Introduction

Investigations into the use of dietary natural plant products have been increasingly been investigated as inexpensive supplements to potentially act as growth promoters to various farmed fish and crustacean species (Reverter et al., 2014). Some of these have included garlic (Shalaby et al., 2006), ginger (Haghighi and Rohani, 2013), flowers (Aly and Mohamed, 2010) and different herbal and seed extracts (Punitha et al., 2008; Kareem et al., 2016) with largely good success. However, their applications to crustaceans are limited, particularly with regards to crabs.

Abstract

A two-part experiment was performed to determine whether dietary peppermint oil could improve the growth and/or decrease aggression among blue swimmer crab, Portunus pelagicus early juveniles. A total of five isonitrogenous diets were made that contained increasing peppermint oil levels of 0.00, 0.05, 0.10, 0.50 or 1.00%. These diets were fed to 45 replicate crabs in each treatment (total of 225 crabs) for 12 days, the final sizes and weights were measured, and then placed in 3 replicate containers (30 in total/treatment) to allow the opportunity for cannibalism over 10 days. After 10 days, the remaining crabs were examined for any histopathological changes in gills or hepatopancreas. Results showed dietary peppermint oil, at the tested levels, had no effect on the growth or cannibalism, in either experiments (p > 0.05). However, there were substantial changes in the hepatopancreatic histopathology that included thinner tubules and significantly less B- and R-cells from 0.10% dietary peppermint oil and above. The unaffected growth or cannibalism indicate that the levels of dietary peppermint oil used were insufficient and further investigations are required, particularly on the implications to the hepatopancreatic changes.

Key words

Cannibalism, Crabs, Essential oil, Histopathology, Mentha, Pulegone

Effects of dietary peppermint (Mentha piperita) essential oil on survival, growth, cannibalism and hepatopancreatic histopathology of Portunus pelagicus juveniles

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Asian seabass (*Lates calcarifer*) and resistance to *Vibrio harveyi* (Talpur, 2014), while ethanolic extract of peppermint has been found to enhance growth and immunity in Caspian white fish (*Rutilus kutum*) (Adel *et al.*, 2015). It was suggested this might be due to different compounds in peppermint acting as immunostimulants and/or antimicrobials (Talpur, 2014; Adel *et al.*, 2015).

Peppermint oil contains various compounds including menthol, menthone and methyl acetate as the dominant types (Dubick, 1986). However, of the many compounds naturally present in peppermint, pulegone may of particular interest for crustacean farming, since it is known to be a strong octopamine blocker. This could have implications to the crab industry since octopamine is a neurotransmitter found in arthropods, mollusks and some worms that is the equivalent to adrenaline in vertebrates and thus, may influence their behavior (Roeder, 1999). Although octopamine plays several roles in crustaceans such as metabolism, appetite and potentially memory, their influence on aggression is species specific such as increasing dominate cannibalistic and aggression appears to be motivated by molting stage and size discrepancies (Marshall *et al.*, 2005). In fact, this behavior is recognized as one of the biggest limiting factors of this industry (Shelley and Lovatelli, 2011; Ravi and Manisseri, 2012), and any method to reduce aggression would likely have important implications to their farming.

Among commercially important crustacean species, the blue swimmer crab, *Portunus pelagicus*, is especially cannibalistic and aggression appears to be motivated by molting stage and size discrepancies (Marshall *et al.*, 2005). In fact, this behavior is recognized as one of the biggest limiting factors of this industry (Shelley and Lovatelli, 2011; Ravi and Manisseri, 2012), and any method to reduce aggression would likely have important implications to their farming.

The aim of the present study was to feed newly settled *P. pelagicus* juveniles with diets containing increasing peppermint oil (0.00, 0.05, 0.10, 0.50 and 1.00%) on survival and growth when individually cultured for 12 days. To assess any effect on cannibalism, the crabs were then cultured communally over 10 days and then examined histopathologically for gills and hepatopancreas.

### Materials and Methods

#### Experimental diets:
Five isonitrogenous diets were formulated to contain different levels of peppermint oil (77411, Sigma), at the expense of cellulose, at 0 (control), 0.05, 0.10, 0.50 and 1.00% and the dietary formulation is shown in Table 1. This broad range was chosen since, to the best of our knowledge, no other experiment has investigated on the diets of crustaceans. The diets were made according to Romano *et al.* (2012), with the exception that the diets were dried overnight at 50°C. The diets were kept in air-tight plastic bags until further use and the proximate composition was measured according to standard methods of AOAC (1996), and were generally similar among the diets (Table 1).

#### Source of experimental animals and setup:
The crabs were larvicultured according to Romano and Zeng (2006) except that 1,000 L tanks were used. Upon settling to the first crab stage (within 12 hrs of metamorphosis), they were siphoned out and a total of 225 intact and apparently healthy crabs were individually placed within 500 ml clear plastic containers to prevent cannibalism. Each crab was therefore a replicate, which yielded 45 replicates in each treatment.

Each day the crabs were fed their respective diets to apparent satiation twice, as well as checked for any mortalities or molts. The crabs received a daily 30% water exchange with natural filtered (5 μm and UV sterilized) seawater (32 – 33 ppt) and the temperature was ambient (26 ± 2°C). After 12 days, the carapace width and length was measured using a digital caliper (0.01 cm) and wet weights were measured using a digital balance (0.01g). After each crab was measured, they were placed back in their respective individual container in order to conduct the cannibalism experiment for the following day.

A day after the crabs were measured for their respective sizes, a total of 29 crabs/treatment were distributed into three replicate flat-bottomed circular containers filled with 50 l seawater. Two replicate containers had ten crabs, while the third had nine crabs and this distribution was the same among all treatments, and the crabs were fed their respective diets. No shelters were provided to prevent the crabs from hiding during molting. Each day, the crabs were counted as well as general observations for aggression and movements. When one treatment only had four crabs remaining, the experiment ended and all the crabs were fixed in FAACC formalin (4% formaldehyde, 5% acetic acid, and 1.3% calcium chloride) for 3 days and then 70% ethanol until
Dietary peppermint oil on crab juveniles

Although the experimental duration was relatively short, this duration was sufficient to observe significant differences in nutritional studies of *P. pelagicus* juveniles (Romano et al., 2012; Noordin et al., 2015). Nevertheless, a longer time frame may yield a different finding, while higher levels of peppermint oil may also be investigated. For example, growth of Asian seabass increased with increasing dietary levels of peppermint in a dose-dependent manner from 0 to 5% (Talpur, 2014). Meanwhile, Adel et al. (2015) found that 1% of peppermint extract had no effect on the growth of Caspian white fish, but higher levels of 2 and 3% significantly increased their growth and improved feeding efficiencies. One of the reasons for relatively conservative levels used in the current study was the presence of pulegone, which as previously mentioned, acts as a strong blocker to octopamine and might have induced lethality.

However, over the course of 12-day feeding trial, no noticeable difference was observed in their activity or feeding rates. Moreover, when the crabs were placed together in the second experiment that provided equal opportunities for cannibalism, there was no significant difference in the survival. Octopamine is considered to change the behavior of various crustacean species (Lingstone et al., 1980; McRae, 1996; McPheen and Wilkens, 1989; Tricarico and Gherardi, 2013).
Fig. 2: Gill histopathology from the blue swimmer crab, Portunus pelagicus, early juveniles in control treatment (A) and 1% dietary peppermint oil treatment (B). Note the prevalence of pillar cells (Pi) and occasional hemocytes (Hem). Magnification × 40. H & E

Fig. 3: Hepatopancreatic histopathology from the blue swimmer crab, Portunus pelagicus, early juveniles in control treatment (A, B) and those in 1% dietary peppermint oil treatment (C, D). Note the reduced prevalence of B-cells (B) and R-cells (R) and higher prevalence of E-cells (E) in 1% dietary peppermint oil treatment. The amount of F-cells (F) were similar among all treatments. Magnification × 40. H & E
Dietary peppermint oil on crabs juveniles

Table 2: Mean survival (%) and specific growth rates (SGR) (±SE) for wet weight, carapace length and carapace width (% day\(^{-1}\)) and molt interval (days) for the C1 – C2 and C2 – C3 stages of early *Portunus pelagicus* juveniles when fed diets with increasing amounts of peppermint oil after 12 days.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.05%</th>
<th>0.1%</th>
<th>0.5%</th>
<th>1.0%</th>
<th>0.05%</th>
<th>0.1%</th>
<th>0.5%</th>
<th>1.0%</th>
</tr>
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<tbody>
<tr>
<td>Survival</td>
<td>82.20</td>
<td>68.80</td>
<td>80.00</td>
<td>84.40</td>
<td>84.40</td>
<td></td>
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<tr>
<td>SGR weight</td>
<td>6.96 ± 0.70</td>
<td>6.95 ± 0.71</td>
<td>5.63 ± 0.56</td>
<td>5.74 ± 0.47</td>
<td>6.53 ± 0.37</td>
<td>6.96 ± 0.70</td>
<td>6.95 ± 0.71</td>
<td>5.63 ± 0.56</td>
<td>5.74 ± 0.47</td>
</tr>
<tr>
<td>SGR length</td>
<td>2.27 ± 0.19</td>
<td>2.33 ± 0.25</td>
<td>2.11 ± 0.15</td>
<td>1.95 ± 0.13</td>
<td>2.50 ± 0.19</td>
<td>2.27 ± 0.19</td>
<td>2.33 ± 0.25</td>
<td>2.11 ± 0.15</td>
<td>1.95 ± 0.13</td>
</tr>
<tr>
<td>SGR width</td>
<td>4.02 ± 0.16</td>
<td>4.36 ± 0.24</td>
<td>3.88 ± 0.19</td>
<td>3.65 ± 0.19</td>
<td>3.95 ± 0.16</td>
<td>4.02 ± 0.16</td>
<td>4.36 ± 0.24</td>
<td>3.88 ± 0.19</td>
<td>3.65 ± 0.19</td>
</tr>
<tr>
<td>C1 – C2</td>
<td>2.94 ± 0.29</td>
<td>3.21 ± 0.42</td>
<td>2.84 ± 0.39</td>
<td>3.07 ± 0.41</td>
<td>3.19 ± 0.27</td>
<td>2.94 ± 0.29</td>
<td>3.21 ± 0.42</td>
<td>2.84 ± 0.39</td>
<td>3.07 ± 0.41</td>
</tr>
<tr>
<td>C2 – C3</td>
<td>7.81 ± 0.44</td>
<td>7.94 ± 0.71</td>
<td>8.04 ± 0.55</td>
<td>8.15 ± 0.76</td>
<td>7.89 ± 0.54</td>
<td>7.81 ± 0.44</td>
<td>7.94 ± 0.71</td>
<td>8.04 ± 0.55</td>
<td>8.15 ± 0.76</td>
</tr>
</tbody>
</table>

1Statistical analysis or data variability on survival data could not be performed since each crab was one replicate. 2SGR weight = (LN(final weight) – LN(initial weight))/number of days) × 100, 3SGR length = (LN(final length) – LN(initial length))/number of days) × 100, 4SGR width = (LN(final width) – LN(initial width))/number of days) × 100. No significant differences for specific growth rates (SGR) or molt intervals were detected (p > 0.05).

Table 3: Mean (±SE) prevalence of B-cells, R-cells and E-cells within the hepatopancreatic tubules of early *Portunus pelagicus* juveniles when fed diets with increasing amounts of peppermint oil after 22 days.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>0.05%</th>
<th>0.1%</th>
<th>0.5%</th>
<th>1.0%</th>
<th>0.05%</th>
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<tbody>
<tr>
<td>B-cells</td>
<td>8.22 ± 0.69</td>
<td>7.93 ± 0.78</td>
<td>7.41 ± 0.54</td>
<td>4.19 ± 0.71</td>
<td>2.14 ± 0.33</td>
<td>8.22 ± 0.69</td>
<td>7.93 ± 0.78</td>
<td>7.41 ± 0.54</td>
<td>4.19 ± 0.71</td>
</tr>
<tr>
<td>R-cells</td>
<td>2.37 ± 0.11</td>
<td>2.54 ± 0.24</td>
<td>1.27 ± 0.13</td>
<td>1.03 ± 0.08</td>
<td>0.72 ± 0.02</td>
<td>2.37 ± 0.11</td>
<td>2.54 ± 0.24</td>
<td>1.27 ± 0.13</td>
<td>1.03 ± 0.08</td>
</tr>
<tr>
<td>E-cells</td>
<td>4.26 ± 0.31</td>
<td>4.61 ± 0.49</td>
<td>5.66 ± 1.36</td>
<td>8.52 ± 0.64</td>
<td>10.44 ± 1.43</td>
<td>4.26 ± 0.31</td>
<td>4.61 ± 0.49</td>
<td>5.66 ± 1.36</td>
<td>8.52 ± 0.64</td>
</tr>
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2007; Pedetta *et al.*, 2010), this again indicates that the dietary peppermint oil levels used were insufficiently high enough. It may also be possible that pulegone volatilized during the feed preparation/drying process, although the dietary peppermint oil extract did have a substantial effect on the hepatopancreatic structure, as well as cell prevalence.

For crabs in control treatment, the hepatopancreatic tubules were characterized as having predominately B-cells with similar amount of F- and E-cells and finally occasional R-cells. B-cells are responsible for enzyme secretion/enzyme secretion/nutrient absorption, F-cells synthesize enzymes, R-cells store lipids and glycogen and the E-cells are undifferentiated (Longo and Diaz, 2015). This general structure and prevalence of epithelial cells drastically changed with increasing dietary peppermint oil, which included thinner tubule lumen, as well as reduced and increased B- and E-cell prevalence, respectively. There are many active compounds in peppermint oil such as menthol, menthone, saponins, phenols, etc. that may have been responsible for altering the hepatopancreas. However, a reduction to these cells, particularly for R-cells, may indicate lower nutrient reserves (Vogt *et al.*, 1985; Simon and James, 2007; Romano *et al.*, 2015), although this was not evidenced based on the unaffected survival or growth of the crabs. It is likely worth noting that Adel *et al.* (2015) found that dietary peppermint oil did alter the leucocyte cell type to Caspian white fish and the authors stated that cause for this was unclear. Finally, regardless of the dietary peppermint oil levels, this had no effect on gill histopathology.

In conclusion, the unaltered growth or cannibalism rates of crabs fed dietary peppermint oil might be related to the levels not being high enough and/or insufficient duration. On the other hand, 0.1% dietary peppermint oil did induce significant alterations in the histopathological hepatopancreatic studies, it is worthwhile to investigate spraying peppermint oil directly onto feed pellets to minimize the potential for volatile compounds to evaporate and further research is warranted.

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References


