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Response to different dietary carbohydrate and protein levels of pearl oysters (*Pinctada fucata martensii*) as revealed by GC–TOF/MS-based metabolomics

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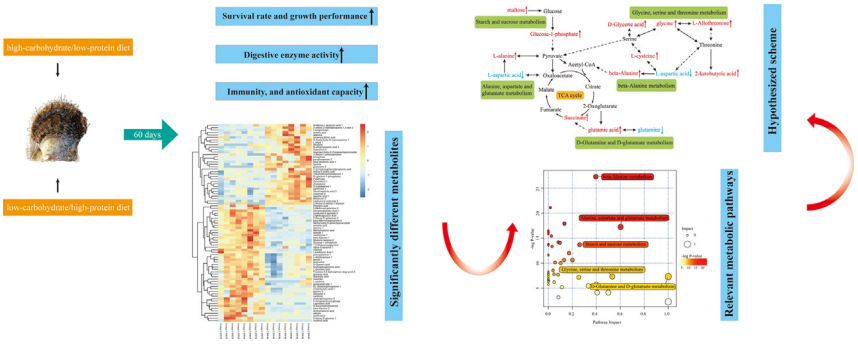
HIGHLIGHTS

- The study is the first to explore the optimum balance of dietary carbohydrates and proteins of pearl oyster.
- Dietary carbohydrates were used as the main energy source for pearl oyster.
- C45P25 enhanced starch and sucrose metabolism to meet the energy demand.
- C45P25 regulated glycine, serine and threonine metabolism to reduce β-oxidation.
- C45P25 modified alanine, aspartate and glutamate metabolism to promote protein synthesis.

GRAPHICAL ABSTRACT



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ABSTRACT

Land-based culturing can avoid the effects of environmental pollution and natural disasters, thus ensuring food safety for shellfish. However, food availability, in this case, is limited. To achieve the optimum balance of dietary carbohydrates and proteins and explore the mechanisms behind the phenomenon, we formulated five isoenergetic and isolipidic diets (C30P40, C35P35, C40P30, C45P25, and C50P20) with different levels of carbohydrates (C) and proteins (P). There were five experimental groups (C30P40, C35P35, C40P30, C45P25, and C50P20) and two control groups (CG1 and CG2). CG1 was fed with mixed powders of yeast and *Chlorella* sp., and CG2 was cultured in natural sea. After 60-day feeding, the highest rates of survival and absolute growth appeared in C45P25. C45P25 exhibited significantly higher activities of amylase, protease, alkaline phosphatase, acid phosphatase, superoxide dismutase, catalase, glutathione peroxidase, and phenoloxidase and significantly lower malondialdehyde content than C30P40, C35P35, C40P30, C50P20, and CG1. No significant differences were observed between C45P25 and CG2. Furthermore, the total antioxidant capacity of the pearl oysters in C45P25 was significantly higher than that in C30P40, C35P35, C40P30, and C50P20. On the basis of these results, the optimal balance of proteins and carbohydrates for pearl oysters was the C45P25 diet. Metabolomics-based profiling of the pearl oysters fed with high-carbohydrate/low-protein diet (C45P25) and low-carbohydrate/high-protein diet (C30P40) revealed 80 significantly different metabolites (VIP > 1 and $P < 0.05$). Furthermore, integrated key metabolic pathway analysis showed that C45P25 regulated starch and sucrose metabolism, alanine, aspartate and glutamate metabolism and glycine, serine and threonine metabolism to meet the energy demand and increase the glucogenic amino acid, thereby promoting protein synthesis and reducing fatty acid β-oxidation in comparison with C30P40. This finding helps elucidate the underlying mechanisms leading to the

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high-carbohydrate/low-protein diet characteristic of the optimal dietary carbohydrate and protein levels of *P. f. martensii*.

1. Introduction

Pearl oyster (*Pinctada fucata martensii*) is a filter-feeder animal and one of the most represented pearl oyster species cultured for marine pearl production in China and Japan. Pearl oysters are traditionally cultured through the raft method, which depends on natural microalgae and is highly susceptible to natural disasters and environmental pollution (Wang et al., 2016). These disadvantages can be avoided through industrial farming, where, unfortunately, the food demand is high and the available formulated diets for bivalves are limited. Optimal diets should ensure the nutritional needs of aquatic animals and promote maximal growth, without increasing production costs and nitrogen input into the system. Hence, efficient feeding formulations and protocols are critical and demanding steps in pearl oyster culture. However, the progress of studies on the nutritional requirements and artificial feeds of bivalve mollusks is slower than those on fishes, shrimps, and crabs. Few studies have reported on the natural diet replacements for several bivalve species (Yang et al., 2017a). Formulated diets partially or completely replaced the microalgae in filter-feeding bivalves successfully (Nevejan et al., 2009; Gui et al., 2016; Wang et al., 2016; Willer and Aldridge, 2017; Yang et al., 2017a, 2017b). Yang et al. (2017b) discussed the protein source utilization in pearl oysters. Incorporation of the optimal level of carbohydrates in diets helps improve feed efficiency and growth rate in aquatic animals. Therefore, the optimum levels of carbohydrates and proteins in the diets must be carefully evaluated and determined, especially for the unstudied species.

When fed with diets of different quality, animals will have different metabolic responses. Understanding the effect of these nutritional regimes on the metabolome is essential to optimize new diets and ensure the quality of the end product for farmed aquatic species. Metabolomics is an effective technique for detection of the overall complexity and determination of essential changes in metabolites; this technique provides a "snapshot" profile of the metabolites in a biological system and has been suggested ideal for metabolic studies (Liu et al., 2016; Cappello et al., 2017, 2018). Low-molecular-weight metabolites, including lipids, sugars, and amino acids, can be quantified in biological samples by utilizing metabolomics approaches, such as gas chromatography–mass spectrometry, liquid chromatography–mass spectrometry, and nuclear magnetic resonance (Jarak et al., 2018; Maherizo et al., 2017; Nguyen et al., 2018; Venter et al., 2018a). The identification and integrative analysis of metabolites may enable the comprehensive characterization of metabolic mechanisms on the molecular and cellular levels under internal or external stimulating conditions. This potential has been explored to assess the effects of food shortage (Tuffnail et al., 2009; Kullgren et al., 2010; Baumgarner and Cooper, 2012), nutrient supplementation (Andersen et al., 2015; Wagner et al., 2014), differences in nutrient levels (Jin et al., 2015; Xu et al., 2018), and dietary protein or lipid substitution (Abro et al., 2014; Cheng et al., 2016; Ma et al., 2017; Yang et al., 2018a) in aquatic animals via metabolomic approaches. Moreover, among the available metabolomic technologies, gas chromatography–time-of-flight mass spectrometry (GC–TOF/MS)-based metabolomics is a promising approach (Hao et al., 2018; Yang et al., 2018a, 2018b) because of its high resolution, high detection sensitivity, and numerous open-access spectral libraries.

Thus, this study aimed to determine the optimum balance of dietary carbohydrates and proteins and compare the metabolomic responses of the pearl oysters fed with different dietary carbohydrate and protein levels by using the GC–TOF/MS-based metabolomics approach. Results enhance our understanding of the different mechanisms underlying the responses and contribute to exploring optimal nutritional requirements and feeding regimes.

2. Materials and methods

2.1. Experimental diet and procedures

Five isoenergetic and isolipidic experimental diets (C30P40, C35P35, C40P30, C45P25, and C50P20) with different levels of carbohydrates (C) and proteins (P) were formulated based on previous studies (Wang et al., 2016; Yang et al., 2017a, b, 2018a, b). The ingredients, proximate composition, and amino acid profiles of the experimental diets are shown in Supplementary Tables 1 and 2. All diets were stored at −20 °C until use. Lipids were obtained from fish oils, whereas proteins were obtained from yeast powder, soybean meal and fish meal. Pearl oysters (43.78 ± 0.95 mm in mean shell length (SL)) were randomly assigned to five experimental groups (C30P40, C35P35, C40P30, C45P25, and C50P20) and two control groups (CGs) (CG1 and CG2). CG1 was fed with mixed yeast powder and *Chlorella* sp. powder, and CG2 was cultured in the natural sea. Three tanks were prepared for each group. Each tank had 360 pearl oysters, the volume of water was 1000 L. Diet doses were specified according to a previous work (Wang et al. 2016); the pearl oysters were fed every 4 h. Water (300 L) was replaced daily. The experimental period lasted 60 days, and the following water parameters were maintained: dissolved oxygen at 5.00 mg/L, temperature at 27.5–29.5 °C, and salinity at 30‰.

2.2. Survival rate and growth rate

At the beginning and end of the experiment, the total number and growth performance of the pearl oysters in each replicate were determined. SL, shell height (SH), and shell width (SW) were measured with a digital caliper (0.02 mm accuracy). Total weight (TW) was obtained with an electronic balance (0.01 g accuracy), and survival rates

Table 1
Survival rate and absolute growth rates (AGRs) of pearl oyster *P. f. martensii* in the experimental groups and control groups.

Group	Survival rate	AGRs			
		Shell length	Shell width	Shell height	Total weight
C30P40	93.89 ± 1.36 a	5.47 ± 0.97 b	2.01 ± 0.59 c	3.73 ± 1.58 c	4.50 ± 1.01 b
C35P35	93.89 ± 3.90 a	5.47 ± 0.85 b	2.06 ± 0.41 c	3.99 ± 0.38 c	4.48 ± 1.10 b
C40P30	95.56 ± 4.04 a	5.62 ± 1.25 b	2.25 ± 0.07 bc	3.58 ± 1.15 c	5.57 ± 0.23 ab
C45P25	98.33 ± 1.83 a	10.41 ± 0.88 a	3.54 ± 0.60 a	9.30 ± 1.75 a	7.58 ± 1.86 a
C50P20	97.22 ± 3.28 a	7.47 ± 0.90 b	2.86 ± 0.33 ab	6.47 ± 1.53 b	5.50 ± 0.51 ab
CG1	80.56 ± 6.47 b	0.99 ± 0.82 d	1.14 ± 0.38 d	0.15 ± 0.05 d	1.26 ± 1.24 c
CG2	83.89 ± 2.51 b	3.55 ± 2.44 c	1.99 ± 0.70 c	4.03 ± 1.88 c	5.59 ± 2.27 ab

Means with the same letters within a column are not significantly different ($P > 0.05$).