Mitigating Hypoxic Stress in Juvenile Abalone (*Haliotis squamata*) through Optimized Carbohydrate Diets: Insights into Survival and Biochemical Adaptations

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Abstract

Abalone (Haliotis squamata) is a high value marine mollusk with significant aquaculture potential in Indonesia. However, the production of this species faces major constraints, especially during the grow-out phase, due to high juvenile mortality during transport, primarily caused by hypoxic stress. This study investigated the effect of high carbohydrate diets on the physiological response and survival of juvenile abalone under simulated dry shipping-induced hypoxia. A total of 120 juveniles were fed five diets (Gracilaria sp. as a control, and formulated feeds with 25%, 35%, 45%, and 55% carbohydrate content) for 30 days prior the hypoxia treatment. The juveniles were then subjected to hypoxic conditions for 6, 12, and 24 h. All abalones survived the feeding period, but only individuals fed with 55% carbohydrate survived after 24 h of hypoxia. Abalones receiving 45% and 55% carbohydrate diets showed the highest protein and carbohydrate accumulation, respectively. Biochemical analysis revealed that hypoxia triggered a decrease in protein content, stable lipid levels, and fluctuating carbohydrate reserves. These results highlight the critical role of carbohydrates in supporting abalone under hypoxic stress and suggest that carbohydrate enriched diets could reduce mortality during dry transport and improve aquaculture outcomes.

Keywords: *carbohydrate, survival, Haliotis squamata, biochemical composition, hypoxia.*

Introduction

One of the marine mollusks with a high economic value and high potential to be further developed in Indonesia is abalone (*Haliotis* spp.). The price of abalone reaches up to IDR 700,000 kg^{-1} in Indonesia, with the market value reached USD 700,000 in 2019 (Dwi and Hollanda, 2023). International market demand for Abalone from Indonesia has been reported to increase, with the USA is the main importer country (about 85% from Indonesia's total export) (Cook, 2019; Dwi and Hollanda, 2023). Abalone has a distinctive meat flavor and high nutritional value with a good nutritional content of 71.99% protein, 3.2% fat, 5.6% crude fiber, and 0.6% water content (Sososutiksno *et al*., 2017). Globally, total abalone production in 2017 reached 174,162 tons, an increase of 34.7% from total production in 2015. The data was derived from the global abalone sector based on catches from nature and the results of aquaculture activities (Cook, 2019). In 2020, Indonesian abalone exports amounted to 262,362 kg with a value of 4.8 million USD (BPBLL, 2021). Considering these conditions, there is a need to develop abalone commodities in Indonesia to position them as premium species for export in the marine aquaculture sector.

Some abalone farmers are beginning to develop their operations, however, they often face difficulties ranging from hatchery challenges to grow-out process. Most abalone production still relies on capture activities from the wild (Taridala *et al*., 2021). Currently, there have been some established abalone hatcheries in Indonesia, mainly located in Karangasem (Bali) and Sekotong (Lombok). These hatcheries have regularly supplied abalone seeds distributed to abalone farmers across Indonesia. However, one of the main challenges faced by those abalone farmers is high mortality of abalone juveniles during transfer from hatcheries to the grow-out site. It has been reported that the highest mortality rate

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occurs when juveniles are transferred from the substrate to the grow-out site (Nguyen *et al*., 2022). Factors of abalone juvenile mortality during seed transfer may occur due to internal (body energy availability) and external (inaccurate handling methods) factors (Gao *et al*., 2023). Transportation stress can stimulate and suppress the immune system of this species (Setyaningsih *et al*., 2020).

Abalone seed delivery methods in Indonesia usually use dry, semi-wet, and wet methods (Sawangwong *et al*., 2019). From all of these delivery methods, dry shipping system is highly used due to its easy and cheap operation. However, shipping abalone seeds using a dry system increases the chance of placing this species in hypoxia conditions. In dry shipping activities, the mortality rate is quite high, ranging between 20-30%, and can even reach 50-100% (Gao *et al*., 2023). Hypoxia is a stress condition characterized by oxygen deficiency at the tissue level, which can be caused by low blood sugar supply or low oxygen content in the blood (Gossman *et al*., 2019). Therefore, abalone relies heavily on anaerobic metabolism to partially compensate for energy production during times of oxygen deprivation (Venter *et al*., 2018a). Abalones, such as *Haliotis midae* and *Haliotis discus hannai*, show a metabolic shift towards anaerobic metabolism during hypoxia, both species utilizing various metabolites for energy production (Shen *et al*., 2019).

Under hypoxic conditions, the increased energy demands lead to the release of metabolites from cellular stores, which are then transported to various organs to support essential functions (Venter *et al*., 2018b). Carbohydrate, in form of glucose molecules has been reported to be primary energy source for animals during hypoxic condition (Guo *et al*., 2022). In the absence of oxygen, animals including abalone undergo anaerobic metabolism through anaerobic glycolysis which provide energy to maintain basal metabolism for survival mechanism (de Zwaan and Wijsman, 1976). Prior studies in hybrid abalone *H. discus hannai* × *H. fulgens* showed that this hybrid species mobilized more carbohydrates when facing reduced oxygen conditions and used greater energy reserves in hypoxia conditions (Venter *et al*., 2018a; Shen *et al*., 2021). This suggests that carbohydrate is essential nutritional component in feed in response to hypoxic stress, a common condition during abalone shipping.

Acknowledging the importance of carbohydrate as essential energy source during anaerobic condition of abalone shipping, there is a lack of study investigating the role of high carbohydrate feeding on physiological responses of juvenile abalone of *Haliotis squamata* abalone seeds exposed to hypoxic conditions. Therefore, this research aimed to examine the role of high concentrations of carbohydrate feeding on the physiological response of juvenile abalone *H. squamata* exposed to hypoxia conditions in a dry shipping simulation setup. The results are expected to stimulate the development of technological innovation in abalone feed to reduce high mortality during seed shipping, thereby optimization of abalone production in Indonesia can be achieved.

Materials and Methods

Materials used in this research include 1.75 x 0.5 x 0.7 m^3 concrete tanks for experimental feeding, a 20L Styrofoam container box for hypoxia experimental challenge, Stereo Microscope (Nikon, SMZ-745, USA), experimental container with 2 m diameter and 50 cm height, analytical balance, ABJ 120-4NM, KERN, Germany), tweezers with < 1 mm tip (Jakemy, JM-T2_14), dropper pipette of 1 ml (A3K, Indonesia), aerator (Resun, LP 100, Indonesia), thermometer (AquaOne), round of Polyvinyl chloride (PVC) pipe of diameter size 3-inch with 12 cm length and equipped with mesh net size of 1 inch at both edge holes.

Approximately 120 juvenile abalone *Haliotis squamata* with an average length of ~2-3 cm and weight of ~2.8±0.15 g in this study were obtained from Karangasem Superior Shrimp and Shellfish Broodstock Production. These abalone were fed with both natural and artificial diets which high in carbohydrate concentrations. The natural diet which play as control was seaweed *Gracillaria* sp, meanwhile, and the artificial diets were pellet with various concentrations of carbohydrate, *i.e*., 25%, 35%, 45%, and 55% (Tabel 1). Sea water was used as the media to rear juvenile abalone at acclimation time prior to the hypoxia experiment.

This experiment was performed based on approval by the laboratory of aquatic animals at Marine aquaculture research center licensed by ISO (No.17025:2017 LP - 566 IDN) and did not require animal ethics following national guidance of research ethics clearance established by the Indonesian Institute of Sciences No. 1528, 2019.

Abalone conditioning and experimental feeding system

Prior to the experiment, abalone were reared in five concrete tanks (1.75 x 0.5 x 0.7 m^3), with a water flow system, each holding 437 L for conditioning. Abalone were placed in experimental plastic baskets of 0.35 x 0.25 x 0.08 m^3 with of 5 x 5 mm^2 of perforation. Shelters were made by halving a five-inch piece of PVC pipe. To place the feeds, a 0.7 mm mesh net was cut to fit inside of each abalone basket. To ensure abalone baskets remained in place in the concrete tank, each basket was secured with a piece of wood positioned across it and weighted down with a stone placed on top. To maintain the dissolved oxygen (DO) level, aeration stones were placed at the tank's bottom. Each tank's water supply was kept at 43.7 L per minute. Feeding experiments were also conducted using this conditioning-rearing method.

Abalone were reared for 13 days of conditioning prior to feeding experiment. The first three day of conditioning, all juvenile abalones were fed with 130 g dry weight of *Gracilaria* sp. macroalgae at ~ 5% from its biomass. At day 4 of conditioning, experimental formulated pellets were introduced for the next 10 days of acclimation time at ~ 5% from its biomass. Salinity, pH, DO, and water temperature were maintained at approximately 33 ppt, 8, 5 $mg.L^{-1}$, and 29°C, respectively. To examine the dry matter loss of both *Gracilaria* sp. and pellets, *Gracilaria* sp. and four feeding experiment meals were tested in different experimental tanks (without abalone), in a triplicate set up. *Gracilaria* sp. and pellets were then collected after 24 h and dried for 24 h at 105 °C in an oven (O'Mahoney *et al*., 2014).

After the acclimatization, juvenile abalone of *Haliotis squamata* were then fed with five experimental diets, consisting of 100% *Gracilaria* sp.(control) and four formulated diets with different levels of carbohydrate, i.e., 25%, 35%, 45%, and 55%. Experimental feedings were conducted for 30 d. The composition of formulated diets with different carbohydrate levels were available on Table 1. After 30 d of the experimental feeding, biochemical profiles (*i.e*., protein, lipid, and carbohydrate) of abalone from each feeding treatment were measured as the baseline (0 h) energy profile of juvenile abalone prior to hypoxia condition treatments.

Hypoxia experimental design

The hypoxia test in the current experiment was set up by placing abalone into a round of PVC pipe with a diameter of 3 inches and length of 12 cm. Both PVC pipe holes were covered by using 1 inch of mesh net to ensure abalone remain inside the pipes. Abalone from each feeding treatment (*i.e*., control, 25%, 35%, 45% and 55% carbohydrate pellet) were placed the pipe at a density of 15 abalones. The pipes containing juvenile abalone were then placed into a styrofoam box of 70×40×30 cm without water. Ice cube were placed inside the styrofoam box to maintain the temperature ~<15°C. The styrofoam containing juvenile abalone were then kept in room temperature for hypoxia experimental test (Figure 1).

Table 1. Feed composition and proximate analysis of artificial diets for juvenile abalone *Haliotis squamata*

Feed Composition					
Feed source ($g.100\ g^{-1}$)	Carbohydrate concentrations				
	Control (0%)	25%	35%	45%	55%
Fish meal	0	36	35	11	4
Head shrimp meal	0	5	10	28	5
Spirulina meal	0	10	5	5	15
Soybean meal	0	35	20	14	5
Wheat meal	0	2	10	16	27
Corn meal	0	2	10	16	34
Alginate meal	0	3	3	3	3
Fish Oil	0	1	1	1	1
Squid Oil	0	1	1	1	1
Vitamin	0	1	1	1	1
Mineral	0	1	1	1	1
Binder	0	2	2	2	2
MSG (Monosodium Glutamate)	0	1	1	1	1
Total	0	100	100	100	100
Proximate analysis					
Nutrients	Carbohydrate concentrations				
	Control	25%	35%	45%	55%
Protein (%)	20,12	33,21	33,24	26,02	20,4
Fat (%)	4,7	12,05	9,36	9,04	7,02
Carbohydrate (%)	33,50	25,37	35,56	45,52	55,33
Others (%)	41,68	29,37	21,84	19,42	17,25
Total (%)	100	100	100	100	100

The hypoxia duration used were 6, 12, and 24 h. Survival rate of abalone was assessed by counting live and death abalone every hypoxia duration (6, 12, 24 h). Any death abalone was removed from the styrofoam box. The calculation of the survival of juvenile abalone was measured based on (Sanjayasari and Jeffs, 2019). To measure the biochemical profiles (protein, lipid and carbohydrate content) of abalone, a triplicate of abalone meat from each feeding treatment were sampled at every 6, 12 h. Abalone meat samples were then kept in -20°C for later analysis.

Biochemical test

All juvenile abalone samples from all feeding treatment prior the hypoxia condition (0 h) and from all hypoxia treatments of 6, and 12 h were obtained and analyzed for their biochemical profile (*i.e*., protein, lipid, and carbohydrate). However, due to limited sample in the 24 h of hypoxia treatments, the biochemical composition from 24 h of hypoxia experimental set up was excluded in the statistical analysis and the result, therefore the statistical analysis and the biochemical result only for 0 h (prior the hypoxia treatment), 6 h and 12 h of the hypoxia treatments. All these samples were freeze-dried for 48 h in a Labconco 187508 freeze dryer and ground into powder before being subjected to biochemical analysis. A triplicate powder samples of 20–50 mg dry weight from each feeding treatment and hypoxia duration treatment were subjected to protein analysis using the bicinchoninic acid (BCA) method, with bovine serum albumin (BSA) standard serving as a reference following Supono *et al*. (2023). Using an Elisa Reader (BioTek Elx800, USA), the extracted protein in abalone samples were read against the BSA standard at a wavelength of 562 nm. For lipid analysis, triplicate samples of 50 mg dry weight from each feeding treatment were subjected to lipid analysis following the method of (Wang *et al*., 2014) based on the methanol-chloroform extraction method. For carbohydrate content measurement, a triplicate dry weight samples (20-50 mg) were used following Supono *et al*. (2023). The carbohydrate content was analyzed using a phenol-sulfuric acid method (Dubois *et al*., 1956), with a D-glucose standard as a reference at a wavelength at 490 nm (Masuko *et al*., 2005). Triplicate dry-weight samples (20-50 mg) we used, following (Supono *et al*., 2023). The analysis of AFDW (ash-free dry weight) was conducted by burning a triplicate of 25 mg of dry weight sample in a muffle furnace (Nabertherm L 15/12 B400, Germany) at 550°C for 4 h. Ash samples were weighed and AFDW was employed as a correction factor for proximate content, which was computed for each sample to calculate the value of protein, lipid, and carbohydrate.

Statistical analysis

All the mean of variables measured i.e., survival, and biochemical profile (i.e., protein, lipid, and carbohydrate) of the body of juvenile abalone across different feeding treatments prior to hypoxia treatments and after each hypoxia duration where analyzed their normality and homogeneity of variance. All percentage data were arcsine square root transformed prior to analysis. A one-way analysis of variance (ANOVA) was used when all data met normality and homogeneity of variance. A Tukey post-hoc test was used at a significant level of 0.05 for further testing when analysis showed statistical differences among different treatments.

Results and Discussion

The mean survival percentage of juvenile abalone after 30 days of experimental feeding prior the hypoxia treatments (0 h of hypoxia) was not statistically different among feeding treatments ($P>0.05$). All abalone from five feeding treatments showed 100% survival. This suggests that formulated feed tested at a range of 25% to 55% carbohydrate contents in this study can be used to replace natural feed (*Gracilaria* sp.) without compromising abalone survivals. Similar response on abalone survival fed with an increased carbohydrate content in formulated feed has also been reported in Japanese abalone *Haliotis discus hannai,* with 38% carbohydrate content in feed showing the highest survival rate. The appropriate level of carbohydrate content in aquatic animal feed has been found to enhance growth, and feed utilization, reduce protein and lipid catabolism, and provide metabolic substances for the synthesis

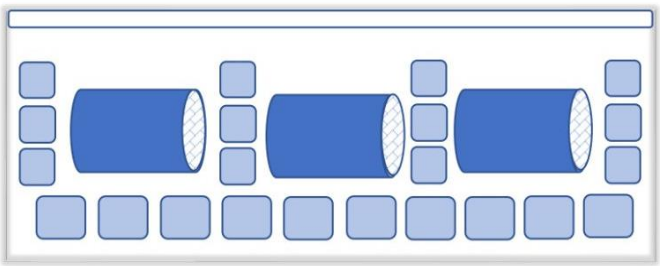

Figure 1. The experimental setup of juvenile abalone inside the Styrofoam box for each feeding treatment and hypoxia duration.

of important compounds (Thongrod *et al*., 2003). On the other hand, the survival of juvenile abalone tends to decrease as the longer of the hypoxia exposure (P<0.05) (Figure 2). All of the juvenile *H. squamata* survived during the 6 h of hypoxia exposure with 100% survival across all the initial feeding treatments. The survival of juvenile abalone *H. squamata* decreased with range of 40-50% at 12 h across all the initial feeding treatments and continue to decrease on the 24 h of hypoxia exposure. Study on Pacific abalone *H. discus* revealed the longer hypoxia period stimulated lower growth performance, weakened the immune system and escalated the oxidative stress (Nam *et al*., 2020), this may lead to the vulnerability of the abalone to the disease (Shen *et al*., 2019). Higher mortality observed on juvenile of the red abalone (*Haliotis rufescens*) after exposed to long period of hypoxia (Kim *et al*., 2013). On the other hand, the other types of mollusks such as Pacific oyster (*Crassostrea gigas*) and Manila clams (*Ruditapes philippinarum*) showed more robust response on the exposure of hypoxia. The Pacific oyster tends to represent high tolerance on the hypoxic condition by delaying the apoptotic and inflammatory responses of the cells (Haider *et al*., 2020). In the same way, Manila clams exhibited tremendous tolerance to 20-day of hypoxic conditions; however, the mitochondrial cells experienced damage and collapse as the compensatory performance of adaptation to hypoxia (Li *et al*., 2019).

The biochemical profiles of abalone at the end of 30 day of feeding treatment (0 h of hypoxia treatment) showed that carbohydrate content of above 45% in feed increased protein and carbohydrate deposition in abalone meat (Table 2, Figure 3). Abalone fed with 45% carbohydrate pellet in this study showed two times higher of meat protein content (444 mg) than abalone fed with natural macroalgae *Gracilaria* sp. (187 mg). Meanwhile, abalone fed with 55% carbohydrate content in feed demonstrated the highest carbohydrate content in meat (125 mg) among abalone from other feeding treatments (<80 mg). Improving nutritional condition prior to transfer to grow-out sites has been suggested to be important for mollusks, in order to improve ability to adapt with environmental change and stressors (Supono *et al*. 2021).

The importance of nutritional state of abalone in dealing with hypoxic condition was presented in this study where abalone fed with high carbohydrate content in feed (>45%) did not only show high nutritional content deposition, but 11% of abalone from this treatment remained survive following 24 h of hypoxia exposure. The immediate effects of hypoxia can negatively impact the survival of aquatic organisms, while sublethal exposure may disrupt aerobic metabolism and affect their reproductive and physiological functions (Diaz *et al*., 2008). Increased severity in survival was also shown by Pacific abalone (*Haliotis discus*) which was exposed to longer hypoxia conditions at both 2.5 and 4 mg O_2 L^{-1} with a response of survival < 40% (Nam *et al*., 2020).

The present study also demonstrated energy utilization of abalone under hypoxic condition. The concentration of protein content in the body of juvenile abalone was significantly influenced by hypoxia for all of the feeding treatments provided

Table 2. Biochemical profile of the body of juvenile abalone after hypoxia exposure

Hypoxia duration	Feeding Treatments				
	Control	25%	35%	45%	55%
Protein (mg.g^{-1})					
0 h	189.68±85.09^{a}	247.59±73.82^{ab}	427.78±95.92^{ab}	444.78±37.38^{b}	293.37±3.78^{ab}
6 h	211.59±34.48^{ab}	391.05±161.56^{b}	106.56±15.5^{a}	150.41±65.26^{ab}	176.15±47.30^{ab}
12 h	389.63±44.09^{b}	245.46±36.35^{ab}	139.54±40.05^{a}	255.55±99.46^{ab}	246.89±71.19^{ab}
Lipid (mg.g^{-1})					
0 h	96.54±9.37	81.88±33.47	89.10±4.25	111.91±9.92	92.39±5.611
6 h	63.52±11.18	74.37±5.56	56.30±12.35	65.06±3.63	49.92±0.41
12 h	41.79±8.35	38.37±12.08	48.07±7.79	65.26±18.15	73.09±16.23
Carbohydrate (mg.g^{-1})					
0 h	76.00±14.55	45.21±21.35	46.79±5.17	64.33±10.16	125.98±2.61
6 h	127.19±54.87	69.34±8.61	138.69±26.81	156.23±59.81	227.96±62.36
12 h	34.25±16.7^{a}	46.56±9.904^{a}	90.19±24.72^{ab}	53.91±5.88^{a}	177.23±47.75^{b}

Different letters following biochemical values within rows showed significance levels (Tukey test, *P*<0.05)

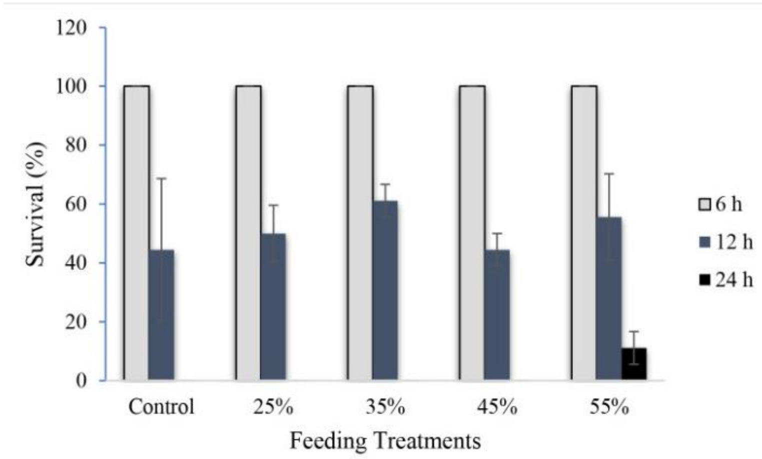

Figure 2. The means of survival of juvenile abalone supplied with different feeding regimes in three hypoxia durations (i.e., 6, 12, and 24 h).

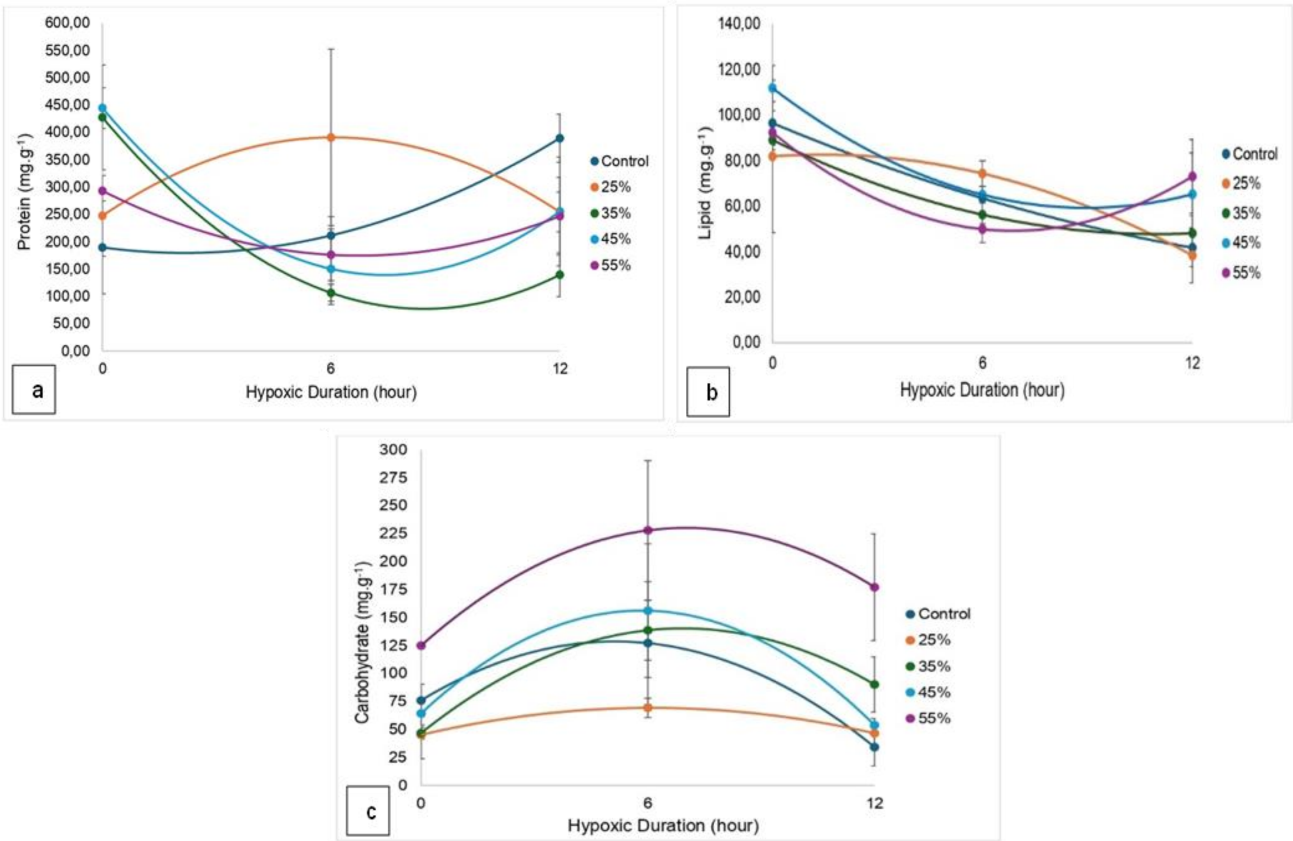

Figure 3. The mean biochemical profile pattern of the body of juvenile abalone for all diet treatments in hypoxia exposure; a) protein concentration, b) lipid concentration, c) carbohydrate concentration.

(Table 2., Figure 3a.). This research showed that during the high energy states (*i.e.*, ~>35% of carbohydrate content in feed), juvenile abalone tends to use the protein as the main energy source to survive. These were revealed by the decreased concentration of protein in higher energy states of juvenile abalone from hypoxia duration of 0 to 6 h (Figure 3a). A similar response had been acclaimed for *Haliotis midae* which experienced an elevation in the protein concentration during hypoxia (Venter *et al*., 2018b). Another result showed that tissues exhibit high concentrations of amino acids during hypoxia in invertebrates, indicating that protein serves as a source for energy metabolism, facilitating conversion to other amino acids or glucose (Shao *et al*., 2015). Protein can be considered a source of energy production during hypoxia. Conversely, under low-energy conditions (*i.e.*, ~25% carbohydrate concentration in feed), juvenile abalone exhibited a slight increase in body protein concentration during the hypoxia period from 0 to 6 h. In order to have an optimum growth, abalone *Haliotis discus hanai* requires moderate to high protein levels, however, if there was limited amount of carbohydrate, this species will eagerly use protein as their main energy (Li *et al*., 2021). Similar responses on the variation of protein composition were also found in abalone of *Haliotis midae* which was exposed to environmental hypoxia. The investigation revealed that there was an increase in the amount of amino acid in some organs *i.e.*, gills and adductor muscle when exposed to hypoxia (Venter *et al*., 2018b). The elevation of protein content during the low energy states could be due to the activation of adenosine monophosphate (AMP) which activated the protein catabolism (Venter *et al*., 2018a), thereby this may lead to the increase of protein content in some organs of *H. midae* (Venter *et al*., 2018b) and the body of abalone in current research.

Noticeable response on metabolic strategies under hypoxia revealed by other mollusks such as mussels, scallop and clams. Compared to abalone, mussels tend to reduce energy-demanding processes, boost anaerobic glycolysis, suppress aerobic metabolism, preserve ATP levels, and switch to anaerobic metabolism during hypoxia (Haider *et al*., 2020; Kokhan *et al*., 2023; Montúfar-Romero *et al*., 2024). Meanwhile clams, during the hypoxia will significantly reduce the metabolic rate, increase oxidation of substrates, inhibit ATP-consuming processes (ion transport, protein synthesis), and maintain the energy balance through the use of glycogen and amino acid metabolism (Ivanina *et al*., 2016; Li *et al*., 2019; Wang *et al*., 2024). Different adaptation to hypoxia performed by scallop which observed to have limited capacity in reducing energy consumption, diminished efficacy of metabolic suppression, heightened oxidative stress, upregulation of glycolytic enzymes while accumulating harmful byproducts, as a result, it might elevate the mortality rates (Götze *et al*., 2020; Ivanina *et al*., 2016; Q. Li *et al*., 2020; Stevens and Gobler, 2018) . The distinct metabolic strategies to cope with hypoxia (low oxygen exposure), reflecting their varying tolerance levels. Mussels and clams are generally more tolerant to hypoxia, relying on their metabolic rate depression and shifts to anaerobic metabolism, while scallops are more sensitive and less able to restrain energy demands under hypoxia condition (Ivanina *et al*., 2016; Kokhan *et al*., 2023; Stevens and Gobler, 2018).

The concentration of lipids in the body of juvenile abalone manifested to be stable during hypoxia conditions (*i.e.*, 0, 6, and 12 h) across all the feeding treatments (Table 2.). The trend on the line graph of the lipid profile on the body of juvenile abalone showed that there was only a slight decrease from the beginning of the hypoxia condition of 0 to 6 h and continued to slightly increase the 12 h of hypoxia (Figure 3b). Although statistically not proven, this phenomenon appeared in juvenile abalone which has a higher state of energy when supplied with a high concentration of carbohydrate (*i.e.*, ~>35%). The current result showed that there was limited lipid change in its concentration during hypoxia. This might be due to abalone are metabolically adaptable compare to other mollusks. Abalone *Haliotis discus hannai* can efficiently use carbohydrates for energy, with optimal growth observed at dietary carbohydrate levels around 37–38%. High carbohydrate diets may enhance glycolysis and stimulate lipid synthesis, while diminishing gluconeogenesis and lipolysis (Guo *et al*., 2022). A similar result on the stability of lipid concentration in abalone, when exposed to hypoxia, was on *H. midae*. The research showed that there were unchanged fatty acid concentrations in the structure of abalone both prior to hypoxia and after hypoxia conditions (Venter *et al*., 2018a). The limited change of lipid concentration in the muscle of juvenile abalone could be due to the activity of lipid catabolism being limited during cellular energy production which may lead to a limited fraction of fatty acid and sterols assimilating in abalone during hypoxia (Venter *et al*., 2018b).

In contradiction to protein and lipid utilization pattern following hypoxia, carbohydrate content of abalone from all feeding treatments in this study increased from their initial state (0 h) up to at 6 h of hypoxic condition and then showed a decreasing trend at 12 h of hypoxia exposure regardless of the feeding treatments (Table 2., Figure 3c). Similar findings were also found in *H. midae* which experienced the elevation of carbohydrate concentration in its muscle following hypoxia conditions (Venter *et al*., 2018a). This may be linked to the physiological mechanisms in abalone, where glycolysis and other carbohydrate pathways play an increasingly vital role during hypoxia, particularly in supporting anaerobic energy production. Hence, it may lead to the release of carbohydrates from glycogen stores and glycoproteins, as well as in the process of gluconeogenesis (Roznere *et al*., 2014).

Providing abalone with greater carbohydrate feed sources can enhance resilience and reduce mortality during stressful events such as hypoxia under transportation set up. Abalone can effectively use carbohydrate sources, and adequate carbohydrate levels in feed can improve survival, development, and stress tolerance (Wang *et al.*, 2024; Thongrod *et al.*, 2003). Previous study on *Haliotis discus hannai Ino* revealed that compared to other sources, dextrin and pregelatinized wheat starch are more efficient as carbohydrate sources at promoting the abalone growth and has better metabolic responses than potato starch. Additionally, these sources raise muscular glycogen, which could aid this species in managing stress during transportation set up (Wang *et al.*, 2024). Therefore, formulating abalone diet with suitable amount of readily digested carbohydrates might potentially improve growth, enhance stress resilience, and decrease mortality during hypoxia. This approach is feasible, effective and efficient to be implemented in abalone culture industry.

Conclusion

Overall, this study showed that hypoxic condition of maximum 24 h caused negative impacts to juvenile abalone, from altering energy reserve utilization to compromising abalone's survival. Feeding abalone with high carbohydrate content (>45%) improved initial nutritional condition (protein and carbohydrate content) and enhanced survival of abalone under hypoxic stressor. Future studies will include combining feeding treatment design used in this study with different shipping methods to increase survival rate of abalone under extended period of more than 24 h of hypoxia.

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