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Is artificial feed suitable for juvenile green turtles (*Chelonia mydas*)?

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ABSTRACT

Artificial feed would make it easier to rear juvenile green turtles (*Chelonia mydas*) in Thailand, but the benefits and potential risks for growth and health of this endangered species need to be assessed. The effects of three dietary treatments on survival, growth, feed efficiency, fecal digestive enzymes, and blood parameters of juvenile green turtles were investigated in this study. The initially 10-day-old turtles (25.38 ± 1.29 g initial body weight) were fed with two conventional feeds, namely fresh feed from minced fresh fish and vegetable (diet 1), and fresh feed from minced fish fillet, vegetable and artificial feed (diet 2). The third diet 3 was artificial feed only. Experiments were run in a completely randomized design with triplicates (3 treatments × 3 replicates × 10 subjects per replication) for 6 months. The survivals were not significantly ($P < 0.05$) different between the dietary treatments. The growth characteristics body weight, average daily gain, and specific growth rate, were significantly higher with diets 2 and 3 than with diet 1. Feed intake and feed conversion ratio were lower with diet 3 than with diet 2. Fecal carbohydrate- and protein-digesting enzymes, as well as feces microstructure, indicated significant adaptations to digestion and utilization of diet 3. The blood parameters determined, namely packed cell volume, hemoglobin, red blood cell count, and white blood cell count, were unaffected by dietary treatment. These findings indicate that artificial feed is suitable for rearing juvenile green turtles as partial or full replacement of a conventional feed, while further improvements could be sought by optimizing the amount of replacement or the artificial feed.

1. Introduction

The population of green turtles (*Chelonia mydas*) is in serious decline throughout tropical and subtropical seas. Husbandry of these turtles for *in situ* conservation is routine for the Department of Marine and Coastal Resources, Ministry of Natural Resources and Environment, in Thailand. Earlier developments of artificial feeds for rearing some turtles have been reported (Bau et al., 1992; Chen and Huang, 2011; Hadjichristophorou and Grove, 1983; Huang et al., 2010; Nuangsaeng and Boonyaratapalin, 2001; Zhou et al., 2013). However, the use of artificial feeds might have negative effects on survival, growth and development, when compared with conventional feeds. There is a lack of scientific information on such diets, making their use a risk. Practically only natural diets are used during propagation, the diets consisting of small aquatic animals, fresh whole fish, fish fillet, seagrass, and mangrove leaves and fruits. The green turtles are believed to be omnivorous, which correlates well with the food items found in their alimentary tracts (Amorocho and Reina, 2008; Arthur et al., 2009). On the other hand, Bjorndal (1997) and Brand-Gardner et al. (1999) have observed also herbivorous behavior of this species. Thongprajukaew et al. (2011) reported upregulation of carbohydrate-digesting enzymes coinciding with an increase in growth rate, for the carnivorous Siamese fighting fish (*Betta splendens*) that was fed a gelatinized diet. These animals could adapt their digestive physiology to successfully digest and utilize the no-choice diet. Therefore, artificial feeds might support the culturing of green turtles in the future, provided scientific studies inform such decisions and appropriate practices.

Digestive enzymes from the alimentary tract are excellent markers indicative of feed utilization and growth in aquatic animals (Rungruangsak-Torrissen et al., 2006; Thongprajukaew et al., 2011). Digestive or accessory glands produce the enzymes that are secreted into gut lumen for digestion, and then enclosed in membranes and excreted in feces. For shrimp the presence of fecal digestive enzymes, especially their active forms, correlates well with the enzymes in the mid gut (CÓrdova-Murueta et al., 2003), so that sampling of feces may inform about the gut function. The forensic investigation of feces can be used in human and veterinary medicine (Kita et al., 1989), sex and species identifications (Tolleson et al., 2005), and has become an important tool for biochemical, physiological and ecological studies (CÓrdova-Murueta et al., 2003, 2004). Moreover, the visual assessment of fecal characteristics has been used for nutritional evaluation in many organisms (Amirkolaie et al., 2006; Varo and Amat, 2008). The fecal

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characteristics are affected both by the food, and by its processing in the alimentary tract.

This study aimed to investigate whether a diet of artificial feed only would be competitive with conventional feeds in growing juvenile green turtles. Fresh feed from fresh fish and vegetables is conventional in the rearing of green turtles in Thailand, while fish fillet, vegetables and artificial feed are combined to prevent intestinal inflammation. These conventional feeds were comparatively studied with a diet of artificial feed only. The growth and gut health of juvenile turtles were evaluated with non-invasive observations, avoiding ethical concerns or any conflicts with ethical standards. The findings from present study are needed to make informed decisions, accounting for benefits and risks that might be caused by a diet, for practical rearing of juvenile green turtles and for further development of their diets.

2. Materials and methods

2.1. Rearing of green turtles

Five-day-old green turtles since hatching were obtained from Marine Endangered Species Unit (MESU), Phuket Marine Biological Center (PMBC), Thailand. The juvenile turtles that were offspring of one and the same mother (85 cm curve carapace width and 96 cm curve carapace length) were acclimatized in round fiberglass tanks containing 3000 L sea water, until absence of yolk (10-day-old). Subsequently, the turtles (25.38 ± 1.29 g initial body weight) were randomly distributed and reared at a density of about 10 turtles m^{-2} in round fiberglass tanks (100 cm diameter × 100 cm height) containing 265 L sea water each. The experiment was run for a duration of 6 months, with three dietary treatment groups in triplicates, and ten turtles per tank. The juvenile green turtles were fed *ad libitum*, twice daily at 10.00 and 17.00 h, with fixed dietary treatment for each tank, as shown in Table 1. The diurnal cycle during experimentation was 12-h light/12-h dark. Water was entirely changed daily before beginning the first meal. The ranges of water quality parameters during the study were: pH 7.25–8.30, temperature 27.50–31.50 °C, pH 7.25–8.30, salinity 29–34 ppt, total alkalinity 112–121 ppm $CaCO_3$, and dissolved oxygen 5.61–7.42 mg L^{-1}. Mortality and morbidity were monitored daily over the duration of the experiment. Observations on growth and feed utilization were done and recorded monthly.

2.2. Chemical composition of experimental diets

Samples of each experimental diet were dried at 105 °C for 24 h and their chemical compositions analyzed for crude protein (p. 127), lipid (p. 132), fiber (p. 134) and ash (p. 125), according to standard methods of AOAC (1980). All analyses were performed in triplicates and are reported on a dry matter basis. Available carbohydrate (nitrogen free extract) was calculated from the differences in chemical constituents.

2.3. Fecal digestive enzyme studies

2.3.1. Extraction of digestive enzymes

Feces were collected within 6 h after the first meal. The fresh feces were rinsed carefully to remove contaminating dirt, and then homogenized in distilled water (1: 2 *w*/*v*) using a micro-homogenizer (THP-220; Omni International, Kennesaw GA, USA). The homogenate was centrifuged at 15,000 ×*g* and 4 °C for 30 min, and the supernatant was kept at −20 °C until assaying of digestive enzymes.

2.3.2. Digestive enzyme assay

The optimal conditions used for assaying fecal digestive enzymes of green turtles were chosen based on preliminary experiments. The conditions used were pH 2 at 35 °C for pepsin (EC 3.4.23.1), pH 10 at 40 °C for trypsin (EC 3.4.21.4), pH 6 at 55 °C for amylase (EC 3.2.1.1), and pH 5 at 45 °C for cellulase (EC 3.2.1.4). Pepsin activity was assayed according to the method of Rungruangsak and Utne (1981), using casein as substrate. The quantity of digested casein was measured spectrophotometrically against *L*-tyrosine standard. *N*-benzoyl-*L*-arginine-*p*-nitroanilide (BAPNA) was used as substrate for assaying trypsin activity according to Rungruangsak-Torrissen et al. (2006). Units of hydrolysis were calculated against *p*-nitroanilide standard at 410 nm. The activities of carbohydrate-digesting enzymes α-amylase and cellulase were determined based on Areekijseree et al. (2004) and Mendels and Weber (1969), using soluble starch and carboxymethylcellulose (CMC) as the substrates, respectively. The activities of both these enzymes were determined spectrophotometrically at 540 nm, by comparison to standard maltose and glucose, respectively.

2.3.3. Determination of protein in crude enzyme extracts

Protein concentration was determined using the method of Lowry et al. (1951). Bovine serum albumin (BSA) was used as protein standard.

2.4. Feces microstructure

Collected feces were dried using a freeze dryer (Delta 2–24 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 48 h and then kept in polyethylene bags. Microscopy with imaging was done with a scanning electron microscope (Quanta 400, FEI, Czech Republic). Each sample was mounted with two-sided adhesive tape on an aluminum stub and coated with gold. The magnifications used were 50, 2000 and 10,000×, with accelerating voltage set at 15 kV.

Table 1
Ingredient formulations (% wet weight) and proximate chemical compositions of diets used for rearing green turtles. The data from triplicate determinations are expressed as % of dry matter (DM).

Ingredient and composition	Diet 1	Diet 2	Diet 3
Ingredient			
Fresh fish (Longtail tuna, *Thunnus tonggol*)	50	–	–
Vegetable (Chinese cabbage, *Brassica pekinensis*)	50	25	–
Fish fillet (Longtail tuna, *T. tonggol*)	–	25	–
Artificial feed[a]	–	50	100
Composition			
Moisture (%)	84.98 ± 0.72	61.55 ± 0.71	7.35 ± 0.15
Crude protein (% DM)	54.13 ± 0.30	50.14 ± 0.06	44.81 ± 0.08
Crude lipid (% DM)	6.20 ± 0.12	6.21 ± 0.02	7.99 ± 0.10
Crude fiber (% DM)	5.30 ± 0.06	3.13 ± 0.01	0.96 ± 0.05
Ash (% DM)	10.43 ± 0.01	11.48 ± 0.03	10.25 ± 0.07
Nitrogen free extract (% DM)	23.94 ± 0.33	29.04 ± 0.07	35.99 ± 0.15

[a] Artificial feed for median size of marine fish (Hi-grade 9773; Charoen Pokphand PCL., Thailand).