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Mule deer do more with less: comparing their nutritional requirements and tolerances with white-tailed deer

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Congeneric species often share ecological niche space resulting in competitive interactions that either limit co-occurrence or lead to niche partitioning. Differences in fundamental nutritional niches mediated through character displacement or isolation during evolution are potential mechanisms that could explain overlapping distribution patterns of congenerics. We directly compared nutritional requirements and tolerances that influence the fundamental niche of mule (*Odocoileus hemionus*) and white-tailed deer (*O. virginianus*), which occur in allopatry and sympatry in similar realized ecological niches across their ranges in North America. Digestible energy and protein requirements and tolerances for plant fiber and plant secondary metabolites (PSMs) of both deer species were quantified using in vivo digestion and intake tolerance trials with six diets ranging in content of fiber, protein, and PSMs using tractable deer raised under identical conditions in captivity. We found that compared with white-tailed deer, mule deer required 54% less digestible protein and 21% less digestible energy intake per day to maintain body mass and nitrogen balance. In addition, they had higher fiber, energy, and dry matter digestibility and produced glucuronic acid (a byproduct of PSM detoxification) at a slower rate when consuming the monoterpene α-pinene. The mule deers' enhanced physiological abilities to cope with low-quality, chemically defended forages relative to white-tailed deer might minimize potential competitive interactions in shared landscapes and provide a modest advantage to mule deer in habitats dominated by low-quality forages.

Key words: detoxification, digestibility, fiber, fundamental niche, *Odocoileus hemionus*, *Odocoileus virginianus*, plant secondary metabolites, sympatry, tannins

Food provides the energy and nutrients needed by animals to survive and reproduce, but nutritious food is often in limited supply in natural communities. The competitive exclusion principle states that two species with identical ecological niches (i.e., the environmental conditions and resources used by a species; Hutchinson 1957; Leibold 1995) cannot coexist indefinitely (Gause 1934). Food resources therefore often are at the core of competitive interactions, particularly between congeneric species occupying similar niches (Harper et al. 1961). In theory, competition for food resources could result in competitive exclusion, where the superior competitor dominates its counterpart and forces it to extinction or to abandon their shared niche (Harper et al. 1961), thereby segregating their use of it in space or time (e.g., *Mazama* spp., Ferreguetti et al. 2015). Alternatively, species might coexist by sufficiently partitioning the niche where they overlap (i.e., syntopy) to avoid competition (e.g., anurans in rainforest leaf litter; Toft 1980). Over evolutionary time, niche partitioning by competition might result in character displacement in morphology and physiology related to feeding (i.e., a change in their fundamental nutritional niche). A classic example occurred in Darwin's finches (*Geospiza* spp.), whereby the evolution of character displacement was tracked by means of the morphological changes in jaws and beaks of two ground finch species as a result of interspecific competition for food (Schluter et al. 1985; Grant and Grant 2006). However, this well-studied example focuses on species that specialize in certain types and sizes of seeds. In contrast, understanding the nature and results of competitive interactions between dietary generalists like mule deer (*Odocoileus hemionus*) and white-tailed deer (*O. virginianus*)

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that consume hundreds of different plants across their ranges can be more difficult.

Detecting differences in the fundamental nutritional niche that might have been driven by character displacement requires a particularly in-depth analysis for closely related, widespread generalist species (DeGabriel et al. 2014). The fundamental niche (i.e., "requirement niche"; Leibold 1995) can be defined as the multivariate space with axes that comprise those parts of an organism's environment that influence its potential to survive and successfully reproduce (i.e., its fitness; Kearney 2006), and thus can be envisioned by the animal's fitness response in relation to a series of niche axes (*n*-dimensional hypervolume; Hutchinson 1957, 1978). The fundamental *nutritional* niche consists of niche axes that pertain to nutrients such as protein and carbohydrates (i.e., macronutrient niche; Machovsky-Capuska et al. 2016) and antinutrients such as plant fiber and plant secondary metabolites (PSMs) that influence the value of the nutrients and the animal's ability to acquire them (Van Soest 1982; Robbins 1995). The overlap in food items actually consumed in a particular place and time (i.e., diet composition; the realized dietary niche; Hutchinson 1957) between herbivore species might not precisely reflect differences in the fundamental nutritional niche that could be used to predict competitive ability and potential use of food resources in sympatry and allopatry. For example, Behmer and Joern (2008) used an experimental geometric framework to show that seven closely related, co-occurring generalist grasshopper species (*Melanoplus* spp.) had species-specific macronutrient niches differing in the absolute and relative amount of plant protein and carbohydrates, even though they ate the same plant species. Understanding differences in the fundamental nutritional niche in herbivores therefore requires controlled comparative studies that measure physiological requirements and tolerances as related to fitness for a range of macronutrients (e.g., energy, protein) and antinutrients (e.g., fiber, PSMs) as well as the mechanics of harvesting plants (Kearney 2006; Shipley et al. 2009; Machovsky-Capuska et al. 2016).

Mule deer and white-tailed deer are congeneric browsing ruminants that consume a generalist diet and occupy a wide range of habitats both sympatrically and allopatrically (Anderson and Wallmo 1984; Hygnstrom et al. 2008). These deer species, the only two extant species in their genus, are segregated over much of North America; however, they overlap across a broad zone of sympatry, primarily along the Rocky Mountains and in the surrounding Plains regions (Geist 1998; Jacobson 2003; Jacobson 2004; Lyman 2006, Hygnstrom et al. 2008; Berry et al. 2019). Plants they consume vary greatly in nutritional value because fibrous cell walls delay and reduce digestion and their PSMs can be toxic or reduce the nutritional quality of the plant (Robbins 1994). Overlap in diets consumed by mule and white-tailed deer where they co-occur have varied greatly (from <50% to >90% similarity) across their ranges (Martinka 1968; Anthony and Smith 1977; Krausman 1978; Mackie et al. 1998; Whittaker and Lindzey 2004; Brunjes et al. 2009; Whitney et al. 2011). In two different study systems, the greatest overlap was documented in habitats where nutritious food was scarce (Whittaker and Lindzey 2004; Brunjes et al. 2009), a situation that could lead to competition in these shared habitats. However, these studies were carried out using fecal or stomach contents of free-ranging animals, and therefore were unable to establish whether dietary differences reflected (i) differences in nutritional requirements and tolerances, thus differences in their fundamental nutritional niches, or (ii) that different foods were available in different habitats selected by the deer species, thus differences in their realized dietary niches. For example, mule deer tend to use steeper, more open habitats at higher elevation than do white-tailed deer, which often contain different plant assemblages (Lingle and Pellis 2002; Dellinger et al. 2019; Staudenmaier et al. 2021).

Previous research suggests that mule deer and white-tailed deer might differ, at least modestly, in their nutritional requirements and tolerances. Berry et al. (2019) observed that when foraging together in the same 0.5 ha forest stands, mule deer consumed less-diverse diets containing more deciduous and evergreen shrubs with higher levels of protein precipitation caused by tannins and lower dry matter digestibility than did white-tailed deer. Although energetics and nutritional requirements of both species have been studied for many decades (e.g., Robbins et al. 1975; Holter et al. 1977, 1979; Parker et al. 1984; Parker and Robbins 1984; Wickstrom et al. 1984; Mautz et al. 1985; Aoki 1987; Sargeant et al. 1994; Tollefson et al. 2010, 2011), few studies have compared directly their digestive morphology and physiology, or their nutritional requirements or tolerances. The two studies that have compared directly the aspects of the digestive morphology between the two deer species (Zimmerman et al. 2006; Clauss et al. 2009) reported slightly higher surface enlargement factors for the ruminal papillae of mule deer than white-tailed deer, suggesting increased ruminal absorption capacity. Zimmerman et al. (2006) also documented that mule deer had longer intestines with greater digesta capacity than white-tailed deer, which might allow them to better process more fibrous foods. However, digestive efficiency in ruminants like deer is relatively plastic and can be enhanced when consuming higher fiber diets through the development of increased absorptive surfaces in the rumen, larger digesta loads and increased rumen-reticulum capacity (e.g., Bonnin et al. 2016). In addition, mule deer might have a slight advantage over white-tailed deer because larger ruminants can retain digesta longer, allowing for more thorough digestion (Gordon and Illius 1994; Robbins et al. 1995; Barboza and Bowyer 2000), and mule deer have been reported to be 10–20% larger than white-tailed deer in some parts of their range (Zimmerman et al. 2006). This digestive plasticity and body size variation could confound comparisons of nutritional niches between deer species but also could reflect a degree of resource partitioning leading to character displacement between these closely related ungulates.

Mule and white-tailed deer might also differ in their ability to tolerate PSMs, such as phenolics and monoterpenes that are common in the woody browses and forbs that form the bulk of their diets (Martinka 1968; Anthony and Smith 1977; Berry et al. 2019). PSMs can impose an energetic cost

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(Sorensen et al. 2005b) as they are absorbed, metabolized, and excreted, by the animal (Dearing et al. 2005). Condensed tannin, a type of phenolic, reduces protein digestibility of plants by binding with digestive enzymes and dietary protein during chewing to form an indigestible complex (Robbins et al. 1987a, 1987b; Hagerman et al. 1992; DeGabriel et al. 2009a). However, both deer species have moderately large parotid salivary glands (Austin et al. 1989; Hagerman and Robbins 1993; Mole et al. 1990) and produce tannin-binding salivary proteins that can reduce the effects of condensed tannins and gallotannins on protein digestibility (Robbins et al. 1987b, 1991; Hagerman and Robbins 1993). Mule deer are known to produce salivary proteins that bind both linear and branched-chain condensed tannins without being induced by the consumption of tanniferous forages, a trait not yet studied in white-tailed deer and not documented in other animal species studied (Austin et al. 1989; Hagerman and Robbins 1993; Shimada 2006).

Information is scarce about the tolerances of either deer species for another common type of PSM, monoterpenes, which are found in many evergreen shrubs and conifers. Monoterpenes are common components of volatile or essential oils, which act as aromatic substances in plants and affect both detection and potential use by herbivores (Elliott and Loudon 1987). Monoterpenes can inhibit digestion by ruminants, and overingestion can result in toxicosis or death (Freeland and Janzen 1974; Fowler 1983); thus, plants high in monoterpenes often are avoided by herbivores (Connolly et al. 1980; Duncan et al. 2001; Vourc'h et al. 2002a). In areas of sympatry, mule deer consumed more terpenoid-rich plants than white-tailed deer (Martinka 1968; Anthony and Smith 1977; Whitney et al. 2011). However, white-tailed deer will consume diets high in monoterpenes and other PSMs when forage is scarce, such as in winter, when forage often is limited to conifers (Casabon and Pothier 2007; Bonnin et al. 2016). Servello and Schneider (2000) measured higher ratios of glucuronic acid (GA), a metabolic product of detoxification that represents loss of endogenous energy (Sorensen et al. 2005a, 2005b), to creatinine, a waste product of muscle metabolism that is excreted at a constant rate over time (DelGiudice et al. 1996), in the urine of white-tailed deer when they consumed greater amounts of conifer browse that contained high levels of monoterpenes, phenolics, and other PSMs.

Because no direct comparisons of the fundamental nutritional niche have been made between mule and white-tailed deer, in this study, we compared their energy and nitrogen (protein) balances under identical conditions to determine whether nutrient requirements differed between the two deer species, potentially allowing one species to subsist on nutritionally poorer diets. We also examined the ability of mule and white-tailed deer to digest fibrous plant diets and to tolerate and detoxify tannins and monoterpenes common in plants they both consume. To do so, we undertook simultaneous *in vivo* digestion trials with diets ranging in fiber content and condensed tannins using captive mule and white-tailed deer. In addition, we compared their behavioral and physiological tolerance for α-pinene, a common monoterpene found in evergreen plants consumed by both deer species, by monitoring intake during feeding trials and by measuring GA in their urine. We hypothesized that if mule deer are better adapted to consume more "difficult" forages (i.e., foods with higher fiber and PSMs and lower protein; Shipley et al. 2009), they would require less digestible energy and protein for maintenance of body mass and would have higher digestibility of dry matter, energy, protein, and fiber on diets with and without condensed tannins compared to white-tailed deer. In addition, we expected mule deer to voluntarily consume more monoterpenes and condensed tannins than white-tailed deer and rely on less expensive detoxification mechanisms as evidenced by producing less GA in the urine relative to PSM ingestion.

Materials and Methods

In vivo digestion trials.—To directly compare requirements for energy and protein, and the ability of mule and white-tailed deer to digest plant nutrients and fiber, we carried out a series of in vivo digestion trials in which we fed hand-raised, tractable female deer (seven mule deer and six white-tailed deer) diets that varied in the amount and type of plant fiber, nutrients (i.e., energy and protein), and condensed tannins (Table 1). Deer ranged from 3 to 12 years old and were nonlactating and not reproductive. Of the animals used in the trials, four mule deer and six white-tailed deer were born in the wild and raised together under identical conditions at the Wild Ungulate Facility at Washington State University, Pullman, Washington. The other

Table 1.—Composition of pelleted diets and willow (*Salix lasiandra*) fed to captive mule (*Odocoileus hemionus*, MD) and white-tailed deer (*Odocoileus virginianus*, WTD) at Washington State University, Pullman, Washington. ADL, acid detergent lignin; HFMP, high-fiber, moderate-protein pelleted diet; LFLP, low-fiber, low-protein pelleted diet; LFLP+Tannin, low-fiber, low-protein pelleted diet plus 1.5% condensed tannins as powdered extract from bark of the quebracho colorado tree (*Schinopsis* spp.); MFHP, moderate-fiber, high-protein pelleted diet; NDF, neutral detergent fiber

Diets	Trial date	Number of animals (MD/WTD)	Crude protein (%)[a]	Gross energy (kJ/g)	Condensed tannin content (%)[a]	Protein precipitation (mg protein/g diet)	NDF (%)[a]	ADL (%)[a]
MFHP	June 2018	6/5	18.13	17.72	0	0	29.85	4.03
HFMP	May 2019	6/5	13.18	18.84	0	0	47.49	6.90
LFLP	May 2020	6/4	11.97	18.09	0	0	23.52	0.95
LFLP+Tannin	November 2019	5/6	12.17	17.76	1.5	75	23.34	1.03
Willow	July 2019	6/6	16.53	18.68	6	300	32.16	6.92

[a]Values calculated on a percent dry matter basis.

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three mule deer were born at the Wild Ungulate Facility to wild-born mothers and raised similarly to the other animals. All housing and experimental procedures for the animals were approved by Washington State University's Institutional Animal Care and Use Committee (protocols #4161, #6267) and followed the guidelines of the American Society of Mammalogists for the use of wild mammals in research (Sikes et al. 2016).

We undertook a series of five total-collection, single-diet digestion trials from July 2018 to May 2020 (Table 1). We selected diets that spanned typical ranges of neutral detergent fiber (NDF, 26–45%) and crude protein (7–16%) content of natural diets consumed by mule and white-tailed deer in northeastern Washington during the summer (Hull 2018). These natural diets also contained a range of PSMs, including monoterpenes and condensed tannins. The moderate-fiber, high-protein (MFHP) pelleted diet was the basal maintenance diet fed to the captive deer and consisted of 28.8% NDF (all of which was from grain-based sources, mostly rice hulls) and 18.1% crude protein. The high-fiber, moderate-protein (HFMP) diet was a commercially produced pelleted diet (Mazuri Moose Diet #5658, MAZURI Exotic Animal Nutrition, St. Louis, Missouri), which consisted of 47.5% NDF (of which 55% was contributed by aspen [*Populus* spp.] and 31% from beet pulp) and 13.2% crude protein. The low-fiber, low-protein diet (LFLP) also was produced by MAZURI (Mazuri Experimental Low Protein Deer Diet #562G) and consisted of 23.5% NDF (of which 23% was contributed by aspen, 13% from beet pulp, and 47% from grain-based sources) and 12.0% crude protein.

We also tested diets with two types of condensed tannins and two ways to administer them (extracted tannin powder added to pellets and whole leaves). First, we used a powdered extract of the quebracho colorado tree (*Schinopsis* spp., Anacardiaceae; UNITAN Saica, Buenos Aires, Argentina; Silvateam, Mondovi, Italy) to create a low-fiber, low-protein tannin diet (LFLP+Tannin) similar to the methods of Clauss et al. (2003) and DeGabriel et al. (2009b). Quebracho tannin is a branched-chain 5-deoxyproanthocyanidin (Reid et al. 2013). We applied a light mist of water to the pellets and then mixed in the prescribed amount of quebracho powder at a concentration of 5% by wet mass (i.e., 4.7% dry mass as confirmed by tannin assays described below). Quantitative assessment of the quebracho powder showed that it contained 30% condensed tannins and 70% nontannin phenolics and precipitated 1.5 µg protein per µg quebracho powder. Thus, the LFLP+Tannin diet was 1.5% tannin by dry mass and was predicted to precipitate 75 mg protein/dry g of diet.

Second, we carried out a digestion trial with the leaves of the Pacific willow tree (*Salix lasiandra*, Salicaceae), a preferred forage of deer in the region, which contained 26.4% NDF and 16.5% crude protein by dry mass. Our tannin assays (described below) confirmed that this willow contained 6% condensed tannins based on linear polymers of (epi)catechin (Schofield et al. 1998). Based on the protein-precipitating capacity of other tannins (5 µg protein per µg tannin), willow was expected to precipitate 300 mg protein/dry g of diet. During this trial, fresh willow, harvested within 48 h of feeding from a nearby riparian area, was offered twice daily. Only current annual growth was collected and composited from various trees for feeding, the woody component of the willow branches was minimized, no flowers or inflorescences were included, and all leaves were of a similar growth stage over the 2-week course of the trials. Animals on trial at the same time were given similar mixtures of leaves to ensure both deer species received forage of comparable quality. When not used in experiments, animals were housed in large outdoor pens and fed the basal maintenance diet (MFHP) supplemented by pasture grass such as meadow foxtail (*Alopecurus pratensis*, Poaceae) and alfalfa (*Medicago sativa*, Fabaceae). Tap water, trace mineral salt blocks, and the MFHP diet were provided ad libitum when off trial.

During the trials, each animal was housed individually in one of six (1.9 × 1.9 m) covered outdoor digestion crates constructed of chain link fence with rubberized, porous flooring and deer-safe materials. To reduce the animals' stress while participating in trials, they were acclimated to the digestion crates periodically throughout the 2-year period during which we undertook our experiment. The digestion crates allowed us to collect feces, urine, and orts (i.e., remaining food) separately and without loss. Feces fell into metal screens below each digestion crate and urine was funneled into a container containing ~40 ml of acetic acid ($C_2H_4O_2$) to reduce the loss of nitrogen as ammonia. Each trial consisted of two sequential sets of six deer (three of each species at a time). For all trials, the deer were offered food and water daily ad libitum to determine their voluntary food intake. If an animal ate less than 500-g fresh weight of food/day (~7.5 g/kg body mass/day), approximately 30% of an animal's average daily intake, for 2 days in a row they were removed from the trial. In addition, data from one animal was removed post hoc from two trials because of chronic digestive inflammation unrelated to the experiment that resulted in abnormal digestion and diarrhea. Therefore, not all trials were completed with six animals of each species. All animals were weighed immediately before and after each trial.

Digestion trials lasted 7 days, preceded, and followed by approximately 2 weeks of gradual transition from their normal basal diet of MFHP to the trial diet and back again while in their outdoor enclosures. The first 2 days of each trial served as acclimation days; we did not include data from those days in our analyses. During days 3–7 of the trial, we weighed the amount of food offered to each animal each morning and collected two fresh samples of the food for dry matter correction and for nutritional analysis. On the subsequent day, we collected and weighed all orts and feces, and we collected and determined the volume of urine produced. We collected two fresh samples of orts and feces—one for dry matter correction and one for nutritional analysis, and we collected one urine sample for nutritional and GA analysis. Samples collected for dry matter were weighed fresh, then oven-dried at 100°C for ≥24 h to a constant weight, and reweighed, to determine % dry matter. All calculations were done on a dry matter basis. All samples collected for nutritional analysis were immediately frozen and stored.

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Food, orts, and feces were immediately frozen after collection for nutritional analysis and later were oven dried at 60°C for 3 days, except for the LFLP+Tannin and willow diets, which were freeze-dried for future tannin analysis. All samples were ground to pass a 1-mm mesh in a Wiley Mill. Processed diet and fecal samples were analyzed for sequential detergent fiber (NDF [%], acid detergent fiber [%], acid detergent lignin [ADL] [%], and acid insoluble ash [%]) including α-amylase and sodium sulfite (Goering and Van Soest 1970; Mould and Robbins 1981) using an Ankom Fiber Analyzer 200/220 (Ankom Technology, Fairport, New York). Gross energy (kJ/g) of diet, fecal, and urine samples was determined for all samples using a bomb calorimeter (various models) in the Wildlife Habitat and Nutrition Lab at Washington State University and at Dairy One (Ithaca, New York). Nitrogen content (N, %) of diet, fecal, and urine samples was determined using a Carbon-Nitrogen TruSpec analyzer (LECO, St. Joseph, Michigan) in the Soil Plant and Waste Analytical Lab at Washington State University. Crude protein content (%) was estimated as 6.25 × N content (Robbins 1994).

The following calculations followed standard approaches used in nutritional ecology (Robbins 1994). Daily dry matter intake (g/day) was calculated from the difference between the dry mass of food offered and the dry mass of orts. We calculated apparent dry matter digestibility (%) as follows: $\frac{(\text{dry matter intake} - \text{dry feces produced})}{\text{dry matter intake}}$. Apparent protein, energy, and NDF digestibility (%) were calculated similarly by multiplying the nutrient concentration of the food (crude protein, gross energy, or NDF) by dry matter intake and the nutrient concentration of the feces by the amount of feces produced (Robbins 1994). Digestible energy intake (DEI, kJ/kg body mass/day) was the product of dry matter intake corrected for body mass (g/kg/day, hereafter referred to as DMI), gross energy, and apparent energy digestibility. Digestible protein intake (DPI, g protein/kg body mass/day) was the product of DMI, crude protein, and apparent protein digestibility.

We determined the amount of digestible energy required per day to maintain body mass of mule and white-tailed deer in the digestion crates similarly to Robbins (1994) from the x-intercept of the regression of average daily change in body mass during each digestion trial as a function of DEI for each diet. The slope of this line represented body mass change per unit of additional DEI. We also estimated their N requirements (the amount of N an animal must consume to counteract the minimum constant N losses from feces and urine) from the x-intercept of the regression line for N balance (N ingested − N excreted via urine and feces, mg N/kg/day) against dietary N intake (mg N/kg/day). Minimum dietary protein requirements were derived from the following equation: [(EUN + MFN (DMI) − 6.25)/DMI/0.74] (Robbins 1994). Metabolic fecal nitrogen (MFN, g N/100 g feed, the amount of N eliminated in the feces when an animal is consuming no protein), for both deer species was estimated as the absolute value of the negative y-intercept of the line of regression of digestible N (g N/100 g feed) against dietary N (%) for nontannin diets. Similarly, we used an exponential model (Asleson et al. 1996) of the y-intercept of the regression of the natural log of urinary N (mg N/kg/day) against dietary N intake (mg N/kg body mass/day to estimate the endogenous urinary nitrogen (EUN, mg N/kg body mass/day, the amount of N eliminated in the urine when an animal is consuming no protein). Data from tannin diets were not included in MFN, EUN, and N balance calculations because of the effects of tannins on protein digestion (Robbins 1994). In addition, only data from animals that did not lose >2 kg (approximately twice the resolution of our weighing scale) body mass over the course of each of the MFHP, HFMP, and LFLP trials were included in N balance calculations (Robbins 1994).

Tannin assays.—Composited samples of diets containing tannins (LFLP+Tannin and willow) were analyzed for condensed tannins using an acid butanol assay at the Hagerman Lab at Miami University, Oxford, Ohio, as detailed in Hagerman (2011). Briefly, freeze-dried and ground samples were extracted with 70% acetone (acetone:water 70:30, v/v), centrifuged, and an aliquot of extract was combined with acid butanol reagent and ferric ammonium sulfate reagent. The absorbance at 550 nm was compared with standard curves based on quebracho tannin powder, willow extract, or purified, well-characterized condensed tannin prepared from *Sorghum* grain (Hagerman 2011).

We also measured protein precipitation of a composite sample of the LFLP+Tannin diet in the Hagerman Lab using the protein-precipitable phenolics method as detailed in Hagerman (2011). To create the calibration curve, quebracho powder or *Sorghum* purified condensed tannin was dissolved in methanol and appropriate aliquots were added to the sodium lauryl sulfate/triethanolamine reagent, vortexed, and combined with ferric ammonium sulfate solution before reading the absorbance values at 510 nm. To determine protein precipitability, aliquots of each tannin sample were mixed with a buffer containing bovine serum albumin (fatty-acid free, Sigma-Aldrich Canada, Oakville, Ontario, Canada), incubated, centrifuged, and the supernatants aspirated. The remaining precipitates were redissolved in the sodium lauryl sulfate/triethanolamine reagent, vortexed, and mixed with ferric ammonium sulfate solution before determining absorbance at 510 nm. The slope of the response curve with and without bovine serum albumin was compared to quantify the fraction of the total phenolics that was precipitable. We also estimated the protein precipitation capacity of the willow diet composited across 5 days using an extract of the composited sample of the willow forage.

Monoterpene intake tolerance trial.—To compare their tolerance for α-pinene, a monoterpene prevalent in many evergreen plants consumed by both deer species, we measured how six deer of each species modified their DMI and production of GA as the concentration of α-pinene increased from 0 to 4%. This range spans the amounts of total monoterpenes measured in plants consumed by deer in the western USA, including sagebrush (*Artemisia tridentata*, Asteraceae; Kelsey et al. 2006), western red cedar (*Thuja plicata*, Cupressaceae), western hemlock (*Tsuga heterophylla*, Pinaceae), and Douglas-fir (*Pseudotsuga menziesii*, Pinaceae; Vourc'h et al. 2002b; Burney and Jacobs 2011). These trials lasted up to

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12 days depending on how long it took an animal's intake to drop below 500 g/day with increasing α-pinene. We fed the basal diet MFHP without α-pinene (0%) for the first three trial days as acclimation and control, and then α-pinene was increased sequentially from 1%, 2%, 3%, and 4%, for 2 days at each level.

Each day, just before feeding the animals, we prepared the monoterpene diet by spritzing the MFHP pelleted diet with the desired concentration by weight of α-pinene, a volatile oil (Sigma-Aldrich, Canada, Oakville, Ontario, Canada), using a handheld garden sprayer with an adjustable tip. The oil was then immediately and rapidly mixed in a lined feed trough to ensure even coverage of oil onto the pellets. This method was repeated until all measured oil had been sprayed onto the pellet mixture. Actual concentrations of oil in prepared feed samples were not determined in a lab, rather inferred from the ratio of the mass of oils applied to total food. These trials were carried out during late fall, when ambient temperatures were relatively low (–0.3 to 8.5°C) to minimize loss of volatile oils from the prepared diet and concentrations were consistent between deer species for each day of the trial.

Each day of the trials, deer were fed the MFHP diet with the specified monoterpene concentration ad libitum. As described for the in vivo digestion trials, we measured the mass of food offered and orts each day, collected and measured the volume of urine produced on the second day at each level of monoterpene in the diet, and corrected diet and orts for dry matter content. To avoid potential confounding effects with GA measurements, we did not include acetic acid in the urine collection containers for this trial. On the second day for each level of α-pinene, we collected and immediately froze a sample of urine for GA analysis.

GA assay.—We compared evidence for detoxification capacity between deer species by measuring the amount of GA produced (mM/kg body mass/day) relative to the amount of PSM consumed (g/kg body mass/day) during the monoterpene, LFLP+Tannin, and willow trials. Willow contains condensed tannins and a variety of other PSMs such as phenolic glycosides, salicylates, and flavonoids, that we did not directly measure (Schofield et al. 1998; Julkunen-Tiitto and Sorsa 2001; Thines et al. 2008). GA is a major pathway for detoxification of PSMs in vertebrates that is related to the amount of toxin an herbivore consumed per unit time and detoxified (Guglielmo et al. 1996; Servello and Schneider 2000; Sorensen et al. 2005b). GA therefore is an index of the toxin load experienced by an herbivore and, because the glucuronidation pathway requires endogenous glucose, it represents a form of energy loss (Sorensen et al. 2005a). We determined the concentration of GA in composite urine samples (days 3–7 combined) for each deer on the LFLP+Tannin and willow trials and for each level of α-pinene separately on the monoterpene trial. Detailed methods for determining GA are found in Mangione et al. (2001). Briefly, the assays were undertaken directly on the thawed urine samples. Urine samples were mixed with a solution of borax and sulfuric acid and heated, producing a colorimetric reaction with phenylphenol in the samples. The samples absorbance values then were compared with a standard curve created using pure GA (Sigma-Aldrich Canada, Oakville, Ontario, Canada). GA assays were completed in duplicate for each sample and values were averaged. To determine the total amount of GA produced per day, we multiplied its concentration (mM/L) by daily urine volume (L).

Statistical analyses.—We compared body size (kg), DMI, DEI, DPI, and apparent dry matter, protein, energy, and NDF digestibility between deer species and among diets using a linear mixed-effects models, with individual deer as a random effect, using the "lme" function of the "nlme" package (version 3.1-140; Pinheiro et al. 2019) in the program R (R Core Team 2019). For each model, we tested first for significant interactions between species and diet, and nonsignificant interactions were removed. Model fit was confirmed visually using residual plots. For linear mixed-effects models, we used the maximum likelihood method for estimation. Significance ($\alpha = 0.10$) of main effects in each linear mixed-effects model was determined using the "Anova" function in the "car" package (version 3.3-3; Fox and Weisberg 2019) when any categorical main effect had more than three levels. This function generated analysis of deviance tables for each model with chi-square (χ^2) test statistics. Type II sums of squares was used for analysis of deviance in the absence of an interaction. Type III sums of squares was used if an interaction was retained in the model. Linear contrasts then were calculated for main effects with three or more levels (i.e., diet) through the "lsmeans" function in the "lsmeans" package (version 2.30-0; Lenth 2016) using the Tukey adjustment for inferences for the estimated marginal means. To compare MFN, EUN, N balance, and energy balance between species, we used the same linear mixed-effects modeling framework as in the preceding analyses, without using linear contrasts because no factor had more than two levels.

To examine tolerance for PSMs and detoxification capacity between mule and white-tailed deer, we compared (i) the rate of decline in DMI with increasing α-pinene concentration (0–4%); (ii) the nonlinear change in α-pinene intake with increasing α-pinene concentration (0–4%) in the diet; (iii) the rate of GA production with increasing α-pinene intake; and (iv) the rate of GA production with intake of tannins while consuming the LFLP+Tannin and willow diets. For each analysis, we used the same linear mixed-effects modeling framework as in the preceding analyses. We expected α-pinene intake to initially increase and then level off when reaching the deer's physiological threshold based on maximum detoxification capacity (McLean et al. 2008). We therefore explored this predicted nonlinear relationship by comparing model estimates for α-pinene among α-pinene concentration levels used as a categorical variable. We also modeled the concentration of α-pinene in the diet as a continuous variable with a linear relationship to DMI. Estimates were interpreted directly from the models for DMI and GA production because categories for main effect levels did not exceed two. For all results, reported means represent arithmetic means unless otherwise labeled, and standard errors are reported as ±*SE*.

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Results

Intake and in vivo digestion.—Body mass at the beginning of digestion trials did not differ significantly between mule deer ($\bar{x}$ = 70.3 ± 10.4 kg) and white-tailed deer ($\bar{x}$ = 63.6 ± 6.7 kg; χ^2_1 = 2.4, P = 0.12). However, the starting body mass of deer varied with diet trials (χ^2_4 = 95.1, P < 0.001; Fig. 1A). Deer were heavier at the start of the LFLP+Tannin trial, which took place in the late fall, than before any other trials, which took place in the spring to late summer. The effect size for the LFLP+Tannin trial was 7.2 ± 1.2 kg (t_{37} = 6.1, P < 0.001; Fig. 1A).

Mule and white-tailed deer consumed the same amount of food relative to their body mass each day (χ^2_1 = 0.4, P = 0.51), but DMI varied with diet (χ^2_4 = 28.5, P < 0.001). Deer ate more MFHP than the other pelleted diets, but a similar amount as the willow diet (Fig. 1B). Mule deer and white-tailed deer also had similar apparent protein digestibility (χ^2_1 = 0.04, P = 0.84; Fig. 1C) across diets. In contrast, apparent NDF digestibility across diets consumed by mule deer was greater than that of white-tailed deer (χ^2_1 = 7.6, P = 0.0060; Fig. 1D), with an effect size of 4.0 percentage points (± 1.5 percentage points) greater apparent NDF digestibility across diets for mule deer (t_{12} = −2.6, P = 0.024). Mule deer also had higher apparent dry matter digestibility than white-tailed deer (χ^2_1 = 3.5, P = 0.061; Fig. 1E), with an effect size of 1.6 ± 0.9 percentage points (t_{12} = −1.8, P = 0.10; Fig. 1E), and a higher apparent energy digestibility than white-tailed deer (χ^2_1 = 4.7, P = 0.031; Fig. 1F), with an effect size of 1.7 ± 0.9 percentage points (t_{12} = −2.04, P = 0.06; Fig. 1F).

Likely because of its low fiber content, the LFLP diet had a higher apparent dry matter, energy, and NDF digestibility than the other diets, and the HFMP diet, which had the highest fiber content, had one of lowest levels of digestibility (Fig. 1, linear contrasts in Appendix A). MFHP had the highest apparent protein digestibility (Fig. 1C; Appendix A). Apparent protein digestibility of the LFLP+Tannin diet was estimated to be 8.7 percentage points (± 2.0 percentage points) lower on average than the LFLP diet (linear contrast, t_{37} = 4.5, P < 0.001; Fig. 1C; Appendix A), demonstrating the ability of the added quebracho powder to reduce protein digestibility. Willow, which also contained tannins, had an intermediate apparent protein digestibility (Fig. 1C; Appendix A). Mule and white-tailed deer had similar DEI (χ^2_1 = 0.9, P = 0.34) and DPI (χ^2_1 = 0.3, P = 0.56), but DEI (χ^2_4 = 30.1, P < 0.001) and DPI (χ^2_4 = 194.5, P < 0.001) varied among diets (Fig. 1G and H). When consuming the MFHP diet, which contained the highest amount of protein, deer had the highest DPI and DEI, whereas deer consuming the LFLP+Tannin had the lowest DPI (Fig. 1G and H; Appendix A).

The amount of digestible energy required to maintain body mass differed between species (t_{12} = −2.3, P = 0.040). Mule deer required 283.9 kJ digestible energy/kg body mass/day and white-tailed deer required 358.0 kJ digestible energy/kg body mass/day to maintain their body mass when residing in digestion crates in spring, summer, and fall (Fig. 2). MFN did not differ between species (t_8 = −0.6, P = 0.58) and was 1.1 g N/100 g feed for mule and 1.2 g N/100 g dry matter intake of feed for white-tailed deer (Fig. 3A). However, when we back transformed the results of the EUN analysis, we found that mule deer had approximately half the EUN (76.4 mg N/kg/day) of white-tailed deer (140.3 mg N/kg/day; t_8 = 2.6, P = 0.034, Fig. 3B). Mule deer also had a lower N balance, and thus required about half the minimum nitrogen (i.e., crude protein) intake (238.6 mg N/kg body mass/day) as did white-tailed deer (517.6 mg N/kg body mass/day, t_8 = −3.1, P = 0.015, Fig. 3C). We found no significant interactions between species and diet for any intake, digestibility, or energy or protein requirement variable.

Monoterpene tolerance.—We found that mule and white-tailed deer responded similarly to α-pinene concentrations in their diet. We used six mule and five white-tailed deer that maintained sufficient DMI through the days of the trial in which 0–3% α-pinene was fed to remain on trial, but only five mule deer and two white-tailed deer ate enough to remain through the final concentration of 4% α-pinene in the diet. DMI of mule and white-tailed deer on the monoterpene diet declined linearly with concentration of α-pinene; for every percentage point increase of monoterpene in the diet, deer were predicted to consume 2.4 g/kg (±0.3) less food (t_{101} = −8.3, P < 0.001; Fig. 4A). However, this relationship between DMI and α-pinene concentration did not vary between mule and white-tailed deer (t_9 = 0.05, P = 0.96; Fig. 4A).

Mule and white-tailed deer consumed a similar amount of α-pinene (χ^2_1 = 0.004, P = 0.95) per day, but daily α-pinene intake varied among α-pinene concentrations in the diet (χ^2_4 = 361.2, P < 0.001; Fig. 4B). Mean daily intake of α-pinene differed significantly between the α-pinene concentrations from 0% to 1% (t_{98} = −6.8, P < 0.001; Fig. 4B) and 1% to 2% (t_{98} = −4.9, P < 0.001; Fig. 4B). However, intake of α-pinene did not differ significantly between 2% and 3% (t_{98} = −2.5, P = 0.11; Fig. 4B) and 3% and 4% (t_{98} = −1.3, P = 0.69; Fig. 4B), suggesting that intake of α-pinene was reaching a threshold between 2% and 4%.

Detoxification of monoterpenes and tannins.—Detoxification of monoterpenes differed between species based on measures of GA in the urine, but detoxification of tannins did not. The GA concentration (mM/kg/day) in the urine increased linearly with intake of α-pinene (g/kg/day, t_{38} = 10.5, P < 0.001), and with the species × α-pinene intake interaction (t_{38} = 2.1, P = 0.046), but not the main effect of species (t_9 = −0.6, P = 0.58). For every unit increase (g/kg/day) of α-pinene intake, GA production was predicted to increase by 2.0 (± 0.2) mM/kg/day for both species (t_{38} = 10.5, P < 0.001), but white-tailed deer were predicted to produce an additional 0.7 (± 0.3) mM/kg/day more GA per unit increase of α-pinene than mule deer (t_{38} = 2.1, P = 0.046, Fig. 5). GA concentration in the urine (mM/kg body mass/day) also increased with the intake of condensed tannin (g/kg/day; t_9 = 8.2, P < 0.001) and associated nontannin phenolics, but was not different between the two tannin diets, LFLP+Tannin and willow (t_9 = 1.5, P = 0.18), between species (t_{10} = −0.6, P = 0.55; Fig. 5), nor was the species × diet interaction significant.

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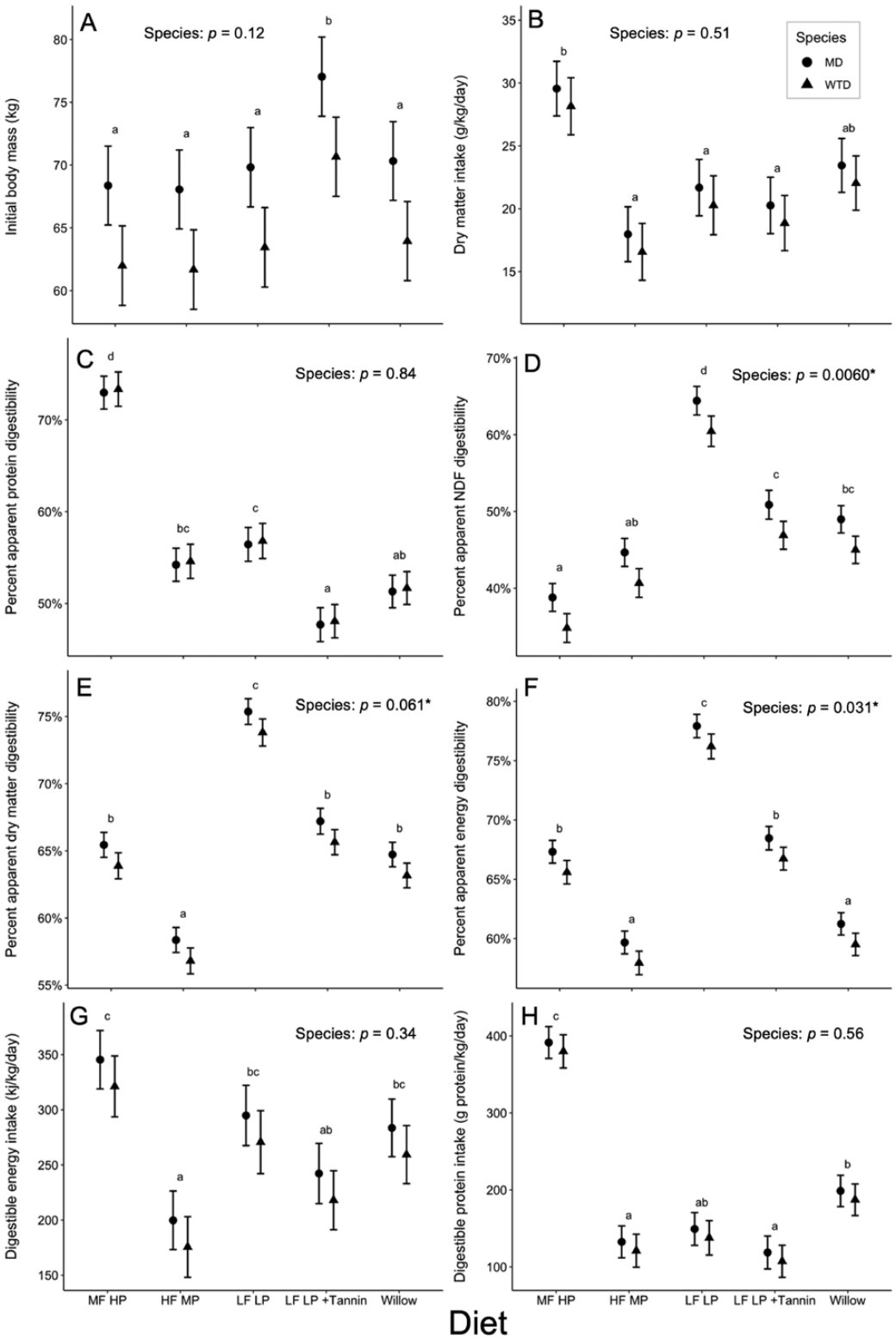

Fig. 1.—Estimated marginal means of linear mixed-effects models ± *SE* bars for seven mule deer (*Odocoileus hemionus*, circles) and six white-tailed deer (*O. virginianus*, triangles) across five single-diet, total-collection in vivo digestion trials conducted from July 2018 to May 2020 at Washington State University, Pullman, Washington. Because no interactions between diet and species were significant ($\alpha = 0.10$), lowercase letters on the graphs represent significant differences between diets calculated using linear contrasts (Appendix A). Species *P*-values with asterisks represent significant differences ($\alpha = 0.10$) between deer species for that response. A random effect for individual animal was included in each model. All digestibility measures are apparent, and not true measures of digestibility. The willow diet consisted of the leaves of the Pacific willow (*Salix lasiandra*). Diets are defined as follows: MFHP, moderate-fiber, high-protein pelleted diet; HFMP, high-fiber, moderate-protein pelleted diet; LFLP, low-fiber, low-protein pelleted diet; LFLP+Tannin, the low-fiber, low-protein pelleted diet with 1.5% condensed tannins added as a powdered extract from bark of the quebracho colorado tree (*Schinopsis* spp.); NDF, neutral detergent fiber.

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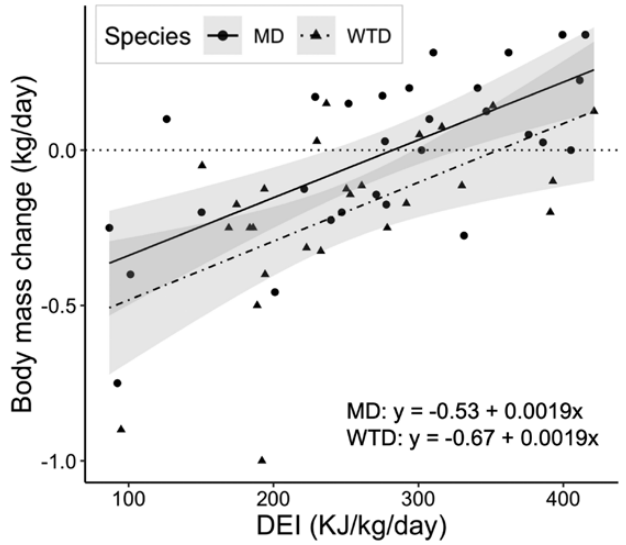

Fig. 2.—Mean daily change in body mass (kg/day) as a function of digestible energy intake (DEI, kJ/kg body mass/day) of captive mule deer (*Odocoileus hemionus*, MD, circles and solid line) and white-tailed deer (*O. virginianus*, WTD, triangles and dashed line) when consuming pelleted diets and willow (*Salix lasiandra*) during in vivo digestion trials conducted from July 2018 to May 2020 at the Wild Ungulate Facility at Washington State University, Pullman, Washington. Daily digestible energy requirements per kg of body mass are found at the intercepts of the x-axis, denoted by a dotted line (MD: 283.9 kJ/kg/day, WTD: 358.0 kJ/kg/day). Confidence bands (95%) are shaded in gray; darker areas represent band overlap between the two species. A significant difference was found between deer species at $\alpha = 0.10$ ($P = 0.040$). A random effect for individual animal was included in the model.

Discussion

In a controlled experimental setting, we directly compared nutritional requirements and tolerances (i.e., characteristics affecting their fundamental nutritional niches) of mule and white-tailed deer across diets ranging in fiber, protein, and PSMs. Although we found many similarities between the species in intake and protein digestibility, we also found notable differences in nutrient requirements, energy and fiber digestibility, and detoxification of monoterpenes. Relative to their body mass, the deer voluntarily consumed the same amounts of dry matter, digestible energy, digestible protein, condensed tannins, and α-pinene, and had similar apparent protein digestibility across five diets. Detoxification rates of condensed tannins and nontannin phenolics also were similar between species, and the two types of condensed tannin (branched and linear) had similar effects on protein digestion in both species. However, mule deer required less digestible energy to maintain body mass and less protein to maintain N balance than white-tailed deer, and were better able to digest dry matter, NDF, and energy in their diets. In addition, mule deer produced less energetically expensive GA when detoxifying monoterpenes. These findings support our general hypotheses that mule deer are better able to tolerate less-nutritious, more "difficult," foods and might explain observed differences in habitat and diet selection between mule and white-tailed deer (Anthony and Smith 1977; Smith 1987; Woods et al. 1989; Avey et al. 2003; Whittaker and Lindzey 2004; Brunjes et al. 2006; Walter et al. 2009; Whitney et al. 2011; Dellinger et al. 2019).

We found no differences in daily intake between deer species for any of the diets when corrected for body mass, although both species consumed less of the nutritionally challenging diets (i.e., high fiber, low protein, added tannins) than the high-quality basal diet (MFHP). Similarly, when some of the same individual animals foraged together in natural forested habitats during the summer 3 years previously, their mass-specific DMI and DEI intake did not differ (Berry et al. 2019). Larger animals are expected to consume more food, but even though mule deer in our study (and in Berry et al. 2019) tended to be larger, body mass of mule (55.2–90.2 kg) and white-tailed deer (53.6–75.8 kg) varied greatly among individuals and moderately across trials; thus, we did not detect significant differences in body mass between species during our experiments. Body mass of mule and white-tailed deer across their ranges can vary to an even greater degree because of genetics, habitat, forage quality, and region. For example, white-tailed deer generally follow Bergmann's rule, increasing in body size with more northern latitudes and with greater net primary productivity (Wolverton et al. 2009). On the other hand, mule deer body size does not seem to vary as drastically across the north-south gradient of their range, but does vary with environmental conditions (Anderson and Wallmo 1984). Our animals (or their mothers if they were born at the Wild Ungulate Facility) came from the wild in locations across eastern Washington where mule and white-tailed deer co-occur, and thus likely reflect some of the variability in stature present in this region, but this variation would be expected to be less than that encountered across the extent of their latitudinal ranges. To better understand the potential effects of body size on competition across the zone of sympatry, more comparative studies between deer found in the same areas at the same time are needed to decipher the confounding effects of diet and habitat versus phylogeny.

Our results suggest that mule and white-tailed deer differ in their energy and protein requirements, even when raised and fed under identical conditions. Although they were similar in body size and voluntary nutrient intake, nonlactating adult female white-tailed deer in our experiments required 26% more digestible energy/kg body mass to maintain body mass than did their mule deer counterparts while confined to digestion crates. Our estimate of 358.0 kJ digestible energy/kg body mass/day for white-tailed deer matched previous estimates of digestible energy requirements for body maintenance of yearling white-tailed deer in captivity (355.4 kJ/kg/day; Holter et al. 1977). Mule deer in our study were estimated to require significantly less DEI for maintaining body mass (283.9 kJ/kg/day) and were more similar to the digestible energy intake requirements measured for mule deer fawns in winter (254 kJ/kg/day, Baker et al. 1979). The lower digestible energy requirements of mule deer might reflect a lower basal metabolic rate than white-tailed deer as an adaptation for living in habitats with poorer quality food. Support for this hypothesis is provided by the 30% lower

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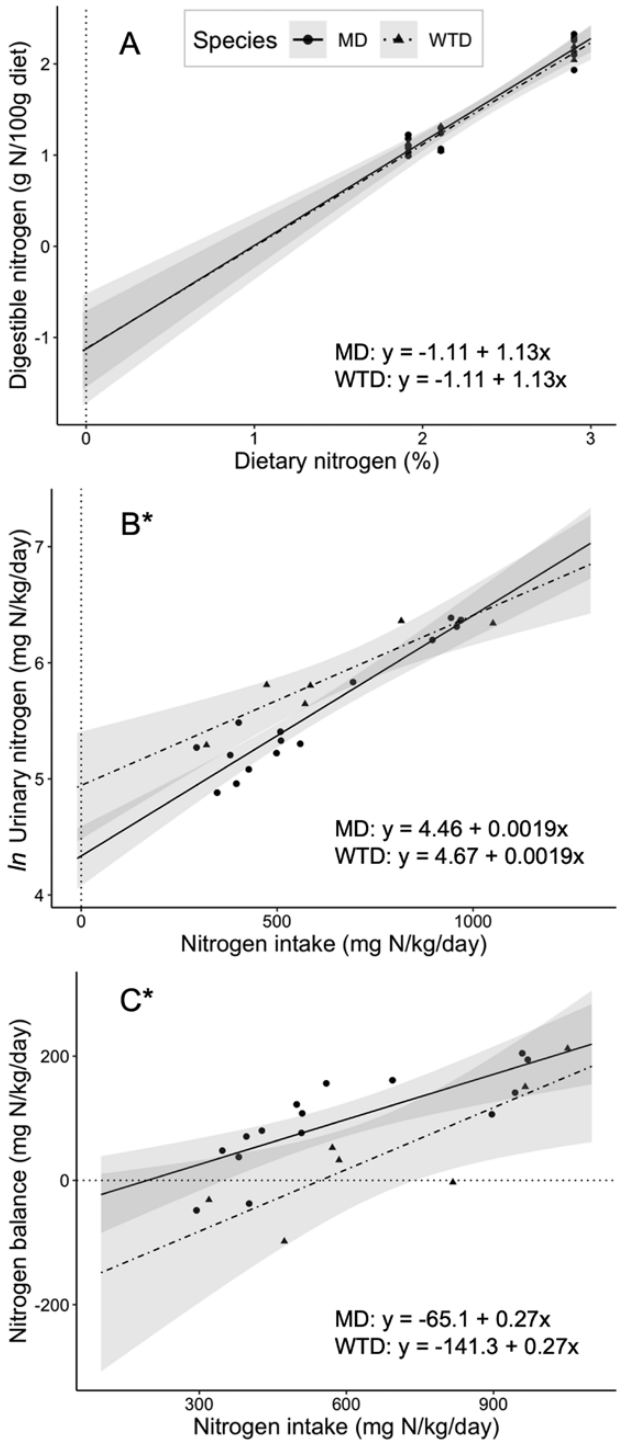

Fig. 3.—Nitrogen intake and excretion of captive mule deer (*Odocoileus hemionus*, MD, circles and solid line) and white-tailed deer (*O. virginianus*, WTD, triangles and dashed line) that lost <2 kg body mass when consuming pelleted diets absent of condensed tannins during in vivo digestion trials conducted from July 2018 to May 2020 at the Wild Ungulate Facility at Washington State University, Pullman, Washington. Asterisks indicate significant differences ($\alpha = 0.10$) between species. Confidence bands (95%) are shaded in gray; darker areas represent band overlap between the two species. A random effect for individual animal was included in each model. Mean daily metabolic fecal nitrogen (MFN, g N/100 g feed) was estimated as 1.1 g N/100 g feed for both species from the absolute value of the negative y-intercept of the line of regression of digestible N (g N/100 g feed) against dietary N (%) for nontannin diets (A). Mean daily endogenous urinary nitrogen (EUN) was estimated as 76.4 mg N/kg body mass/day for mule deer and 140.3 mg N/kg body mass/day for white-tailed deer from (B) the back transformed y-intercept of the regression of the natural log of urinary N (mg N/kg body mass/day) against dietary N intake (mg N/kg body mass/day). Daily minimum nitrogen requirements per kg of body mass were 238.6 mg N/kg/day for mule deer and 517.6 mg N/kg/day for white-tailed deer, as the x-intercept of (C) the mean daily nitrogen balance (mg N/kg body mass/day) as a function of daily nitrogen intake (mg N/kg body mass/day).

metabolic heat production measured for mule deer than for Columbian black-tailed deer (*O. h. columbianus*) within their thermal neutral zones in both summer and winter (Parker and Robbins 1984; Parker 1988), and the lower metabolic rate of mule deer than white-tailed deer when temperatures declined beyond their lower critical temperatures (Mautz et al. 1985). Because animal movement was restricted to some degree by the digestion crates, and some deer were calmer and moved less while confined than others, the magnitude of actual differences between the species in terms of digestible energy requirements of free-ranging deer for moving, thermoregulating, and reproducing might not correspond directly to those we measured in our experiments.

The differences in protein requirements for maintenance were even more dramatic than those of digestible energy between the deer species in our experiments. White-tailed deer required twice the amount of nitrogen/kg body mass/day than did mule deer to maintain N balance. Our estimates of minimum protein requirements for white-tailed deer (517.6 mg N/kg/day) were similar to those found by Holter et al. (1979) for yearling white-tailed deer in captivity (540.0 mg N/kg/day). Protein requirements for mule deer were significantly lower (238.6 mg N/kg/day) than white-tailed deer in our study, but MFN and EUN have not been previously measured for mule deer. Nitrogen excreted in feces was similar between deer species, but mule deer excreted about half as much nitrogen in urine as white-tailed deer. This pattern suggests that mule deer might have a more efficient mechanism for urea recycling that do white-tailed deer, which might allow them to conserve nitrogen and survive on foods with lower protein content (Smith et al. 1975). In ruminants, nitrogen-rich urea is recycled from the blood into the gastrointestinal tract where it is hydrolyzed to ammonia and then can be synthesized into useable protein by rumen microbes. The rate of urea recycling is higher when deer eat lower protein diets (Robbins et al. 1974). Although energy and protein requirements for activity (Parker et al. 1984), thermoregulation (Parker and Robbins 1984), fawn growth (Robbins and Moen 1975a; Sadleir 1980), pregnancy and lactation (Robbins and Moen 1975b; Sadleir 1982; Tollefson et al. 2010), and antler growth (Asleson et al. 1996) have been measured for either white-tailed deer or mule deer, these requirements have yet to be compared between the deer species.

As we hypothesized, mule deer digested cell wall fiber (NDF) more effectively than did white-tailed deer, which also

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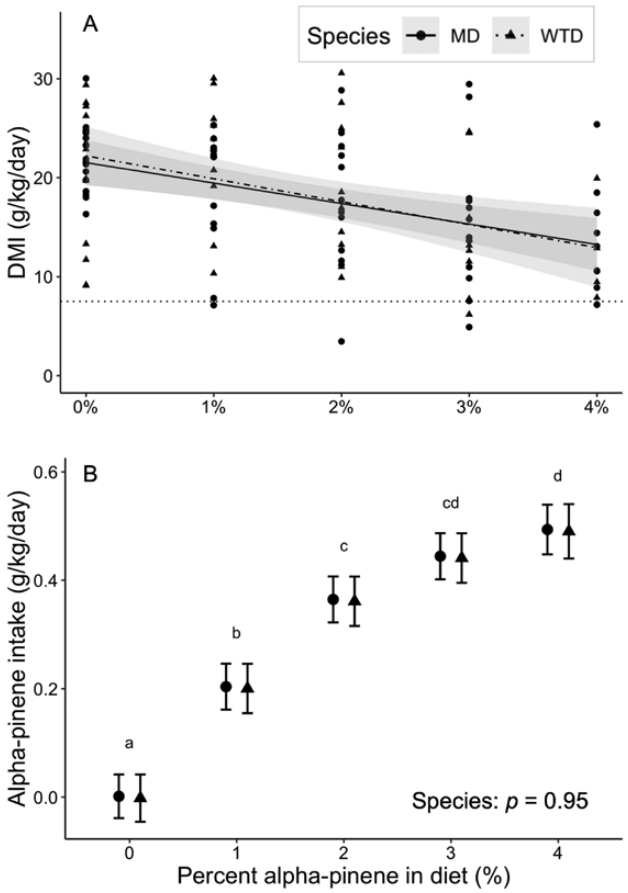

Fig. 4.—Daily dry matter intake (DMI, g/kg body mass/day (A) and daily α-pinene intake (B) of six mule deer (*Odocoileus hemionus*, MD, circles and solid line) and five white-tailed deer (*O. virginianus*, WTD, triangles and dashed line) when consuming diets with increasing concentrations of the monoterpene α-pinene (0–4%) in November of 2018 at the Wild Ungulate Facility at Washington State University, Pullman, Washington. The dotted horizontal line (A) represents the minimum DMI requirement for all animals (7.5 g/kg/day) to remain on the trial. If an animal did not eat enough to meet that threshold 2 days in a row, they were removed from the trial. Confidence bands are shaded in gray; darker areas represent band overlap between the two deer species. Because no interactions between α-pinene concentration and species were significant ($\alpha = 0.10$), lowercase letters (B) represent significant differences between levels of α-pinene concentration calculated using linear contrasts. A random effect for individual animal was included in each model.

resulted in higher apparent dry matter and energy digestibility. However, both species showed the same trends in digestibility among diets. MFHP, which contained mostly rice hulls as its fiber source, had lower apparent NDF digestibility than all of the other pelleted diets, which contained fiber mostly from aspen sawdust, beet pulp, and willow, despite a lower level of indigestible ADL content. This finding suggests that deer are able to digest fiber from browse sources such as willow and aspen more readily than from a novel, graminoid seed source (i.e., rice hulls), which is unlikely to be encountered by free-ranging deer in North America. These results also provide functional confirmation of the predictions of Zimmerman et al. (2006) and Clauss et al. (2009) who documented, based on rumen and digestive tract anatomy, that mule deer have higher absorptive and digesta capacity than white-tailed deer. In addition, our findings explain why mule deer selected natural diets with slightly, but significantly, lower apparent dry matter digestibility than those selected by white-tailed deer in a common garden experiment, even during the summer with relatively abundant nutritious forage (Berry et al. 2019).

Because mule deer voluntarily consumed natural forage diets higher in tannins than did white-tailed deer (Berry et al. 2019), we expected that mule deer would have an enhanced mechanism for processing tannins and have higher protein digestibility for tannin diets. Contrary to our hypothesis, however, apparent protein digestibility did not differ between the two deer species, even when consuming condensed tannins that reduced protein digestibility in the lowest-protein diet (LFLP+Tannin) and in willow. However, our protein digestibility results were consistent with those of Robbins et al. (1987a) who did not find differences in protein digestion between species when data from different studies in which mule and white-tailed deer consumed early-season grasses and cultivated legumes were combined. This result is not particularly surprising because both species are known to have relatively large parotid salivary glands and are capable of producing tannin-binding salivary proteins that reduce the negative effects of tannins on protein digestibility (Austin et al. 1989; Mole et al. 1990; Shimada 2006). Despite their tannin defense mechanisms, apparent protein digestibility (9% decrease) and daily intake of digestible protein (22% decrease) were depressed in both species when tannin-containing quebracho powder was added to the LFLP diet at a concentration of only 1.5% tannin. Although we were unable to measure the reduction in protein digestibility caused by the linear tannins in willow relative to same plant without tannins, it is noteworthy that protein digestibility was similar between the willow diet and the LFLP+Tannin, even though willow had four times higher tannin concentration and precipitated four times more protein. North American deer might be poorly adapted to the chemically distinct type of condensed tannins (5-deoxy) found mainly in the southern hemisphere (De Bruyne et al. 1999), and applying quebracho as a dry powder to formulated food provides a very different presentation to the digestive system than the willow tannin that is integral to the forage. Regardless of the type of tannin, our results showed both deer species seem to tolerate tannins and digest protein equally effectively. However, the higher nitrogen balance (thus higher minimum protein requirements) of white-tailed deer might predispose them to selecting diets with higher protein and lower tannins, as was documented by Berry et al. (2019). Monteith et al. (2019) also found that the use of condensed tannins resulted in a strong avoidance of feeding and reduction of crop depredation by white-tailed deer, although mule deer were not included in their study. A direct comparison of the size and histology of the parotid salivary glands, and concentrations of tannin-specific salivary binding

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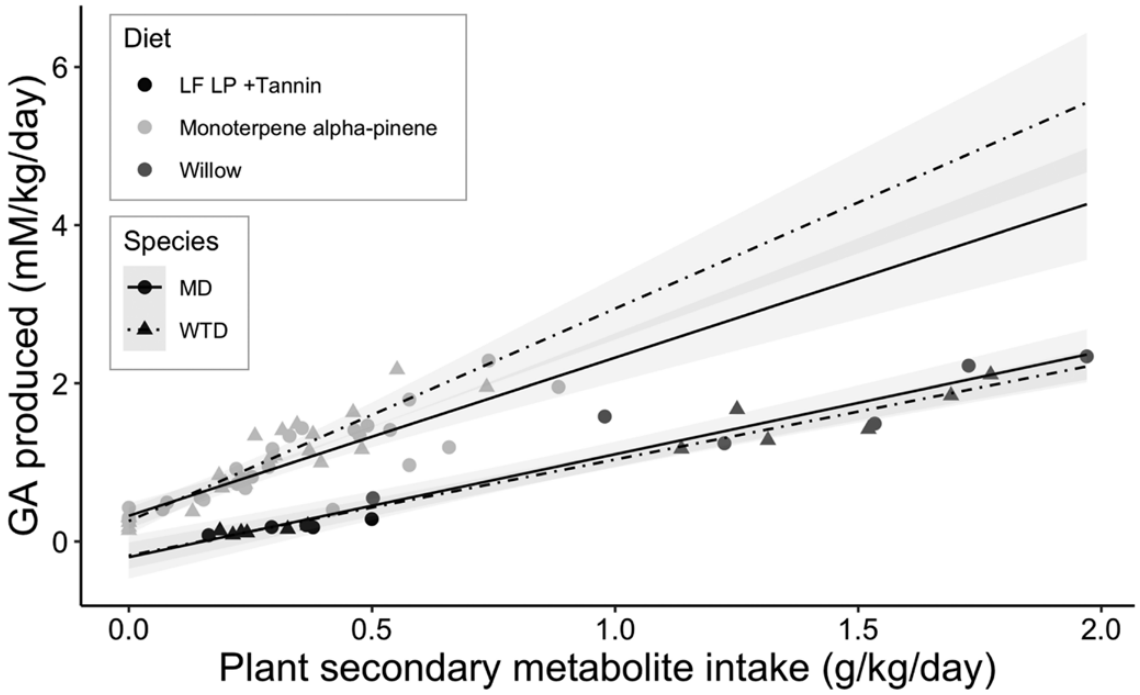

Fig. 5.—Production of a byproduct of detoxification, glucuronic acid (GA, mM/kg body mass/day), with increasing daily intake of two types of plant secondary metabolites of mule deer (*Odocoileus hemionus*, MD, circles and solid line) and white-tailed deer (*O. virginianus*, WTD, triangles and dashed line) at the Wild Ungulate Facility at Washington State University, Pullman, Washington. In separate experiments, deer were fed a (i) medium-fiber, high-protein pelleted diet with the monoterpene α-pinene added in increasing amounts (0–4%, light gray symbols); (ii) a low-fiber, low-protein pelleted diet with 5% powdered extract from the quebracho colorado tree (*Schinopsis* spp., LFLP+Tannin, black symbols, 1.5% condensed tannins); and (iii) the leaves of Pacific willow (*Salix lasiandra*, medium gray points, 6% condensed tannins). The species × α-pinene intake interaction was significant (α = 0.10) suggesting a faster rate of GA production in WTD, but no difference between species or an interaction was detected for condensed tannins. Confidence bands (95%) are shaded in gray; darker areas represent band overlap between the two species. A random effect for individual animal was included in each model.

proteins they produce, would provide additional information about whether one of the deer species has a greater potential to tolerate dietary condensed tannins.

Voluntary intake for diets containing both monoterpenes and condensed tannins was similar between deer species and lower when compared with the basal diet (MFHP) intake. These reductions in intake confirm that the diets we provided had biologically relevant concentrations of PSMs and that both mule and white-tailed deer were willing to consume the same amounts of these PSMs to acquire energy, protein, and other nutrients from the food. Dry matter intake decreased linearly as α-pinene in the diet increased, which allowed deer to regulate the total amount of this PSM ingested to a threshold of about 0.5 g α-pinene/kg/day, which was reached at a concentration around 3–4%. However, because we incrementally increased α-pinene concentration of the diet over a period of up to 10 days, we may have induced increased functionality of detoxification pathways such as Cytochrome P450 enzymes that metabolize PSMs (Dearing et al. 2005), allowing the deer to consume relatively more α-pinene as the trial progressed. Average food intake by the deer declined to less than half their normal intake at a dietary concentration of 4% α-pinene. We suspect that neither deer species is likely to consume a diet with >4% monoterpene concentration in natural habitats, which is approximately the upper limit of total monoterpene concentration in most deer forages (e.g., conifers, sagebrush; Kelsey et al. 2006; Vourc'h et al. 2002b; Burney and Jacobs 2011). For example, free-ranging mule deer accept plants with high monoterpene content (~3–4%) in their diets and were estimated to consume up to 20% of their total diet consisting of forages containing high monoterpene content before serious inhibition of digestive function developed (Wallmo et al. 1977). We acknowledge that natural diets contain multiple individual monoterpenes, not to mention a variety of other PSMs like phenolics and alkaloids (Schwartz et al. 1980; Servello and Schneider 2000), and free-ranging deer may differ from captive deer in their functional intestinal microbiota (Guan et al. 2017; Sun et al. 2019), which may influence physiological tolerances to PSMs. Our results with α-pinene therefore served as a method to compare PSM tolerances of mule and white-tailed deer with fewer confounding factors, but might not reflect the levels they would naturally consume of other individual or combinations of monoterpenes found in natural forages.

Voluntary intake of PSMs by herbivores depends on the rates at which absorbed PSMs are detoxified via metabolizing enzymes (e.g., Cytochrome P450, UDP-glucuronosyltransferases; Dearing et al. 2005; McLean and Duncan 2006; Sorensen and Dearing 2006). PSMs can be conjugated with polar molecules such as GA, an energetically expensive process that depletes glucose. When consuming diets that contain α-pinene, the

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rate of detoxification via conjugation with GA differed between the deer species in our experiments, but not when they consumed tannins and nontannin phenolics. Mule deer produced less GA in their urine relative to α-pinene consumption than did white-tailed deer, which suggests that they might use less costly methods of detoxification, such as oxidation (Boyle et al. 1999, 2000). Similarly, herbivores that specialize on diets high in monoterpenes, like Stephen's woodrat (*Neotoma stephensi*; Sorensen et al. 2005b), and pygmy rabbits (*Brachylagus idahoensis*; Shipley et al. 2012), have developed more effective and energy efficient mechanisms to detoxify and limit PSM absorption than their generalist herbivore counterparts, including producing less GA in their urine (Sorensen and Dearing 2003; Sorensen et al. 2004). Although we did not identify the specific metabolites of α-pinene in the deer's urine, Sorensen et al. (2005b) and Shipley et al. (2012) found a correspondence between higher GA production and more conjugated and fewer oxidized metabolites for the detoxification of monoterpenes in other mammalian herbivores. Lower reliance on GA for detoxification might also be attributed to low absorption of PSMs, a physiological mechanism expressed in herbivores that specialize on plants defended by high concentrations of PSMs (Sorensen et al. 2004; Thacker et al. 2012). Because mule deer likely use less energy to detoxify monoterpenes and require less digestible energy to maintain body mass, they might be better equipped to subsist on forages high in monoterpenes than white-tailed deer (Welch et al. 1983; Wambolt 1996; Vourc'h 2002b), particularly when energy is limited (e.g., winter). In addition, although they both are considered dietary generalists, mule deer might have a greater capacity to tolerate ingested PSMs such as monoterpenes than white-tailed deer. Regardless, this finding provides further evidence of differentiation in the fundamental nutritional niches of mule and white-tailed deer.

Although we detected a difference in the rate of detoxification of α-pinene between the deer species, we did not detect a similar difference when they consumed diets with tannins and nontannin phenolics. Furthermore, when comparing the diets containing PSMs, for each gram of diet consumed, α-pinene induced a two-fold increase in GA production when compared to the GA production induced by the tannin and nontannin phenolics in the LFLP+Tannin (quebracho powder) and willow diets. Other studies with white-tailed deer (Servello and Schneider 2000) and common brushtail possums (*Trichosurus vulpecula*; DeGabriel et al. 2009b) indicated that these species responded more strongly to diets containing monoterpenes than those containing condensed tannins and nontannin phenolics in terms of GA production and voluntary intake. The greater production of GA when consuming a monoterpene and lower tolerance to monoterpenes compared with phenolics could reflect differences in the relative absorption of these chemical classes, where monoterpenes are lipophilic and more readily absorbed in most species compared to water-soluble phenolics. Highly polymerized tannins are not well absorbed in the small intestine (Smeriglio et al. 2017), and tannin-binding salivary proteins of deer are expected to further reduce their absorption.

The differences in nutritional requirements and tolerances we found suggest that mule deer can "do more with less," because they can meet their requirements when consuming less protein and energy and can tolerate higher levels of monoterpenes than can white-tailed deer. This, in addition to the mule deer's ability to better digest plant fiber might, in turn, allow diet or habitat segregation that could potentially reduce direct competition in areas of sympatry (Whittaker and Lindzey 2004; Brunjes et al. 2009). However, because we observed relatively small effect sizes for some variables (e.g., 1–4 percentage points for digestibility), the mule deer's advantage relative to lower nutrient requirements and higher digestive efficiency would likely be more pronounced when nutritious, low-fiber forage is scarce, such as during winter, at high elevations, in some arid habitats, or in areas where mule deer may compete with white-tailed deer for limited forage. Lower nutrient and energy requirements coupled with more efficient digestion of fiber in part could explain why mule deer are more likely to occupy arid deserts and rangelands (Eberhardt et al. 1984, Marshal et al. 2010) where they might have a greater chance of survival and reproduction because they can access a wider breath of the available nutritional niche, especially on the more "difficult" side of the niche axes (Shipley et al. 2009), than can white-tailed deer. However, whether the mule deer's enhanced ability to extract more nutrients from plant diets relative to their nutritional requirements translates into competitive advantage that leads to a greater population productivity relative to white-tailed deer depends on (i) the relationship between intake requirements and predation (e.g., less exposure to predation if they need to acquire fewer nutrients—McArthur et al. 2014) and (ii) how efficiently each species can turn nutrients from available forages into new offspring that survive to recruitment, both when forage resources are abundant and nutritious and when they are scarce and of poor quality. Although DE and DP requirements for pregnancy and twinning have been measured in mule deer (Tollefson et al. 2010, 2011), similar data are not yet available for white-tailed deer. However, evidence of range expansion of white-tailed deer towards the north and west into traditional mule deer range (Hanberry and Hanberry 2020), coupled with the possible concomitant decline of mule deer populations in parts of their range (Ballard et al. 2001), suggests that white-tailed deer might have higher reproduction than mule deer, especially when nutritious forage resources are relatively abundant.

Although repeated and complex glaciation events in North America have destroyed most evidence of early evolution of *Odocoileus*, mule and white-tailed deer are thought to have diverged in the mid-Pleistocene to late Pleistocene between 750,000 and 3.7 million years ago when isolated by geographic barriers created by recurring glaciations (Heffelfinger 2011). The current geographic overlap along the Rocky Mountains represents a secondary contact between the two species after their post-Pleistocene range expansion (Heffelfinger 2011). Mule deer might have evolved a more efficient digestive anatomy when isolated from white-tailed deer in biomes with lower-quality plant communities, or might have secondarily

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evolved broader fundamental nutritional niche that includes more "difficult," less-nutritious plants (i.e., character displacement) to reduce intensive competition with white-tailed deer in areas where they were sympatric over millennia. A deeper understanding of the paleogeography of these species might provide greater insight to this question, and the relationship between nutritional adaptations and differences in other morphological and physiological traits between the two species that likely shape axes of their fundamental niches and drive much of the observed habitat segregation by the congeneric deer both in allopatry and sympatry (Anthony and Smith 1977; Smith 1987; Woods et al. 1989; Avey et al. 2003; Whittaker and Lindzey 2004; Brunjes et al. 2006; Walter et al. 2009; Whitney et al. 2011; Dellinger et al. 2019; Staudenmaier et al. 2021). For example, differences in escape gaits might explain the use of steeper, more rugged slopes by mule deer than white-tailed deer (Lingle and Pellis 2002; Dellinger et al. 2019), and the onset of thermal stress at colder temperatures in mule deer than white-tailed deer that might allow them to tolerate colder temperatures at higher elevations (Mautz et al. 1985).

Despite general difficulties in clearly demarcating upper and lower niche boundaries (Angilletta et al. 2019), our results provide a way to detect boundaries for some axes of the fundamental nutritional niche based on levels of nutrients and antinutrients that allow animals to meet energy and protein balance. This analysis suggests that mule deer have lower niche boundaries for minimum DE and DP content of food, and higher niche boundaries for maximum fiber content (NDF, ADL) of food. However, further research is needed to be able to identify and compare levels of these nutrients/antinutrients required for reproduction, a more conservative definition of the fundamental niche space where fitness ≥ 1 (Kearney 2006). Regardless, our findings provide a quantitative and direct comparison of the nutritional requirements and tolerances of mule and white-tailed deer in a controlled setting. The enhanced physiological abilities of mule deer that allow them to survive on less digestible energy and protein and more effectively cope with "difficult" forages relative to white-tailed deer minimize potential competitive interactions in shared landscapes and provide a modest advantage to mule deer in habitats dominated by low-quality forages.

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Appendix A

Results of linear contrasts between diets for linear mixed-effects models comparing mule deer (*Odocoileus hemionus*) and white-tailed deer (*O. virginianus*) across five single-diet, total-collection in vivo digestion trials conducted from July 2018–May 2020 at Washington State University, Pullman, Washington. DEI, digestible energy intake; DPI, digestible protein intake; NDF, neutral detergent fiber

Response	Main effects	Factor levels[a]	EM mean (*SE*)[b]	Confidence interval
Initial body mass (kg)	Diet	MFHP	65.2^{A} (2.29)	60.2–70.2
		HFMP	64.9^{A} (2.29)	59.9–69.8
		LFLP	66.6^{A} (2.30)	61.6–71.6
		LFLP+Tannin	73.8^{B} (2.29)	68.9–78.8
		Willow	67.1^{A} (2.27)	62.2–72.1
Dry matter intake (g/kg/day)	Diet	MFHP	28.9^{B} (1.92)	24.7–33.0
		HFMP	17.3^{A} (1.92)	13.1–21.5
		LFLP	21.0^{A} (2.00)	16.6–25.3
		LFLP+Tannin	19.6^{A} (1.92)	15.4–23.7
		Willow	22.7^{AB} (1.84)	18.7–26.8
Dry matter digestibility (%)	Diet	MFHP	64.7^{B} (0.84)	62.8–66.5
		HFMP	57.6^{A} (0.84)	55.8–59.4
		LFLP	74.6^{C} (0.88)	72.7–76.5
		LFLP+Tannin	66.4^{B} (0.84)	64.6–68.3
		Willow	63.9^{B} (0.80)	62.2–65.7
Protein digestibility (%)	Diet	MFHP	73.2^{D} (1.54)	69.8–76.5
		HFMP	54.5^{BC} (1.54)	51.0–57.8
		LFLP	56.6^{C} (1.60)	53.1–60.1
		LFLP+Tannin	47.9^{A} (1.54)	44.5–51.2
		Willow	51.5^{AB} (1.49)	48.3–54.7
Energy digestibility (%)	Diet	MFHP	66.5^{B} (0.88)	64.5–68.4
		HFMP	58.8^{A} (0.88)	56.9–60.7
		LFLP	77.1^{C} (0.92)	75.1–79.1
		LFLP+Tannin	67.6^{B} (0.88)	65.7–69.5
		Willow	60.4^{A} (0.84)	58.5–62.2
NDF digestibility (%)	Diet	MFHP	36.8^{A} (1.7)	33.2–40.5
		HFMP	42.7^{AB} (1.7)	39.0–40.5
		LFLP	62.4^{D} (1.8)	58.6–66.3
		LFLP+Tannin	48.9^{C} (1.7)	45.2–52.5
		Willow	47.0^{BC} (1.6)	43.5–50.5
DEI (MJ/kg/day)	Diet	MFHP	333^{C} (23.4)	282–384
		HFMP	188^{A} (23.4)	137–239
		LFLP	283^{BC} (23.4)	230–336
		LFLP+Tannin	230^{AB} (23.4)	179–281
		Willow	271^{BC} (23.4)	223–320
DPI (g/kg/day)	Diet	MFHP	386^{C} (1.7)	346–426
		HFMP	127^{A} (18.4)	87–167
		LFLP	144^{AB} (19.2)	102–185
		LFLP+Tannin	113^{A} (18.4)	73–153
		Willow	193^{B} (17.6)	155–231

[a]Factor levels include the five diet types from in vivo digestion trials: MFHP, moderate-fiber, high-protein pelleted diet; HFMP, high-fiber, moderate-protein pelleted diet; LFLP, low-fiber, low-protein pelleted diet, LFLP+Tannin, low-fiber, low-protein pelleted diet plus 1.5% condensed tannins as powdered extract from bark of the quebracho colorado tree (*Schinopsis* spp.).

[b]Capital letters denote significant differences between given estimated marginal (EM) means and *SE* of diet levels ($\alpha = 0.10$).

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