Aquaculture 428–429 (2014) 97–103

Contents lists available at ScienceDirect

Aquaculture

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

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

Hirun Kanghae [a], Karun Thongprajukaew [b,c,*], Alisa Madlee [b], Kongkiat Kittiwattanawong [a]

[a] Phuket Marine Biological Center, Phuket 83000, Thailand
[b] Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand
[c] Biochemical Research Unit for Feed Utilization Assessment, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand

ARTICLE INFO

Article history:
Received 17 January 2014
Received in revised form 28 February 2014
Accepted 28 February 2014
Available online 11 March 2014

Keywords:
Artificial feed
Chelonia mydas
Digestive enzyme
Feces
Green turtle

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

* Corresponding author at: Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand. Tel.: +66 74288562; fax: +66 74446681.
E-mail address: karun.t@psu.ac.th (K. Thongprajukaew).

http://dx.doi.org/10.1016/j.aquaculture.2014.02.039
0044-8486/

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).

2.5. Blood collection and determination

At the end of the experiment, the turtles were starved for 24 h before collecting blood samples from dorsal cervical sinus. The samples were mixed into 1% ethylenediaminetetracetic acid (EDTA) and kept at refrigerated temperature. Hemoglobin and packed cell volume (hematocrit) were determined according to the method of Larsen and Snieszko (1961). Red and white blood cell counts from diluted samples were determined based on the method of Blaxhall and Daisley (1973).

2.6. Statistical analysis

A completely randomized design with triplicate observations was used for the experiments. Data were analyzed using SPSS Version 14 (SPSS Inc., Chicago, USA), and results are summarized as mean ± SE. Significance of differences between means was tested with Duncan's multiple range test with threshold set at $P = 0.05$.

3. Results

3.1. Proximal compositions of experimental diets

Chemical compositions of the experimental diets are shown in Table 1. The artificial feed differed significantly from the other diets in each type of constituent tabulated. Of the diets the artificial feed (diet 3) was highest in lipids and available carbohydrates (nitrogen free extract), and lowest in crude protein, fiber and ash. The main differences between diets 1 and 2 were that diet 1 was comparatively high in protein and fiber, while it was low in carbohydrates. Diet 1 could be considered the baseline, diet 2 as partial substitution of carbohydrate rich artificial feed, and diet 3 as full replacement relative to baseline.

3.2. Survival of green turtles

There was no mortality over the first 3 months (Fig. 1a). The mortalities accumulated by the end of experiment were not significantly different between treatments ($P > 0.05$). Turtles fed with diet 1 had 100% survival, followed by 96.67 ± 3.33% and 90.00 ± 5.77% for diets 3 and 2, respectively.

3.3. Growth of green turtles

There were no significant differences in body weight between the three diets at 4 months of treatment (Fig. 1b). The differences at 6 months were also insignificant between diets 2 and 3, but diet 1 gave significantly lower weights. Average daily gain (ADG, Fig. 1c) and specific growth rate (SGR, Fig. 1d) had similar patterns with significant differences only on month 6. In this respect, diets 2 and 3 fared better than diet 1 without artificial feed.

3.4. Feed utilization of green turtles

Feed intake (FI) varied during the experiment (Figs. 2a and b). FI on wet weight basis in the first 4 months was significantly ($P < 0.05$, Fig. 2a) higher in the green turtles fed diet 1 than in the other treatment groups, with diets 2 and 3 following in this order. At months 5 and 6, the turtles ingested similar amounts of diets 1 and 2, but significantly less artificial feed diet. On a dry weight basis, in the latter half of the treatments, dietary group 2 consistently ingested more food than the other groups (Fig. 2b). Feed conversion ratio (FCR) in Fig. 2c was consistently lowest with artificial feed only (diet 3), indicating that this diet required the least amount of feed relative to growth. Essentially, the artificial feed was most efficiently used for growth.

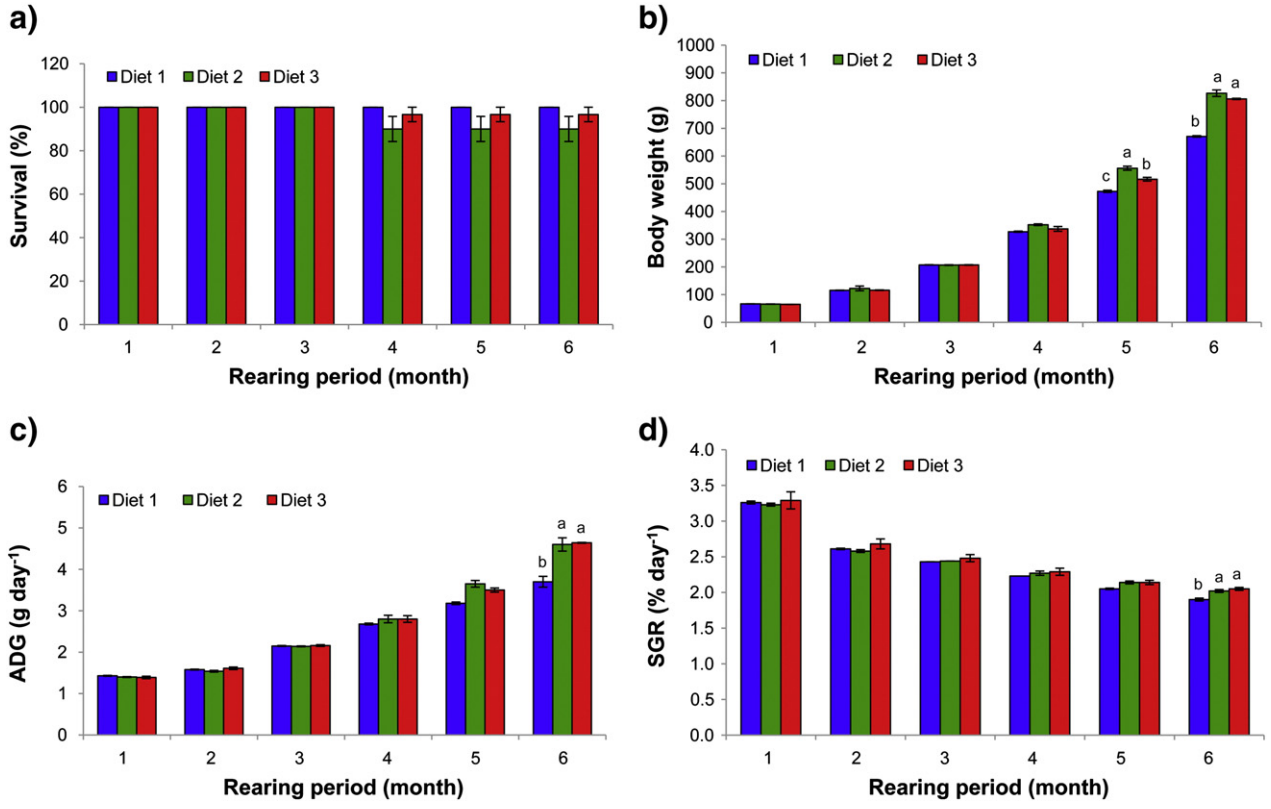

Fig. 1. Observed survival (a), body weight (b), average daily gain (c), and specific growth rate (d) of juvenile green turtles subjected to various dietary treatments. The duration of treatments was six months. Data are expressed as mean ± SE from triplicate observations. Different superscripts within a month indicate significant differences between the groups ($P < 0.05$).

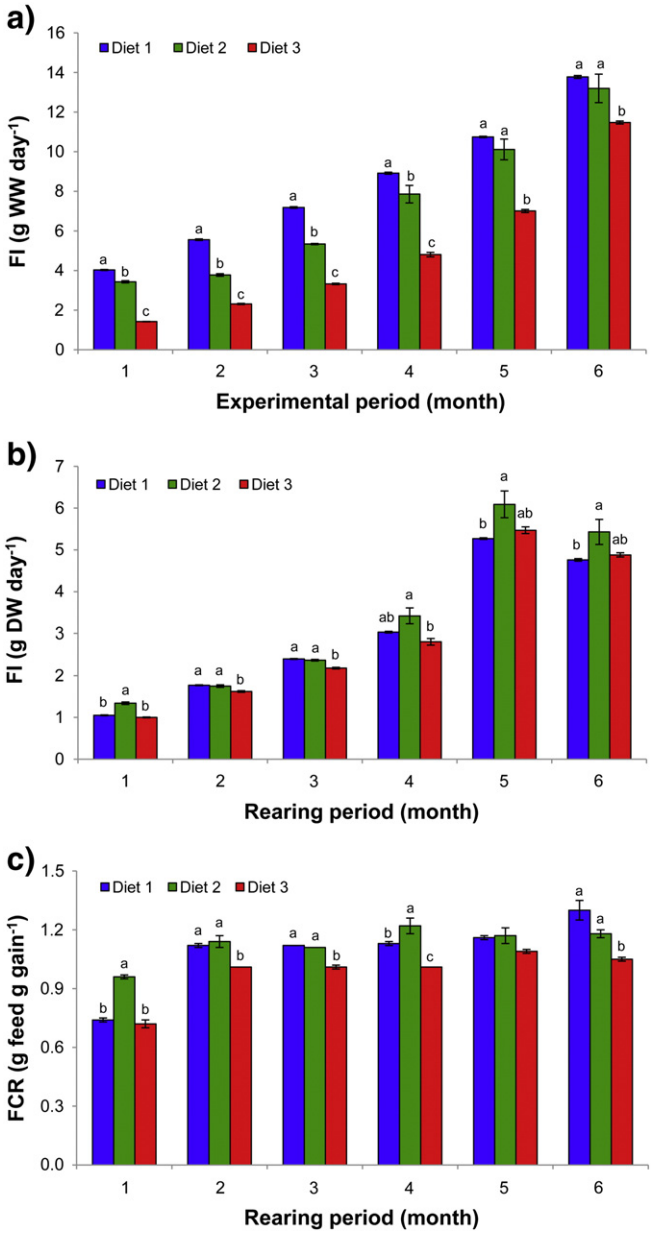

Fig. 2. Feed intake on wet (a) and dry weights (b), and feed conversion ratio (c) of juvenile green turtles undergoing dietary treatments. The experimental duration was six months. Data are expressed as mean ± SE from triplicate observations. Different superscripts within a month indicate significant differences between groups ($P < 0.05$).

Table 2
Specific activities of fecal digestive enzymes from green turtles undergoing dietary treatments. The experiment was performed in triplicates over 6 months, and these data are for the fecal samples at the end of those 6 months.

Fecal digestive enzyme	Dietary treatment[a]			Pooled SEM
	Diet 1	Diet 2	Diet 3	
Amylase (U mg protein^{-1})	3.41	4.65	6.45	0.29
Cellulase (U mg protein^{-1})	56.89^{c}	116.15^{b}	142.55^{a}	21.23
Pepsin (U mg protein^{-1})	0.10^{b}	0.09^{b}	0.15^{a}	<0.01
Trypsin (U mg protein^{-1})	24.15	21.92	17.93	2.96
Activity ratio of amylase to trypsin (A/T ratio)	0.26^{b}	0.21^{b}	0.30^{a}	<0.01

Significant differences in each row are indicated by different superscripts ($P < 0.05$).
[a] Green turtles were fed either by fresh feed containing minced fresh fish and vegetable (1:1, Diet 1); fresh feed containing minced fish fillet, vegetable and artificial feed (1:1:2, Diet 2); or artificial feed only (Diet 3).

3.5. Specific activities of fecal digestive enzymes

The fecal specific activities of digestive enzyme differed between the dietary treatments at the end of experiment (Table 2). Amylase specific activity was highest with diet 3 though without statistical significance, while cellulose and pepsin specific activities were significantly ($P < 0.05$) increased by this treatment. Trypsin specific activity was comparatively high with conventional feeds 1 and 2. The activity ratio of amylase to trypsin (A/T ratio) was consequently highest with diet 3 that provided only artificial feed.

3.6. Feces microstructure

Feces microstructures subjectively differed between the dietary treatments (Fig. 3). The rows represent treatment group, with diet 1 on top, while the columns each have a fixed magnification as indicated at the bottom of the figure. The first column suggests that diet 1 gave the coarsest morphology in terms of particle sizes. The last column with highest magnification suggests that, at this scale, the artificial feed produced powdery appearing components in the feces, while diet 1 without it gave more platelet like and less powdery constitution to the feces.

3.7. Blood characteristics

The blood characteristics determined, namely packed cell volume, hemoglobin, red blood cell and white blood cell counts, are shown in Table 3. The sampling was done at the end of the experiment, and represents state after 6 months of dietary treatment. No statistically significant differences were found between the three dietary groups.

4. Discussion

Artificial feed has significant potential in the rearing of juvenile green turtles. In the current experiments it gave superior growth and feed utilization, with a high survival. The sampling of feces was non-intrusive, non-invasive, and without ethical concerns, while it supported studying the nutrient utilization of turtles. Data from fecal digestive enzyme assays suggests that there is metabolic flexibility that allows high carbohydrates in a dietary treatment. Apparent digestibility of cellulose in green turtles is 72–91% when feeding on seagrass (*Thalassia testudinum*) and 82–88% in mixtures of omnivorous diet (fish and fresh leaves of *Araceae*, *Moraceae* and *Bombaceae*) (Amorocho and Reina, 2008; Bjorndal, 1980). This digestibility does not depend on turtle weight (Bjorndal, 1980), but could depend on prior adaptation to diet, as suggested by significant upregulation of cellulase specific activity with artificial feed in the current study. Bjorndal (1997) proposed that the adaptation of gut microflora may indicate what type of forage would be optimal for a green turtle. Artificial feed containing a mixture of plant and animal raw materials may lead to an adaptation of the microbial community from what it would be with a natural diet.

Direct observation of fecal materials can help identify dietary ingredients (Amorocho and Reina, 2008). Fecal microstructures exhibit constituents of plant cell walls (cellulose, hemicelluloses and lignin) not digested to nutrients. An increase of feces particle size has been reported in water bird fed a high fiber diet (Varo and Amat, 2008). The flat and long shapes of feces from first dietary group were probably due to a large amount of indigestible elements in the feed. Ji et al. (2008) reported that the roughness increases surface-to-volume of materials, which favors enzyme loading. Higher roughness in feces might therefore act as evidence from enzymatic hydrolysis along the alimentary tract of turtles.

Amylase specific activity and the A/T ratio are indicators related to carbohydrate utilization (Hofer and Schiemer, 1981; Thongprajukaew et al., 2011). Green turtles with artificial feed appeared to adapt towards carbohydrate (nitrogen free extract) digestion more than with the conventional feeds. The enzymatic changes and growth performance

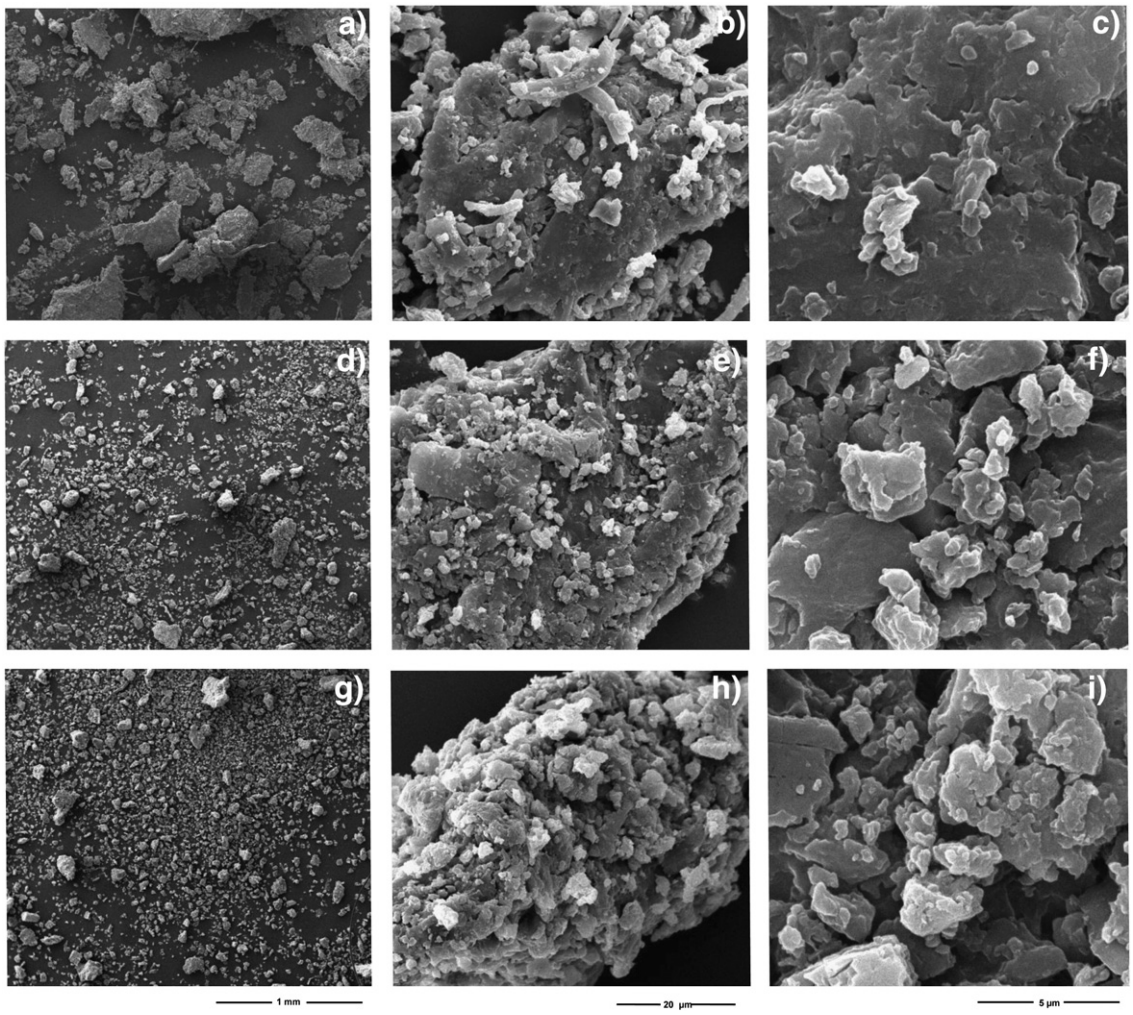

Fig. 3. Fecal sample microstructures of green turtles on diets 1 (a–c), 2 (d–f) or 3 (g–i) at 6 months. The images have magnifications 50× (left), 2000× (middle) and 10,000× (right).

observed indicate that green turtles adapted well even to diet 3 with only artificial feed. Further gains in growth, without negative effects on health, might be possible by optimization of artificial feed composition, or its mixing ratio with other available feeds. In any case, significant usage of artificial feed is feasible in rearing green turtles. Thongprajukaew et al. (2013) reported that the quality of carbohydrates plays a key role in their digestibility and complements their quantity in nutrition. The induction of carbohydrate-digesting enzymes in turtle feces suggests that enzymatic hydrolysis was used to digest the artificial feed. The starch in the artificial feed pellets is partly gelatinized during extrusion, improving nutritional properties and digestibility (Thongprajukaew et al., 2011), as well as promoting the microbial fermentation in the distal part of intestine (Amirkolaie et al., 2006).

Table 3
Blood characteristics of green turtles undergoing dietary treatments. The experiments were performed in triplicates over 6 months, and the blood samples collected at the end.

Blood parameter	Dietary treatment[a]			Pooled SEM
	Diet 1	Diet 2	Diet 3	
Packed cell volume (%)	25.50	29.67	27.67	6.68
Hemoglobin (g dL^{-1})	7.10	9.87	9.20	1.15
Red blood cell (× 10^5 μL^{-1})	2.47	3.77	3.63	0.40
White blood cell (× 10^4 μL^{-1})	1.94	1.60	1.45	0.10

Significant differences in each row are indicated by different superscripts ($P < 0.05$).
[a] Green turtles were fed either by fresh feed containing minced fresh fish and vegetable (1:1, Diet 1); fresh feed containing minced fish fillet, vegetable and artificial feed (1:1:2, Diet 2); or artificial feed only (Diet 3).

Pepsin is present in the gut of both soft-shell turtle, *Trionyx sinensis* (Long and Bai, 1997) and red-eared slider turtle, *Trachemys scripta elegans* (Sun et al., 2007). This enzyme is effective in cleaving peptide bonds between hydrophobic and preferably aromatic amino acids, such as phenylalanine, tryptophan, and tyrosine (Dunn, 2001). Detection of pepsin in feces of juvenile green turtles indicates extracellular digestion in their guts (Salze et al., 2012). The pepsin specific activity in feces of turtles fed entirely with artificial feed was the highest, although this dietary treatment had the lowest protein in proximate composition (Table 1). Then the acidic digestion of proteins depended on protein quality but not on the amount of protein. This suggests that in *in vitro* digestibility studies using fecal digestive enzymes, both acid and alkaline conditions need to be assessed.

Colorimetric detection and gel electrophoresis of fecal trypsin and chymotrypsin have been reported (CÓrdova-Murueta et al., 2003, 2004). These enzymes had identical responses to feed protein stressor as observed in mid gut gland (CÓrdova-Murueta et al., 2003). Similarly, the increase in fecal trypsin specific activity in the current study matches the protein content which was high in conventional feeds relative to the artificial feed alone. Trypsin cleaves the carboxyl side of charged polar R groups (including lysine and arginine) in polypeptide chains. Therefore, the use of fecal trypsin as a marker related to feed protein response in green turtles could be possible. Sun et al. (2007) reported a higher expression of alkaline proteases in pancreas than in the

three main parts of the intestine (anterior, posterior and middle, respectively). These organs contain a mixture of active enzymes, zymogens and inhibitors (Albuquerque et al., 2002) that could affect enzymatic activity, while feces contain only active enzymes.

Protein inclusion has a significant impact on overall feed costs. The 48 baht kg^{-1} variable cost of artificial feed in the current study was dramatically lower than of the conventional feeds: 108 baht kg^{-1} for diet 1 and 78 baht kg^{-1} for diet 2. However, the cost reduction needs to be weighed against growth and health effects, affected by both protein and carbohydrate contents. Commercial feeds containing 40–50% protein (Hadjichristophorou and Grove, 1983) are optimal for rearing over 1-year-old green turtles. This range covers the 41% protein in Purina Trout Chow®5V-VO5 (Purina Mills, LLC, St. Louis, MO, USA) feed used in a study on the metabolic rate in this species (Jones et al., 2009; Price et al., 2013). For rearing juvenile soft-shelled turtles (3.7–21.0 g initial weight) the optimal protein content is about 42.20–46.48% (Bau et al., 1992; Nuangsaeng and Boonyaratapalin, 2001; Zhou et al., 2013) which well overlaps the basal protein contents (41.5–44.3%) of casein-based feeds optimized for inclusion (Chen and Huang, 2011; Huang et al., 2010). The about 45% protein content of the artificial feed in the current study matches the above range for juveniles. More investigations on optimal feed ingredients and dietary protein levels are needed to further improve the quality of husbandry with this species.

Hematological characteristics have been used for indentifying health categories (Flint et al., 2010) and feeding or fasting status (Price et al., 2013) of green sea turtles. The similar blood characteristics observed in the current study indicate similar health statuses of the dietary treatment groups at the end of the 6-month experiment. The packed cell volume and hemoglobin of turtles in this study agree with Bolten and Bjorndal (1992), Al Kindi and Mashmoud (2002) and Fong et al. (2010). Therefore, the current artificial feed appears to be suitable for good growth and feed utilization efficiency, and without negative effects on general hematological parameters. More detailed hematological profiles of green turtles undergoing dietary treatments are the topic of an ongoing study, to be reported later.

5. Conclusions

The experimental results indicated significant potential of artificial feed in rearing juvenile green turtles. Partial substitution or full replacement by artificial feed improved growth and feed utilization relative to a fresh feed, without negative impacts observed on health or mortality. The fecal digestive enzyme activities and feces microstructure suggested that the turtles adapted to utilize their no-choice diets, by physiological responses in digestion. The induction of enzyme expression by various feedstuff ingredients deserves further studies that can be non-invasive and only sample feces. Studies to clarify the best practices in replacement of conventional feed by artificial feed, partially or fully, in the routine rearing of green turtles in Thailand, are currently underway.

Acknowledgments

This work was financially supported in part by the PMBC, Phuket, Thailand. The authors would like to thank Dr. Pinsak Suraswadi, Director of Marine and Coastal Resources Research and Development Institute; Mr. Ukkrit Satapoomin, Director of PMBC, for kindly consulting during experimentation; Mr. Visoot Autthasab, Miss Sasiporn Jatupornpituchat and Mrs. Yaowaluk Hemthanon, for help in turtle rearing; and Phang-Nga Naval Base, Royal Thai Navy, for kindly providing the green turtles. We also gratefully acknowledge Assoc. Prof. Dr. Seppo Karrila, from the Publication Clinic, Research and Development Office, Prince of Songkla University, for help in manuscript proofreading.

References

Al Kindi, A.Y.A., Mashmoud, I.Y., 2002. Hematological survey in two species of sea turtles in the Arabian Sea during nesting season. Pak. J. Biol. Sci. 5, 359–361.
Albuquerque, C., García-Carreño, F., Navarrete-del-Toro, M., 2002. Trypsin and trypsin inhibitors from Penaeid shrimp. J. Food Biochem. 226, 233–251.
Amirkolaie, A.K., Verreth, J.A.J., Schrama, J.W., 2006. Effect of gelatinization degree and inclusion level of dietary starch on the characteristics of digesta and faeces in Nile tilapia (*Oreochromis niloticus* (L.)). Aquaculture 260, 194–205.
Amorocho, D.F., Reina, R.D., 2008. Intake passage time, digesta composition and digestibility in East Pacific green turtles (*Chelonia mydas agassizii*). J. Exp. Mar. Biol. Ecol. 360, 117–124.
AOAC, 1980. Official Methods of Analysis of AOAC International, 13th ed. Association of Official Analytical Chemists, Arlington, VA.
Areekijseree, M., Engkagul, A., Kovitvadhi, U., Thongpan, A., Mingmuang, M., Pakkong, P., Rungruangsak-Torrissen, K., 2004. Temperature and pH characteristics of amylase and proteinase of adult freshwater pearl mussel, *Hyriopsis* (*Hyriopsis*) *bialatus* Simpson 1900. Aquaculture 234, 575–587.
Arthur, K.E., Mcmahon, K.M., Limpus, C.J., Dennison, W.C., 2009. Feeding ecology of green turtles (*Chelonia mydas*) from Shoalwater Bay, Australia. Mar. Turt. Newsl. 123, 6–12.
Bau, J., Liu, C., Gao, X., Gu, Z., Yang, X., 1992. The daily requirement of nutrient and optimum calorie-protein ratio in the diet for juvenile turtle (*Trionyx sinensis*). J. Fish. China 16, 365–371 (in Chinese).
Bjorndal, K.A., 1980. Nutrition and grazing behavior of the green turtles *Chelonia mydas*. Mar. Biol. 56, 147–154.
Bjorndal, K.A., 1997. Foraging ecology and nutrition of sea turtles. In: Lutz, P.L., Musick, J.A. (Eds.), The Biology of Sea Turtles. CRC Marine Science Series, Florida, pp. 199–231.
Blaxhall, P.C., Daisley, K.W., 1973. Routine haematological methods for use with fish blood. J. Fish Biol. 5, 771–781.
Bolten, A.B.., Bjorndal, K.A., 1992. Blood profiles for a wild population of green turtle (*Chelonia mydas*) in the Southern Bahamas: size-specific and sex-specific relationships. J. Wildl. Dis. 28, 407–413.
Brand-Gardner, S.J., Lanyon, J.M., Limpus, C.J., 1999. Diet selection by immature green turtles, *Chelonia mydas*, in subtropical Moreton Bay, south-east Queensland. Aust. J. Zool. 47, 181–191.
Chen, L.P., Huang, C.H., 2011. Effects of dietary ß-carotene levels on growth and liver vitamin A concentrations of the soft-shelled turtle, *Pelodiscus sinensis* (Wiegmann). Aquacult. Res. 42, 1848–1854.
CÓrdova-Murueta, J.H., García-Carreño, F.L., Navarrete-del-Toro,, M.A., 2003. Digestive enzymes present in crustacean feces as a tool for biochemical, physiological, and ecological studies. J. Exp. Mar. Biol. Ecol. 297, 43–56.
CÓrdova-Murueta, J.H., García-Carreño, F.L., Navarrete-del-Toro, M.A., 2004. Effects of stressors on shrimp digestive enzymes from assays of feces: an alternate method of evaluation. Aquaculture 233, 439–449.
Dunn, B.M., 2001. Overview of pepsin-like aspartic peptidases. Curr. Protoc. Protein Sci. http://dx.doi.org/10.1002/0471140864.ps2103s25.
Flint, M., Morton, J.M., Limpus, C.J., Patterson-Kane, J.C., Murray, P.J., Mills, P.C., 2010. Development and application of biochemical and haematological reference intervals to identify unhealthy green sea turtles (*Chelonia mydas*). Vet. J. 185, 299–304.
Fong, C.I., Chen, H.C., Cheng, I.J., 2010. Blood profiles from wild populations of green sea turtles in Taiwan. J. Vet. Med. Anim. Health 2, 008–010.
Hadjichristophorou, M., Grove, D.J., 1983. A study of appetite, digestion and growth in juvenile green turtle (*Chelonia mydas* L.) fed on artificial diets. Aquaculture 30, 191–201.
Hofer, R., Schiemer, F., 1981. Proteolytic activity in the digestive tract of several species of fish with different feeding habits. Oecologia 48, 342–345.
Huang, S.C., Chen, S.M., Huang, C.H., 2010. Effects of dietary zinc levels on growth, serum zinc, haematological parameters and tissue trace elements of soft-shelled turtles, *Pelodiscus sinensis*. Aquacult. Nutr. 16, 284–289.
Ji, J., Zhang, Y., Zhou, X., Kong, J., Tang, Y., Liu, B., 2008. Enhanced protein digestion through the confinement of nanozeolite-assembled microchip reactors. Anal. Chem. 80, 2457–2463.
Jones, T.T., Hastings, M.D., Bostrom, B.L., Andrews, R.D., Jones, D.R., 2009. Validation of the use of doubly labeled water for estimating metabolic rate in the green turtle (*Chelonia mydas* L.): a word of caution. J. Exp. Biol. 212, 2635–2644.
Kita, J., Lechowski, R., Degorski, A., 1989. Rotavirus infection in newborn calves: I. Evaluation trials of certain enzymes in feces. J. Vet. Med. 36, 21–26.
Larsen, H.N., Snieszko, S.F., 1961. Comparison of various methods of determination of haemoglobin in trout blood. Prog. Fish Cult. 23, 8–17.
Long, L.Q., Bai, D.Q., 1997. Distribution of three major digestive enzymes of digestive tissue of *Trionyx sinensis*. Chin. J. Zool. 32, 23–26.
Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.
Mendels, M., Weber, J., 1969. The product of cellulose in cellulase and their application. Adv. Chem. Ser. 95, 39–443.
Nuangsaeng, B., Boonyaratapalin, M., 2001. Protein requirement of juvenile soft-shell turtle *Trionyx sinensis* Wiegmann. Aquacult. Res. 32, 106–111.
Price, E.R., Jones, T.T., Wallace, B.P., Guglielmo, C.G., 2013. Serum triglycerides and ß-hydroxybutyrate predict feeding status in green turtles (*Chelonia mydas*): evaluating a single blood sample method for assessing feeding/fasting in reptiles. J. Exp. Mar. Biol. Ecol. 439, 176–180.
Rungruangsak, K., Utne, F., 1981. Effect of different acidified wet feeds on protease activities in the digestive tract and on growth rate of rainbow trout (*Salmo gairdneri* Richardson). Aquaculture 22, 67–79.

Rungruangsak-Torrissen, K., Moss, R., Andresen, L.H., Berg, A., Waagbo, R., 2006. Different expressions of trypsin and chymotrypsin in relation to growth in Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. 32, 7–23.

Salze, G., McLean, E., Craig, S.R., 2012. Pepsin ontogeny and stomach development in larval cobia. Aquaculture 324–325, 315–318.

Sun, J.Y., Du, J., Qian, L.C., Jing, M.Y., Weng, X.Y., 2007. Distribution and characteristics of endogenous digestive enzymes in the red-eared slider turtle, *Trachemys scripta elagans*. Comp. Biochem. Physiol. 147, 1125–1129.

Thongprajukaew, K., Kovitvadhi, U., Kovitvadhi, S., Somsueb, P., Rungruangsak-Torrissen, K., 2011. Effects of different modified diets on growth, digestive enzyme activities and muscle compositions in juvenile Siamese fighting fish (*Betta splendens* Regan, 1910). Aquaculture 322–323, 1–9.

Thongprajukaew, K., Yawang, P., Dudae, L., Bilanglod, H., Dumrongrittamatt, T., Tantikitti, C., Kovitvadhi, U., 2013. Physical modification of palm kernel meal improved available carbohydrate, physicochemical properties and *in vitro* digestibility in economic freshwater fish. J. Sci. Food Agric. 93, 3832–3840.

Tolleson, D.R., Randel, R.D., Stuth, J.W., Neuendorff, D.A., 2005. Determination of sex and species in red and fallow deer by near infrared reflectance spectroscopy of the faeces. Small Rumin. Res. 57, 141–150.

Varo, N., Amat, J.A., 2008. Differences in food assimilation between two coot species assessed with stable isotopes and particle size in faeces: linking physiology and conservation. Comp. Biochem. Physiol. 149A, 217–223.

Zhou, F., Ding, X.Y., Feng, H., Xu, Y.B., Xue, H.L., Zhang, J.R., Ng, W.K., 2013. The dietary protein requirement of a new Japanese strain of juvenile Chinese soft shell turtle, *Pelodiscus sinensis*. Aquaculture 412–413, 74–80.