS[2]	CL[2]	MT[2]	SC[2]	WF[3]	*U. pinnatifida*
Ingredients (%)								
Fish meal	28	28	28	28	28	28	28	
Corn gluten meal	10	10	10	10	10	10	10	
Soybean meal	8	8	8	8	8	8	8	
Carbohydrate source	12	12	12	12	12	12	12	
Sea tangle	13	13	13	13	13	13	13	
Squid liver oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
Soybean oil	0.5	0.5	0.5	0.5	0.5	0.5	0.5	
Sodium alginate	22	22	22	22	22	22	22	
Mineral premix[4]	4	4	4	4	4	4	4	
Vitamin premix[5]	2	2	2	2	2	2	2	
Nutrients (%)								
Dry matter	79.6	82.0	86.1	88.6	84.0	82.9	87.4	79.8
Crude protein0	35.6	35.9	35.7	35.0	36.3	36.2	35.2	27.2
Crude lipid	4.0	3.8	3.8	3.8	3.9	3.9	3.7	1.7
Carbohydrate	44.3	43.5	44.8	44.5	43.7	43.7	45.8	45.8
Ash	16.1	16.8	15.7	16.7	16.1	16.2	15.3	25.3

[1]DT was purchased from Duksan Pure Chemicals (Ansan-si, Kyungki-do, Korea).

[2]GC, CS, CL, MT, and SC were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA).

[3]WF was supplied by CJ CheilJedang Corp. (Seoul, Korea).

[4]Mineral premix contained the following ingredients (g/kg mix): NaCl, 10; $MgSO_4 \cdot 7H_2O$, 150; $NaH_2PO_4 \cdot 2H_2O$, 250; KH_2PO_4, 320; $CaH_4(PO_4)_2$. H_2O, 200; Ferric citrate, 25; $ZnSO_4 \cdot 7H_2O$, 4; Ca-lactate, 38.5; CuCl, 0.3; $AlCl_3 \cdot 6H_2O$, 0.15; KIO_3, 0.03;

$Na_2Se_2O_3$, 0.01; $MnSO_4 \cdot H_2O$, 2; $CoCl_2 \cdot 6H_2O$, 0.1.

[5]Vitamin premix contained the following amount, which were diluted in cellulose (g/kg mix): L-ascorbic acid, 200; α-tocopheryl acetate, 20; thiamin hydrochloride, 5; riboflavin, 8; pyridoxine, 2; niacin, 40; CaD-pantothenate, 12; myo-inositol, 200; D-biotin, 0.4; folic acid, 1.5; p-amino benzoic acid, 20; K3, 4; A, 1.5; D 3, 0.003; choline chloride, 200; cyanocobalamin, 0.003.

2.3 Analytical procedures of the diets and carcasses

Twenty abalone at the start and 10 abalone from each container at the termination of the feeding trial were sampled and frozen at –40℃ for chemical analysis. Prior to examination, all samples were thawed slightly, and the shell was separated from the soft body tissue. Shell length, shell width, and shell height were measured to a precision of 1.0 mm using a digital caliper (Mitutoyo Corporation, Kawasaki, Japan), and the ratio of soft body weight to total body weight (the soft body weight + the excised shell weight) was calculated to determine a condition index. Specific growth rate (SGR, % body weight gain/day) was calculated using Britz et al. (1996): SGR = $[(\ln(W_f) - \ln(W_i))/\text{days of feeding}] \times 100$, where $\ln(W_f)$ is final mean weight and $\ln(W_i)$ is initial mean weight.

The soft body tissue of the 10 abalone from each container was then homogenized and used for proximate analysis. Crude protein content was determined using the Kjeldahl method (Auto Kjeldahl System, Buchi B-324/435/412, Switzerland); crude lipid content was determined using an ether-extraction method; moisture content was determined by oven-drying at 105℃ for 24 h; and ash content was determined using a muffle furnace at 550℃ for 4 h. All methods were based on AOAC (1990) practices.

2.4 Statistical analysis

Differences between treatments were tested for significance using one-way analysis of variance (ANOVA) and Duncan's multiple range test (Duncan, 1955) in SAS 9.3 (SAS Institute, Cary, NC). All percentage data were

arcsine-transformed prior to statistical analysis.

3. Results

3.1 Growth performance of abalone

Abalone survival was consistently greater than 93.8%, but not significantly different ($P > 0.05$) among the diets (Table 7). Weight gain and SGR of abalone fed the CL diet were significantly greater ($P < 0.05$) than those of abalone fed the all other diets. In addition, weight gain and SGR of abalone fed the experimental diets were significantly ($P < 0.05$) greater than those of abalone fed the *U. pinnatifida*, except for the GC and MT diets.

Table 7 Survival (%), weight gain (g/abalone) and SGR (%/day) of juvenile abalone fed the experimental diets containing the various sources of carbohydrate for 16 week

Experimental diets	Initial weight (g/abalone)	Final weight (g/abalone)	Survival (%)	Weight gain (g/abalone)	SGR[1] (%/day)
DT	1.67 ± 0.0002	4.33 ± 0.063^{b}	94.3 ± 0.82^{a}	2.65 ± 0.062^{b}	0.83 ± 0.012^{b}
GC	1.68 ± 0.0003	4.17 ± 0.126^{bc}	93.8 ± 2.65^{a}	2.49 ± 0.124^{bc}	0.79 ± 0.025^{bc}
CS	1.68 ± 0.0003	4.26 ± 0.044^{b}	94.3 ± 1.65^{a}	2.58 ± 0.044^{b}	0.81 ± 0.009^{b}
CL	1.68 ± 0.0004	4.80 ± 0.800^{a}	94.3 ± 1.43^{a}	3.12 ± 0.098^{a}	0.91 ± 0.017^{a}
MT	1.68 ± 0.0004	4.12 ± 0.061^{bc}	94.3 ± 1.43^{a}	2.44 ± 0.058^{bc}	0.78 ± 0.011^{bc}
SC	1.67 ± 0.0001	4.29 ± 0.059^{b}	96.2 ± 1.72^{a}	2.61 ± 0.059^{b}	0.82 ± 0.012^{b}
WF	1.67 ± 0.0001	4.35 ± 0.158^{b}	95.7 ± 1.43^{a}	2.68 ± 0.159^{b}	0.83 ± 0.032^{b}
U. pinnatifida	1.68 ± 0.0020	3.95 ± 0.031^{c}	95.7 ± 0.82^{a}	2.27 ± 0.033^{c}	0.74 ± 0.008^{c}

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

[1]SGR (%/day) = $[(\ln(W_f) - \ln(W_i))/\text{days of feeding}] \times 100$, where $\ln(W_f)$ = natural log of the final mean weight of abalone and $\ln(W_i)$ = natural log of the initial mean weight of abalone.

Shells were largest (in terms of length, width, and height) and soft body weight was greatest in abalone fed the CL diet, followed by the WF, DT, SC, CS, GC, and MT diets, and *U. pinnatifida* (Table 8). Abalone fed the CL, WF, DT, SC, GC, and MT diets, however, had a significantly ($P < 0.05$) higher ratio of soft body weight to total body weight than abalone fed the CS diet and *U. pinnatifida*.

Table 8 Shell length (mm), shell width (mm), shell height (mm), soft body weight (g/abalone), and the ratio of soft body weight to total weight of abalone fed the experimental diets containing the various sources of carbohydrate for 16 week

Experimental diets	Shell length (mm)	Shell width (mm)	Shell height (mm)	Soft body weight (g)	Soft body weight/total weight
DT	34.5 ± 0.06^{b}	23.7 ± 0.07^{b}	6.8 ± 0.07^{b}	2.6 ± 0.07^{b}	0.67 ± 0.007^{a}
GC	33.5 ± 0.05^{dc}	23.2 ± 0.05^{c}	6.4 ± 0.05^{cd}	2.3 ± 0.02^{de}	0.65 ± 0.003^{a}
CS	33.8 ± 0.04^{cd}	23.3 ± 0.04^{c}	6.5 ± 0.05^{c}	2.4 ± 0.03^{cd}	0.63 ± 0.008^{b}
CL	35.3 ± 0.11^{a}	24.7 ± 0.10^{a}	7.1 ± 0.07^{a}	3.0 ± 0.05^{a}	0.67 ± 0.004^{a}
MT	33.3 ± 0.03^{e}	23.2 ± 0.08^{c}	6.3 ± 0.04^{d}	2.2 ± 0.01^{e}	0.65 ± 0.009^{a}
SC	34.0 ± 0.21^{c}	23.5 ± 0.03^{b}	6.6 ± 0.06^{c}	2.5 ± 0.10^{c}	0.67 ± 0.015^{a}
WF	34.6 ± 0.21^{b}	23.8 ± 0.12^{b}	6.9 ± 0.09^{ab}	2.7 ± 0.06^{b}	0.67 ± 0.004^{a}
U. pinnatifida	32.5 ± 0.17^{f}	22.3 ± 0.15^{d}	6.1 ± 0.08^{e}	2.0 ± 0.04^{f}	0.62 ± 0.005^{b}

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

3.2 Proximate composition of the soft body of abalone

The moisture content of the soft body of abalone fed the CS diet was significantly ($P < 0.05$) greater than that of abalone fed the GC, SC, CL, and WF diets, but not significantly ($P > 0.05$) different from the DT and MT diets, and *U. pinnatifida* (Table 9). The crude protein content was significantly ($P < 0.05$) greater in abalone fed the MT diet than in those fed the all other diets, and in abalone fed the GC, CS, and SC diets than those fed the DT, CL, and WF diets, and *U. pinnatifida*. The crude lipid content of abalone fed the all experimental diets was significantly ($P < 0.05$) greater than that fed *U. pinnatifida*. The ash content of the soft body of abalone did not, however, differ significantly ($P > 0.05$) among the diets.

Table 9 Chemical composition (%, wet weight basis) of the soft body of abalone fed the experimental diets containing the various sources of carbohydrate for 16 week

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
DT	71.6 ± 0.07^{ab}	17.0 ± 0.02^{c}	1.1 ± 0.05^{ab}	2.6 ± 0.05^{a}
GC	71.5 ± 0.06^{bc}	17.4 ± 0.02^{b}	1.1 ± 0.03^{ab}	2.7 ± 0.03^{a}
CS	71.7 ± 0.02^{a}	17.3 ± 0.02^{b}	1.2 ± 0.02^{a}	2.6 ± 0.02^{a}
CL	71.5 ± 0.04^{b}	17.1 ± 0.05^{c}	1.1 ± 0.04^{ab}	2.6 ± 0.04^{a}
MT	71.5 ± 0.04^{ab}	17.6 ± 0.04^{a}	1.1 ± 0.04^{ab}	2.7 ± 0.02^{a}
SC	71.5 ± 0.05^{b}	17.4 ± 0.02^{b}	1.1 ± 0.03^{ab}	2.6 ± 0.06^{a}
WF	71.3 ± 0.05^{c}	17.1 ± 0.05^{c}	1.1 ± 0.04^{b}	2.6 ± 0.02^{a}
U. pinnatifida	71.6 ± 0.06^{ab}	16.4 ± 0.05^{d}	0.7 ± 0.01^{c}	2.6 ± 0.04^{a}

Values (means of triplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

4. Discussion

The greater weight gain and SGR of abalone fed the experimental diets, compared with those of abalone fed on *U. pinnatifida*, was consistent with previous findings that a single MA produces a poorer growth performance in abalone than does a formulated diet (Lee, 1998; Lee et al., 1998a, 1998b, 1998c; Cho et al., 2006; Garcia-Esquivel and Felbeck, 2006; Cho et al., 2008; Garcia-Esquivel and Felbeck, 2009; Dang et al., 2011; Myung et al., 2016).

The fact that weight gain and SGR did not differ significantly among carbohydrate sources (except for the CL diet achieving the best weight gain and SGR) was partially consistent with the study of Lee et al. (1998c) showing that weight gain of juvenile abalone (*H. discus hannai*) was not affected by the type of carbohydrate included in the diet (they tested diets containing 24.2% WF, 20% DT, 20% SC, 10% each of α- and β-potato starch, 15% α-potato starch, 20% α-potato starch, or 25% α-potato starch). They concluded that abalone can use carbohydrates with various degrees of polymerization (i.e., mono-, di-, and polysaccharide) relatively well. Erasmus et al. (1997) also detected alginate lyase, carboxymethylcellulase, laminarinase, agarase, carragenase in the hepatopancrease of abalone (*H. midae*) and proposed that bacteria resident in the digestive system of *H. midae* assisted in the digestion of alginate, laminarin, agarose, carrageenan, and cellulose in diet. Careful consideration must be given in feeding trials, in which CL was used as filler in abalone feed and considered to have no

effect on abalone, because abalone fed the CL diet outgrew those fed the other carbohydrate source in this chapter. Garcia-Esquivel and Felbeck (2006) observed high cellulase activity in the digestive glands of red abalone (*H. rufescens*) when they were fed formulated diets and kelp (*Macrocystis pyrifera*). In earlier work, Nakagawa and Nagayama (1988) showed that high activities of xylanase and carboxymethylcellulase were found in the extracts of abalone (*H. discus hannai* and *H. sieboldii*) and demonstrated that distribution of polysaccharidases might be related to the food habit of marine invertebrates, although the levels of activity is highly variable between species and types of cellulose. Cellulase was also found in the gut of abalone (*H. gigantea* and *H. japonica*) (Yokoe and Yasumasu, 1964). The high cellulose activity in abalone may explain why the dietary inclusion of CL produced the best growth performance in this chapter.

Walton and Cowey (1982) reported that the effective utilization of dietary carbohydrates in aquatic animals seems to be closely related to the capacity of their digestive and metabolic systems to adapt to different aquatic environments and different quantities and complexities of dietary carbohydrates. Tan et al. (2006) reported that gibel carp (*C. auratus gibelio*) performed better on a CL diet, followed by soluble starch, SC, DT, and GC diets (each of which included 20% of its respective carbohydrate), on the other hand, Chinese longsnout catfish (*L. longirostris*) responded better to a DT diet, followed by SC, CL, soluble starch, and GC diets (each of which included 6% of its respective carbohydrate). They concluded that omnivorous and carnivorous fishes achieved different abilities to use complex

carbohydrates. It is also known that an increased amount of fiber stimulates the activity of cellulase and the growth of microflora in the gut of the Tilapia (*Oreochromis mossambicus* Peters) (Manju and Dhevendaran, 2002). Several researchers, however, have reported reduced weight gain in fish fed the diets containing CL (Leary and Lovell, 1975; Hilton et al., 1983; Anderson et al., 1984; Lee et al., 2003). Hilton et al. (1983), in particular, showed that CL is a water-insoluble dietary fiber, and that it increases gastric emptying time in rainbow trout (*Salmo gairdneri*). Abalone fed the CL diet in this chapter had the largest shells and the heaviest soft bodies. All morphometric criteria measured, except the ratio of soft body weight to total body weight, appeared to be relatively well reflected by growth rate, and were also consistent with previous studies showing that morphometric measurements of abalone agreed with growth rate (Bautista-Teruel et al., 2003; Cho, 2010; Myung et al., 2016).

The chemical composition of the soft bodies was affected by the type of carbohydrate in the diet in this chapter. The greater crude protein and lipid content of abalone fed the formulated diets, relative to abalone fed on *U. pinnatifida* was partially consistent with previous studies showing that dietary nutrient content affects the proximate composition of abalone (Mai et al., 1995a, 1995b; Thongrod et al., 2003; Cho et al., 2008; Gracia-Esquivel and Felbeck, 2009; Cho, 2010; Kim et al., 2016).

In conclusion, inclusion of any type of carbohydrate in the diet produced a better growth performance than did a diet consisting solely of *U. pinnatifida*. The inclusion of CL in the diet was, therefore, the most

effective strategy for increasing the growth of abalone among the various types of carbohydrates tested. This information is practically helpful in formulating abalone feed.

Chapter 4.

Effects of substitution of fish meal and macroalgae with soybean meal and rice bran in a commercial juvenile abalone (*Haliotis discus hannai*) diet on growth performance

Abstract

The effects of FM and MA substitution with fermented soybean meal and rice bran were tested on a commercial diet in juvenile abalone. Abalone (21,600) individuals were distributed equally to four diet treatments. Four experimental diets were prepared: a Std diet, FM50, FM50+MA50 and FM50+MA100. The standard diet (Std) consists of FM (14%), fermented soybean meal (25%), corn gluten meal (3.4%), shrimp meal (3%), wheat flour (20%), dextrin (5%) and MA (25%). *Undaria* and *Saccharina* were also prepared. The feeding trial lasted for 16 weeks. Survival of abalone fed the formulated diets was higher than that of abalone fed the *Undaria* and *Saccharina*. Weight gain and specific growth rate of abalone fed the Std diet were higher than all other diets. Substitutability of the fermented soybean meal for FM seemed to be rather limited in the commercial diet for abalone farms. However, another 50% MA in the commercial diet could be substituted with rice bran as long as 50% MA were substituted with rice bran.

1. Introduction

Annual abalone aquaculture production in Korea was about 20 metric tons in 2000 and reached 20,053 metric tons in 2018 (KOSIS, 2019). This trend is likely to continue due to its continuing high consumer demand and continued farm expansions to meet the demand.

FM, which has been commonly used as a primary protein source in aquafeeds, prices have increased 285% since 2001 (FAO, 2014) and are projected to continue increasing due to a shortage of catchable FM sources in wild and continuing expansion of global fish meal fed aquaculture. MA, which has been used as a replacement or feed additive, prices are also becoming more costly due to expansion of the biofuel industry to develop MA for ethanol extraction.

In Korea, abalone farmers are likely to continue to feed abalone on MA such as *U. pinnatifida* or *S. japonica* during the winter season on their farms as these MA are grown naturally and harvested in the wild. Although its nutrient content, such as protein (amino acid) and lipid (fatty acid) do not satisfy the complete dietary requirement for abalone (*H. discus hannai* Ino) (Uki et al., 1986a; Mai et al., 1995a, 1995b), farmers prefer feeding abalone on MA for the convenience of farm management. This may result in poorer growth rates and increase the production cost of abalone over commercial feeds. The dry or salted MA is commonly fed to abalone during the summer. However, the nutrients in the dry or salted MA are easily destroyed in the drying or salting process and this can also result in poor

growth rate.

One of the largest production costs in aquaculture operations is feed and a compromise needs to be made between a nutrition-balanced and cost-effective feed (Neori and Nobre, 2012). As FM and MA are the largest components of commercial abalone feeds and prices continue to increase, alternatively sources need to be identified and their performances evaluated against existing commercial feeds. Globally research has shown that a nutrition-balanced feed will produce better growth rates that a single algal species diet (Lee, 1998; Cho et al., 2006; Kim et al., 1998; Cho et al., 2008; Naidoo et al., 2006; Gracia-Esquivel and Felbeck, 2009; Dang et al., 2011; Kim et al., 2016; Myung et al., 2016).

Studies by Uki et al. (1985a, 1985b, 1986a) have shown that plant protein (soybean and cottonseed meal) are good alternative sources for casein, and can produce the best weight gain of abalone (*H. discus hannai*) compared to other plant proteins tested (Lee et al., 1998a). Cho et al. (2008) also reported that growth of abalone fed a combined fish and soybean meal diet or a combined fish, soybean and crustacean meal diet was comparable to that of abalone fed a casein-basal diet. In addition, FM at 35% could be completely replaced with soybean meal at 58% with an additional 0.5% methionine supplementation, as it is lacking in the plant protein source (Cho, 2010). Therefore, soybean meal seems to be a promising alternative protein source for FM in abalone feed.

Fermentation of soybean meal increases protein content, eliminates trypsin inhibitors, and reduces peptide size (Hong et al., 2004; Kook et al., 2014).

Dietary inclusion of fermented plant protein sources, such as soybean and cottonseed meals also produced a promising effect on performance of Nile tilapia (*O. niloticus* Linnaeus) and black sea bream (*Acanthopagrus schlegelii* Bleeker) (Lim and Lee, 2011; Zhou et al., 2011). A study by Kim et al. (2016) on abalone (*H. discus* Reeve) revealed that 100% substitution of *S. japonica* with rice bran, [an agriculture byproduct and rich in the nutrients such as crude protein and vitamins (Gao et al., 2008)], at 20% in the diet was successful in terms of abalone weight gain. However, the work needed to be trialed under commercial farm conditions.

The aim of this paper is to investigate the substitution effect of FM and MA with fermented soybean meal and rice bran in commercial abalone diet on growth performance of juvenile abalone at commercial farm.

2. Materials and methods

2.1 Preparation of abalone and rearing conditions

Juvenile abalone, *H. discus hannai*, were purchased from a private hatchery and transferred to Joeun abalone farm (Wando, Jeollanamdo, Korea). Abalone were acclimated for two weeks and fed with the dry *S. japonica* once a day at the ratio of 2–3% of total biomass. A 6 ton concrete flow-through raceway tank (120 × 690 × 50 cm; water volume: 4.14 ton) at a flow rate of 42.3 L/min with sand filtered seawater (temperature range: 17.9 to 22.8℃ (mean ± SD: 20.2 ± 0.01℃) was divided into 3 sections (120 × 230 × 50 cm; water volume: 1.38 ton) by a plastic-framed net (mesh size of 5 mm), and 4 concrete tanks were used for this experiment. Juvenile abalone (n = 21600) individual averaging 3.6 g were randomly distributed into each section (n = 1800 per section). Aeration was supplied into each raceway and the photoperiod followed natural conditions. The experimental diets were fed to abalone once a day (17:00 h) at a satiation level with a little leftover (about 2–3% of biomass). Dead abalone were removed daily and the tanks were cleaned twice a week. The feeding trial lasted for 16 weeks. At the end of the feeding trial, 200 abalone were randomly sampled from each treatment and collectively weighed.

2.2 Preparation of the experimental diets

Four experimental diets and the dry *U. pinnatifida* and *S. japonica* were prepared (Table 10). Four diets are referred to as follows: a Std, FM50, FM50+MA50 and FM50+MA100 diets. The standard diet (Std) is a commercial juvenile abalone diet and consists of FM and shrimp meal, fermented soybean, what flour and MA (25%, a mixture of *U. pinnatifida* and *Sargassum fusiforme* Harvey at a ratio of 1:1) as the protein and carbohydrate sources and was formulated to satisfy dietary nutrient requirements for (Uki et al., 1986a; Mai et al., 1995a, b). The 50% FM (7% of diet), and combined 50% FM and 50% MA (12.5% of diet), and combined 50% FM and 100% MA (25% of diet) were substituted with the same amount of fermented soybean meal, and combined fermented soybean meal and rice bran, respectively, in the Std diet, referred to as FM50, FM50+MA50 and FM50+MA100 diets. Four diets were pelletized by an extruded pelleter (Jyoda, Japan) in Ewha Oil and Fat Industry Co. Ltd. (Busan, Korea). Pellets were round-shape, and their sizes were 5 mm in diameter (1.5 mm in thickness). *U. pinnatifida* and *S. japonica* were prepared to compare effect of the experimental diets with that of MA on the growth performance of abalone.

Table 10 Feed formulation of the experimental diets (%, DM basis)

	Experimental diets					
	Std	FM50	FM50 + MA50	FM50 + MA100	*U. pinnatifida*	*S. japonica*
Ingredients (%)	14	7	7	7		
Fish meal (FM)[1]	14	7	7	7		
Fermented soybean meal[2]	25	32	32	32		
Shrimp meal	3	3	3	3		
Wheat flour	20	20	20	20		
Spirulina	0.5	0.5	0.5	0.5		
Macroalgae (MA)[3]	25	25	12.5			
Rice bran			12.5	25		
Yeast	1	1	1	1		
Dextrin	5	5	5	5		
Others	4	4	4	4		
Vitamin premix[4]	0.5	0.5	0.5	0.5		
Mineral premix[5]	2	2	2	2		
Nutrients (%)						
Dry matter	98.7	98.8	97.8	98.1	86.0	88.7
Crude protein	36.3	36.1	38.0	38.3	20.7	9.0
Crude lipid	1.4	1.4	3.7	6.8	0.8	0.4
Carbohydrate[6]	42.4	43.5	46.4	46.7	42.4	58.1
Ash	19.9	19.0	11.9	8.2	36.1	32.5

[1]Fish meal (FM): Alaska pollack meal.

[2]Fermented soybean meal was purchased from CJ CheilJedang Corp. (Seoul, Korea)

[3]Macroalgae (MA) were the mixture of *U. pinnatifida* and *Sargassum fusiforme* at a ratio 1:1

[4]Vitamin premix contained the following amount which were diluted in cellulose (g/ kg mix): excipient, 317; riboflavin, 23.8; pyridoxine, 4.7; niacin, 95.2; Ca-pantothenate, 33.3; inositol, 476.9; folic acid, 1.5; p-amino benzoic acid, 47.6.

[5]Mineral premix contained the following ingredients (g/kg mix): Excipient, 45.5; $MgSO_4$, 140.8; NaH_2PO_4, 92.4; KH_2PO_4, 246; $Ca(H_2PO_4)_2$, 139.5; $ZnSO_4$, 22.5; Ca-lactate, 310; $AlCl_3$, 0.15; KI, 0.15; $MnSO_4$, 2; $CoCl_2$, 1.

[6]Carbohydrate was calculated as the difference between 100 and the sum of crude protein, crude lipid and ash contents.

2.3 Analytical procedures of the diets and carcass

Abalone from each treatment were sampled and frozen for chemical analysis (100 at the beginning and 50 at the trial termination). Prior to examination, all samples were slightly thawed, followed by separation of the shell and soft-body tissue. Shell length and width were measured to 1.0 mm using a digital caliper (Mitutoyo Corporation, Kawasaki, Japan), and the ratio of soft body weight to body weight (the soft body weight + the excised shell's weight) was calculated to determine a condition index for abalone. Specific growth rate (SGR, %/body weight gain day) was calculated using the formula:

$SGR = [(\ln(W_f) - \ln(W_i))/\text{days of feeding}] \times 100,$

where $\ln(W_f)$ = natural log of the final mean weight of abalone and $\ln(W_i)$ = natural log of the initial mean weight of abalone Britz (1996).

The pooled separated soft body tissue from the sampled abalone from each diet was then homogenized and used for proximate analysis. Crude protein content was determined by the Kjeldahl method (Auto Kjeldahl System, Buchi B324/435/412, Switzerland), crude lipid was determined using an ether-extraction method, moisture was determined by oven drying at 10 5℃ for 24 h and ash was determined using a muffle furnace at 550℃ for 4 h. all methods were according to AOAC (1990) practices. Amino acid composition of the experimental diets were determined by using a high speed amino acid analyzer (Hitachi L-8800, Tokyo, Japan) after which the samples were hydrolyzed in 6 N HCl for 24 h at 110℃.

2.4 Statistical analysis

One-way ANOVA and Duncan`s multiple range test (Duncan, 1955) were used to determine the significance of the differences among the means of treatments by using SAS version 9.3 program (SAS Institute, Cary, NC, USA). Percentage data was arcsine-transformed prior to statistical analysis.

3. Results

3.1 Amino acids and fatty acids profiles of experimental diets

Amino acid profiles of the experimental diets are given in Table 11. The essential amino acids, such as arginine, isoleucine, lysine, methionine, threonine and valine were relatively low in the FM50 diet, but arginine, histidine, isoleucine, leucine, phenylalanine and valine were relatively high in the FM50+MA50 and FM50+MA 100 diets compared to the Std diet.

Table 11 Amino acid profiles of the experimental diets (%, DM basis)

	Experimental diets					
	Std	FM50	FM50 + MA50	FM50 + MA100	*U. pinnatifida*	*S. japonica*
Alanine	1.90	1.81	1.90	1.87	1.93	0.47
Arginine	2.13	2.05	2.22	2.29	0.73	0.26
Aspartic	3.40	3.44	3.64	3.58	1.40	1.56
Cystine	0.45	0.46	0.50	0.51	0.14	0.10
Glutamic	5.95	6.02	6.43	6.41	1.98	2.56
Glycine	1.95	1.73	1.83	1.80	0.82	0.34
Histidine	0.74	0.75	0.83	0.85	0.22	0.10
Isoleucine	1.44	1.42	1.57	1.55	0.64	0.24
Leucine	2.69	2.69	2.89	2.85	1.11	0.41
Lysine	1.81	1.69	1.82	1.80	0.72	0.28
Methionine	0.57	0.53	0.56	0.61	0.28	0.11
Phenylalanine	1.61	1.63	1.75	1.72	0.37	0.29
Proline	1.84	1.81	1.92	1.96	0.65	0.31
Serine	1.68	1.69	1.70	1.68	0.60	0.28
Threonine	1.42	1.40	1.43	1.40	0.64	0.30
Tyrosine	1.06	1.05	1.13	1.17	0.42	0.18
Valine	1.62	1.59	1.76	1.75	0.78	0.33

Fatty acid profiles of the experimental diets are presented in Table 12. A sum of n-3 highly unsaturated fatty acid (HUFA) content were relatively high in the Std diet compared to that of the FM50, FM50+MA50 and FM50+MA100 diets, especially linolenic acid (18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexanoic acid (DHA, 22:6n-3) decreased in proportion to an amount of substitution of FM and MA with the fermented soybean meal and rice bran, respectively, but linoleic acid (18:2n-6) content increased. No EPA and DHA was also found in MA (*U. pinnatifida* and *S. japonica*) diets.

Table 12 Fatty acid compositions of the experimental diets (% of lipid in the experimental diets)

	Experimental diets					
	Std	FM50	FM50 + MA50	FM50 + MA100	*U. pinnatifida*	*S. japonica*
14:0	3.61	3.21	1.62	1.00	5.59	8.84
15:0	n.d	n.d	n.d	n.d	0.96	n.d
16:0	19.20	19.48	17.42	17.29	27.36	15.50
18:0	2.51	2.54	1.98	1.86	2.70	1.32
20:0	n.d	n.d	0.41	0.46	0.63	n.d
21:0	n.d	n.d	n.d	n.d	0.58	n.d
22:0	n.d	n.d	0.28	0.29	n.d	n.d
24:0	n.d	n.d	0.45	0.49	n.d	n.d
Saturates	25.32	25.23	22.16	21.39	37.82	25.66
14:1n-9	0.29	0.32	n.d	n.d	0.71	0.57
16:1n-9	3.56	2.74	1.33	n.d	0.81	2.75
16:2n-6	n.d	n.d	n.d	0.82	n.d	n.d
17:1n-9	n.d	n.d	n.d	n.d	0.57	0.60
18:1n-9	17.63	17.01	29.37	33.59	11.51	21.06
18:1n-11	4.26	3.88	2.00	1.48	n.d	n.d
20:1n-9	2.98	1.90	1.21	0.95	n.d	n.d
22:1n-9	1.87	1.02	0.48	0.28	n.d	0.62
24:1n-9	0.29	n.d	n.d	n.d	n.d	n.d
Monoenes	30.88	26.87	34.39	37.12	13.60	25.60
C18:2n-6	26.02	31.24	34.40	36.31	6.11	8.51
C22:2n-6	n.d	n.d	n.d	n.d	0.57	n.d
C18:3n-3	3.07	3.94	2.65	2.18	6.19	6.44
C18:3n-6	n.d	n.d	n.d	n.d	0.89	3.14
C18:4n-3	1.39	1.55	0.94	0.36	10.27	7.03
C20:3n-3	0.53	0.51	0.42	n.d	10.68	14.58
C20:3n-6	n.d	n.d	n.d	n.d	0.52	0.60
C20:4n-3	0.29	n.d	n.d	n.d	0.49	n.d
C20:5n-3	6.09	5.24	2.68	1.45	n.d	n.d
C22:5n-3	0.73	0.50	n.d	n.d	5.76	6.28
C22:6n-3	5.00	4.34	2.08	1.19	n.d	n.d
∑n-3 HUFA	12.64	10.59	5.18	2.64	17.45	21.46
Unknown	0.69	0.56	0.27	n.d	7.09	2.15

3.2 Growth performance of abalone

Survival of abalone fed the all formulated diets was significantly higher ($P < 0.0001$) than that of abalone fed the *U. pinnatifida* and *S. japonica* (Table 13). Survival of abalone fed the *U. pinnatifida* was significantly higher ($P < 0.05$) than that of abalone fed the *S. japonica*. Weight gain (g/abalone) and SGR (%/day) of abalone fed the Std diet were significantly higher ($P < 0.0001$) than those of abalone fed the all other diets, and followed by the FM50, FM50+MA50 and FM50+MA100 diets, *U. pinnatifida* and *S. japonica* in that in order.

The longest shell length was obtained in abalone fed the Std diet, followed by the FM50, FM50+MA50 and FM50+MA100 diets, *S. japonica*, and *U. pinnatifida* (Table 14). The widest shell width was observed in abalone fed the Std diet, and followed by the FM50, FM50+MA50 and FM50+MA100 diets, *U. pinnatifida*, and *S. japonica*. Shell height of abalone fed the formulated (Std, FM50, FM50+MA50 and FM50+MA100) diets were not significantly different among treatments, but significantly taller ($P < 0.0009$) than that of abalone fed the *U. pinnatifida* and *S. japonica*. The soft body weight of abalone fed the Std diet was significantly heavier ($P < 0.002$) than all other diets, except for the FM50 diet. However, the ratio of the soft body weight to total weight of abalone was not significantly ($P > 0.4$) different among the diets.

Table 13 Survival (%), weight gain (g/abalone) and specific growth rate (SGR) of juvenile abalone fed the experimental diets for 16 week

Experimental diets	Initial weight (g/abalone)	Final weight (g/abalone)	Survival (%)	Weight gain (g/abalone)	SGR[1] (%/day)
Std	3.6 ± 0.00	7.5 ± 0.01[a]	85.1 ± 0.48[a]	3.87 ± 0.011[a]	0.63 ± 0.001[a]
FM50	3.6 ± 0.00	6.5 ± 0.01[b]	84.8 ± 0.75[a]	2.94 ± 0.005[b]	0.52 ± 0.000[b]
FM50 + MA50	3.6 ± 0.00	6.1 ± 0.01[c]	84.0 ± 0.48[a]	2.49 ± 0.014[c]	0.46 ± 0.002[c]
FM50 + MA100	3.6 ± 0.00	6.0 ± 0.02[c]	84.9 ± 0.11[a]	2.40 ± 0.021[c]	0.44 ± 0.003[c]
U. pinnatifida	3.6 ± 0.00	5.6 ± 0.04[d]	80.4 ± 0.18[b]	2.00 ± 0.038[d]	0.38 ± 0.006[d]
S. japonica	3.6 ± 0.00	4.6 ± 0.06[e]	72.4 ± 0.27[c]	1.02 ± 0.063[e]	0.22 ± 0.012[e]

Values (means of duplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

[1]Specific growth rate (SGR) = $[(\ln(W_f) - \ln(W_i))/\text{days of feeding}] \times 100$, where $\ln(W_f)$ = natural log of the final mean weight of abalone and $\ln(W_i)$ = natural log of the initial mean weight of abalone.

Table 14 Shell length (mm), shell width (mm), shell height (mm), soft body weight (g/individual) and the ratio of soft body weight to total weight of abalone fed the experimental diets for 16 week

Experimental diets	Shell length (mm)	Shell width (mm)	Shell height (mm)	Soft body weight (g)	Soft body weight/total weight
Std	41.7 ± 0.14^{a}	28.5 ± 0.01^{a}	8.6 ± 0.14^{a}	4.3 ± 0.05^{a}	0.61 ± 0.004
FM50	40.6 ± 0.05^{b}	28.0 ± 0.33^{ab}	8.6 ± 0.01^{a}	4.0 ± 0.02^{ab}	0.60 ± 0.003
FM50 + MA50	40.0 ± 0.10^{c}	27.2 ± 0.41^{b}	8.6 ± 0.20^{a}	3.8 ± 0.21^{b}	0.60 ± 0.013
FM50 + MA100	39.7 ± 0.00^{d}	27.1 ± 0.09^{b}	8.3 ± 0.12^{a}	3.7 ± 0.04^{b}	0.61 ± 0.004
U. pinnatifida	38.1 ± 0.08^{e}	25.9 ± 0.02^{c}	7.2 ± 0.05^{b}	3.1 ± 0.08^{c}	0.61 ± 0.002
S. japonica	38.2 ± 0.00^{e}	25.9 ± 0.05^{c}	7.2 ± 0.13^{b}	3.1 ± 0.05^{c}	0.60 ± 0.006

Values (means of duplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

3.3 Proximate composition of the soft body of abalone

Moisture and ash content of the soft body of abalone was not significantly ($P > 0.6$) different among the diets (Table 15). However, crude protein content of the soft body of abalone fed the FM50+MA100 diet was significantly higher ($P < 0.05$) than that of abalone fed the all other diets. Crude protein content of the soft body of abalone fed the FM50+MA50 diet was also significantly higher ($P < 0.0003$) than that of abalone fed the *U. pinnatifida* and *S. japonica*, but not significantly different from that of abalone fed the Std and FM50 diets. Crude lipid content of the soft body of abalone fed the FM50+MA50 and FM50+MA100 diets was significantly higher ($P < 0.0002$) that of abalone fed the all other diets. Crude lipid content of the soft body of abalone fed the Std and FM 50 diets was also significantly higher that of abalone fed the *U. pinnatifida* and *S. japonica*. Crude protein and lipid content of the soft body of abalone was directly reflected from dietary protein and lipid contents.

Table 15 Chemical composition (%, wet weight basis) of the soft body of abalone at the end of the 16-week feeding trial

Experimental diets	Moisture	Crude protein	Crude lipid	Ash
Std	77.3 ± 0.20[a]	16.8 ± 0.16[bc]	0.9 ± 0.04[b]	2.8 ± 0.12[a]
FM50	77.8 ± 0.12[a]	16.7 ± 0.08[bc]	0.8 ± 0.08[b]	2.7 ± 0.04[a]
FM50 + MA50	77.5 ± 0.69[a]	17.2 ± 0.08[b]	1.6 ± 0.08[a]	2.5 ± 0.00[a]
FM50 + MA100	76.9 ± 0.08[a]	18.4 ± 0.12[a]	1.7 ± 0.08[a]	2.5 ± 0.04[a]
U. pinnatifida	77.0 ± 0.04[a]	16.3 ± 0.16[c]	0.6 ± 0.04[c]	2.9 ± 0.04[a]
S. japonica	77.1 ± 0.00[a]	15.8 ± 0.12[d]	0.6 ± 0.04[c]	2.7 ± 0.04[a]

Values (means of duplicate ± SE) in the same column sharing the same superscript letter are not significantly different ($P > 0.05$).

4. Discussion

The several essential amino acids, such as arginine, isoleucine, lysine, methionine, threonine and valine were low in the FM50 diet, but arginine, histidine, isoleucine, leucine, phenylalanine and valine were high in the FM50+MA50 and FM50+MA 100 diets compared to the Std diet (Table 11). All essential amino acids were found, but relatively low in the *U. pinnatifida* and *S. japonica* in this chapter, agreeing with Daweczynski et al. (2007)'s study. Mai et al. (1994) also reported that the several essential amino acids, such as arginine, methionine, threonine and histidine were the limiting factor in 6 species of MA, *Ulva lactuca* Linnaeus, *Chondrus crispus* Stackh, *Palmaria palmata* Linnaeus, *Alaria esculenta* (Linnaeus) Greville, *Laminaria digitata* Hudson and *S. latissima* (Linnaeus) C. E. Lane, C. Mayes, Druehl & G.W. Saunders, for abalones, *H. tuberculata* Linnaeus and *H. discus hannai*. Histidine was also reported to be a limiting factor when fish meal was substituted with various sources of animal and/or plant protein sources (Cho, 2010).

A sum of n-3 HUFA content were high in the Std diet compared to that of the FM50, FM50+MA50 and FM50+MA100 diets, especially linolenic acid, EPA and DHA decreased in proportion to an amount of substitution of FM and MA with the fermented soybean meal and rice bran, respectively, but linoleic acid content increased. Similar EPA and DHA contents in soybean and rice bran oils were reported by Krishna et al. (2006) and Grisdale-Helland et al. (2002). No EPA and DHA was also found in MA, *U. pinnatifida* and *S. japonica*, diets in this chapter, partially agreeing with

Dawezynski et al. (2007)'s study showing that 13.2 and 16.2% of EPA were detected in *U. pinnatifia* and *S.* spp but no DHA was detected in all seaweeds investigated. The requirement of n-3 HUFA was reported to be 1% in the diet containing 5% lipid, being equivalent to 0.05% in the diet for abalone (*H. discus hannai*) (Uki et al., 1986b). However, the requirement of n-3 HUFA was all satisfied in the all diets, and ranged from 0.09% (*S. japonica*) to 0.19% (FM50+MA50 diet). Mai et al. (1996) reported that EPA played a prominent role for 2 species of abalone (*H. tuberculata* and *H. discus hannai*) and n-3 and n-6 polyunsaturated fatty acid (PUFA) seemed to be essential for abalone (*H. discus hannai*).

Poor performance in survival, weight gain and SGR of abalone fed the *U. pinnatifida* and *S. japonica* could be explained by the poor nutritional quantity and quality of essential amino and fatty acids in these MA (Tables 11 and 12). Similarly, MA commonly used as feed in wild did not produce the optimal performance of abalone (Lee, 1998; Cho et al., 2006; Kim et al., 1998; Naidoo et al., 2006; Cho et al., 2008; Gracia-Esquivel and Felbeck, 2009; Dang et al., 2011; Myung et al., 2016).

Poorer weight gain of abalone fed the FM50, FM50+MA50 and FM50+MA100 diets was observed compared to that of abalone fed the Std diet in this chapter. However, unlike this chapter, Cho (2010) showed that FM at 35% could be completely replaced with soybean meal at 58% with 0.5% methionine supplementation in the diet for juvenile abalone. The differences in performance of abalone between this chapter and Cho (2010)'s study could have resulted from the lack of methionine supplementation in

this chapter. However, Lee et al. (1998a) reported that the plant protein sources (soybean meal or cotton seed meal) without supplementation of amino acids and are likely to be deficient in plant protein sources could replace casein or FM in the diet for abalone (*H. discus hannai*). Fish meal could also be successfully replaced with a combined animal and plant protein sources in abalone diets (Guzman and Viana, 1998; Bautista-Teruel et al., 2003; Cho, 2010).

Another reason for the poorer weight gain of abalone fed the FM50, FM50+MA50 and FM50+MA100 diets compared to that of abalone fed the Std diet could be due to the fermentation of soybean meal in this chapter. Abalone may not utilize the fermented soybean meal well. This is why all 50% fish meal substituted diets with the fermented soybean meal regardless of substitution of MA with rice bran produced poorer weight gain in this chapter. Similarly, juvenile abalone (*H. discus*) fed the FM basal diet with 25% fermented soybean meal achieved poorer weight gain than that fed the FM basal diet with 25% soybean meal in the 16-week feeding trial (Kim et al., 2017). Nutritional values of fermented soybean meal varied depending on physical conditions of fermentation (Kook et al., 2014). More studies to elucidate the reason why abalone fed the diet containing fermented soybean meal showed poorer growth performance than that fed diet containing soybean meal is needed. Unlike this chapter, however, dietary inclusion of the fermented plant protein sources, such as soybean meal and cottonseed meal produced promising effect on performance of fish (Lim and Lee, 2011; Zhou et al., 2011).

Unlike Kim et al. (2016)'s study, 50% and 100% substitution of MA with rice bran in the FM50+MA50 and FM50+MA100 diets, respectively, produced poorer weight gain of abalone compared to the Std and FM50 diets in this chapter. The differences could have resulted from those in MA used in both studies. MA used in this chapter are the mixture of *U. pinnatifida* and *S. fusiforme* at a ratio of 1:1, but a single *S. japonica* was used in the former. Abalone fed the diets (Std and FM50 diets) containing the mixture of *U. pinnatifida* and *S. fusiforme* in this chapter outgrew over that fed the control diet containing *S. japonica* in Kim et al. (2016)'s study. The mean SGR was 0.58%/day in this chapter, but 0.46%/day for the latter. Therefore, substitution effect of MA with rice bran in this chapter was probably masked. The combined MA produced slightly, but not significantly, improved weight gain of abalone (*H. discus hannai* and *H. laevigata* Donovan) over a single MA (Qi et al., 2010; Dang et al., 2011).

No difference in weight gain and SGR of abalone fed the FM50+MA50 and FM50+MA100 diets (Table 13) in this chapter could indicate that another 50% MA in the commercial diet could be substituted with rice bran as long as 50% MA were substituted with rice bran. Abalone are known to be herbivorous and feed mostly on MA, which is usually low in protein and lipid, but high in carbohydrate, 40-50% in the wild (Thongrod et al., 2003). Unlike this chapter, however, Kim et al. (2016) reported that 40% substitution of *S. japonica* with rice bran at 20% in the diet achieved the best specific growth rate of abalone, *H. discus* and concluded that 100% substitution of *S. japonica* with rice bran at 20% in the diet was

successfully made without retardation of weight gain when juvenile abalone were fed on the experimental diets trialed over *S. japonica*. However, a 16 week trial may not be a sufficiently long enough time give that abalone remain on farms for considerably longer periods. Similarly, the leaf meal (*Moringa oleifera* Lamarck) and freshwater aquatic fern (*Azolla pinnata* R. Brown) are promising alternative feed ingredients for *H. asinina* (Linnaeus) culture (Reyes and Fermin, 2003).

Biological criteria of abalone measured in this chapter (shell length, shell width, shell height and soft body weight), except for the ratio of the soft body weight to total weight seemed to be closely related to growth rate of abalone. Similarly, biological criteria of abalone agreed with growth rate of abalone (Bautista-Teruel et al., 2003; Cho, 2010; Myung et al., 2016).

Crude protein and lipid content of the soft tissue directly related to dietary protein and lipid contents in this chapter and agreed with other studies (Mai et al., 1995a, 1995b; Thongrod et al., 2003; Cho et al., 2008; Garcia-Esquivel and Felbeck, 2009; Cho, 2010; Kim et al., 2016; Myung et al., 2016).

In conclusion, the substitutability of fermented soybean meal for fish meal seems to be rather limited in EP in commercial abalone farm. However, another 50% MA in the commercial diet could be substituted with rice bran as long as 50% MA were substituted with rice bran in EP.

Chapter 5.

General Discussion

Abalone feeding with well-formulated diets is highly recommended over feeding MA alone because the former diets are available year-around and their nutrient contents can be easily manipulated to satisfy the requirements. Growth performances of abalone have been improved by balanced diets in several studies that make comparisons with MA feeding alone (Lee 1998; Kim et al., 1998; Cho et al., 2006, 2008; Jung et al., 2016; Lee et al., 2016; Myung et al., 2016; Kim et al., 2017; Lee et al., 2018).

The natural diet of abalone comprises 40–50% carbohydrates, which is an important nonprotein energy source for abalone. The digestive and metabolic systems of animal help to utilize dietary carbohydrates effectively and thus helps to adapt with different aquatic environments and various levels of carbohydrates (Bergot, 1979; Walton and Cowey, 1982). Montano-Vargas et al. (2005) reported that carbohydrates are preferred over lipids as energy sources in pink abalone, and lipid levels are optimally minimized to levels that meet essential fatty acids requirements. Moreover, it has been shown that high dietary lipid contents negatively affect the performance of abalone by limiting the consumption of feed (Mai et al., 1995a; Britz and Hecht, 1997; Gómez-Montes et al., 2003; Thongrod et al., 2003). Hence, low lipid content (<5%) is commonly recommended for commercial abalone feed (Fleming et al., 1996; Bansemer et al., 2014).

In the first study, the highest survival and weight gain (SGR) were obtained in abalone fed the C48:L2 and C47:L3 diets, respectively, indicating that the optimal dietary carbohydrate : lipid ratio was estimated to be in the region of 48:2 and 47:3 for performance of juvenile abalone. These values (15.7–24.0) are relatively high when compared with those reported for herbivorous fish (4.0–11.2) and carnivorous fish (2.0–3.2), as shown in Table 5. The fastest weight gain of abalone fed the C47:L3 diet also corresponded with the largest values for other biological criteria including shell length, shell width, shell height, soft body weight, and the ratio of soft body weight to total weight of abalone. Crude protein and lipid and ash content retained slowly decreased with time throughout 48 h observation, except for the crude lipid content in the C49:L1 and C48:L2 diets.

The effects of carbohydrate supplements on aquatic animals remain controversial. In a study of 5–25% cellulose, glucose, maltose, and dextrin supplements, olive flounder achieved better weight gains on diets containing 15% maltose and 15–25% dextrin than on diets containing 15% cellulose and 5% dextrin over 45 days (Lee et al., 2003). Rahman et al. (2016) also showed that potato starch and dextrin are more effectively used as energy sources by olive flounder than wheat flour, corn starch, Na alginate and carboxymethyl cellulose. Although weight gain and SGR of gilthead sea bream were not affected by various sources of carbohydrate (maltose, dextrin, starch or glucose), administration of maltose, dextrin or starch would be better than glucose as energy source based on feed utilization (Enes et al., 2010). In addition, Tan et al. (2006) compared the performances of gibel

carp and Chinese longsnout catfish fed on diets containing glucose, dextrin, soluble starch, or α−cellulose, and concluded that the dietary availabilities of these carbohydrate sources varied between food habitats. Abalone have high levels of the digestive enzymes protease, amylase, cellulase and alginase, but low levels of lipases (Emerson, 1967; Gomez-Pinchetti and Garcia-Reina, 1993; Britz et al., 1994; Garcia-Esquivel and Felbeck, 2006). Therefore, determination of optimal carbohydrate sources are required to improve growth performances of abalone.

In this chapter, weight gain and SGR of abalone fed the experimental diets were greater than those of abalone fed the *U. pinnatifida*. The results that weight gain and SGR did not differ among carbohydrate sources, except for the CL diet achieving the greatest weight gain and SGR of abalone, was partially consistent with Lee et al. (1998c)'s study showing that weight gain of juvenile abalone (*H. discus hannai*) was not affected by the type of carbohydrate in the diet (they tested diets containing 24.2% WF, 20% DT, 20% SC, 10% each of α- and β-potato starch, 15% α-potato starch, 20% α -potato starch or 25% α-potato starch). Abalone fed the CL diet had the greatest shell growth and the heaviest soft bodies.

Abalone farmers remain likely to feed their cultures with MA alone, particularly as the MA (*U. pinnatifida* and *S. japonica*) are easy to manage in farms in Korea. Hence, the development of alternative sources for FM and MA in abalone feed has received much scientific attention and interests. Fermented soybean meal was used as a substitute for FM in several species, including black sea bream (Zhou et al., 2011; Azarm and Lee, 2014),

rainbow trout (*Oncorhynchus mykiss* Walbaum) (Barnes et al., 2012), and fresh prawn (*Macrobrachium nipponense* De Haan) (Ding et al., 2015). Lee et al. (1998a) demonstrated that soybean meal, casein, FM, and cotton seed meal are good protein sources for abalone (*H. discus hannai*). Kim et al. (2016) also replaced up to 100% of MA (*S. japonica*) in feed with rice bran and showed no retardation of abalone (*H. discus*) growth performance when 20% *S. japonica* was included. These data urgently require validation of suitability and feasibility for practical applications to EP in a commercial abalone farm.

In the third study, poorer weight gain of abalone fed the FM50, FM50+MA50 and FM50+MA100 diets was observed compared to that of abalone fed the Std diet. Unlike Kim et al. (2016)'s study, 50% and 100% substitution of MA with rice bran in the FM50+MA50 and FM50+MA100 diets, respectively, produced inferior weight gain of abalone to abalone fed the Std diet. In considering the market price of feed ingredient used on May 2019, however, the feed cost is decreased with the increased amount of substituted fermented soybean meal and rice bran for FM and MA (Fig. 4) by approximately 1, 12 and 23% feed cost in the FM50, FM50+MA50 and FM50+MA100 diets, respectively, expressed as 100% of feed cost in the Std diet. Although inferior growth performance (weight gain and SGR) of abalone receiving the FM50, FM50+MA50 and FM50+MA100 diets to that of abalone fed the Std diet were achieved, it would be beneficial to abalone farmers to consider feed cost of EP substituting the fermented soybean meal and rice bran with FM and MA, respectively.

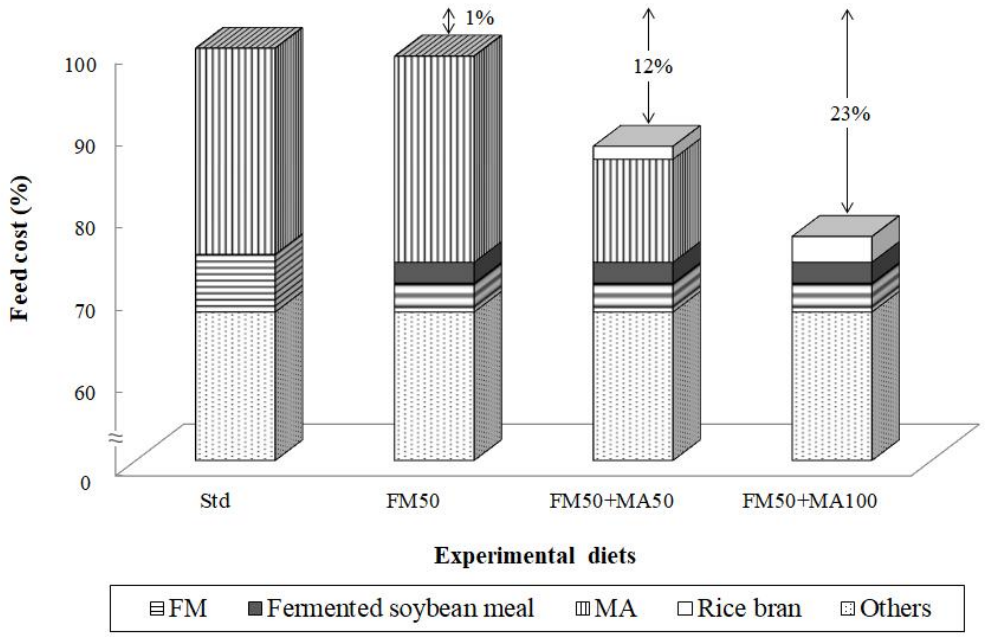

Fig. 4. Feed cost of the EP substituting the fermented soybean meal and rice bran with FM and MA, respectively used in the 3rd study.

In conclusion, the optimal dietary carbohydrate : lipid ratio was estimated to be 48:2 and 47:3 based on weight gain and SGR of abalone, respectively. Higher than 3% lipid content in diet deteriorated performance of abalone in the first study. Moreover, among various dietary carbohydrates, α-celllose was the most effective at improving growth performance of abalone. In the third study, substitutability of fermented soybean meal for fish meal seems to be rather limited in the extruded pellet, but further 50% MA in the extruded pellet could be substituted with rice bran as long as 50% MA were substituted with rice bran in commercial abalone farm. These informations will be practically helpful to develop the nutrition-balanced feed for abalone culture.

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