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Development of a water-stable agar-based diet for the supplementary feeding of cleaner fish ballan wrasse (*Labrus bergylta*) deployed within commercial Atlantic salmon (*Salmon salar*) net-pens

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ABSTRACT

The aim of this project was to develop a water-stable and palatable diet for the supplementary feeding of wrasse deployed in salmon sea-pens using a gelling agent mixed with a manufactured dry-feed component. Three binders (gelatine from cold water fish skin, beef gelatin and agar-agar) were compared for water-gel strength over a range of concentrations. Gel formed using agar was found to be significantly stronger than the other binders tested. An experimental aqua-feed made using a grinded, dry ingredient mix binded with 20 g/L agar solution at 1/1.6 (w/v) ratio and offered as blocks within individual feeders was water-stable for 7 days when deployed fresh or following a week of preservation at -20 °C. Farmed ballan wrasse in tanks fed on the agar-based diet within 2 days of deployment. Wild wrasse stocked in salmon sea-pens at low density (1.2–2.1%), up to 4 weeks prior the start of the trial and not previously fed a manufactured diet first ingested the agar feed within 2 weeks and total feed intake significantly increased afterwards. Feed intake was significantly higher from feeders placed within a small feeding shelter made of artificial kelp than within the large wrasse shelter. No nutrient leaching after water immersion and no alterations in the fatty acid profile after preparation of the experimental feed was found. A manufactured grinded ingredient mix binded with 20 g/L agar solution at a 1/1.6 (w/v) ratio and offered within static feeders is proposed as the basis of a novel supplementary feeding methodology for cleaner fish wrasse deployed in salmon sea-pens. This methodology has the potential to facilitate wrasse feeding and to allow the monitoring of feed intake to safeguard the health, welfare and delousing activity of the biological stock over the salmon rearing cycle.

1. Introduction

Cleaner fish, first tested in late 1980s (Rae, 2002), are increasingly acknowledged as an effective and sustainable biological treatment against sea-lice (Skiftesvik et al., 2013; Leclercq et al., 2014) with clear benefits over the use of chemotherapeutants. Cleaner fish are typically deployed preventively within commercial salmon net-pens and seldom exposed to high sea-lice density due to mandatory or voluntary treatment at pre-defined trigger levels. Net bio-fouling, known to act as an

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alternative feed source for the cleaner fish stock (Deady et al., 1995) is typically kept minimal to maintain biological delousing and the performance of the rearing system. Under such conditions, a decrease in the condition factor and body-mass of ballan wrasse (*Labrus bergylta*) was evident within 6 weeks of deployment despite a relatively high initial lice density (9 lice/salmon) and documented delousing activity (Skiftesvik et al., 2013). In a comparative tank trial, farmed ballan wrasse stocked at 5 wrasse/100 salmon and exposed to as much as 12 motile lice per salmon consumed over 75% of available sea-lice, i.e. ~180 sea-lice per wrasse, within 24 h (Leclercq et al., 2014). Such high lice consumption levels were not negatively affected by supplementary feeding on fresh crushed blue-mussels and did not satisfy satiation based on the functional predatory response of wrasse to sea-lice density. It is becoming increasingly evident that the supplementary feeding of cleaner fish deployed within commercial salmon pens is necessary to maintain the nutritional condition, welfare and efficacy of the biological controls over the Atlantic salmon grow-out cycle typically lasting 18–22 months. Therefore, a feed source adapted to the species grazing feeding habit and to the salmon net-pens rearing environment has first to be developed.

Fresh seafood (e.g. locally collected crushed blue mussels) and manufactured extruded pellets formulated to labrids requirements have been offered using submerged nets and video evidences of consumption were obtained. However, the provision of sufficient fresh seafood is logistically prohibitive while raising biosecurity concerns. Manufactured dry-pellets (extruded) delivered in fine mesh bags were found to disintegrate within hours in water leading to significant wastage further compromising validation and quantification of feed intake. A practical feed for cleaner fish within salmon net-pens should combine a manufactured base providing a complete and standardised nutrient profile, biosecurity and ease of procurement with high water stability for distribution as grazing substrate.

Hydrocolloid agents have been used as binders to produce practical aqua-feed with high water stability mostly for slow-feeding, i.e. grazing crustaceans and echinoderms (Tacon, 1987). Gelatine (Panreac® Aditio 80-100 Blooms) used to bind frozen shrimp and squid was found palatable and not to compromise growth rate and feed conversion in the common octopus (*Octopus vulgaris*; Quintana et al., 2008). Gelatin at a concentration of 20–30 g/L was deemed a suitable binder of microbound diet for barramundi (*Lates calcarifer*) larvae (Partridge and Southgate, 1999). Natural gum extracted from seaweed such as agar, alginate and carragennan have also been successfully applied (Teshima et al., 1984; Cho et al., 1985). Caltagiorne et al., 1992 reported better binding performance of agar compared to gelatine, carboxymethyl cellulose and sodium alginate. In another study, agar prevented the disintegration of a manufactured diet for up to six days (Fabbrocini et al., 2012) and had positive effects on the growth rate of crustaceans at inclusions of 20–30 g/L (Palma et al., 2008; Volpe et al., 2008; Coccia et al., 2010). However contrasting comparative performances of various binders have been reported across studies which may be due to the type of feed used, variations in the formulation and assessment of the practical feed (Ruscoe et al., 2005; Paolucci et al., 2012). Gelatine is derived from the collagen extracted from the bone and skin of terrestrial and aquatic animals, it is rich in amino acids, odourless and tasteless (Paolucci et al., 2012). Gelatine is soluble in hot water, typically used at concentration over 5 g/L and forms a thermo-reversible gel which increases in viscosity when cooled below 25 °C (Karim and Bhat, 2009). Agar is a polysaccharide composed of agarose and agaropectine extracted from agarophyte seaweed (Usov, 1998). It dissolves at water temperature above 90 °C, forms a gel at concentrations as low as 3 g/L and is typically used at 20–30 g/L in aqua-feed (Fabbrocini et al., 2012). The gelling point of agar solution is lower than its melting point (hysteresis) conferring good gel strength at ambient temperature. The aim of this study was to develop a water stable and well accepted cleaner-fish feed based on a commercially available dry-feed with the view to facilitate feed management and sustain the nutritional condition and welfare of cleaner fish in commercial salmon net-pens.

2. Materials and methods

2.1. Water-gel strength

Three binders selected based on apparent efficacy and practicality were used: Beef gelatine (BG; 200–250 g high Bloom strength, Dr Oetker(UK)^{Ltd}, Leeds, UK), fish gelatine from cold water fish skin (FG; 90–110 g low Bloom-strength; G7765, Sigma–Aldrich, Dorset, UK) and agar-agar (AA; Special Ingredients^{Ltd}, Chesterfield, UK). The water gel strength of each binder was tested in duplicate under standard conditions, at two temperatures (5 and 20 °C) and over a range of concentrations (n = 7 concentrations/binder) selected according to the general recommendations of each product.

Stock solutions of BG (40 and 50 g/L), FG (150 and 200 g/L) and AA (25 and 40 g/L) were prepared as follow. Binders were weighted (±0.001 g) and transferred into tap-water (pH: 6.9; 35 °C). BG and FG were kept at 35 °C under constant stirring until dissolution within 10 min. AA was left to hydrate for 10 min at 35 °C prior to boiling for 2 min. Stock solutions were then serially diluted, transferred into 100 mL vials and left to solidify for 24–28 h at 5 °C. Gel strength was measured at 5 °C (immediately after refrigeration) or at 20 °C (after 4 h of room conditioning) on 2 vials/temperature/concentration/binder using an in-house piston system. Pressure was applied at 10 g/cm^2 increments until breakage of the gel surface which was recorded as a quantitative measure of gel-strength.

2.2. Water stability of the experimental feed

Binder solutions showing satisfactory water-gel strength (AA 10 and 20 g/L, BG 50 g/L) were selected for preparation of the experimental feed using two types of complete dry-feed components: manufactured extruded pellets (EP; Ø 14 mm Symbio wrasse maintenance; BioMar(UK)^{Ltd}, Grangemouth, UK) and its corresponding grinded ingredient mix provided