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Effects of dietary lipids on the fatty acid composition and lipid metabolism of the green sea urchin *Strongylocentrotus droebachiensis*

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Abstract

The effects of different dietary lipids (hydrogenated coconut oil, corn oil, linseed oil, and mixtures of corn oil, linseed oil, menhaden oil, and/or soy lecithin) on the fatty acid composition of the sea urchin *Strongylocentrotus droebachiensis* were investigated. Dietary lipid compositions were reflected in the fatty acid profiles of sea urchin guts, gonads and shells. The shells had the highest level of 20:4*n*-6 and 20:5*n*-3, while the highest levels of monounsaturated fatty acids were seen in the guts. Tissue levels of 20:4*n*-6 and 20:5*n*-3 were influenced by the dietary levels of 18:2*n*-6 and 18:3*n*-3. The addition of soy lecithin to the diet (CLnML) enhanced the production and incorporation of 20:4*n*-6 in shell and gonad. Sea urchin also synthesized and incorporated 20 and 22 carbon non-methylene-interrupted dienoic fatty acids (NMIDs) in all tissue lipids. The NMID content varied among the different tissues and was influenced by the dietary lipid source. The hydrogenated coconut oil (HCO) diet resulted in the greatest NMID content, while the lowest levels were seen in sea urchins fed diets containing a mixture of corn oil, linseed oil, menhaden oil and lecithin (CLnML). The NMID content was lowest in gut and highest in the shell. Significant levels of mead acid (20:3*n*-9) were detected in the shells of sea urchins fed the EFA-deficient diet (HCO). The enzymatic capabilities of sea urchins to desaturate and elongate 18:2*n*-6 to 20:4*n*-6 and 18:3*n*-3 to 20:5*n*-3, as well as the capabilities to *de novo* synthesize NMID were hypothesized. Possible physiological roles and mechanisms for formation of NMID are discussed.

Keywords: Sea urchin; Nutrition; Lipids; Fatty acid; Metabolism; Aquaculture; NMID

1. Introduction

As exploitation of wild sea urchin populations is declining, countries like Chile, Japan, and Canada have started developing technologies for the culture of juvenile and enhancement of the sea urchin gonad quality by supplementary feeding of naturally occurring macroalgae and through the use of manufactured feeds. On the Atlantic Coast of Canada, and in the Maine region of the USA, for example, the green sea urchin *Strongylocentrotus droebachiensis* has been examined for its potential for aquaculture development. Most of the research on this species has been focused on larvae culture, culture system development, roe enhancement, and feed development (De Jong-Westman et al., 1995; Hooper et al., 1997; Kennedy et al., 2000; Vadas et al., 2000; Pearce et al., 2002a,b). Attempts to improve gonad quality of poorly developed sea urchins through supplementation of either macroalgae or artificial diets have been reported (González et al., 1993; De Jong-Westman et al., 1995; Floreto et al., 1996; Fernández and Pergent, 1998; McBride et al., 1997; Russell 1998; Watts et al., 1998; Meidel and Scheibling, 1999; Pantazis et al., 2000; Vadas et al., 2000; Pearce et al., 2002a,b).

Although sea urchin lipid metabolism has been investigated since the 70s, relatively little information is available on the EFA requirements and lipid metabolism of the sea urchin species that have been considered for commercial culture. Preliminary studies have shown that sea urchins have the ability to elongate and

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desaturate fatty acids of the *n*-3 and *n*-6 families (Bell et al., 2001; Castell et al., 2004). In *S. droebachiensis* for instance, 20:4*n*-6 and 20:5*n*-3 were reported to be the principal polyunsaturated fatty acids (PUFA) (Liyana-Pathirana et al., 2002a,b). Furthermore the *de novo* synthesis of an unusual type of PUFA, known as non-methylene-interrupted dienoic fatty acid (NMID) was reported. In fact, NMIDs have been reported in a number of marine invertebrates (Ackman and Hooper, 1973; Takagi et al., 1979; Cook et al., 2000; Bell et al., 2001; Castell et al., 2004), however, their specific functions are not yet known. Generally, an understanding of the requirements of dietary lipids and fatty acid metabolism is essential in formulating nutritionally balanced diets for the culture of the sea urchins.

This study was designed to test the effects of different dietary lipids on the fatty acid metabolism of juvenile *S. droebachiensis* through the analysis of the fatty acid compositions of gut, gonad, and shell. The enzymatic abilities for desaturation and elongation and the specific selectivity of C18 fatty acid precursors in terms of synthesized products e.g. 20:4*n*-6 and 20:5*n*-3 and/or 22:6*n*-3 (for 18:2*n*-6 and 18:3*n*-3, respectively) are investigated by determining the effects of dietary lipids on the juvenile sea urchin tissue fatty acid compositions over the course of a 188-day feeding experiment. This paper also presents information on modification of tissue fatty acid profiles throughout time, discussion of blocking mechanisms for the *de novo* synthesis of specific fatty acid, such as 20:4*n*-6 or 20:5*n*-3, and hypotheses on the possible metabolic role of NMIDs, and mechanism of their formation. Juveniles are used as the rate of tissue growth and relative dilution of original lipids is much greater during the experimental period than would be experienced with adult sea urchins. As previous studies (Stephens, 1972) had indicated that optimum growth of juvenile green sea urchins occurs between 12 and 19 °C, the animals in this study were acclimated to 12 °C prior to initiating experimental feeding in order to maximize responses to dietary differences within the experimental period of 188 days.

2. Materials and methods

2.1. Juvenile sea urchin source

Sea urchins, *S. droebachiensis*, (700, test diameter <2.54 cm) were collected by SCUBA diving from the St Croix River in waters adjacent to the St Andrews Biological Station, during the first week of May 2001. Sea urchins, with shell diameters ranging from 0.86–1.99 cm, were maintained for 4 weeks in 3 circular 0.5-m^3 tanks. In order to increase the rate of tissue growth, temperature was raised over a period of 2 days from 9 °C to 12 °C at 1.5 °C/day. The juveniles were accli mated to laboratory conditions for 4 weeks and were starved during this time to ensure a depleted nutritional condition for all juveniles. After that, an analysis for normal distribution of the weights and shell diameters was carried out and animals were rearranged to ensure equivalent size distribution among the different treatments.

2.2. Laboratory set-up

The study was conducted at the St Andrews Biological Station (SABS) in St Andrews, New Brunswick, Canada. Sea urchins with test diameter ranging from 0.86–1.99 cm were maintained in 3 circular fiberglass tanks of 0.5 m^3 capacity each. After the period of starvation the sea urchins were fed a control EFA-deficient diet containing hydrogenated coconut oil as the principal lipid source for the next 28 days, in order to ensure same nutritional level as well as low levels of polyunsaturated fatty acids (PUFA). On day 35, 630 sea urchins were moved from the conditioning tanks, and placed in the individual containers.

During the feeding trials, sea urchins were housed in individual containers (110×25-mm PVC pipes) with holes drilled in the side for water circulation and plastic mesh on the bottom to contain the sea urchins. To hold the containers in place on a 2.5-cm thick Styrofoam® floating support, 3 to 4 rubber bands were positioned at 1 cm from the top edge of the PVC tube. Pipes were changed for bigger ones (110×50 mm) as sea urchin shell diameter increased. The seawater (salinity 30±2‰) was filtered through a Hayward sand filter (IS-210T-00-HC) with washed silica sand (filtered to 20 μm) purchased from Valox, Ltd, then through 2 pleated polypropylene filter cartridges of 1 and 10 μm, purchased from Atlantic Purification Systems, a UV filter, through a degassing column (to prevent nitrogen gas supersaturation), and finally into a reservoir tank. From the reservoir, water was gravity fed to the 18 experimental tanks, each equipped with an air stone to ensure uniform water circulation within the tank. The seawater temperature was maintained at 12±1 °C. Water flow rates were the same in each of the tanks (~2.60 l/min). Three replicate tanks, each housing 25 individual sea urchin containers, were used for each of the 6 experimental diet treatments, resulting in 75 replicates for each treatment and 450 sea urchins for the entire experiment. The 25 containers within the same tank were fed a similar diet.

Experimental feeds were supplied twice a day (8:00 and 19:00 h) during 188 days at a ratio of 1.5% of the average sea urchin wet weight of each tank. Rations were adjusted to the sea urchin weights and leftovers. Uneaten fed was removed daily by flushing the containers with pressurized seawater. Removal of the leftovers occurred during the evening before the 19:00 h feeding. In order to estimate tissue dry matter, ash and lipid composition, 90 sea urchins were randomly selected (15 for each treatment, 5 from each tank), and sacrificed on day 94. Similar samples were also collected at the end of the feeding trials (day 188).

For moisture and lipid analyses a total of 180 sea urchins were sacrificed by cracking them open on their aboral surface and inverting for ~5 min, this time allows the coelomic fluid to drain. A roe scoop and tweezers were used to removed gonad, gut and test. Tissues were placed into labeled aluminum dishes to be weighed, samples were then placed into 2 ml plastic containers to be frozen and stored at −40 °C for further lipid extraction.

2.3. Diet preparation

The sea urchin base diet formulation was based on the protocol developed by Robinson et al. (2002). Formulations of the diets are show in Table 1. The lipid source mixtures are given in Table 2. Diets were produced to include the

Table 1
Composition of the basal diet

Ingredients	% dry mass
Soybean protein concentrate [a]	45.0
Potato starch [b]	24.5
Sodium alginate [c]	2.0
Vitamin mix [d]	2.0
Modified Bernhart–Tomarelli salt mix [e]	15.0
Algro [f]	1.2
Gelatin [g]	5.0
Etoxyquin [h]	0.2
Lipid source	5.0

[a] Supplied by ShurGain/Maple Leaf Foods Inc.
[b] Supplied by ICN Biochemicals, Inc., USA, Costa Mesa, California.
[c] (grade prime F-200) Supplied by from Multi-Kem Corp. NB. Canada.
[d] Vitamin mix: vitamin A (500,000 IU/g) 0.13%, vitamin D3 (400,000 IU/g) 0.07%, vitamin E (500 IU/g) 3.33%, vitamin K (menadione sodium bisulphite) 0.30%, thiamin ·HCl 0.50%, riboflavin 0.40%, D-calcium pantothenate 1.20%, biotin (1%) 0.80%, folic acid 0.16%, vitamin B12 (0.1%) 0.66, niacin 1.33%, pyridoxine · HCl 0.40%, ascorbic acid (stay c) 6.67%, inositol 1.33%, etoxyquin (75%) 0.88%, wheat middlings 81.80%.
[e] Supplied by Purina, Inc, USA, Richmond, Indiana.
[f] An algae based beta-carotene source supplied by Cognis Pty. Ltd. Chentenham, Australia. 1.2% Algro = 250 mg beta-carotene/kg dry diet.
[g] Supplied by United States Biochemical Corporation (USB) Cleveland, Ohio.
[h] Supplied by Sigma-Aldrich Canada Ltd., Oakville, Ontario.