Aquaculture 318 (2011) 335–342

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

The effect of artificial diets on gonad colour and biomass in the edible sea urchin *Psammechinus miliaris*

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ARTICLE INFO

Article history:
Received 27 January 2011
Received in revised form 24 May 2011
Accepted 25 May 2011
Available online 31 May 2011

Keywords:
Psammechinus miliaris
Carotenoid
Artificial diet
Gonad colour
Sea urchin
Aquaculture

ABSTRACT

During two 12 week trials, groups of edible sea urchins, *Psammechinus miliaris,* were fed artificial diets containing carotenoid pigments. The aim was to improve both the biomass and the colour of the sea urchin gonad in terms of its acceptability as a human food-stuff in the European market place. The pigmented artificial diets, based on the formula used by Robinson et al. (2002), increased gonad index (GI), pigment deposition and improved gonad colour from that of the initial samples. In Trial I gonad β-carotene levels increased >2 fold, echinenone and total carotenoid >7 fold. Trial II showed greater increases. Diets containing high levels of β-carotene (500 mg per kg dry weight of diet) gave rise to the highest percentages of marketable gonad colours (61–73%), and GI of 17.87–19.81%, (Trial I and II respectively). There was some variation in the results for this particular diet treatment across the two trials presumably reflecting individual urchins varying capacity to ingest, deposit or express the carotenoids in their diet. Providing additional lipids in the diets gave no improvement to gonad colour (56% acceptable) or GI (15.95%) suggesting the lipid content of the basic formulation is adequate. Utilizing an esterified form of lutein and zeaxanthin as a pigment source gave no significant improvement in gonad colour (30–63% acceptable) suggesting that this form of xanthophyll cannot be assimilated by *P. miliaris*. Female urchins had acceptable gonad colouration more often than males. The dominant carotenoid in the successful diets was β-carotene and this was successfully metabolized into echinenone, the dominant carotenoid in all gonad samples. Total levels of echinenone positively correlated with acceptable gonad colour scores. This study demonstrates that 12 weeks is sufficient to effect the desired change in gonad biomass and colour in cultivated *P. miliaris*.

1. Introduction

The gonads of edible sea urchins are highly prized as a luxury food. The gonads of both sexes are eaten, and are termed 'roe' regardless of gender. Many wild populations of edible sea urchins have been seriously over exploited to meet market demand (Andrew et al., 2002) and as a consequence there is a continued effort into developing economically viable culture methods. Several studies have been conducted to examine the aquaculture potential of *Psammechinus miliaris*, a small regular echinoid locally abundant on the west coast of Scotland (Kelly et al., 2007). Although smaller in test diameter (TD) than the more commonly consumed *Paracentrotus lividus* it has a pleasantly flavoured roe and has been shown to be robust when cultured on a range of diets (Cook et al., 1998; Otero-Villanueva, 2003); in a hatchery context (Kelly et al., 2000; Kelly, 2002) and in polyculture (Kelly et al., 1998). In this opportunistic and omnivorous sea urchin (Kelly et al., 2007) the gonad biomass increases rapidly, independently of reproductive state or season when it is provided with a suitably nutritious diet (Otero-Villanueva et al., 2004; Cook; Kelly et al., 2007), so it clearly has potential as an aquaculture species. Its relatively smaller size may not be such as disadvantage as there is some evidence of a trend toward new and even smaller sized seafoods, with the advent of 'cock-tail size' abalone (Jarayabhand and Paphavasit, 1996) and princess scallops (Gordon Goldsworthy, AM Seafoods Ltd., personal communication).

Gonad colour is an important criterion for obtaining the best price. Colour is successfully manipulated by the use of dietary carotenoids in other well established seafoods such as salmon and trout flesh. The influence of dietary carotenoids on gonad colour has been investigated for some sea urchin species (*Strongylocentrotus droebachiensis* — Robinson et al., 2002; *P. lividus* — Shpigel et al., 2005; 2006), and preliminary studies have been made for *Psammechinus miliaris* (McLaughlin and Kelly, 2001). Further investigation is required to fully understand the deposition and expression of dietary carotenoids

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doi:10.1016/j.aquaculture.2011.05.042

Table 1
Experimental parameters for Trials I and II.

	Trial I	Trial II
Total number of urchins	180	96
Initial test diameter (mm)	23.60 ± 1.41	25.85 ± 1.54
Final test diameter (mm)	25.99 ± 2.23	27.92 ± 2.00
Number of urchins per tank	10	8
Replicate tanks per treatment	3	3
Number/available surface area: nm^{-2}	54	43
Biomass/available surface area: gm^{-2}	47.04	59.05
Temperature (°C)	12.26 ± 0.97	13.19 ± 1.62
Date started	3rd October 2005	11th September 2006
Date completed	9th January 2006	8th December 2006
Experimental duration (days)	99	98

in *P. miliaris* roe. The objective of this study was to test the effectiveness of artificial diet formulations combined with a variety of naturally derived carotenoids in producing a large gonad biomass of the desired colour in *P. miliaris*.

2. Materials and methods

2.1. Sea urchin source and maintenance

Two separate experiments were conducted over the last quarter of 2005 (Trial I) and 2006 (Trial II). *P. miliaris* were collected during low spring tides from the intertidal zone of Loch Creran, on the west coast of Scotland and transferred to aquaria at the Scottish Association for Marine Science (SAMS), Oban. The urchins (size and sample numbers in Table 1) were selected at random and distributed between eighteen (Trial I) and twelve (Trial II) 9 l aquaria. Each aquarium had an independent supply of air and 10 μm filtered seawater of ambient temperature (Table 1) and salinity. Ambient photoperiod was also maintained for the duration of the experiment utilizing natural spectrum fluorescent lighting. The urchins acclimated to the aquarium conditions for a period of 14 days prior to the experiment, during which time they were starved to standardise their nutritional status (Vadas, 1977). The urchins were then fed one of six (Trial I) and four (Trial II) experimental diets at a constant rate of approximately 5% of their mean body weight every 2–3 days. Uneaten food and faeces were removed carefully by siphoning around the urchins twice weekly and before new feed was introduced.

2.2. Diet preparation

The treatments (Table 2) in triple replication were allocated randomly to the aquaria using Minitab's (Statistical Software™ Version 14) random block design. The diet formula used was similar to that described by Robinson et al. (2002) and termed the St. Andrews Biological Station (SABS) diet (Table 3). The main ingredients were commercially available raw materials; the pigments incorporated into these SABS diets were commercial sources of naturally derived carotenoids manufactured by Cognis©: Algro Natural® (β-carotene: β,β-carotene, BC)) and Xangold 10% Beadlets® (lutein and zeaxanthin: β,ε-carotene-3,3′-diol and β,β-carotene-3,3′-diol, LZ)). Algro Natural® contains a minimum of 2% (dry weight) total carotenoids primarily in the form of β-carotene and is derived from spray dried microalgae *Dunaliella salina*. Xangold 10% (dry weight) Beadlets® are a gelatine-free microencapsulated tablet grade powder containing natural mixed carotenoid esters (lutein and zeaxanthin) isolated from marigold flowers (*Tagetes erecta*). In Trial I Algro Natural® was used at one of three different inclusion rates (low, medium and high; Table 2). The SABS diets with the medium inclusion rate of Algro Natural® was also formulated into a high lipid diet, containing approximately four times the quantity of linseed oil as the other diets. Carotenoids are lipid soluble compounds and in some animals lipid is important for their metabolism and deposition (Schiedt, 1998). A diet of SABS with no added pigments was used as a control and the final treatment was of the control SABS for eight

Table 2
Experimental diets and associated codes for Trials I and II.

Trial I		Trial II	
Treatment	Code	Treatment	Code
SABS + Algro® 100 mg β-carotene kg^{-1} dry ingredients (Low)	SA-L	SABS + High Algro®	SA-H
SABS + Algro® 250 mg β-carotene kg^{-1} dry ingredients (Medium)	SA-M	SABS + Xangold® 250 mg Lutein and Zeaxanthin kg^{-1} dry ingredients	S-LZ
SABS + Algro® 500 mg β-carotene kg^{-1} dry ingredients (High)	SA-H	SABS + High Algro® + Xangold® 250 mg pigments kg^{-1} dry ingredients	SA-HLZ
SABS + Medium Algro® + High Lipid	SA-ML	Seaweed	Alg
SABS without pigment	SA-0		
SABS without pigment to wk 8/ seaweed from wk 8–12	SA-0Swd		

Table 3
SABS based dietary treatment compositions (%), adapted from Robinson et al. (2002).

Ingredients %	Trial I					Trial II		
	SA-L	SA-M	SA-H	SA-ML	SA-0	SA-M	SA-LZ	SA-MLZ
Soybean meal	21.27	21.27	21.27	21.27	21.89	21.27	21.27	21.27
Wheat (Flour)	23.51	22.82	21.27	16.54	21.89	22.82	23.61	22.36
Canola meal (Rapeseed Meal)	21.27	21.27	21.27	21.27	21.89	21.27	21.27	21.27
Potato Starch (processed)	19.84	19.84	19.84	19.84	20.41	19.84	19.84	19.84
Gelatine (bovine skin)	5.60	5.60	5.60	5.60	5.76	5.60	5.60	5.60
Sodium alginate	2.24	2.24	2.24	2.24	2.30	2.24	2.24	2.24
Linseed oil (Flax Oil)	2.24	2.24	2.24	8.38	2.30	2.24	2.24	2.24
Lecithin	2.24	2.24	2.24	2.24	2.30	2.24	2.24	2.24
Vitamin premix	0.56	0.56	0.56	0.56	0.58	0.56	0.56	0.56
Mineral premix	0.34	0.34	0.34	0.34	0.35	0.34	0.34	0.34
Inositol	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
Stabilised vitamin C	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09
Paradigmox	0.22	0.22	0.22	0.22	0.23	0.22	0.22	0.22
Algro® (β-Carotene)	0.56	1.25	2.80	1.40	0.00	1.25	0.00	1.25
Xangold® (Lutein + Zeaxanthin)	–	–	–	–	–	0.00	0.46	0.46