RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2016.42.237

ScienceAsia 42 (2016): **237–246**

Faecal characteristics as markers of *Chelonia mydas* feeding

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Received 23 Feb 2015
Accepted 13 Jul 2016

ABSTRACT: Faecal sampling can provide an unobtrusive monitoring of feed utilization. We demonstrate that faecal digestive enzymes and physicochemical characteristics correlate with the feed conversion efficiency in green turtle (*Chelonia mydas*). The 10-day-old juvenile turtles (25.4 ± 1.3 g body weight) were fed with either fresh feed containing minced fresh fish and vegetable (diet 1); fresh feed containing minced fish fillet, vegetable and fish pellet diet (diet 2); or only fish pellet diet (diet 3) for four months in a completely randomized design (3 treatments × 3 replicates × 10 subjects per replication). Analysis of faecal digestive enzymes (pepsin, trypsin, amylase, and activity ratio of amylase to trypsin) based on colorimetric determinations of specific activity suggested that carbohydrate digestion is a key indicator for feed utilization efficiency in terms of intake and conversion ratio. Physicochemical characteristics to enhance enzymatic hydrolysis, namely, microstructure (scanning electron microscope), thermal transition properties (differential scanning calorimeter), and relative crystallinity (X-ray diffractometer) indicate that the faeces of turtles fed with diet 3 provide a superior feed utilization efficiency. The observed faecal characteristics varied in response to different diets over the duration of the experiments. Thus analysis of faecal digestive enzymes and physicochemical characteristics appear to provide useful unobtrusively sampled indicators for determining feed utilization and can be applied in studies of nutritional status in endangered species without conflicting ethical standards.

KEYWORDS: digestive enzyme, faeces, feed utilization, physicochemical characteristics

INTRODUCTION

Green turtle (*Chelonia mydas*) is listed as an endangered species by the International Union for conservation of Nature (IUCN) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The green turtle is distributed throughout tropical and subtropical seas, although its populations are globally declining[1]. In Thailand, head-started propagation of turtles prior to release in natural ecosystem is conducted under the Marine and Coastal Resources Research Centre, Ministry of Natural Resources and Environment[2]. For such efforts, optimization of artificial diet for improving growth and feed utilization efficiency is essential to reduce indoor captivity time under ex situ conservation program. Thus non-lethal and non-invasive approaches to assess feed utilization efficiency in terms of faecal characteristics (digestive enzymes and physicochemical properties), in comparison to measured feed intake and conversion ratio from in vivo trials, are desired and these would not conflict with ethical standards.

We have previously suggested that feed utilization efficiency in aquatic animals is typically studied through digestive enzymes extracted from the alimentary tracts[3]. In higher animals, faecal analysis can be used for monitoring feed quality in domestic livestock[4], and for identifying sex and species of organisms present in the wild[5]. Recently, digestive enzymes from faeces have become an important tool for biochemical, physiological and ecological studies of crustaceans[6,7]. The enzymes are produced by digestive or accessory glands and secreted into gut lumen. After digestion, the enzymes are excreted in the faeces, and are protected by membranes that prevent leakage[6]. Excreted digestive enzymes in faeces may therefore help determine recent feeding history. Similarly, digestive enzymes extracted from alimentary tract could be used to determine growth[1,8] and feed conversion efficiency[9], as indicated by a positive relationship between them. This

indicates a close connection between the nutritional status, based on digestive enzyme interpretation, and the growth of animals.

Physicochemical characteristics of feed could be used to estimate the utilization efficiency in animals[10]. The characteristics relating to enzymatic hydrolysis, in vitro and probably also in vivo, include microstructure, thermal transition properties, and crystallinity[10–13]. As the hydrolysis directly affects digestion and absorption along the alimentary tract, these physicochemical characteristics of feed impact feed utilization, as well as the digestion of feed by the digestive enzymes observable in the faeces[1,2]. The main objective of this study was to evaluate whether digestive enzymes present in turtle faeces, as well as faecal physicochemical characteristics, can be used to predict feed utilization. The optimal conditions for assaying faecal digestive enzymes in this species have however not yet reported. Thus a preliminary study on the pH and temperature characteristics was conducted in order to establish the condition for the subsequent experiment. In this study, the dietary treatment groups were (1) fresh fish and vegetable, which is traditional in the rearing of green turtles in Thailand; (2) fish fillet, vegetable and fish pellet diet, in which the fillet is believed to prevent intestinal inflammation[1,14]; and (3) fish pellet diet, which is a potential future alternative in rearing this species. The present work might lead to unobtrusive and non-invasive approaches for forensic investigations into foraging success of various endangered species.

MATERIALS AND METHODS

Collection and extraction of faecal digestive enzymes

Fresh faeces were collected by dip-net from 4-month-old green turtles ($n = 3$) at the Phuket Marine Biological Centre (PMBC), Phuket, Thailand, after starvation for 24 h. These turtles were reared in a round fibreglass tank (100 cm diameter and 100 cm height) containing 265 l of sea water. They were fed ad libitum by conventional minced mixtures between fresh fish and vegetable (1:1 on wet weight basis), twice daily at 10:00 and 17:00 h. Their faeces samples were packed in polyethylene bags, kept in ice, and then transported to the Department of Applied Science, Faculty of Science, Prince of Songkla University. The fresh faeces were rinsed to remove contaminating dirt and homogenized in cool distilled water (1:2 w/v) using a micro-homogenizer (THP-220; Omni International, Kennesaw GA, USA). The homogenate was centrifuged at 15 000g at 4 °C for 30 min before collecting the supernatant. Small samples of these faeces extracts were kept at −20 °C until use in characterizations of digestive enzymes.

Characterizations of faecal digestive enzymes

Characterizations of faecal digestive enzymes were performed at varying pH values achieved by buffering: KCl-HCl buffer for pH 1–2, citrate-phosphate buffer for pH 3–5, phosphate buffer for pH 6–8, $NaHCO_3$-Na_2CO_3 buffer for pH 9–10, Na_2HPO_4-NaOH for pH 11, and KCl-NaOH for pH 12. The temperature effects on determinations at optimal pH were assessed in the range of 25–80 °C. Two proteolytic enzymes, namely, pepsin (EC 3.4.23.1) and trypsin (EC 3.4.21.4), which are digestive enzymes from stomach and intestine, were determined according to the method described in Rungruangsak-Torrissen et al[15]. The substrates used in these assays were 1% casein and 1.25 mmol/l benzoyl-*L*-arginine-*p*-nitroanilide (BAPNA), respectively. The detected amounts of products, including *L*-tyrosine and *p*-nitroanilide for pepsin and trypsin, were used to calculate relative activities expressed as conversion per min. The determination of α-amylase (EC 3.2.1.1) was based on Areekijseree et al[16], with slight modifications. The reaction mixture contained 25 µl of 5% soluble starch, 62.5 µl of buffer, 37.5 µl of 20 mmol/l NaCl, and 125 µl of faecal enzyme extract. This mixture was incubated for 15 min at ambient temperature; the reactions arrested by adding 250 µl of 1% dinitrosalicylic acid, the mixture boiled at 100 °C for 5 min, cooled to room temperature, and mixed with 2.5 ml of distilled water. The activity of amylase was measured spectrophotometrically at 540 nm against standard maltose.

Hatching, husbandry and rearing

One hundred and six eggs were collected from a turtle (85 cm curved carapace width and 96 cm curved carapace length) under PMBC's project at Huyong Island, Phang-Nga, Thailand. Ninety-nine turtles hatched after incubation at 28.7 ± 1.1 °C for 54 days, and were transported to the Marine Endangered Species Unit, PMBC, at day 5 after hatching. The hatchlings were acclimatized in round fibreglass tanks containing 3000 l sea water, until absence of yolk. Subsequently, ten turtles (25.4 ± 1.3 g body weight/individual) were reared in each round fibreglass tank (100 cm diameter and 100 cm height) containing 265 l sea water each. There were nine

tanks in total, corresponding to three dietary treatment groups with three replicates. The turtles were fed ad libitum by fresh feed containing minced mixtures between fresh fish and vegetable (1:1 on wet weight basis, diet 1); fresh feed containing minced mixtures of fish fillet, vegetable and carnivorous fish pellets (1:1:2 on wet weight basis, diet 2); or carnivorous fish pellets only (diet 3), twice daily at 10:00 and 17:00 h. Water was changed at 100% daily before beginning the first meal. Parameters of water recorded during experimental period were pH 7.25–8.30, temperature 27.50–31.50 °C, salinity 29–34%, total alkalinity 112–121 ppm $CaCO_3$, and dissolved oxygen 5.61–7.42 mg/l. The experiment was conducted for 4 months with 12-h light/12-h dark. Survival, body weight, straight carapace width, straight carapace length, feed intake (FI), and feed conversion ratio (FCR) were recorded weekly while the significant data were presented at the end of experiment. The FI and FCR were estimated by the differences in actual mass (on a dry matter basis) between the diet offered and any recovered uneaten diet. The uneaten diet was immediately removed by a dip-net after satiation and then dried at 60 °C to eliminate the moisture until constant weight. Both values were calculated for each individual turtle. Practical guidelines during experimentation were conducted based on the Ethical Principles and Guidelines for the Use of Animals for Scientific Purposes (Sections 4.1–4.5), National Research Council, Thailand. The parameters describing growth and feed utilization were calculated as follows:

$$\begin{aligned}
\text{SGR} &= (\ln W_t - \ln W_0)/(t - t_0),\\
\text{ADG} &= (\text{net weight gain})/(\text{rearing period}),\\
\text{FI} &= (\text{dry feed consumed})/(\text{rearing period}),\\
\text{FCR} &= (\text{dry feed consumed})/(\text{wet weight gain}),
\end{aligned}$$

where SGR is the specific growth rate, W_t is the mean weight at day t, W_0 is the mean weight at day t_0, and ADG is the average daily gain.

Faeces collection

The pooled fresh faeces (three samples per treatment) were collected from the last meal at the end of experiment. The faeces used in digestive enzyme analysis were kept at −20 °C until extraction. For microstructural studies, the faeces were dried using a freeze dryer (Delta 2–24 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 48 h, sieved and then kept in polyethylene bags until use. For studies on thermal transition properties and X-ray diffraction pattern, the freeze-dried faeces were ground to homogeneity and then prepared as described below.

Determination of diet chemical compositions

The experimental diets were dried at 105 °C for 24 h to measure the moisture content. Crude protein, lipid, ash and fibre were determined according to standard methods of AOAC[17]. The nitrogen free extract (NFE) was calculated from the differences in chemical components. All the chemical analyses were done in triplicate, and the values are reported on dry matter basis.

Determination of the specific activity of the faecal digestive enzymes

Faecal digestive enzymes were extracted and assayed as described above. Optimal conditions selected from preliminary studies on digestive enzyme characterization were used for determining enzyme specific activities: pH 2 at 35 °C for pepsin, pH 10 at 40 °C for trypsin, and pH 6 at 55 °C for amylase. Soluble protein concentration in the enzyme extract was determined according to Lowry et al[18] using bovine serum albumin as a standard. These data were used for proportionally quantifying specific activities of the faecal enzymes (U/mg protein). The amylase to trypsin activity ratio (A/T ratio) was calculated.

Analysis of faecal physicochemical characteristics

To analyse the microstructure, dried faeces were mounted with two-sided adhesive tape on an aluminium stub coated with gold. Imaging was performed using a scanning electron microscope (Quanta 400, FEI, Brno, Czech Republic) at 50, 2000, and 10 000 × magnifications, with accelerating voltage set at 15 kV. The assessment of microstructure included general morphology, particle size, porosity, and roughness. The characteristics of fine morphology, small particle size, high porosity, and high roughness would contribute positively to enzymatic hydrolysis[10–13].

Thermal transition properties including onset (T_o), peak (T_p), and conclusion (T_c) temperatures, melting temperature range ($T_c - T_o$), and transition enthalpy (ΔH), were measured with a differential scanning calorimeter (DSC7, Perkin Elmer, Waltham, Massachusetts, USA). Three milligrams of sample were placed in an aluminium pan, sealed, allowed to equilibrate at room temperature for 1 h, and then heated from 40 °C to 400 °C at a rate

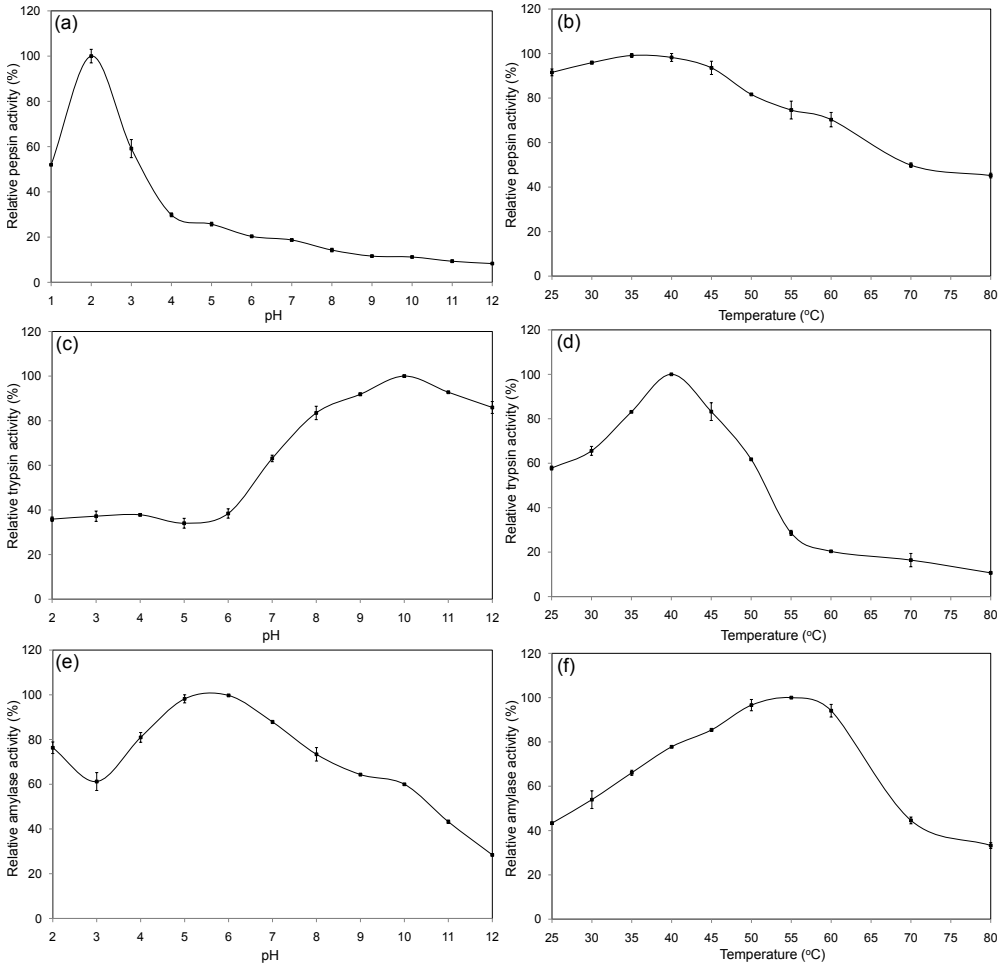

Fig. 1 Activities of faecal digestive enzymes from green turtles. The dependence of activity on pH for (a) pepsin, (c) trypsin, and (e) amylase, at ambient temperature. The optimum pH was selected to maximize activity for each enzyme, and the dependence of activity on temperature determined at this pH, for (b) pepsin, (d) trypsin, and (f) amylase. All assays were performed in triplicate.

of 10 °C/min. The thermal transition properties indicate changes in the enthalpy of samples[19]. A high ΔH would be positive for the feed utilization efficiency, with the feeds as well as the faeces containing a large amount of available nutrients[20].

X-ray diffraction patterns of turtle faeces were determined using an X-ray diffractometer (X′ Pert MPD, Philips, Amsterdam, Netherlands), operated at 40 kV voltage and 30 mA current. The diffractograms were recorded for 2θ from 2° to 90° with a scanning rate of 1°/min. Relative crystallinity was calculated using EXCEL 2007 (Microsoft Corp., Redmond, WA, USA). This measurement indicates molecular level changes, especially in crystalline regions. Diffraction patterns in the range of 10–35° (2θ) is related to the presence of the starch that the animal enzymes hydrolyse well[20].

Statistical analysis

Data analysis was performed using SPSS Version 14 (SPSS Inc., Chicago, USA), and results are summarized as mean ± SD from the triplicate determinations. The variable was analysed using a one-way ANOVA. If there was a significant F-test, comparisons of treatment means were tested with Duncan's multiple range test, requiring $p < 0.05$.

RESULTS

Preliminary study on characterization of faecal digestive enzymes

The highest activity of pepsin was found at pH 2 (Fig. 1a) with maximal relative activity at 35 °C (Fig. 1b). The relative activity of pepsin was however in excess of 90% over the range of 25–45 °C.

Table 1 Proximate chemical compositions of the diets used for rearing green turtles.

Chemical composition	Diet 1	Diet 2	Diet 3
Moisture (%)	85.0 ± 1.2	61.5 ± 1.2	7.35 ± 0.26
Crude protein (% DM)	54.13 ± 0.52	50.14 ± 0.10	44.81 ± 0.14
Crude lipid (% DM)	6.20 ± 0.21	6.21 ± 0.03	7.99 ± 0.17
Crude fibre (% DM)	5.30 ± 0.10	3.13 ± 0.02	0.96 ± 0.09
Crude ash (% DM)	10.43 ± 0.02	11.48 ± 0.05	10.25 ± 0.12
Nitrogen free extract (% DM)	23.94 ± 0.57	29.04 ± 0.12	35.99 ± 0.27

The data from triplicate determinations are expressed as % of dry matter.

Trypsin activity was higher in alkaline than in acidic conditions (Fig. 1c). The optimal condition with maximal activity was found at pH 10 and 40 °C (Fig. 1d), and the activity decreased by about 70% at 55 °C. Amylase was active in weak acidic conditions with pH 5–6, and its activity decreased with pH in the range of 7–12 (Fig. 1e). The optimal temperatures for assaying faecal amylase at pH 6 were in the range of 50–60 °C, with maximal activity at 55 °C (Fig. 1f).

Chemical compositions of experimental diets

The diet 3 differed significantly from the others in each type of constituent tabulated (Table 1). This was the highest in lipids and available carbohydrates (NFE), and the lowest in crude protein, fibre and ash. The main differences between diets 1 and 2 were that diet 1 was comparatively high in protein and fibre, while it was low in NFE.

Changes in soluble protein and faecal digestive enzyme activity

Soluble protein in the faeces extract differed significantly between the dietary groups ($p < 0.05$, Table 2). The highest values were found in turtles fed by diet 3, followed by diets 2 and 1, respectively. Turtles fed by diets 2 and 3 had a similar level of pepsin activity; the values were significantly higher than for turtles fed by diet 1. The highest trypsin activity was observed in turtles fed by diet 2, followed by diets 1 and 3, respectively, ($p < 0.05$). Amylase activity had similar patterns as amylase to trypsin activity ratio (A/T ratio). Turtles fed by diet 3 had the highest amylase activity and the A/T ratio, followed by diets 2 and 1 ($p < 0.05$).

Faecal microstructure

The dietary treatments affected faecal microscopic structure (Fig. 2). A coarse morphology with flat, long and irregular shapes was observed in faeces of turtles fed by diet 1 (Fig. 2a to Fig. 2c). For turtles fed diets 2 (Fig. 2d to Fig. 2f) and 3 (Fig. 2g to Fig. 2i), the general morphologies of faeces were finer grained but also irregular. Porosity and roughness of faeces were mainly observed for diets 2 and 3.

Table 2 Protein content and digestive enzyme specific activities in the faeces of green turtles.

Parameter	Dietary treatment			p value
	Diet 1	Diet 2	Diet 3	
Protein (mg/ml)	13.9 ± 4.6^{c}	32.0 ± 2.8^{b}	52 ± 10^{a}	0.001
Pepsin†	0.10 ± 0.02^{b}	0.21 ± 0.03^{a}	0.20 ± 0.02^{a}	0.028
Trypsin†	20.0 ± 1.5^{b}	28.41 ± 0.74^{a}	13.7 ± 2.9^{c}	0.004
Amylase†	3.26 ± 0.95^{c}	7.1 ± 1.5^{b}	15.8 ± 3.1^{a}	0.001
A/T ratio‡	0.13 ± 0.02^{c}	0.23 ± 0.04^{b}	0.83 ± 0.07^{a}	< 0.001

† U/mg protein.
‡ Activity ratio of amylase to trypsin.
The determinations were done in triplicate for samples collected at month 4 of treatment. Significant differences within each row are indicated by different superscripts ($p < 0.05$).

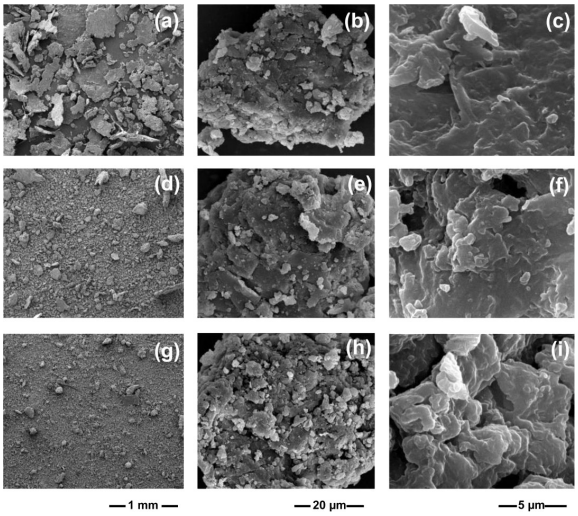

Fig. 2 Microstructures of green turtle faeces sampled at 4 months of dietary treatment. The turtles were fed by fresh feed containing (a–c) minced fresh fish and vegetable (diet 1); (d–f) fresh feed containing minced fish fillet, vegetable, and fish pellet diet (diet 2); or (g–i) fish pellets only (diet 3). Microscopic imaging at magnifications 50 × (left), 2000 × (middle) and 10 000 × (right).

Faecal thermal transition properties

The thermal transition parameters of turtle's faeces varied between dietary treatments (Table 3). The highest T_o, T_p, and T_c values were mostly for turtles

Table 3 Thermal transition properties of green turtle faeces collected on month 4.

Thermal parameter	Dietary treatment			p value
	Diet 1	Diet 2	Diet 3	
T_o (°C)	75.69 ± 0.02^b	75.78 ± 0.35^b	79.96 ± 0.03^a	0.034
T_p (°C)	89.33 ± 0.40^c	93.33 ± 0.21^b	100.67 ± 0.29^a	< 0.001
T_c (°C)	107.93 ± 0.10^b	106.31 ± 0.16^c	112.37 ± 0.40^a	0.001
$T_c - T_o$ (°C)	32.24 ± 0.16^a	30.53 ± 0.05^b	32.41 ± 0.21^a	0.028
ΔH (J/g)	16.08 ± 0.29^c	28.16 ± 0.21^b	69.37 ± 0.66^a	< 0.001

T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; $T_c - T_o$, melting temperature range; ΔH, enthalpy.
Significant differences within each row are indicated by different superscripts ($p < 0.05$).

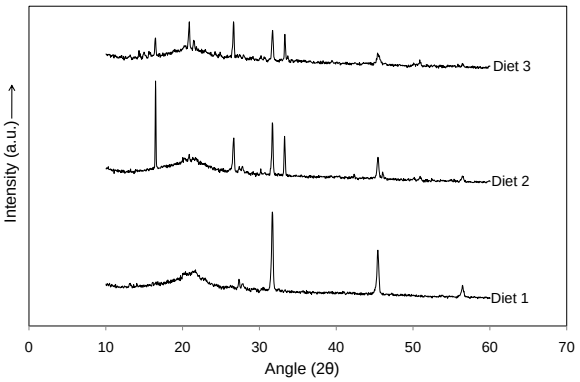

Fig. 3 Diffractograms of green turtle faeces at 4 months of dietary treatment. The turtles were fed by fresh feed containing minced fresh fish and vegetable (diet 1); fresh feed containing minced fish fillet, vegetable and fish pellet diet (diet 2); or fish pellets only (diet 3). Diffraction patterns are shown for 2θ range 10–60°.

fed by diet 3, followed by diets 2 and 1. The $T_c - T_o$ was high and similar for diets 1 and 3 whereas the lowest value was observed for diet 2. The trends of ΔH differed dramatically between the treatment groups. The highest value was found in turtles fed by diet 3, followed by diets 2 and 1, respectively.

X-ray diffraction patterns

Diffraction patterns and peaks of turtle faeces were mainly found in the 2θ angle range of 10–60° (Fig. 3). The main peaks of starch (10–35°) were prominently found in faeces of turtles fed by diet 3, followed by diet 2. Relative crystallinity in diet 3 ($19.18 \pm 0.01\%$) was significantly higher than in diets 2 ($18.72 \pm 0.03\%$) and 1 ($15.97 \pm 0.04\%$).

Growth and feeding performance

No differences in survival, body weight, straight carapace width, straight carapace lengths, SGR and ADG were observed among treatments ($p > 0.05$, Table 4). Under satiation feeding condition, FI was the highest in turtles fed by diet 2, followed by diets 1 and 3. This leaded to the lowest FCR in turtles fed by diet 3 ($p < 0.05$) and followed by diets 1 and 2, respectively.

DISCUSSION

Characterization of faecal digestive enzymes

The digestive enzymes in turtle faeces were affected by dietary treatment, and the sampling was non-intrusive and non-invasive. Two proteolytic enzymes indicated protein digestion in stomach and intestine. The optimal analysis conditions of faecal pepsin were similar to those reported for analysis of samples from digestive organs of aquatic species, such as pH 2.2 when assayed at 37 °C in soft-shell turtle, *Trionyx sinensis*[21], pH 2.0–2.5 at 40 °C in red-eared slider turtle, *Trachemys scripta elegans*[22] and pH 3.0–3.5 at 45 °C in pectoral rattail, *Coryphaenoides pectoralis*[23]. For intestinal enzymes, pH 10 and 40 °C were optimal for trypsin, whereas for alkaline protease found in red-eared slider turtle pH 7.0–8.5 at 50 °C were optimal[22]. Stability over a range of pH and temperature values is common to most trypsin isoforms reported in fish[24], and similar optimal conditions at pH 10.5 and 40 °C in grey triggerfish, *Balistes capriscus*[25] and pH 10 at 30–35 °C in Siamese fighting fish, *Betta splendens*[26] have also been reported. This enzyme is most active in pancreas, followed by anterior, middle and posterior sections of intestine[22]. Moreover, this enzyme is also observed in faeces of white shrimp, *Penaeus vannamei*, when assayed using colorimetric method and confirmed by gel electrophoresis[6,7]. Faeces might therefore serve as an important source of proteolytic enzymes in order to screen appropriate protein sources. In vitro digestibility, based on faecal enzyme extracts in the presence of various substrates from feedstuffs, is an alternative for developing specific feed formulation for green turtles.

Amylase present in faeces suggests the available carbohydrate in the diet is mainly digested with the animals' own enzymes, whereas structural carbohydrates (such as cellulose) are digested with the help of intestinal microflora. The pH and temperature characteristics of amylase from green turtles were similar to those in some other aquatic species, such as pH 7.0–7.5 in sea bream, *Sparus aurata* and turbot, *Scophthalmus maximus*, with an optimal temperature range of 35–45 °C[27], and pH 7–8 at

Table 4 Survival, growth and feed utilization efficiency of green turtles undergoing dietary treatments.

Parameter	Dietary treatment			*p* value
	Diet 1	Diet 2	Diet 3	
Survival (%)	100.0 ± 0.0	90 ± 10	96.7 ± 5.8	0.126
Final body weight (g)	327.1 ± 3.3	352.2 ± 5.1	337 ± 15	0.289
Straight carapace width (cm)	10.89 ± 0.16	10.93 ± 0.12	10.38 ± 0.48	0.113
Straight carapace length (cm)	12.40 ± 0.07	12.60 ± 0.12	11.80 ± 0.48	0.056
SGR (%/day)	2.23 ± 0.02	2.27 ± 0.05	2.29 ± 0.09	0.308
ADG (g/day)	2.68 ± 0.16	2.80 ± 0.16	2.80 ± 0.14	0.315
FI (g/day)	3.03 ± 0.03^{ab}	3.42 ± 0.33^{a}	2.81 ± 0.14^{b}	0.029
FCR (g feed/g gain)	1.13 ± 0.02^{b}	1.22 ± 0.07^{a}	1.01 ± 0.02^{c}	0.001

SGR, specific growth rate; ADG, average daily gain; FI, feed intake; FCR, feed conversion ratio.
Significant differences within each row are indicated by different superscripts ($p < 0.05$).

40–50 °C in red-eared slider turtle[22].

Specific activity of faecal digestive enzymes in relation to feed utilization

Protein concentrations in the feacal extract were not proportionally correlated with the diet protein content. The concentrations measured probably indicate the presence of enzymatic proteins induced by the different diets. The experimentally observed activities of protein- and carbohydrate-digesting enzymes might indicate the omnivorous feeding habits of this species. The high flexibility of the enzyme response when fed by various types of diet supports this presumption. This correlates well with the food items found in their alimentary tracts[28]. On the other hand, herbivorous feeding habit of this species has been reported[29,30]. Analysis of pepsin and trypsin activities demonstrated that the turtles have the capacity to adapt to diets 2 and 1 better than to diet 3. This proteolytic upregulation might be due to high total protein from fresh fish and vegetable (54%) and fish fillet, vegetable and fish pellets (50%); while fish pellet diet has only 45%. These activities were not correlated with all growth (body weight, straight carapace width, straight carapace length, SGR and ADG) and feed utilization parameters (FI and FCR) of the turtles. Thus protein-digesting enzymes appear unsuitable to predict feed utilization efficiency in green turtles. However, prior research has found identical responses to feed stressor of protease compositions (trypsin and chymotrypsin) in faeces and mid gut of shrimp[7], suggesting that sampling of faeces might be representative of whole gut function. The use of proteolytic enzyme specific activities, such as trypsin and activity ratio of trypsin and chymotrypsin (T/C ratio), for predicting growth in aquatic species have been reported[8,15].

For carbohydrate digestion, amylase activity and the A/T ratio, which is used as indicator of metabolic flexibility to utilize carbohydrate-protein[31], appeared to be indicative of FCR in the experiments. The significant increase in amylase specific activity in turtles fed by diet 3 might be due to the high amount of available carbohydrate in the fish pellet diet (36%), while the other carbohydrate contents of feed constituents were 24% in fresh feed containing minced fresh fish and vegetable, and 29% in fresh feed containing minced fish fillet, vegetable, and fish pellet diet. Moreover, pellet diet contains a high amount of gelatinized starch, which is utilized better than native starch in fresh raw materials. Thongprajukaew et al[3] reported a positive relationship between A/T ratio and SGR in a carnivorous fish. This was probably due to dietary carbohydrate, which appears necessary for improving growth as well as protein utilization[32]. Thus optimizing carbohydrate content for maximal feed utilization efficiency in green turtle might have significant growth effects.

Faecal physicochemical characteristics in relation to feed utilization

Visual judgement of faecal characteristics have been used for nutritional evaluation in many organisms[33–35]. The microstructure of faeces responded to dietary treatment. Roughness increases surface-to-volume ratio, which favours high enzyme loading[36] when hydrolysing the digested foods. This finding is in agreement with roughness improving the in vitro digestion of raw materials[10]. Thus the presence of roughness in faeces might act as evidence of enzymatic hydrolysis along the alimentary tract of animals. Varo and Amat[35] reported

increased size of feacal particles when waterbird feed had high fibre content, suggesting that high fibre reduced the digestion of food. The flat and long shapes of faeces from turtles fed 50% vegetable (5% crude fibre), in dietary Group 1, might be due to elements from plant cell walls that were not digestible. This finding appears potentially useful for evaluating feed utilization efficiency in indoor experiments, with diets that mainly contain carbohydrate inclusions. Further studies such as faecal fibre characteristics in wild turtles could be of interest, because their natural diets contain large amounts of indigestible elements.

Differences in thermal transition properties were due to the raw material constituents in feed affecting the faeces. Despite the various raw materials, the thermal transition peaks in this range of observed temperatures appear to come from gelatinization temperatures of carbohydrates. Bao and Corke[37] suggested that $T_c - T_o$ might increase with heterogeneity of crystallites in rice starch. Thus the broadly distributed peaks for turtles fed by diets 1 and 3 might indicate heterogeneity in the cleaved carbohydrate chain lengths after digestion. Cooke and Gidley[19] suggested that changes in ΔH primarily reflect loss of double helical order in starch, so the constituents would require more energy for transformation. Hence, the highest ΔH of turtle faeces for Group 3 might be due to the abundance of available carbohydrate in diet, affecting thermal response of faeces.

The changes in diffraction patterns reflect the crystallinity of faeces. The presence of starch peaks with the highest relative crystallinity in turtles fed by diet 3 indicates a large amount of available carbohydrates in the faeces. This result matches the observed highest ΔH from DSC. Kaur et al[12] reported a positive correlation between the relative crystallinity and in vitro digestibility of resistant starch in lentil, while a negative relationship was found for rapidly and slowly digestible starches. The turtles fed by diet 3 can probably utilize available carbohydrates with amorphous structure best, while the more crystalline structures are less digestible and emerge in the faeces. This correlates with the higher carbohydrate utilization in pellet diet, as the starch in pellets is partly gelatinized during extrusion.

Our findings from the current study indicate that there is a significant relationship between the feacal characteristics and feed utilization (FI and FCR) under satiation condition. The most desirable characteristics of the faeces, in relation to enzymatic hydrolysis of the available carbohydrates, of turtles fed by diet 3 can reduce FI and FCR due to the high capacity to release the available nutrients. Prolonging the feeding experiments might discriminate between the responses in growth performances contributed by both parameters.

CONCLUSIONS

Digestive enzyme analysis and physicochemical characteristics of faeces appear to have potential as indicators of feed utilization efficiency in green turtles, as indicated by FI and FCR, when no prior information of nutritional background is available. The sampling of faeces is unobtrusive and non-invasive, without ethical concerns, and therefore is an attractive option especially with endangered species. Various diets that simulate the natural diet (e.g., without gelatinized starch, but with a high fibre content) need to be assessed for further applications to wild turtle populations. These current techniques are directly applicable for studying feed utilization in captive animal experiments to improve the quality of husbandry for the head-started turtles, as well as in public displays in zoos or aquaria, when the commercial pellet diets are supplied. Collection of the faeces from landforms along the coast of an ocean or sea, as well as the collection by anal suction may be an alternative method for studying wild green turtles.

Acknowledgements: This work was financially supported in part by the PMBC, Phuket, the Office Strategies Management for Southern Province Cluster and Laguna Resorts & Hotels PCL. The authors would like to thank Dr Pinsak Suraswadi for kindly consulting during experimentation; Ms Sasiporn Jatupornpitukchatand and Ms Phattira Chumsawad, for help in turtle rearing; and Third Naval Area Command, Royal Thai Navy, for kindly providing the green turtles. Finally, we also gratefully acknowledge the Publication Clinic, research and Development Office, Prince of Songkla University and Dr Brian Hodgson of the Faculty of Pharmaceutical Sciences for assistance with English proofreading.

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