RUMEN-RETICULUM ORGAN MASS AND RUMEN MUCOSA SURFACE AREA

OF WHITE-TAILED DEER (*ODOCOILEUS* VIRGINIANUS)

CONSUMING TWO DIETS

by

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DEDICATION

I would like to dedicate this thesis to my father Wayne Allen Spilinek.

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ABSTRACT

Ungulate diets display spatial and temporal variation. To accommodate dietary variation, elasticity, organ mass, and absorptive capacity presumably change in the rumen-reticulum. The gastrointestinal organs, where most of the capacity and where most digestion occurs. I measured rumen-reticulum organ mass and absorptive capacity in white-tailed deer (*Odocoileus virginianus*) collected at Kerr Wildlife Management Area, Texas. I hypothesized that nutrient poor diets would result in greater mass-specific food intake because of low nutrient concentrations. The consequence would be heavy rumen-reticulum organs and low absorptive capacity. Since weaning, deer were fed a pelleted diet, ad libitum, of 1.77 or 2.67 kcal/gm digestible energy. In December 2017, 4.5- and 5.5-year-old deer were euthanized, the rumen-reticulum was extracted, thoroughly rinsed, wrung out, and weighted. Four, 1 x 3 cm samples were cut from four regions of the rumen. The samples were fixed in ExCell plus^{TM} for 24 hours, then preserved in 70% ethanol until measured. For each 1 cm^2 sample, I measured length and width of 10 randomly selected papillae, counted papillae density, and calculated a surface enlargement factor (SEF). My surrogate of food intake was first molar height measured on the right side of the jaw. Diet consumed by deer was unbeknownst to the measurer. Analyses of general linear models indicated that deer consuming the low energy diet had higher food intake, heavier rumen-reticulum organs, and lower SEF than deer consuming

the higher energy diet. White-tailed deer adjust rumen-reticulum morphology to maintain digestive functions when diets vary in energy content.

I. RUMEN-RETICULUM ORGAN MASS AND RUMEN MUCOSA SURFACE AREA OF WHITE-TAILED DEER (*ODOCOILEUS VIRGINIANUS*) CONSUMING TWO ENERGY DIETS

Introduction

Adjustments to the morphology of the rumen and reticulum because of variation in energy concentration of the diet must differ from the hind gut. The small and large intestines are tubular in morphology. When the diet is highly digestible, length of the intestines tends to increase to provide greater absorptive surface area to optimize nutrient assimilation (Sibly 1981, Taghon 1981, Weckerly 1989, Jenks et al. 1994, Zimmerman et al. 2006). In contrast, the rumen-reticulum cannot simply increase or shorten in length because these organs are voluminous, not tubular (Van Soest 1994).

The rumen-reticulum is the part of the gut where most fermentation and digestion occur (Van Soest 1994, Cronje et al. 2000). The disproportionate amount of digestion that occurs in the rumen-reticulum is facilitated by the process of selective particle delay. Ingested particles are not passed to the hind gut until they are smaller in size (Robbins 2013). Consequently, larger particles are regurgitated to the buccal cavity for further comminution. As a result, ingesta is retained in the rumen-reticulum for a considerable period of time and there is flow of ingesta and fluids in multiple directions in the rumen-reticulum (Van Soest 1994, Barboza et al. 2009).

Adjustments to rumen-reticulum anatomy in response to variation in dietary energy concentration can occur in at least three ways. One means is simply through variation in stretch of the organs to allow a greater volume to house more ingesta and fluids. The rumen-reticulum appears to have considerable stretch (Luna et al. 2012). The

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ability to stretch seems to be needed to provide spare capacity to accommodate short-term predictable and unpredictable changes in food intake that can arise from food limitations and climatic fluctuations that affect animal appetite (Weckerly 2010, Luna et al. 2012, Thompson and Barboza 2013, Aiken et al. 2014). Thus, considerable stretch is inherent to a functioning rumen-reticulum.

The rumen-reticulum organs might also vary in mass in response to changes in dietary bulk (gm/volume) (Weckerly et al. 2018). One way that bulk can increase is when the diet is difficult to digest and food intake is high. Consequently, forage is retained longer, there is more ingesta, and rumen fill is greater. Rumen-reticulum organs might then be heavier to provide reinforcement and maintain rumen motility (Veiberg et al. 2009, Weckerly et al. 2018).

Another possible response by the rumen-reticulum is from the layer of mucosa that lines the lumen. One of the functions of the mucosa is passive absorption of nutrients across the rumen wall (Hofmann et al. 1988, Van Soest 1994). Adjustments to the amount of absorptive surface area is driven by variation in size and density of papilla, collectively summarized by the surface enlargement factor (SEF). Variation in SEF is positively associated with variation in volatile fatty acid concentration in the rumen. When the diet is more digestible and presumably nutrient rich, volatile fatty acid concentration is high and more SEF is needed for absorptive capacity (Schären et al. 2016). When the diet is less digestible, mucosal surface area should decline to reduce energy demands of maintaining energetically demanding mucosal tissue (Baldwin et al. 1998)

I examined morphological adjustments to rumen-reticulum organ mass and SEF of white-tailed deer that consumed two pelleted diets that differed in digestible energy. The rumen-reticulum might respond to variation in digestible energy in two ways. There might be a positive response in both organ mass and SEF to increased digestible energy. In free-ranging deer food intake and dietary nutrition is usually low in winter whereas the opposite occurs in summer. Consequently, to accommodate increased food intake and greater bulk, rumen-reticulum organ mass is heavier, and SEF is also greater because of increased VFA production. Seasonal changes in rumen-reticulum fill, dietary nutrition, and SEF of free-ranging animals support this possibility (Hoffmann and Nygren 1992, Jenks et al. 1994, Zimmerman et al. 2006). Alternatively, there might be an inverse response between organ mass and SEF when the concentration of digestible energy is consistently low or high. When the concentration of digestible energy in the diet is low across seasons, deer can increase food intake to attempt to compensate for lower nutrient concentration (Verme and Ozoga 1980). Referred to as the instantaneous response (Meyer et al. 2010). The increased food intake should result in greater rumen-reticulum organ mass because of rumen fill. But greater SEF is not needed because VFA concentrations are low. Conversely, when animals consume a readily digestible, high energy diet an elevated food intake is not needed. Therefore, rumen-reticulum organ mass should be light, but SEF should be high to increase absorptive capacity because of higher VFA concentrations.

The objective of my study was to examine morphological changes to the rumen-reticulum caused by variation in energy concentrations of the diet. Specifically, I tested whether there was a positive or inverse relationship between rumen-reticulum organ mass

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and SEF to low and high energy diets. The findings from my study should increase the understanding about digestive functions in the rumen-reticulum when diets vary in energy concentrations and factors that prompt morphological plasticity in these organs.

Methods

Captive colony

The deer for this study were housed at the research pens (hereafter pens) located at Kerr Wildlife Management Area (30.0633° N, 99.5066° W), Kerr County, Texas. The pens totaled 6.5 ha. There were six 0.3 ha breeding pens, three 1.6 ha rearing pens, and 24 holding pens for handling animals (Harmel et al. 1989). Every pen was surrounded by a 2.5 m tall fence. The breeding and holding pens have housed deer since 1978 and, consequently, there was little natural forage available in the pens from 2013 to 2017 which was the time my study was conducted (Weckerly et al. 2018). The climate in the area was semi-arid. Temperatures ranged from an average low of 17.3 ℃ to a high of 34.4 ℃ during summer months and an average low of 1 ℃ to a high of 17.6 ℃ during winter months. Annual precipitation averaged 80 cm (Luna et al. 2012).

Diets

The deer were fed one of two kinds of pelleted feed that differed in digestible energy (Table 1). The low and standard energy diets contained about 1.77 kcal/gm and 2.67 kcal/gm of digestible energy, respectively (Appendix A). Across the time of this study, each of the seven batches of the standard and low energy pelleted diets fed to deer

were analyzed by Dairy One[TM], Ithaca, New York. Each week pelleted diets were supplemented with 1 kg of either alfalfa or straw, per deer, to provide roughage to maintain rumen function. Since weaning, every deer in the two populations were fed the same energy diet.

Data collection

Animals were euthanized in either a CO_2 chamber (n =66, Kinsey et al. 2016) or they were shot in the neck or head with a center fire rifle (n = 8). The self-contained CO_2 chamber was on a 4.3 m trailer. The protocol for use of the CO_2 chamber required that animals fasted 24 hr. before sacrifice. Animals were dispatched on December 4, 8, and 11, 2017. There were 11 females (6 – 4.5 yrs. old, 5 – 5.5 yrs.) and 15 males (8, 7) that consumed the standard energy diet and 23 females (12, 11) and 25 males (19, 6) that consumed the low energy diet. Every deer was ear-tagged at birth for individual identification, so I had known ages of every animal. Height of the first molar (M_1) from the gum line to the highest cusp was measured to the nearest 0.01 mm on the labial side of the right side of the mandible using an electronic caliper. I used M_1 height as an indicator of tooth wear and forage intake. Deer that ingest large amounts of high fiber food to meet demands require more chewing to comminute forage and should have more tooth wear than deer consuming a diet with low fiber content where a low food intake can meet demands (Veiberg et al. 2007, Perez-Barberia et al. 2015). Antlers from male deer were removed just above the pedicle shortly after antler velvet had been rubbed off the previous October. The removed antlers were weighed to the nearest 0.01 kg. If the

standard energy diet is, in fact, more nutritious then these males should have heavier antlers, relative to body mass, and greater body mass. Females consuming the low energy diet might also be lighter in mass than females on the standard energy diet if they have not had time to grow and reach asymptotic body mass (Weckerly 1998, Barboza and Bowyer 2000, Keyser et al. 2005).

Dissection of every animal occurred within 1 hour of euthanasia. A mid-ventral incision was made from the upper sternum to the anus in sacrificed deer and internal organs were extracted. Dressed body mass was the mass of the animal minus internal organs and blood loss and was weighed to the nearest 0.1 kg. Dressed body mass was used as our measure of body mass to reduce heterogeneity from diurnal variation in fill of the gastrointestinal tract. The rumen-reticulum was separated from the remaining gastrointestinal tract by severing the esophagus 5 cm above where the esophagus joins the reticulum and another ligation at the reticulo-omasal sphincter (Luna et al. 2012). The rumen-reticulum was inverted and rinsed thoroughly to remove plant particles adhering to rumen-reticulum mucosa, reverted and wrung out to remove excess water, and then weighted to the nearest 0.1 kg. Next, four, 1 x 3 cm strips were cut out of the rumen wall from four regions: the atrium ruminis, dorsal sac, ventral sac, and the caudal-dorsal blind sac (Luna 2013). These regions were chosen so that I sampled anterior, posterior, dorsal and ventral regions of the rumen. Each strip was fixed in ExCell plus[TM], (Clark et al. 2012), a formalin alternative consisting of a mixture of glyoxial, ethanediol, and ethanol, for 12 to 24 hours. The rumen strips were then preserved in 70% Ethanol until papillae were measured.

Papillae measurement

Using a Nikon SMZ 745 stereo dissecting microscope and crosshair grid of 0.1 mm measurements, 10 papillae were chosen at random in each of the 1 x 3 cm rumen strips. Papillae were collected in two rows along the 1 x 3 cm strip. A random quadrant was assigned using the crosshair grid to randomize papillae selection. For each papilla, I measured length and width at the top and bottom. I then counted all papillae within a 1 x 1 cm square *(base surface)* to quantify the density *(papillae number)*. In each region of the rumen, the surface enlargement factor (SEF) was calculated using the following equation:

$$\frac{[(2 \ x \ papillae \ surface) \ x \ papillae \ number + base \ surface]}{base \ surface}$$

where papillae surface is the product of papillae length and width and base surface area is 1 cm^2 (Hoffmann and Nygren 1992, Luna 2013). The SEF measured in each region was averaged for each white-tailed deer. Lastly, I did not know the diet consumed by a deer until after I had taken SEF measurements from all deer.

Statistical analyses

I used general linear models to analyze response variables (Dobson and Barnett 2018). The response variables were antler mass, dressed body mass, M_1 height, rumen-reticulum organ mass, and SEF. The first set of analyses examined whether age or an interaction involving diet influenced response variables. Age might confound responses from diet on rumen-reticulum organ mass and SEF (Veiberg et al. 2009, Duarte et al.

2011). There might also be an interaction between diet and dressed mass or diet and sex on response variables (Bartoskewitz et al. 2003, Weckerly 2010, Bonin et al. 2016). For antler mass, as an example, the regression model was

$$Antler\ mass = \beta_o + \beta_1 Dr.mass + \beta_2 Diet + \beta_3 Dr.mass \times Diet + \beta_4 Age + \epsilon$$

where ϵ is residual variance. For antler mass, a second model was analyzed which included dressed mass and diet and the interaction which was statistically significant ($P < 0.05$, see Results). The same general plan was followed for the other response variables. For rumen-reticulum organ mass, the predictors were identical to equation 1. Because antler mass and rumen-reticulum organ mass covary with sex and dressed body mass, dressed body mass and not sex was included as a predictor for these two response variables. For dressed mass, M_1 height, and SEF, the predictors were sex, diet, and the interaction between sex and diet.

Results

For the 74 white-tailed deer collected, the ranges of dressed body mass were 27 to 68 kg, 0.5 to 1.5 kg for rumen-reticulum organ mass, 3.19 to 11.55 for SEF, and 3.67 to 12.60 mm for M1 height (Table 2). For the low and high energy diets, the crude protein contents were similar (Table 1) but fiber and ash concentrations were higher in the low energy diet. The percent dry matter digestibility for the low and standard energy diets were 41.40 and 61.50, respectively. Also, the consistency in nutritional attributes among batches of each diet were similar as coefficients of variation ranged from 0.02 to 0.12.

All response variables were analyzed to determine if age and interactions involving diet were influential. Age had no influence on any response variable (Appendix

B). For antler and rumen-reticulum organ masses the interaction between diet and dressed body mass was statistically non-significant. In contrast, the interaction between diet and sex was influential for dressed body mass, M_1 height, and SEF. In further analyses age and all non-significant interactions were removed.

For antler mass, diet and dressed body mass were influential (Table 3). Controlling for dressed body mass, antler mass was lighter in males that consumed the low energy diet. Findings from analyses of dressed body mass were more complex. There was a statistically significant interaction between sex and diet. The magnitude of the difference in dressed body mass was greater in males than females. Nonetheless, for both sexes deer that consumed the high energy diet were heavier. There was also a statistically significant interaction between sex and diet for M_1 height. For M_1 height, however, the magnitude of the difference was greater in females than males (Fig. 1). Females had greater tooth wear (lower M_1 heights) than females that consumed the standard energy diet. Males that consumed the low energy diet tended to have greater tooth wear but mean M_1 heights were not statistically different from males that consumed the standard energy diet. The response of SEF to diet and sex was also complex because of an interaction between diet and sex (Fig. 2). Females that consumed the high energy diet had higher SEF than females that consumed the low energy diet, but males had similar SEF regardless of the diet consumed. There was also an effect from diet and dressed body mass on rumen-reticulum organ mass (Fig. 3). Regardless of dressed body mass, deer that consumed a low energy diet had a heavier rumen-reticulum organ mass than deer that consumed the high energy diet.

Discussion

My findings are consistent with an inverse relationship between rumen-reticulum organ mass and SEF. The height of M_1 molars, on average, was shorter in deer fed the low energy diet; indicating greater tooth wear from mastication than by deer fed the standard energy diet (Veiberg et al. 2007). Greater mastication might have been associated with higher food intake to compensate for lower ingestion of energy, the instantaneous response (Meyer et al. 2010). Greater food intake might have increased rumen-reticulum fill (Aiken et al. 2014). When rumen-reticulum fill is greater the rumen-reticulum organ mass should be heavier to maintain rumen motility. The low energy diet should have lower volatile fatty acid production in the rumen than the standard energy diet. Thus, less mucosal surface area is needed in the rumen for nutrient absorption (Titus and Ahearn 1992, Dijkstra et al. 1993, Mathiesen et al. 2000, Soveri and Nieminen 2007, Schären et al. 2016).

Tooth wear in ungulates has been observed to differ between the sexes as predicted by the disposable-soma hypothesis of senescence (Kirkwood et al. 1985). This hypothesis is founded in tissue repair that is correlated to life expectancy and reproduction. Males display greater tooth wear than females in polygynous species, presumably because of a greater investment in reproductive quests than to longevity of life (Van Deelen et al. 2000, Carranza et al. 2004). In my study I recorded a higher rate of tooth wear in males for both the low and standard energy diets. No difference in tooth wear was detected between the two diets in males, this is thought to be due to less tissue

repair of tooth enamel, or softer enamel, in males than females. As such wear from mastication affects males of the two diets in a similar fashion.

Contractions of the rumen-reticulum occur multiple times in a minute in white-tailed deer and function to provide rumen motility (Dziuk et al. 1963). In *Cervus elaphus* rumen-reticulum contractions did not differ across seasons when food intake and dietary bulk did differ (Stafford et al. 1993). With a constant rate of contractions, a more muscular rumen-reticulum is probably needed to maintain rumen motility of the bulkier gut contents. An increase in muscle mass of the rumen-reticulum should lead to a heavier rumen-reticulum organ mass (Álvarez-Rodríguez et al. 2012, Lima et al. 2019).

A prior study examining rumen-reticulum organ mass compared browse and pellet diet types that differed in bulk from differences in particle sizes (Weckerly et al. 2018). In this study, both the low and standard energy diets were pelleted and, thus, similar types of diets with, presumably, similar particle sizes. Bulk probably differed from food intake of deer was likely due to energy concentration of the diets and not differences from particle sizes. The consequence might have been greater bulk from higher rumen fill. The mass of the rumen-reticulum organs therefore can vary in a variety of ways depending upon how bulk can manifest in the rumen (Knott et al. 2004, Weckerly et al. 2018).

In previous studies of free ranging cervids at high latitudes, the SEF varied with seasonal changes in diet quality (Hofmann et al. 1988, Jiang and Hudson 1996, Jiang et al. 2003, Zimmerman et al. 2006, Clauss et al. 2009).The SEF was low in winter and high in summer. Also rumen-reticulum organ mass followed the same seasonal pattern, light winter and heavy in summer. In summer and autumn when nutritious food had a greater

availability, rumen fill tended to be high as well (Hofmann et al. 1988, Jenks et al. 1994, Zimmerman et al. 2006). In this setting, it is not surprising that rumen-reticulum organ mass and SEF are positively related. When there is low intake of poor quality forage there is little need for a heavy rumen-reticulum organ or increased SEF.

One facet to variation in SEF in the rumen is diet type (Wang et al. 2017). Changes in SEF because of diet type is rarely mentioned. Not only might SEF vary because of dietary changes that prompt rumen-reticulum changes in volatile fatty acids, but also there might be a role of diet type. In my study, it is likely that SEF differed because of diet type. Deer that consume low and standard energy diets both consumed a pelleted ration or the same type of diet.

For my study, mean surface enlargement factor did not differ in males between the low and standard energy diets. It is thought that the collection date could have influenced similar SEF between the low and standard energy diets. Data was collected in early December, which coincides with the latter part of the mating season (Weckerly et al. 2018). Hypophagia in the mating season might be why there were no significant differences in SEF between the two pelleted diets for males (McMillin et al. 1980). Due to short term adaptations of the rumen-reticulum, from hypophagia, papillae probably regressed (Knott et al. 2004). Males appeared to respond to the demands of the mating season more than to energy differences in the diet.

If the low energy diet was limiting to deer, then body mass should be lighter in females and males as should antler mass in males. I found a noticeable difference in dressed body mass and antler mass between the two diets. Specifically, males and females that consumed the standard energy diet had heavier body mass and males had

heavier antler mass relative to body mass. In my study, the low energy diet appeared to be limiting to the extent that deer did not appear to be able to compensate for the low energy dietary energy concentration by consuming more feed.

The voluminous rumen-reticulum seems to adjust to changes in the amount of ingesta in the lumen and the energy concentration of that ingesta. The amount of ingesta appears to influence the rumen-reticulum organ mass and the energy concentration in that ingesta appears to influence the mucosal surface area. Plasticity in rumen-reticulum morphology appears to be quite large in white-tailed deer (Luna et al. 2012, Bonin et al. 2016). The enormous plasticity appears, in part, because these organs respond to multiple dietary factors.

Table 1. Means, standard deviations (Sd), coefficient of variations (CV), and ranges of crude protein, fiber variables, and ash concentrations of seven batches of low energy and high energy pellets fed to a captive colony of white-tailed deer from 2013 to 2017, Kerr Wildlife Management Area, Texas. Also, displayed is the descriptive statistics of trials estimating dry matter digestibility. Summaries of pooled and un-pooled t-tests comparing crude protein, fiber variables, and ash concentrations of the low and high energy pellets is reported.

| Variable | **Low energy** | | | | | **High energy** | | | | | | | |
	Mean	Sd	CV	Range	*n*	Mean	Sd	CV	Range	*n*	*t*	*df*	*P*
Crude protein	17.83	0.44	0.02	16.90-18.30	7	18.04	0.29	0.02	17.70-18.60	7	-1.08	10.36	0.304
Acid detergent fiber	28.10	3.49	0.12	23.80-33.40	7	19.21	1.45	0.08	16.90-21.40	7	19.46	6.00	>0.001
Neutral detergent fiber	36.93	2.34	0.06	33.10-39.60	7	27.40	2.08	0.08	25.20-30.60	7	8.04	12.00	>0.001
Ash	19.81	0.57	0.03	19.08-20.41	7	11.46	0.53	0.05	10.93-12.34	7	28.50	12.00	>0.001
Dry matter digestibility	41.40	5.10			4	61.50	3.37			3			

Table 2. Summaries of dressed body mass (DBM), rumen-reticulum organ mass (RROM), surface enlargement factor (SEF), and first molar height (M_1) of white-tailed deer collected from captive colony at the Kerr Wildlife Management Area, Texas, USA. Summaries were displayed by sex and low (L) or high (H) energy pelleted diets. 11 females (6 – 4.5 yrs. old, 5 – 5.5 yrs.) and 15 males (8, 7) that consumed the high energy diet and 23 females (12, 11) and 25 males (19, 6) that consumed the low energy diet.

Sex	Diet	DBM			RROM			SEF			M_1		
		Mean	Sd	Range	Mean	Sd	Range	Mean	Sd	Range	Mean	Sd	Range
F	H	35.591	3.316	32.0-41.6	0.696	0.149	0.50-0.90	6.975	1.999	4.145-11.553	9.436	1.734	6.90-12.30
M	H	55.667	6.602	44.5-68.1	0.803	0.163	0.60-1.15	5.512	1.512	3.481-8.127	6.646	1.140	4.80-8.80
F	L	33.835	2.936	27.8-38.5	0.741	0.149	0.50-0.95	5.244	1.296	3.191-8.005	7.173	1.120	5.70-9.60
M	L	50.252	4.956	43.3-59.7	0.934	0.189	0.60-1.50	5.928	1.570	3.640-9.323	5.859	1.254	3.67-8.56

Table 3. Summaries of analyses of antler mass, dressed body mass (DBM), M_1 height, surface enlargement factor, and rumen-reticulum organ mass for white-tailed deer from a captive colony at the Kerr Wildlife Management Area, Texas, USA. The reference categories were female and high energy diet. The colon denotes an interaction.

Predictors	Estimate	SE	t	P
Antler mass				
Intercept	0.513	0.599	0.856	0.398
DBM	0.017	0.010	1.703	0.097
Diet	-0.295	0.138	-2.135	0.039
Dressed body mass				
Intercept	35.591	1.395	25.505	<0.001
Diet	-1.756	1.697	-1.035	0.304
Sex	22.076	1.837	12.016	<0.001
Diet:Sex	-5.559	2.272	-2.446	0.017
M_1 height				
Intercept	9.436	0.384	24.566	<0.001
Diet	-2.263	0.467	-4.845	<0.001
Sex	-2.790	0.506	-5.518	<0.001
Diet:Sex	1.476	0.626	2.359	0.021
Surface Enlargement Factor				
Intercept	6.975	0.467	14.930	<0.001
Sex	-1.464	0.615	-2.380	0.020
Diet	-1.731	0.568	-3.048	0.003
Diet:Sex	2.148	0.761	2.824	0.006
Rumen-reticulum organ mass				
Intercept	0.286	0.090	3.186	0.002
Diet	0.141	0.039	3.614	<0.001
DBM	0.010	0.002	5.580	<0.001
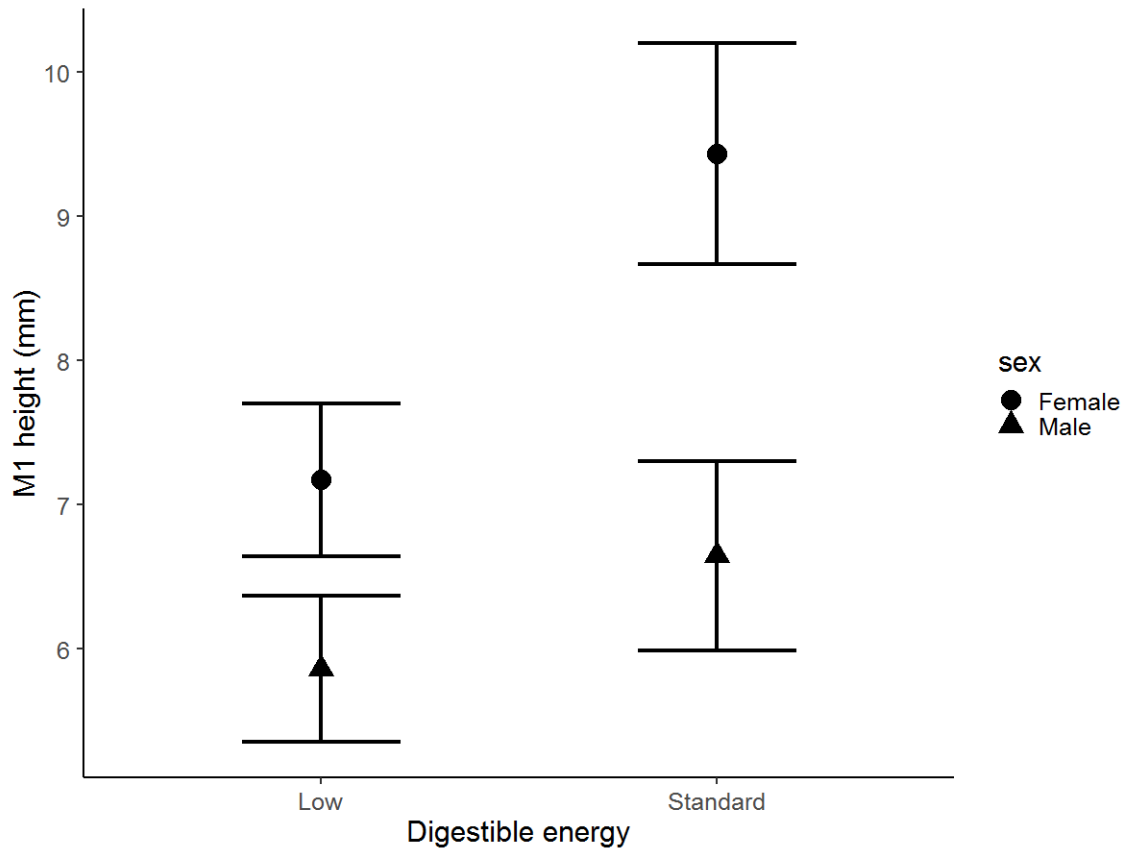
Figure 1. Means and 95 percent confidence intervals of female and male M_1 heights of a captive colony of white-tailed deer that consumed a low and standard energy diet at Kerr Wildlife Management Area, Texas, USA.

Figure 2. Means and 95 percent confidence intervals of females and male surface enlargement factor (SEF) of a captive colony of white-tailed deer that consumed a low or standard energy diet at Kerr Wildlife Management Area, Texas, USA.

*Mean SEF was calculated from four different regions of the rumen.

Figure 3. Mean and 95 percent confidence intervals of rumen-reticulum organ mass (kg) relative to dressed body mass was in a captive colony of white-tailed deer that consumed low or standard energy diet, Kerr Wildlife Management Area, Texas, USA.

APPENDIX A

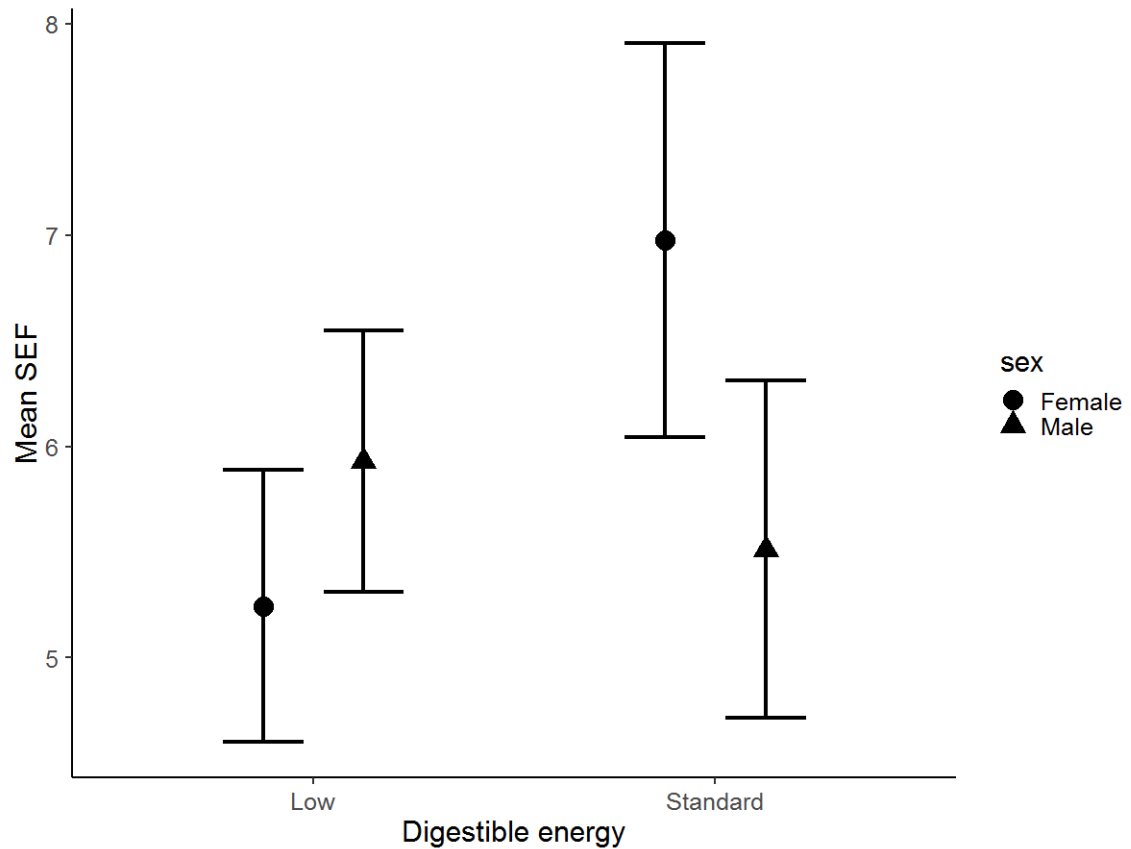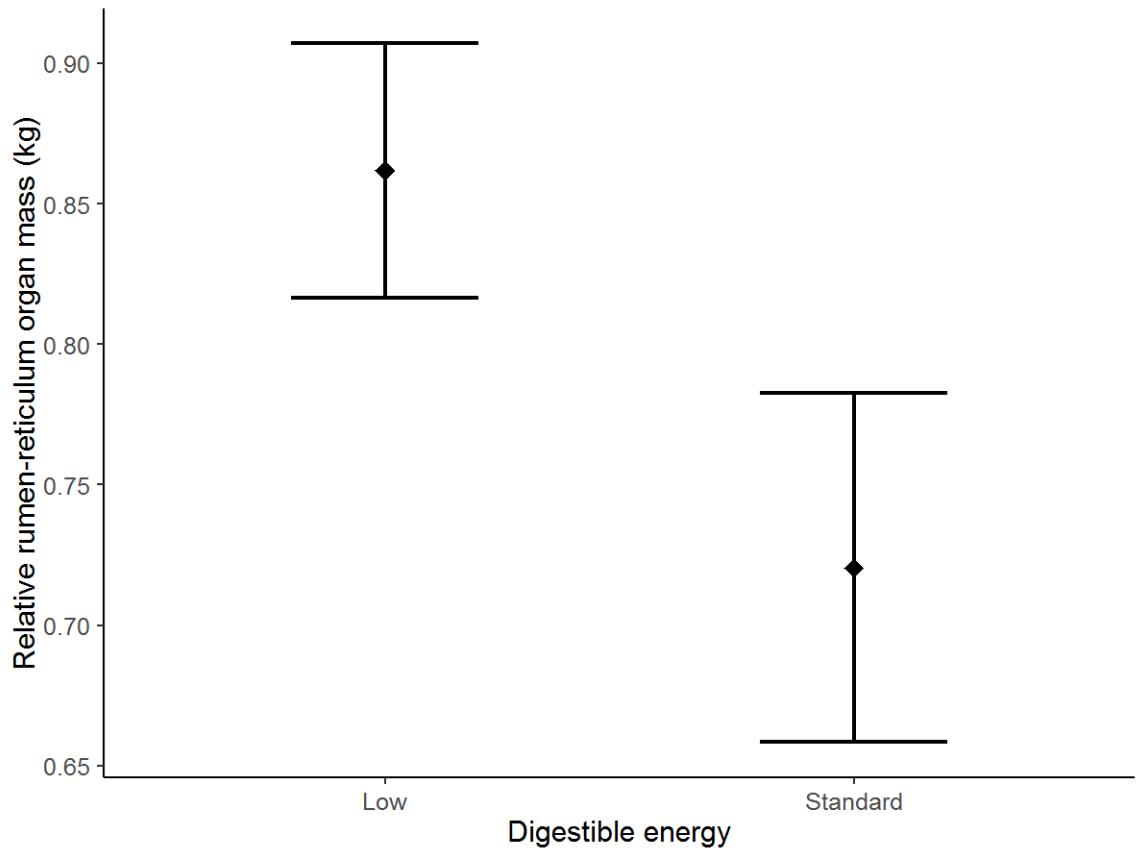**Table A1**. Studies from the literature estimating digestible dry matter (DDM) and digestible energy (DE) of browse or pellet diets in digestion trials of white-tailed deer. Browse diets were included to increase sample size and because DDM was low for these diets. Browse diets were a mix of woody species trying to mimic a possible diet consumed by free ranging animals. Digestion trials were conducted with tame animals using the mass balance method. For each experimental animal gross energy content of the fed diet, food intake, and fecal output was measured to estimate DDM and DE (Barboza et al. 2009)

Author	DDM	DE	Diet
Thompson et al. 1973	70.00	3.01	Pellet
Thompson et al. 1973	71.00	3.06	Pellet
Thompson et al. 1973	74.00	3.13	Pellet
Thompson et al. 1973	77.00	3.32	Pellet
Thompson et al. 1973	83.00	3.58	Pellet
Wheaton and Brown 1983	54.95		Pellet
Campbell and Hewitt 2005	64.20	2.70	Pellet
Campbell and Hewitt 2005	56.50	2.43	Browse
Campbell and Hewitt 2005	49.90	2.17	Browse
Campbell and Hewitt 2005	44.30	1.94	Browse
Holter et al. 1977	64.90	2.84	Pellet
Holter et al. 1977	64.70	2.70	Pellet
Holter et al. 1977	68.00	2.92	Pellet
Holter et al. 1977	68.00	2.90	Pellet
Holter et al. 1977	67.10	2.93	Pellet
Holter et al. 1977	67.30	2.88	Pellet
Holter et al. 1977	64.70	2.84	Pellet
Holter et al. 1977	68.00	2.99	Pellet
Holter et al. 1977	64.30	2.82	Pellet
Holter et al. 1977	64.40	2.79	Pellet
Holter et al. 1977	57.70	2.54	Pellet
Holter et al. 1977	59.20	2.49	Pellet
Holter et al. 1977	68.10	2.94	Pellet
Holter et al. 1977	68.90	3.00	Pellet

Author	DDM	DE	Diet
Holter et al. 1977	65.80	2.83	Pellet
Amman et al. 1973	72.31	3.22	Pellet
Amman et al. 1973	62.64	2.78	Pellet
Amman et al. 1973	56.19	2.49	Pellet
Amman et al. 1973	52.96	2.35	Pellet
Amman et al. 1973	49.74	2.20	Pellet
Amman et al. 1973	43.29	1.91	Pellet
Ullrey et al. 1971	65.40	2.75	Pellet
Gray and Servello 1995	42.10	1.95	Browse
Gray and Servello 1995	42.70	2.07	Browse
Gray and Servello 1995	44.50	2.12	Browse
Gray and Servello 1995	48.30	2.24	Browse
Gray and Servello 1995	47.20	2.23	Browse
Gray and Servello 1995	48.80	2.30	Browse
Gray and Servello 1995	49.20	2.29	Browse
Gray and Servello 1995	49.20	2.39	Browse
Gray and Servello 1995	68.30	3.01	Pellet

Ammann, A. P., R. L. Cowan, C. L. Mothershead, and B. Baumgardt. 1973. Dry matter and energy intake in relation to digestibility in white-tailed deer. The Journal of Wildlife Management:195-201.

Campbell, T. A., and D. G. Hewitt. 2005. Nutritional value of guajillo as a component of male white-tailed deer diets. Rangeland ecology & management **58**:58-64.

Gray, P. B., and F. A. Servello. 1995. Energy-intake relationships for white-tailed deer on winter browse diets. Journal of Wildlife Management **59**:147-152.

Holter, J., and H. Hayes. 1977. Growth in white-tailed deer fawns fed varying energy and constant protein. The Journal of Wildlife Management:506-510.

Thompson, C. B., J. B. Holter, H. H. Hayes, H. Silver, and J. W. E. Urban. 1973. Nutrition of white-tailed deer. I. Energy requirements of fawns. Journal of Wildlife Management **37**:301-311.

Ullrey, D., W. Youatt, H. Johnson, L. Fay, D. Purser, B. Schoepke, and W. Magee. 1971. Limitations of winter aspen   browse for the white-tailed deer. The Journal of Wildlife Management:732-743.

Table A2. Attributes of individual white-tailed deer where dry matter digestibly (DDM, %) was measured for the low or high energy pellets fed to deer at the pens, Kerr Wildlife Management Area, Kerr County, Texas. The digestion trials were conducted at Texas A&M University, Kingsville by Dr. David Hewitt in April, 2014.

Deer age	Sex	Body mass (kg)	Low energy	High energy
4	Female	42.18	X	
5	Female	42.18	X	X
6	Male	65.32	X	
3	Male	58.97	X	X
5	Male	43.09		X

Table A3. Summary of linear mixed effects model examining influences from diet (browse, pelleted) and digestible dry matter (DDM) on digestible energy. Reported are means and 95 percent confidence intervals (LB, UB) of fixed effects and standard deviations and confidence intervals of standard deviations of study (random intercept)

Fixed effects	Estimate	LB	UB
Intercept	0.0177	-0.052	0.210
Diet pellet	-0.122	-0.188	-0.053
DDM	0.044	0.041	0.046
Random effects	**Sd**	**LB**	**UB**
Residual	0.037	0.029	0.047
Study	0.070	0.034	0.129

and residual. Browse diet was the reference category.
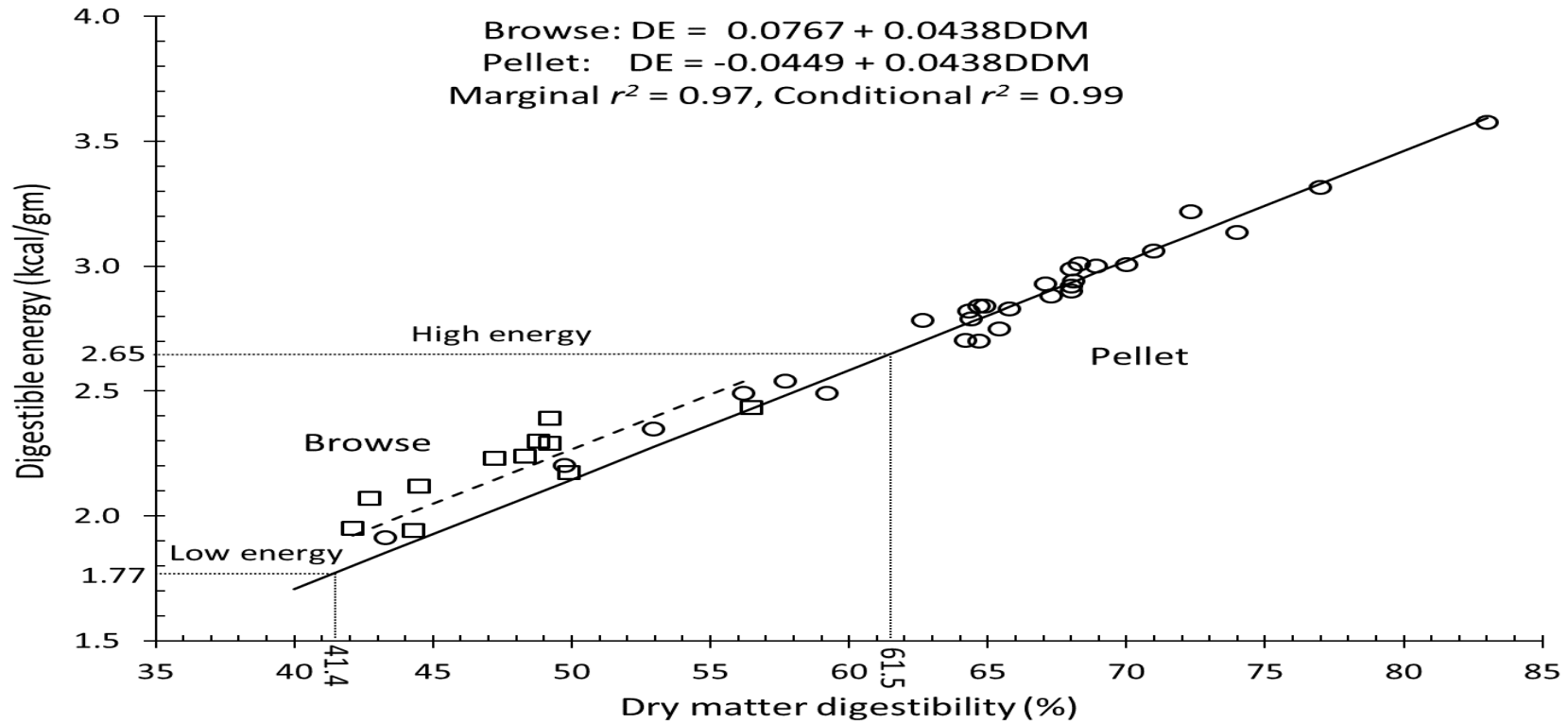
Figure A1. Scatterplot of data and with regressions, and marginal and conditional r^2 values, estimated from linear mixed effects model (Table A3). Also displayed are the predicted values of digestible energy with digestible dry matter estimated for the low and standard energy diets. The marginal r^2 estimated the proportion of variation in digestible energy accounted for by the fixed effects of dry matter digestibility and diet, and the conditional r^2 estimated the influence of both fixed and random effects.

Table B1. Preliminary analysis looking at all predictors and interactions for antler mass, dressed body mass (DBM), M_1 height, and surface enlargement factors for white-tailed deer from a captive colony at the Kerr Wildlife Management Area, Kerr County Texas, USA. The reference category for diet is high energy pellets, sex is female. Age represents the true age, and colons denote interactions.

Predictors	Estimate	SE	t	P
Antler mass				
Intercept	-0.239	1.140	-0.210	0.835
DBM	0.025	0.015	1.698	0.098
Diet	0.578	1.174	0.493	0.625
Age	0.063	0.129	0.484	0.631
Diet:DBM	-0.016	0.021	-0.743	0.463
Dressed body mass				
Intercept	38.775	5.216	7.434	<0.001
Diet	-1.854	1.711	-1.084	0.282
Sex	21.971	1.853	11.860	<0.001
Age	-0.620	0.978	-0.634	0.5283
Diet:Sex	-5.601	2.283	-2.453	0.017
M_1 height				
Intercept	9.131	1.946	4.692	<0.001
Diet	-2.251	0.480	-4.692	<0.001
Sex	-2.867	0.898	-3.194	0.002
Age	0.033	0.273	0.123	0.903
DBM	0.004	0.033	0.112	0.911
Diet:Sex	1.499	0.662	2.264	0.027
Surface Enlargement Factor				
Intercept	9.131	1.946	4.692	<0.001
Diet	-2.251	0.480	-4.692	<0.001
Sex	-2.867	0.898	-3.194	0.002
Age	0.033	0.271	0.123	0.903
DBM	0.004	0.033	0.112	0.911
Diet:Sex	1.500	0.662	2.264	0.027
Rumen-reticulum organ mass				
Intercept	0.170	0.216	0.786	0.435
DBM	0.007	0.002	2.924	0.005
Diet	-0.112	0.161	-0.699	0.487
Age	0.047	0.032	1.470	0.146
Diet:DBM	0.006	0.003	1.704	0.093

LITERATURE CITED

Aiken, M., A. Duarte, R. Luna, D. Wolcott, and F. Weckerly. 2014. Daytime declines in rumen–reticulum fill of male white-tailed deer (Odocoileus virginianus) from south Texas. Canadian Journal of Zoology **92**:637-642.

Álvarez-Rodríguez, J., E. Monleón, A. Sanz, J. Badiola, and M. Joy. 2012. Rumen fermentation and histology in light lambs as affected by forage supply and lactation length. Research in veterinary science **92**:247-253.

Baldwin, A. L., L. M. Wilson, and J. E. Valeski. 1998. Ultrastructural effects of intravascularly injected polyethylene glycol-hemoglobin in intestinal mucosa. American Journal of Physiology-Heart and Circulatory Physiology **275**:H615-H625.

Barboza, P. S., and R. T. Bowyer. 2000. Sexual segregation in dimorphic deer: a new gastrocentric hypothesis. Journal of Mammalogy **81**:473-489.

Barboza, P. S., K. L. Parker, and I. D. Hume. 2009. Integrative wildlife nutrition.

Bartoskewitz, M. L., D. G. Hewitt, J. S. Pitts, and F. C. Bryant. 2003. Supplemental feed use by free-ranging white-tailed deer in southern Texas. Wildlife Society Bulletin:1218-1228.

Bonin, M., J.-P. Tremblay, and S. D. Côté. 2016. Contributions of digestive plasticity to the ability of white-tailed deer to cope with a low-quality diet. Journal of Mammalogy **97**:1406-1413.

Carranza, J., S. Alarcos, C. B. Sánchez-Prieto, J. Valencia, and C. Mateos. 2004. Disposable-soma senescence mediated by sexual selection in an ungulate. Nature **432**:215.

Clark, C. A., R. Vatsyayan, U. Hedner, C. T. Esmon, U. R. Pendurthi, and L. V. Rao. 2012. Endothelial cell protein C receptor-mediated redistribution and tissue-level accumulation of factor VIIa. J Thromb Haemost **10**:2383-2391.

Clauss, M., J. Adamczewski, and R. R. Hofmann. 2009. Surface enlargement in the rumen of free-ranging muskoxen (*Ovibos moschatus*). European Journal of Wildlife Research **56**:181-185.

Cronje, P., E. Boomker, P. Henning, W. Schultheiss, and J. van der Walt. 2000. Ruminant Physiology. Digestion, metabolism, growth and reproduction. UK: Cabi.

Dijkstra, J., H. Boer, J. Van Bruchem, M. Bruining, and S. Tamminga. 1993. Absorption of volatile fatty acids from the rumen of lactating dairy cows as influenced by volatile fatty acid concentration, pH and rumen liquid volume. British Journal of Nutrition **69**:385-396.

Dobson, A. J., and A. G. Barnett. 2018. An introduction to generalized linear models. CRC press.

Dziuk, H., B. Fashingbauer, and J. Idstrom. 1963. Ruminoreticular pressure patterns in fistulated white-tailed deer. American journal of veterinary research **24**:772-783.

Harmel, D. E., J. D. Williams, and W. E. Armstrong. 1989. Effects of genetics and nutrition on antler development and body size of white-tailed deer.*in* W. D. Texas Parks and Wildlife Department, Austin, editor.

Hoffmann, R. R., and K. Nygren. 1992. Ruminal mucosa as indicator of nutritional status in wild and captive moose. Alces **1**:77–83.

Hofmann, R. R., K. R. A., L. J., and A. H. 1988. Seasonal changes in rumen papillary development and body condition in free ranging Chinese water deer. Journal of Zoology **216**:103-117.

Jenks, J. A., D. M. L. Jr., R. L. Lochmiller, and M. A. Melchiors. 1994. Variation in Gastrointestinal Characteristics of Male and Female White-Tailed Deer: Implications for Resource Partitioning. Journal of Mammalogy **75**:1045-1053.

Jiang, Z., and R. J. Hudson. 1996. Digestive responses of wapiti *Cervus elaphus canadensis* to seasonal forages. Acta Theriologica **45**:415-423.

Jiang, Z. W., S. Takatsuki, W. Wang, J. Li, K. Jin, and Z. Gao. 2003. Seasonal changes in parotid and rumen papillae in Mongolian gazelle (*Procapra gutturosa* Pallas). Ecological Research **18**:65-72.

Keyser, P. D., D. C. Guynn, and H. S. Hill. 2005. Population density-physical condition relationships in white-tailed deer. The Journal of Wildlife Management **69**:356-365.

Kirkwood, T., C. Finch, and E. Schneider. 1985. Handbook of the Biology of Aging. Van Nostrand Reinhold Co., New York.

Knott, K. K., P. S. Barboza, R. T. Bowyer, and J. E. Blake. 2004. Nutritional development of feeding strategies in arctic ruminants: digestive morphometry of reindeer, Rangifer tarandus, and muskoxen, Ovibos moschatus. Zoology (Jena) **107**:315-333.

Lima, T. J., R. G. Costa, G. R. de Medeiros, A. N. de Medeiros, N. L. Ribeiro, J. S. de
Oliveira, R. R. Guerra, and F. F. R. de Carvalho. 2019. Ruminal and
morphometric parameters of the rumen and intestines of sheep fed with increasing
levels of spineless cactus (Nopalea cochenillifera Salm-Dyck). Tropical animal
health and production **51**:363-368.

Luna, R. S. 2013. Body size, rumen-reticulum functions, and dietary nutrition of white-
tailed deer. Texas State University- San Marcos.

Luna, R. S., A. Duarte, and F. W. Weckerly. 2012. Rumen–reticulum characteristics,
scaling relationships, and ontogeny in white-tailed deer (*Odocoileus virginianus*).
Canadian Journal of Zoology **90**:1351-1358.

Mathiesen, S. D., O. E. Haga, T. Kaino, and N. J. C. Tyler. 2000. Diet composition,
rumen papillation and maintenance of carcass mass in female Norwegian reindeer
(*Rangifer tarandus tarandus*) in winter. Journal of Zoology **251**:129-138.

McMillin, J. M., U. Seal, and P. Karns. 1980. Hormonal correlates of hypophagia in
white-tailed deer. Pages 2964-2968 *in* Federation proceedings.

Meyer, K., J. Hummel, and M. Clauss. 2010. The relationship between forage cell wall
content and voluntary food intake in mammalian herbivores. Mammal Review
40:221-245.

Perez-Barberia, F. J., S. L. Ramsay, R. J. Hooper, E. Perez-Fernandez, A. H. J.
Robertson, A. Aldezabal, P. Goddard, and I. J. Gordon. 2015. The influence of
habitat on body size and tooth wear in Scottish red deer (Cervus elaphus).
Canadian Journal of Zoology **93**:61-70.

Robbins, C. T. 2013. Wildlife feeding and nutrition. Academic press, INC., London.

Schären, M., G. M. Seyfang, H. Steingass, K. Dieho, J. Dijkstra, L. Huther, J. Frahm, A. Beineke, D. von Soosten, U. Meyer, G. Breves, and S. Danicke. 2016. The effects of a ration change from a total mixed ration to pasture on rumen fermentation, volatile fatty acid absorption characteristics, and morphology of dairy cows. J Dairy Sci **99**:3549-3565.

Sibly, R. 1981. Strategy of digestion and defecation. Physiological ecology: an evolutionary approach to resource use.

Soveri, T., and M. Nieminen. 2007. Papillar morphology of the rumen of forest reindeer (*Rangifer tarandus fennicus*) and semidomesticated reindeer (*R. t. tarandus*). Anat Histol Embryol **36**:366-370.

Stafford, K., C. Reid, T. Barry, and J. Suttie. 1993. Rumino-reticular motility in red deer (Census elaphus) fed chaffed lucerne hay during winter and summer. New Zealand Journal of Agricultural Research **36**:465-473.

Taghon, G. L. 1981. Beyond Selection: Optimal Ingestion Rate as a Function of Food Value. The American Naturalist **118**:202-214.

Thompson, D., and P. Barboza. 2013. Responses of caribou and reindeer (Rangifer tarandus) to acute food shortages in spring. Canadian Journal of Zoology **91**:610-618.

Titus, E., and G. A. Ahearn. 1992. Vertebrate gastrointestinal fermentation: transport mechanisms for volatile fatty acids. American Journal of Physiology - Regulatory, Integrative and Comparative Physiology **262**:R547-R553.

Van Deelen, T. R., K. M. Hollis, C. Anchor, and D. R. Etter. 2000. Sex affects age
    determination and wear of molariform teeth in white-tailed deer. The Journal of
    Wildlife Management:1076-1083.

Van Soest, P. J. 1994. Nutritional ecology of the ruminant. Cornell University Press.

Veiberg, V., A. Mysterud, E. Bjorkvoll, R. Langvatn, L. E. Loe, R. J. Irvine, C.
    Bonenfant, F. Couweleers, and N. C. Stenseth. 2007. Evidence for a trade-off
    between early growth and tooth wear in Svalbard reindeer. Journal of Animal
    Ecology **76**:1139-1148.

Veiberg, V., A. Mysterud, R. Irvine, W. Sørmo, and R. Langvatn. 2009. Increased mass
    of reticulo-rumen tissue and contents with advancing age in Svalbard reindeer.
    Journal of Zoology **278**:15-23.

Verme, L. J., and J. J. Ozoga. 1980. Influence of protein-energy intake on deer fawns in
    autumn. The Journal of Wildlife Management:305-314.

Wang, B., D. Wang, X. Wu, J. Cai, M. Liu, X. Huang, J. Wu, J. Liu, and L. Guan. 2017.
    Effects of dietary physical or nutritional factors on morphology of rumen papillae
    and transcriptome changes in lactating dairy cows based on three different forage-
    based diets. BMC genomics **18**:353.

Weckerly, F. W. 1989. Social bonding and aggression in female Roosevelt elk. Canadian
    Journal of Zoology **77**:1379-1384.

Weckerly, F. W. 1998. Sexual-size dimorphism: influence of mass and mating systems in
    the most dimorphic mammals. Journal of Mammalogy **79**:33-52.

Weckerly, F. W. 2010. Allometric scaling of rumenâ□"reticulum capacity in white-tailed
    deer. Journal of Zoology **280**:41-48.

Weckerly, F. W., G. Bhaskar, A. Duarte, R. S. Luna, and H. D. Starns. 2018. Heavier

rumen-reticulum organs in white-tailed deer (Odocoileus virginianus) is

consistent with dietary bulk not quality. Canadian Journal of Zoology **96**:748-752.

Zimmerman, T. J., J. A. Jenks, and D. M. Leslie. 2006. Gastrointestinal morphology of

female white-tailed and mule deer: effects of fire, reproduction, and feeding type.

Journal of Mammalogy **87**:598-605.