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In Vivo Apparent Digestibility of Fiber in Florida Manatees (*Trichechus manatus latirostris*) Under Human Care

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Abstract

The Florida manatee (*Trichechus manatus latirostris*) is a threatened herbivorous marine mammal with a long intestinal tract and prolonged intestinal transit time that should allow symbiotic microbes in their large intestine to thoroughly digest otherwise indigestible fiber in their diet. Nevertheless, the apparent digestibility (AD) of fiber has not been well documented in Florida manatees. This study measured the AD of dry matter (DM), crude protein, neutral detergent fiber (NDF), and acid detergent fiber (ADF) in three adult and two subadult manatees offered diets that are typically fed to rehabilitating manatees recovering from illness or injury. These diets were composed principally of romaine lettuce with or without added vegetables and fruit. Acid detergent lignin (ADL) was used as a marker; hemicellulose was calculated as NDF-ADF, and cellulose as ADF-ADL. Acid insoluble ash (AIA) proved unsuitable as a marker because concentrations were lower in feces than in food. The AD of all the nutrients measured was high (> 61%) and was very high for cellulose (> 86%). The AD of all nutrients except hemicellulose decreased as ADL in the food increased ($r \leq -0.74$, $p \leq 0.02$). Intake of DM increased both as ADL in food increased and AD of DM decreased ($r^2 \geq 0.77$, $p \leq 0.002$). Our results demonstrate that evaluations of nutrient and energy requirements of manatees during rehabilitation must take account of the high AD of fiber in typical diets. Furthermore, measuring ADL in the diet may help to more accurately estimate energy availability from the diet of manatees.

Key Words: Florida manatee, *Trichechus manatus latirostris*, apparent digestibility, fiber, protein, lignin

Introduction

The Florida manatee (*Trichechus manatus latirostris*; Order Sirenia) is a "threatened" marine mammal that is susceptible to illness and injury. Sirenians are the only herbivorous marine mammal and have a unique digestive strategy that appears to efficiently utilize otherwise indigestible plant fiber in their diet by combining hindgut fermentation (Burn, 1986; Reynolds & Rommel, 1996; Goto et al., 2008) with a long (average of 7 d) intestinal transit time (Larkin et al., 2007). Plant fiber includes carbohydrates, such as hemicellulose and cellulose, which are not digested by mammalian enzymes in the small intestine but can be metabolized, given time, by large intestinal microbes to volatile fatty acids and gases such as methane (Goto et al., 2004). Volatile fatty acids are mostly absorbed and metabolized as a source of energy, whereas gases are released as flatus or by exhalation. Fiber also contains a phenolic compound, lignin, which is neither metabolized nor absorbed.

To help maintain the free-ranging population, injured and sick manatees are rescued and cared for in participating oceanaria within the Manatee Rescue/Rehabilitation Partnership until they are deemed ready for release back into their natural habitat (http://public.wildtracks.org/about). Whereas the diet of free-ranging manatees is composed mostly of fresh and saltwater grasses and other submerged aquatic vegetation (Reep & Bonde, 2006), during rehabilitation, manatees under professional management are fed a diet composed mostly of romaine lettuce because it is available in sufficient quantities and appears to support recovery. Romaine lettuce contains much less ash, lignin, and structural carbohydrates, measured as neutral detergent fiber (NDF) and acid detergent fiber (ADF), on a dry matter (DM) basis than seagrasses commonly consumed by manatees (Siegal-Willott et al., 2010). Romaine lettuce also contains more ether-extracted crude fat (EE), crude protein (CP), and non-structural carbohydrates (NSC) than seagrasses on a DM basis (Siegal-Willott et al., 2010). Nevertheless, how much nutrient is available to a manatee depends on the digestibility of the nutrient as well as the amount of diet consumed. Similarly, how much a manatee needs to consume to maintain body condition depends on how much energy the manatee can obtain from its diet. Some of that energy can come from protein, digestible NSC, and fat, but much of the energy in an herbivore's diet comes from fiber. Thus, understanding how effectively manatees process fiber in their diet should help to improve manatee management and care.

Apparent digestibility (AD), the percent of each nutrient in a diet that is not present in the feces, represents a practical measure of nutrient availability. It can be measured by comparing the total fecal excretion of a nutrient with total dietary intake (the total collection method) or by comparing the concentration of a nutrient in food and feces relative to a marker that is neither absorbed nor metabolized during intestinal transit. Acid insoluble ash (AIA), acid detergent lignin (ADL), and manganese have been used as markers to measure AD in endangered and exotic species because they are normal components of the diet (Worthy & Worthy, 2014). AIA has been used successfully as a marker to measure AD in terrestrial hindgut fermenters such as horses (*Equus ferus caballus*) and elephants (genus *Loxodonta*), and has yielded similar estimates of AD compared to the total collection method (Miraglia et al., 1999; Clauss et al., 2003; Bergero et al., 2004; Pendlebury et al., 2005). ADL is the preferred method for forages (Jung et al., 1999), but manganese has been shown recently to give similar results to ADL in manatees (Worthy & Worthy, 2014).

Very few studies have measured the AD of nutrients consumed by sirenians, whether free-ranging and consuming their natural higher fiber diet or under managed care when consuming lower fiber, lettuce-based diets. The AD of DM in a single adult female Florida manatee was reported to be about 83% for water hyacinth and 91% for lettuce, both of which are lower fiber feeds, but details of the methods used and the AD of other nutrients were not reported (Lomolino & Ewel, 1984). Two studies have estimated the AD of nutrients by comparing the nutrient composition of the contents of the stomach and rectum of carcasses of free-ranging sirenians. The food consumed by these animals before they died is unknown, but the stomach analysis suggests that they were consuming higher fiber aquatic vegetation. Both studies found fiber AD to be quite high compared to other herbivores: the ADs of organic matter (OM), CP, EE, and cellulose obtained from Florida manatee carcasses were 50 to 89% (Burn, 1986); and the mean ADs of CP, NDF, and ADF in dugong (*Dugong dugon*) carcasses were 70 to 84% (Murray et al., 1977). The accuracy of using material from carcasses to measure AD has not been demonstrated, but using the total collection method, the AD of DM, OM, CP, and fiber was found to be very high (> 88%) in two healthy dugongs fed higher fiber eelgrass (*Zostera marina*), which is part of the natural diet consumed by free-ranging dugongs (Goto et al., 2008).

The AD of DM has also been reported recently in Florida manatees *in situ* and *ex situ* using manganese as a marker (Worthy & Worthy, 2014). The average AD of DM was high (84%) in *ex situ* manatees consuming a romaine lettuce/vegetable diet, slightly lower (78%) in carcasses of *in situ* manatees that had been consuming freshwater vegetation, and lower still (47%) in carcasses of *in situ* manatees that had been consuming marine vegetation. Nevertheless, measuring AD of DM does not account for differences in AD and energy available from the various nutrients that make up the DM. This is relevant to manatees because the ash and fiber content varies widely among the foods consumed by free-living manatees and manatees under managed care (Siegal-Willott et al., 2010). Ash provides no energy, and the availability of energy from fiber depends on the degree of fermentation that it undergoes within the intestinal tract. Estimates of energy availability in manatees depend, therefore, on the AD of various nutrients that make up their diet.

We are unaware of any studies of the AD of fiber in diets typically consumed by manatees under human care, so this study sought to measure the AD of two typical diets fed to Florida manatees while under professional management. The first diet consisted almost exclusively of romaine lettuce; the second diet also consisted primarily of romaine lettuce but included a variety of fruits, vegetables, and other foods. We also hoped to identify a useful internal marker for application in digestibility studies of manatees.

Seasonal differences in nutrient concentrations have been observed in romaine lettuce and seagrasses commonly consumed by manatees

(Dawes & Lawrence, 1980; Siegal-Willott et al., 2010). Seasonal variation in digestibility of eelgrass related to the lignin content of the diet has been reported in dugongs (Aketa et al., 2003) and terrestrial herbivores (Aagnes et al., 1995, 1996). We, therefore, compared AD between spring and winter because we hypothesized that seasonal differences in the nutrient composition of the diet might affect AD.

Methods

This work was performed under a permit obtained from the U.S. Fish and Wildlife Service (#MA038448-3) and with approval from the University of Florida Institutional Animal Care and Use Committee (#200902762). The AD of fiber was measured in healthy Florida manatees under human care at Mote Marine Laboratory in Sarasota, Florida (Facility A) and The Seas, Epcot®, Walt Disney World® Resort, Lake Buena Vista, Florida (Facility B). Life stage of the manatees was determined using length-age class parameters established by the U.S. Geological Survey's (USGS) Sirenia Project (Bonde et al., 2012) or by known amount of time under professional management. Manatees with a straight length of greater than 266 cm were considered to be adults, whereas those in the length range of 236 to 265 cm were considered to be growing subadults. Facilities maintained a water temperature of 25 to 26ºC and salinity of 30 to 34 ppt.

Representative samples of each of the ingredients of the diet offered at each facility were collected during a 3-wk food collection period. Small samples were collected every day, or larger samples every 3 d, subject to staff availability. Food samples were placed in plastic bags and frozen at -80 or -20ºC until the end of the collection period. Then, food samples were transported back to the laboratory on wet ice and kept at -20ºC until analysis.

Intake was also measured during these 3 wks by visually observing each of the dietary ingredients being ingested. All of the foods offered to the manatees were weighed before feeding to determine offered amount. Heads of romaine lettuce were observed to be consumed whole by each of the manatees, and any remnants were considered to be negligible in quantity. Supplemental fruits and vegetables were hand-fed as part of husbandry practices and were wholly consumed.

Fecal samples were collected over a period of 5 to 7 d following the food collection period, with the goal of obtaining a total of at least 100 g of dried feces. Fecal samples were collected non-invasively from the holding tank within 5 min of defecation. Feces were collected using a pool net before feces reached the bottom of the pool, then placed and sealed either in polypropylene tubes (Nunc 50 mL; Thermo Fisher Scientific, Waltham, MA, USA) or plastic bags, and frozen at -80 or -20ºC. Fecal samples were only collected from the water column because the holding tanks had sand on the bottom, which could contaminate the samples. Samples were then transported back to the lab and stored at -80ºC until analysis.

Individual samples of all dietary ingredients and fecal samples were dried to a stable weight over 7 to 10 d in a forced air oven (Model 05015-58; Cole-Parmer, Niles, IL, USA) at 55ºC. Samples were weighed before and after drying to determine percent of DM. Dried food and fecal samples were then individually ground using a Wiley mill (3383-L10 Series; Thomas Scientific, Swedesboro, NJ, USA) to pass through a 1-mm screen. Samples from each individual were then combined, with an effort to include equal amounts of dried feces from each day of the collection period to form a single sample for each animal for each collection period. Daily samples of each dietary ingredient were combined to form a single sample for each facility and for each ingredient for each collection period. Then, these composites were sealed inside plastic bags until analysis.

Nutrient Analysis

Individual dietary ingredients and fecal samples were analyzed for CP (Hansen, 1989), ADF/NDF (Van Soest, 1978), ADL, and AIA. Elemental nitrogen content was measured in triplicate using a modified Dumas combustion method (Hansen, 1989) with thermal conductivity detection in a protein analyzer (N cube, Model Vario Max CN; Elementar Americas, Inc., Mt. Laurel, NJ, USA) and multiplied by 6.25 to obtain CP concentrations. ADF, NDF, and ADL were measured sequentially in duplicate using a fiber analyzer (ANKOM 200; ANKOM Technology Corp., Fairport, NY, USA). Approximately 0.5 g of sample material was weighed into 3 × 5 cm bags made of polyester filter material (Unibond HK-250-N; Midwest Filtration, Cincinnati, OH, USA) and heat sealed. Filter bags were inserted into 24 slots of the bag suspender, and this entire apparatus was then held down by a small weight inside the fiber analyzer vessel to keep it submerged in 1.9 to 2 L of neutral detergent solution (30 g sodium dodecyl sulfate, USP; 18.61 g disodium ethylenediaminetetraacetate dihydrate; 6.81 g sodium borate; 4.56 g anhydrous dibasic sodium phosphate; and 10 mL triethylene glycol in 1 L distilled water) to which 20 g of anhydrous sodium sulfite (all Fisher Scientific, Hampton, NH, USA) and 4 mL of heat-stable alpha-amylase (17,400 Liquefon Units/mL; ANKOM Technology Corp.) had been added. Samples were exposed to agitation and heat for 75 min, followed by 3- to 5-min rinses with

70 to 90ºC water. Four mL of alpha-amylase were included in the first two rinses. The filter bags were removed from the vessel, gently squeezed, and then soaked in acetone (Certified ACS; Fisher Scientific) for 5 min to remove any excess water. After air drying to evaporate the acetone, filter bags were dried in the forced-air oven at 102ºC for 4 h and then reweighed to determine post-NDF dry weight and to calculate % NDF. The NDF-extracted filter bags were then once again inserted into the bag suspender and submerged in 1.9 to 2 L of acid detergent solution (20 g cetyl trimethylammonium bromide [CTAB] in 1 L of 1 N sulfuric acid) within the fiber analyzer vessel and exposed to agitation and heat for 60 min. Bags were then rinsed for 3- to 5-min cycles with 70 to 90ºC water and soaked in acetone for 5 min, dried, and weighed, as described above, to determine % ADF by difference. Two blank bags were included and weighed before and after each run to calculate a blank bag correction factor (C1) and determine whether there was any particle loss from the filter bags.

ADL was determined using a rotary incubator (Daisy II; ANKOM Technology Corp.). The sample filter bags previously used for NDF and ADF determination were first dried in the oven at 102ºC for 2 h and then allowed to cool to ambient temperature in a desiccator (MoistureStop weigh pouch, Model F39; ANKOM Technology Corp.). Next, the filter bags were randomly sorted and placed into three of the jars in the rotary incubator with 500 mL of 72% sulfuric acid to cover the bags. Jars were allowed to rotate for 3 h, after which the sulfuric acid was decanted. Bags were then rinsed using distilled water until a neutral pH was reached, soaked in acetone to remove excess water, dried in a 102ºC oven for 4 h, and weighed.

AIA was measured at an analytical laboratory (Dairy One Cooperative Inc., Ithaca, NY, USA), using the 2 N hydrochloric acid method (Van Keulen & Young, 1977). Five grams of sample was weighed into porcelain crucibles and dried in an oven (Isotemp Model 501; Thermo Fisher Scientific) at 135ºC for 2 h to determine dry weight. Samples were then ashed overnight in these same crucibles at 450ºC in a muffle furnace (Thermolyne Model 30400; Thermo Fisher Scientific). The ash residue was allowed to cool and then rinsed into a 600 mL Berzelius beaker with 2 N hydrochloric acid up to a total volume of 100 mL; the mixture was boiled for 5 min. The hot solution was filtered through ashless filter paper (Whatman grade 41; Thermo Fisher Scientific) and rinsed three times with boiling water (85 to 100ºC). The filter paper and any remaining ash were then transferred back to the crucible and ashed overnight once again. Once samples were cooled in a desiccator, they were weighed to obtain a final AIA weight.

Calculations and Statistical Analysis

The percent intake of each nutrient or indicator in food was calculated from the concentration of each nutrient or marker in each ingredient and the proportion of each ingredient within the total amount of food consumed. Hemicellulose content was estimated as the difference between NDF and ADF; cellulose content was estimated as the difference between ADF and ADL. Apparent digestibility for each nutrient was then calculated from the relative concentrations of marker and nutrient in feed using the following equation:

$$AD = 100 - [100 \times (\%\ \text{indicator in feed}/\%\ \text{indicator in feces}) \times (\%\ \text{nutrient in feces}/\%\ \text{nutrient in feed})] \quad \text{(Equation 1)}$$

Results are reported as means ± 1 standard deviation. Statistical analyses were performed using *SAS*® (*SAS*® *for Windows*®, Version 9.3; SAS Institute Inc., Cary, NC, USA). Replicate values of nutrient concentrations from food and feces were averaged because the coefficient of variation was acceptable (≤ 10% for fiber analyses and < 4% for CP). Apparent digestibility was compared between seasons in the four manatees with both spring and winter measurements using a Wilcoxon signed rank test. Digestibilities were not compared among locations or diets because sample sizes were too small. Post-hoc, a linear regression was performed to determine whether there was a correlation between low AIA concentrations and amount of feces collected. Nutrient AD was regressed against the ADL content of the food, and DM intake was regressed with ADL and AD of DM. A probability of type 1 error < 0.05 was considered significant.

Results

The AD of fiber was measured in two adult male manatees living at Facility A that were born at the Miami Seaquarium, and three recently rehabilitated male manatees (one adult and two subadults) receiving secondary care at Facility B (Table 1). These recently rehabilitated individuals had been rescued and had received critical care at primary rehabilitation facilities previously for boat injuries and cold stress. One of the adults at Facility A is inbred but to date has shown no evidence of being unfit. Whether the rehabilitating animals at Facility B were inbred is unknown. Digestibility was measured during both winter and spring in four manatees and during a second winter in the adult at Facility B. One subadult manatee from Facility B was released into its natural habitat before a spring measurement could be performed.

Table 1. Life stage, body weight, and daily dry matter (DM) intake of manatees (*Trichechus manatus latirostris*) and measurement period when food intake was documented and feces were collected to assess apparent digestibility; DM intake was averaged over 3 wks of observation.

Animal	Life stage	Body weight (kg)	Facility	Season	Collection month/ Year	Daily DM intake (g)	(g/kg)	($g/kg^{0.75}$)
1	Adult	786	A	Spring	April-May 2010	3,925	5.0	26
1		786*	A	Winter 1	December 2009-January 2010	3,664	4.7	25
2	Adult	514	A	Spring	April-May 2010	3,804	7.4	35
2		514*	A	Winter 1	December 2009-January 2010	3,654	7.1	34
3	Subadult	275	B	Spring	April 2010	2,320	8.4	34
3		356	B	Winter 2	December 2010-January 2011	2,688	7.6	33
4	Subadult	376	B	Winter 1	December 2009-January 2010	1,951	5.2	23
5	Adult	626	B	Spring	April 2010	1,797	2.9	14
5		646	B	Winter 1	December 2009-January 2010	1,816	2.8	14
5		655	B	Winter 2	December 2010-January 2011	2,431	3.7	19

*Manatees were only weighed once.

A varied diet that included romaine lettuce, kale, apples, carrots, beets, and monkey biscuits (Zupreem®; Premium Nutritional Products, Inc.©, Shawnee, KS, USA) was offered to manatees at Facility A, so each ingredient was analyzed. Dietary items other than romaine lettuce were offered only sporadically at Facility B, and quantities offered were less than 1% of the total diet on an as-fed basis, so romaine lettuce was the only dietary ingredient collected and analyzed for Facility B.

Mean daily DM intake varied among the five manatees and between seasons ranging from 1.8 to 3.9 kg or 14 to 35 $g/kg^{0.75}$ (Table 1). Fiber and CP concentrations in each dietary ingredient differed among ingredients but appeared similar within each ingredient between seasons at each facility (Table 2). The diets at the two facilities were very similar with differences in concentration of < 2% DM for all nutrients (Table 3); in relative terms, however, the romaine lettuce diet at Facility B contained on average 10% more CP, 10% less NDF, 3% less ADF, 27% less hemicellulose, only 1% less cellulose, and 24% less ADL than the more varied diet at Facility A.

Only AD measurements using ADL as a marker are reported. AIA proved unsuitable as a marker because concentrations of AIA were lower in feces than in the food for all but two measurements of AD, and the mean concentration of AIA in feces (0.2%) was less than half that in food (0.46%). Poor recovery of AIA was not related to the amount of feces collected because there was no evidence of a correlation between AIA concentrations and amount of feces collected ($r = 0.54$, $p = 0.11$).

Average AD (%) of DM, CP, NDF, ADF, and cellulose were slightly lower at Facility A (70, 72, 70, 70, and 87, respectively) than at Facility B (79, 80, 74, 75, and 89, respectively), but the mean AD of hemicellulose was 69% at Facility A and slightly lower (67%) at Facility B (Table 4). There was no evidence of a difference in AD between spring and winter seasons for any nutrient ($p \geq 0.5$). The AD of DM across all measurements was negatively correlated with the ADL in food ($p < 0.0001$) because the AD of CP, NDF, ADF, and cellulose all decreased as ADL increased in food ($p < 0.02$). Of the nutrients measured, only the AD of hemicellulose was not correlated with ADL in food ($r = 0.38$, $p = 0.3$). Neither the AD of DM, CP, or any fiber correlated with NDF in the diet ($r = -0.5$ to 0.1, $p > 0.1$). Daily DM intake (g) increased as ADL in food increased ($r = 0.9$, $p < 0.0001$) and AD of DM decreased ($r = -0.9$, $p = 0.0009$), but there was no evidence of a correlation ($p = 0.2$) between daily DM intake relative to metabolic body weight ($g/kg^{0.75}$) and either ADL in food or AD of DM ($r = 0.4$ and -0.4, respectively).

Table 2. Nutrient composition of dietary components fed to manatees at each facility during each season; nutrient values are expressed as percentage dry matter (DM) for crude protein (CP), neutral detergent fiber (NDF), and acid detergent fiber (ADF), and are reported as the mean of replicates ± 1 standard deviation.

Diet component	Facility	Season	CP	NDF	ADF
Romaine lettuce	A	Spring	23.9 ± 0.2	28.2 ± 1.1	20.9 ± 0.6
Romaine lettuce	A	Winter 1	23.9 ± 0.2	25.1 ± 0.7	18.8 ± 0.2
Monkey biscuit	A	Spring	22.5 ± 0.1	10.1 ± 0.3	2.9 ± 0.2
Monkey biscuit	A	Winter 1	23.1 ± 0.3	8.6 ± 0.9	2.7 ± 0.3
Kale	A	Spring	27.9 ± 0.1	18.9 ± 0.0	14.5 ± 0.0
Kale	A	Winter 1	25.9 ± 0.2	16.9 ± 1.2	11.9 ± 0.1
Beet	A	Spring	13.0 ± 0.4	16.9 ± 0.3	9.6 ± 0.0
Beet	A	Winter 1	9.5 ± 0.1	15.3 ± 0.0	8.1 ± 0.2
Baby carrot	A	Spring	7.6 ± 0.2	15.2 ± 0.2	11.5 ± 0.2
Baby carrot	A	Winter 1	8.7 ± 0.1	13.8 ± 0.0	10.7 ± 0.0
Apple	A	Spring	2.6 ± 0.1	14.5 ± 0.0	10.6 ± 0.2
Apple	A	Winter 1	2.3 ± 0.1	11.0 ± 0.2	8.0 ± 0.0
Romaine lettuce	B	Spring	25.4 ± 0.2	21.0 ± 0.4	16.9 ± 0.2
Romaine lettuce	B	Winter 1	21.6 ± 0.2	19.2 ± 0.1	15.3 ± 0.0
Romaine lettuce	B	Winter 2	26.5 ± 0.1	25.9 ± 0.4	19.8 ± 0.0

Table 3. Nutrient composition of complete diets offered to manatees at each facility during each season

		Nutrient content (%)*							
Facility	Season	DM	CP	NDF	ADF	Hemi-cellulose	Cellulose	ADL	AIA
A	Spring	22.0	25.7	18.8	22.0	25.7	18.8	3.69	0.48
A	Winter 1	22.8	23.1	17.1	6.0	13.9	22.8	3.16	0.36
B	Spring	25.4	21.0	16.9	4.1	14.5	25.4	2.42	0.38
B	Winter 1	21.6	19.2	15.3	3.9	12.7	21.6	2.60	0.16
B	Winter 2	26.5	25.9	19.8	6.1	17.0	26.5	2.83	0.90

*With the exception of dry matter (DM), which is expressed as percentage as fed, nutrient content is expressed as a % DM for crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and acid insoluble ash (AIA), with hemicellulose estimated as NDF-ADF and cellulose estimated as ADF-ADL. Facility B fed romaine lettuce almost exclusively, whereas Facility A fed a diet with a wider range of ingredients as shown in Table 2.

If an ash correction was applied to ADL, ADF, and NDF by subtracting AIA from measured fiber values in food and feces, AD of all nutrients was on average about 3% higher, and correlations between AD of nutrients and ADL were improved slightly, but the overall tenor of the results did not change.

Discussion

Herbivores have symbiotic relationships with microorganisms in their intestines that help the host to obtain energy from the otherwise indigestible carbohydrates in their diet. Foregut fermenters are generally considered to be more efficient than

Table 4. Apparent digestibility of nutrients in diets consumed by manatees under professional management, and regression of apparent digestibility with acid detergent lignin (ADL) in food

			Apparent digestibility (%)*					
Animal	Facility	Season	DM	CP	NDF	ADF	Hemi-cellulose	Cellulose
1	A	Spring	67.6	71.5	70.6	69.3	74.2	86.1
1	A	Winter 1	74.5	75.0	70.2	72.6	63.1	89.1
2	A	Spring	67.8	72.5	68.6	68.9	68.2	85.5
2	A	Winter 1	69.3	69.1	70.7	71.2	69.3	87.4
3	B	Spring	80.8	81.4	73.0	75.2	63.9	87.6
3	B	Winter 2	79.1	83.5	73.3	77.1	60.9	89.9
4	B	Winter 1	77.6	75.3	73.4	74.2	70.1	89.4
5	B	Spring	81.7	83.7	75.3	77.2	67.0	90.2
5	B	Winter 1	77.6	79.9	72.0	73.2	67.9	88.1
5	B	Winter 2	76.4	79.1	75.2	76.5	71.0	89.3
Mean‡	A and B	Both	75.1	76.5	72.1	73.4	67.7	88.3
Regression line parameters of relationship between apparent digestibility with ADL in food†								
All	A and B	Intercept	106.1	101.6	83.1	89.5	58.2	95.5
		Slope	-10.5	-8.3	-3.7	-5.4	3.2	-2.5
		R	-0.94	-0.77	-0.80	-0.84	0.38	-0.73

*Apparent digestibilities are expressed as a percentage of nutrient intake for nutrients with abbreviations as in Table 3.
†Regression lines were obtained using all measurements, with AD expressed as % intake and ADL in food as % DM—for example, apparent digestibility of NDF (%) = 83.1 - 3.7 × ADL (% DM).
‡$n = 5$

hindgut fermenters such as manatees (Van Soest, 1994). The AD of DM in manatees in this study ranged from 68 to 82%, which is higher than for most other hindgut fermenters but slightly lower than that reported in other manatees consuming a lettuce ± vegetable diet (Lomolino & Ewel, 1984; Worthy & Worthy, 2014). DM AD for horses usually averages around 40 to 60%, depending on the forage being tested (Miraglia et al., 1999; Bergero et al., 2004). DM AD in African elephants (*Loxodonta africana*) averaged 35 to 38% (Pendlebury et al., 2005), whereas DM digestibility by Asian elephants (*Elephas maximus*) ranged from 25 to 41% (Clauss et al., 2003). Manatees have an extended digesta passage time of 6 to 10 d (Larkin et al., 2007). This is similar to the digesta passage time of 6 to 7 d for the dugong (Lanyon & Marsh, 1995), but longer than for horses or elephants, which have average digesta passage times of 40 h (Van Weyenberg et al., 2006) and 24 to 48 h (Dierenfeld, 2006), respectively. An extended passage time allows more time for fermentation, which could explain the high digestibility values found for manatees in this study. Sloths, which have prolonged intestinal retention times of several days, were found to have an even higher AD of DM (about 95%) than manatees (Vendl et al., 2016). Koalas (*Phascolarctos cinereus*), which have an extended digesta passage time of 4 to 8 d on average (Caroline et al., 2003), have been reported to have similar but slightly lower DM digestibility to these manatees, ranging from 54 to 70% (Ullrey et al., 1981). Manatees consuming marine vegetation are reported to have a lower AD of DM (< 50%; Worthy & Worthy, 2014), but these measurements do not allow for the high ash content of some submerged aquatic vegetation.

Fiber digestibility was also higher in these manatees than in most terrestrial herbivores. The 69 to 77% digestibilities of NDF and ADF in these manatees were almost twice the AD values (40 to 50%) for NDF and ADF reported in horses (Miraglia et al., 1999) and much more than the AD of ADF (13 to 36%) in Asian elephants (Clauss et al., 2003). However, the forages fed to horses and elephants had higher concentrations of NDF and ADF than romaine lettuce. In sloths consuming a diet containing comparable amounts of fiber to that fed to manatees in this study, and which have long intestinal passage times, the AD of NDF was higher (89 to 95%) than in manatees (Vendl et al., 2016), whereas lower fiber digestibility has been linked to rapid digesta passage rates in both giant pandas (*Ailuropoda melanoleuca*; Sims et al., 2007) and black lemurs (*Eulemur macaco*; Schmidt et al., 2005b). In koalas, which have longer passage times, the digestibilities of NDF (ranging from 22 to 57%) and of ADF (ranging from 8 to 54%) were also lower than in the manatees in the present study, even though the koalas were consuming a diet containing similar concentrations of NDF and ADF (Ullrey et al., 1981). On the other hand, in dugongs, which also have a long intestinal transit time, the average AD of NDF was much higher (95%), even though dugongs were eating eelgrass containing twice as much NDF (44% DM) as the romaine lettuce-based diets fed to the manatees here.

The AD of cellulose was of similar magnitude in these Florida manatees (88%) as the AD of cellulose estimated from the intestinal contents of carcasses of Florida manatees (80%) and dugongs (91%), but the AD of hemicellulose in the Florida manatees in our study (68%) was much lower than the AD of hemicellulose (95%) deduced from dugong carcasses (Murray et al., 1977; Burn, 1986). The reason for the low AD of hemicellulose is unclear because the stomachs of the dugongs contained 36% DM of NDF, which suggests that these dugongs must have been consuming a diet containing more fiber than the Florida manatees under human care in this study before the dugongs died (Table 5).

In the study reported herein, the digestibilities of DM, CP, and fiber decreased as ADL increased in the diet as has been reported previously in dugongs (Aketa et al., 2003) and as NDF increases in the diet in black lemurs (Schmidt et al., 2005b). Lignin has been identified as the cell wall component that most influences the digestibility of plant material (Thayer et al., 1984; Van Soest, 1994) because lignin often chemically bonds with the cellulose and hemicellulose, creating a complex that cannot be easily digested (Bjorndal, 1980). These diets fed to manatees while under managed care were very low in fiber compared to the high fiber diets normally consumed by free-ranging manatees (Siegal-Willott et al., 2010), hindgut fermenters such as horses (National Research Council [NRC], 2007), and elephants (Clauss et al., 2003). It is possible, therefore, that these low fiber manatee diets were more digestible because they contain less fiber. On the other hand, the high AD of ADF and NDF reported in other sirenians

Table 5. Diet composition and apparent digestibility of crude protein (CP) and fiber* in sirenians

Sirenian	Diet	Parameter†	CP	NDF	ADF	Hemicellulose	Cellulose	References
Florida manatee	Romaine lettuce	Diet composition	24.5	22.0	17.3	4.7	14.7	Present study
		Apparent digestibility	80.5	73.7	75.6	66.8	89.1	
Florida manatee	Mixed diet	Diet composition	22.4	24.4	17.9	6.5	14.5	Present study
		Apparent digestibility	72.0	70.0	70.5	68.7	87.1	
Florida manatee	Unknown	Stomach contents	17.9		34.9		25.9	Burn, 1986
		Apparent digestibility	61.0		68.7		79.6	
Dugong	Unknown	Stomach contents	19.4	36.0	29.0	7.0	26.0	Murray et al., 1977
		Apparent digestibility‡	70.0	84.0	82.0	95.2	90.9	
Dugong	Eelgrass	Diet composition	16.3	45.4				Goto et al., 2008
		Apparent digestibility	77.6	94.2				

*See Table 3 for nutrient abbreviations.

†Diet composition (% DM) either from diet consumed by live animals or assumed from stomach content of carcasses, or apparent digestibility (AD, %)

‡Calculated from the means provided in reference of composition of distal large intestine and stomach using lignin as marker

(Murray et al., 1977; Burn, 1986; Goto et al., 2008), some consuming diets with much higher lignin content (Table 5), suggests that high fiber digestibility may be a characteristic of sirenians.

It is possible, however, that differences between manatees or between the two facilities other than the amount of fiber in the diet could also have been responsible for this apparent association between AD and ADL because both of the manatees consuming the diet containing more supplementary ingredients and more fiber were at Facility A, whereas all the manatees consuming the lower fiber lettuce diet were at Facility B. Thus, any difference between the facilities could have been responsible for the lower AD values for DM, CP, NDF, and ADF (68 to 75%) at Facility A than those at Facility B (72 to 84%). For example, higher intake can decrease AD if increased intake reduces intestinal residence time (Edwards & Ullrey, 1999; Clauss et al., 2007). Digestibility did decrease as DM intake increased as in other herbivores (Meyer et al., 2010), but age, size, and individual variation could explain this association: two of the manatees at Facility B that were consuming the slightly more digestible diet were probably consuming less because they were smaller subadults, and the single adult at that facility also consumed less food than the adult manatees at Facility A. There was no evidence of a relationship between AD and DM intake when DM intake was expressed relative to metabolic body weight ($Mass^{0.75}$). There was also no evidence that manatees digested their diets differently between spring and winter seasons. Digestibility differed between seasons in two dugongs fed eelgrass (Aketa et al., 2003), but the composition of eelgrass differed between seasons. In the study presented herein, the composition of the produce-based diets did not change very much between seasons, and water temperature did not change as it might do in the wild because the manatees were maintained in a controlled environment with consistent water temperature and salinity year round.

The amount of dietary fiber is not the only dietary factor which can affect AD. For example, the AD of CP increases in all animals without any change in true digestibility as CP increases in the diet until the AD of CP reaches a plateau close to the true digestibility of CP. This occurs because fecal nitrogen includes endogenous nitrogen secreted into the intestine as well as undigested exogenous nitrogen from the diet. This endogenous nitrogen is the difference between AD and true digestibility and that difference becomes a smaller proportion of nitrogen intake as CP increases in the diet (Donkoh & Moughan, 1994). Thus, the slightly higher AD of CP at Facility B (75 to 84%) than at Facility A (69 to 72%) might result from the slightly higher CP content of the diet at Facility B (24% DM) than at Facility A (22% DM). Comparison of the AD of CP with the CP content of the diet in these manatees and other sirenians suggests that, with the exception of very high AD of CP in dugongs consuming eelgrass, AD of CP does tend to decrease as CP increases in the diet (Table 5; Figure 1). The change in AD of CP with the concentration of CP in the diet is large, which suggests that another factor such as the amount of dietary fiber is also playing a role in this relationship, but the AD of CP is comparable to that in other herbivores consuming similar amounts of CP in their diet (Richard et al., 2017).

Percent CP, NDF, and ADF composition values for the dietary ingredients collected were generally similar to those previously reported for apples, carrots, and kale (Schmidt et al., 2005a). CP concentrations of romaine lettuce were similar to those reported by Siegal-Willott et al. (2010), but about 6 to 9% higher than the value of 18% from Schmidt et al. (2005a). Plant fiber concentrations of romaine lettuce from both facilities were higher than values published by Schmidt et al. (2005a) and Siegal-Willott et al. (2010), with NDF 3 to 12% DM higher and ADF 1 to 6% DM higher than these authors' published values; however, these differences may be explained by differences in growing locations and conditions such as fertilization, moisture, soil, and light (Van Soest, 1978). Composition concentrations of NDF, ADF, and lignin of foods in this study were determined using the same assay and equipment (ANKOM A200; ANKOM Technology Corp.) as Siegal-Willott et al. (2010), except that a correction for residual ash was made by Siegal-Willott et al. A residual ash correction can only explain a small part of the difference, however, because the concentration of AIA in lettuce was low. A potential source of error may have resulted from samples in this study being oven-dried at 55ºC, instead of being lyophilized as they were in the other two studies. Heat can sometimes alter the fiber and protein structures (Parissi et al., 2005), but a lyophilizer was not available. Lignin values for romaine lettuce were similar to those reported by Siegal-Willott et al. (2010).

AIA proved not to be a useful marker for measuring AD in manatees. AIA has been used to measure AD in both African and Asian elephants, which are the manatee's closest living non-sirenian relatives (Clauss et al., 2003; Pendlebury et al., 2005). Manatees retain their digesta for longer periods of time than elephants, however, and could be absorbing some of the AIA. Alternatively, insufficient fecal material may have been collected to be representative or analytical precision may have been low because the concentrations of AIA in romaine

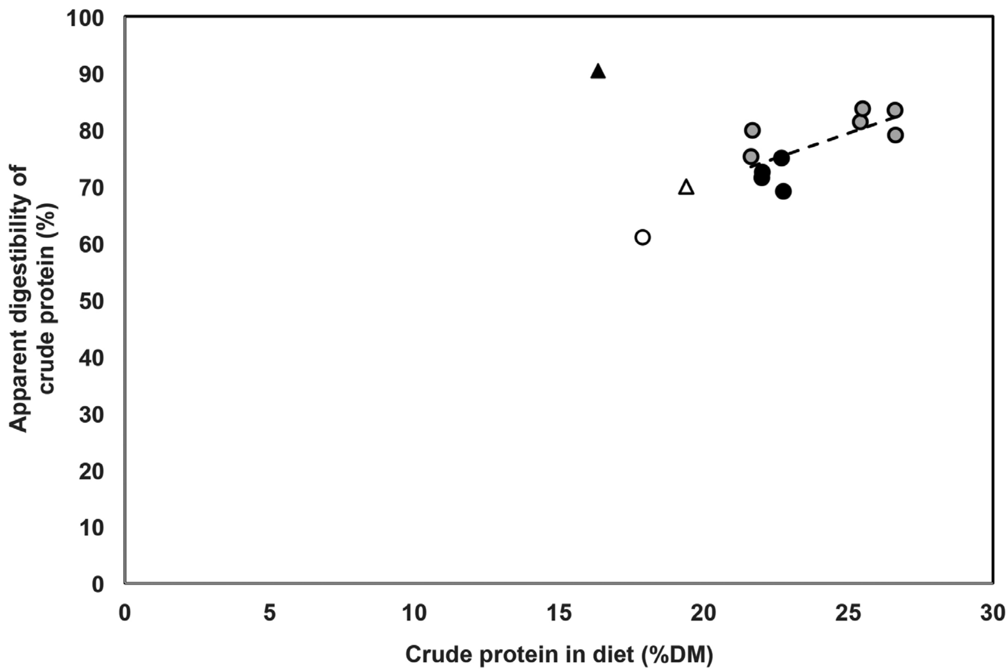

Figure 1. Relationship between apparent digestibility of acid detergent fiber (ADF) and lignin content of the diet in sirenians. Solid black circles represent values from Florida manatees (*Trichechus manatus latirostris*) consuming a mixed diet at Facility A, and circles with grey centers represent values from Florida manatees consuming only romaine lettuce at Facility B in the present study. The open circle represents the mean value obtained from Florida manatee carcasses (Burn, 1986), and the open triangle represents the mean value from dugong carcasses (Murray et al., 1977).

lettuce were very low (Sales & Janssens, 2003; Guevara et al., 2008).

In summary, the CP and fiber in typical diets consumed by Florida manatees under managed care were highly digestible as has been reported previously in other sirenians, and digestibility was slightly inversely related to the lignin content of the diet. Fiber digestibility was higher than has been previously reported in some terrestrial herbivores, but manatees were consuming diets with comparatively less fiber and lignin than other herbivores. This high fiber digestibility should be taken into account when comparing the energy and nutrient intakes of manatees under managed care and free-ranging manatees. There was no evidence of seasonal differences in digestibility, but seasonal differences in nutrient composition and living conditions were small. Future studies should involve more individuals, of more than one sex, maintained in water of different temperatures, and consuming higher fiber diets that more closely mimic the living conditions and diet of free-ranging manatees to better understand the nutritional and physiological needs of this threatened species.

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Literature Cited

Aagnes, T. H., Blix, A. S., & Mathiesen, S. D. (1996). Food intake, digestibility and rumen fermentation in reindeer fed baled timothy silage in summer and winter. *The Journal of Agricultural Science*, *127*, 517-523. https://doi.org/10.1017/S0021859600078746

Aagnes, T. H., Sormo, W., & Mathiesen, S. D. (1995). Ruminal microbial digestion in free-living, in captive lichen-fed, and in starved reindeer (*Rangifer tarandus*) in winter. *Applied and Environmental Microbiology*, *61*(2), 583-591.

Aketa, K., Asano, S., Wakai, Y., & Kawamura, A. (2003). Apparent digestibility of eelgrass (*Zostera marina*) by captive dugongs (*Dugong dugon*) in relation to the nutritional content of eelgrass and dugong feeding parameters. *Mammal Study*, *28*(1), 23-30. https://doi.org/10.3106/mammalstudy.28.23

Bergero, D., Miraglia, N., Abba, C., & Polidori, M. (2004). Apparent digestibility of Mediterranean forages determined by total collection of faeces and acid-insoluble ash as internal marker. *Livestock Production Science*, *85*(2-3), 235-238. https://doi.org/10.1016/S0301-6226(03)00137-4

Bjorndal, K. A. (1980). Nutrition and grazing behavior of the green turtle *Chelonia mydas*. *Marine Biology*, *56*(2), 147-154. https://doi.org/10.1007/BF00397131

Bonde, R. K., Garrett, A., Belanger, M., Askin, N., Tan, L., & Wittnich, C. (2012). Biomedical health assessments of the Florida manatee in Crystal River: Providing opportunities for training during the capture, handling, and processing of this endangered aquatic mammal. *Journal of Marine Animals and Their Ecology*, *5*(2), 17-28.

Burn, D. M. (1986). The digestive strategy and efficiency of the West Indian manatee, *Trichechus manatus*. *Comparative Biochemistry and Physiology A: Physiology*, *85*(1), 139-142. https://doi.org/10.1016/0300-9629(86)90475-5

Caroline, J., Gross, M. S., & Spillman, D. M. (2003). Fiber digestion in mammals. *Pakistan Journal of Biological Sciences*, *6*(17), 1564-1573. https://doi.org/10.3923/pjbs.2003.1564.1573

Clauss, M., Loehlein, W., Kienzle, E., & Wiesner, H. (2003). Studies on feed digestibilities in captive Asian elephants (*Elephas maximus*). *Journal of Animal Physiology and Animal Nutrition*, *87*, 160-173. https://doi.org/10.1046/j.1439-0396.2003.00429.x

Clauss, M., Streich, W. J., Schwarm, A., Ortmann, S., & Hummel, J. (2007). The relationship of food intake and ingesta passage predicts feeding ecology in two different megaherbivore groups. *OIKOS*, *116*, 209-216. https://doi.org/10.1111/j.0030-1299.2007.15461.x

Dawes, C. J., & Lawrence, J. M. (1980). Seasonal changes in the proximate constituents of the seagrasses *Thalassia testudinum*, *Halodule wrightii*, and *Syringodium filiforme*. *Aquatic Botany*, *8*(4), 371-380. https://doi.org/10.1016/0304-3770(80)90066-2

Dierenfeld, E. S. (2006). Nutrition. In M. E. Fowler & S. K. Mikota (Eds.), *Biology, medicine, and surgery of elephants* (pp. 229-307). Oxford, UK: Blackwell Publishing Ltd.

Donkoh, A., & Moughan, P. J. (1994). The effect of dietary crude protein content on apparent and true ileal nitrogen and amino acid digestibilities. *British Journal of Nutrition*, *72*(1), 59-68. https://doi.org/10.1079/BJN19940009

Edwards, M. S., & Ullrey, D. E. (1999). Effect of dietary fiber concentration on apparent digestibility and digesta passage in non-human primates. II. Hindgut- and foregut-fermenting folivores. *Zoo Biology*, *18*(6), 537-549. https://doi.org/10.1002/(SICI)1098-2361(1999)18:6<537::AID-ZOO8>3.0.CO;2-F

Goto, M., Ito, C., Yahaya, M. S., Wakamura, K., Asano, S., Wakai, Y., & Kataoka, T. (2004). Effects of age, body size and season on food consumption and digestion of captive dugongs (*Dugong dugon*). *Marine and Freshwater Behaviour and Physiology*, *37*(2), 89-97. https://doi.org/10.1080/1023624042000199935

Goto, M., Watanabe, A., Karita, S., Tokita, N., Yamamoto, Y., Wakaki, Y., . . . Furuta, M. (2008). Nutrient and energy consumption of captive mature dugong (*Dugong dugon*) consuming eelgrass at the Toba Aquarium. *Marine and Freshwater Behaviour and Physiology*, *41*(3), 169-177. https://doi.org/10.1080/10236240802284445

Guevara, P., Claeys, T., & Janssens, G. P. J. (2008). Apparent digestibility in meat-type guinea pigs as determined by total collection or by internal marker. *Veterinarni Medicina*, *53*(4), 203-206. https://doi.org/10.17221/1917-VETMED

Hansen, B. (1989). Determination of nitrogen as Elementary-N, an alternative to Kjeldahl. *Acta Agriculturae Scandinavica*, *39*(2), 113-118. https://doi.org/10.1080/00015128909438504

Jung, H. J. G., Varel, V. H., Weimer, P. J., & Ralph, J. (1999). Accuracy of Klason lignin and acid detergent lignin methods as assessed by bomb calorimetry. *Journal of Agricultural and Food Chemistry*, *47*(5), 2005-2008. https://doi.org/10.1021/jf981250q

Lanyon, J. M., & Marsh, H. (1995). Digesta passage times in the dugong. *Australian Journal of Zoology*, *43*(2), 119-127. https://doi.org/10.1071/ZO9950119

Larkin, I. L. V., Fowler, V. F., & Reep, R. L. (2007). Digesta passage rates in the Florida manatee (*Trichechus manatus latirostris*). *Zoo Biology*, *26*(6), 503-515. https://doi.org/10.1002/zoo.20150

Lomolino, M. V., & Ewel, K. C. (1984). Digestive efficiencies of the West Indian manatee *Trichechus manatus*. *Florida Scientist*, *47*(3), 176-179.

Meyer, K., Hummel, J., & Clauss, M. (2010). The relationship between forage cell wall content and voluntary food intake in mammalian herbivores. *Mammal Review*, *40*, 221-245. https://doi.org/10.1111/j.1365-2907.2010.00161.x

Miraglia, N., Bergero, D., Bassano, B., Tarantola, M., & Ladetto, G. (1999). Studies of apparent digestibility in horses and the use of internal markers. *Livestock Production Science*, *60*(1), 21-25. https://doi.org/10.1016/S0301-6226(99)00043-3

Murray, R. M., Marsh, H., Heinsohn, G. E., & Spain, A. V. (1977). The role of the midgut caecum and large intestine in the digestion of sea grasses by the dugong (Mammalia Sirenia). *Comparative Biochemistry and Physiology*, *56A*, 7-10. https://doi.org/10.1016/0300-9629(77)90432-7

National Research Council (NRC). (2007). *Nutrient requirements of horses* (6th rev. ed.). Washington, DC: National Academies Press.

Pendlebury, C., Odongo, N., Renjifo, A., Naelitz, J., Valdes, E., & McBride, B. (2005). Acid-insoluble ash as a measure of dry matter digestibility in captive African elephants (*Loxodonta africana*). *Zoo Biology*, *24*(3), 261-265. https://doi.org/10.1002/zoo.20052

Reep, R., & Bonde, R. K. (Eds.). (2006). *The Florida manatee: Biology and conservation*. Gainesville, FL: University Press of Florida.

Reynolds III, J. E., & Rommel, S. A. (1996). Structure and function of the gastrointestinal tract of the Florida manatee, *Trichechus manatus latirostris*. *Anatomical Record*, *245*(3), 539-558. https:/doi.org/10.1002/(SICI)1097-0185(199607)245:3<539::AID-AR11>3.0.CO;2-Q

Richard, O. K., Codron, D., Hagen, K. B., Südekum, K-H., & Clauss, M. (2017). Little differences in digestive efficiency for protein and fat in mammals of different trophic guilds and digestive strategies: Data constraints or fundamental functional similarity? *Journal of Animal Physiology and Animal Nutrition*, *101*(Supp. 1), 127-141. https://doi.org/10.1111/jpn.12657

Sales, J., & Janssens, G. P. J. (2003). Acid-insoluble ash as a marker in digestibility studies: A review. *Journal of Animal and Feed Sciences*, *12*(3), 383-401. https://doi.org/10.22358/jafs/67718/2003

Schmidt, D. A., Kerley, M. S., Porter, J. H., & Dempsey, J. L. (2005a). Structural and nonstructural carbohydrate, fat, and protein composition of commercially available, whole produce. *Zoo Biology*, *24*(4), 359-373. https://doi.org/10.1002/zoo.20057

Schmidt, D. A., Kerley, M. S., Porton, I. J., Porter, J. H., Dempsey, J. L., Griffin, M. E., & Sadler, W. C. (2005b). Fiber digestibility by black lemurs (*Eulemur macaco macaco*). *Journal of Zoo and Wildlife Medicine*, *36*(2), 204-211. https://doi.org/10.1638/04-018.1

Siegal-Willott, J. L., Harr, K., Hayek, L. A. C., Scott, K. C., Gerlach, T., Sirois, P., & Hill, R. C. (2010). Proximate nutrient analyses of four species of submerged aquatic vegetation consumed by Florida manatee (*Trichechus manatus latirostris*) compared to romaine lettuce (*Lactuca sativa* var. *longifolia*). *Journal of Zoo and Wildlife Medicine*, *41*(4), 594-602. https://doi.org/10.1638/2009-0118.1

Sims, J. A., Parsons, J. L., Bissell, H. A., Sikes, R. S., Ouellette, J. R., & Rude, B. J. (2007). Determination of bamboo-diet digestibility and fecal output by giant pandas. *Ursus*, *18*(1), 38-45. https://doi.org/10.2192/1537-6176(2007)18[38:DOBDAF]2.0.CO;2

Thayer, G. W., Bjorndal, K. A., Ogden, J. C., Williams, S. L., & Zieman, J. C. (1984). Role of larger herbivores in seagrass communities. *Estuaries*, *7*(4A), 351-376. https://doi.org/10.2307/1351619

Ullrey, D. E., Robinson, P. T., & Whetter, P. A. (1981). Eucalyptus digestibility and digestible energy-requirements of adult male koalas, *Phascolarctos cinereus* (Marsupialia). *Australian Journal of Zoology*, *29*(6), 847-852. https://doi.org/10.1071/ZO9810847

Van Keulen, J., & Young, B. A. (1977). Evaluation of acid-insoluble ash as a natural marker in ruminant digestibility studies. *Journal of Animal Science*, *44*(2), 282-287. https://doi.org/10.2527/jas1977.442282x

Van Soest, P. J. (1978). Dietary fibers: Their definition and nutritional properties. *The American Journal of Clinical Nutrition*, *31*(10 Supp.), S12-S20. https://doi.org/10.1093/ajcn/31.10.S12

Van Soest, P. J. (Ed.). (1994). *Nutritional ecology of the ruminant* (2nd ed.). Ithaca, NY: Comstock Publishing Associates.

Van Weyenberg, S., Sales, J., & Janssens, G. P. J. (2006). Passage rate of digesta through the equine gastrointestinal tract: A review. *Livestock Science*, *99*(1), 3-12. https://doi.org/10.1016/j.livprodsci.2005.04.008

Vendl, C., Frei, S., Dittmann, M. T., Furrer, S., Osmann, C., Ortmann, S., . . . Clauss, M. (2016). Digestive physiology, metabolism and methane production of captive Linné's two-toed sloths (*Choloepus didactylus*). *Journal of Animal Physiology and Animal Nutrition*, *100*, 552-564. https://doi.org/10.1111/jpn.12356

Worthy, G. A., & Worthy, T. A. (2014). Digestive efficiencies of *ex situ* and *in situ* West Indian manatees (*Trichechus manatus latirostris*). *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*, *87*(1), 77-91. https://doi.org/10.1086/673545