Comparative Biochemistry and Physiology, Part A 155 (2010) 264–270

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Non-additive dietary effects in juvenile slider turtles, *Trachemys scripta*

Sarah S. Bouchard [a,b,*], Amber K. Murphy [a], Jennifer A. Berry [a]

[a] Life and Earth Sciences Department, Otterbein College, Westerville, OH 43081, USA
[b] Department of Zoology, University of Florida, Gainesville, FL 32611, USA

ARTICLE INFO

Article history:
Received 29 July 2009
Received in revised form 13 November 2009
Accepted 14 November 2009
Available online 24 November 2009

Keywords:
Associative effects
Diet selection
Digestibility
Freshwater turtle
Mixed diets
Nutritional ecology
Ontogenetic diet shift
Reptile

ABSTRACT

Non-additive dietary effects occur when nutritional gains from a mixed diet are greater than or less than that predicted by summing the gains from individual diet items. Both positive and negative effects occur in adult slider turtles, *Trachemys scripta*. Such effects may also be important to juvenile *T. scripta* as they ontogenetically switch from carnivorous to herbivorous diets. The purpose of this study was to determine if juveniles experience non-additive effects and to assess the underlying mechanism. Two feeding trials were conducted. In Trial 1, juveniles were fed 100% duckweed, *Lemna valdiviana*, 100% grass shrimp, *Palaemontes paludosus*, or a mixed diet containing 81% duckweed and 19% shrimp. In Trial 2, juveniles were fed 100% duckweed, *Lemna minor*, 100% cricket, *Acheta domesticus*, or one of three mixed diets containing duckweed and cricket in varying percentages (22%, 39% and 66% cricket). Similar to adults, a negative non-additive effect was demonstrated on the 19% shrimp and 22% cricket diets. However, the positive effect found in adults was not observed. Intake varied dramatically between the plant and animal diets, resulting in differences in transit time that could explain the non-additive effect. These results offer some insight into understanding ontogenetic diet shifts in turtles.

1. Introduction

Non-additive dietary interactions occur when energy and nutrient gains from a mixed diet are greater than or less than that predicted by summing the gains from individual diet items. Both positive and negative interactions have been documented in a wide variety of species, including insects, mammals, birds, and reptiles (summarized in Bouchard and Bjorndal, 2006a). Depending on the animal and the diet items involved, different mechanisms have been proposed to explain such interactions. For example, in a beetle, *Tetraopes tetraophthalamus*, a negative non-additive effect between milkweed, *Asclepias syriaca*, foliage and flowers was attributed to secondary compounds in the foliage depressing digestion of the flowers (Matter et al., 1999). Alternatively, in the Galapagos cactus finch, *Geospiza fortis*, a positive effect between cactus, *Opuntia echios*, pollen and nectar was due to nectar in the crop stimulating germination of the pollen, making it more digestible (Grant, 1996).

Positive and negative non-additive effects may also occur through effects of diet items on microbial gut symbiont populations. These symbionts ferment structural carbohydrates of the diet, producing short-chain fatty acids as a by-product that the host absorbs as an energy source. Bjorndal (1991) found a positive non-additive effect in adult yellow-bellied slider turtles, *Trachemys scripta*, fed a diet composed of 77% duckweed, *Spirodela punctata*, and 23% mealworm larvae, *Tenebrio* sp. (dry matter basis). Like other herbivorous reptiles, this turtle maintains microbial symbionts in its large intestine (Bouchard and Bjorndal, 2005), and she hypothesized that the effect was due to increased nitrogen from the larvae stimulating growth of the microbial population which could then ferment the plant cell walls more efficiently. A similar positive effect was found in a rodent, *Abrothrix longipilis*, fed a mixed diet of unspecified insect larvae and fungi, *Boletus edulis* (Bozinovic and Muñoz-Pedreros, 1995).

Other studies investigating non-additive effects in *T. scripta* reveal that the effects may be complex. Turtles fed a diet composed of duckweed, *Lemna valdiviana*, and freshwater grass shrimp, *Palaemontes paludosus*, experienced either a positive or a negative effect depending on the ratio in which the diet items were fed (positive effect: 67% duckweed, 33% shrimp; negative effect: 14% duckweed, 86% shrimp, dry matter basis) (Bouchard and Bjorndal, 2006a). Differences in transit time between duckweed and shrimp were hypothesized to explain the opposing effects produced with the same diet items at different ratios. The addition of animal matter to a plant diet could decrease digestibility by accelerating transit time and decreasing time digesta is exposed to gut symbionts. Conversely, the addition of plant matter to an animal diet could increase digestibility by slowing transit time and exposing digesta to endogenous enzymes for more time (Bouchard and Bjorndal, 2006a). Differences in transit time between diet items have been proposed to explain positive effects in elk, *Cervus elaphus*, and mountain sheep, *Ovis canadensis*,

* Corresponding author. Life and Earth Sciences Department, Otterbein College, Westerville, OH 43081, USA. Tel.: +1 614 823 1119; fax: +1 614 823 3024.
E-mail address: SBouchard@otterbein.edu (S.S. Bouchard).

1095-6433/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2009.11.013

fed a combination of browse stems, *Vaccinium* sp., and grass hay, *Bromus inermis* (Baker and Hobbs, 1987) and negative effects in Hingeback tortoises, *Kinixys spekii*, fed kale, *Brassica oleracea*, and millipedes, *Alloporus* sp. (Hailey et al., 1998).

Non-additive effects may be particularly important to the nutrition of juvenile *T. scripta*. Like many freshwater turtles, they experience an ontogenetic diet shift. As juveniles, they are carnivores that consume aquatic invertebrates, including gastropods, decapods, and isopods, as well as a variety of aquatic insects, such as coleopterans, hemipterans, dipterans, and odonates. As the turtles develop, they become opportunistic omnivores that primarily consume plants, including *Naja* sp., *Potamogeton* sp., and a variety of duckweeds, such as *Lemna* sp., *Spirodela* sp., and *Wolffia* sp. (Parmenter and Avery, 1990). Depending on the population, the diet shift can occur gradually (Hart, 1983) or suddenly (Clark and Gibbons, 1969). For those populations where the shift occurs gradually, juvenile turtles consume a broad range of plant to animal ratios as they mature, and non-additive effects could play a significant role during this important life stage.

The purpose of this study was to quantify non-additive dietary effects in juvenile *T. scripta*. Like adult *T. scripta*, juveniles use microbial gut symbionts in their large intestines to ferment plant cell wall components (Bouchard and Bjorndal, 2005). They are able to digest plants to the same extent as do adults (65% dry matter digestibility), although intake of plant material is limited compared to adults. Additionally, juveniles are extremely efficient at digesting animal material and do so to a greater extent than do adults (97 vs. 89% dry matter digestibility; Bouchard and Bjorndal, 2006b). Based on the similarities and differences in digestive processing between these age groups, it is difficult to predict if juveniles will experience the same non-additive effects as adults. We therefore performed two independent feeding trials to assess non-additive effects in juveniles. In Trial 1, we fed juvenile turtles either 100% duckweed, *L. valdiviana*, 100% grass shrimp, *P. paludosus*, or a mixed diet containing 81% *L. valdiviana* and 19% *P. paludosus*, a combination known to produce a negative effect in adult *T. scripta* (Bouchard and Bjorndal, 2006a). We measured intake, digestibility, and turtle growth rate on each diet. In Trial 2, we selected two food items not previously tested for non-additive effects. We fed juveniles one of five diets containing 100% duckweed, *Lemna minor*; 100% cricket, *Acheta domesticus*; and three mixed diets containing 34% duckweed and 66% cricket, 61% duckweed and 39% cricket, and 78% duckweed and 22% cricket (dry mass basis). During this trial, we measured turtle growth rate and diet transit time. Together, these two trials allowed us to determine (1) if juveniles experience the same non-additive effect as adult turtles fed duckweed and shrimp, (2) if juveniles experience positive and negative effects when fed the same diet items at different ratios and (3) if these effects may be related to differences in diet transit time as has been suggested (Bouchard and Bjorndal, 2006a).

2. Materials and methods

2.1. Feeding Trial 1

The first feeding trial took place at the University of Florida in Gainesville, Fl. from 28 August to 2 October 2000. *Trachemys scripta* hatchling turtles were obtained from a commercial turtle farm in Port Mayaka, Florida, in mid June. Before the trial began, turtles were fed feces from locally caught wild turtles to inoculate their guts with microbial symbionts (Troyer, 1984). Turtles were randomly assigned to either 100% duckweed diet, *L. valdiviana* ($n=7$), 100% grass shrimp, *P. paludosus* ($n=7$), or a mixed diet by dry mass of 81% duckweed, 19% shrimp ($n=8$). Mean turtle mass at the beginning of the trial was 11.24 g (range: 8.94–14.05 g). Duckweed was collected from a local pond in Gainesville, Florida, and grass shrimp was purchased from a bait shop that obtained the shrimp from Gainesville area lakes. Because some turtles did not eat the anterior portion of the shrimp containing the eyes and antennae or the posterior portion containing the caudal fin, these parts were removed before shrimp were fed to turtles. This ensured all animals consumed the same diet. The nutrient composition of all diets is described in Table 1.

Turtles were housed individually in square Rubbermaid™ containers (18×18 cm) placed within larger Nalgene tanks (45×60 cm). Each Nalgene tank was equipped with a 75 W floodlight and a 20-W full spectrum natural light fluorescent bulb, and during the trials, turtles experienced a 12-hour photoperiod and water temperatures between 25 and 26 °C. Rubbermaid™ containers were rotated within and between Nalgene tanks to ensure all turtles experienced the same conditions throughout the trial. To determine digestibility, all feces produced during the experimental periods were collected and quantified. Turtles were therefore fitted with fecal collection devices as described in Bouchard (2004). These devices consisted of a small Nalgene tubing connector that was attached to the posterior end of the turtle by a piece of wire wrapped around the connector and threaded through two holes placed in the posterior marginal scutes of the turtle. A water balloon was fastened to the end of the tubing connector not attached to the turtle. All turtles were acclimated to the experimental diets for two weeks before the three week fecal collection period began. Feces were collected daily and dried overnight at 60 °C. Urinary wastes were not subtracted from the fecal samples because freshwater turtles excrete ammonia and urea, which were lost either in the water or during drying. All fecal samples collected from each turtle were combined to produce a composite sample for each turtle.

Turtles were not provided a basking platform because we did not want the fecal collection devices to snag on anything and possibly tear. Therefore, during the trial, water was drained from tanks every morning at 0800 h so turtles could bask. At 1000 h, feces were collected, and tanks were refilled with water. At 1100 h, turtles were fed a known mass of either duckweed or shrimp, with turtles on the mixed diet receiving only the duckweed fraction of their diet. Turtles fed *ad libitum* for 6 h until 1700 h when orts (remaining food) were collected with a small dip net and weighed. Turtles on the mixed diets were then fed a quantity of shrimp that resulted in the appropriate ratio of duckweed to shrimp depending on the amount of duckweed consumed that day. This ensured they consumed a constant ratio of plant to animal matter despite daily fluctuations in duckweed intake. Turtles were weighed once a week to determine growth rate during the trial.

Data from turtles fed the 100% duckweed and shrimp diets have been previously published in Bouchard and Bjorndal (2006b). Those data are presented here for comparison to the mixed diet data to determine the presence or absence of a non-additive effect. Additionally, those data are compared to the results of Trial 2.

Table 1
Nutrient composition of diets. All values except energy are presented on a percent dry matter basis. NDF represents the cell wall component of the duckweed (cellulose, hemicellulose, lignin and cutin), and ADF represents the lingo-cellulose and cutin component. ADF represents the exoskeleton (primarily chitin) fraction of the shrimp and cricket diets.

	Organic matter (%)	Fiber		Nitrogen (%)	Energy (kJ g^{-1} DM)
		NDF	ADF		
Trial 1					
100% shrimp (*P. paludosus*)	88.0	–	6.4	12.6	21.75
19% shrimp, 81% duckweed	86.7	33.4	17.2	6.4	19.11
100% duckweed (*L. valdiviana*)	86.4	41.2	19.7	5.0	18.49
Trial 2					
100% cricket (*A. domesticus*)	95.8	–	12.1	11.0	23.58
66% cricket, 34% duckweed	93.1	13.4	12.4	8.1	21.03
39% cricket, 61% duckweed	91.1	24.2	12.7	5.7	19.01
22% cricket, 78% duckweed	89.8	31.0	12.9	4.3	17.74
100% duckweed (*L. minor*)	88.1	39.7	13.1	2.4	16.09