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Performance of Head-Started Green Turtle, *Chelonia mydas* (Linnaeus 1758) fed a commercial diet

HIRUN KANGHAE[1], SANTI NINWAT[2], KARUN THONGPRAJUKAEW[3,4*], ATICHAT INTONGCOME[5] and KONGKIAT KITTIWATTANAWONG[1]

[1]Phuket Marine Biological Center, Phuket 83000, Thailand
[2]Marine and Coastal Resources Research Center, Lower Gulf of Thailand, Songkhla 90100, Thailand
[3]Department of Applied Science, Faculty of Science, Prince of Songkla University, Songkhla 90112, Thailand
[4]Biochemical Research Unit for Feed Utilization Assessment, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand
[5]Marine and Coastal Resources Research Center, The Central Gulf of Thailand, Chumphon 86000, Thailand

Abstract

The selection of an appropriate diet for the head-started juvenile green turtles *Chelonia mydas* (Linnaeus 1758) conservation needs to be assessed. Growth performance, feed utilisation, behaviour, and faecal characteristics of 45-day-old green turtles (91.88±0.08 g initial weight) were compared among dietary carnivorous fish diet (CFD), omnivorous fish diet (OFD), carnivorous shrimp diet (CSD), and a conventional fresh diet (FD) of fresh fish and vegetable as control. The completely randomised design had five replicates (4 treatments × 5 replicates × 5 subjects per replication) and the feeding trial was conducted for 6 weeks. No mortality was observed in any of the treatment groups. Green turtles fed with CFD exhibited superior growth (weight gain 282.74±8.96 g and specific growth rate 2.64±0.16 %·day^{-1}) and feed utilisation (feeding rate 2.41±0.06 % body weight·day^{-1} and feed conversion ratio 1.34±0.04 g feed·g gain^{-1}). In terms of behaviour, reduced aggression as indicated by low incidence of bitten hind limbs was noted along with good appetite in this group fed CFD. These findings suggest that CFD is a suitable choice among the commercially available diets for head-started juvenile green turtles conservation.

Introduction

Green turtle, *Chelonia mydas* (Linnaeus 1758) is an endangered marine species listed by the International Union for Conservation of Nature (IUCN) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). In Thailand, head-started juvenile turtles propagation (within one year after hatching) prior to their release into the natural habitats is routine for the Department of Marine and Coastal Resources, Ministry of Natural Resources and Environment. During husbandry, the diet is an important factor contributing to the survival and

*Corresponding author. E-mail address: karun.t@psu.ac.th

growth of the juvenile turtles. Small aquatic animals, fresh whole fish, seagrass, and mangrove leaves and fruits have been reported in the natural diet of turtles (Bjorndal 1980, 1997; Amorocho and Reina 2008; Kanghae et al. 2014).

A commercially available carnivorous fish diet, Purina Trout Chow®5V-VO5 (Purina Mills, LLC, St. Louis, MO, USA) has been used for rearing juvenile green turtles in indoor experiments, as well as at Cayman Turtle Farm Ltd., Cayman Islands, BWI, UK (Jones et al. 2009; Price et al. 2013). An artificial diet containing 41% protein resulted in inferior survival and growth in the same species, compared to feeding fish fillet containing approximately 82% protein of dry matter (Ruangkaew and Buakaew 2005). On the other hand, artificial diet containing 45% protein of dry matter can promote growth, feed conversion ratio, and capacity for digestion of protein and carbohydrate (quantified by measuring faecal digestive enzymes) better than the conventional diet consisting of 54% protein of dry matter combining whole fish and vegetable (Kanghae et al. 2014). Clearly, the available information is conflicting, probably due to varying husbandry techniques and ages of the reared turtles, as well as the types of commercial diets that prevent replication of the results. Thus, the nutritional information on this species is limited for indoor rearing systems and does not provide an alternate choice of diet. The aim of this study was to select a suitable commercial diet for rapid growth and successful propagation of juvenile green turtles. Three commercial diets used for marine animals were compared with a traditional fresh diet (FD). Carnivorous fish (CFD) and omnivorous fish (OFD) diets, with their main difference in protein and carbohydrate contents, were chosen to evaluate the survival, growth performance, feed utilisation, and feeding habits of juvenile turtles in an indoor environment. The carnivorous sinking shrimp diet (CSD) is an alternative that might suit a bottom feeder, and contains similar amounts of protein as used for rearing green turtles (see Ruangkaew and Buakaew 2005; Jones et al. 2009; Price et al. 2013; Kanghae et al. 2014). The findings from this study might support further development of a commercial diet for culturing juvenile green turtles.

Materials and Methods

Egg collection and acclimatisation of juvenile turtles

Turtle eggs were collected from one mother (81 cm curved carapace width and 93 cm curved carapace length) at Huyong Island, Phang-Nga, Thailand. The eggs were incubated for 50 days at Third Naval Area Command, Royal Thai Navy, and after hatching 5-day-old green turtles (2.00-2.20 g) were transported to Phuket Marine Biological Center (PMBC). The juvenile turtles were acclimatised indoors for 40 days in a round fiberglass tank (2 m diameter × 1.5 m height) containing 500 L seawater. Water was renewed 100% daily and water temperature during acclimatisation ranged from 27 to 30 ^{o}C. The turtles were fed with a commercial diet containing 40% crude protein (Higrade 9773, Charoen Pokphand Foods PCL., Thailand). Bacterial and external parasitic infection were diagnosed in all turtles and treated by dipping the turtles in 60 ppm polyvinylpyrrolidone-iodine for 20 min prior to use in the feeding experiment.

Feeding experiment of the juvenile turtles

The 45-day-old turtles (91.88±0.08 g initial weight) were randomly distributed into four dietary treatments with five replicates and five turtles per tank. The turtles were reared for 6 weeks in round fiberglass tanks (150 cm diameter × 75 cm height) containing 177 L sea water each. The dietary treatments were with CFD, OFD and CSD (Charoen Pokphand Foods PCL., Thailand). These diets were prepared by soaking 100 g of the pellet in 30 mL of water before use. For the control treatment with FD, the frozen fish (redtail scad, *Decapterus kurroides* Bleeker 1855) were defrosted, cut to small size (2 × 2 cm) pieces and fed to the turtle. This was followed by feeding an equal amount (in wet weight) of Chinese cabbage, *Brassica rapa* L. spp. *pekinensis* (Lour.). Both raw materials were fed alternately to reduce the selective feeding behaviour until the end of the meal. All turtles were fed twice daily *ad libitum*, at 10.00 and 17.00 h. The diurnal cycle was set at 12-h light/12-h dark. Water was changed daily before the first feeding. The water quality parameters during the study was as follow: pH 7.30-8.30, temperature 27-30 ^{o}C, salinity 27-33 ppt, total alkalinity 112-118 ppm $CaCO_3$, and dissolved oxygen 5.67-6.33 $mg{\cdot}L^{-1}$. Prophylactic treatment for diseases was provided by dipping the turtles in 60 ppm polyvinylpyrrolidone-iodine for 20 min and then cleaning them with a soft sponge, every other week. Mortality and morbidity were monitored daily over the duration of the experiment. Turtles in the same tank were individually distinguished based upon a combination of characteristics that include size priority, scale (on snout), and scute colours (on carapace). Weight gain, curved carapace width, and curved carapace length of each turtle were recorded weekly. The individual feed consumption was measured during every meal based on the actual mass (on a dry matter basis). The feeding rate (FR) and feed conversion ratio (FCR) were monitored closely by temporarily enclosing each turtle with plastic net during feeding.

Observation of feeding performance and aggressive behaviour

Feeding performance was observed throughout the duration of the experiment. Levels of appetite were evaluated for comparisons between the dietary groups, by determining the average time taken by the turtles to start feeding the given feed for the respective treatments, and these were ranked as highest, high, moderate and lowest. At the end of the experiment, the aggressive behaviour of juvenile turtles was evaluated by counting the numbers of bitten hind limbs, and converted to percentages.

Determination of chemical compositions of diets

The experimental diets were dried at 105^{o}C for 24 h before analysing their chemical compositions. Crude protein, lipid, ash and fibre, were determined according to standard methods of AOAC (2000). The nitrogen free extract (NFE) was calculated from the results. All the chemical analyses were done in triplicates, and the values are reported on dry matter basis.

Faecal microstructure

Pooled fresh faeces from the five samples per treatment were collected at the end of the experiment. The faecal samples were dried in a freeze dryer (Delta 2-24 LSC, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 24 h and then kept in a desiccator until use. The faecal microstructure characteristics were studied from scanning electron micrographs (Quanta 400, FEI, Brno, Czech Republic). The dried samples were mounted using double-sided adhesive tape on an aluminum stub and coated with gold. The images were taken at 50×, 2000× and 10000× magnifications using 15 kV accelerating voltage.

Statistical analysis and calculations

A completely randomised design (4 treatments × 5 replicates × 5 subjects per replication) was used in this study. Data are expressed as tank mean±SEM from five replicate observations. Significant differences between means at a 95% significance level were determined using Duncan's multiple range test, with the software SPSS Version 14 (SPSS Inc., Chicago, USA). Growth and feed utilisation parameters were calculated as follows:

Specific growth rate (SGR, $\%\cdot day^{-1}$) = 100 $[(lnW_t-lnW_0)/(t-t_0)]$

Where W_t = mean weight (g) at day t, W_0 = mean weight (g) at day t_0.

Weight gain (WG, g) = Final body weight (g) - initial body weight (g)

Condition factor (CF, $kg\cdot cm^{-3}$) = [Body weight (kg)/curved carapace length $(cm)^3$] × 10^4

Feeding rate (FR, % body $weight\cdot day^{-1}$) = 100 [Daily dry feed consumed (g)/body weight (g)]

Feed conversion ratio (FCR, $g\ feed\cdot g\ gain^{-1}$) = Dry feed consumed (g)/wet weight gain (g)

Results

Proximate chemical compositions of the experimental diets

The proximate chemical compositions of the four experimental diets are shown in Table 1. The three artificial diets differed significantly from the traditional diet in each type of constituent tabulated. A comparison of the artificial diets shows that the two carnivorous diets (CFD and CSD) were similar in their chemical compositions with a large amount of protein (42-49% of dry matter) and lipid (6.8-7.0% of dry matter). Their contents of crude fibre (1.77-1.78% of dry matter) and available carbohydrates (30-36% of dry matter) were comparatively low relative to the omnivorous OFD. These differences are expected and consistent with the distinction between carnivorous and omnivorous diets.

Table 1. Proximate chemical compositions (% of dry weight) of the experimental diets used for rearing juvenile green turtles. Data from triplicate analyses are expressed as mean ± SEM.

Chemical composition	Diet type			
	FD	CFD	OFD	CSD
Crude protein	54.13 ± 0.46^{a}	48.75 ± 0.05^{b}	18.52 ± 0.09^{d}	42.45 ± 0.06^{c}
Crude lipid	6.20 ± 0.38^{ab}	6.98 ± 0.22^{a}	3.83 ± 0.07^{b}	6.75 ± 0.11^{a}
Crude fibre	5.30 ± 0.11^{b}	1.77 ± 0.12^{c}	12.31 ± 0.44^{a}	1.78 ± 0.15^{c}
Crude ash	12.44 ± 0.01^{c}	12.14 ± 0.02^{d}	12.75 ± 0.01^{b}	13.25 ± 0.01^{a}
Nitrogen free extract	21.93 ± 0.61^{d}	30.36 ± 0.26^{c}	52.59 ± 0.45^{a}	35.77 ± 0.20^{b}

FD, fresh diet; CFD, carnivorous fish diet; OFD, omnivorous fish diet; CSD, carnivorous shrimp diet.
Different superscripts within the same row indicate significant differences ($P < 0.05$).

Growth performance of green turtles

Green turtles in all dietary treatments had 100% survival throughout the experiment. Weight gain was highest at the end of the experiment in turtles fed with CFD, followed by FD and CSD, respectively (Fig. 1a). Curved carapace width of CFD group at the end of the experiment did not differ significantly from FD (Fig. 1b, $P > 0.05$) whereas their lengths did not differ from both FD and CSD groups (Fig. 1c, $P > 0.05$). The lowest growth performance was observed in turtles fed with OFD. The specific growth rate (SGR) was similar for turtles fed FD and CFD during the first week (Fig. 2a, $P > 0.05$). Thereafter, the group differences in SGR became significant, and the turtles fed with CFD had faster growth rates. The final SGRs for the turtles fed with FD and CSD were similar ($P > 0.05$). The lowest growth performance was observed in turtles fed with OFD. For condition factor (CF), the values did not differ significantly among the dietary groups during the experiment ($P > 0.05$, data not shown).

Feed utilisation of green turtles

The FR of individual green turtles, within any of the dietary groups, decreased with time (Fig. 2b). The FD group had a significantly higher FR than the other groups. The FCR of turtles fed with CFD or CSD were significantly lower than with FD or OFD group (Fig. 2c). Both these measures suggest significantly better feed utilisation capacity with CFD and CSD than with FD or OFD.

Feeding and aggressive behaviour of green turtles

Swimming, feeding (Table 2) and aggressive behaviour (Fig. 3) of green turtles were also observed in this study. All turtles spent a lot of time swimming close to the water surface. Bottom feeding behaviour was observed in turtles fed with FD and CSD, whereas surface feeding was found with CFD and OFD. The highest appetite was observed in turtles fed with FD, followed by high and moderate levels with CFD and CSD, respectively. A longer time taken to start feeding was recorded in turtles fed with OFD. As for aggression, the turtles fed with FD had significantly more bite marks than with any of the commercial diets. Bitten hind limbs were found in 25% of the OFD group, and in 10% of CFD and CSD groups.

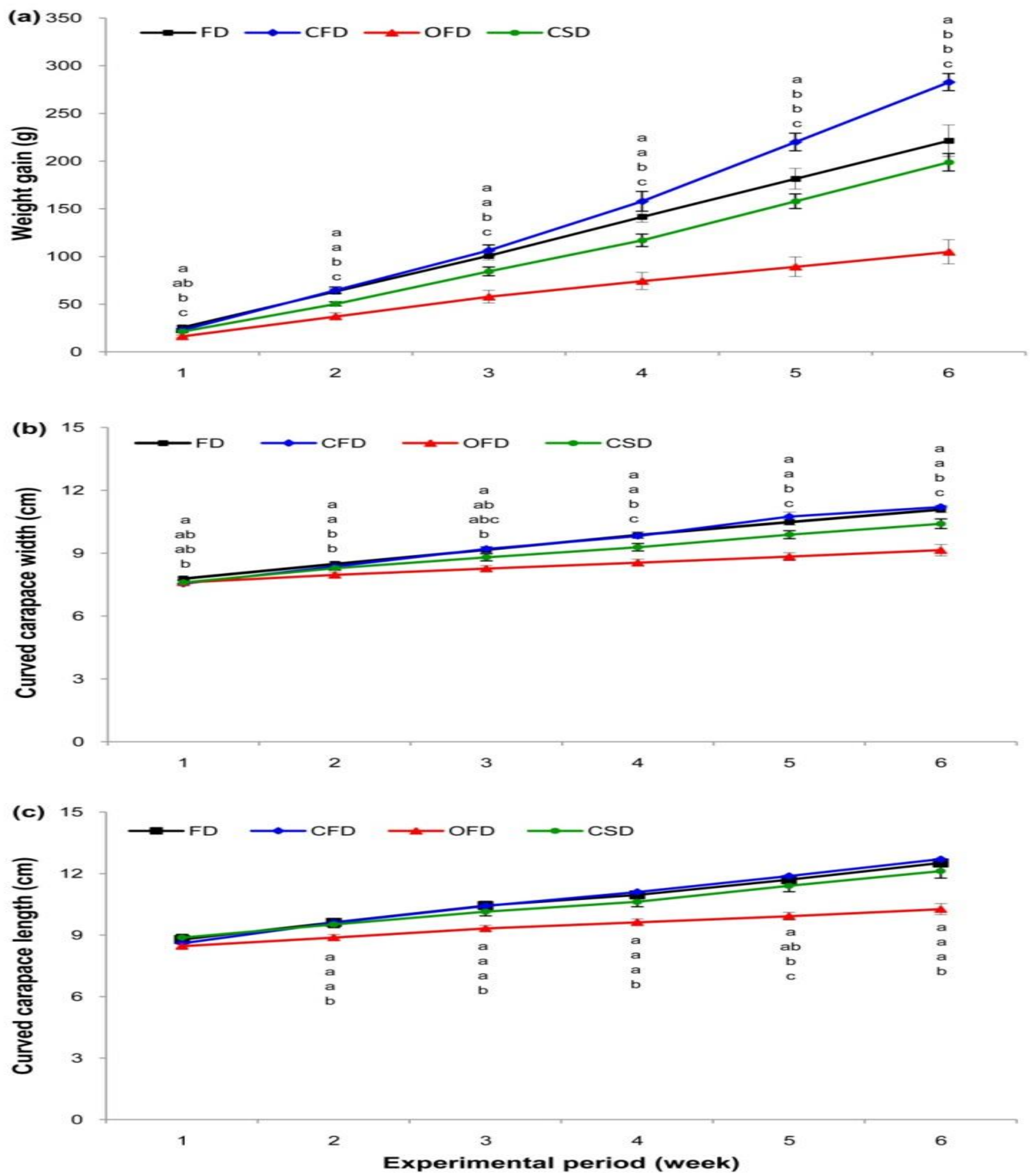

Fig.1. Weight gain (a), curved carapace width (b), and curved carapace length (c) of juvenile green turtles subjected to four dietary treatments. The duration of treatments was 6 weeks. Data are expressed as tank mean ± SEM from five replicate observations. Different superscripts within a week indicate significant differences between the groups (*P* < 0.05).

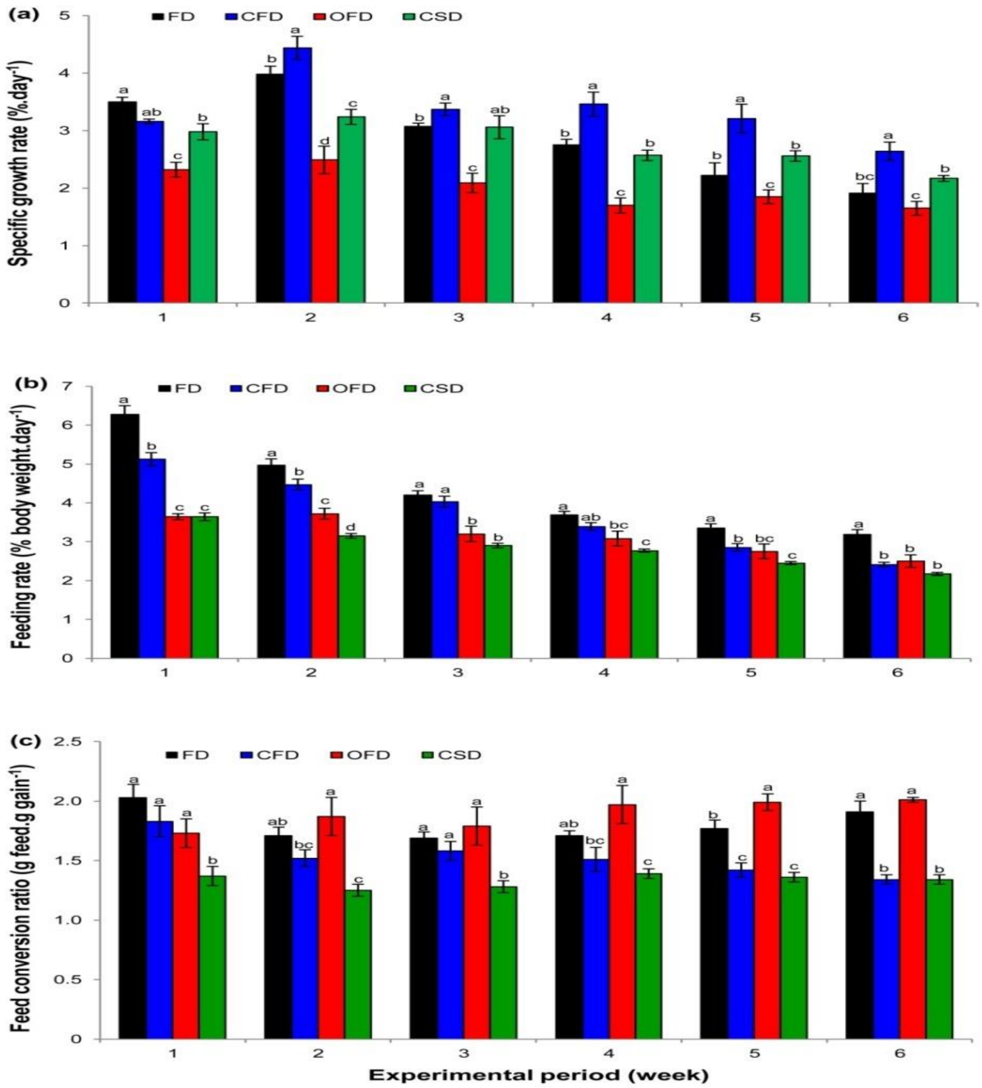

Fig. 2. Specific growth rate (a), feeding rate (b), and feed conversion ratio (c) of juvenile green turtles subjected to the four dietary treatments. The duration of treatments was 6 weeks. Data are expressed as tank mean ±SEM from five replicate observations. Different superscripts within a week indicate significant differences between the groups ($P < 0.05$).

Table 2. Behavioural observations of juvenile green turtles fed with different diets.

Diet type	Behavioural parameter		
	Swimming	Feeding	Appetite level
FD	Surface	Bottom	Highest
CFD	Surface	Surface	High
OFD	Surface	Surface	Lowest
CSD	Surface	Bottom	Moderate

FD, fresh diet; CFD, carnivorous fish diet; OFD, omnivorous fish diet; CSD, carnivorous shrimp diet.

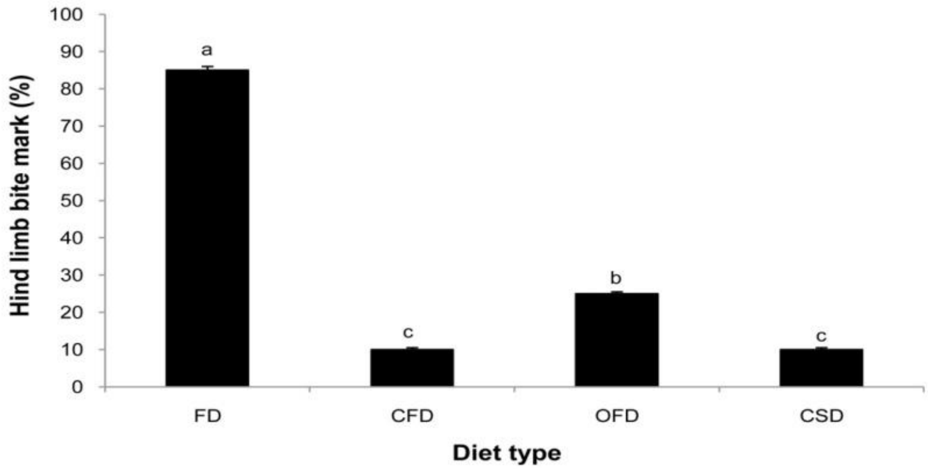

Fig. 3. Hind limb bite marks (% of turtle population) in green turtles fed with various diets. Data are expressed as tank mean ± SEM from five replicate observations. Significant differences are indicated by different superscripts ($P < 0.05$).

Faecal characteristics of turtles fed with FD and CFD

Micrographs of the green turtles' faeces when fed with FD and CFD are shown in Fig. 4. The faeces from CFD group had coarse surfaces with powdery components (Figs. 4d-4f). On the other hand, faeces of turtles from the FD group had constitutions that were more platelet-like and less powdery. (Figs. 4a-4c).

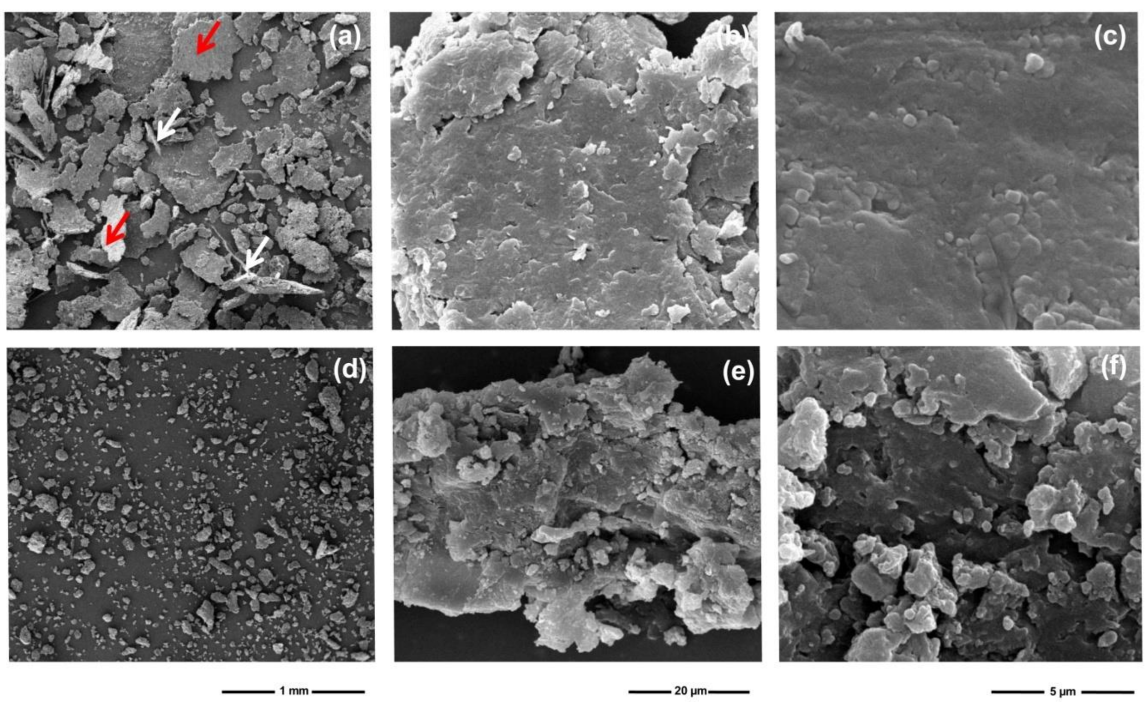

Fig. 4. The faecal microstructures of juvenile green turtles fed with FD (a-c) or with CFD (d-f). The SEM images have magnifications 50× (left), 2000× (middle) and 10,000× (right). White and red arrows designate indigestible elements and platelet like structure in the faeces.

Discussion

Most mortality of sea turtles occurs at the hatchling stage, and mortality levels off by month 6 (Higgins 2003). A most interesting observation during this experiment was the absence of mortality over 6 weeks, and all the turtles were healthy at the end of the experiment. Prior findings have also reported 100% survival of juvenile green turtles (25.38±1.29 g initial body weight) when fed with the CFD over 6 weeks, and then reduced to 97% at 6 months (Kanghae et al. 2014). On the other hand, green turtles (26.47±2.28 g initial body weight) fed with soft-shelled turtle or herbivorous fish diet survived approximately 60-65% over the same time (Ruangkaew and Buakaew 2005). These differences might be due to the new technique used for preparation of the artificial diets prior to feeding. Sea turtles overfed a commercially prepared pellet diet can develop floating-bloating problems (Fontaine et al. 1985). Pre-soaking such diets in water might have reduced the abdominal bloating of turtles, which is a presumptive factor that lowers survival of juveniles (unpublished data). Overall, the experimental data indicate superior growth and feed utilisation in juvenile turtles fed with CFD, whereas the weight-length relationship as indicated by CF was unaffected by treatment. This is in agreement with prior work where turtles were fed an artificial diet for carnivorous fish (Kanghae et al. 2014). Based on the chemical compositions, the artificial diet contained a mixture of plant and animal raw materials. Such no-choice diet may cause an acclimatisation of the microbial community along the alimentary tract for digestion of food (Kanghae et al. 2014), as well as promoting the microbial fermentation in the distal part of intestine (Amirkolaie et al. 2006). Moreover, hydrothermal processing (such as boiling and extrusion) during feed production can improve the nutritional quality of a diet (Thongprajukaew et al. 2011). Hadjichristophorou and Grove (1983) reported an improved SGR when one-year-old turtles were fed with a commercial diet containing 40-50% protein, when compared with Aberdeen flatfish diet (containing 71% of white fish meal). Similarly, the Purina Trout Chow®5V-VO5 (Purina Mills, LLC, St. Louis, MO, USA) containing 41% protein has been used to study the metabolic rate in green turtles (Jones et al. 2009; Price et al. 2013). For well-known turtle species, an optimal protein range of 42.2-46.5% is used to feed juvenile soft-shelled turtles (Bau et al. 1992; Nuangsaeng and Boonyaratapalin 2001; Chen and Huang 2011; Zhou et al. 2013). The approximately 45% protein content (on wet basis) of the CFD used in the current study matches the above range considered optimal for juveniles. Our results indicate that juvenile green turtles can utilise a no-choice diet with a high amount of protein (CFD and CSD) better than a low protein diet (OFD). However, an appropriate artificial diet for marine species (CFD or CSD) and a diet for brackish water species, like soft-shelled turtles, could be compared in further studies.

The differences in floating tendencies of the feeds affected feeding behaviour of the turtles, causing surface feeding with CFD and OFD. Bjorndal (1997) explains that the hatchlings and pelagic turtles typically consume what is available at the surface, whereas older or larger turtles consume food throughout the water column with emphasis on benthos. The floating tendency of these feeds provides an opportunity for food intake at the water surface, preferred by juveniles. On the other hand, diets settling to the benthic area of a water column may reduce the feeding opportunity and survival of unhealthy turtles. However, no effects of diet on survival were observed in this study despite the differences in floating tendency, which might be due to the shallow water level (10 cm). The shortest time taken to start consuming the feed, an indication of best appetite, was seen in turtles fed with FD, and the second best in those fed with CFD. Both these diets contain a high amount of feeding attractants and are rich in the main proteins from animal raw materials, especially from the fish meal in CFD. This leads to significantly increased feed intake in both dietary groups (8.45±0.39 and 7.83±0.50 $g \cdot day^{-1}$ for FD and CFD, respectively) relative to CSD (5.31±0.36 $g \cdot day^{-1}$) or OFD (4.96±0.25 $g \cdot day^{-1}$) during experiment. Similar finding on FR was also observed within the first 3 weeks of experimentation, when comparing the FD (5.15±0.61 % body $weight \cdot day^{-1}$ on average) and the CFD (4.54±0.32 % body $weight \cdot day^{-1}$ on average) groups to the others (3.38±0.15 % body $weight \cdot day^{-1}$ on average). This is in agreement with an earlier observed vigorous appetite when green turtles were fed floating trout pellets. (Hadjichristophorou and Grove 1983). The present findings showed that OFD had the lowest animal protein content due to a high fraction of plant raw materials.

The effects of diet on aggressive behaviour that can affect turtle health and quality were also observed in this study. Generally, the biting of hind limbs during propagation of turtles can induce infections and inflammation (Higgins 2003), affecting health. Moreover, the hind limbs are used to dig holes, and any injury to them can affect the depth of egg laying and their subsequent hatching as temperature influences sex differentiation (Fontaine et al. 1985). High aggressive behaviour was seen in the turtles that were fed with FD, with dramatically less damage in the other dietary treatment groups. It is possible that FD was able to induce carnivorous behaviour due to an increase proteolytic enzyme activity as suggested by Kanghae et al. (2014). Moreover, the high appetite of turtles in this group may have encouraged a competitive behaviour. On the other hand, hunger from low feed consumption might also have caused competition in the turtles that were fed with OFD. The biting tendencies of the turtles in this group might have been induced by the herbivorous diet (Marshall et al. 2014).

The faecal characteristics, based on direct observation (Amorocho and Reina 2008) or SEM photography (Kanghae et al. 2014; present work), can help evaluate foraging success in juvenile green turtles. Platelet like and less powdery constitution of the turtle faeces when fed with FD indicate the presence of indigestible cell wall constituents (cellulose, hemicellulose and lignin) after digestion (Kanghae et al. 2014). This finding is in agreement with an increase in the faecal particle size in water birds fed with a high fibre diet (Varo and Amat 2008). Therefore, the coarse surface and the powdery appearance of faeces when turtles were fed with a low fibre diet (CFD) might indicate a higher capacity for digestion, due to increased surface-to-volume ratio of the feed, which favours digestive enzyme loading (Ji et al. 2008). Wood and Wood (1981) reported that 14-month-old turtles can on average digest 83% of dry matter and 85% of protein in a commercially prepared pelleted diet. The apparent cellulose digestibility in turtles fed with seagrass was in the range of 72-91% (Bjorndal 1980) and 82-88% when fed mixtures containing fish and fresh leaves (Amorocho and Reina 2008). Based on our experiment, juvenile green turtles appear to better utilise diets with a low amount of fibre (CFD and CSD) than those with a high fibre content (OFD). However, the digestibility may change by acclimatisation to a diet due to the significant increase of major carbohydrate-digesting enzymes (amylase and cellulase) in turtles fed with artificial diet containing higher amount of carbohydrates, when compared with the lower amount from fresh diets (Kanghae et al. 2014).

Feed cost is an important factor to consider when rearing animals. The 64 baht·kg^{-1} of CFD and CSD in the current study was dramatically lower than the 158 baht·kg^{-1} for FD (dry matter basis). At 35 baht·kg^{-1}the OFD was very cheap but provided poor growth. Feeding with an artificial diet is easy to manage and may have the benefit of commercial quality control of the feed. On the other hand, the fresh diet (FD) creates water quality problems, and may induce intestinal inflammation in small turtles (Kanghae et al. 2014). Overall our findings suggest that CFD is appropriate for head-started juvenile green turtles propagation, and further experiments are underway on some effects of the water content prior to feeding with this artificial diet.

Conclusion

The feeding of juvenile green turtles with a CFD showed superior growth performance and feed utilisation relative to a conventional FD and two other commercial feed diets. This CFD diet reduced aggressive behaviour of the turtles and induced a good appetite. These findings suggest that a CFD diet is well suited for the head-started juvenile green turtles propagation. The conservation of this species may benefit from further work on its diet, specifically on improving the nutrient composition and the pre-processing of feed.

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