Suitability of three red macroalgae as a feed for the abalone *Haliotis tuberculata coccinea* Reeve

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Abstract

A 60-day feeding trial was conducted to assess the suitability of three red algae, *Hynea spinella*, *Hynea musciformis* and *Gracilaria cornea*, as potential feed for the culture of juvenile abalone, *Haliotis tuberculata coccinea* R. Seaweeds were reared in a biofiltration unit with fishpond waste water effluents. The three algal species were found to contain high protein contents which would be related to its production under the high nitrogen culture conditions of the biofilter system. Protein and carbohydrate contents were highest in *H. musciformis* and lowest in *G. cornea*. Survival rates of juvenile abalone were very good, regardless of the algae fed. Feed intake of *H. spinella* was highest, followed by *H. musciformis*. Growth rates of abalone were within the range obtained under commercial conditions, final shell length and weight being significantly highest in animals fed *H. spinella* and lowest in those fed *G. cornea*. Feeding *G. cornea* lead to the lowest growth performance due to the lowest feed intake, whereas feed conversion ratios were significantly highest for *H. musciformis* and protein efficiency ratios were higher for both *H. spinella* and *G. cornea*. This study suggested the good potential of any of the three red seaweeds tested—successfully produced by the biofilter system, their nutritional composition being similar to other macroalgae used as feed for abalone and matching the abalone protein and lipid requirements—hence promoting growth and survival. Nevertheless, the biofilter produced macroalgae *H. spinella* showed the highest dietary value for juvenile of *H. tuberculata coccinea*.

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1. Introduction

Commercial aquaculture in the Canary Islands is limited to the production of marine fish species such as gilthead seabream and European sea bass, with

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species diversification being a challenge for further local aquaculture development. The potential as a new species for aquaculture of the Canarian abalone *Haliotis tuberculata coccinea* R., whose natural population is becoming depleted due to overfishing, relies on the high demand for abalone both in local and external markets, as well as on the high degree of development achieved in some other species of the family Haliotidae (*Haliotis discus hannai*, *Haliotis asinina*, *Haliotis rufescens*, etc.), in particular of the European ormer *Haliotis tuberculata tuberculata* L., with close biological characteristics. Besides, there is public interest in developing the culture techniques of this species to contribute to the recovery of wild populations. Studies on *H. tuberculata coccinea* are scarce and have been focused only on spawning (Peña, 1985, 1986; Viera et al., 2003), ecology (Pérez and Moreno, 1991; Espino and Herrera, 2002) and culture techniques (Toledo et al., 2000).

Food preferences differ among abalone species around the world, depending on habitat and food availability (Barkai and Griffiths, 1986; Dunstan et al., 1996). In the wild, abalone consumes different macroalgae species, obtaining their required nutrients from a combination of algal species. Although *H. tuberculata coccinea* feeds on a diverse assemblage of macroalgae (Espino and Herrera, 2002), its nutritional needs and the relative importance of these algae are unknown. Algal diets have been extensively used for the production of abalone (Clarke, 1988). This herbivorous gastropod can consume seaweed at a rate close to 35% of its body weight per day. Hence sustaining of growth requires a large amount of fresh macroalgae (Tahil and Juinio-Menez, 1999). Since wild algae are not commercially valuable in the Canary Islands, and hence not harvested, availability of this natural resource is scarce and insufficient to sustain the commercial production of abalone, which would require the replacement of wild seaweed as a main diet component.

In an aquaculture integrated system, nitrogenous enriched waste water of intensively cultured organisms such as fish, shrimp, and abalone may be transformed into a valuable algal biomass, seaweeds production being an added income as feed for shellfish (Evans and Langdon, 2000; Schuenhoff et al., 2003; Neori et al., 2004). Besides, several studies have shown that culture of *Ulva* spp. in nutrient-rich

waters increases its protein content from 11% to over 32% in dry weight (Shpigel et al., 1999; Boarder and Shpigel, 2001). Thus, this biofilter produced *Ulva* has been shown to provide good growth rates for *H. tuberculata* (Neori et al., 1998; Shpigel et al., 1999), *H. discus hannai* (Corazani and Illanes, 1998; Shpigel et al., 1999) and *H. roei* (Boarder and Shpigel, 2001). Previous experiences in our laboratory have also shown the potential of using enriched *Ulva rigida* as a suitable feed for *H. tuberculata coccinea* (Viera and co-workers, unpublished data). But in the wild, Canarian abalone seem to have a higher preference for red algae (Espino and Herrera, 2002) as it has also been shown for several abalone species such as *H. iris* and *H. australis* (Poore, 1972; Shepherd, 1973; Wells and Keesing, 1989; Shepherd and Steinberg, 1992; Fleming, 1995), *H. tuberculata* or *H. discus hannai* (Mai et al., 1995a). Hence, in order to diversify the offer of macroalgae to cultured abalone in the Canary Islands, this study focused the evaluation of the suitability of three local red macroalgae cultivated in a biofiltration unit as a potential feed for the Canarian abalone *H. tuberculata coccinea*.

2. Materials and methods

2.1. Algal culture

Gracilaria cornea J. Agardh, *Hypnea spinella* (C. Agardh) Kützinger and *Hypnea musciformis* (Wulfen) J. V. Lamouroux were grown in a flow-through integrated system consisting of two intensive fishponds stocked with gilthead sea bream and a macroalgal biofilter. Effluents were channelled from the fishponds to a 11 m³ sedimentation pond for the removal of suspended particles and, then, pumped at a flow rate of 10 m³ h⁻¹ to the seaweed tanks located in a greenhouse, where maximum irradiance was close to 1600 μmol photons m⁻² s⁻¹. Semi-circular fiberglass tanks with a surface of 1.8 m², volume of 0.75 m³ and aeration supplied by a bottom-central linear pipeline were used for the cultivation of macroalgae. Algal stocking densities were adjusted to the optimal values obtained from previous experiments (6 g l⁻¹). Water exchange rate in the seaweed tanks was 4 vol day⁻¹ and TAN (total ammonia nitrogen) inflow into the biofilter ranged between 10 and 400 μM.

2.2. Abalone and rearing conditions

Abalones (*H. tubercalata coccinea*) were produced within the experimental hatchery production unit of the Instituto Canario de Ciencias Marinas, (Canary Islands, Spain). A total of 360 juvenile abalones (40/replicate) with an average shell length and weight of 11.18 ± 1.27 mm and 0.17 ± 0.06 g, respectively, were selected for the trial.

Animals were initially fed a diet of *Navicula* sp. and *Nitzschia* sp. for 4–5 months. Feeding of all abalone was then switched to the green macroalgae *U. rigida* cultured at the laboratory biofiltration system for a period of 1 month prior to the beginning of the experiment.

Individuals were blot dried, weighed to the nearest 0.1 mg (total wet body weight: TWBW), measured with manual caliper with 0.1 mm accuracy (total shell length: SL) and assigned to an experimental unit. Abalones were distributed among replicates so that there were no significant differences in SL or TWBW. Each algal type was fed for 8 weeks to the experimental abalone and tested in triplicates (9 experimental units plus 3 control units). The experimental unit consisting of a 1 l lidded (plastic net of 2 mm mesh) PVC plastic container (20 × 14 cm), located in a 100-l cylindrical tank filled with 50 µm filtered seawater, was provided with constant aeration. Water flowed in at approximately 2.4 l/min. Water temperature ranged between 23.9 and 25.4 °C. Abalones were subjected to a natural photoperiod of approximately 12 h L / 12 h D.

2.3. Growth and algal consumption

SL and TWBW of each animal were measured every four weeks.

Three experimental units that contained algae but no abalone were used as controls for changes in algal weight. Feed was changed once a week during the growth trial and supplied in excess to guarantee ad libitum feeding along the whole experiment. To determine feed intake, freshly collected algae to be fed to the abalone were blotted dry and accurately weighed as well as the remaining algae at the end of the week. The weight of unconsumed food was deducted from the total weekly ration. Besides, weight

of uneaten algae was corrected by calculating the natural change in weight of the algae in the control units during the same feeding period.

The average daily intakes by individual abalones during the entire feeding trial were calculated by dividing the algal biomass eaten each week by the feeding days and the number of abalones in each experimental unit.

The following nutritional indices were calculated for all treatments at the end of the trial:

Shell growth rate = $(L_2 - L_1) / \text{days of culture} \times 1000$

Specific growth rate, SGR = $(\ln W_2 - \ln W_1) / t \times 100$

Weight gain (%) = $((W_2 - W_1) / W_1) \times 100$

Food conversion ratio = $\text{total feed intake (g wet)} / \text{total weight gain (g wet)}$

Protein efficiency ratio = $(\text{increase in body wet weight (g)}) / (\text{protein intake (g)})$

where L_1 is the initial mean length of animals, L_2 is the final mean length of animals, $\ln W_2$ is the natural logarithm of weight at time t (days of culture), and $\ln W_1$ is the natural logarithm of the initial weight.

2.4. Nutritional analysis

Homogenized samples of each type of algae provided to the abalones were analyzed in triplicate for nutrient composition. The samples collected were cleaned, washed with freshwater to remove salts and epizoots, frozen in –80 °C freezer, freeze-dried and finely ground using an electric fine mill (sieve size < 0.1 mm). The dry matter was determined by drying at 110 °C for 24 h. Ash content was determined by incinerating samples at 600 °C for 24 h. Protein content was analyzed according to AOAC (1995) standard methods. Total lipids were extracted with chloroform–methanol (2:1) mixture as described by Folch et al. (1957).

2.5. Statistical analysis

Statistical analysis was conducted using one-way ANOVA and Tukey test for multiple comparison of means with 5% significance level was applied ($P < 0.05$). When data did not have normal distribu-

Table 1

Proximate composition and caloric content of the three red macroalgae (g/100 g DW) (mean $\pm$ S.D.) fed to abalone along the experimental trial

	Algal species		
	<i>H. musciformis</i>	<i>H. spinella</i>	<i>G. cornea</i>
Moisture	8.63 $\pm$ 0.27 ^a	8.11 $\pm$ 0.17 ^b	7.29 $\pm$ 0.12 ^c
Crude protein	27.13 $\pm$ 0.2 ^a	25.9 $\pm$ 0.1 ^b	21.54 $\pm$ 0.08 ^c
Crude lipid	2.45 $\pm$ 0.08 ^a	1.99 $\pm$ 0.01 ^c	2.36 $\pm$ 0.11 ^b
Carbohydrate ^c	32.79 $\pm$ 0.33 ^a	30.97 $\pm$ 0.27 ^b	27.7 $\pm$ 0.1 ^c
Ash	37.63 $\pm$ 0.12 ^c	41.14 $\pm$ 0.28 ^b	48.39 $\pm$ 0.17 ^a
GE ^b (kcal g ⁻¹)	3.1	2.9	2.6
Protein:energy ratio ^a	87.4	89.3	82.7

Values in the same row with different letters are significantly different ($P < 0.05$) $n = 3$.

^a Metabolizable energy was calculated based on the physiological values at 5.6 kcal g⁻¹ protein, 9.5 kcal g⁻¹ lipid and 4.1 kcal g⁻¹ carbohydrates (Cho et al., 1982).

^b Gross energy.

^c Calculated by difference (AOAC, 1995).

tion, a non-parametric one-way ANOVA on ranks of Kruskal–Wallis was tested (Zar, 1984).

3. Results

3.1. Algal nutritional composition

Nutritional composition and caloric content of the three red seaweeds are shown in Table 1. Protein energy ratios and gross energy were very similar among the different algae. However, there were

significant differences ($P < 0.05$) among the proximate compositions of the algae fed. Protein content was significantly highest in *H. musciformis*, whereas *G. cornea* showed the lowest. Similarly, *H. musciformis* showed the highest carbohydrate content and *G. cornea* the lowest. The three algal species were low in lipid content, the highest being also found in *H. musciformis* and the lowest in *H. spinella*. The ash content varied inversely to protein and carbohydrate contents and was highest in *G. cornea* and lowest in *H. musciformis*.

3.2. Growth and consumption

Survival, growth performance and feed utilization of juvenile *H. tuberculata coccinea* fed the macroalgae *H. musciformis*, *H. spinella* and *G. cornea* are shown in Table 2. Survival rates were very good and no significant differences were found among the different feeding regimes. Daily feed intake on different algal rations recorded for 8 weeks showed that all three macroalgae were very well accepted by the abalone, but a significantly higher feed intake of *H. spinella*, followed by *H. musciformis*, was registered. Hence, final shell length and weight were significantly highest in animals fed *H. spinella* and lowest in those fed with *G. cornea*. Accordingly, shell growth rate, specific growth rate and weight gain followed the same pattern, all these parameters being significantly highest in animals fed *H. spinella* and lowest in those fed *G. cornea*. Study of the feed

Table 2

Growth, feed utilization and survival of juvenile Canarian abalone (*H. tuberculata coccinea*) at the beginning of the experiment and after being fed the selected macroalgae for 60 days under laboratory conditions

	Algae species fed to abalone		
	<i>H. musciformis</i>	<i>H. spinella</i>	<i>G. cornea</i>
Initial length (mm)	11.18 $\pm$ 1.27	11.2 $\pm$ 1.29	11.17 $\pm$ 1.27
Final length (mm)	15.92 $\pm$ 1.52 ^b	18.54 $\pm$ 1.73 ^c	14.75 $\pm$ 1.75 ^a
Shell growth rate (μ m day ⁻¹)	81.79 $\pm$ 3.23 ^b	126.61 $\pm$ 5.92 ^c	61.71 $\pm$ 1.73 ^a
SGR (% day ⁻¹)	1.89 $\pm$ 0.03 ^b	2.66 $\pm$ 0.11 ^c	1.47 $\pm$ 0.05 ^a
Initial weight (g)	0.17 $\pm$ 0.06	0.17 $\pm$ 0.06	0.16 $\pm$ 0.06
Final weight (g)	0.51 $\pm$ 0.15 ^b	0.78 $\pm$ 0.21 ^c	0.38 $\pm$ 0.12 ^a
Weight gain (%)	178.41 $\pm$ 8.63 ^b	348.65 $\pm$ 30.35 ^c	123.64 $\pm$ 4.43 ^a
Feed intake (mg abalone ⁻¹ day ⁻¹)	165.54 $\pm$ 4.96 ^b	215.63 $\pm$ 0.61 ^c	78.98 $\pm$ 1.79 ^a
FCR	30.49 $\pm$ 1.64 ^b	20.94 $\pm$ 1.49 ^a	22.09 $\pm$ 1.15 ^a
PER	1.1 $\pm$ 0.06 ^a	1.68 $\pm$ 0.1 ^b	1.75 $\pm$ 0.09 ^b
Survival (%)	93.33 $\pm$ 2.89	95.83 $\pm$ 1.44	95.75 $\pm$ 5.1

Values in the same row with different letters are significantly different ($P < 0.05$) $n = 40 \times 3$.

efficacy of feed utilization showed that feed conversion ratios were significantly higher for *H. musciformis*, whereas protein efficiency ratios were higher for both *H. spinella* and *G. cornea*.

4. Discussion

The nutritional value of food rations depends on many factors including nutrient composition, bioavailability, palatability and digestibility (Serviere-Zaragoza et al., 2001). This study examined the nutritional value of three algal diets for juvenile abalone, *H. tuberculata coccinea*, by measuring biochemical composition and energy content of the algae and relating this to growth performance and feed intake of the animals.

The proximate composition of the algae was in the range of values reported for other species of seaweeds used as feed for abalone (Jackson et al., 2001; McBride et al., 2001). Protein content (22–27% DW) of the three algae was within the range for red seaweeds (10–47%) (Fleurence, 1999; Wong and Cheung, 2000) and was higher than the level found in the red seaweed, *Palmaria palmata* (18.4%), which has been found to be a good algal diet for *H. tuberculata* (Mercer et al., 1993; Mai et al., 1995a). Furthermore, protein content of *H. spinella* and *H. musciformis* was higher than that reported for other species of *Hypnea* (4.2–19% DW) (Portugal et al., 1983; Wong and Cheung, 2000). These high levels of protein content observed in the algae of the present study would be related to its production under the high nitrogen culture conditions of the biofilter system, as it has been also observed in *Ulva* spp. cultured as macroalgal biofilters (Tenore, 1976; Neori, 1996; Shpigel et al., 1996a,b, 1999). Hence, protein content of the three macroalgae used in the present experiment would match the protein requirements described for several species of *Haliotis* such as *H. discus* (20%) (Ogino and Kato, 1964), *H. discus hannai* (20–30%) (Uki et al., 1986a), *H. kamtschaticana* (30%) (Taylor, 1992) or for *H. tuberculata* and *H. discus hannai* (25–35%) (Mai et al., 1995b), although it would be lower than that required for *H. midae* (47%) (Britz, 1996).

All the algae were low in lipid (2–2.4% DW) but within the range of other species of red seaweeds

(1–3% DW) reported previously (Mabeau and Fleurence, 1993; Wong and Cheung, 2000). Nevertheless, abalone species show low lipid requirement, typical of herbivore molluscs and fish (Mai et al., 1995a). This low lipid requirement has been associated by some authors (Durazo-Beltrán et al., 2004) with the low use of dietary lipids as energy source by abalone based upon its low metabolic rate. Indeed, high levels of dietary lipid seem to negatively affect abalone growth (Thongrod et al., 2003). However, high levels of carbohydrate enhance growth (Thongrod et al., 2003) of abalone which has various enzymes capable of hydrolysing complex carbohydrates (Fleming et al., 1996) and a good capacity to synthesize non-essential lipids from carbohydrates. In the present study, carbohydrate values (28–33% DW) were high and comparable to those obtained for other algal species (Kaehler and Kennish, 1996; Foster and Hodgson, 1998).

Ash content of the *Hypnea* species was higher than that of other species of the same genus *H. charoide* (23–35% DW), *H. pannosa* (15% DW), and *H. japonica* (19% DW) (Portugal et al., 1983; Wong and Cheung, 2000). High ash content in algae results from the calcium carbonate, which limit the other nutrients' presence and reduce nutrient digestibility (Horn, 1989; Hay et al., 1994).

It is generally accepted that balanced levels of protein (>15%), lipid (3–5%) and carbohydrate (20–30%), with no toxic substances in natural algae, are essential for optimal growth performance of abalone (Mercer et al., 1993). From this point of view, the three algae tested appear to be able to match the abalone requirements.

Growth rates of abalone fed the dietary treatments in this study were within the range of 62–127 $\mu\text{m day}^{-1}$, close to those obtained by other authors in similar species (50–100 $\mu\text{m day}^{-1}$; Viana et al., 1996, 2000; Guzmán and Viana, 1998; Gómez-Montes et al., 2003) under similar experimental conditions and to that obtained under commercial conditions (80 $\mu\text{m day}^{-1}$; Gómez-Montes et al., 2003). SGR values were also high (1.5–2.7%) in comparison with those reported by Mai et al. (1996), who studied the effect of five species of macroalgae (*P. palmata*, *Alaria esculenta*, *Ulva lactuca*, *Laminaria digitata* and *Laminaria saccharina*) and reported SGR values of

1.03–1.31% for *H. tuberculata* and 0.7–1.25% for *H. discus hannai*.

Gracilaria species have been reported to promote high growth and survival in other species of abalone such as *H. asinina* (Upatham et al., 1998; Bautista-Teruel and Millamena, 1999; Capinpin et al., 1999; Reyes and Fermin, 2003). In the present study feeding *G. cornea* lead to the lowest growth performance due to the lowest feed intake registered, since feed utilization in terms of FCR and PER was as good as those of the highest growing abalone fed *H. spinella*. Indeed, consumption rate in abalone may be influenced by factors other than the nutritional quality of food, such as its toughness or presence of antinutritional chemicals (Fleming, 1995). Hence, the harder texture and ash content of *G. cornea* may have affected its consumption by *H. tuberculata* since, in the wild, abalone prefer soft texture macroalgae (Shepherd and Steinberg, 1992). This preference seem to be related to the little capacity of the rhipidoglossan radula to penetrate the algal surface (Steneck and Watling, 1982) as its teeth have limited ability to exert force against the substrate.

The high FCR of the three treatments agrees well with those reported for *H. discus hannai* and *H. tuberculata* using seaweeds as food (Shpigel et al., 1999) and *H. asinina* fed *G. fisheri* (Kunavongdate et al., 1995). The higher food conversion rate (FCR) attained for abalone fed *H. musciformis* in the present study does not seem to be explained by the general composition of the algae, or by the protein:energy ratio but to a significantly lower protein efficiency ratio (PER) than the rest of the treatments, suggesting that differences in response could be attributed to differential amino acid composition. Optimum growth is achieved through proper balance of dietary nutrients and fulfillment of requirements of essential nutrients and energy (Smith, 1989; Gómez-Montes et al., 2003).

In conclusion, this study suggested the good potential of using any of the three red seaweeds tested—successfully produced by the biofilter system, their nutritional composition being similar to other macroalgae used as feed for abalone and being able to match the protein and lipid requirements of abalone—hence promoting good growth and survival. Nevertheless, based on growth performance, food conversion ratio, protein efficiency ratio and protein:energy

ratio, the macroalgae *H. spinella* produced by the biofilter showed the highest dietary value for juvenile of *H. tuberculata coccinea*.

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