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Energy and nutrient utilization of juvenile green abalone (*Haliotis fulgens*) during starvation

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Abstract

Juvenile green abalone *Haliotis fulgens* (mean length = 65.15 mm; mean weight = 38.77 g) were fed a standard formulated diet for 15 days (acclimation period), and then starved for 27 days. Following the starvation period, wet weight loss was 13.4%. Crude protein and NFE accounted for 69.9% and 31.5%, respectively, of the total net loss in dry weight that was calculated to be a total loss of 3.57 kcal/organism. Lipid content of the tissue increased by 1.0% (dry weight) while protein content remained unchanged.

Plasma levels of free amino acid, soluble protein and glucose were determined on days 0, 7, 14, 21 and 27. The level of plasma glucose significantly decreased after the first 7 days, 34 to 10 μm/ml, and then leveled at 15 μm/mL. Soluble protein in the plasma decreased from 3.33 to 2.6 mg per ml during the starvation period. Taurine was the principal free amino acid among the plasma amino acids, comprising approximately 50–65% and showing a net decrease of almost 75% at 27 days. For the essential amino acids, gross levels of histidine and arginine decreased significantly and correspondingly produced substantially higher net decreases. For the non-essential amino acids, plasma levels of alanine, proline, tyrosine, glutamic acid, and serine decreased significantly by day 27 or earlier, but proportional composition remained similar.

After starvation, changes in the gross amounts (μg/mg total dry weight) of some essential and non-essential amino acids in the muscle and visceral tissue occurred, but their relative proportions remained essentially unchanged. The ratio of the concentration of non-essential to essential amino acids in both the muscle and viscera (~1.9) did not change after starvation. The mean net change of non-essential amino acid content per abalone (within muscle tissue) was higher (−41.8) than that of the essential amino acids (−8.9). In contrast to all other amino acids, little or no loss of arginine, histidine and threonine occurred.

Plasma levels of carbohydrates and protein in starved abalone dramatically decrease within the first two days and then decrease very slowly. Muscle protein is the principal source of energy, and non-essential amino acids are preferentially used. Retention of arginine in the muscle tissue may reflect its need in arginophosphate for muscle contraction. By converting the decrease in weight to energy loss, the amount of energy used for basal metabolism during starvation was estimated to be 2.99 cal g org^{-1} day^{-1}.

Keywords: Starvation; Abalone; *Haliotis fulgens*; Aminoacids; Basal metabolism

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1. Introduction

For the successful culture of an organism, a principal goal is to develop a diet that meets all nutritional requirements. Protein is the crucial nutrient, for its importance in growth and metabolism, and its being the nutrient that proportionately represents the highest cost of the diet. Among the most common amino acids, 11 of them have been identified as essential in the red abalone (*H. rufescens*) because they cannot be synthesized (Allen and Kilgore, 1975).

Mollusks, as part of their natural life cycle, are able to withstand long periods of time without food, being well adapted to mobilize their metabolic reserves and body constituents to survive periods of food deprivation. Carefoot et al. (1993) reported that no mortality of the abalone (*H. kamtschatkana*) was observed after 27 days of starvation and that a significant weight loss occurred under basal metabolism conditions. Upon resumption of feeding, a return to normal metabolism occurred. Durazo-Beltrán et al. (2004) reported an 18.8% decrease in the weight of juvenile abalone (*H. fulgens*) with no mortality after 60 days of starvation. Tissue lipids were mainly conserved, leading to the conclusion that carbohydrate and protein are used as the initial sources of energy. The results of a recent study in our laboratory suggest that use of lipid as an energy source does not begin to occur until at least 70 days of starvation.

Amino acid metabolism can be studied by monitoring and comparing profiles of amino acids in tissue, as well as free amino acid composition in the muscle and plasma. Protein mobilization seems to be quite similar for several species of fish whereby protein reserves are commonly spared at the beginning of fast. Proteolysis occurs after more readily available energy reserves like liver glycogen and lipid stores have been mobilized. When protein utilization for energy begins, the level of use can be species specific as illustrated by the marked differences reported for degradation can be different as has been reported for mackerel (*Scomber scombrus*) compared to cod (*Gadus morhua*) or carp (*Cyprinius carpio*) (Navarro and Gutiérrez, 1995). For muscle tissue, contractile and soluble proteins are removed preferentially, while connective tissue proteins are used to a lesser extent. As a result, connective fibers rich in collagen are almost entirely spared from metabolic degradation, and relative proportions of glycine, proline and hydroxyproline in muscle tissue accordingly increase during fasting.

With the knowledge of the importance of protein as an energy source in abalone, an investigation of amino acid profiles of tissue under starvation conditions can provide useful information. Knowledge of the abalone's nutritional physiology under conditions of starvation should include the utilization of energy and nutrients, particularly protein/amino acids and carbohydrates. This information may help to understand the requirements for particular amino acids of abalone by understanding their relative use in metabolic activities other than growth. Requirements of essential amino acids would be underestimated if estimation of essential amino acid requirements was based upon close proportional relationships to the profile of muscle tissue, the ideal protein ratio (Cowey and Tacon, 1983).

The objective of the present work is to develop an amino acid profile of tissue of juvenile green abalone and to focus on energy and nutrient utilization, particularly amino acids, during and after a 27-day period of starvation.

2. Material and methods

2.1. Experimental procedure

Twenty juvenile green abalone (*Haliotis fulgens*; mean length = 65.15 ± 0.66 mm and mean weight = 38.77 ± 1.20 g) selected from a group provided by BCAbalone farm SA de CV (Erendira, Baja California, Mexico). All were individually tagged and equally distributed among four 4L buckets (experimental units) and maintained at constant air and water flow, and water temperature (21.5 ± 0.5 °C) in a flow-through system, equipped with a 700 L reservoir and heat pump, throughout the duration of the experiment. In an attempt to standardize the organisms relative to a baseline amino acid content, a conditioning period of 4 weeks was observed when all abalone were fed the conditioning diet *ad libitum* (5% of weight) each day at 2000 h. Uneaten food was removed at 0800 h. At the conclusion of the conditioning period, one abalone per replicate was selected to conduct proximate analysis and amino acid compositional analysis of muscle and viscera. Also, prior to the beginning of the 27 days of starvation, the weight and length of each abalone were determined. During the 27-day period of starvation, samples of plasma (100 μL) were collected with a No. 10 syringe directly from the celomic cavity of each of the four abalone of each replicate and then pooled. Plasma was collected on days 0, 7, 14, 21 and 27 days for amino acid analysis, and at 0, 1, 2, 3, 14 and 27 days for analysis of total soluble protein and glucose. At the conclusion of the experiment, four abalone were sacrificed (one per replicate) to perform proximate analysis and the amino acid analysis of tissue.