**Title: Review: Using physiologically-based models to predict population responses to phytochemicals by wild vertebrate herbivores**

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**Journal.**  *animal*

**Supplementary Material S1**

**Estimating parameters for physiologically-based models.** Estimates of each parameter in our physiological models described in the main text (Table 1) can be independently assessed using *in vitro* and *in vivo* studies.

*Estimating food intake* (*l(t), l0*). In generic formulations of consumer-resource interactions, intake rate is related to the product of encounter rate and food selection. For some browsing vertebrate herbivores, the density of forage is high enough that intake rates tend to be insensitive to encounter rates. In such cases, intake rate depends largely on bite size (Shipley et al., 1999; Cohen et al., 1999; Pastor et al., 1999; Shipley, 2007). Direct measurements of bite size at foraging patches can be estimated from bite diameters of woody species at the point of browse by herbivores. For example, twig diameter is related to the biomass of the portion of the twig consumed by moose by an allometric relationship (Risenhoover, 1987). Estimates of encounter rate, food selection, and bite diameters require observations of feeding rates of free ranging animals coupled with measuring biomass of plants consumed. As such, this type of data is rare for free-ranging herbivores due to the practical difficulties and costs involved (Felton et al., 2018). Novel digital approaches used to estimate food intake in humans could be adopted by ecologists where foraging patches are photographed before and after a browser is allowed access to the food patch (Martin et al., 2014). Although for free-ranging individuals, this type of observation would not capture the complete daily food intake. Intake during short periods of time in the life of the animal can also be estimated from biomass of food in stomach of mammals (Zeman et al., 2016) or crops of birds (Mortensen et al., 1983) accounting for time of day and food passage rates. These lethal approaches should be used to validate behavioral observations and DNA-based assessment of feces (Pompanon et al., 2012), but may not be feasible for some species. Captive trials are non lethal and provide the most accurate assessment of food intake (e.g., (Guglielmo et al., 1996; Sorensen et al., 2005b; a; McLean et al., 2007). However, captive trials may not be feasible for some species and cannot capture the range of food intake associated with diet mixing that occurs in natural habitats (DeGabriel et al., 2014).

Phytochemical exposure (*p, TG(t), NG (t)*), *TB(t), NB (t)*), *E+(t)*). To estimate and validate predictions of phytochemical exposure and rates of absorption and metabolism, biological samples should be obtained from herbivores interacting with phytochemicals. Nutritional phytochemicals, such as energy used in our model examples, can be quantified from food using standard techniques available at most forage labs (e.g., energy, nitrogen for crude protein, Karasov and del Rio, 2007; Rothman et al., 2012). Concentrations of toxic phytochemicals in food as well as parent and metabolized phytochemicals in gut compartments and blood can be quantified and characterized following established metabolomics methods (Jones et al., 2012; Wu et al., 2014; Kurita et al., 2015; Richards et al., 2015; Vinaixa et al., 2016; Wang et al., 2017; Wishart et al., 2017). While we emphasize phytochemical interactions within the gut and blood compartments, concentration-dependent functions could be assessed in any organ using similar assays and experiments.

Rates of phytochemical absorption and metabolism (*kaG, kmG, kmL, kaB*). Controlled *in vivo* feeding or dosing trials can be used to measure changes in concentrations of phytochemicals over time and provide estimates of rates of absorption and metabolism. Metabolites can be determined from body compartments of euthanized free-ranging animals or from collection of blood and excreta from captive animals consuming known doses of phytochemicals (e.g., Sorensen and Dearing, 2003; Sorensen et al., 2004, 2006; McLean et al., 2007). In whole animals, the proportion of phytochemicals that remain unchanged or are metabolized in the small intestine and remain within sequential gut compartments can be used to determine the extent and the location where phytochemicals are absorbed and metabolized. However, this assumes that diet composition and intake and passage rates are at steady state since only one time point is assessed for each body compartment. Absorption and metabolism parameters can also be estimated from *in situ* and *in vitro* assays. Absorption rates of phytochemicals can be estimated using intestines from herbivores of interest (Starck et al., 2000; Green et al., 2005) and intestinal cell lines from model organisms (e.g., CaCo2 cells, Wu et al., 2017). Metabolism of toxic phytochemicals can be estimated using microsomes and cytosol fractions (e.g., Shou et al., 2005; Karlsson et al., 2013; Yamagata et al., 2017) isolated from intestinal lining of gut compartments or livers of herbivores. These metabolic stability assays compare rates of loss of parent phytochemicals or formation of new metabolites exposed to enzymes. These assays also isolate the physiological function of herbivore enzymes from the microbiome, which is not possible in whole organism studies. To isolate the physiological function of microbes, short-term batch *in vitro* assays can be used to determine the metabolism of phytochemicals by microbial communities from different sections of the gut (Wu et al., 2017). These *in vitro* assays can simulate interactions between microbial communities and diverse phytochemicals and measure the rate of change of phytochemicals over time (Zoetendal et al., 2008).

Digestive and metabolic efficiency (*keG*, *keB*). To estimate and validate predictions of physiological function,*in vivo* and *in vitro* assays can be used similar to those described for absorption and metabolism. Because digestion and metabolism of nutrients can be influenced by toxic phytochemicals, it is important to investigate how nutrients are assimilated with and without presence of toxic phytochemicals. For *in vivo* estimates, metabolic chambers that separate intake and excreta can be used to determine apparent digestion and metabolism of nutrients (e.g., Guglielmo et al., 1996; Sorensen et al., 2005b). *In situ* assays use entire digestive tracts to assess uptake of specific nutrients across the intestinal tract (Starck et al., 2000; Karasov et al., 2012; Lozoya-Agullo et al., 2015), but generally do not include processes of digestion prior to uptake. Digestive efficiency can be estimate*d* using *in vitro* digestibility assays that vary in biological relevance. For example, commercially available α-amylase with either cellulase or a fibrolytic enzyme mixture (e.g. Viscozyme) can approximate digestive capacity of the herbivore microbiome (VanSomeren et al., 2014). In addition, the use of polyethylene glycol that accounts for tannin-blocking agents can better integrate effect of tannins on digestion (DeGabriel et al., 2008). These assays are correlated with *in vivo* digestibility by ruminants (VanSomeren et al., 2014) and allow researchers to rank the digestibility of diets that vary in composition or phytochemical concentration. However, they do not account for relative difference in digestibility associated with functional enzymes of herbivores and their microbiome.

To supplement traditional *in vitro* digestibility assays, the activity of functional enzymes isolated from herbivores should be assessed using standard enzyme activity assays. The choice of enzymatic assays are diverse, but may include the activity of maltase, sucrase, or aminopeptidase-N (Kohl and Dearing, 2011; Kohl et al., 2015). Microbiome-directed *in vitro* assays that use preserved microbial communities (Olsen et al., 1997, 1999; Storeheier et al., 2002) can also be used to estimate digestibility of nutritional phytochemicals similar to those described for estimating metabolism of toxic phytochemicals. Quantifying changes in phytochemicals and their metabolites and digestive products (e.g., sugars, volatile fatty acids, protein, methane, etc.) over time can be compared to *in vivo* profiles to validate parameter estimates for models.

Biomarkers of absorption, metabolism, and body size. While *in vitro* assays using tissues collected from species can help identify phytochemical exposure and physiological responses to those phytochemicals, *in vitro* assays do not always predict *in vivo* outcomes (Karlsson et al., 2013; Tan et al., 2017). In addition, obtaining *in vivo* parameters using field observations, lethal collection, or captive studies may not be possible for some species due to their large body size, threatened conservation status, or cryptic behavior. Therefore, there is great need to identify measures of rates of phytochemical absorption, metabolism and demographics that do not require euthanizing animals or working with captive animals that may lose physiological function (Kohl et al., 2014; Clayton et al., 2016). One approach is to use metabolites in excreta deposited by free-ranging animals to estimate *in vivo* absorption and metabolism rates. A relative measure of *in vivo* absorption can be determined from the percentage of phytochemicals detected unchanged in feces compared to those in the plants consumed. However, fecal excretion of unchanged phytochemicals represents the minimal amount of ingested phytochemicals that are not absorbed because it does not account for metabolism of parent compound by the host or microbiome prior to excretion. Concentration of metabolites in the urine can be used to estimate rates of detoxification of ingested and absorbed toxic phytochemicals. For example, global metabolomics of urinary metabolites can reveal the metabolic fate of polyphenolics (Van Duynhoven et al., 2010) and predict detoxification of alkaloids by specific metabolizing enzymes in humans (Tay-Sontheimer et al., 2014).  In vertebrate herbivores, the concentration of glucuronic acid (GA) and other conjugation metabolites in urine can serve as a biomarker of the concentration of toxic phytochemicals absorbed and metabolized. Conjugation with GA is a major pathway for metabolism of phytochemicals in vertebrates that is related to the amount of phytochemical that is consumed, absorbed and metabolized (Servello and Schneider, 2000; Sorensen et al., 2005b; Sauvé and Côté, 2006). Glucuronic acid and other metabolites represent a biomarker of intrinsic exposure to phytochemicals, that can be indexed by the ratio of metabolites to creatinine (GA:C) in urine of mammalian herbivores and fecal droppings of avian herbivores. For example, recent work demonstrated that higher GA:C in the urine of moose from Isle Royale was associated with lower nutritional condition and is spatially and temporally variable (Melody, 2017; Parikh et al., 2017).The likely explanation for that linkage is that the production of GA is energetically expensive (Sorensen et al., 2005b). Inter-annual variation in GA:C or other metabolites can therefore be used to evaluate intake rates of phytochemicals in free-ranging herbivores relative to the genome and availability of phytochemicals on the landscape.

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**Supplementary Material S2**

**Study System Characteristics.** We recognize that not all vertebrate systems are amenable to estimating parameters and testing the proposed models. Working with wild systems poses challenges concerning largely unknown variation in phytochemicals, microbial communities, and physiology among individuals sampled. Nonetheless, we have identified some characteristics of study systems that could help capture functional physiological diversity, while also minimizing confounding variation. These study systems account for evolutionarily diverse vertebrate lineages, how those lineages interact with phytochemicals from single or multiple plant species, and variation in thresholds to nutritional phytochemicals.

System 1: Separate evolutionarily distinct vertebrate lineages interacting with phytochemicals from a single plant species. This system type focuses on different vertebrate species that specialize on the same plant. Specialization is defined as a relatively narrow diet comprised of plants with relatively challenging phytochemical characteristics (e.g., high toxins or fiber, or low nutrients, Shipley et al., 2009). One example includes sage-grouse (*Centrocercus urophasianus*) and pygmy rabbits (*Brachylagus idahoensis*) lineages that have separately evolved to specialize on the same toxic plant - sagebrush (*Artemisia spp,* Kelley et al., 1992). Sage-grouse and pygmy rabbits often co-occur and consume the same patches of sagebrush as defined by unique browsing characteristics and fecal pellets (personal observation). In winter, these species consume more than 90% sagebrush for several consecutive months compared to spring when diets are relatively diverse and less toxic (Ulappa, 2011; Frye, 2012; Crowell, 2015; Fremgen, 2015; Nobler, 2016). These species do not appear to tolerate other toxic plants that co-occur across their distributions (e.g., juniper [*Juniperus* spp.] and rabbitbrush [*Ericameria nauseosa*] Crowell, 2015) even though these plants contain the same or similar classes of toxic phytochemicals. Although not co-existing, lemurs and pandas represent distinct lineages that consume a similar class of cyanogenic toxins in bamboo (Ballhorn et al., 2016; Huang et al., 2016). Within the lemurs, the Golden bamboo (*Hapalemur aureus*) and Greater bamboo (*Prolemur simus*) lemur both consume up to 90% of Giant bamboo (*Cephalotachyum vigueri*) (Glander et al., 1989; Tan, 1999) and the Giant (*Ailuropoda melanoleuca*) and Red (*Ailurus fulgens*) panda both consume up to 95% bamboo (*Fargesia* and *Gelidocalamus spp*.) (Shan et al., 2018). Within Marsupials, koalas (*Phascolarctos cinereus*) and common ringtail possums (*Pseudocheirus peregrinus*) both consume up to 93% of Eucalyptus spp (Pahl, 1987; Moore and Foley, 2000) that is also known to have toxic phytochemicals (Moore et al., 2005; Barbosa et al., 2016). These systems provide opportunities to identify the molecular and physiological mechanisms through which distinct vertebrate herbivores (Avian versus Mammalian, Primate versus Carnivora, and two species within Marsupialia) tolerate the same phytochemicals.

System 2: Single evolutionary vertebrate lineage interacting with diverse phytochemicals from distinct plant species. This system focuses on a single vertebrate species that locally specialize on distinctly different plants. One example includes desert woodrats (*Neotoma lepida)* where populations within an individual evolutionary genetic unit of *N. lepida* (Matocq et al., 2007; Patton et al., 2007) locally specialize on either juniper or creosote, each of which contains distinct plant toxins (Skopec et al., 2015). Another example are rock ptarmigan from Svalbard (*Lagopus muta hyperboreus*) that consume primarily Saxifraga and Salix polaris (Unander et al., 1985) versus those (*Lagopus muta*) that consumed *Rhododendron ferrugineum* in France (García-González et al., 2016), and birch (*Betula pubescence*) in Iceland (Nielsen, 2014) in winter. Through collaboration with hunters, researchers have a unique opportunity for large-scale sampling of free-ranging individuals that belong to several different populations consuming different plants. As habitat quality and management often differs greatly within a species’ range, such a system can be seen as a natural experiment where the taxa are held constant, but diet and therefore phytochemicals differ. Another option is to sample semi-free ranging individuals inhabiting large enclosures that naturally vary in forage (Ulappa, 2015) or are modified experimentally (Felton et al., 2017). This system type provides opportunities to identify the molecular and physiological mechanisms through which a single vertebrate lineage and population tolerates exposure to distinct phytochemicals. In addition, these systems create opportunities for natural or experimental manipulation of food availability and quality. The interest in management of game species also offers advantages of legacy data on demographic traits of herbivores and monitoring habitat quality and food availability across the landscape allows predictions to be scaled up over time and space.

System 3: Related vertebrate lineages with different rules of compromise and interacting with diverse phytochemicals. As explained above, when faced with restricted choices, food selection and intake by different species may be governed by different rules of compromise, i.e. which compromise the animal makes between over-eating the constituent in high concentration against undereating the constituent in low concentration. Good examples exist among primates, sprung out of research using the Geometric Framework. For example, when food options seasonally fluctuate, and gorillas (*Gorilla beringei*) are not able to compose a diet with the preferred balance, they try to maintain a stable intake of carbohydrates and lipids while allowing protein intake to fluctuate (Rothman et al., 2011).Observations of howler monkeys (*Allouatta pigra*), who share several of the digestive characteristics of gorillas, indicate that they have a similar rule of compromise (Behie and Pavelka, 2012). Although it might be assumed that sugars and starches would influence diet choice in ripe-fruit specialists, spider monkeys do not in fact appear to use carbohydrates as the foremost cue in food selection, but instead prioritize to maintain a stable intake of protein when options are scarce (Felton et al., 2009). As such, the daily energy intake of spider monkeys is dictated by the protein content of the food, which interestingly is a similar rule of compromise as that of humans (Raubenheimer et al., 2015). By applying the approach we describe in this paper, researchers could investigate the physiological mechanisms behind these interesting differences between two relatively closely related taxa expressing different rules of compromise. By combining physiologically-based models with the Geometric Framework (e.g., by combining detailed observations and sample collection of plants, feces and urine), and by leveraging insight from human health research, individual variation within taxa can also be investigated and placed into a larger ecological context.

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