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Flavour enhancement of freshwater farmed barramundi (*Lates calcarifer*), through dietary enrichment with cultivated sea lettuce, *Ulva ohnoi*

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ABSTRACT

Nutrition plays an important role in shaping the organoleptic properties of fish as key flavour compounds are principally sequestered from the diet. Conventional aquaculture feeds are primarily formulated to maximise somatic growth with little consideration given to the effect on organoleptic attributes such as flavour, taste and aroma. We assessed the efficacy of the marine macroalgae *Ulva ohnoi* to alter the organoleptic properties of cultured freshwater barramundi (*Lates calcarifer*) by feeding a short term finishing diet. Barramundi were finished on diets containing 0%, 10%, 20% and 30% of dried *U. ohnoi* for 7, 14 and 21 days. Following harvest the organoleptic properties of fish were subjected to human sensory assessment. The flavour of barramundi finished on diets containing ≥20% inclusion level of *U. ohnoi* was described as being more 'desirable', 'sweeter', and 'rich and complex' when compared to fish finished on a standard commercial rearing diet. Fish finished with *U. ohnoi* also developed a crab-like/seafood flavour and a cooked crab aroma, resulting in significant increases in these organoleptic attributes.

Fillets were subsequently analysed for bromophenol compounds and dimethylsulfide. Bromophenols were not detected at any time in fillets of barramundi that were finished on either *U. ohnoi* or a standard commercial diet. However, dimethylsulfide was found to be more elevated (~8 fold) in fish fed *U. ohnoi*.

Feeding cultured freshwater fish a diet containing the marine algae *U. ohnoi* was effective in altering the organoleptic profile of fillets, imparting a rich and complex seafood-like flavour. The potent flavour compound dimethylsulfide was associated with this change and appears to be a key flavour compound in this instance. The application of a finishing diet that enhances flavour and aroma properties pre-harvest would enable aquaculture growers to exert direct control over these properties. This is especially important for freshwater finfish species as growers could conceivably produce fish that have organoleptic similarity with wild caught marine species.

Statement of relevance: A functional feed providing a mechanism to produce low cost freshwater fish with flavours that are more characteristic of wild caught marine species is presented. This diet caused farmed freshwater fish to develop marine aromas and flavours and increased sweetness and flavour complexity during 21 days of feeding.

1. Introduction

The organoleptic quality of wild caught seafood and aquaculture products is profoundly influenced by dietary factors occurring over the pre-harvest period (Ackman et al., 1972; Boyle et al., 1993; Levasseur et al., 1994; Ma et al., 2005; Whitfield et al., 2002). The diet of wild-caught seafood is often diverse and this is thought to contribute to the complex and distinctive flavour of seafood products (Boyle et al., 1992; Whitfield et al., 1997; Whitfield et al., 1998). Aquaculture systems however typically utilise manufactured feeds, accordingly fish reared in captive systems lack the diversity of wild ecological diets. Resultantly, the flavour of fish reared in aquaculture systems can be noticeably different from their wild-caught counterparts (Carton and Jones, 2013; Grigorakis et al., 2003; Grigorakis, 2007; Ma et al., 2005; Whitfield et al., 1997).

Whilst the flavour quality of aquaculture products is highly acceptable, consumers have reported a difference between the flavour of some aquaculture fish and wild-caught fish (Carton and Jones, 2013; Grigorakis et al., 2003; Grigorakis, 2007; Ma et al., 2005; Whitfield et al., 1997). The organoleptic quality of some aquaculture products has been described as less complex and lacking ocean or 'sea-fresh' characteristics (Ma et al., 2005; Whitfield et al., 1997). There have however been recent attempts to enhance the organoleptic attributes of aquaculture products by enriching manufactured artificial diets with critical flavour compounds (Fuller et al., 2008; Kim et al., 2007; Ma et al., 2005).

A wide array of compounds has been implicated in the organoleptic quality of aquatic animals. Of particular interest are bromophenol

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compounds (BPs) and dimethylsulfide (DMS). These compounds appear to be unique in their ability to produce a characteristic sea-like or sea-fresh aroma, have been credited with giving the sea its distinctive smell and are known to contribute desirable flavour characteristics to seafood (Ackman and Hingley, 1968; Boyle et al., 1993; Brooke et al., 1968; Iida and Tokunaga, 1986 cited in Hill et al., 2000; Kim et al., 2007; Ma et al., 2005; White, 1982 cited in Van Alstyne et al., 2009; Whitfield et al., 1997; Wolfe, 2014).

Several studies have investigated the importance of bromophenols in seafood and aquaculture products. For example Whitfield et al. (1997) identified that farmed prawns lacked the distinctive ocean-flavour of wild-caught prawns, this was attributed to the absence of bromophenols in the diet. However Kim et al. (2007) enhanced bromophenol levels in cultured green grouper by enriching diets with marine algae whilst Ma et al. (2005) were successful in replacing ocean-flavour in cultured silver sea bream, again through dietary enrichment with marine algae high in BPs.

At present, dietary enrichment with DMS for the purposes of organoleptic enhancement remains unexplored. Many species of marine algae are known to synthesise a high concentration of dimethylsulphoniopropionate (DMSP), which cleaves to form DMS (Kwint and Kramer, 1996) and acrylic acid (Iida, 1988 cited in Kawai and Sakaguchi, 1996). It is also well understood that DMS and DMSP are passed through the food chain by herbivory and predation (Ackman et al., 1966; Ackman et al., 1972; Levasseur et al., 1994). Although DMS has been identified as a flavour volatile in several species of marine fish and prawns (Mansur et al., 2003), the implications of feeding captive fish diets containing marine algae rich in DMSP or DMS remain unexplored.

Ulva is a genus of coastal marine green algae distributed throughout temperate and tropical regions (Kirkendale et al., 2013; Lawton et al., 2013). Species within this genus are known to synthesise a large number of volatile organic compounds and olfactory compounds including BPs, DMSP and DMS (Flodin et al., 1999; Sugisawa et al., 1990; Whitfield et al., 1999b). *Ulva ohnoi* is a species of the genus *Ulva* which is successfully cultivated in aquaculture systems (Mata et al., 2015) and is considered an ideal species to target bioremediation in land based aquaculture farms (Lawton et al., 2013). *U. ohnoi* is also used as a bioremediation tool in abalone waste water tanks with the produced biomass being used as a feed for abalone (Bolton et al., 2009). Diets incorporating a relatively high inclusion level of *Ulva* are readily consumed by finfish (Marinho et al., 2013; Pereira et al., 2012; Wassef et al., 2013) and may have the potential to alter organoleptic properties of cultured species. The ability to manipulate these properties has the potential to increase marketability and financial returns to producers. In addition, as aquaculture feed manufacturers seek to replace wild-sourced fish meal and oil with terrestrial plant based products, organoleptic quality issues may become significant as such diets could possibly be devoid of key flavour and aroma compounds.

This study explores the application and potential of the green seaweed *U. ohnoi* in a short-term finishing diet for the purpose of altering the organoleptic attributes of cultured freshwater fish prior to harvest. Short term finishing diets are used to actively modify traits that have high consumer appeal. For example finishing diets high in fish oil have been shown to restore highly desirable fatty acid profiles of farmed Atlantic salmon, red sea bream and Senegalese sole previously reared on plant oil based diets (Bell et al., 2004; Glencross et al., 2003; Reis et al., 2014).

Lates calcarifer, known as Asian sea bass or barramundi, was selected to assess the efficacy of an organoleptic enhancing diet enriched with *Ulva*. Barramundi is an important food fish in tropical regions with a total global harvest approaching ~164,000 tonnes per annum, 40% (~66,000 t) of which originates from captive aquaculture production (FAO, 2012). Whilst farmed barramundi is generally acknowledged to possess a favourable fresh fish flavour (Carton and Jones, 2013; Jones et al., 2013) wild-caught barramundi are known to possess significantly stronger shellfish (prawn) characteristics (Frank et al., 2009) and typically achieve a higher sale price in Australian fish markets.

The objectives of this study were to:

1. Investigate the palatability of *Ulva* in aquaculture diets and determine the maximum inclusion rate by quantifying feed intake at varying dietary levels.
2. Determine if feeding fish diets enriched with *Ulva* affects aroma and flavour, characterise any organoleptic changes and investigate key flavour compounds in the muscle tissue of *Ulva* fed barramundi.
3. Determine the optimal inclusion level of *Ulva* in the diet, with respect to organoleptic changes, and to explore the temporal response of key flavour attributes to dietary manipulation.

2. Methods

2.1. Preparation and formulation of experimental diets

Dried, powdered *U. ohnoi* was supplied by MBD Energy and was used at four inclusion levels to formulate experimental diets. *U. ohnoi* was initially harvested from the Pacific Reef Fisheries production facility (MBD Energy, Ayr, Australia) and subsequently grown in continuous culture in 10,000 l parabolic tanks (Mata et al., 2015). A sufficient volume of the macroalgae was harvested and oven dried (24 h at 60 °C) before being milled and screened (<1 mm) prior to use in the experimental diets. Cold-pressed diets were prepared by milling a commercially available barramundi grower diet (Ridley Aquafeed, Narrangba, Australia) to a fine powder (<1 mm) and reconstituting with pre-gel maize starch (10%) and dried, powdered *Ulva*. Inclusion levels of *Ulva* were 0% (reference diet), 10%, 20%, 30% and 50%. Water was incorporated to form a pliable dough and pressure pelleted through a 10 mm die (Hobart Corp, Troy, USA). Pellets were manually cut to ~10 mm length prior to oven drying at 60 °C until a constant weight was achieved. After 3 days of storage 100 randomly selected pellets from each diet were individually weighed to determine average pellet weight. Diets incorporating 10, 20 and 30% *Ulva* were used to characterise the flavour profile, and undertake sensory evaluations. The 30% and 50% diets were used to test the palatability of *Ulva* as a feed ingredient.

2.2. Fish supply

All fish in this study were supplied by a local freshwater barramundi farm (Good Fortune Bay Fisheries, Kelso, Australia), fish were netted from growout ponds and transported to the Marine Aquaculture Research Facilities Unit (MARFU) at James Cook University.

2.3. Preliminary assessment of Ulva as a feed additive

A total of 27 market sized barramundi (800–1000 g) were initially acclimated for a period of 7 days in 9 × 500 l fibreglass tanks ($n = 3$ fish per tank) supplied with continuous flow-through dechlorinated municipal water at ambient temperature. All fish were fed a cold pressed pelleted diet (0% *Ulva*) during the period of acclimation. Following this, diets comprising of 0% (reference diet), 30% and 50% *Ulva* were randomly allocated to each tank such that each diet was fed to a total of 3 tanks ($n = 9$). Fish were hand fed to satiation once daily for a period of 30 days. Daily feed consumption was determined as the difference between the number of pellets supplied to each tank and the number of uneaten pellets remaining after feeding activity had ceased. Average pellet weight was used to calculate the weight of feed consumed per tank, and average daily feed consumption over the 30 day feeding period was used to evaluate the relative palatability of each diet. Following 30 days of feeding, all fish were euthanized according to Australian industry standards (see Carton and Jones, 2013) and filleted on both sides within 30 min of harvest. Fillets were immediately frozen