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Mule Deer Do More with Less: Comparing Their Nutritional Requirements and Tolerances with White-Tailed Deer

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Comparing the Fundamental Nutritional Niches of Mule (*Odocoileus hemionus*) and White-Tailed Deer (*O. virginianus*)

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Abstract

Congeneric species often share ecological niche space resulting in competitive interactions that either limit co-occurrence or lead to niche partitioning. We propose that differences in fundamental nutritional niches are a potential mechanism that could explain overlapping distribution patterns of congenerics. We directly compared the fundamental nutritional niches of mule (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) that occur in sympatry and allopatry in similar realized ecological niches across their ranges in North America. Nutritional niches of mule and white-tailed deer were quantified using *in vivo* digestion and intake tolerance trials with six diets ranging in content of fiber, protein, and plant secondary metabolites (PSMs) using tractable deer raised under identical conditions in captivity. We found that compared to white-tailed deer, mule deer had higher fiber, energy, and dry matter digestibility, produced glucuronic acid (a byproduct of PSM detoxification) at a slower rate when consuming the monoterpene α-pinene, and required 54% less digestible protein and 21% less digestible energy intake per day to maintain body mass and nitrogen balance. 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.

Keywords: deer, detoxification, digestibility, *Odocoileus hemionus*, *Odocoileus virginianus*, plant secondary metabolites, niche, nutrient requirements, sympatry, tannins

Introduction

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). Therefore, food resources are often at the core of competitive interactions, particularly between congeneric species occupying similar niches (Harper 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 1961), thus 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.), through which the evolution of character displacement was tracked via 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 (*Odocoileus virginianus*), that consume hundreds of different plants across their ranges can be more difficult.

Detecting competition and differences in the fundamental nutritional niches (i.e, the full range of nutrients and food resources that allows a species to maintain viable populations without interference from other species or other limits to access food access [Hutchinson 1957]; the "requirement niche" [Leibold 1995]) of widespread generalist herbivores requires a particularly in-depth analysis as suggested by DeGabriel et al. (2014). The overlap in food resources 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 nutritional niches differing in the absolute and relative amount of plant protein and carbohydrates, even though they ate the same plant species. Therefore, measuring differences in the fundamental nutritional niche in herbivores requires controlled comparative studies that measure physiological requirements and tolerances for a range of nutrients (e.g., energy, protein) and anti-nutrients (e.g., fiber, plant secondary metabolites [PSMs]) and the mechanics of harvesting plants.

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 conducted using fecal or stomach contents of free-ranging animals, and therefore were unable to establish whether dietary differences reflected differences in nutritional requirements and tolerances (i.e., differences in their fundamental nutritional niche) or reflected that different foods were available in different habitats selected by the deer species (i.e., differences in their realized dietary niche). 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).

Previous research suggests that mule deer and white-tailed deer might differ, at least modestly, in their fundamental nutritional niche and digestive physiology. Berry at 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 directly compared their morphology, physiology, nutritional requirements or PSM tolerances. The two studies that have directly compared aspects of the digestive morphology between the two deer species (Zimmerman et al. 2006, Clauss et al. 2009) both 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 white-tailed deer is relatively plastic and can be enhanced when consuming higher fiber diets through 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 could also 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 (Sorensen et al. 2005b) as they are absorbed, metabolized and excreted by the animal (Dearing et al. 2005). Condensed tannins, a type of phenolic, reduce 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, Robbins et al. 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 on 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 that affect both detection and potential use by herbivores (Elliott and Loudon 1987). Monoterpenes can inhibit digestion by ruminants and over-ingestion can result in toxicosis or death (Freeland and Janzen 1974, Fowler 1983), thus plants high in monoterpenes are often 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 is often limited to conifers like balsam fir (*Abies balsamea*) and spruce (*Picea* spp.) (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.

Despite previous research on their digestive efficiency, nutrient requirements, and dietary tolerances, no comprehensive, direct comparisons of the fundamental nutritional niche have been made between mule and white-tailed deer under identical conditions. Therefore, in this study we compared the ability of mule and white-tailed deer to digest plant diets and to tolerate and detoxify tannins and monoterpenes common in plants they both consume. We also compared their nitrogen and energy balances to assess if nutrient requirements differed between the two deer species, potentially allowing one species to subsist on nutritionally poorer diets. To do so, we conducted 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 deer, 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 have higher digestibility of dry matter, energy, protein, and fiber on diets with and without condensed tannins, and would require less digestible energy and protein for maintenance of body mass 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 the ability of mule and white-tailed deer to digest plant nutrients and fiber, we conducted 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 ranged in the amount and type of plant fiber, nutrients (i.e., energy and protein), and condensed tannins (Table 1). Deer ranged from 3-12 years old and were non-lactating 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, WA. The other 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 outlined by the American Society of Mammalogists for the use of wild mammals in research (Sikes et al. 2016).

We conducted a series of five total-collection, single-diet digestion trials from July 2018 – 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, MO), 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) was also 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.; 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 (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% non-tannin 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 conducted a digestion trial with the leaves of the Pacific willow tree (*Salix lasiandra*), 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 hours 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 two-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*) and alfalfa (*Medicago sativa*). 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 x 1.9 m) covered outdoor digestion crates constructed of chain link fence with rubberized, porous flooring and deer-safe materials. Animals were acclimated to the digestion crates periodically throughout the two-year period during which we conducted our experiment to

reduce the animals' stress while participating in trials. 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 two days in a row they were removed from the trial. In addition, data from one animal was removed from two trials *post hoc* 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 seven days, preceded and followed by approximately two 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 two days of each trial served as acclimation days; we did not include data from those days in our analyses. During days three through seven 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 $\geq$ 24 hours 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.

Food, orts, and feces were immediately frozen after collection for nutritional analysis and were later oven dried at 60° C for three 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 [%], 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, USA). 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, NY). Nitrogen content (N, %) of diet, fecal, and urine samples was determined using a Carbon-Nitrogen TruSpec analyzer (LECO, St. Joseph, Michigan, USA) 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 $\frac{(\text{dry matter intake} - \text{dry feces produced})}{\text{dry matter intake}}$. 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 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 non-tannin 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, OH, 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 to 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 LFLP+Tannin diet's composited sample using the protein precipitable phenolics method as detailed in (Hagerman 2011) in the Hagerman Lab. To create the calibration curve, quebracho powder or *Sorghum* purified condensed tannin was dissolved in methanol and appropriate aliquots were added to the SDS/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), incubated, centrifuged, and the supernatants aspirated. The remaining precipitates were re-dissolved in the SDS/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 were 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 five days using an extract of the composited sample of the willow forage.

Monoterpene Intake Tolerance Trial

We compared the tolerance for α-pinene, a monoterpene prevalent in many evergreen plants, between deer species by measuring 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 U.S., including sagebrush (*Artemisia tridentata*, Kelsey et al. 2006), western red cedar (*Thuja plicata*), western hemlock (*Tsuga heterophylla*), and Douglas-fir (*Pseudotsuga menziesii*) (Vourc'h et al. 2002b, Burney and Jacobs 2011). These trials lasted up to 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 two 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) 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 conducted during late fall, when ambient temperatures were relatively low (-0.3 – 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.

Glucuronic Acid 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, Julkunenen-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 is therefore 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 conducted directly from 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 were then compared with a standard curve created using pure GA (Sigma-Aldrich Canada, Oakville, Ontario). 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 Analysis

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 first tested for significant interactions between species and diet, and non-significant interactions were removed. Model fit was visually confirmed 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-squared (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 were then 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 modelling 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 1) the rate of decline in DMI with increasing α-pinene concentration (0-4%), 2) the non-linear change in α-pinene intake with increasing α-pinene concentration (0-4%) in the diet, 3) the rate of GA production with increasing α-pinene intake, and 4) 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 modelling framework as in the preceding analyses. We expected alpha-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). Therefore, we explored this predicted non-linear relationship by comparing model estimates for α-pinene among α-pinene concentration levels used as a categorical variable. We also modelled the concentration of α-pinene in the diet as a continuous variable with a linear relationship to DMI. Estimates were directly interpreted 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 labelled and standard errors are reported as ± standard error.

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 percentage points (± 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 percentage points (± 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, 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, 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 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 ($\chi^2_1 = 0.004$, $p = 0.95$) per day, but daily α-pinene intake varied among α-pinene concentrations in the diet ($\chi^2_4 = 361.2$, $p < 0.001$; Fig. 4b). Mean daily intake of α-pinene differed significantly between the α-pinene concentrations from 0% – 1% ($t_{98} = -6.8$, $p < 0.001$; Fig. 4b) and 1% – 2% ($t_{98} = -4.9$, $p < 0.001$; Fig. 4b). However, intake of α-pinene did not differ significantly between 2% – 3% ($t_{98} = -2.5$, $p = 0.11$; Fig. 4b) and 3% – 4% ($t_{98} = -1.3$, $p = 0.69$; Fig. 4b) suggesting intake of α-pinene was reaching a threshold between 2% and 4%.

Detoxification of Monoterpenes and Tannins

Detoxification of monoterpenes differed between species based upon 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 non-tannin 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.

Discussion

In a controlled experimental setting, we directly compared aspects of the 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 non-tannin phenolics were also 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 were better able to digest dry matter, NDF, and energy in their diets and required less digestible energy to maintain body mass and less protein to maintain N balance. 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 body size can directly influence intake, and although mule deer in our study (and in Berry et al. 2019) tended to be larger, body mass of mule (55.2 to 90.2 kg) and white-tailed deer (53.6 to 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 and 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 vs. 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, non-lactating 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 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 gastro-intestinal 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 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 acid detergent lignin 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 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) based on rumen and digestive tract anatomy, who documented 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 we observed relatively small effect sizes (1-4 percentage points), the mule deer's advantage relative to 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. 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). Lower nutrient and energy requirements coupled with more efficient digestion of fiber could explain, in part, why mule deer are more likely to occupy arid deserts and rangelands (Eberhardt et al. 1984, Marshal et al. 2010).

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- to late Pleistocene between 750,000 – 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 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.

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 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, the fact that protein digestibility was similar between the willow diet and the LFLP+ Tannin, despite the fact that willow had 4 times higher tannin concentration and precipitate four times more protein, is noteworthy. 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 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, though 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 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 to 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. Therefore, our results with α-pinene 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 et al. 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 rate of detoxification via conjugation with GA differed between the deer species in our experiments, but not when they consumed tannins and non-tannin 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), especially when energy is limited (e.g., winter). In addition, although they are both 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 non-tannin 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 non-tannin 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 in terms of GA production and voluntary intake to diets containing monoterpenes than those containing condensed tannins and non-tannin phenolics. The greater production of GA when consuming a monoterpene and lower tolerance to monoterpenes compared to 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.

Our findings provide the first quantitative evaluation and direct comparison of the fundamental nutritional niche of mule and white-tailed deer in a controlled setting. Previous work has identified differences other aspects of the fundamental niches of the two species, including differences in escape gaits that explain the use of steeper, more rugged slopes by mule 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). These differences likely 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). However, we identified significant differences in fiber, energy, and dry matter digestion, monoterpene detoxification, and daily energy and protein requirements between the species that could provide additional means for segregation, in terms of not only different habitats but also in terms of different diets within the same habitats. These mechanisms are likely to be most important when food is scarce or of low quality (such as in arid environments or high elevations), at which point mule deer 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 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 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. Reproductive performance relative to diet quality has not yet been directly compared between the deer species, but evidence of range expansion of white-tailed deer towards the north and west (Hanberry and Hanberry 2020), coupled with the hypothesized 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 when nutritious forage resources are relatively abundant. White-tailed deer might also be superior competitors to mule deer where sympatric because of apparent competition mediated by cougars (*Puma concolor)* that favors white-tailed deer (Robinson et al. 2002, Cooley et al. 2008, Keehner et al. 2015) and predator defense behavior by mule deer that might disproportionally benefit the survival of white-tailed deer fawns at the expense of mule deer (Lingle et al. 2005, Bonar et al. 2015). Future studies that compare production and survival of offspring in relation to forage quality and other aspects of the fundamental niche would further elucidate mechanisms contributing to differences in distribution or habitat use and the potential for competition between these deer species.

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Literature Cited

Anderson, A. E., and O. C. Wallmo. 1984. *Odocoileus hemionus*. Mammalian Species 219:1–9.

Anthony, R. G., and N. S. Smith. 1977. Ecological relationships between mule deer and white-tailed deer in southeastern Arizona. Ecological Monographs 47:255–277.

Aoki, I. 1987. Entropy balance of white-tailed deer during a winter night. Bulletin of Mathematical Biology 49:321–327.

Asleson, M. A., E. C. Hellgren, and L. W. Varner. 1996. Nitrogen requirements for antler growth and maintenance in white-tailed deer. Journal of Wildlife Management 60:744–752.

Austin, P. J., L. A. Suchar, C. T. Robbins, and A. E. Hagerman. 1989. Tannin-binding proteins in saliva of deer and their absence in saliva of sheep and cattle. Journal of Chemical Ecology 15:1335–1347.

Avey, J. T., W. B. Ballard, M. C. Wallace, M. H. Humphrey, P. R. Krausman, F. Harwell, and E. B. Fisch. 2003. Habitat relationships between sympatric mule deer and white-tailed deer in Texas. Southwestern Naturalist 48:644–653.

Baker, D. L., D. E. Johnson, L. H. Carpenter, O. C. Wallmo, and R. B. Gill. 1979. Energy requirements of mule deer fawns in winter. Journal of Wildlife Management 43:162–169.

Ballard, W. B., D. Lutz, W. Keegan, L. H. Carpenter, and J. C. DeVos Jr. 2001. Deer-predator relationships: a review of recent North American studies with emphasis on mule and black-tailed deer. Wildlife Society Bulletin 29:99–115.

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

Behmer, S. T., and A. Joern. 2008. Coexisting generalist herbivores occupy unique nutritional feeding niches. PNAS 105:1977-1982.

Berry, S. L., L. A. Shipley, R. A. Long, and C. Loggers. 2019. Differences in dietary niche and foraging behavior of sympatric mule and white-tailed deer. Ecosphere 10:e02815.10.1002/ecs2.2815.

Bonar, M., M. Manseau, J. Geisheimer, T. Bannatyne, and S. Lingle. 2016. The effect of terrain and female density on survival of neonatal white-tailed deer and mule deer fawns. Ecology and Evolution 6:4387-4402.

Bonnin, M., J. P. Tremblay, and S. D. Cote. 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.

Boyle, R., S. McLean, W. J. Foley, and N. W. Davies. 1999. Comparative metabolism of dietary terpene, p-cymene, in generalist and specialist folivorous marsupials. Journal of Chemical Ecology 25:2109–2126.

Boyle, R., S. McLean, W. J. Foley, and N. W. Davies. 2000. Biotransformation of 1,8-cineole in the brushtail possum (*Trichosurus vulpecula*). Xenobiotica 30:915–932.

Brunjes, K. J., W. B. Ballard, M. H. Humphrey, F. Harwell, N. E. McIntyre, P. R. Krausman, and M. C. Wallace. 2006. Habitat use by sympatric mule and white-tailed deer in Texas. Journal of Wildlife Management 70:1351–1359.

Brunjes, K. J., W. B. Ballard, M. H. Humphrey, F. Harwell, N. E. McIntyre, P. R. Krausman, and M. C. Wallace. 2009. Home ranges of sympatric mule deer and white-tailed deer in Texas. Southwestern Naturalist 53:253–260.

Burney, O. T., and D. F. Jacobs. 2011. Ungulate herbivory of regenerating conifers in relation to foliar nutrition and terpenoid production. Forest Ecology and Management 262:1834–1845.

Casabon, C., and D. Pothier. 2007. Browsing of tree regeneration by white-tailed deer in large clearcuts on Anticosti Island, Quebec. Forest Ecology and Management 253:112–119.

Clauss, M., K. Lason, J. Gehrke, M. Lechner-Doll, J. Fickel, T. Grune, and W. J. Streich. 2003. Captive roe deer (*Capreolus capreolus*) select for low amounts of tannic acid but not quebracho: fluctuations of preferences and potential benefits. Comparative Biochemistry and Physiology Part B 136:369-382.

Clauss M., R. R. Hofmann, J. Fickel, W. J. Streich, and J. Hummel. 2009. The intraruminal papillation gradient in wild ruminants of different feeding types: implications for rumen physiology. Journal of Morphology 270:929–942.

Connolly, G. E., B. O. Ellison, J. W. Fleming, S. Geng, R. E. Kepner, W. M. Longhurst, J. H. Oh, and G. F. Russell. 1980. Deer browsing of Douglas-fir trees in relation to volatile terpene composition and in vitro fermentability. Forest Science 26:179–193.

Cooley, H. S., H. S. Robinson, R. B. Wielgus, and C. S. Lambert. 2008. Cougar prey selection in a white-tailed deer and mule deer community. Journal of Wildlife Management 72:99–106.

Dearing, M. D., W. J. Foley, and S. McLean. 2005. The influence of plant secondary metabolites on the nutritional ecology of herbivorous terrestrial vertebrates. Annual Review of Ecology, Evolution, and Systematics 36:169–189.

De Bruyne, T., L. Pieters, H. Deelstra, and A. Vlietinck. 1999. Condensed vegetable tannins: Biodiversity in structure and biological activities. Biochemical Systematics and Ecology 27:445–459.

DeGabriel, J. L., B. D. Moore, W. J. Foley, and C. N. Johnson. 2009a. The effects of plant defensive chemistry on nutrient availability predict reproductive success in a mammal. Ecology 90:711-719.

DeGabriel, J. L., B. D. Moore, L. A. Shipley, A. K. Krockenberger, I. R. Wallis, C. N. Johnson, and W. J. Foley. 2009b. Inter-population differences in tolerances of a marsupial folivore to plant secondary metabolites. Oecologia 161:539–548.

DeGabriel, J. L., B. D. Moore, A. M. Felton, J. U. Ganzhorn, C. Stolter, I. R. Wallis, C. N. Johnson, and W. J. Foley. 2014. Translating nutritional ecology from the laboratory to the field: milestones in linking plant chemistry to population regulation in mammalian browsers. Oikos 123:298-308.

DelGiudice, G.D., M. A. Asleson, L. Varner, E. C. Hellgren, and M. R. Riggs. 1996. Creatinine ratios in random sampled and 24-hour urines of white-tailed deer. Journal of Wildlife Management 60:381–387.

Dellinger, J. A., C. R. Shores, A. Craig, M. R. Heithaus, W. J. Ripple, and A. J. Wirsing. 2019. Habitat use of sympatric prey suggests divergent anti-predator responses to recolonizing gray wolves. Oecologia 189:487–500.

Duncan, A. J., S. E. Hartley, M. Thurlow, S. Young, and B. W. Staines. 2001. Clonal variation in monoterpene concentrations in Sitka spruce (*Picea sitchensis*) saplings and its effect on their susceptibility to browsing damage by red deer (*Cervus elaphus*). Forest Ecological Management 148:259–269.

Eberhardt, L. E., E. E. Hanson, and L. L. Cadwell. 1984. Movement and activity patterns of mule deer in the Sagebrush-Steppe region. Journal of Mammalogy 65:404–409.

Elliott, S., and A. Loudon. 1987. Effects of monoterpene odors on food selection by red deer calves (*Cervus elaphus*). Journal of Chemical Ecology 13:1343–1349.

Ferreguetti, A. C., W. M. Tomas, and H. G. Bergallo. 2015. Density, occupancy, and activity pattern of two sympatric deer (*Mazama*) in the Atlantic Forest, Brazil. Journal of Mammalogy 96:1245–1254.

Fowler, M. E. 1983. Plant poisoning in free-living wild animals: a review. Journal of Wildlife Diseases 19:34–43.

Fox, J., and S. Weisberg. 2019. An {R} Companion to Applied Regression. 3rd ed. SAGE Publishing. Thousand Oaks, California.

Freeland, W. J., and D. H. Janzen. 1974. Strategies in herbivory by mammals: the role of plant secondary compounds. American Naturalist 108:269–289.

Gause, G. F. 1934. The struggle for existence. The Williams and Wilkins Company. Baltimore, Maryland.

Geist, V. 1998. White-tailed deer and mule deer. Pp. 255–414 in Deer of the world: Their evolution, behavior, and ecology. Stackpole Books, Mechanicsburg, Pennsylvania, USA.

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analysis (apparatus, reagents, procedures and some applications). U.S. Department of Agriculture Research Station. Agriculture Handbook 379. U.S. Government Printing Office, Washington, D.C., USA.

Gordon, I. J., and A. W. Illius. 1994. The functional significance of the browser-grazer dichotomy in African ruminants. Oecologia 98:167–175.

Grant, P. R., and B. R. Grant. 2006. Evolution of character displacement in Darwin's finches. Science 5784:224–226.

Guan, Y., H. Yang, S. Han, L. Feng, T. Wang, and J. Ge. 2017. Comparison of the gut microbiota composition between wild and captive sika deer (*Cervus nippon hortulorum*) from feces by high-throughput sequencing. AMB Express *7*:1–13.

Guglielmo, C. G., W. H. Karasov, and W. J. Jakubas. 1996. Nutritional costs of a plant secondary metabolite explain selective foraging by ruffed grouse. Ecology 77:1103–1115.

Hagerman, A. E. 2011. Tannin Handbook. www.users.muohio.edu/hagermae/. Accessed 25 June 2020.

Hagerman, A. E., C. T. Robbins, Y. Weerasuriya, T. C. Wilson, and C. McArthur. 1992. Tannin chemistry in relation to digestion. Journal of Rangeland Management 45:57–62.

Hagerman, A. E., and C. T. Robbins. 1993. Specificity of tannin-binding salivary proteins relative to diet selection by mammals. Canadian Journal of Zoology 71:628–633.

Hanberry, B. and P. Hanberry. 2020. Rapid digitization to reclaim thematic maps of white-tailed deer density from 1982 and 2003 in the conterminous US. PeerJ 8:e8262.

Harper, J. L., J. N. Clatworthy, I. H. McNaughton, and G. R. Sagar. 1961. The evolution and ecology of closely related species living in the same area. Evolution 15:209–227.

Heffelfinger, J. R. 2011. Taxonomy, evolutionary history, and distribution. Pp. 3-39 in Biology and management of white-tailed deer (D. G. Hewitt, ed.). CRC Press, Boca Raton, FL.

Holter, J. B., W. E. Urban Jr., and H. H. Hayes. 1977. Nutrition of northern white-tailed deer throughout the year. Journal of Animal Science 45:365–376.

Holter, J. B., W. E. Urban Jr., and H. H. Hayes. 1979. Predicting energy and nitrogen retention in young white-tailed deer. Journal of Wildlife Management 43:880–888.

Hull, I. T. 2018. Influences of fuel reduction treatments on the nutritional ecology of deer in northeastern Washington. M.S. Thesis, Washington State University, Pullman.

Hutchinson, G. E. 1957. Concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology 22:415-427.

Hygnstrom, S. E., S. R. Groepper, K. C. VerCauteren, C. J. Frost, and J. R. Boner. 2008. Literature review of mule deer and white-tailed deer movements in western and midwestern landscapes. Great Plains Research 18:219–231.

Jacobson, J. A. 2003. Identification of mule deer (Odocoileus hemionus) and white-tailed deer (*Odocoileus virginianus*) postcranial remains as a means of determining human subsistence strategies. Plains Anthropologist 48:287–297.

Jacobson, J. A. 2004. Determining human ecology on the Plains through the identification of mule deer (*Odocoileus hemionus*) and white-tailed deer (*Odocoileus virginianus*) postcranial remains. Dissertation, University of Tennessee, Knoxville, USA.

Julkunen-Tiitto, R., and S. Sorsa. 2001. Testing the effects of drying methods on willow flavonoids, tannins, and salicylates. Journal of Chemical Ecology 27:779–789.

Keehner, J. R., R. B. Wielgus, and A. M. Keehner. 2015. Effects of male targeted harvest regimes on prey switching by female mountain lions: Implications for apparent competition on declining secondary prey. Biological Conservation 192:101–108.

Kelsey, R. G., J. R. Stephens, and F. Shafizadeh. 2006. The chemical constituents of sagebrush foliage and their isolation. Journal of Range Management Archives 35:617–622.

Krausman, P. R. 1978. Forage relationships between two deer species in Big Bend National Park, Texas. Journal of Wildlife Management 42:101–107.

Leibold, M. A. 1995. The Niche Concept revisited: Mechanistic models and community context. Ecology 76:1371-1382.

Lenth, R.V. 2016. Least-Squares Means: The R Package lsmeans. Journal of Statistical Software 69:1–33.

Lingle, S., and S.M. Pellis. 2002. Fight or flight? Antipredator behavior and the escalation of coyote encounters with deer. Oecologia 131:154–164.

Lingle, S., S. M. Pellis, W. F. Wilson. 2005. Interspecific variation in antipredator behaviour leads to differential vulnerability of mule deer and white-tailed deer fawns early in life. Journal of Animal Ecology 74:1140–1149.

Lyman, R. L. 2006. Late prehistoric and early historic abundance of Columbian white-tailed deer, Portland Basin, Washington and Oregon, USA. Journal of Wildlife Management 70:278–282.

Mackie, R. J., D. F. Pac, K. L. Hamlin, and G. L. Dusek. 1998. Ecology and management of mule deer and white-tailed deer in Montana. Montana Fish, Wildlife and Parks, Wildlife Division. Helena, Montana.

Mangione, A. M., D. Dearing, W. and Karasov. 2001. Detoxification in relation to toxin tolerance in desert woodrats eating creosote bush. Journal of Chemical Ecology 27:2559–2578.

Marshal, J. P., V. C. Bleich, P. R. Krausman, M. L. Reid, and N. G. Andrew. 2010. Factors affecting habitat use and distribution of desert mule deer in an arid environment. Wildlife Society Bulletin 34:609–619.

Martinka, C. J. 1968. Habitat relationships of white-tailed and mule deer in northern Montana. Journal of Wildlife Management 32:558–565.

Mautz, W. W., P. J. Pekins, and J. A. Warren. 1985. Cold temperature effects on metabolic rates of white-tailed, mule, and black-tailed deer in winter coat. Biology of deer production. Royal Society of New Zealand Bulletin 22:453–457.

McLean, S., and A. J. Duncan. 2006. Pharmacological perspectives on the detoxification of plant secondary metabolites: Implications for ingestive behavior of herbivores. Journal of Chemical Ecology 32:1213–1228.

McLean, S., S. Brandon, R. R. Boyle, and N. L. Wiggins. 2008. Development of tolerance to the dietary plant secondary metabolite 1,8-cineole by the brushtail possum (*Trichosurus vulpecula*). Journal of Chemical Ecology 34:672-680.

Mole, S., L. G. Butler, and G. Iason. 1990. Defense against dietary tannin in herbivores—a survey for proline-rich salivary proteins in mammals. Biochemical Systematics and Ecology 18:287–293.

Monteith, K. B., K. L. Monteith, and J. A. Jenks. 2019. Condensed tannins as a deterrent to crop depredation by white-tailed deer: Effects of concentration and learning. Wildlife Society Bulletin 43:693–700.

Mould, E. D., and C. T. Robbins. 1981. Evaluation of detergent analysis in estimating nutritional value of browse. Journal of Wildlife Management 45:937–947.

Parker, K. L. 1988. Effects of heat, cold, and rain on coastal black-tailed deer. Canadian Journal of Zoology 66:1409–1422.

Parker, K. L., and C. T. Robbins. 1984. Thermoregulation in mule deer and elk. Canadian Journal of Zoology 62:1409–1422.

Parker, K. L., C. T. Robbins, and T. A. Hanley. 1984. Energy expenditures for locomotion by mule deer and elk. Journal of Wildlife Management 48:474–488.

Pinheiro J., D. Bates, S. DebRoy, D. Sarkar, and R Core Team. 2019. Nlme: Linear and Nonlinear Mixed Effects Models. R package version 3.1–140.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.

Reid, D. G., S. L. Bonnet, G. Kemp, J. H. van der Westhuizen. 2013. Analysis of commercial proanthocyanidins. Part 4: solid state (13)C NMR as a tool for in situ analysis of proanthocyanidin tannins, in heartwood and bark of quebracho and acacia, and related species. Phytochemistry 94:243-248.

Robbins, C. T. 1994. Wildlife feeding and nutrition. 2nd ed. Academic Press. London.

Robbins, C. T., and A. N. Moen. 1975a. Milk consumption and weight gain of white-tailed deer. Journal of Wildlife Management 39:355–360.

Robbins, C. T., and A. N. Moen. 1975b. Uterine composition and growth in pregnant white-tailed deer. Journal of Wildlife Management 39:684–691.

Robbins, C. T., R. L. Prior, A. N. Moen, and W. J. Visek. 1974. Nitrogen metabolism of white-tailed deer. Journal of Animal Science 38:186–191.

Robbins, C. T., P. J. Van Soest, W. M. Mautz, and A. N. Moen. 1975. Feed analyses and digestion with references to white-tailed deer. Journal of Wildlife Management 39:67–79.

Robbins, C. T., S. Mole, A. E. Hagerman, and T. A. Hanley. 1987a. Role of tannins in defending plants against ruminants: reduction in protein availability. Ecology 68:1606–1615.

Robbins, C. T., S. Mole, A. E. Hagerman, and T. A. Hanley. 1987b. Role of tannins in defending plants against ruminants: reduction in dry matter digestion. Ecology 68:98–107.

Robbins, C. T., A. E. Hagerman, P. J. Austin, C. McArthur, T. A. Hanley. 1991. Variation in mammalian physiological responses to a condensed tannin and Its ecological implications. Journal of Mammalogy 72:480–486.

Robbins, C. T., D. E. Spalinger, and W. van Hoven. 1995. Adaptation of ruminants to browse and grass diets: are anatomical-based browser-grazer interpretations valid? Oecologia 103:208–213.

Robinson, H. S., R. B. Wielgus, and J. C. Gwilliam. 2002. Cougar predation and population growth of sympatric mule deer and white-tailed deer. Canadian Journal of Zoology 80:556–568.

Sadleir, R. M. F. S. 1980. Energy and protein intake in relation to growth of suckling black-tailed deer fawns. Canadian Journal of Zoology 58:1347–1354.

Sadleir, R. M. F. S. 1982. Energy consumption and subsequent partitioning in lactating black-tailed deer. Canadian Journal of Zoology 60:382–386.

Sargeant, G. A., L. E. Eberhardt, and J. M. Peek. 1994. Thermoregulation by mule deer (*Odocoileus hemionus*) in arid rangelands of Southcentral Washington. Journal of Mammalogy 75:536–544.

Schluter, D., T. D. Price, and P. R. Grant. 1985. Ecological character displacement in Darwin's finches. Science 4690:1056–1059.

Schofield, J. A., A. E. Hagerman, and A. Harold. 1998. Loss of tannins and other phenolics from willow leaf litter. Journal of Chemical Ecology 24:1409–1421.

Schwartz, C. C., J. G. Nagy, and W. L. Regelin. 1980. Juniper oil yield, terpenoid concentration, and antimicrobial effects on deer. Journal of Wildlife Management 44:107–113.

Servello, F. A. and J. W. Schneider. 2000. Evaluation of urinary indices of nutritional status for white-tailed deer: Tests with winter browse diets. Journal of Wildlife Management 64:137–145.

Shimada, T. 2006. Salivary proteins as a defense against dietary tannins. Journal of Chemical Ecology 32:1149–1163.

Shipley, L. A., J. S. Forbey, and B. D. Moore. 2009. Revisiting the dietary niche: when is a mammalian herbivore a specialist? Integrative and Comparative Biology 49:274–290.

Shipley, L. A., E. M. Davis, L. A. Felicetti, S. McLean, and J. S. Forbey. 2012. Mechanisms for eliminating monoterpenes of sagebrush by specialist and generalist rabbits. Journal of Chemical Ecology 38:1178–1189.

Sikes, R. S., and the Animal Care and Use Committee of the American Society of Mammalogists. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. Journal of Mammalogy 97:663−688.

Smeriglio, A., D. Barreca, E. Bellocco, and D. Trombetta. 2017. Proanthocyanidins and hydrolysable tannins: occurrence, dietary intake and pharmacological effects. British Journal of Pharmacology 174: 1244−1262.

Smith, S. H., J. B. Holter, H. H. Hayes, and H. Silver. 1975. Protein requirement of white-tailed deer fawns. Journal of Wildlife Management 39:582−589.

Smith, W. P. 1987. Dispersion and habitat use by sympatric Columbian white-tailed deer and Columbian black-tailed deer. Journal of Mammalogy 68:337–347.

Sorensen, J. S., and M. D. Dearing. 2003. Elimination of plant toxins by herbivorous woodrats: revisiting an explanation for dietary specialization in mammalian herbivores. Oecologia 134:88–94.

Sorenson, J. S., C. A. Turnbull, and M. D. Dearing. 2004. A specialist herbivore (*Neotoma stephensi*) absorbs fewer plant toxins than does a generalist (*Neotoma albigula*). Physiological and Biochemical Zoology 77:139–148.

Sorensen, J. S., E. Heward, and M. D. Dearing. 2005a. Plant secondary metabolites alter the feeding patterns of a mammalian herbivore (*Neotoma lepida*). Oecologia 146:415–422.

Sorensen, J. S., J. D. McLister, and M. D. Dearing. 2005b. Plant secondary metabolites compromise the energy budgets of specialist and generalist mammalian herbivores. Ecology 86:125–139.

Sorenson, J. S., and M. D. Dearing. 2006. Efflux transporters as a novel herbivore counter mechanism to plant chemical defenses. Journal of Chemical Ecology 32:1181–1196.

Sun, C-H, Liu H-Y, Liu B., B-D Yuan, and Lu C-H. 2019. Analysis of the gut microbiome of wild and captive Père David's deer. Frontiers in Microbiology 10:2331.

Thacker, E. T., D. R. Gardner, T. A. Messmer, M. R. Guttery, and D. K. Dahlgren. 2012. Using gas chromatography to determine winter diets of greater sage-grouse in Utah. Journal of Wildlife Management 76:588–592.

Thines, N. J., L. A. Shipley, J. H. Bassman, J. R. Slusser, and W. Gao. 2008. UV-B effects on nutritional chemistry of plants and responses of a mammalian herbivore. Oecologia 156:125–135.

Toft, C. A. 1980. Feeding ecology of thirteen syntopic species of anurans in a seasonal tropical environment. Oecologia 45:131–141.

Tollefson, T. L., L. A. Shipley, W. L. Myers, D. H. Keisler, and N. Dasgupta. 2010. The influence of summer and autumn nutrition on body condition and reproduction in lactating mule deer. Journal of Wildlife Management 74:974–986.

Tollefson, T. L., L. A. Shipley, W. L. Myers, and N. Dasgupta. 2011. Forage quality's influence on mule deer fawns. Journal of Wildlife Management 75:919–928.

Torregrossa, A. M., and M. D. Dearing. 2009. Nutritional toxicology of mammals: regulated intake of plant secondary compounds. Functional Ecology 23:48–56.

Vourc'h, G., B. Vila, D. Gillon, J. Escarré, F. Guibal, H. Fritz, T. P. Clausen, and J. L. Martin. 2002a. Disentangling the causes of damage variation by deer browsing on long-lived saplings: a chemical and dendrochronological approach. Oikos 98:271–283.

Vourc'h, G., J. Russel, J. L. Martin. 2002b. Linking deer browsing and terpene production among genetic identities in *Chamaecyparis nootkatensis* and *Thuja plicata* (Cupressaceae). Journal of Heredity 93:370–376.

Wallmo, O. C., L. H. Carpenter, W. L. Regelin, R. B. Gill, and D. L. Baker. 1977. Evaluation of deer habitat on a nutritional basis. Journal of Range Management 30:122–127.

Walter, W. D., T. J. Zimmerman, D. M. Leslie Jr., and J. A. Jenks. 2009. Dietary response of sympatric deer to fire using stable isotope analysis of liver tissue. Natural Resource Management Faculty Publications. 172. http://openprairie.sdstate.edu/nrm_pubs/172.

Wambolt, C. L. 1996. Mule deer and elk foraging preference for 4-sage-brush taxa. Journal of Range Management 49:499–503.

Welch, B. L., E. D. McArthur, and J. N. Davis. 1983. Mule deer preference and monoterpenoids (essential oils). Journal of Range Management 36:485–487.

Whitney, L. W., R. G. Anthony, and D. H. Jackson. 2011. Resource partitioning between sympatric Columbian white-tailed and black-tailed deer in western Oregon. Journal of Wildlife Management 75:631–645.

Whittaker, D. G., and F. G. Lindzey. 2004. Habitat use patterns of sympatric deer species on Rocky Mountain Arsenal, Colorado. Wildlife Society Bulletin 32:1114–1123.

Wickstrom, M. L., C. T. Robbins, and T. Hanley. 1984. Food intake and foraging energetics of elk and mule deer. Journal of Wildlife Management 48:285–301.

Wolverton, S., M. A. Huston, J. H. Kennedy, K. Cagle, and J. D. Cornelius. 2009. Conformation to Bergmann's rule in white-tailed deer can be explained by food availability. The American Midland Naturalist 162:403–17.

Woods, A. K., R. J. Mackie, and K. L. Hamlin. 1989. Ecology of sympatric populations of mule and white-tailed deer in a prairie environment. Montana Department of Fish, Wildlife, and Parks. Color World Printers, Bozeman, Montana.

Zimmerman, T. J., J. A. Jenks, and D. M. Leslie Jr. 2006. Gastrointestinal morphology of female white-tailed and mule deer: effects of fire, reproduction, and feeding type. Journal of Mammalogy 87:598–605.

Figures

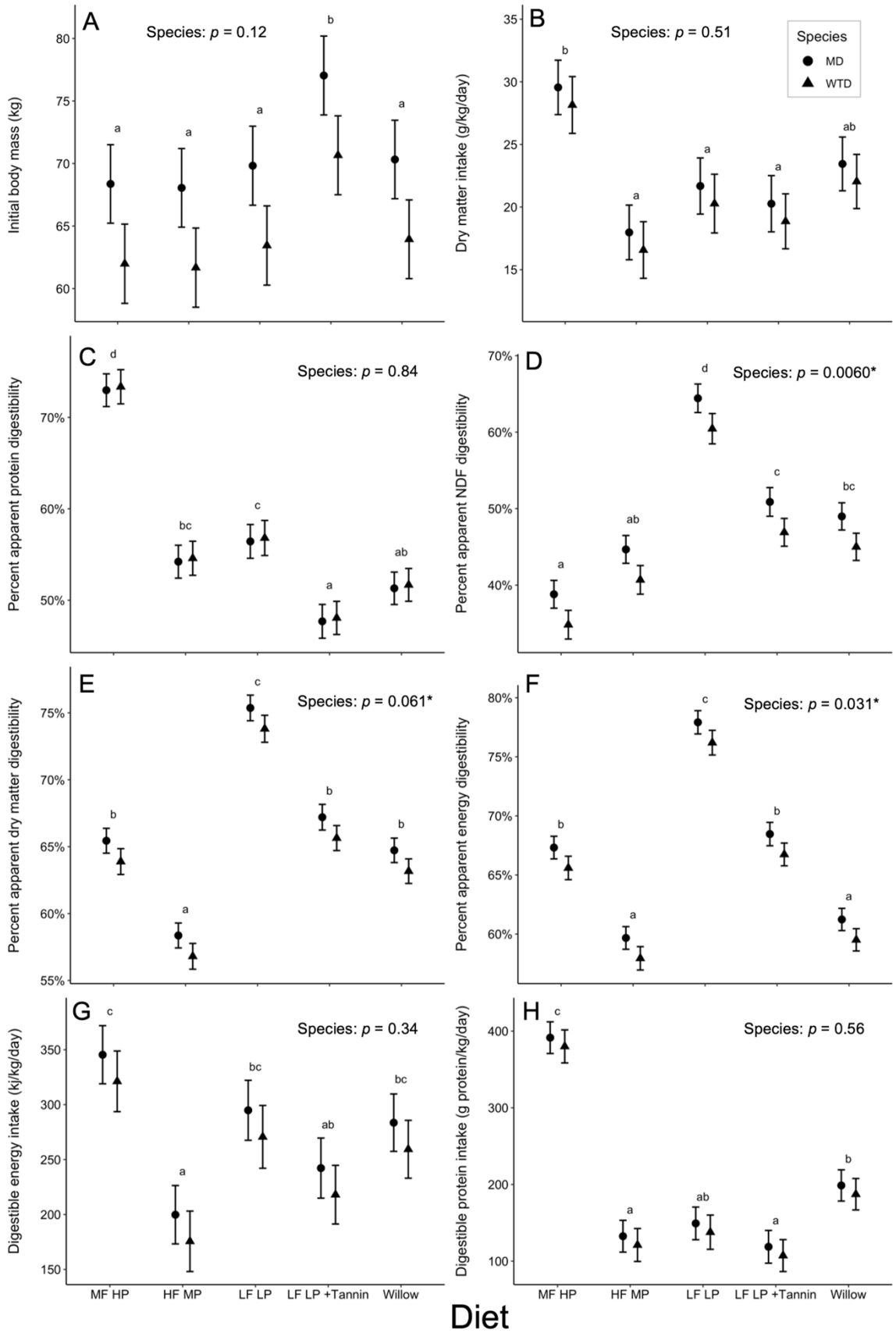

Figure 1. — Estimated marginal means of linear mixed-effects models ± standard error bars for seven mule deer (*Odocoileus hemionus*, circles) and six white-tailed deer (*O. virginianus*, triangles) across five single-diet, total-collection *in vivo* digestion trials conducted from July 2018 – May 2020 at Washington State University, Pullman, USA. 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.

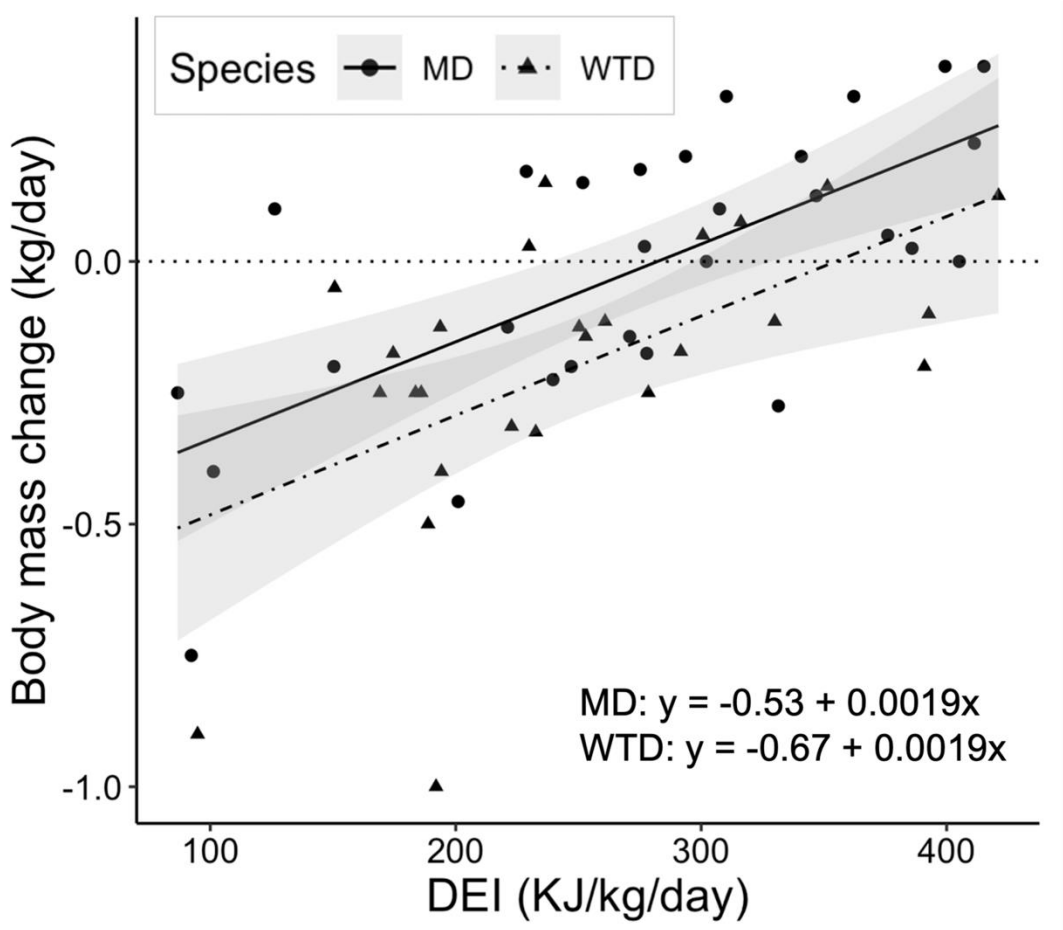

Figure 2. — Mean daily change in body mass (kg/day) as a function of digestible energy intake (DEI, KJ/kg body mass/day) of captive mule deer (*Odocoileus hemionus*, MD, circles and solid line) and white-tailed deer (*O. virginianus*, WTD, triangles and dashed line) when consuming pelleted diets and willow (*Salix lasiandra*) during *in vivo* digestion trials conducted from July 2018–May 2020 at the Wild Ungulate Facility at Washington State University, Pullman, USA. 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 grey; 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.

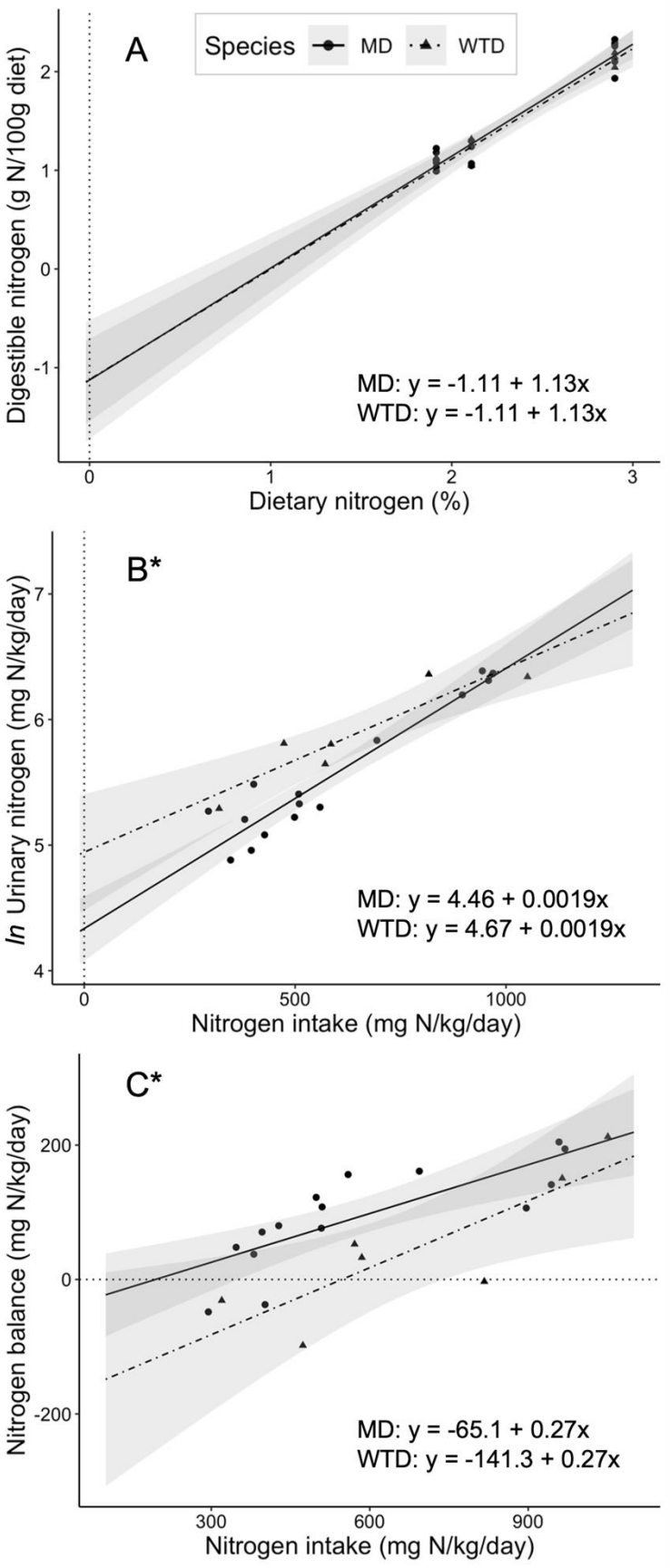

Figure 3. — Nitrogen intake and excretion of captive mule deer (*Odocoileus hemionus*, MD, circles and solid line) and white-tailed deer (*O. virginianus*, WTD, triangles and dashed line) that lost < 2 kg body mass when consuming pelleted diets absent of condensed tannins during *in vivo* digestion trials conducted from July 2018–May 2020 at the Wild Ungulate Facility at Washington State University, Pullman, USA. Asterisks indicate significant differences (α = 0.10) between species. Confidence bands (95%) are shaded in grey; 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 1.1g 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 non-tannin 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).

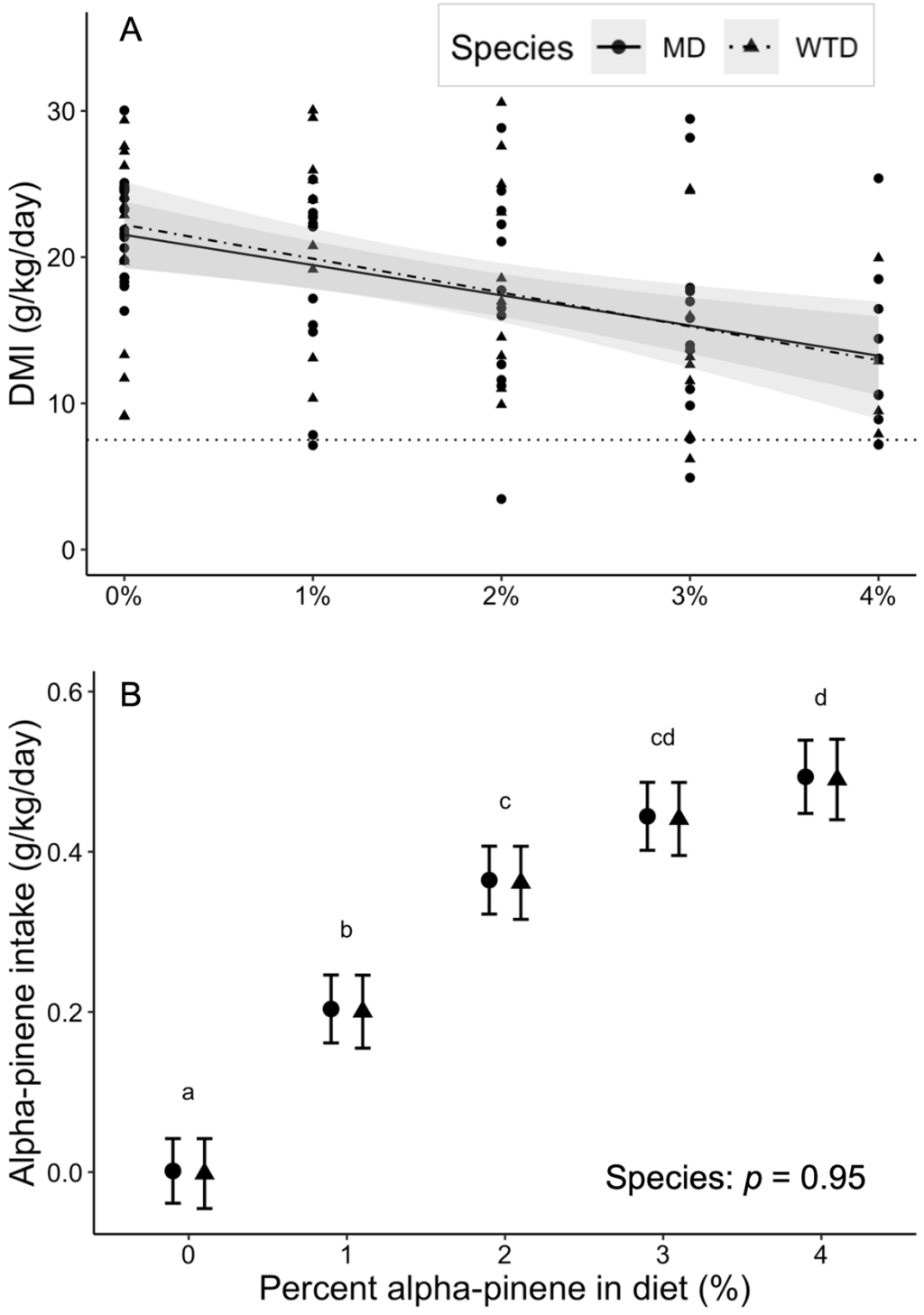

Figure 4. — Daily dry matter intake (DMI, g/kg body mass/day (A) and daily alpha-pinene intake (B) of six mule deer (*Odocoileus hemionus*, MD, circles and solid line) and five white-tailed deer (*O. virginianus*, WTD, triangles and dashed line) when consuming diets with increasing concentrations of the monoterpene alpha-pinene (0-4%) in November of 2018 at the Wild Ungulate Facility at Washington State University, Pullman, USA. 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 two days in a row they were removed from the trial. Confidence bands are shaded in grey; darker areas represent band overlap between the two deer species. Because no interactions

between alpha-pinene concentration and species were significant ($\alpha = 0.10$), lowercase letters (B) represent significant differences between levels of alpha-pinene concentration calculated using linear contrasts. A random effect for individual animal was included in each model.

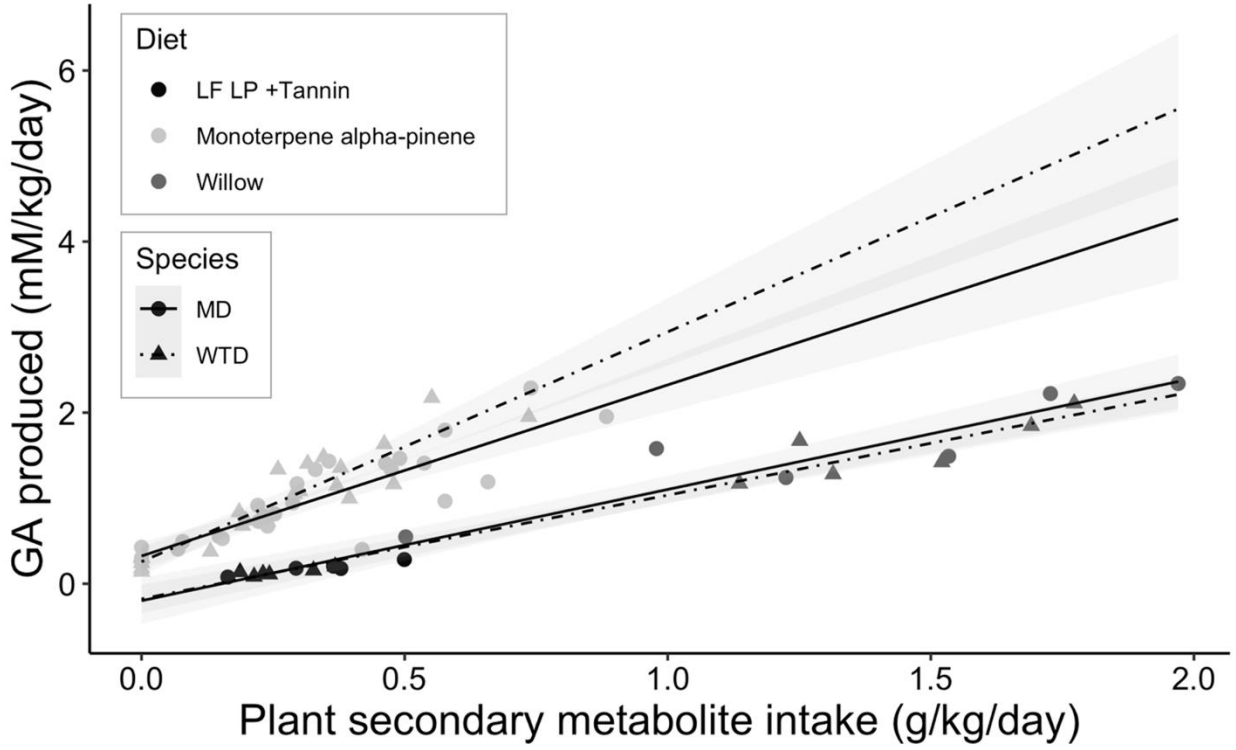

Figure 5. — Production of a byproduct of detoxification, glucuronic acid (GA, mM/kg body mass/day), with increasing daily intake of two types of plant secondary metabolites of mule deer (*Odocoileus hemionus*, MD, circles and solid line) and white-tailed deer (*O. virginianus*, WTD, triangles and dashed line) at the Wild Ungulate Facility at Washington State University, Pullman, WA, USA. In separate experiments, deer were fed a 1) medium fiber, high protein pelleted diet with the monoterpene alpha-pinene added in increasing amounts (0-4%, light grey symbols), 2) 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 3) the leaves of Pacific willow (*Salix lasiandra*, medium grey points, 6% condensed tannins). The species × alpha-pinene intake interaction was significant ($\alpha = 0.10$) 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 grey; darker areas represent band overlap between the two species. A random effect for individual animal was included in each model.

Tables

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, WA, USA.

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[b] (%)[a]	ADL[c] (%)[a]
MFHP[d]	June 2018	6/5	18.13	17.72	0	0	29.85	4.03
HFMP[e]	May 2019	6/5	13.18	18.84	0	0	47.49	6.90
LFLP[f]	May 2020	6/4	11.97	18.09	0	0	23.52	0.95
LFLP +Tannin[g]	Nov. 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
[b] Neutral Detergent Fiber
[c] Acid Detergent Lignin
[d] Moderate fiber, high protein pelleted diet
[e] High fiber, moderate protein pelleted diet
[f] Low fiber, low protein pelleted diet
[g] Low fiber, low protein pelleted diet plus 1.5% condensed tannins as powdered extract from bark of the quebracho colorado tree (*Schinopsis* spp.)

Appendices

Appendix 1. — Results of linear contrasts between diets for linear mixed-effects models comparing mule deer (*Odocoileus hemionus*) and white-tailed deer (*O. virginianus*) across 5 single-diet, total-collection *in vivo* digestion trials conducted from July 2018 – May 2020 at Washington State University, Pullman, WA, USA.

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 to 70.2
		HFMP	64.9^{A} (2.29)	59.9 to 69.8
		LFLP	66.6^{A} (2.30)	61.6 to 71.6
		LFLP +Tannin	73.8^{B} (2.29)	68.9 to 78.8
		Willow	67.1^{A} (2.27)	62.2 to 72.1
Dry matter intake (g/kg/day)	Diet	MFHP	28.9^{B} (1.92)	24.7 to 33.0
		HFMP	17.3^{A} (1.92)	13.1 to 21.5
		LFLP	21.0^{A} (2.00)	16.6 to 25.3
		LFLP +Tannin	19.6^{A} (1.92)	15.4 to 23.7
		Willow	22.7^{AB} (1.84)	18.7 to 26.8
Dry matter digestibility (%)	Diet	MFHP	64.7^{B} (0.84)	62.8 to 66.5
		HFMP	57.6^{A} (0.84)	55.8 to 59.4
		LFLP	74.6^{C} (0.88)	72.7 to 76.5
		LFLP +Tannin	66.4^{B} (0.84)	64.6 to 68.3
		Willow	63.9^{B} (0.80)	62.2 to 65.7
Protein digestibility (%)	Diet	MFHP	73.2^{D} (1.54)	69.8 to 76.5
		HFMP	54.5^{BC} (1.54)	51.0 to 57.8
		LFLP	56.6^{C} (1.60)	53.1 to 60.1
		LFLP +Tannin	47.9^{A} (1.54)	44.5 to 51.2
		Willow	51.5^{AB} (1.49)	48.3 to 54.7

Energy digestibility (%)	Diet	MFHP	66.5^{B} (0.88)	64.5 to 68.4
		HFMP	58.8^{A} (0.88)	56.9 to 60.7
		LFLP	77.1^{C} (0.92)	75.1 to 79.1
		LFLP +Tannin	67.6^{B} (0.88)	65.7 to 69.5
		Willow	60.4^{A} (0.84)	58.5 to 62.2
NDF digestibility (%)[c]	Diet	MFHP	36.8^{A} (1.7)	33.2 to 40.5
		HFMP	42.7^{AB} (1.7)	39.0 to 40.5
		LFLP	62.4^{D} (1.8)	58.6 to 66.3
		LFLP +Tannin	48.9^{C} (1.7)	45.2 to 52.5
		Willow	47.0^{BC} (1.6)	43.5 to 50.5
DEI (MJ/kg/day)[d]	Diet	MFHP	333^{C} (23.4)	282 to 384
		HFMP	188^{A} (23.4)	137 to 239
		LFLP	283^{BC} (23.4)	230 to 336
		LFLP +Tannin	230^{AB} (23.4)	179 to 281
		Willow	271^{BC} (23.4)	223 to 320
DPI (g/kg/day)[e]	Diet	MFHP	386^{C} (1.7)	346 to 426
		HFMP	127^{A} (18.4)	87 to 167
		LFLP	144^{AB} (19.2)	102 to 185
		LFLP +Tannin	113^{A} (18.4)	73 to 153
		Willow	193^{B} (17.6)	155 to 231

[a] Factor levels include the 5 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 standard errors (SE) of diet levels ($\alpha = 0.10$)

[c] Neutral detergent fiber

[d] Digestible energy intake

[e] Digestible protein intake