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ARTICLE

Use of rumen–reticulum fill to examine nutrient transfer and factors influencing food intake in white-tailed deer (*Odocoileus virginianus*)

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Abstract: Estimating relationships of gut fill in mammalian herbivores is useful to understanding digestive functions. Large animals might have more fluid in the gut to facilitate nutrient transfer between the gut lumen and the gut wall. Furthermore, relationships between concentrations of dietary refractory and indigestible fiber (CRIF) and gut fill might indicate whether chemostatic factors or physical distension of the gut affects food intake. We collected white-tailed deer (*Odocoileus virginianus* (Zimmermann, 1780); 122 males, 152 females) from three sites in central and south Texas that varied in diet quality as indexed by rumen–reticulum crude protein concentrations. Large animals did not have more fluid in their rumina–reticula than small animals because the scalar between body mass and wet mass of rumen–reticulum contents was not greater than the scalar estimated for dry mass of rumen–reticulum contents. We expected a positive or an inverse relationship when rates of forage comminution, digestion, and particle passage were high or low, respectively. At the site where deer had access to a high-quality pelleted diet, we detected a positive relationship between CRIF and dry mass. At sites with free-ranging deer and lower quality diets, relationships between CRIF and dry fill were inversely related. Food intake of deer was probably influenced by chemostatic factors at the site with a high-quality pelleted diet and by physical distension of the gut at the other two sites.

Key words: dietary fiber, gastrointestinal tract, *Odocoileus virginianus*, Texas, white-tailed deer.

Résumé : L'estimation des relations du contenu intestinal chez les herbivores mammaliens est utile pour comprendre les fonctions digestives. Les grands animaux pourraient avoir plus de liquide dans leur intestin pour faciliter le transfert de nutriments entre la lumière et la paroi de ce dernier. En outre, les relations entre la concentration de fibres alimentaires réfractaires et non digestibles (CRIF) et le contenu intestinal pourraient indiquer si des facteurs chimiostatiques ou la distension physique de l'intestin ont une incidence sur l'ingestion d'aliments. Nous avons prélevé des cerfs de Virginie (*Odocoileus virginianus* (Zimmermann, 1780); 122 mâles, 152 femelles) de trois sites du centre et du sud du Texas présentant de la nourriture de différents niveaux de qualité de la nourriture indiqués par les concentrations de protéines brutes dans le rumen–réticulum. Les grands individus n'avaient pas plus de liquide dans leur rumen–réticulum que les petits individus parce que le scalaire entre la masse corporelle et la masse humide du contenu du rumen–réticulum n'était pas plus grand que le scalaire estimé pour la masse sèche du contenu du rumen–réticulum. Nous nous attendions à une relation positive ou négative quand les taux de communition et de digestion du fourrage et de passage des particules étaient élevés ou faibles, respectivement. Au site où les cerfs avaient accès à des aliments en boulettes de haute qualité, nous avons noté une relation positive entre les CRIF et la masse sèche. Aux sites où les cerfs étaient en liberté et caractérisés par des aliments de moins bonne qualité, les relations entre les CRIF et le contenu sec étaient négatives. L'ingestion d'aliments par les cerfs était probablement influencée par des facteurs chimiostatiques au site caractérisé par des aliments en boulettes de haute qualité, et par la distension physique de l'intestin aux deux autres sites. [Traduit par la Rédaction]

Mots-clés : fibre alimentaire, tube digestif, *Odocoileus virginianus*, Texas, cerf de Virginie.

Introduction

Mammalian herbivores display considerable variation in the fill of the gastrointestinal tract or gut. Gut fill refers to the wet and dry masses from ingested forage, products from fermentation, and endogenous secretions. Variation in gut fill probably reflects adjustments in digestive functions to accommodate heterogeneity in the quality and quantity of food supplies, as well as seasonal variation in animal production demands. When animal production demands are high, there is a concomitant increase in food intake and gut fill (Short 1975; Jenks et al. 1994; Zimmerman et al. 2006). Concentrations of structural carbohydrates and antinutritional compounds that impede the rate of digestion differ in response to change in the life stage of forage plants such as when forage plants grow, mature, and senesce (Everitt and Gonzalez 1981). When forages are growing, they are usually described as high quality because leaves of growing plants have high concentrations of readily digestible fiber, and food particles have shorter retention time in the gut (Jenks et al. 1994; Duarte et al. 2011; Luna et al. 2012; Lane et al. 2014).

In the gastrointestinal tract of ruminants, the voluminous rumen–reticulum is where most fermentation products are

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absorbed (Van Soest 1994; Barboza et al. 2006; Ramzinski and Weckerly 2007; Barboza et al. 2009). Symbiotic microbes in the rumen are proficient at digesting plant carbohydrates, although inefficient in the reduction of forage particle size (Spalinger et al. 1986; Maulfair et al. 2010). The forage particles are mixed by contraction and relaxation of the reticulorumen wall and comminuted to smaller particles through rumination (Van Soest 1994). Forage particles are retained in the rumen by selective particle delay (Van Soest 1994), in which predominantly smaller particles leave the rumen by passing through the reticulo-omasal sphincter (Barboza et al. 2009).

Examination of the digestive process via variation in rumen–reticulum fill of free-ranging animals is a largely unexplored field (Aiken et al. 2014). Most studies of gut fill in free-ranging animals estimate correlations between surrogates of animal demands (i.e., lactation, age) and rumen–reticulum fill, or describe temporal and spatial variations in gut fill (Jenks et al. 1994; Zimmerman et al. 2006; Duarte et al. 2011; Luna et al. 2012). Understanding the digestive processes of ungulates is usually examined through controlled laboratory settings, where animals habituated to handling are presented a consistent diet. In controlled laboratory settings, precise measurements of food intake, forage particle breakdown, digestion, as well as liquid and forage particle retentions, are possible (Spalinger et al. 1986, 1992; Baker and Hobbs 1987; Barboza et al. 2006; Kammes and Allen 2012). It is problematic, however, to apply such findings to free-ranging animals. Free-ranging animals often have access to a large variety of forages that vary temporally and spatially. Moreover, the activity patterns and production demands of free-ranging animals can differ substantially from tame animals in a laboratory setting.

Rumen–reticulum fill measured from free-ranging animals can be broken down into two components: dry mass and wet mass. Because these two measures of rumen–reticulum fill seem to correlate with slightly different factors, they may shed insight into different aspects of digestion (Luna et al. 2012). Dry mass is presumably influenced by rates of forage intake, digestibility, and rate of particle passage (Holleman and White 1989; Müller et al. 2013). On the other hand, wet mass seems to be affected by diet type, whether free water was ingested, forage particle size, microbial activity, and facilitating nutrient transport in addition to the factors that influence dry mass (Barboza et al. 2006; Kammes and Allen 2012; Luna et al. 2012; Müller et al. 2013).

It is well documented that rumen–reticulum fill scales positively with body size (Demment 1982; Weckerly et al. 2003; Weckerly 2010; Luna et al. 2012). The positive scalar reflects the fact that larger animals have greater absolute food intake than smaller animals to meet greater absolute energetic requirements (Weckerly 2013). Yet, the scalars for each measure of fill (i.e., wet and dry masses) might differ (Müller et al. 2013). Large animals have a larger rumen–reticulum and therefore might require more ruminal fluid to facilitate movement of nutrients between fermented plant material and absorptive sites on the rumen wall. Consequently, wet mass should have a scalar greater than the scalar for dry mass.

Understanding what factors affect food intake is useful because the rate of digestion, not finding enough to eat, is usually what limits nutrient assimilation in herbivores (Crawley 1983). The relationship between the concentration of refractory and indigestible fiber (CRIF) in the rumen–reticulum and its fill might signal whether food intake is governed by chemostatic factors or physical distension of the gut, particularly for dry mass (Van Soest 1994; Barboza et al. 2009). Clearance of plant material from the rumen–reticulum is a result of the rates of forage particle breakdown, fermentation, and forage particle retention (Kammes and Allen 2012). We hypothesize that when a diet is quickly comminuted into small particles and fiber is rapidly fermented, the particle passage rate through the rumen–reticulum should also be quick (Holand 1994; Jiang and Hudson 1996). Such diets, however, will have forages that vary in CRIF. When animals consume forage with greater CRIF, dry mass will be greater than when forages have less CRIF. If food intake is regulated by chemostatic factors (Barboza et al. 2009), then CRIF in the rumen–reticulum should be positively related to dry mass. Also, we hypothesize that when food intake is regulated by physical distention of the gut, CRIF in the rumen–reticulum should be negatively related to dry mass. Lower quality diets should take longer to comminute, ferment, and retention of particles should be for a long enough period of time that gut fill and food intake is dictated by the time it takes to comminute and ferment forage. Yet, forages will vary in CRIF and digestibility (Gray and Servello 1995). In this setting, more digestible diets should clear the rumen–reticulum more rapidly, allowing for elevated food intake and dry rumen–reticulum fill.

We estimated relationships between body mass, CRIF in the rumen–reticulum, and dry and wet masses of rumen–reticulum fill for white-tailed deer. Deer were collected from sites in central and south Texas, USA, that had access to variable dietary qualities. We tested the following: (*i*) whether the scalar between body mass and rumen–reticulum fill was greater for wet than dry masses, (*ii*) whether predictions about relationships between CRIF and dry mass differed due to dietary quality, and (*iii*) whether those relationships were similar for dry and wet masses.

Materials and methods

Study area

Deer were collected from two separate areas on the Kerr Wildlife Management Area (WMA). Kerr WMA is located in Kerr County within the Edwards Plateau ecoregion that is managed by the Texas Parks and Wildlife Department (TPWD). Kerr County has a mean annual precipitation of 70 cm, which varies from year to year (Luna et al. 2012). During the years that data were collected (2009, 2010, and 2011), respective precipitation was 62.74, 76.6, and 33.3 cm. Daytime temperatures were hot and often exceeded 35 °C in the summer, while temperatures in the winter were mild and ranged from 10 to 22 °C. One area of the property encompasses a free-ranging white-tailed deer population. These deer have access to 2628 ha of natural habitat that is surrounded by a 2.4 m high fence. Another area of the property consisted of breeding pens used by TPWD personnel to conduct deer research. In this area, there was a 6.5 ha facility containing six 0.3 ha breeding pens, three 1.6 ha rearing pens, and 24 small pens for handling research animals (Harmel et al. 1989). Hereafter, we will refer to the area with free-ranging deer as the WMA and the area with penned deer as the Pens. Warren and Krysl (1983) reported the primary forage consumed by deer on the WMA consists of various oaks (genus *Quercus* L.), Ashe juniper (*Juniperus ashei* J. Buchhoz), silverleaf nighshade (*Solanum elaeagnifolium* Cav.), bladderpods (genus *Physaria* (Nutt. ex Torr. & A. Gray) A. Gray), whorled nodding violet (*Hybanthus verticillatus* (Ortega) Baill.), common horehound (*Marrubium vulgare* L.), spurges (genus *Euphorbia* L.), redseed plantain (*Plantago rhodosperma* Decne.), globemallows (genus *Sphaeralcea* A. St.-Hil.), filaree (genus *Erodium* L'Hér. ex Aiton), and Texas wintergrass (*Nassella leucotricha* (Trin. & Rupr.) R.W. Pohl). Conversely, the Pens consisted of bare ground, mostly limestone, and live oak (*Quercus virginiana* Mill.) that provided 25%–50% canopy cover (Lockwood et al. 2007). Foliage of live oak within the Pens was primarily out of reach of deer and was not available for forage. At the Pens, deer were fed a commercial pelleted ration (16% crude protein and 18.5% acid detergent fiber) ad libitum and approximately 1 kg (dry mass) of alfalfa (*Medicago sativa* L.) per week per animal (Parra et al. 2014). Thus, deer from the Pens did not have access to natural forage and deer from the WMA did not have access to pelleted feed.

Deer were also collected from a private ranch (hereafter, the Ranch), which is located in Jim Hogg County, within the South Texas Brushlands ecoregion. The Ranch encompassed 2994 ha and was also surrounded by a 2.4 m high fence. Precipitation at the Ranch averaged 60.5 cm annually (Parra et al. 2014). During the years that data were collected (2010 and 2011), precipitation was

91.16 and 24.82 cm, respectively. The subhumid environment was hot in summer, with daytime temperatures often exceeding 35 °C, and mild in winter, where the daytime temperatures averaged 11 °C. The primary forage consumed by white-tailed deer in this ecoregion consisted of pricklypear (genus *Opuntia* Mill.), perennial Riddell's dozedaisy (*Aphanostephus riddellii* Torr. & Gray), western ragweed (*Ambrosia psilostachya* DC.), Indian blanket (*Gaillardia pulchella* Foug.), and Drummond's clematis (*Clematis drummondii* Torr. & A. Gray) (Everitt and Drawe 1974; Arnold and Drawe 1979; Barnes et al. 1990). White-tailed deer in this area, however, also consume annual Arkansas dozedaisy (*Aphanostephus kidderi* S.F. Blake = *Aphanostephus skirrhobasis* var. *kidderi* (S.F. Blake) B.L. Turner), granjeno (*Celtis pallida* Torr.), prostrate sandmat (*Euphorbia prostrata* Aiton), desert lantana (*Lantana achyranthifolia* Desf.), and honey mesquite (*Prosopis glandulosa* Torr.). At the Ranch, gravity feeders (1 per 107 ha) provided protein feed from January through October.

Sample collection

White-tailed deer were collected from the WMA in November 2009 and 2010, March 2010 and 2011, and September 2010. Sampling during these months allowed us to collect deer when reproductive demands and diets varied across seasons. All deer were dispatched with high-powered rifles. Deer collected in November were harvested during annual either sex deer hunts and TPWD personnel collected deer in other months. Texas Parks and Wildlife Department personnel also collected deer in November 2011 from the Pens. White-tailed deer were culled from the Ranch on 16–17 October 2010 and 12–13 November 2011. At the Ranch, deer were netted from a helicopter, restrained, and transported to a processing area within 45 min. Collection procedures followed the Institutional Animal Care and Use protocol from Texas State University (permit No. 00933_09_06-03141BF15D).

All deer were processed within 3 h of being dispatched. Time of kill was when the animal was dispatched. Deer were weighed, sexed, and aged using tooth eruption and wear (Severinghaus 1949). Body mass, minus blood loss, was recorded to the nearest 0.1 kg. Depth of back fat was measured to the nearest 1.0 mm, from an incision near the L3–L4 lumbar vertebrae. Lactation status was determined by the presence or absence of milk in the udders. The animal was eviscerated and the carcass weighed to the nearest 0.1 kg to measure dressed body mass. The gastrointestinal tract was separated from the remaining visceral organs and the rumen–reticulum was excised 5 cm anterior to the junction of the esophagus and reticulum and at the reticulo-omasal sphincter. The rumen–reticulum was then excised from the omasum and abomasum. The rumen–reticulum containing the digesta was weighed to the nearest 0.1 kg. The rumen was emptied of fluid and forage particles, inverted, and thoroughly rinsed with tap water to remove particles that were on papillae and the rumen wall. The organ mass was then recorded. Wet mass of the rumen–reticulum fill was the difference between the rumen–reticulum organ with digesta and rumen–reticulum organ without digesta.

A wet digesta sample of approximately 800 g was collected from each animal, dried at 60 °C for 48 h, then reweighed (Jiang et al. 2009). The mass of dry rumen–reticulum fill was calculated as wet mass of the rumen–reticulum fill × percent dry matter (i.e., dry digesta sample/wet digesta sample). Another part of the wet digesta sample was then ground to ≤1 mm particles. A 1 g sample of the dried particulate was analyzed with a nitrogen gas analyzer using an induction furnace and thermal conductivity using a Leco FP-528 apparatus to determine nitrogen content in the digesta sample (method No. 973.18 in AOAC 1997). Crude protein (CP) concentration was the nitrogen concentration (expressed as proportion) × 6.25. Another 1 g sample of the ground and dried particulate digesta was placed in a filter bag and immersed in a hexadecyl-trimethyl-sulphuric acid solution. The sample was then rinsed three times in boiling water followed by a final rinse with acetone. When the sample was dry, it was weighed and acid detergent fiber (ADF) determined from a nitrogen gas analyzer and Leco FP-528 (AOAC 1997). Acid detergent fiber was also expressed as a proportion. Digesta samples for nutrition analyses were analyzed by A&L Plains Laboratory, Inc. (Lubbock, Texas, USA).

Crude protein measured from rumen–reticulum samples includes nitrogen from forage, micro-organisms, and endogenous secretions (Van Soest 1994; Lukas et al. 2005). Crude protein from micro-organisms is positively influenced by the concentration of fermentable carbohydrates (Lukas et al. 2005). Therefore, CP concentration in the rumen–reticulum was used as our indicator of diet quality. Acid detergent fiber in the rumen–reticulum was used as an indicator of CRIF in the rumen–reticulum. Acid detergent fiber measures cellulose, which is often refractory to digestion, and indigestible material such as lignin and cutin.

Data analysis

A single-factor analysis of variance (ANOVA) followed by a Tukey's honest significant difference (HSD) multiple comparison procedure was used to test for differences in CP across sites (Sokal and Rohlf 2012). To ameliorate heteroscedasticity, the response variable was the natural logarithm of CP.

We constructed a series of models to estimate the variation in two response variables: wet rumen–reticulum and dry rumen–reticulum fill. Covariates were selected to estimate relationships of interest and to account for confounding influences on the relationships of interest. The covariates were dressed body mass (hereafter body mass), sex, lactation status, type of diet (predominantly pelleted or browse), site (Pens, Ranch, WMA), depth of back fat, and ADF in the rumen–reticulum. We used dressed body mass to remove the problem of having response variables as part of a covariate (Parra et al. 2014). We chose to consider variation in diet with categorical covariates for predominantly pelleted or browse diets, as well as a categorical covariate for site. Pelleted forage is designed to be high in protein and digestible fiber and therefore easy to comminute in comparison with forage available to free-ranging animals. Besides ease of comminution, however, other factors might influence fill. Those other latent factors might be captured by a categorical covariate for site. Furthermore, categorical variables for diet and site allowed us to assess if the relationship between CRIF and response variables differed between diets and across sites (see next paragraph). Lactation and sex were also categorical covariates. To satisfy the assumption of homoscedasticity and to estimate scaling relationships that can be nonlinear, we logarithmically transformed body mass and rumen–reticulum response variables in every model (Sokal and Rohlf 2012).

We fit 10 models to estimate the relationships between body mass, ADF, site, or diet on wet and dry masses of the rumen–reticulum fill. Every model had body mass, sex, female lactation status, and time of kill because these covariates invariably influence dry and wet masses (Short et al. 1969; Weckerly et al. 2003; Weckerly 2010; Luna et al. 2012; Duarte et al. 2014). We also included an interaction between time of kill and sex in every model because diel foraging patterns usually differ between males and females (Aiken et al. 2014; Duarte et al. 2014). Models were built to assess whether ADF and diet, or site, were related to rumen–reticulum fill in an additive or multiplicative manner. Considering a multiplicative influence between ADF and categorical variables for diet and site allowed us to critically test our objectives. We also considered whether back fat and diet, or site, were related to rumen–reticulum fill in an additive or multiplicative manner (Table 1). We conducted an information–theoretic model selection analysis with the second-order Akaike's information criterion (AIC_c) to select a model or identify competing models (Burnham and Anderson 2002). After calculating the AIC_c, we computed the ΔAIC_c (AIC_c – minimum AIC_c, where minimum AIC_c refers to the model with the smallest AIC_c) for each of the 10 models. Competing models were identified as $\Delta AIC_c \leq 2$. We used the "model.avg" function within the MuMIn package in R to estimate coefficients and standard errors (SE) averaged among competing models (Barton

Table 1. Summary of variables from sampled white-tailed deer (*Odocoileus virginianus*) on Kerr Wildlife Management Area (with free-ranging deer (WMA) and with penned deer (Pens)) and a private ranch (with free-ranging deer (Ranch)).

Variable	Years	Months	Site	Mean	Range	*n*
Wet mass	2009, 2010, 2011	Sept., Nov., Mar.	WMA	3.02	0.9–7.9	154
	2011	Nov.	Pens	2.60	1.9–3.3	40
	2010, 2011	Oct., Nov.	Ranch	4.55	2.1–9.2	80
Dry mass	2009, 2010, 2011	Sept., Nov., Mar.	WMA	0.89	0.1–2.6	154
	2011	Nov.	Pens	0.75	0.2–1.4	40
	2010, 2011	Oct., Nov.	Ranch	0.86	0.4–1.8	80
Body mass	2009, 2010, 2011	Sept., Nov., Mar.	WMA	29.75	12.6–59.8	154
	2011	Nov.	Pens	38.27	27.6–70.2	40
	2010, 2011	Oct., Nov.	Ranch	56.10	24.0–78.9	80
Back fat	2009, 2010, 2011	Sept., Nov., Mar.	WMA	1.63	0–4.75	154
	2011	Nov.	Pens	0.47	0–1.9	40
	2010, 2011	Oct., Nov.	Ranch	2.01	0–3.18	80
Acid detergent fiber	2009, 2010, 2011	Sept., Nov., Mar.	WMA	0.42	0.2–0.6	154
	2011	Nov.	Pens	0.47	0.4–0.6	40
	2010, 2011	Oct., Nov.	Ranch	0.48	0.3–0.6	80
Time of kill	2009, 2010, 2011	Sept., Nov., Mar.	WMA	14.16	6.7–23.1	154
	2011	Nov.	Pens	11.3	9–13.3	40
	2010, 2011	Oct., Nov.	Ranch	12.84	8.8–17.6	80

Note: Table shows months and years when all variables were sampled, mean and range of wet and dry masses (kg) of rumen–reticulum fill, body mass (kg), depth of back fat (cm), acid detergent fiber (proportion), and time of kill (hours, with minutes expressed as proportion).

2009). We then calculated 95% confidence intervals (CI) of coefficients and SE, as well as the adjusted coefficient of determination (r^2). A coefficient was statistically significant if the 95% CI excluded 0.

Results

A total of 274 deer were collected: 17 males and 23 females were taken from the Pens, 38 males and 116 females were taken from the WMA, and 67 males and 13 females were taken from the Ranch. Rumen–reticulum CP varied among sites ($F_{[2,261]}$ = 56.4, P < 0.001). Interpreting a Tukey's HSD indicated that CP in the rumen–reticulum was greater at the Pens (untransformed mean = 0.260, untransformed standard deviation (SD) = 0.043) than at the WMA (P < 0.001, 0.188, 0.033) and at the Ranch (P < 0.001, 0.195, 0.024). No difference was detected (P = 0.171) in rumen–reticulum CP between deer from the WMA and the Ranch.

Body masses of deer tended to be lighter at the WMA than at the Pens or at the Ranch (Table 1). On average, back fat was least for deer from the Pens, whereas deer from the WMA and the Ranch had a wide range of back fat. Range in rumen–reticulum ADF was 0.2–0.6 for deer from the WMA, 0.4–0.6 for deer from the Pens, and 0.3–0.6 for deer from the Ranch.

The model selection analyses indicated models with the site covariate, not the diet covariate, had the strongest influence on both wet and dry masses of rumen–reticulum fill (Table 2). For wet mass, three models were competing ($\Delta AIC_c \leq 2$). These models indicated there was a multiplicative influence of site and ADF on wet mass and that perhaps back fat was also influential. For dry mass, the findings from the model selection analysis were clear-cut. Dry mass was influenced by the multiplicative influence from ADF and site, as well as from the multiplicative influence of back fat and site.

The body mass scalar for wet mass was greater in value than the scalar for dry mass (Table 3). Yet, the broad overlap in CI for the two scalars suggested no statistical difference. For the dry mass regression, body mass, sex, lactation status, back fat, time of kill, and interactions between deer from the Pens and ADF and between back fat and the two site covariates were statistically significant. For the model-averaged wet mass regression, body mass, lactation status, time of kill, and the interactions between sex and time of kill and between ADF and the Ranch were statistically significant. Plotting regressions between ADF and dry mass revealed an inverse relationship for deer from the WMA and the Ranch and a positive relationship for deer from the Pens (Fig. 1). These relationships for each site were not evident for wet mass. The only relationship that we detected between ADF and wet mass was an inverse relationship for deer from the Ranch.

Discussion

Contrary to our prediction, the body mass scalar for wet mass was not greater than the dry mass scalar. Our predictions about rumen–reticulum CRIF and dry mass, however, were met. Penned deer had the highest quality diet and we also detected a positive relationship between rumen–reticulum CRIF and dry mass. Free-ranging deer at the WMA and the Ranch had a similar, yet lower dietary quality and deer from these two sites also displayed an inverse relationship between rumen–reticulum CRIF and dry mass. Presumably, food intake of deer from the Pens was influenced by chemostatic factors, whereas food intake of deer from the WMA and the Ranch was affected by physical distension of the gut. Regarding the third prediction, we found that rumen–reticulum wet and dry masses had slightly different sets of covariates and parameter estimates. Thus, our estimated relationships of wet and dry masses were not similar.

Our index of diet quality, CP in the rumen–reticulum, is reliable as long as it captures digestible fiber concentration. When cattle consume grasses with low concentrations of secondary compounds, the concentration of CP in the gut is a reliable measure of diet quality (Lukas et al. 2005). Browsers such as white-tailed deer consume forages in central and south Texas that contain phenolic amines, alkaloids, condensed tannins, and other secondary compounds (Campbell and Hewitt 2005; Adams et al. 2013). The high concentration of CP in the rumen–reticulum of deer from the Pens probably reflected a diet high in digestible fiber because pellets and alfalfa presumably had little if any secondary compounds. It is likely that free-ranging deer from both the WMA and the Ranch ingested secondary compounds, which probably reduced diet digestibility (Campbell and Hewitt 2005). Lower fiber digestibility, owing to the presence of secondary compounds, should result in a lower concentration of CP in the rumen–reticulum.

A greater scalar for wet mass than dry mass was found among herbivorous, mammal species that spanned over four orders of magnitude in body mass (Müller et al. 2013). Wet and dry masses of gut contents also ranged over four orders of magnitude in that study. In comparison, our intraspecific study had body masses and

Table 2. Model selection summary for wet and dry masses of rumen–reticulum fill in white-tailed deer (*Odocoileus virginianus*) sampled from Kerr Wildlife Management Area and a private ranch, Texas.

		Response variables					
		ln (wet mass)			ln (dry mass)		
Model predictors	n_{Par}	Δ	Akaike weight	r^2	Δ	Akaike weight	r^2
BM, ST, SEX, LACT, KT, KT:SEX	9	0.66	0.25	0.48	19.65	<0.01	0.34
BM, ST, SEX, LACT, KT, KT:SEX, ADF	10	2.80	0.08	0.48	20.80	<0.01	0.34
BM, ST, SEX, LACT, KT, KT:SEX, ADF, ADF:ST	12	0.00	0.34	0.49	20.57	<0.01	0.35
BM, ST, SEX, LACT, KT, KT:SEX, ADF, ADF:ST, BF	13	1.51	0.16	0.49	10.59	<0.01	0.38
BM, ST, SEX, LACT, KT, KT:SEX, ADF, ADF:ST, BF, BF:ST	15	2.70	0.09	0.49	0.0	0.79	0.41
BM, DT, SEX, LACT, KT, KT:SEX	8	4.64	0.03	0.47	17.58	<0.01	0.35
BM, DT, SEX, LACT, KT, KT:SEX, ADF	9	6.77	0.01	0.47	18.72	<0.01	0.35
BM, DT, SEX, LACT, KT, KT:SEX, ADF, ADF:DT	11	5.62	0.02	0.47	16.81	<0.01	0.35
BM, DT, SEX, LACT, KT, KT:SEX, ADF, ADF:DT, BF	12	7.76	0.01	0.47	9.04	<0.01	0.38
BM, DT, SEX, LACT, KT, KT:SEX, ADF, ADF:DT, BF, BF:DT	14	8.88	<0.01	0.47	2.75	0.21	0.39

Note: Predictor labels are natural logarithm of body mass (BM), diet type (DT), lactation (LACT), acid detergent fiber (ADF), time of kill (KT), sex (SEX), site (ST), and depth of back fat (BF). The change (Δ) in Akaike's information criterion corrected for small sample size (AIC_c) between a model and the model with the smallest AIC_c, number of parameters estimated (n_{Par}), and adjusted r^2 are provided.

Table 3. Parameter estimates, standard errors (SE), and confidence intervals (lower bound (lb), upper bound (ub)) of wet mass (model-averaged) and dry mass of rumen–reticulum fill in white-tailed deer (*Odocoileus virginianus*) sampled from Kerr Wildlife Management Area (Kerr Pen) and a private ranch (Ranch), Texas.

	Response variable							
	ln (wet mass)				ln (dry mass)			
Coefficients	Estimate	SE	lb	ub	Estimate	SE	lb	ub
ln (intercept)	−1.80	0.33	−2.46	−1.15	−2.62	0.39	−3.38	−1.86
ln (body mass)	0.72	0.09	0.54	0.90	0.57	0.11	0.36	0.79
Ranch	0.51	0.34	−0.16	1.17	−0.25	0.32	−0.37	0.88
Pens	−0.14	0.43	−0.99	0.71	−0.83	0.48	−1.78	0.12
Male	0.21	0.15	−0.08	0.50	0.57	0.17	0.24	0.91
LACT	0.32	0.05	0.22	0.43	0.32	0.07	0.19	0.45
BF	0.02	0.02	−0.03	0.06	0.14	0.03	0.08	0.20
KT	0.02	0.006	0.006	0.03	0.02	0.007	0.009	0.04
ADF	0.14	0.35	−0.56	0.83	−1.25	0.42	−2.06	−0.44
ADF:Ranch	−1.22	0.62	−2.45	−0.007	1.35	0.77	−0.17	2.86
ADF:Kerr Pen	1.14	0.89	−0.61	2.90	2.74	1.04	0.70	4.77
KT:male	−0.02	0.01	−0.04	0.0008	−0.04	0.01	−0.07	−0.02
BF:Ranch	—	—	—	—	−0.14	0.05	−0.25	−0.04
BF:Kerr Pen	—	—	—	—	−0.35	0.12	−0.59	−0.12

Note: The intercept is in natural logarithmic scale. Females were the reference category for sex, the WMA was the reference for site, and nonlactating animals were the reference category for lactation (LACT). Covariates are statistically significant if confidence intervals exclude 0. ADF, acid detergent fiber; BF, back fat; KT, time of kill.

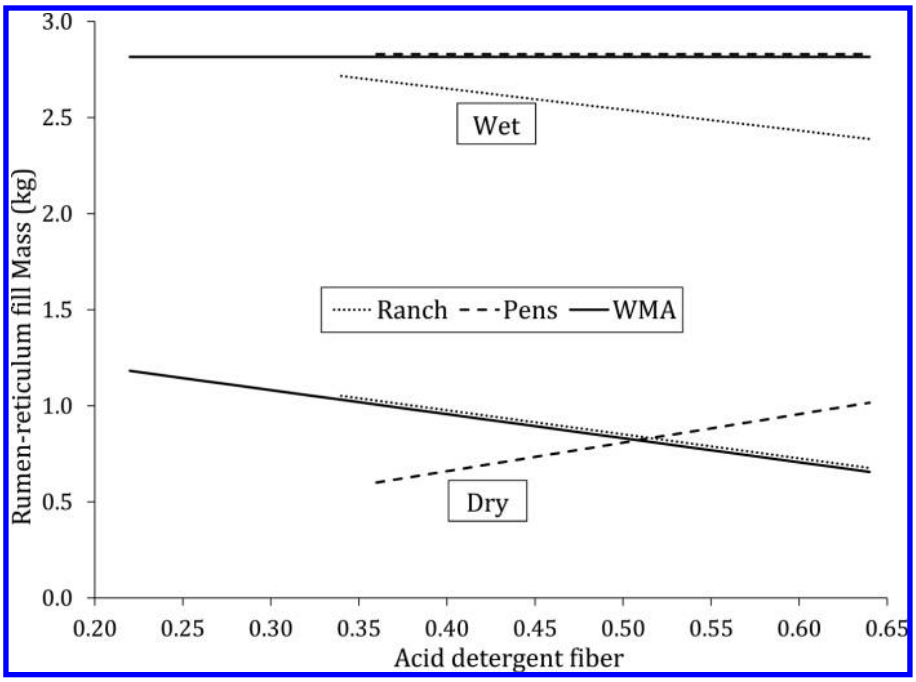
wet or dry masses of rumen–reticulum fill that ranged a paltry one order of magnitude or less. Large rumina–reticula of white-tailed deer probably do not require additional fluid solely for the function of facilitating nutrient transfer between the gut lumen and the gut wall.

The positive relationship between rumen–reticulum CRIF and dry mass for deer from the Pens might have reflected times when animals were consuming pellets and other times when animals were consuming more alfalfa. Pellets can be comminuted quickly, the particles are rapidly fermented, and it is likely that indigestible material passes through the rumen–reticulum quickly. In support of particles being rapidly comminuted to small particles is the finding that rumination time is often drastically curtailed when ruminants consume a pelleted diet (Jiang and Hudson 1996). Consequently, rumen–reticulum dry mass should be light unless animals are collected immediately after a large meal. Alfalfa, however, does take time to ruminate and contains higher amounts of CRIF than pellets (Jiang and Hudson 1996; Lockwood et al. 2007; Kammes and Allen 2012). One reason deer probably consumed alfalfa was to promote rumination. Chewing stimulates salivary secretion and the saliva contains pH-buffering sodium bicarbonate (Maulfair et al. 2010). When dairy cows consume a nutrient-rich diet that does not require much rumination, digestive upset from rumen acidosis is common (Krause et al. 2002; Couderc et al. 2006; Maulfair et al. 2010).

We suspect that the inverse relationship between CRIF and dry mass for deer from the WMA and the Ranch is because particle breakdown in general is slow. Free-ranging deer consume a variety of leaves, fruits, and acorns that have to be masticated through rumination. It takes longer for deer to comminute natural forage particles to a small enough size so that they pass through the reticulo-omasal sphincter. Also, the time that it took to comminute forage particles to a small size probably covaried positively with digestible fiber content of the diet. When the diet was more digestible, rumen–reticulum CRIF was probably less and dry mass greater because of higher food intake (Robbins 1983; Gray and Servello 1995). Food intake was probably affected by physical distension of the gut.

The ADF coefficient that estimated the relationship between rumen–reticulum ADF and dry mass for free-ranging deer at the WMA was not statistically different from the slope adjustment

Fig. 1. Regressions between acid detergent fiber and wet or dry mass of rumen–reticulum fill in white-tailed deer (*Odocoileus virginianus*) sampled from three sites in central and south Texas. Relationships were estimated for nonlactating females with mean values for body mass (39.03), time of kill (13.22), and back fat (1.57). Regressions with horizontal lines are not statistically significant (wet mass: the WMA (wildlife management area with free-ranging deer), the Pens (area with penned deer)). The wet mass regressions for the WMA and the Pens and the dry mass regressions for the WMA and the Ranch were statistically similar but off-set slightly for purposes of display.



coefficient for free-ranging deer at the Ranch (ADF:Ranch interaction, Table 3). Which is why we concluded that the ADF – dry mass relationship did not differ between the two sites. Nonetheless, the slope adjustment coefficient for the deer from the Ranch was noticeably greater than the ADF coefficient that estimated the relationship for deer from the WMA. The magnitude of the slope adjustment coefficient for deer from the Ranch might help explain why the model with the site covariate had a noticeably smaller AIC_c than models with a diet covariate. The diet covariate reflected whether the diet was predominantly pelleted or not pelleted. It is plausible that we might have detected statistical differences between these two sites in the relationships between ADF and dry mass if we had samples from the Ranch that spanned as wide a time span, and hence a wider range of environmental heterogeneity, as we did for deer sampled at the WMA. It should also be pointed out that the differing ways that deer were collected between the two sites might also have influenced our findings.

We did not find agreement in the relationships between rumen–reticulum ADF and wet mass or rumen–reticulum ADF and dry mass. Evidently, dry mass of rumen–reticulum fill reflects rate of food intake, fermentation, and forage particle passage, but wet mass also appears to be influenced by diet type, forage particle size, and microbial activity (Luna et al. 2012). Diets varied among sites. Deer from the Ranch had access to much more opuntia than deer from the WMA, which had access to acorns and leaves of woody plants and forbs (Arnold and Drawe 1979; Everitt and Gonzalez 1981; Warren and Krysl 1983). These forages were assumed to be unavailable to deer at the Pens. The size distribution of forage particles that pass through the reticulo-omasal sphincter has not been studied in free-ranging white-tailed deer. In domestic sheep, fecal particle size is not constant but varies with reproductive state and diet (Helander et al. 2014). A small particle size for an animal consuming a predominantly opuntia diet, for example, might not be the same as small particle sizes for animals consuming a predominantly leafy browse and acorn diet.

Relationships between body mass and CRIF with dry and wet masses might have been distorted if sex, whether females were lactating or not, time of day that animals were killed, and back fat were not considered. Sex and time of kill, as well as the interaction between sex and time of kill, seem to be needed because males and females often differ in feeding times, which determines time since the last meal (Short et al. 1969; Luna et al. 2012; Aiken et al. 2014; Duarte et al. 2014). Time of kill might also be useful for controlling for variation in composition of ADF when estimating relationships between ADF and response variables (Fig. 1). The longer that ingesta is in the rumen–reticulum, the more likely it is that ADF contains more indigestible fiber and less cellulose. Back fat, a measure of animal condition, is often not considered in studies of gut variation (Weckerly et al. 2003; Weckerly 2010). Yet, the findings from two other studies suggest that animal condition might influence food intake and, concomitantly, gut fill (Luna et al. 2012; Duarte et al. 2014).

Detecting digestive functions in free-ranging animals from gut fill is challenging. Large sample sizes and measuring appropriate covariates is necessary because gut fill is influenced by body mass, diet, reproductive state, animal condition, and time since the last meal. Yet, if animals from a location are collected under a wide range of environmental conditions, then it is possible to estimate relationships between dietary CRIF and rumen–reticulum fill that can be used to infer whether food intake is influenced by chemostatic factors or physical distension of the gut. In the past, study of the factors that governed food intake required the use of tame animals that were usually in highly controlled environmental settings (Robbins 1983; Barboza et al. 2009). What usually limits nutrient assimilation in mammalian herbivores is not finding enough forage to consume, but rather the rate at which food is digested (Crawley 1983). Thus, knowledge of what drives dietary CRIF – gut fill relationships might have implications for what influences food intake. Fundamental to evaluating growth and

production constraints in free-ranging herbivores is what governs food intake.

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