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Partial Substitution of Fresh Microalgae with Baker's Yeast (*Saccharomyces cerevisiae*) Enhances the Growth of Juvenile *Ostrea edulis* and *Ruditapes decussatus* †

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Abstract: The hatchery culture of bivalve mollusks depends on feeding with fresh microalgae which represent up to 50% of the production costs. We investigated the growth performance of juvenile *Ostrea edulis* and *Ruditapes decussatus* under 15% and 30% replacement of microalgae with *Saccharomyces cerevisiae*. Metabolic indices were measured along with weight-specific growth rate and condition index for 28 days. 15% substitution led to great results, whereas 30% yeast-fed treatments displayed poor growth and a depressed metabolism.

Keywords: aquaculture; bivalves; yeast; microalgae substitution

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1. Introduction

Microalgae production is the main limiting factor impeding the industrial growth of the bivalve aquaculture industry since it corresponds to 30–50% hatchery production's operating costs [1,2]. Diets aiming to substitute live microalgae have been implemented in the early stages of shellfish culture, with varying outcomes [3–5]. Yeast cells possess the capability for mass production, are highly stable in water, have an appropriate size for consumption, and high levels and quality of protein. All these favorable characteristics indicate yeast as a promising substitute for live algal feeds [1,6]. Despite their advantageous aspects, yeast cells present low digestibility and contain limited amounts of polyunsaturated fatty acids [4,7]. Therefore, yeast should be provided to bivalves accompanied by live microalgae, which contain highly unsaturated fatty acids [7]. This study investigated the effects of a partial microalgae replacement (15% and 30%) with baker's yeast on the feeds of juvenile *Ostrea edulis* and *Ruditapes decussatus* by assessing the activities of two key metabolic enzymes (citrate synthase and hydroxyacylCoA dehydrogenase) and the functioning of the respiratory chain through the activity of the electron transport system (ETS). Moreover, the specific growth rate (SGR) of weight and the condition index (CI) were measured.

2. Materials and Methods

Wild juvenile *Ostrea edulis* and *Ruditapes decussatus*, weighting approximately 2 and 4 g, respectively, were placed in rectangular aquaria (50 L) containing natural seawater. Bivalves were fed a live microalgae diet consisting of the marine flagellates *Tisochrysis lutea* (CCAP 927/14) and *Tetraselmis* spp. (Mediterranean strain) as well as the diatom *Chaetoceros* *calcitrans* (CCAP 1085/3) at a 2:1:1 dry weight ratio. Yeast cells, *Saccharomyces cerevisiae* NCPF 3191 (Sigma-Aldrich, St. Louis, MO, USA), were cultured in a YPD medium and included in two treatments so as to represent 15% and 30% substitution of the microalgae. Treatments were tested in triplicates. Four samplings were performed in a 28-day period on day 1, day 4, day 12, and day 28. At each sampling, the mantle tissue from 6 animals from each treatment was dissected for biochemical analyses. Twelve specimens at the beginning and another twelve at the end of the experiment were used for the condition index calculation, as described by Irisarri et al. [8]. SGR and CI were calculated as follows:

- (SGR) = 100 $\times$ (($\ln W_2 - \ln W_1$)/t), where W_1 and W_2 are the initial and final weights (g) of the bivalves and t is the number of feeding days;
- (CI) = (flesh dry weight/shell dry weight) $\times$ 100.

The activities of the metabolic enzymes citrate synthase (CS, EC 4.1.3.7) and hydroxyacylCoA dehydrogenase (HOAD, EC 1.1.1.35) were assessed spectrophotometrically based on well-established protocols [9], while ETS activity was determined according to Haider et al. [10]. The results of all the above indices were expressed as means $\pm$ standard deviation. One-way analysis of variance (ANOVA) was applied, followed by Tukey's HSD post hoc comparisons to define the statistically significant differences at $p < 0.05$.

3. Results

The condition of Ostrea edulis was similar to all treatments after 28 days, while Ruditapes decussatus fed on 30% yeast exhibited statistically significant lower conditions compared to the 0% and 15% treatments (Table 1). The growth rate of both species was significantly higher in treatments fed on 15% yeast and lower in replicates subjected to a 30% substitution of algae (Table 1).

Table 1. Condition index (CI) and specific growth rate of weight (SGR).

	Ostrea edulis		***Ruditapes decussatus***	
Condition index	mean	SD	mean	SD
Initial CI	1.86 ^{a}	0.24	18.35 ^{a}	1.84
Final CI—0% yeast	2.05 ^{a}	0.21	18.46 ^{a}	1.78
Final CI—15% yeast	1.92 ^{a}	0.12	18.31 ^{a}	1.43
Final CI—30% yeast	1.94 ^{a}	0.17	16.68 ^{b}	0.98
SGR of weight	mean	SD	mean	SD
SGRw 0% yeast	0.102 ^{a}	0.011	0.07 ^{a}	0.005
SGRw 15% yeast	0.12 ^{b}	0.008	0.108 ^{b}	0.013
SGRw 30% yeast	0.085 ^{c}	0.007	0.05 ^{c}	0.006

$^{a, b, c}$ Depict statistically different means by ANOVA ($p < 0.05$).

CS and HOAD in Ruditapes decussatus displayed generally similar activities among all treatments until day 4. On days 12 and 28, the activities of both enzymes significantly increased in both yeast-fed replicates, where the activity of these enzymes was similar (Figure 1A,B). Ostrea edulis demonstrated minor differences in the activity of CS at days 1 and 4, regardless of the feed composition. At day 12, both yeast-fed treatments presented a statistically significant increase in activity. At day 28, the 15% treatment had significantly greater activity, and the 30% treatment exhibited decreased activity compared to the control (Figure 2A). Concerning the HOAD in Ostrea edulis, 15% replacement of microalgae resulted in similar or greater control activities, while the 30% replacement led to a generally significantly reduced activity (Figure 2B).

The ETS activity displayed a clear pattern in both bivalves. When fed on 15% yeast, the two species exhibited similar to the control treatment activity of the electron transport system, but when fed on 30% yeast, the activity was significantly reduced (Figures 1C and 2C).

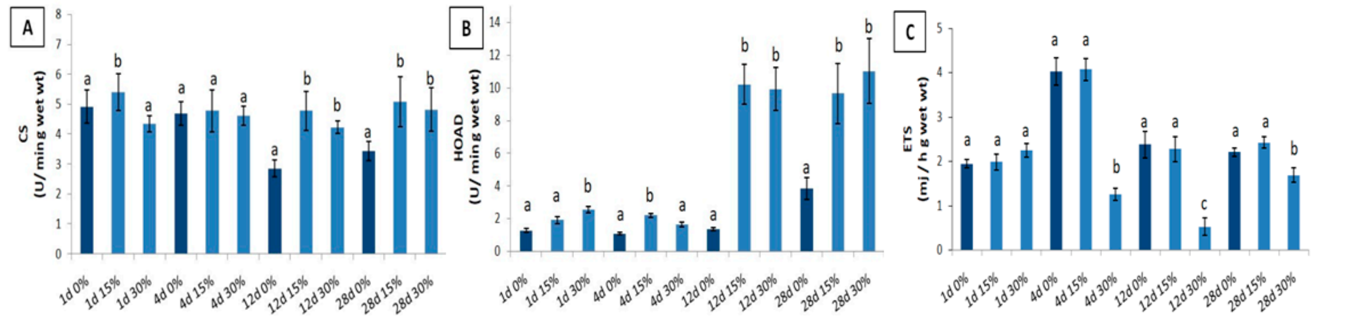

Figure 1. Activity of citrate synthase (**A**), hydroxyacylCoA dehydrogenase (**B**), and electron transport system (**C**) in the mantle tissue of *Ruditapes decussatus*. Dark blue indicates the control treatment. Values are means ± SD. Lower-case letters depict statistically significant differences ($p < 0.05$).

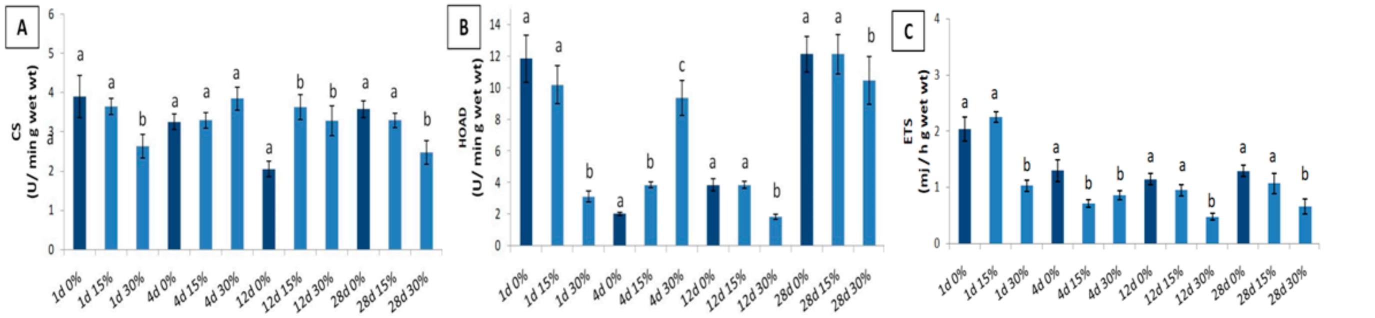

Figure 2. Activity of citrate synthase (**A**), hydroxyacylCoA dehydrogenase (**B**), and electron transport system (**C**) in the mantle tissue of *Ostrea edulis*. Dark blue indicates the control treatment. Values are means ± SD. Lower-case letters depict statistically significant differences ($p < 0.05$).

4. Discussion

Citrate synthase is a key metabolic enzyme that is associated with an organism's capacity for energy production (ATP generation) [11], while hydroxyacylCoA dehydrogenase is involved in fatty acid metabolic processes. Feeding regulates ETS activity, which can be used as an instantaneous index of oyster metabolism [12]. An increased ETS activity is also indicative of a higher rate of ATP production. On the other hand, ETS activity may decrease to conserve cellular resources, which might have happened in the case of 30% algae substitution in both species.

The highest growth rate of weight was detected at 15% yeast-fed replicates at both bivalves. Moreover, the same condition index as well as the similar or increased metabolic intensity in comparison to 100% algae-fed treatment indicate that a 15% substitution of algae enhances the growth of *Ruditapes decussatus* and *Ostrea edulis* juveniles. 30% replacement resulted in depressed ETS activity in both species, decreased activities of CS and HOAD in *Ostrea edulis*, and a lower condition index in *Ruditapes decussatus*. Encouraging results have been reported by many authors when using manipulated yeasts as an algal substitute [3,6] or by using efficiently digested mutant yeast cells [13], reaching substitution percentages of 50–80%.

5. Conclusions

A 15% percentage of fresh microalgae substitution with baker's yeast could be applied in the nursery stages of *Ruditapes decussatus* and *Ostrea edulis* to enhance their growth and eliminate production costs. Longer experiments, which will also include intermediate percentages of algae replacement (e.g., 20% and 25%) and/or different microalgae species, are necessary to assess the highest level of fresh microalgae substitution that can be achieved with *Saccharomyces cerevisiae* for these bivalve mollusks.

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