Aquaculture 464 (2016) 364–373

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Effects of dietary grape seed extract, green tea extract, peanut extract and vitamin C supplementation on metabolism and survival of greenlip abalone (*Haliotis laevigata* Donovan) cultured at high temperature

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ARTICLE INFO

Article history:
Received 17 February 2016
Received in revised form 27 April 2016
Accepted 8 July 2016
Available online 9 July 2016

Keywords:
Haliotis laevigata
Grape seed extract
Peanut skin extract
Green tea extract
High temperature
Survival

ABSTRACT

A major problem confronting the abalone farming industry in Australia is elevated mortality during summer months. Recent research suggests that temperature-induced tissue breakdown allows bacteria entry to the host, and nutritional supplementation can alleviate this breakdown. The aim of this study was to alleviate mortality of greenlip abalone, *Haliotis laevigata*, cultured at high summer water temperatures (25 °C) by dietary intervention using graded levels of peanut skin extract (PE; 0.5, 1.0, 2.5 and 5%), green tea extract (GTE; 0.5, 1.0, 2.5 and 5%) and vitamin C (1.0% vitamin C; 1.0% vitamin C + 1.0% GTE; and 1.0% vitamin C + 1.0% PE) in a commercial diet; these supplements contain antioxidant and bioactive compounds. The commercial diet containing 5.0% Australian grapeseed extract (GSE) fed at 25 °C was also included as a negative temperature/positive diet control due to improved survival and health of abalone at high temperatures in our previous study. Three-year-old abalone (49.21 g; 70.26 mm) were fed the commercial diet at 22 °C (positive temperature control), and the commercial diet (negative temperature control) and test diets at 25 °C for 38 days. Abalone survival was 85% for the commercial diet at 22 °C, whereas survival of abalone was significantly reduced to 40% when fed the commercial diet at 25 °C. There were no significant differences in survival of abalone fed the commercial diet at 22 °C and those fed 5.0% GSE, 0.5% GTE and 2.5% GTE diets at 25 °C. Supplements did not significantly affect oxygen consumption, ammonia excretion rates and total hemocyte count. Abalone fed 5.0% GTE had significantly lower phagocytic activity than those fed the commercial control diet at 22 °C. Supplementation with PE and vitamin C had no beneficial effects on survival, while GTE holds promise as a potential dietary additive to enhance the survival of abalone at higher water temperature. This study confirms that supplementation of 5.0% GSE in the commercial diet also improves the survival of greenlip abalone cultured at high summer water temperature in the laboratory setting.

Statement of relevance: Purported to have diverse health benefits including antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic properties, 5% Australian grape extract, graded levels of green tea extract, peanut skin extract and vitamin C were supplemented in a commercial abalone diet in order to improve survival of greenlip abalone at high water temperature.

1. Introduction

The greenlip abalone, *Haliotis laevigata,* is an important species in Australian aquaculture and is grown mainly in land-based systems (Stone et al., 2013, 2014b). Abalone are classified as thermoconformers as their body temperature varies depending on their surrounding environment (Prosser, 1991). Abalone cannot maintain constant body temperature when the ambient temperature changes and a fluctuation of one or two degrees will have significant repercussions on metabolism (Van Barneveld, 2008). Feeding, respiration and growth rates generally increase as water temperature increases, until the temperature reaches a level that causes stress, and ultimately death of the animal (Mozqueira, 1996).

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http://dx.doi.org/10.1016/j.aquaculture.2016.07.011
0044-8486/

Abalone farmers often report higher mortalities over the summer months, when temperatures approach or exceed the thermal limits of abalone. Higher water temperatures accelerate adverse water quality problems such as lower dissolved oxygen and elevated bacteria levels (Vandepeer, 2006; Van Barneveld, 2008). Greenlip abalone are highly sensitive to ammonia, which depresses growth and food consumption but increases energy expenditure, as indicated by oxygen consumption rate (Harris et al., 1998a; Harris et al., 1998b). Oxygen depletion can occur quite rapidly during periods of low water flow or high temperatures (Mozqueira, 1996), and low dissolved oxygen is known to reduce the growth and food consumption rates of juvenile greenlip abalone (Harris et al., 1999). Additionally, proliferation of bacteria may pose another major threat to cultured abalone at high temperatures (Hooper et al., 2007).

In order to reduce mortality rates and optimise production efficiency, recent studies have focused on the effectiveness of adding various supplements to abalone diets, such as macro and microalgae (Dang et al., 2011; Lange et al., 2014; Stone et al., 2014b) and grape seed extract (GSE) (Lange et al., 2014). The results from later studies demonstrated that those dietary supplements had positive effects on survival of abalone. However, incorporating GSE into abalone diets may not be economically viable, so alternatives need to be explored.

Green tea extract (GTE), GSE and peanut skin extract (PE) have been recognized to have diverse health benefits including antioxidant, antimicrobial, anti-inflammatory, and anticarcinogenic properties (Saito et al., 1998; Yu et al., 2005; Perumalla and Hettiarachchy, 2011). In terrestrial animal models, GTE has been tested in rat, mice and rabbits (Inagake et al., 1995; Löest et al., 2002; Yokozawa et al., 2002; Bursill et al., 2007; Bose et al., 2008; Bruno et al., 2008), while GSE has been examined in mice, rat, rabbit, horse and hamsters (Bagchi et al., 1998; Koga et al., 1999; Yamakoshi et al., 1999; Davies et al., 2009; Décordé et al., 2009; Yousef et al., 2009). In aquaculture, GTE has been evaluated in fish. Dietary inclusion of GTE significantly improved growth and feed utilization, while it was effective in lowering serum lipoprotein cholesterol and glutamic oxaloacetic transaminase in olive flounder, *Paralichthys olivaceus* (Cho et al., 2007). Furthermore, dietary inclusions of <1.0% GTE was optimal in improving growth, feed utilization and stress recovery in black rockfish, *Sebastes schlegeli* (Hwang et al., 2013).

Similarly, positive health benefits from dietary GSE supplementation have also been identified in several fish species and the greenlip abalone. Dietary inclusion of 0.2 g GSE kg^{-1} feed had positive effects on growth and body composition, and ameliorated serum biochemistry parameters of tilapia, *Oreochromis niloticus* (Zhai et al., 2014). Kao et al. (2010) reported significant reductions in inflammatory responses and mortality in zebrafish, *Danio rerio*, infected with *Staphylococcus aureus* pre-incubated with GSE. Specifically relevant to abalone, Lange et al. (2014) reported improved survival and feed intake at 26 °C in greenlip abalone fed a commercial diet containing 5% GSE.

Peanut skin is a by-product of the production of peanut oil, peanut butter, snack peanuts and confectionary, and is mainly used as a low-value stock feed ingredient (Yu et al., 2006). It has a low economic value despite its relatively high concentration of antioxidants including catechins, procyanidins (mainly A1), and other phenolic compounds (Nepote et al., 2005; Yu et al., 2005). It has been reported that one gram of dry peanut skin contained 90–125 mg total phenolics (Yu et al., 2005) and 16, 111, 221 and 296 mg 100 g^{-1}, respectively of total catechins, procyanidin dimers, trimers and tetramers in directly peeled peanut skin (Yu et al., 2006). Therefore, extracts from peanut skin are suggested as an inexpensive source of antioxidants for use as functional ingredients in foods or as dietary supplements (Yu et al., 2006). Limited data are available regarding the use of PE to improve animal health and survival.

High water temperature and low oxygen levels can cause oxidative stress in aquatic animals by disrupting the oxidant-antioxidant equilibrium (Lushchak, 2011). Vitamin C is a recognized exogenous dietary antioxidant and is capable of preventing oxidation of molecules in humans and animals (Frei et al., 1988; Cederberg et al., 2001). It was reported that supplementation of 2 g vitamin C kg^{-1} diet enhanced the resistance to stress conditions and bacterial infections in shrimp post larvae (Merchie et al., 1997). Similarly, positive effects on stress resistance in salinity stress tests have been observed in European sea bass, *Dicentrarchus labrax*, fed high concentrations of vitamin C (Merchie et al., 1995). Also for turbot, *Scophthalmus maximus*, feeding vitamin C-enriched diets reduced mortality by 10% after a challenge with *Vibrio anguillarum* (Merchie et al., 1996). Recently, vitamin C has also been shown to influence the immune response and disease resistance of large yellow croaker, *Pseudosciaena crocea* (Ai et al., 2006) and Indian major carp, *Labeo rohita* (Misra et al., 2007). However, little work has been carried out on the effects of vitamin C in molluscs, particularly in abalone (Mai, 1998).

Despite the reported health benefits of GSE, GTE, PE and vitamin C in some animal and fish species, limited information is available on the application of these products to enhance the health and survival of abalone at high water temperatures (Lange et al., 2014). The aim of this study was to examine the effects of graded levels of GTE; PE and vitamin C supplementation in a commercial abalone diet on survival, feed intake, oxygen consumption, ammonia excretion and immunity of greenlip abalone at high water temperature.

2. Materials and methods

2.1. Experimental diets

Eyre Peninsula Aquafeeds provided the Abgrow diet mash. PE and GTE (Shenzhen Naturactive Inc., Shenzhen, Guangdong, People's Republic of China) were formulated into the Abgrow mash at graded levels of 0.5, 1.0, 2.5 and 5.0%, while vitamin C (ROVIMIX® STAY-C® 35; DSM, Heerlen, the Netherlands) supplementations were 1.0% vitamin C; 1.0% vitamin C + 1.0% GTE; and 1.0% vitamin C + 1.0% PE. A positive control diet (Positive control diet B) containing 5% Australian GSE (GSeedEX grape seed tannin, Tarac Technologies Pty Ltd., Nuriootpa, SA, Australia) was also formulated into the Abgrow mash based on previous research (Lange et al., 2014).

To manufacture the diets, the required amounts of dry Abgrow mash, vitamin/mineral premix and fish oil were weighed out and mixed in a Hobart mixer (Hobart Corp., Troy, OH, USA) for 5 min. The vitamin/mineral premix and fish oil inclusions were kept constant in all diets at 2 and 15 g kg^{-1}, respectively. Water (30%) and the sodium alginate binder (0.36%) were then added to the diet mash and mixed for a further 3 min. The diets were cold pelleted using a TR110 pasta machine (MacchinePer Pasta SRL, Molina Di Malo, VI, Italy) to produce a 5 mm flat sinking pellet. Diets were then dried at 50 °C for approximately 48 h until the diets were <10% moisture.

2.2. Experimental design and feeding

The study used the temperature challenge method developed by Stone et al. (2014a, 2014b) to investigate the effects of the inclusion of graded levels of GTE or PE (0.5, 1.0, 2.5 and 5.0%) and vitamin C supplementation (1.0% vitamin C; 1.0% vitamin C + 1.0% GTE; and 1.0% vitamin C + 1.0% PE) in a commercial diet (5 mm commercial Abgrow diet chips (Eyre Peninsula Aquafeed Pty Ltd. [EPA], Lonsdale, South Australia, Australia)) on the survival rate, feed intake, oxygen consumption, ammonia excretion and immunity of greenlip abalone at 25 °C water temperature. Since the optimal water temperatures for growth for greenlip abalone originating from South Australian waters to be 22 °C in experimental set up, abalone fed a commercial diet without dietary supplements and maintained at 22 °C served as the positive control-positive control A (Stone et al., 2013), whereas abalone fed the same diet and maintained at 25 °C served as a negative control. Abalone in the remaining treatment groups were all maintained at 25 °C. Furthermore, abalone fed a commercial diet supplemented with 5.0% GSE

and maintained at 25 °C served as an additional positive control (positive control B), as it has been demonstrated to significantly increase the survival of greenlip abalone at 26 °C (Lange et al., 2014).

Abalone were fed to excess (1% body weight (bw) day^{-1}) at 16:00 daily. Cleaning and collection of uneaten food was performed at 08:30 daily by straining the entire tank contents through a fine mesh. The uneaten feed was weighed, first stored frozen at −20 °C and then was dried in an oven at 105 °C for 16 h. The proportion of uneaten feed that was lost through leaching was estimated in a tank without animals and the correction factor was used to calculate the apparent feed intake. Feed intake was determined and reported as g kg^{-1} abalone day^{-1}.

2.3. Experimental system

The experiment used two identical water temperature controlled systems (22 and 25 °C) previously described in Stone et al. (2013) with 30 μm sand-filtered, and UV treated seawater. Each system was comprised of a 780-L sump, 780-L intermediate tank, and 780-L header tank (Solid Nally MegaBins, MS7800; Viscount Plastics Pty Ltd., Hawthorn East, Vie, Australia). The systems consisted of 56 blue plastic experimental tanks (12.5 L, Nally IH305, Viscount Plastics Pty Ltd.; length, 39.2 cm; width, 28.8 cm; depth, 11.0 cm; and bottom surface area, 1129 cm^2), with a water depth of 5 cm (as appropriate level for good growth of greenlip abalone and also similar to what is seen on greenlip abalone farms (Stone et al., 2013, 2014a; Lange et al., 2014) controlled by a standpipe resulting in a tank water volume of 5.4 L with a mesh screen (nominal mesh size, 0.8 mm) at the outlet to retain uneaten food. Since income water was 19 °C during experimental period, to archive and maintain water temperature at 25 °C, 3-kW immersion heaters (240 V, 3 kW; JQ20, Austin & Cridland, Carlton, NSW, Australia) were used, while the room temperature was adjusted to 23 °C in order to archive the water temperature of 22 °C.

2.4. Experimental animals

Three-year-old greenlip abalone were obtained from South Australian Mariculture (Boston Point, Port Lincoln, South Australia). Upon arrival at the South Australian Research and Development Institute (SARDI), the abalone were transferred to 500 L flow through tanks supplied with aeration and seawater at ambient temperature (21 °C) and photoperiod and fed 5 mm commercial Abgrow diet chips (Eyre Peninsula Aquafeed Pty Ltd. [EPA], Lonsdale, South Australia, Australia) for one month prior to the experiment.

2.5. Experimental stocking

Ten abalone at a time were removed from the holding tank using a spatula. They were weighed (wet weight, 49.21 ± 0.05 g), measured (shell length, 70.26 ± 0.09 mm) and systematically interspersed among four replicate tanks per treatment combination. The experiment ran for a total of 38 days, which included a one week acclimation period to slowly raise the water temperature from 21 °C (~1 °C day^{-1}) to the desired treatment temperatures of 22 and 25 °C, followed by a 33 day temperature challenge period. For each treatment, the size and weight of dead abalone were measured and in an attempt to keep stocking densities equal, replaced with tagged abalone of a similar weight and size that had been treated identically held at the same water temperature and fed their respective diets.

2.6. Oxygen consumption and ammonia excretion measurements

At the end of experiment, three abalone from each treatment tank were placed into 1 L chambers supplied with their original temperature controlled seawater for three days to allow their metabolism to recover from handling (Harris et al., 1997, 1998b; Harris et al., 1999). The flow rates provided to the 1 L chamber were sufficient in order ensure that the water quality parameters, which were tested daily, were not at levels that compromised the abalone. Levels for all water quality parameters were appropriate as suggested in Hutchinson and Vandepeer (2004). Abalone were fed 1.0%bw days^{-1} for two days and then starved on the third day. At 09:00 on the fourth day, initial water samples were taken. After that, the chambers were sealed and the water flow was halted for 15 min. After 15 min incubation period, the chamber lids were carefully opened and the dissolved oxygen (% saturation and mg L^{-1}) was determined. At this point water samples were also collected and stored at −20 °C for ammonia analysis. Abalone in each chamber were measured for their weight and length. Oxygen consumption rate was calculated as the difference between levels of oxygen in the seawater in the chamber, before and after incubation, accounting for the volume of the chamber, the incubation time and the biomass to give final measurements in mg O_2 kg^{-1} h^{-1}. Possible interference due to significant bacterial oxygen consumption was standardised by a blank determination of oxygen consumption in an identical chamber with no abalone. To avoid the effects of hypoxia on metabolic rate, the incubation time was restricted to 15 mins to ensure that dissolved oxygen levels in the chambers never fell below 70% oxygen saturation (Harris et al., 1999). The ammonia concentrations in the samples were determined using a salicylate-hypochlorite method for determining ammonia in seawater (Bower and Holm-Hansen, 1980). The rate of ammonia excretion was measured as the difference between the initial and final ammonia concentrations in water samples divided by abalone weight and time.

2.7. Haemolymph sampling

1 mL of haemolymph was collected from each of three abalone per replicate tank (n = 4) at the end of experiment from the cephalic sinus of the abalone using a 23 gauge needle and 1 mL syringe. Haemolymph was removed from the animals within 1 min after abalone were removed from the tank.

2.7.1. Total hemocyte count (THC)

Fresh haemolymph (50 μL) was immediately fixed in a 6% formalin 35 ppt saline solution (100 μL) and kept on ice to prevent hemocytes from clumping. The THC for each individual was determined using a Neubauer-improved haemocytometer counting chamber and expressed in cells mL^{-1}. However, THC may also be affected by moisture content of abalone as haemolymph composes 55% of the weight of abalone (shell excluded), which is consistent with an open circulatory design in which tissues are bathed in haemolymph, but all tissues are not bathed equally. As such haemolymph contributes to large proportion of the moisture content of abalone (Jorgensen et al., 1984). Thus, to measure moisture content of abalone, abalone were weighed and removed from their shells. Wet mass tissue weights were recorded for each animal. The soft tissues from each treatment was freeze dried for 48 h to constant mass.

The moisture content (%) = (100 − dried weight of abalone) / wet weight of abalone × 100%. The ratio of THC to moisture = THC / the moisture of abalone. The ratio of THC to moisture was expressed as 10^5:1.

2.7.2. Phagocytic activity

The haemolymph phagocytic activity was measured using the method of Chen et al. (2005) and Dang et al. (2011) with some modifications. Briefly, a yeast solution was prepared by autoclaving 2.5% baker's yeast (*Saccharomyces cerevisiae*) (Tandaco, Cerebos Foods, Seven Hills, NSW, Australia) in 4% Congo red (Sigma) in filtered seawater (FSW; 0.2 μm). The stained yeast cells were centrifuged at 1500*g* for 10 min, washed three times with FSW and re-suspended in FSW at 1×10^7 cells mL^{-1}. Fresh haemolymph (150 μL) was added to a 1.5 mL Eppendorf tubes at room temperature with 40 μL of the yeast suspension, lightly vortexed then incubated for 10 min in the dark. The tubes were then

vortexed, again before placing two drops (~50 μL) onto three replicate glass slides per abalone (n = 3). Hemocytes were counted in each of the slide smears (30 per slide) and the number of phagocytic hemocytes calculated as a percentage of total hemocytes.

2.8. Biochemical analyses

The biochemical compositions of the diets and test ingredients were analysed according to the methods of the AOAC (1995) and are displayed in Tables 1 and 2. Crude protein (N × 6.25) was determined by the Kjeldahl method. Crude lipid was analysed with a Soxtherm rapid extraction system (Gerhardt GmbH and Co. KG, Konigswinter, Germany) with petroleum liquid (BP 100 °C) as the extracting solvent. Ash was determined using a muffle furnace at 550 °C for 16 h. Carbohydrate was calculated by difference (Table 1). GSE, GTE and PE were assayed for ferric reducing antioxidant power (FRAP) using the methods of Cheah (2011), slightly modified from Xu et al. (2010). The polyphenol composition of GSE, GTE and PE were also assessed qualitatively at the Analytical Research Laboratory, Plant Science, Southern Cross University, Lismore, New South Wales, Australia using an Agilent 1100 HPLC coupled in series to an Agilent PDA (Photo-Diode Array) and an Agilent 1100 MS (Mass Selective) detectors. Test specimens were solubilised in 70:30 ethanol:water and injected onto a reverse phase (Phenomenex Luna C18 100 mm × 4.6 mm ID, 3 μm) over a broad gradient eluting from 10% Acetonitrile (0.005% % TFA): 0.005% TFA (Triflouroacetic acid) to 95% Acetonitrile (0.005% % TFA): 5%, 0.005% TFA over 20 mins.

The composition of each specimen extract was examined using characteristic UV–Vis profiles and MS fragment ions as markers for respective ID of catechins, oligomeric procyanidins and condensed tannins.

2.9. Water quality

Water quality data are presented in Table 3. Water temperature (°C), dissolved oxygen (mg L^{-1} and % saturation), pH and salinity (g L^{-1}) were measured at 12:00 daily using an alcohol filled thermometer, an OxyGuard™ Handygamma dissolved oxygen meter (OxyGuard International A/S, Birkerød, Denmark), a pH meter (Oakton pHtestr 20; Oakton Instruments, Vernon Hills, IL, USA) and a portable salinity refractometer (model RF20, Extech Instruments, Nashua, NH, USA). The dissolved oxygen meter was calibrated daily in air.

Table 2
The composition of grape seed extract (GSE), green tea extract (GTE) and peanut skin extract (PE).

Nutrient composition	GSE	GTE	PE
Moisture (g kg^{-1})	51.0	77.0	86.0
Crude protein (g kg^{-1})	46.0	9.0	10.0
Crude lipid (g kg^{-1})	7.0	12.0	3.0
Ash (g kg^{-1} as fed)	71.0	26.0	40.0
Carbohydrate (g kg^{-1})	852.0	876.0	896.0
NFE (g kg^{-1})[a]	876.0	953.0	946.0
Gross energy (MJ kg^{-1})	15.1	15.1	15.5
FRAP (mmol Fe^{2+} equivalent g^{-1})[b]	6.02	9.78	5.55
HPLC-MS qualitative profile (mAU)			
Gallic acid	1700	NA	NA
Procyanidin	450	NA	NA
Flavan-3-ol (catechin)	1850	NA	250
Procyanidin (dimer Type-B1)	1650	NA	NA
Flavan-3-ol (epicatechin)	2000	NA	350
Multiple procyanidin polymers (>dimeric)	1000	NA	NA
Catechin gallate	NA	250	NA
Epgallocatechin	NA	1800	NA
Catechin	NA	700	NA
Caffeine	NA	300	NA
Epicatechin, epigallo-catechin gallate (EGCG)-mixed peak	NA	2100	NA
Epicatechin gallate (ECG)	NA	2050	NA
Procyanidin (flavan-3-ol dimer)	NA	NA	650
procyanidin (Type-A, two bonds between flavan-3-ol units)	NA	NA	1800
procyanidin (Type-A1)	NA	NA	1350

NA: not available.
[a] NFE = nitrogen-free extract was calculated by difference = 100% − (crude protein% + total fat% + ash%).
[b] FRAP = ferric-reducing antioxidant potential was measured by homogenising the ingredient and feed sample in DMSO and assaying the supernatant. Test ingredient FRAP values (mmol Fe^{2+} equivalent g^{-1}).

2.10. Statistical analysis

Statistics were computed using Statistical Package for the Social Sciences (SPSS) for Windows (version 22, IBM Corp., Armonk, NY, USA). In order to ensure normal distribution, data was transformed where appropriate, while Levene's test for equality of variance was used to assess the homogeneity of variance among means prior to running the ANOVA and the post-hoc comparisons. Survival patterns were assessed using Kaplan-Meier analyses with Log-Rank, Breslow and Cox proportional hazard regression tests. The Dunnett's 2-tailed test was used to compare feed intake, oxygen consumption, ammonia excretion rates, phagocytic activity and THC of greenlip abalone fed the test diets

Table 1
The nutrient composition of diets used in this study.

Diet Test ingredient (%)	Commercial 0.0	GSE 5.0	GTE 0.5	GTE 1.0	GTE 2.5	GTE 5.0	PE 0.5	PE 1.0	PE 2.5	PE 5.0	Vit C 1.0	Vit C 1.0 GTE 1.0	Vit C 1.0 PE 1.0
Ingredient (as fed)													
Commercial diet mash (g kg^{-1})	979.4	929.4	974.4	969.4	954.4	929.4	974.4	969.4	954.4	929.4	969.4	959.4	959.4
GSE (g kg^{-1})	0.0	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
PE (g kg^{-1})	0.0	0.0	0.0	0.0	0.0	0.0	5.0	10.0	25.0	50.0	0.0	0.0	10.0
GTE (g kg^{-1})	0.0	0.0	5.0	10.0	25.0	50.0	0.0	0.0	0.0	0.0	0.0	10.0	0.0
Sodium alginate (g kg^{-1})	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6	3.6
Vitamin C (g kg^{-1})	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.0	10.0	10.0
Vitamin–mineral premix (g kg^{-1})	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Salmon oil (mL kg^{-1})	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Sum	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0	1000.0
Biochemical composition (as fed)													
Moisture (g kg^{-1})	63.0	62.4	63.1	63.1	63.4	66.5	63.1	63.2	63.6	64.2	63.1	63.1	63.1
Crude protein (g kg^{-1})	309.0	295.9	307.5	306.0	301.5	307.9	294.1	306.0	301.6	294.1	306.0	306.0	306.0
Crude lipid (g kg^{-1})	49.0	46.9	48.8	48.6	48.1	49.4	46.7	48.5	47.9	46.7	48.6	48.6	48.6
Ash (g kg^{-1})	62.0	62.5	61.8	61.6	61.1	63.0	60.9	61.8	61.5	60.9	61.6	61.6	61.6
Carbohydrate (g kg^{-1})	517.0	532.4	518.8	520.6	526.0	558.2	536.0	520.8	526.5	536.0	520.6	520.6	520.6
NFE (g kg^{-1})[a]	517.0	532.3	518.8	520.7	525.9	513.2	535.2	520.5	525.4	534.1	520.7	520.7	520.7
Gross energy (MJ kg^{-1})	15.9	15.9	15.9	15.9	15.9	16.6	15.9	15.9	15.9	15.9	15.9	15.9	15.9

[a] NFE = nitrogen-free extract was calculated by difference = 1000 g kg^{-1} - (moisture g kg^{-1} + crude protein g kg^{-1} + crude lipid g kg^{-1} + ash g kg^{-1}).

Table 3
The water quality parameters of all treatments during the 38 day temperature challenge period.

Temperature and diet	Water temperature (°C)		Dissolved oxygen (% saturation)		Dissolved oxygen (mg L^{-1})		pH	
	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range	Mean ± SE	Range
Control series								
22 °C commercial (positive temperature control)	22.2 ± 0.04	21.8–23.0	84.8 ± 0.5	78.5–91.0	5.9 ± 0.04	5.5–6.5	8.17 ± 0.01	8.03–8.28
25 °C commercial (negative temperature control)	24.7 ± 0.14	21.4–25.9	88.0 ± 0.6	80.5–96.0	6.0 ± 0.04	5.6–6.7	8.22 ± 0.01	8.05–8.32
25 °C GSE 5.0% (negative temperature - positive diet control)	24.7 ± 0.14	21.4–25.8	87.3 ± 0.6	80.5–92.5	6.0 ± 0.04	5.5–6.5	8.22 ± 0.01	8.08–8.31
GTE series								
25 °C GTE 0.5%	24.7 ± 0.14	21.4–25.8	87.8 ± 0.6	79.0–94.5	6.0 ± 0.04	5.5–6.6	8.21 ± 0.01	8.06–8.31
25 °C GTE 1.0%	24.7 ± 0.14	21.4–25.8	88.6 ± 0.6	80.0–94.5	6.0 ± 0.04	5.4–6.6	8.22 ± 0.01	8.06–8.32
25 °C GTE 2.5%	24.7 ± 0.14	21.6–25.7	88.1 ± 0.6	79.5–94.5	6.0 ± 0.04	5.5–6.6	8.22 ± 0.01	8.08–8.31
25 °C GTE 5.0%	24.7 ± 0.14	21.6–25.8	90.0 ± 0.6	82.5–96.5	6.1 ± 0.04	5.6–6.7	8.23 ± 0.01	8.07–8.31
PE series								
25 °C PE 0.5%	24.7 ± 0.14	21.4–25.8	88.6 ± 0.8	78.5–97.0	6.1 ± 0.05	5.2–6.6	8.22 ± 0.01	8.04–8.32
25 °C PE 1.0%	24.7 ± 0.14	21.6–25.7	87.0 ± 0.6	79.0–93.0	5.9 ± 0.04	5.4–6.5	8.22 ± 0.01	8.06–8.31
25 °C PE 2.5%	24.7 ± 0.14	21.4–25.8	87.8 ± 0.6	80.5–94.0	6.0 ± 0.04	5.5–6.6	8.22 ± 0.01	8.06–8.32
25 °C PE 5.0%	24.7 ± 0.14	21.4–25.9	89.2 ± 0.6	81.0–95.5	6.1 ± 0.04	5.6–6.7	8.23 ± 0.01	8.07–8.31
Vitamin C series								
25 °C Vit C 1.0%	24.7 ± 0.14	21.6–25.8	87.8 ± 0.6	80.0–94.0	6.0 ± 0.04	5.4–6.6	8.22 ± 0.01	8.04–8.31
25 °C Vit C 1.0% + GTE 1.0%	24.7 ± 0.14	21.6–25.7	88.4 ± 0.6	78.5–94.0	6.0 ± 0.04	5.3–6.6	8.22 ± 0.01	8.06–8.32
25 °C Vit C 1.0% + PE 1.0%	24.7 ± 0.14	21.6–25.7	89.0 ± 0.7	77.5–96.5	6.1 ± 0.05	5.2–6.7	8.23 ± 0.01	8.04–8.32

and the commercial diet at 25 °C against the commercial diet at 22 °C as the control.

3. Results

3.1. General comments and water quality parameters

There were no significant differences in the initial weights and lengths of greenlip abalone at stocking ($P > 0.05$). Over the course of the experiment, greenlip abalone were more active and exhibited normal feeding behaviour at 22 °C compared to those at 25 °C, which exhibited less movement, released mucus from the foot, displayed limited attachment to the tank substrate, and often detached and inverted before mortality occurred.

Water quality was maintained throughout the study at appropriate levels for the growth of greenlip abalone (Hutchinson and Vandepeer, 2004; Table 3). In the 22 °C treatment, water temperature averaged 22.2 °C while in 25 °C treatments, temperatures averaged 24.7 °C. Dissolved oxygen, pH and salinity did not differ ($P > 0.05$) among treatments; the saturation levels were 84.8% in the 22 °C treatment and 89.2% in the 25 °C treatments, while concentrations ranged from 5.9 to 6.1 mg L^{-1}. The water pH value was 8.17 to 8.23 while salinity was between 35 and 36‰.

3.2. Composition of GSE, GTE and PE

The composition of GSE, GTE and PE are presented in Table 2. Protein was the highest in GSE (46 g kg^{-1}), followed by PE (10 g kg^{-1}) and GTE (9 g kg^{-1}). Carbohydrate was high in all ingredients and ranged from 852 g kg^{-1} for GSE to 896 g kg^{-1} for PE. Gross energy values were similar in all ingredients (15.1 to 15.5 MJ kg^{-1}). FRAP values were generally higher in GTE (9.78) than GSE (6.02) and PE (5.55 mmol Fe^{2+} equivalent g^{-1}).

The qualitative polyphenol composition of the ingredients, were analysed using HPLC-MS, reported in Table 2. The antioxidant components of GSE were found as flavan-3-ol (epicatechin), flavan-3-ol (catechin), gallic acid, procyanidin (dimer Type-B1), multiple procyanidin polymers (>dimeric) and procyanidin, while they were epicatechin, epigallo-catechin gallate (EGCG), epicatechin gallate (ECG), catechin gallate, catechin, epgallocatechin and caffeine in GTE. Procyanidin (Type-A, two bonds between flavan-3-ol units), procyanidin (Type-A1), procyanidin (flavan-3-ol dimer), flavan-3-ol (epicatechin) and flavan-3-ol (catechin) were detected in PE.

3.3. Survival

3.3.1. Survival of greenlip abalone fed graded levels of GTE at 25 °C

Survival of greenlip abalone fed the commercial diet at 22 °C was 85% and was significantly reduced to 40% at 25 °C for abalone fed the same diet (n = 40; $P < 0.001$; Kaplan-Meier; Log-Rank test; Fig. 1). In fact, these abalone were 5.5 times more likely to die over the course of the experiment than abalone fed the commercial diet at 22 °C ($P < 0.001$; Cox proportional hazards regression). The survival of greenlip abalone fed the diets containing 1.0% (30%; $P < 0.001$) and 5.0% GTE (47.5%; $P < 0.001$) at 25 °C was significantly lower compared to abalone fed the commercial diet at 22 °C (Kaplan Meier, Log-Rank test; Fig. 1). Greenlip abalone fed the diets containing 1.0% and 5.0% GTE at 25 °C were 7.2 ($P < 0.001$) and 4.4 times ($P = 0.002$) more likely to die over the course of the experiment, respectively, compared to abalone fed the commercial diet at 22 °C (Cox proportional hazards regression). There were no significant differences in the survival of

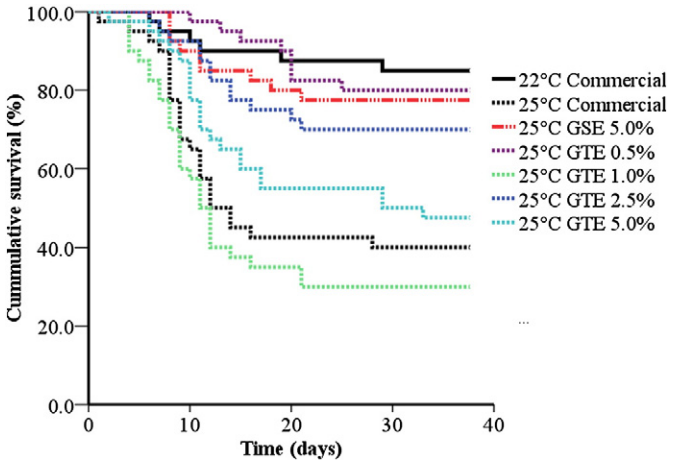

Fig. 1. Kaplan-Meier survival curves of greenlip abalone (*Haliotis laevigata*) fed the commercial diet at 22 °C, or the commercial diet and diets containing 5.0% GSE or graded levels of GTE at 25 °C.

greenlip abalone fed the commercial diet at 22 °C and those fed 5.0% GSE ($P = 0.402$), 0.5% GTE ($P = 0.620$) or 2.5% GTE ($P = 0.119$) at 25 °C. At 25 °C, the survival rate of abalone fed 5.0% GSE (77.5%) was significantly higher than those fed the commercial diet at the same temperature ($P < 0.001$; Kaplan-Meier; Log-Rank test; Fig. 1) and at 1.0% GTE ($P < 0.001$) or 5.0% GTE ($P = 0.007$) inclusion, but not from those fed 0.5% GTE ($P = 0.680$) and 2.5% GTE ($P = 0.462$). Greenlip abalone fed the commercial diet at 25 °C were 3.6 ($P = 0.001$), 4.6 ($P < 0.001$) and 2.7 ($P = 0.006$) times more likely to die than when fed 5% GSE, 0.5% GTE or 2.5% GTE, respectively (Cox proportional hazards regression).

3.3.2. Survival of greenlip abalone fed graded levels of PE at 25 °C

The survival of greenlip abalone fed any of the diets containing PE ranged from 37.5 to 52.5% and were significantly lower than those fed the commercial diet at 22 °C (n = 40; $P < 0.001$; Kaplan-Meier; Log-Rank test; Fig. 2). Greenlip abalone fed the diets containing 0.5, 1.0, 2.5 and 5.0% PE at 25 °C were 5.3 ($P < 0.001$), 3.9 ($P = 0.004$), 5.7 ($P < 0.001$) and 5.2 ($P < 0.001$) times more likely to die over the period of the experiment, respectively, compared to abalone fed the commercial diet at 22 °C (Cox proportional hazards regression). There were no significant differences between the survival rate of greenlip abalone fed any of the diets containing PE and the commercial diet at 25 °C ($P > 0.05$). The survival rate of greenlip abalone fed 5.0% GSE was significantly higher than those fed the diets with graded levels of PE ($P < 0.01$; Kaplan-Meier; Log-Rank test; Fig. 2).

3.3.3. Survival of greenlip abalone fed the diets supplemented with vitamin C at 25 °C

The survival of greenlip abalone fed diets supplemented with vitamin C at 25 °C ranged from 17.5 to 52.5%, which was significantly lower than abalone fed the commercial diet at 22 °C ($P < 0.001$; Kaplan-Meier; Log-Rank test; Fig. 3). In addition, greenlip abalone fed the diets supplemented with 1.0% vitamin C, 1.0% vitamin C + 1.0% GTE and 1.0% vitamin C + 1.0% PE at 25 °C were 5.2 ($P < 0.001$), 3.8 ($P = 0.005$) and 9.8 ($P < 0.001$) times more likely to die over the course of the experiment, respectively, compared to abalone fed the commercial diet at 22 °C (Cox proportional hazards regression). No significant difference was observed in the survival of greenlip abalone fed any of the vitamin C supplemented diets and the commercial diet at the 25 °C ($P > 0.05$; Kaplan-Meier; Log-Rank test; Fig. 3). The survival of greenlip abalone fed the 1.0% vitamin C + 1.0% PE diet was the lowest in this series. Abalone fed 5.0% GSE had significantly higher survival than those fed the diets supplemented with vitamin C ($P < 0.01$; Kaplan-Meier; Log-Rank test; Fig. 2).

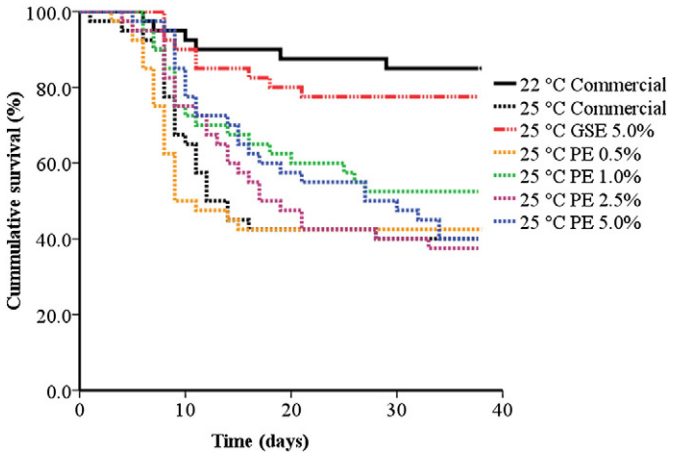

Fig. 2. Kaplan-Meier survival curves of greenlip abalone (*Haliotis laevigata*) fed the commercial diet at 22 °C, or the commercial diet and diets containing 5.0% GSE or graded levels of PE at 25 °C.

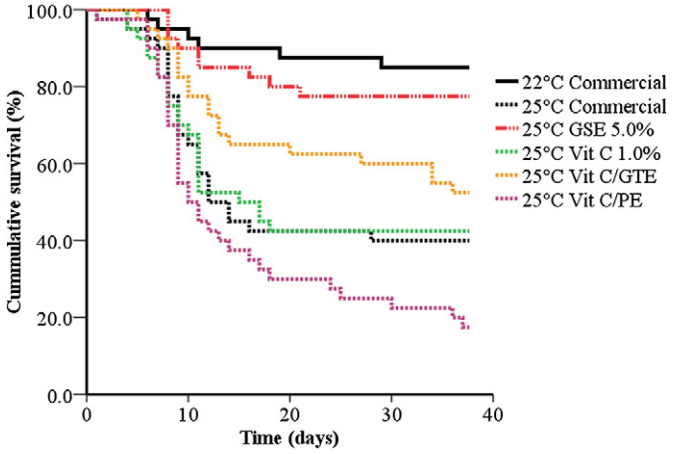

Fig. 3. Kaplan-Meier survival curves of greenlip abalone (*Haliotis laevigata*) fed the commercial diet at 22 °C, or the commercial diet and diets containing 5.0% GSE or supplemented with vitamin C at 25 °C.

3.4. Feed intake rate

3.4.1. Feed intake rate of greenlip abalone fed graded levels of GTE at 25 °C

The feed intake rates of abalone fed the diets containing GTE were significantly affected by temperature. Abalone fed the commercial diet at 22 °C had significantly higher feed intake rates than those fed the commercial diet, 5.0% GSE and GTE supplemented diets at 25 °C ($P < 0.001$; Dunnett's 2-tailed test; Table 4). Among GTE diets, feed intake rate was the highest in abalone fed 1.0% (1.26 g kg abalone^{-1} d^{-1}) 1) and the lowest in those fed 5.0% (0.89 g kg abalone^{-1} d^{-1}).

3.4.2. Feed intake rate of greenlip abalone fed graded levels of PE at 25 °C

A significant effect of temperature on the feed intake rate was also observed for greenlip abalone fed the diets containing graded levels of PE. The feed intake rate of abalone fed the commercial diet at 22 °C was significantly higher than those fed the diets supplemented with PE at 25 °C ($P < 0.001$; Dunnett's 2-tailed test; Table 4). Feed intake rates for greenlip abalone fed the PE diets ranged from 0.82 to 1.49 g kg abalone^{-1} d^{-1}.

3.4.3. Feed intake rate of greenlip abalone fed graded levels of vitamin C at 25 °C

Temperature also had a significant effect on the feed intake rates of greenlip abalone fed diets supplemented with vitamin C. Abalone fed commercial diet at 22 °C had a significantly higher feed intake rate compared to those fed the diets supplemented with vitamin C at 25 °C ($P < 0.001$; Dunnett's 2-tailed test; Table 4). Feed intake rate was similar in abalone fed vitamin C supplemented diets, ranging from 0.88 to 0.96 g kg abalone^{-1} d^{-1}.

3.5. Oxygen consumption rates

There was no significant effect of temperature or diet supplementation on oxygen consumption rates in the current study ($P > 0.05$; Dunnett's 2-tailed test; Table 4). The oxygen consumption rates of greenlip abalone for all treatments ranged from 29.9 to 43.6 mg O_2 kg abalone^{-1} h^{-1}.

3.6. Ammonia excretion rates

The ammonia excretion rate of greenlip abalone was not significantly affected by temperature or antioxidant supplementation ($P > 0.05$; Dunnett's 2-tailed test; Table 4). Ammonia excretion rates ranged from 0.63 to 2.40 mg NH_3 kg abalone^{-1} h^{-1}.

Table 4
Feed intake rates, oxygen consumption rates, ammonia excretion rates, phagocytic activity, total haemocyte count and THC to moisture ration of greenlip abalone (*Haliotis laevigata*) fed commercial diet at 22 °C and the commercial diet and diets supplemented with GSE, GTE, PE and vitamin C at 25 °C.

Temperature and diet	Feed intake rate (g kg abalone^{-1} d^{-1})	Oxygen consumption rate (mg kg abalone^{-1} h^{-1})	Ammonia excretion rate (mg kg abalone^{-1} h^{-1})	Phagocytic activity (%)	Total hemocyte count (cells × 10^6 mL^{-1})	THC:moisture of abalone tissue ratio (10^5:1)
Control series						
22 °C commercial (positive temperature control)	2.41 ± 0.17	36.5 ± 3.91	2.04 ± 0.34	59.0 ± 5.44	6.20 ± 0.90	0.85 ± 0.12
25 °C commercial (negative temperature control)	1.21 ± 0.16**	43.6 ± 1.61	1.72 ± 0.75	55.3 ± 4.34	7.99 ± 2.13	1.08 ± 0.28
25 °C GSE 5.0% (negative temperature-positive diet control)	1.47 ± 0.09**	34.8 ± 3.52	0.74 ± 0.26	44.4 ± 7.32	7.59 ± 2.83	1.02 ± 0.38
GTE series						
25 °C GTE 0.5%	1.00 ± 0.13**	31.0 ± 4.04	0.63 ± 0.31	47.5 ± 3.96	6.83 ± 3.14	0.92 ± 0.42
25 °C GTE 1.0%	1.26 ± 0.17**	30.6 ± 2.31	0.80 ± 0.20	47.8 ± 3.27	5.27 ± 1.63	0.71 ± 0.22
25 °C GTE 2.5%	0.93 ± 0.03**	33.8 ± 2.57	2.03 ± 0.95	51.5 ± 5.15	6.08 ± 1.40	0.82 ± 0.19
25 °C GTE 5.0%	0.89 ± 0.09**	29.9 ± 1.06	1.23 ± 0.28	31.0 ± 5.89*	2.84 ± 1.28	0.38 ± 0.17
PE series						
25 °C PE 0.5%	0.82 ± 0.07**	39.4 ± 2.21	2.40 ± 0.49	40.6 ± 16.1	6.51 ± 1.96	0.87 ± 0.26
25 °C PE 1.0%	1.49 ± 0.14**	35.5 ± 5.99	0.64 ± 0.36	49.9 ± 4.32	5.59 ± 0.58	0.75 ± 0.07
25 °C PE 2.5%	1.18 ± 0.13**	35.4 ± 9.08	1.70 ± 0.41	51.9 ± 3.55	3.93 ± 2.28	0.54 ± 0.03
25 °C PE 5.0%	1.40 ± 0.27**	31.6 ± 5.45	0.78 ± 0.28	55.7 ± 6.35	6.33 ± 2.16	0.87 ± 0.31
Vitamin C series						
25 °C Vit C 1.0%	0.89 ± 0.07**	32.3 ± 3.40	1.45 ± 0.25	43.5 ± 12.3	5.64 ± 1.30	0.76 ± 0.18
25 °C Vit C 1.0%/GTE 1.0%	0.88 ± 0.05**	35.0 ± 1.41	1.22 ± 0.24	42.6 ± 4.47	8.02 ± 1.45	1.07 ± 0.19
25 °C Vit C 1.0%/PE 1.0%	0.96 ± 0.05**	39.6 ± 0.99	1.73 ± 0.22	43.8 ± 8.71	6.30 ± 2.09	0.85 ± 0.27

* $P < 0.05$.
** $P < 0.001$ denotes that value in each column is significantly different compared to the commercial diet at the 22 °C (Dunnett's two-tailed test).

3.7. Total hemocyte count (THC)

There was no significant effect of all treatments on the THC of greenlip abalone compared to abalone fed the commercial control diet at 22 °C ($P > 0.05$; Dunnett's 2-tailed test; Table 4). The THC ranged from 2.84 to 8.02 × 10^6 cells mL^{-1}. Similarly, no significant differences were found in the ratio of THC to moisture between abalone fed dietary treatments at 25 °C and those fed the commercial control diet at 22 °C ($P > 0.05$; Dunnett's 2-tailed test; Table 4). The ratios of THC to moisture varied from 0.38 × 10^5: 1 to 1.08 × 10^5: 1.

3.8. Phagocytic activity

The phagocytic activity of greenlip abalone fed commercial diet at 22 °C was significantly higher than those fed the diets supplemented with 5.0% GTE ($P = 0.02$) and there was no significant difference between any other treatment and the positive temperature control treatment ($P > 0.05$; Dunnett's 2-tailed test; Table 4). The phagocytic activity ranged from 31.0 to 59.0%.

4. Discussion

Abalone cultured in Australian land based system become increasingly stressed and mortality rates rise when temperatures exceed 24 °C (Mozqueira, 1996). Stone et al. (2014b) also reported that increased health problems and significant levels of mortality occur on abalone farms when water temperatures exceed 23 °C in South Australia. In particular, the survival of 3-year-old greenlip abalone fed the EPA commercial diet at 26 °C was significantly reduced in two separate laboratory based experiments by 35% and 50%, respectively (Stone et al., 2014b). Other studies have also investigated summer mortality in greenlip abalone in the laboratory setting and reported similar reductions in survival rate ranging from 50 to 75% during periods of extreme water temperatures of up to 26 °C (Vandepeer, 2006; Lange et al., 2014). In the present study, the survival rate of abalone fed the commercial diet at 22 °C and 25 °C declined from 85% to 40%, respectively. The survival patterns in the present study were consistent with results reported by Mozqueira (1996) and Stone et al. (2014b) for cultured greenlip abalone in land based production systems.

In the current study, feeding greenlip abalone the commercial diet containing 5.0% GSE resulted in a significant improvement in survival compared to the commercial diet at 25 °C. Additionally, there was no statistical difference between the survival of abalone fed 5.0% GSE at 25 °C and those fed the commercial diet at 22 °C. Similarly, Lange et al. (2014) reported that the addition of 5.0% GSE to a commercial abalone diet significantly improved the survival of greenlip abalone chronically exposed to high water temperature (26 °C). The improvement may be due to antioxidant and antibacterial activities and high levels of bioactive polyphenol compounds in GSE (Jayaprakasha et al., 2003; Monagas et al., 2003; Baydar et al., 2006; Delgado Adámez et al., 2012). Previous studies have identified the antioxidant activities in GSE may be due to higher amounts of epicatechin and catechin (Rababah et al., 2004), while the active compound for the inhibition of bacteria was identified as gallic acid (Jayaprakasha et al., 2003). In the current study, flavan-3-ol (catechin - monomer), flavan-3-ol (epicatechin - isomer) and gallic acid were detected in GSE along with other phenolic contents. The improved survival of the abalone in the current study fed 5.0% GSE confirms previous research in this area (Lange et al., 2014).

GSE contains a range of compounds which have antioxidative properties, such as phenols catechins, epicatechins, procyanidins and proanthocyanidins which could improve the antioxidant capacity of abalone exposed to oxidative-stress-inducing culture conditions. In the current study, some important antioxidants such as: gallic acid, procyanidin, flavan-3-ol (catechin), procyanidin (dimer Type-B1), flavan-3-ol (epicatechin), multiple procyanidin polymers (>dimeric) were found in GSE while they were not detected in GTE or only Flavan-3-ol (catechin) and Flavan-3-ol (epicatechin) were found in PE. In addition, although we did not compare feed intake of abalone fed GSE with those fed GTE or PE, visually, the feed intake of abalone fed 5%GSE was higher than those fed other diets. Our preliminary study

also showed that GSE addition also significantly increased serum superoxide dismutase activity, feed intake, and meal acceptance of the abalone ($P < 0.05$) compared to commercial diet at 26 °C (Lange et al., 2014). Thus, it is likely that GSE showed more powerful than other tested products in current study.

High water temperature have been known to cause oxidative stress in abalone by disrupting the oxidant-antioxidant equilibrium (Lushchak, 2011), increases vulnerability of abalone to bacterial infection from pathogenic species (Travers et al., 2010). It is also hypothesised that opportunistic pathogens present may simply be taking advantage of the immune-suppressed abalone (Hooper et al., 2007). GSE has been reported to restore the balance of oxidant-antioxidant status in broiler chickens or enhance the antioxidant status and reduced oxidative-related damage in rodent models (Çetin et al., 2008; Wang et al., 2008; Yousef et al., 2009). GSE addition also significantly increased serum superoxide dismutase activity of the abalone compared to commercial diet at 26 °C (Lange et al., 2014). In addition, GSE also has been reported to protect against opportunistic pathogens in vitro (Jayaprakasha et al., 2003; Al-Habib et al., 2010). In current study, when GSE was supplemented, no significant difference in THC or phagocytic activity of the circulating haemocytes of abalone fed GSE diets at 25 °C compared to those fed the commercial diet at 22 °C (positive control A). Thus, it is possible that the improvement in survival rate of abalone fed GSE in the current study may have been due to improvement in the antioxidant response, reduced oxidative damage in abalone, while inhibited bacterial in the culture tank, on the gills, or in the gut of the abalone (Lange et al., 2014).

Greenlip abalone fed the commercial diet at 25 °C consumed numerically more oxygen compared to those fed the same diet at 22 °C, despite of no significant difference. Our data supports previous observations in that as temperature increases, the rate of physiological reactions of abalone increase which requires more energy and thus more oxygen (Harris et al., 2005; Romo et al., 2010; Morash and Alter, 2015). For example, Lyon (1995) reported that oxygen consumption rates of three different size classes (15, 30, and 50 mm shell length) of South African abalone, *Haliotis midae* at 16, 20 and 23 °C increased with temperature. In current study, we would expect the oxygen consumption of abalone at high temperature reduced by feeding additives. However, no significant difference in oxygen consumption rates was observed between abalone fed the additive diets or commercial diet at 25 °C and those fed the formulated diet at 22 °C. Thus, it is possible that feeding the antioxidant products, which have antioxidative properties, may have little effect on the stress tolerance of greenlip abalone. Thus, it is highly unlikely that any protective survival effects have an oxygen-related component. However, we recommend further research in response to the inclusion of dietary antioxidants is necessary in this aspect of abalone physiology.

The results of present study show that 0.5% GTE dietary inclusion significantly improved survival of abalone at 25 °C. In addition, there were no significant differences in THC or phagocytic activity of abalone fed 0.5% GTE at 25 °C, compared to those fed the commercial diet at 22 °C (positive control A). Green tea has been shown to contain various types of catechins, including >70% polyphenols (Kuo et al., 2005), and GTE has been identified to exhibit antimicrobial activity, anti-inflammatory effects, antitumorigenic, antioxidative, antiproliferative, antiviral, and antiparasitic properties, immunity reinforcement from lysozyme activity and improvements in stress recovery, which may have improved abalone survival in this study (Isogai et al., 2001; Molan et al., 2003; Weber et al., 2003; Crespy and Williamson, 2004; Abdel-Tawwab et al., 2010; Hwang et al., 2013). Abdel-Tawwab et al. (2010) reported that green tea supplement as promising immunostimulant could improve fish performance, health, and prevent tilapia *aermoniosis*. According to Hwang et al. (2013), GTE diet increases survival rate of the juvenile black rockfish, *Sebastes schlegeli* due to improving immunity reinforcement from lysozyme activity and improvements in stress recovery from 2-phenoxyethanol and air exposure. Therefore, similar to GSE in the current study, these benefits of GTE could contribute in the improved survival rate of abalone fed 0.5% GTE. However, abalone fed 5% GTE at 25 °C had significantly lower survival rate and phagocytic activity than those fed the commercial diet and held at 22 °C. Thus, we recommend further research to examine beneficial GTE content of under 1%.

Changes in water temperature has been reported to affect the immune parameters and increase the susceptibility of abalone to microbial infections (Cheng et al., 2004; Travers et al., 2008; Travers et al., 2009; Dang et al., 2012). Previously research has identified an initial depression in haemocyte counts and phagocytosis during stress, followed by an increase in some immune responses such as haemocyte counts or phagocytic ability after the stress, peaking after 2–4 h, followed by a return towards baseline values (Hooper et al., 2007). *H. diversicolor supertexta* transferred from 28 °C to 32 °C showed a lower phagocytic activity, while THC increased after 24 h and no significant difference after 72 and 120 h (Cheng et al., 2004). Similarly, THC of greenlip abalone increased at day 1 and then dropped back to control levels by days 3 and 7 as water temperature increased from 18 to 21 or 24 °C (Dang et al., 2012). Results in current study showed that although there were no significant differences in phagocytic activity between abalone fed all diets at 25 °C and those fed the commercial control diet at 22 °C, phagocytic activity was lower at higher temperature. Similar to two above studies, it is also possible that THC of greenlip abalone increased after first day of experiment and dropped back and remained until the end of trial, and at the same level to the control group. However, the sampling design of this study did not allow for day-by-day changes to be determined.

In conclusion, dietary intervention was once again shown to have the potential to improve the survival of greenlip abalone under the laboratory culture conditions at high summer water temperatures. Information through the use of dietary intervention may be applied under commercial conditions to alleviate the occurrence of summer mortality on-farm in greenlip abalone during summer at high water temperatures. The survival rate of greenlip abalone was influenced by water temperature in a similar pattern as reported on farm under normal production conditions during periods of high summer water temperatures. The addition of 5.0% GSE to a commercial abalone diet was confirmed to significantly improve survival for greenlip abalone chronically exposed to high water temperature (25 °C). The inclusion of 0.5% and 2.5% GTE to the commercial diet also had positive effects on the survival of greenlip abalone at 25 °C. However, the supplementation of PE and vitamin C was not beneficial, and as a result, fortification of both products is not recommended in diets for greenlip abalone at high summer water temperatures to prevent mortality. Overall, this study supports the use of antioxidants as dietary additives to enhance the survival of greenlip abalone during periods of higher summer water temperatures. Further research to confirm the beneficial effects of GSE and GTE on abalone survival is recommended to validate these products under normal production conditions on-farm during periods of high summer water temperatures. Additionally, impacts on sensory quality attributes of abalone, especially flavour, should also be determined for these products before incorporated into greenlip abalone production diets.

Acknowledgments

This study is part of the Thriving Abalone Project (6251) and funding also provided by the Functional Food Focus Program being conducted by SARDI as part of the South Australian Government Primary Industries and Regions South Australia Agribusiness Accelerator Program. Funding for the project was also provided by the Australian Abalone Growers Association (2014/6251). The authors also wish to thank Joel Scanlon (Aquafeeds Australia) and Dr. Tom Coote and Kym Heidenreich (Eyre Peninsula Aquafeeds) for their technical and financial contributions to the supply and manufacture of the diets and Tarac Technologies (Nuriootpa, South Australia) for providing the GSE and financial support. Duong Duong Ngoc would also like to acknowledge the AusAID

Australian Development Scholarship and the Thai Nguyen University of Agriculture and Forestry, Thai Nguyen City, Vietnam for their financial support. The also authors wish to acknowledge Elise Schaefer of Flinders University, and Brett Lange of The University of Adelaide for technical and academic assistance.

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