WILEY ECOSPHERE

Environmental and prey-based factors underpinning variability in prairie dogs eaten by black footed ferrets

Journal:	*Ecosphere*
Manuscript ID	ECS20-0337.R1
Wiley - Manuscript type:	Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Dierenfeld, Ellen; New York Zoological Society, Wildlife Nutrition ; Nottingham Trent University - Brackenhurst Campus, School of Animal, Rural & Environmental Sciences; Ellen S. Dierenfeld, LLC Whitehouse-Tedd, Katherine; Nottingham Trent University - Brackenhurst Campus, School of Animal, Rural and Environmental Sciences Dermauw, Veronique; Institute of Tropical Medicine, Department of Biomedical Sciences Hanebury, Louis; U.S. Fish and Wildlife Service, National Ecology Research Center; U.S. Fish and Wildlife Service , (Retired) Biggins, Dean; U.S. Fish and Wildlife Service, National Ecology Research Center; U.S. Geological Survey, Department of the Interior
Abstract:	The endangered black-footed ferret (Mustela nigripis) has been the focus of intensive captive breeding and reintroduction projects for several decades. To better understand nutritional provision during captivity, primary prey items (prairie dogs) of free-ranging black-footed ferret populations were sampled from 6 native habitat sites in Wyoming and Colorado over a one-year period. Morphometrics and nutritional analyses including proximate composition (water, crude fat, crude protein, ash), vitamins A and E, and select macro- and microminerals were conducted on black-tailed (BT, Cynomys ludovicianus, n=81) and white-tailed (WT, C. leucurus; n=58) prairie dogs. Stomach and intestinal contents were extracted and sampled separately from other carcass components. Multivariate linear modelling of data was used to determine the influence of environmental (season, site) and prey-based (species, age, sex) factors on prey nutritional composition. Seasonality impacted the nutrient profiles of prairie dogs as food for black footed ferrets, affecting carcass, stomach, and intestinal samples in most nutrients evaluated for both species. Carcass and subcutaneous fat concentrations were lowest in spring for both species compared with other seasons. Conversely, fat-soluble vitamin A in carcasses was highest in the spring for both species. Vitamin E was also highest in the spring for WT, but highest in the winter for BT,although no comparative winter data were available for the hibernating WT. Macronutrient composition did not differ between sexes for WT, but carcass fat was higher, hence protein lower, in female vs male BT. Age class and site-specific differences detected for some nutrients suggested possible underlying feeding ecology differences. Given the on-going concerns regarding ex-situ population sustainability

	and the documented role of nutrition in black-footed ferret health and reproduction, these seasonal nutrient profiles provide valuable guidelines for optimizing managed feeding programs for this endangered species, and similar considerations in prey nutrient variability can be applied to feeding programs of other carnivorous species.

Environmental and prey-based factors underpinning variability in prairie dogs eaten by black footed ferrets

Ellen S. Dierenfeld[1, 2, 5]†, Katherine Whitehouse-Tedd[2], Veronique Dermauw[3], Louis R. Hanebury[4, 6] and Dean E. Biggins[4, 7]

[1]Department of Wildlife Nutrition, New York Zoological Society (now Wildlife Conservation Society), Bronx, New York 10460 USA

[2] School of Animal, Rural and Environmental Sciences, Nottingham Trent University, Southwell, Nottinghamshire, NG25 0QF, United Kingdom

[3] Department of Biomedical Sciences, Institute of Tropical Medicine, Nationalestraat 155, 2000 Antwerp, Belgium

[4] U.S. Fish and Wildlife Service, National Ecology Research Center, 1300 Blue Spruce Drive, Fort Collins, CO 80524 USA.

Present affiliations:

[5]Ellen S. Dierenfeld LLC, 4736 Gatesbury Drive, St. Louis, MO 63128 USA

[6]U.S. Fish and Wildlife Service (Retired), Billings, MT 59101 USA

[7]U.S Geological Survey, Department of the Interior, Fort Collins Science Center, 2150 Centre Avenue, Building C, Fort Collins, CO 80526 USA

†Email: ellen.dierenfeld@ntu.ac.uk; Phone: (001) 314.717.3885

Abstract The endangered black-footed ferret (*Mustela nigripis*) has been the focus of intensive captive breeding and reintroduction projects for several decades. To better understand nutritional provision during captivity, primary prey items (prairie dogs) of free-ranging black-footed ferret populations were sampled from 6 native habitat sites in Wyoming and Colorado over a one-year period. Morphometrics and nutritional analyses including proximate composition (water, crude fat, crude protein, ash), vitamins A and E, and select macro- and microminerals were conducted on black-tailed (BT, *Cynomys ludovicianus*, n=81) and white-tailed (WT, *C. leucurus*; n=58) prairie dogs. Stomach and intestinal contents were extracted and sampled separately from other carcass components. Multivariate linear modelling of data was used to determine the influence of environmental (season, site) and prey-based (species, age, sex) factors on prey nutritional composition. Seasonality impacted the nutrient profiles of prairie dogs as food for black footed ferrets, affecting carcass, stomach, and intestinal samples in most nutrients evaluated for both species. Carcass and subcutaneous fat concentrations were lowest in spring for both species compared with other seasons. Conversely, fat-soluble vitamin A in carcasses was highest in the spring for both species. Vitamin E was also highest in the spring for WT, but highest in the winter for BT,although no comparative winter data were available for the hibernating WT. Macronutrient composition did not differ between sexes for WT, but carcass fat was higher, hence protein lower, in female vs male BT. Age class and site-specific differences detected for some nutrients suggested possible underlying feeding ecology differences. Given the on-going concerns regarding ex-situ population sustainability and the documented role of nutrition in black-footed ferret health and reproduction, these seasonal nutrient profiles provide valuable guidelines for optimizing managed feeding programs for this endangered species, and similar considerations in prey nutrient variability can be applied to feeding programs of other carnivorous species.

Keywords: Black-footed ferret; diet; feeding ecology; nutrition; prairie dog; reintroduction; seasonality.

Introduction

The nutrient composition of whole vertebrate prey consumed by carnivores is important to understand from both an ecological and management perspective. However, such datasets are scarce in the published literature(see for example Dierenfeld et al., 2002; Kerr et al., 2014a,b; Kremer et al., 2013). For the most part, analyses have focused on proximate composition (water, crude protein, crude fat, and ash), with energy content and some minerals quantified. More recent studies have started filling data gaps with detailed assays of amino acid (Kerr et al., 2014a; Kremer et al. 2013) and fatty acid profiles (Kerr et al. 2014b), but there is still a dearth of information on essential nutrients such as fat-soluble vitamins. Furthermore, variables known to impact nutritional content of whole prey across species, such as sex and age (see Douglas et al., 1994), diet (Clum et al. 1996), seasonality, and/or the consequences of not analyzing the prey in the same form as eaten (for example, eviscerated vs complete, or with or without fur/feathers/skin) have not been fully explored. Such aspects of whole prey must be considered, as they can critically affect prey nutritional profiles and subsequent health and nutrition of the consuming predator, as exemplified in this study using a model obligate carnivore, the black-footed ferret.

Considered extinct in the late 1970s, the black-footed ferret (*Mustela nigripis*) remains one of the world's most endangered species despite intensive recovery efforts since a small surviving population was discovered in 1981 (Fish and Wildlife Service 2014; Belant et al. 2015). Native to the western North American prairies, the black-footed ferret (herein referred to as the ferret) has been listed as endangered across its entire range since March 1967, with the exception of several reintroduced populations designated as experimental. Latest

population estimates report there are less than 300 wild born mature individuals living in several re-established populations (Belant et al. 2015). Of the 24 reintroduction sites, only a few ferret populations might (optimistically) be considered self-sustaining; the number of breeding adults declined by approximately 40% between 2008 and 2015 (Belant et al. 2015).

Black-footed ferrets rely predominantly on prairie dogs (*Cynomys* spp.) for food, as well as utilizing their burrows for shelter (Wolf et al. 2000; Roelle et al. 2006). As specialist predators, 60-90% of theferret's diet comprises prairie dogs (Sheets, Linder, and Dahlgren 1972; Campbell et al. 1987; Brickner et al. 2014; Biggins and Eads 2017). However, between the late 1800's and 1960, prairie dog numbers dramatically declined due to habitat destruction, expansion of non-native sylvatic plague, and poisoning (Fish and Wildlife Service 2013a). Consequentially, ferret numbers declined precipitously and have been the subject of intensive conservation efforts, including captive breeding and reintroduction, ever since (Biggins et al. 1993).

The 2013 US Fish & Wildlife Revised Recovery Plan for the Black-Footed Ferret (Fish and Wildlife Service 2013b) identified that recovery of black-footed ferrets will depend upon on-going captive breeding efforts to provide suitable animals for release into the wild, alongside *in-situ* conservation efforts. However, the captive population has experienced a substantial loss of fecundity over time (e.g. reduced whelping success from 70% to 46%, and decreased production of normal sperm from 50% to 16% (reviewed in (Santymire et al. 2015)), representing a significant concern for the recovery of this endangered species. The mechanism of this effect has not been elucidated but is postulated to be dietary in origin, including potentially excessive concentrations of vitamin A when consuming commercially-blended horsemeat-based diets, which would have an antagonistic effect on vitamin E status (Santymire et al. 2015). Compounding this concern is the fact that information is limited

regarding dietary requirements of ferrets, and captive diets are still largely based on extrapolation of requirements determined for similar species, particularly mink (*Mustela vison*) (National Research Council 1982). Other health concerns associated with captive diets have also been raised for this species, such as an increased incidence of calculus accumulation and periodontal disease associated with captive diets lacking fibrous material (Antonelli et al. 2016), as per other carnivores (Vosburgh et al. 1982; Hartstone-Rose et al. 2014; Kapoor et al. 2016), making dietary provision a research priority.

As part of the U.S. Fish and Wildlife Service black-footed ferret recovery program, an unpublished government report following a study undertaken during the late 1980's by our research group documented summary nutrient information on the natural diet of ferrets through chemical analysis of black-tailed (*Cynomys ludovicianus)* and white-tailed (*C. leucurus)* prairie dogs (Dierenfeld and McGuire 1989). However, the variability in prey composition between prairie dog species with divergent physiological strategies (i.e. hibernation), among prey at different reintroduction sites, or across seasons may represent important, but as yet unquantified, aspects in the feeding ecology of ferrets. The current study aims to address this knowledge gap by examining the following hypotheses: 1) prey factors (species (hibernating vs non-hibernating), sex, age) impact their nutrient composition, 2) nutrient composition of prairie dogs, eaten as whole prey of ferrets, changes on a seasonal basis and 3) sampling locale may additionaly underlie differences in chemical/nutritional profiles of prey consumed.

Materials and Methods

Animal Acquisition

Black-tailed (BT) and white-tailed (WT) prairie dogs were opportunistically sampled on a quarterly basis between July 1988 and March 1989 from different sites in North America including: Pawnee Grassland (PG), Rocky Mountain Arsenal (RMA), Waverly (WAV), or Wellington (WEL), in Colorado, USA (black-tailed only) and Laramie (LAR) or Medicine Bow (MB) in Wyoming, USA (white-tailed only). Locations were identified on the basis of prairie dog presence, and approximately equal numbers of adult males and females (minimally $n = 3$ per sex) as well the two age classes (considered juvenile if < 1 yr of age vs adults > 1 yr of age; selected by size alone, regardless of sex), were targeted for sampling during each season. No other sampling criteria were applied and none of the sites had ferrets present at the time of the study (all known remaining individuals of this species were in captivity). Spring was defined as dates falling March—May, summer June—August, fall September—November, and winter December—February. No WT prairie dogs were available during winter months as a consequence of their being a hibernating species, and juveniles were not present for either species during spring sampling periods when young had not yet emerged from burrows. Trained marksmen ensured that prairie dogs were shot in a manner that minimized suffering and did not impact sympatric species. Due to their status as a pest species during this time frame (Fish and Wildlife Service 2013b), harvesting permits were not required for collection. The protocol (Cooperative Agreement 14-16-0009-88-952) was approved by the Animal Care and Use Committee at the National Ecology Research Center (NERC, Fort Collins, CO).

Sample Preparation

Each individual was assigned an ID number and species, sex, age class, season and collection site were recorded for each animal in the field. Dead prairie dogs were transported on ice and processed at NERC. Efforts were made to prepare the prairie dog carcasses in a manner

reflecting the portion consumed by ferrets. Although detailed investigations of ferret feeding behavior were lacking at the time of sampling, field observations indicated that the feet and anterior skull (nose and teeth; herein referred to as "face") were often rejected. As such, these were cut off, weighed (all weights to the nearest 0.1 g) and then discarded. Carcasses were skinned and the skin weighed. Since skin was particularly difficult to prepare for chemical analysis (i.e. grinding), only one skin from each age class per location was saved and frozen for further processing/analysis; all other skins were discarded. The remainder of the carcass (i.e. reflecting the consumed components), including brain and digestive tract tissues plus contents, was weighed and recorded.

Next, the entire gastrointestinal tract (esophagus through anus) was removed intact. To examine potential variability in prairie dog diets across sites, stomach contents (when present) were removed *in toto* and weighed separately. Five g of stomach contents were placed into labelled plastic bags with 5 ml of 25% sodium ascorbate solution for vitamin analysis; any residual stomach contents were stored in a separate, labelled bag for later proximate and mineral composition analysis. The intestinal tract was then stripped of contents into a separate container, before the entire gastrointestinal tissue was weighed, and tissue placed back into the carcass sample. Intestinal contents were mixed homogenously; 5 g of intestinal contents were placed into labelled plastic bags with 5 ml of 25% sodium ascorbate solution for vitamin analysis, and 5 g of intestinal content stored in a separate plastic bag for proximate analysis. The entire carcass (including gastrointestinal tract tissues, less contents) was ground through a meat grinder four times into a homogenous mixture; 5 g of carcass mixture was placed into labelled plastic bags with 5 ml of 25% sodium ascorbate solution for vitamin analysis, and a separate 20-g carcass sample was stored in a separate bag for later proximate and mineral composition analyses. Previously frozen skins were ground through a meat grinder, 20-g subsamples taken and placed into labelled plastic bags. All

labels, associated subsamples, and data sheets were double-checked for completeness and consistency, and stored frozen at -20°C for no longer than 6 mo before overnight shipment to the Nutrition Laboratory, Wildlife Health Center at the New York Zoological Society (Bronx, NY).

Laboratory Analyses

Prior to analysis, samples were thawed at room temperature; vitamin assays on both carcass and GI tract samples were performed immediately upon thawing. Subsamples for remaining analyses were freeze-dried within 24 hours, reground using a laboratory mill if necessary, and stored at -20°C until analysis.

Fat-soluble vitamins A and E were quantified in duplicate from carcass and gastrointestinal tract content subsamples following the protocol of Douglas, Pennino, and Dierenfeld (1994). Data analyzed as µg/ml were converted to vitamin activity using conversion factors of 0.3 µg = 1 IU vitamin A (Olson 1984), and 1 mg α-tocopherol = 1.49 IU vitamin E (Machlin 1984). Zero values of vitamin A were quantified and considered true values in both stomach and intestinal content samples, since plant materials contain no preformed vitamin A or retinol.

Percent moisture, crude protein and crude fat values were obtained according to AOAC methodology for meat (Ellis 1984) or dry feed samples (Jones 1984) for carcass and digestive tract (stomach and intestinal tract content) components, respectively. Duplicate samples were weighed, freeze-dried overnight, and percent moisture calculated by difference. Ash values were obtained by incineration at 500°C. Crude protein was analyzed using a macro-Kjeldahl method with a copper catalyst, calculated as total N × 6.25, and crude fat was determined by extraction with petroleum ether (carcass samples only).

Mineral content was determined on carcass and stomach content samples (n=34) by inductively coupled argon plasma emission spectroscopy (ICP-AES) using the methods of Stowe et al. (1985) through Michigan State University Animal Health Diagnostic Laboratory (East Lansing, MI). Minerals included macrominerals (Ca, K, Mg, P, Na), trace elements (Cu, Fe, Mn, Zn) and select heavy metals (Al, Ba, Pb). Mineral analyses were not prioritized, so were the last analyses run following vitamin and proximate nutrient composition. Some of the sampled prairie dogs had empty stomachs (hence no samples available) and others contained inadequate volume for the entire analytical suite, hence a limited number of mineral assays were performed. A summary of various analyses conducted on the different tissues is found in Table A1.

Data Analysis

First, descriptive statistics were used to summarize the data. For the subset of animals in which stomach content samples were available (n=34), mineral concentrations in carcass and stomach contents were analyzed separately since values could not be linked to the other nutrient data. Due to marked skewness, the mineral data were log-transformed, and results are presented as geometric means and errors. Afterwards, multivariate linear models were used to investigate the impact of species, sex, age, season as well as sampling site on detectable tissue nutrient concentrations. All statistical analyses were conducted using R, version 3. 3.6.1 (R Core Team 2019), with $p \leq 0.05$ considered significant.

Results

Proximate nutrient and vitamins A and E composition

The majority of animals sampled for proximate nutrient analysis (n=139) were adult (68.3%), black-tailed (58.3%) prairie dogs. Similar numbers of males and females were sampled (n=73

and 65, respectively, 1 sex unrecorded). Many were sampled in fall (31.7%) and at the RMA site (28.8%). Sample distribution by species is found in Table A2 and sample size for each analysis is found in Table A3 (*n* available for analysis varied as explained previously).

Carcass and tissue weights varied significantly between species, with BT prairie dogs being significantly heavier than WT, and according to sex (heavier males than females) and age class (heavier adults than juveniles) (all $p < 0.001$; data not shown).

Nutritional parameters varied significantly ($p < 0.05$) according to season for the majority of body components and nutrients tested, the exception being stomach content vitamin A. Vitamin A was rarely detected in stomach contents (n= 17/102; 16.7%), but found in the majority of intestinal contents analyzed (n=63/77; 72.4%); mean (± SE) data include analytical values of zero. Significant species differences were detected for carcass dry matter (DM) and carcass vitamin A ($p < 0.05$; both higher in BT vs WT prairie dogs), as well as stomach content protein ($p < 0.05$; BT > WT) and stomach content vitamin E ($p < 0.001$; BT < WT). Intestinal content protein ($p < 0.01$ and skin fat concentrations, $p < 0.05$ were both lower in BT compared with WT samples (Table A3). Ranges for carcass vitamin E measured were similar between species (black-tailed prairie dogs 44 to 76 IU/kg DM, white-tailed 39 to 79 IU/kg DM); highest values were recorded in winter samples for BT versus spring samples for WT prairie dogs.

Due to the inter-specific differences identified, further analyses of proximate nutrients, vitamins A and E were performed on a species-by-species basis (Tables 1a&b and 2a&b).

Add Tables 1a & 1b here

Add Tables 2a & 2b here

When examining compositional data on a species basis, sex differences were relatively minor; BT prairie dog male carcasses contained more protein and less fat than females. Numerically, both species contained lowest mean carcass fat and skin (subcutaneous) fat in the spring, increasing mean fat stores seasonally (spring < summer < fall). The hibernating WT was unavailable for sampling in winter but BT prairie dogs increased in carcass fat % while concurrently decreasing in skin (subcutaneous) fat in winter (Figure 1).

Add Figure 1 here

Sex differences in vitamin A concentrations were not detected in any of the tissues analysed for either species (Table 1a and 2a). Age differences were found in vitamin A concentrations of carcasses (juveniles < adults; p <0.05) and intestinal contents (adults << juveniles; p <0.001) in the WT prairie dogs only (Table 2a). Season had a highly significant effect on vitamin A content in carcasses of both prairie dog species (p <0.01) as well as intestinal contents (p < 0.001) of BT prairie dogs (Figure 2).

Add Figure 2 here

Carcass Vitamin E concentrations differed by species and age (WT only) with adults > juveniles; seasonal contrasts could not be conducted due to the lack of winter samples for hibernating white-tailed prairie dogs (Tables 1a and 2a). Stomach content vitamin E concentrations differed seasonally in BT prairie dogs only (summer > spring > fall > winter; p<0.001). Intestinal contents contained the most vitamin E in samples from both species (Figure 3, Tables 1b and 2b), with numerically the highest values in fall (averaging 174 and 225 IU/kg DM for BT and WT prairie dogs, respectively, and winter the least (47 IU/kg DM, black-tailed only). Neither sex nor age differences in vitamin E concentrations of either stomach or gastrointestinal contents were detected for either species.

Add Figure 3 here

Sampling site had a significant effect on a range of parameters and since prairie dog species were not sympatric these differences reflect species and/or geographical differences (Table 3). Among the BT sampling sites (PG, RMA, WEL), carcass ash and stomach ash were both highest at RMA, carcass protein, stomach protein, and carcass vitamin A were all highest at WEL; stomach content vitamin E was highest at RMA. No site differences were detected for intestinal content nutrients among BT sites. Among WT sampling sites (LAR and MB), ash was the only nutrient to vary by site and was highest in both carcass and stomach contents at MB. The only nutrient to differ in intestinal contents was vitamin A and this was also highest at MB. No site differences were detected for either species in face, feet or skin weight.

Add Table 3 here

Mineral composition

Distribution of tissues sampled for mineral analysis is outlined in Table A4. In line with overall prairie dog analyses, samples for mineral analyses (n=34) comprised primarily black-tailed (65%), adult (71%) prairie dogs, with most sampled in summer (35%) from the RMA site (32%). Mineral composition data for the sub-set of prairie dogs' carcasses and stomachs are provided in Table 4. The heavy metals Al, Ba and Pb were detected in prairie dog carcasses from all sites. Mean Al concentrations ranged from 36 to 86 mg/kg, with prairie dogs at one outlier site having 491 mg/kg. Mean Ba was 8 – 28 mg/kg, whilst Pb was 3 – 9 mg/kg at two sites, but 25 - 53 mg/kg at the remaining 4 sites (data not shown).

Add Table 4 here

Intra-species differences were detected for carcass K and stomach P, Na, Cu, Fe, and Zn (Table A5a). The only sex difference detected was in Fe concentration of stomach contents

(male > female), whilst age influenced carcass K, and stomach K, Mg, Na and P, as well as Fe and Mn (Table A5b). Season had a significant effect on most minerals and body components measured, with the exception of stomach K and Zn (Table 5). Carcass Na composition (only) was influenced by sampling site; however, stomach mineral composition differed among sites for multiple minerals including Ca, Cu, Fe, K, Mg, Mn, and P (Table 6).

Add Tables 5 and 6 here

Discussion

In support of our three hypotheses, the current analyses demonstrate that prairie dog species differ significantly in a range of nutrients, indicating important potential differences in dietary nutrient intake profiles for the endangered black-footed ferret, and other animals utilizing these prey species. Additionally, we have shown, for the first time, that nutrient intake will also be influenced by season of harvesting, and the age and sex of the prairie dog consumed – factors that can impact carnivore population dynamics. The influence of prey-, environmental- and seasonally-based parameters is rarely considered when evaluating *in situ* feeding ecology for extrapolation to captive-animal dietary provision, or during release site assessment in reintroduction projects; oversights which are of concern to a range of carnivore conservation and management programs.

Distinct differences in lipid metabolism and feeding behaviors, previously described for the two species of prairie dogs (Thompson, Agar, and Bintz 1993; Harlow 1995; Lehmer and Van Horne 2001) may explain differences in carcass and skin compositions. Black-tailed prairie dogs are active throughout the winter, feed selectively, and practice intermittent facultative torpor (Lehmer and Van Horne 2001); our findings indicate their body fat stores

peak during winter but decline rapidly, reaching their lowest relative proportion in the spring, likely due to utilization in support of reproductive activities, and/or simply as a primary energy source. At this point in time (spring) ferrets are also entering their reproductive season (Miller and Anderson 1993) and therefore would be predicted to have increased demand for dietary fat. Increased nutritional plane is associated with earlier onset of ovarian activity in females (e.g. goats; (Zarazaga et al. 2005)) and higher quality diets are considered to improve male ejaculate traits (e.g. cheetahs; (Crosier et al. 2007)). As such, our finding of markedly lower BT prairie dog fat composition in springcompared to other seasons warrants consideration. A possible explanation is that ferrets may utilize stored prey items of higher fat content during this critical period. Ferrets have been reported to temporarily cache prairie dog prey in their burrows for possible later consumption over the short term (Richardson et al. 1987), thus the higher-fat late winter kills may be available to ferrets in early spring. However, such caching behaviour is not considered to be long-term (i.e. unlikely to occur inter-seasonally) and research suggests that kleptoparasitic badgers excavate and rapidly consume these stored meals (Biggins et al. 1991; Eads et al. 2013), effectively negating any nutritional advantage of this behavior. Alternatively, ferrets may switch dependence on prairie dogs in favor of other prey species during the late winter and early spring, but ferret activity has been documented to be largely focused on prairie dog colonies in the winter, where other prey such as voles are rare (Richardson et al. 1987).

Ferrets will excavate hibernating WT prairie dogs plugged within their burrows (Biggins, Hanebury, and Fagerstone 2012) and digging intensity by ferrets increases in the winter, suggesting that hibernating WT prairie dogs represent an important dietary component (Richardson et al. 1987; Biggins et al. 2012). However, our sampling strategy was unable to locate this species during hibernation. Nonetheless, similar to BT prairie dogs, WT prairie dogs demonstrated their lowest carcass fat composition in spring, with significantly higher

concentrations in both summer and fall. Since peak fat intake during winter/early spring may be physiologically important for successful reproduction, it is possible ferrets achieve this pre-breeding intake via harvesting WT prairie dogs throughout fall and winter months at sites occupied by this hibernating species. Our field-based findings therefore provide the first insight into seasonal ferret nutrient intake, but further research is warranted to determine the prey preferences and subsequent nutrient intake of ferrets during and prior to reproductive activity.

Despite physiological and ecological differences, both prairie dog species present a similar food package to ferrets in spring, with an overall carcass composition containing about 32% fat and 57% protein. This dietary fat:protein content provides a 1.3:1 fat:protein energy ratio, similar to the dietary macronutrient levels suggested as optimal for other obligate carnivores (cats (*Felis catus,* 36% dietary fat; 56% dietary protein; 1.4:1 fat:protein energy ratio (Hewson-Hughes et al. 2011) and domestic mink (1.4:1 protein:fat energy) (Mayntz et al. 2009)).

Recent data (Biggins, et al., unpublished manuscript) suggest that juvenile prairie dogs are particularly important prey for ferrets during reproductive phases. Selective consumption of this prey age class (comprising 43% protein, 48% fat) would result in a high predicted fat:protein energy ratio of 2.1:1 and 1.8:1 for BT and WT prairie dog carcass-based diets, respectively. This may be important for meeting the higher nutrient demands of reproduction/lactation, and growth of the kits as they emerge. To date, there are no further definitive data on seasonal or prey preferences identified for ferrets from which to extrapolate nutrient intakes/profiles. Other prey rodents containing crude fat contents > ~35% on a dry matter basis (with concurrent lower protein levels) include weanling domestic mice (*Mus musculus*, Douglas et al., 1994; Kerr et al., 2014), domestic guinea pigs (*Porcellus cavia*,

Clum et al., 1996), and free-range pocket gophers (*Thomomys bottae;* Kremen et al., 2013). Therefore, prairie dogs represent a relatively high fat prey item across quadruped prey species for which data exist.

Spring prairie dog carcasses contained the highest protein levels, as well as overall mineral composition in both species. As for fat, these nutrients are also important in supporting reproduction and lactation, such that seasonal dietary nutrient variability is likely critical for optimal reproductive output in ferrets. This is supported by research in mink, for which maintenance requirements of 24% protein increase to 38 - 46% protein for gestation and lactation, dropping to 35 – 38% during the growth period (National Research Council 1982). Prairie dog carcasses of both species appear to meet or exceed these requirements with seasonal changes paralleling the change in physiological status of breeding ferrets.

Other nutrients that may be linked with spring reproductive activity for ferrets include the fat-soluble vitamins A and E. Although vitamin A requirements of ferrets are currently unknown, dietary vitamin A levels between 6 and 10 IU/g DM are suggested for mink and domestic carnivores (National Research Council 1982, 2006); in our study, this range was only met by prairie dog carcasses sampled in spring. While some vitamin A may be stored in and mobilized from adipose tissues to meet rodent (i.e. prairie dog) dietary requirements (~2.5 IU/g DM; NRC 1995), vitamin A is primarily stored in the liver (Frey and Vogel 2011). Thus different tissues may underlie the seasonal changes in prairie dog vitamin A concentrations documented. Further, the development and function of adipose tissue is influenced by vitamin A status, with low status favoring increased white fat deposition (Bonet et al. 2003); different mechanisms may occur with hibernating species and brown fat (Villarroya et al. 1999). Multiple biological tissues, nutrients, species and metabolic interactions must be considered.

The seasonally variable dietary intake of prairie dogs is likely the key driver for this variability in their body (carcass) composition. As such, we also investigated fat-soluble vitamins A and E in prairie dog stomach and intestinal contents. These components are typically excluded from analyses, and therefore disregarded in erroneously termed "whole prey" nutrient composition reports, and yet may provide significant nutritional value to carnivore consumers. This is highlighted by our finding that vitamin A values of intestinal contents in both species of prairie dogs were notably higher than carcasses in non-breeding seasons, and would provide concentrations sufficient to meet predicted requirements for ferrets. Without knowledge of this component's contribution, interpretation of vitamin A intake by ferrets outside of the breeding season based on prairie dog carcass composition alone would be misleading. Very few values for vitamin A content in whole rodent prey are found in the literature (Douglas et al., 1994; Clum et al., 1996; Dierenfeld et al., 2002); captive-reared rodents consistently demonstrated high and widely varying levels of this nutrient, especially compared to values measured in free-range cotton mice (*Peromyscus gossypinus*; Thomas et al., 2004).

The detection of vitamin A in some stomach samples was unexpected as plants contain no preformed vitamin A but rather carotenoid precursors utilized by herbivores to convert into active forms in the intestinal tract. Since measured values from intestinal contents were highest in samples taken from prairie dogs in the fall, it is possible that diet (potentially including cannibalism (Hoogland 1985, 1996)), microbial changes or even altered lipid metabolism in preparation for winter months may have impacted vitamin A synthesis/storage. Regardless of source, our findings reveal that prey intestinal tract contents may represent a critical dietary source of this essential nutrient which must be considered when feeding ferrets (and other carnivores) in breeding or release programs.

Similar to vitamin A, vitamin E is important for supporting reproduction (Tauson 1994) and seasonal foraging on fresh plant materials and/or lipid mobilization of this stored nutrient in BT prairie dogs may explain the levels of vitamin E detected in spring carcasses compared to winter values. Although differences were non-significant, the lower spring values may indicate lower dietary intake of vitamin E *per se* in newly emerging forages, or may be linked with vitamin E depletion related to high polyunsaturated fatty acids (PUFA) in young plants. The antioxidant properties of vitamin E may also be reflected in differential lipid metabolism of the two prairie dog species (Lehmer and Van Horne 2001). The patterns of lipid deposition and use are the opposite in hibernators and non-hibernators (Thompson, Agar, and Bintz 1993), where modulating PUFA intakes and subsequent lipid peroxidation systems are critical for successful hibernation (Frank, Dierenfeld, and Storey 1998). Hence, the seasonal differences observed in carcass vitamin E (and lipid) concentrations between the two prey species in this study may be associated with differential needs for dietary fat intakes as well as lipid antioxidant function between the facultative vs. obligate hibernating prairie dog species. In any case, both species appear to contain adequate vitamin E as food for ferrets compared to estimated dietary requirements of mink or domestic carnivores (~30 IU/kg DM for maintenance, and 80 IU/kg DM for reproduction; National Research Council 1982, 2006). While prairie dog values were similar to those measured in free-ranging cotton mice (Thomas et al., 2004) and within ranges reported for medium-sized prey species such as rats or rabbits, these vitamin E levels measured are markedly higher than vitamin E concentrations reported in domestically-reared small prey rodents (mice, hamsters; Dierenfeld et al, 2002). Prey sourcing therefore requires careful consideration for captive carnivore diet formulation.

As seen for vitamin A, vitamin E in stomach contents also displayed seasonal variability. Stomach content concentrations of vitamin E reflect dietary intake by prairie dogs and varied by season for BT prairie dogs, whilst no seasonality was detectable for WT prairie dogs. In

BT prairie dogs, stomach content vitamin E was highest in summer when plants may be under greatest heat/water stress and known to increase vitamin E content in response to abiotic stressors (Munné-Bosch et al. 1999; Keles and Oncel 2002). However, across all seasons and for both species, vitamin E contribution from either stomach or intestinal contents was notable, such that consumption of the gastrointestinal contents of prairie dog by ferrets can be expected to provide a substantial source of dietary vitamin E in nature. The relatively common practice of eviscerating prey prior to offering to captive carnivores should be evaluated in light of these findings, as it may incur unconsidered impacts on vitamin A and E provision. This is especially relevant concerning the key role these vitamins play in reproduction and in light of the on-going reproductive challenges facing ex-situ populations of ferrets (Santymire et al. 2015) and other endangered carnivores.

Carcass mineral concentrations reflected nutrient values that generally met or exceeded published macro- and trace mineral requirements for reproduction and maintenance of mink (NRC, 1982) as well as domestic carnivores (NRC, 2006), particularly if stomach contents are consumed. Plants eaten by prairie dogs (stomach contents), as well as the carcass itself, contained high Na concentrations (range 0.3 to 4.3% of DM), compared to estimated dietary requirement of ~0.2%. Species-and age-specific differences noted in particular minerals may reflect habitat/food resources, but seasonality impacted all minerals measured in carcasses (being highest in spring) as well as stomachs, which were highest in winter and spring, with the exception of sodium (being highest in summer).

Stomach content protein and several mineral (P, Na, Cu, Fe, Zn) concentrations suggest differences in diet choices by prairie dog species, but may also simply reflect site-specific forage availability. Black-tailed prairie dogs consume grasses, sedges and forbs/succulents, with reported stomach contents ranging from about 24% protein in spring to 7% in fall

(Lehmer and Van Horne 2001); similar ranges were seen across sampling sites in our study (7 to 23%) indicating that environmental conditions may be equally or more important in influencing nutrient availability to ferrets (and their prey) as season. Precipitation, for example, seems to have an influence on prairie dog pup production, which can then extend directly to prairie dog biomass as ferret prey (Eads et al., unpublished manuscript). Compared with BT, the WT prairie dogs in this study appeared slightly more conservative in diet variability, with stomach content protein values averaging 18% in spring, decreasing to 13% in summer, and 7% in fall. Prey diet has been shown to impact whole prey composition in other studies (Clum et al. 1996) and should be considered a critical variable influencing dietary balance for the consumers of these prey.

Wide variability in concentrations of select heavy metals quantified here (Al, Ba, Pb) likely reflect site differences in soil composition and or possibly ingestion of accumulator plant species by prairie dogs. Although sites were confounded by species (no sites included both prairie dog species), differences detected were most prominent in stomach contents rather than carcass content, supporting a site-specific or dietary preference effect. Given the consumption of prairie dog stomach by ferrets, these site differences warrant consideration. Dietary tolerances extrapolated from hindgut-fermenting rabbits (200, 250, and 10 mg/kg for Al, Ba, and Pb, respectively (National Research Council 2005)) suggest that Al loads may have been excessive in diets consumed by prairie dogs in some locations, with elevated values also measured in carcasses. Of possible greater concern are the elevated Pb levels found in prairie dog carcasses. The European Union has set the maximum concentration of lead at 5 mg/kg in pet foods (12% moisture; Bampidis et al., 2013); with prairie dog carcasses containing ~65% moisture, our median value only just met that as-consumed limit, whereas the recorded mean was considerably higher (24 mg/kg on an as-consumed basis). Although prairie dogs are removed from areas considered to be potentially contaminated within

anticipated black-footed ferret ranges (Biggins, personal observation) in order to reduce the risk of a toxicity concern , our findings reveal that 4 out of 6 sites sampled would have exposed ferrets to unacceptable concentrations of lead. Whilst one of these sites (RMA) would likely have been considered as "contaminated" prior to testing due to its historic use as an arsenal storage site, three sites were of unknown classification. Testing of soil and/or prey is therefore advocated for release site evaluation of any species.

Studies of free-ranging ferrets have determined they will consume the heart, lungs and liver of prey within hours of the kill (Biggins and Eads 2017). At the time of sampling, it was assumed that certain body parts were left uneaten and as such these components were discarded from analysis. Field observations have subsequently determined that the skin and feet, front of skull with teeth, and sometimes the lower half of the intestinal tract may actually be eaten, although often last (hence presumably least preferred; Tretton, personal communication, 2019). Hair, bones, and often even claws and paws are also consumed, indicating that the majority, if not entirety, of the prairie dog carcass is consumed (Biggins and Eads 2017). Thus, selective consumption of specific parts does not appear to play a major role in overall nutrition of the free-ranging ferrets, and the dressing of carcasses in captive dietary provision can no longer be considered representative of the wild diet. Rather, consumption of the whole body, with various essential nutrients provided by different components (skin vs. carcass vs. gastrointestinal tract) may be critical to meeting the nutritional requirements of the black footed ferret. Similar detailed consideration of feeding habits and appropriate prey sample preparation would also be critical for evaluation of available nutritional resources for other carnivores.

In summary, prairie dogs were sampled from different sites and seasons, of different ages and sexes, and included vitamin nutrient analyses rarely determined in free-range prey items. In

other species, differences in prey sampling have translated to divergent nutritional profiles and are therefore a better reflection of the variability that likely exists in predator nutrient intake than single season or species sampling. This variability is considered crucial to the ability of many free-living predators to balance their nutrient intake (Kohl, Coogan, and Raubenheimer 2015) but is rarely considered in captive provisioning or even in many conservation release programs. In particular, seasonality greatly impacted differences in nutrient composition of prairie dogs consumed as prey, as did body component. Duplicating seasonal nutrient profiles and provisioning on the basis of truly whole prey composition (as opposed to just the carcass) should be considered important in dietary management for black-footed ferrets.

However, the lack of WT prairie dog samples during winter months represents an important limitation to this study, along with the sampling of prairie dogs being targeted rather than random. Firstly, during each sampling period animals were harvested until pre-determined minimum sample sizes per age group and species per site were achieved (where possible). This approach was chosen to allow for a comprehensive sample of the prairie dog population subgroups whilst taking into account ethical as well as logistical challenges related to sampling of wild animals. Secondly, age was determined only by body size and therefore some misidentification of juvenile animals may have occurred. This may explain the relative lack of age-related differences for some parameters. Thus, evaluation of neonatal prairie dogs as prey for ferrets would be a valuable future study. For the multivariate modelling, we did not adjust *p*-values for multiple comparisons, as our aim was not to provide exact estimates of group means, but rather to give a first indication of the effect of variables such as sex, age, season and sampling site. However, differences between sites containing only BT, or between those containing only WT, suggest site-specific differences may not be simply explained as species-specific differences.

Whilst further research is necessary to address these limiting aspects, our study is the first to integrate behavioral ecology with the chemical characterization of nutrient profiles of prey consumed by ferrets and reveals factors likely to be important in driving variability in predicted nutrient intake for free-ranging populations. Analysis of this previously unpublished dataset fills an important knowledge gap, providing insight into overall nutrient variability of the primary prey items for the free-ranging ferret. Our findings have immediate implications for ferret conservation, including the utilization of *in situ* knowledge to inform *ex situ* dietary management in breed-for-release programs. Moreover, site- and season-specific differences in prey composition can be incorporated into reintroduction site assessments, in order to optimize conservation planning to increase post-release survival. Similarly, consideration of feeding behaviors (species, sexes, ages, and portions of prey/foods consumed), as well as seasonal or locale differences, and inclusion of a broader range of nutrients than simply energy or protein contributions, would provide a more comprehensive understanding of nutrient resources and dynamics for any target species, program or ecosystem under investigation.

Acknowledgements

Numerous United States Department of the Interior's Fish and Wildlife Service technicians were involved in the hot, cold, and dirty work of collection and processing of samples. We are grateful for help and suggestions of Jerry Godbey and Dr. John Oldemeyer of the U.S. Geological Survey, and Dr. Hank Harlow of the University of Wyoming. We appreciate the support of James Doherty, General Curator, as well as the laboratory analytical expertise of Jane McGuire at the New York Zoological Society (now Wildlife Conservation Society). Kayley Bryan is thanked for assistance with an early draft of this manuscript, and the authors are grateful to Dr. Sarah Redgate for recent motivations in re-analyzing this archived dataset.

Lab analyses were originally funded through cooperative agreement number 14-16-0009-88-952 between the New York Zoological Society and the U.S. Fish and Wildlife Service.

Literature Cited

Antonelli, T. S., C. L. Leischner, J. J. Ososky, and A. Hartstone-Rose. 2016. The effect of captivity on the oral health of the critically endangered black-footed ferret (*Mustela nigripes*). Canadian Journal of Zoology 94: 15–22.

Belant, J., D.E. Biggins, D. Garelle, R.G. Griebel, and J.P. Hughes. 2015. *Mustela nigripes*.The IUCN Red List of Threatened Species. 2015. http://dx.doi.org/10.2305/IUCN.UK.2015-4.RLTS.T14020A45200314.en.

Biggins, D.E., and D.A. Eads. 2017. Evolution, natural history, and conservation of black-footed ferrets. Pages 340-356 *in* D.W. Macdonald, C. Newman, and L.A. Harrington,, editors. Biology and conservation of musteloids. Oxford University Press, Oxford, UK.

Biggins, D.E., L.R. Hanebury, and K.A. Fagerstone. 2012. Digging Bbehaviors of radio-tagged black-footed ferrest near Meeteetse, Wyoming, 1981-1984. Western North American Naturalist 72: 148–57.

Biggins, D.E., B. J. Miller, L. R. Hanebury, B. Oakleaf, A. H. Farmer, R. Crete, and A Dood. 1993. A technique for evaluating black-footed ferret habitat *in* Management of prairie dog complexes for the reintroduction of the black-footed ferret. US Fish and Wildlife Service Biological Report 13, 73–88. US Fish and Wildlife Service, Denver, Colorado, USA

Bonet, M.L., J. Ribot, F. Felipe, and A. Palou. 2003. Vitamin A and the regulation of fat reserves. Celluar and Molecular Life Sciences 60: 1311–1321.

Brickner, K.M., M.B. Grenier, A.E.Corsier, and J.N Pauli. 2014. Foraging plasticity in a

ighly specialized carnivore, the endangered black-footed ferret. Biological Conservation 168: 1–5.

Campbell, Thomas M., Tim W. Clark, Louise Richardson, Steven C. Forrest, and Brent R. Houston. 1987. Food habits of Wyoming black-footed ferrets. The American Midland Naturalist 117 (1): 208–10.

Clum, Nancy J., Marianne P. Fitzpatrick, and Ellen S. Dierenfeld. 1996. Effects of diet on nutritional content of whole vertebrate prey. Zoo Biology 15:525--537.

Crosier, Adrienne E., JoGayle Howard, Budhan S. Pukazhenthi, David E. Wildt, Laurie Marker, and Josephine N. Henghali. 2007. Ejaculate traits in the Namibian cheetah (*Acinonyx jubatus*): influence of gge, season and captivity. Reproduction, Fertility and Development 19 (2): 370–82. https://doi.org/10.1071/RD06057.

Dierenfeld, E.S., H.L. Alcorn and K.L. Jacoben. 2002. Nutrient Composition of Whole Vertebrate Prey (Excluding Fish) Fed in Zoos. National Agricultural Library Z7994.Z65. 20 pp. http:/www.nal.usda.gov/awic/zoo/WholePreyFinal02May29.pdf

Douglas, T.C., M. Pennino, and E.S. Dierenfeld. 1994. Vitamins E & A, and proximate composition of whole mice and rats used as feed. Journal of Comparative Biochemistry and Physiology 107A: 419–424.

Eads, D.A., D.E. Biggins, T.M. Livieri, and J.J. Millspaugh. 2013. American badgers selectively excavate burrows in areas used by black-footed ferrets: implications for predator avoidance. Journal of Mammalogy 94: 1364–1370.

Ellis, R.L. 1984. Meat and meat products. Pages 431–43 *in* S. Williams, editor. Official methods of analysis of the association of official analytical chemists, edited by,. Association of Official Analytical Chemists, Inc., Arlington, Virginia, USA.

Fish and Wildlife Service, U.S. Department of the Interior. 2013a. Recovery plan for the black-footed ferret (*Mustela nigripes*). U.S. Fish and Wildlife Service, Denver, Colorado, USA.

———. 2013b. Revised black-footed ferret recovery plan. Vol. 78, U.S. Fish and Wildlife Service, Denver, Colorado, USA.

———. 2014. Black-footed ferret (*Mustela nigripes*). U.S. Fish and Wildlife Service, Denver, Colorado, USA. http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?spcode=A004. [Accessed on 12th January 2014].

Frank, Craig L., Ellen S. Dierenfeld, and Kenneth B. Storey. 1998. The relationship between pipid peroxidation, hibernation, and food. American Zoologist 38: 341–49.

Frey, Simone K. and Sike Vogel. 2011. Vitamin A metabolism and adipose tissue biology. Nutrients 3: 27–39.

Harlow, Henry J. 1995. Fasting biochemistry of representative spontaneous and facultative hibernators: the white-tailed prairie dog and the black-tailed prairie dog. Physiological Zoology 68 (5): 915–34.https://doi.org/10.1086/physzool.68.5.30163938.

Hartstone-Rose, A., H. Selvey, J.R. Villari, M. Atwell, and T. Schmidt. 2014. The three-dimensional morphological effects of captivity. PLoS ONE 9 (11): e113437.

Hewson-Hughes, Adrian K, Victoria L Hewson-Hughes, Andrew T Miller, Simon R Hall, Stephen J Simpson, and David Raubenheimer. 2011. Geometric analysis of macronutrient selection in the adult domestic cat, *Felis catus*. The Journal of Experimental Biology 214 (March): 1039–51. https://doi.org/10.1242/jeb.049429.

Hoogland, J.L. 1985. Infanticide in prairie dogs: lactating females kill offspring of close kin. Science 230 (4729): 1137–40.

———. 1996. *Cynomys udovicianus*. Mammalian Species 535: 1–10.

Jones, C.E. 1984. Animal feeds *in* S. Williams, editor, Official methods of analysis of the association of official analytical chemists, Association of Official Analytical Chemists, Inc., Arlington, Virginia, USA.

Kapoor, Varsha, Tyler Antonelli, Jennifer A. Parkinson, and Adam Hartstone-Rose. 2016. Oral health correlates of captivity. Research in Veterinary Science 107: 213–19. https://doi.org/10.1016/j.rvsc.2016.06.009.

Keles, Y. and I. Oncel. 2002. Response of antioxidative defence system to temperature and water stress combinations in wheat seedlings. Plant Science 163 783–790.

Kerr, K., Kappen, K.L., Garner, L.M. and Swanson, K.S. 2014a. Commercially available avian and mammalian whole prey diet items targeted for consumption by managed exotic and domestic pet felines:true metabolizable energy and amino acid digestibility using the precision-fed cecectomized rooster assay. J. Anim. Sci. 92, 4478–4485. doi:10.2527/jas2013-7246.

Kerr, K., Kappen, K.L., Garner, L.M. Utterback, P.L., Parsons, C.M. and Swanson, K.S. 2014b. Commercially available avian and mammalian whole prey diet items targeted for consumption by managed exotic and domestic pet felines: macronutrient, mineral, and long-chain fatty acid composition. Zoo Biol. 33:327-335.

Kohl, Kevin D., Sean C. P. Coogan, and David Raubenheimer. 2015. Do wild carnivores forage for prey or for nutrients? BioEssays 37 (6): 701–9. https://doi.org/10.1002/bies.201400171.

Kremen, N.A., C.C. Calvert, J.A. Larsen, R.A. Baldwin, T.P Hahn and A.J. Fascetti. 2013. Body composition and amino acid concentrations of select birds and mammals consumed by cats in northern and central California. J Anim Sci 91:1270-1276.

Lehmer, Erin M, and Beatrice Van Horne. 2001. Seasonal changes in lipids, diet, and body composition of free-ranging black-tailed prairie dogs (*Cynomys ludovicianus*). Canadian Journal of Zoology 79 (6): 955–65. https://doi.org/10.1139/z01-060.

Machlin, L.J. 1984. Vitamin E: a comprehensive treatise. Marcel Dekker Inc., New York, USA.

Mayntz, David, Vivi Hunnicke Nielsen, Allan Sørensen, Søren Toft, David Raubenheimer, Carsten Hejlesen, and Stephen James Simpson. 2009. Balancing of protein and lipid intake by a mammalian carnivore, the mink, *Mustela vison*. Animal Behaviour 77 (2): 349–55. https://doi.org/10.1016/j.anbehav.2008.09.036.

Miller, B.J., and S.H. Anderson. 1993. Ethology of the endangered black-footed ferret (*Mustela nigripes*). Advances in Ethology, Vol. 31. Paul Parey Science Publishers.: Berlin, Germany and Hawkory, UK.

Munné-Bosch, S., K. Schwarz K, and L. Alegre. 1999. Enhanced formation of alpha-tocopherol and highly oxidized abietane diterpenes in water-stressed rosemary plants. Plant Physiology 121(3):1047-1052.

National Research Council. 1982. Nutrient requirements of mink and foxes, 2nd Edition. National Academies Press, Washington DC, USA.

———. 1995. Nutrient requirements of laboratory animals. National Academy of Sciences, Washington DC, USA.

———. 2005. Mineral tolerance of animals. National Academy Press, Washington DC, USA.

———. 2006. Nutrient requirements of dogs and cats. National Academy of Sciences, Washington DC, USA.

Olson, J.A. 1984. Serum levels of vitamin A and carotenoids as reflectors of nutritional

status. Journal of the National Cancer Institute 73 (6): 1439–44.

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

Roelle, J. E., B. J. Miller, J.L. Godbey, and D.E. Biggins (Eds). 2006. Recovery of the black-footed ferret- progress and continuing challenges. United States Geological Survey Scientific Investigations Report *2005-5293*, 288. Reston, Virginia, USA.

Santymire, Rachel M., Shana R. Lavin, Heather Branvold-Faber, Julie Kreeger, and Paul Marinari. 2015. Effect of dietary vitamin E and prey supplementation on semen quality in male black-footed ferrets (*Mustela nigripes*). Theriogenology 84 (2): 217–25. https://doi.org/10.1016/j.theriogenology.2015.03.007.

Sheets, R.G., R.L. Linder, and R.B. Dahlgren. 1972. Food habits of two litters of black-footed ferrets in South Dakota. The American Midland Naturalist 87 (1): 249–51.

Stowe, H.D., W.E. Braselton, J.B. Kaneene, and M. Slanker. 1985. Multielement assays of bovine tissue specimens by inductively coupled argon plasma emission spectroscopy. American Journal of Veterinary Research 46 (3): 561–65.

Tauson, AH. 1994. High dietary level of polyunsaturated fatty acids and varied vitamin E supplementation in the reproduction period of mink. Journal of Animal Physiology and Animal Nutrition 72: 1–13.

Thomas, Jasmine, Batsheva Glatt, and Ellen S. Dierenfeld. 2004. Proximate, vitamins A and E, and mineral composition of free-ranging cotton mice (*Peromyscus gossypinus*) from St. Catherines Island, Georgia, Zoo Biology 23: 253-261.

Thompson, Todd A., Mark W. Agar, and Gary L. Bintz. 1993. Lipid deposition and use by black-tailed prairie dogs, *Cynomys ludovicianus*, in the natural environment. Physiological Zoology 66 (4): 561–79.

Villarroya, F., M. Giralt, and R. Iglesias. 1999. Retinoids and adipose tissues: metabolism, cell differentiation and gene expression. International Journal of Obesity 23: 1–6.

Vosburgh, K.M., R.B. Barbiers, J.G. Sikarskie, and D.E. Ullrey. 1982. A soft versus hard diet and oral health in captive timber wolves (*Canis lupus*). The Journal of Zoo and Wild Animal Medicine 13 (3): 104–7.

Wolf, K.N., D.E. Wildt, A. Vargas, P.E. Marinari, M.A. Ottinger, and J.G. Howard. 2000. Reproductive inefficiency in male black-footed ferrets (*Mustela nigripes*). Zoo Biology 19 (6): 517–28.

Zarazaga, L.A., J.L. Guzman, C. Dominguez, M.C. Perez, and R. Prieto. 2005. Effect of plane of nutrition on seasonality of reproduction in Spanish payoya goats. Animal Reproduction Science 87 (3–4): 253–67.

31 – Dierenfeld et al.

Table 1a. Black tailed prairie dog (*Cynomys ludovicianus)* composition according to body component, sex, and age. All values expressed on a dry matter (DM) basis except weight and DM, which are fresh wet basis.

	Sex					Age				
Body component and variable measured	**Female**		**Male**		***p***	**Adult**		**Juvenile**		***p***
	mean	**SE**	**mean**	**SE**		**mean**	**SE**	**mean**	**SE**	
Carcass										
Weight, g	671.3	22.5	769.8	29.3	***	769.7	19.6	544.4	26.8	***
DM, %	38.8	1.6	37.1	1.5		38.9	1.3	35.0	2.1	**
Ash, %	8.4	1.2	11.2	1.6		9.4	1.0	11.0	2.8	
Protein, %	41.4	2.3	45.1	2.5	*	42.3	2.0	46.2	3.2	*
Fat, %	46.0	2.1	41.6	2.6	*	44.2	2.1	42.1	2.6	
VitA, IU/g	6.7	1.8	5.9	1.4		6.8	1.5	4.8	0.8	
VitE, IU/kg	54.8	3.7	57.1	3.7		55.2	2.8	58.6	6.2	
Gastrointestinal tract tissue										
Weight, g	181.4	7.3	181.5	8.7		187.3	6.7	159.6	8.6	**
Stomach contents										
DM, %	20.5	1.0	18.7	1.0		19.4	0.8	20.1	1.6	
Ash, %	19.7	2.0	24.6	2.8		21.5	2.1	24.4	3.3	
Protein, %	15.6	2.0	18.5	2.0		18.0	1.6	14.6	3.1	
VitA, IU/g	0.3	0.1	0.1	0.1		0.2	0.1	0.2	0.2	
VitE, IU/kg	36.1	5.9	37.7	4.9		38.1	4.0	33.5	9.4	
Intestinal contents										
Weight, g	37.2	2.2	41.8	2.6		40.9	2.1	34.8	2.0	
DM, %	22.9	1.3	21.2	0.7		22.6	0.9	20.2	1.1	
Ash, %	24.0	3.4	23.4	2.1		25.0	2.6	19.6	0.8	
Protein, %	19.9	1.5	21.3	2.1		21.9	1.6	16.7	1.5	*
VitA, IU/g	4.3	1.2	3.5	0.9		4.1	0.9	3.3	1.3	
VitE, IU/kg	121.9	27.1	128.3	27.1		136.2	24.1	90.6	20.2	
Face										
Weight, g	42.2	2.1	50.9	2.1	**	48.9	1.8	39.3	2.9	*
Feet										
Weight, g	19.9	0.6	22.7	0.5	***	22.2	0.4	18.1	0.7	***
Skin										
Weight, g	115.2	5.7	126.4	4.6		129.5	3.7	88.9	5.3	
DM, %	40.6	2.4	37.4	2.2		40.3	1.7	36.7	4.3	
Ash, %	9.2	2.1	12.5	3.9		9.6	2.0	13.0	5.1	
Protein, %	73.7	3.7	83.8	3.6	*	78.1	3.6	77.1	3.7	
Fat, %	23.3	2.5	17.0	3.0		20.8	2.5	20.6	3.4	

Notes: DM = dry matter; VitA = vitamin A; VitE = Vitamin E;*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Table 1b. Black tailed prairie dog (*Cynomys ludovicianus)* composition according to body component and season. All values expressed on a dry matter (DM) basis except weight and DM, which are fresh weight basis.

	Season								
Body component and variable measured	**Spring**		**Summer**		**Fall**		**Winter**		***p***
	mean	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	
Carcass									
Weight, g	604.4^{A}	24.9	756.9^{B}	41.2	757.3^{B}	39.9	753.6^{B}	32.5	***
DM, %	29.9^{A}	1.2	34.1^{B}	2.3	40.5^{C}	1.4	45.4^{D}	1.2	***
Ash, %	12.5^{B}	0.6	15.4^{B}	3.2	5.9^{A}	0.5	6.3^{A}	0.7	***
Protein, %	57.7^{C}	2.8	48.5^{B}	3.3	37.2^{A}	1.8	33.0^{A}	1.5	***
Fat, %	29.5^{A}	2.9	40.9^{B}	2.9	45.7^{B}	2.1	55.9^{C}	2.1	***
VitA, IU/g	16.6^{B}	4.3	6.1^{A}	1.3	2.8^{A}	0.3	2.3^{A}	0.3	***
VitE, IU/kg	51.5^{B}	3.4	53.9^{B}	5.9	43.7^{A}	2.4	76.0^{B}	4.3	***
Gastrointestinal tract tissue									
Weight, g	160.2^{A}	8.0	223.2^{B}	8.1	198.4^{B}	8.7	128.6^{A}	7.5	***
Stomach contents									
DM, %	19.0^{AB}	0.8	15.8^{A}	1.9	22.2^{B}	0.2	21.9^{B}	0.8	***
Ash, %	13.2^{AB}	1.8	35.8^{C}	3.8	21.7^{B}	0.6	13.0^{A}	1.7	***
Protein, %	23.8^{B}	1.6	21.2^{B}	3.4	7.0^{A}	0.8	18.1^{B}	1.5	***
VitA, IU/g	0.4	0.2	0.4	0.3	0.0	0.0	0.0	0.0	
VitE, IU/kg	30.0^{A}	3.9	60.4^{B}	9.2	29.2^{A}	4.7	21.6^{A}	3.7	***
Intestinal contents									
Weight, g	28.8^{A}	1.0	55.2^{C}	3.7	41.8^{B}	2.1	28.8^{A}	1.5	***
DM, %	25.0	2.6	18.4	1.0	21.7	0.2	22.2	1.1	
Ash, %	34.8	7.0	18.7	1.4	19.3	0.7	22.8	3.2	*
Protein, %	31.9^{C}	3.3	22.5^{BC}	1.1	17.8^{AB}	0.7	13.6^{A}	1.4	***
VitA, IU/g	2.4^{A}	0.8	0.2^{A}	0.1	9.0^{B}	1.7	1.9^{A}	0.5	***
VitE, IU/kg	149.2	72.2	130.8	33.8	173.9	16.3	46.8	7.3	
Face									
Weight, g	44.8	1.5	46.6	3.6	51.6	3.1	43.0	3.2	
Feet									
Weight, g	19.7^{A}	0.7	22.1^{BC}	0.9	22.0^{C}	0.7	21.1^{B}	0.8	***
Skin									
Weight, g	113.4^{A}	8.2	121.6^{AB}	7.8	134.6^{B}	5.4	111.2^{AB}	6.9	**
DM, %	39.7^{AB}	2.4	35.2^{A}	3.2	35.0^{A}	2.4	47.5^{B}	2.1	*
Ash, %	4.1^{AB}	0.7	21.6^{C}	4.0	11.8^{B}	1.1	2.7^{A}	0.3	***
Protein, %	96.9^{B}	0.6	72.0^{A}	4.3	76.0^{A}	4.7	72.9^{A}	4.3	**
Fat, %	8.8^{A}	3.4	26.1^{B}	2.7	24.1^{B}	1.4	20.1^{AB}	4.6	**

Notes: Abbreviations as in Table 1; different letters for means within rows differ significantly.

33 – Dierenfeld et al.

Table 2a. White-tailed prairie dog (*Cynomys leucurus)* nutrient composition according to body component, sex, and age. All values expressed on a dry matter (DM) basis except weight and DM, which are fresh weight basis.

	Sex					Age				
Body component and variable measured	**Female**		**Male**		***p***	**Adult**		**Juvenile**		***p***
	Mean	**SE**	**Mean**	**SE**		**mean**	**SE**	**mean**	**SE**	
Carcass										
Weight, g	491.8	19.5	634.1	43.1	***	654.0	42.3	464.5	12.5	***
DM, %	36.2	1.6	34.9	1.9		37.1	1.7	32.5	1.6	**
Ash, %	9.1	0.9	9.9	1.5		9.0	0.9	10.2	1.8	
Protein, %	44.4	2.7	47.9	3.5		45.0	3.1	49.7	3.4	
Fat, %	45.1	3.1	44.4	3.7		46.1	3.6	41.4	3.2	
VitA, IU/g	4.4	1.1	3.3	0.7		4.9	0.9	2.2	0.5	*
VitE, IU/kg	55.5	6.2	51.6	4.7		60.6	5.7	43.2	2.3	**
Gastrointestinal tract tissue										
Weight, g	154.7	7.7	161.6	11.9		163.5	12.9	151.5	4.2	
Stomach contents										
DM, %	19.8	1.4	20.5	1.1		18.3	1.0	22.8	1.3	**
Ash, %	21.5	2.1	19.9	1.3		20.5	1.8	20.7	1.5	
Protein, %	12.3	1.8	13.4	1.3		16.1	1.2	8.4	1.2	***
VitA, IU/g	0.2	0.1	0.4	0.3		0.3	0.2	0.3	0.3	
VitE. IU/kg	77.6	9.5	65.5	8.2		84.5	8.0	51.8	7.8	
Intestinal contents										
Weight, g	32.6	1.8	38.9	3.9		39.6	4.0	31.8	1.4	*
DM, %	18.9	1.5	19.1	0.8		17.7	1.2	20.8	0.6	*
Ash, %	23.3	3.6	20.6	1.7		25.4	3.2	17.4	0.5	
Protein, %	31.7	4.5	26.7	2.2		34.9	3.5	21.1	1.8	**
VitA, IU/g	4.3	1.3	6.5	2.1		1.5	0.5	9.6	2.1	***
VitE, IU/kg	185.8	36.4	167.2	25.4		150.4	20.8	203.6	39.7	
Face										
Weight, g	43.3	2.0	47.8	2.8		49.9	2.6	40.6	2.1	*
Skin										
Weight, g	83.2	4.2	112.5	9.2		118.5	8.7	74.9	3.2	***
DM, %	37.5	4.7	38.4	2.4		42.7	1.4	28.4	3.8	**
Ash, %	15.5	5.7	8.5	1.1		7.6	1.1	20.8	7.2	*
Protein, %	68.4	9.0	73.0	9.0		67.8	8.5	76.4	7.7	
Fat, %	29.0	5.4	24.8	6.8		26.6	6.3	27.4	2.9	
Feet										
Weight, g	16.3	0.5	19.8	0.7	***	20.2	0.6	15.7	0.4	

Notes: Abbreviations as in Table 1a.

Table 2b. White-tailed prairie dog (*Cynomys leucurus)* nutrient composition according to body component and season. All values expressed on a dry matter (DM) basis except weight and DM, which are fresh weight basis.

	Season						
Body component and variable measured	**Spring**		**Summer**		**Fall**		
	Mean	**SE**	**mean**	**SE**	**mean**	**SE**	***P***
Carcass							
Weight, g	512.5^{A}	22.0	682.8^{B}	56.2	513.7^{B}	42.2	***
DM, %	30.5^{A}	0.9	37.8^{B}	2.7	36.2^{B}	1.0	***
Ash, %	11.2^{B}	0.8	9.6^{AB}	2.1	7.6^{A}	0.8	***
Protein, %	55.9^{B}	2.3	44.2^{A}	4.9	41.8^{A}	1.6	***
Fat, %	34.1^{A}	3.6	48.2^{B}	4.8	48.6^{B}	2.1	***
VitA, IU/g	7.0	1.5	3.2	0.5	1.3	0.2	**
VitE, IU/kg	79.4^{B}	6.1	38.8^{A}	3.6	46.2^{B}	2.5	***
Gastrointestinal tract tissue							
Weight, g	112.4^{A}	6.8	200.3^{C}	12.3	160.5^{B}	9.4	***
Stomach contents							
DM, %	21.2^{B}	1.1	16.2^{A}	1.1	24.0^{B}	1.4	***
Ash, %	16.5^{A}	2.6	24.3^{B}	1.2	20.2^{AB}	1.9	**
Protein, %	17.8	1.7	13.2	1.6	7.6	1.1	
VitA, IU/g	0.5	0.3	0.1	0.1	0.4	0.4	
VitE. IU/kg	92.8	11.3	72.1	9.8	48.0	7.8	
Intestinal contents							
Weight, g	26.0^{A}	1.2	48.3^{B}	5.4	34.1^{B}	2.5	***
DM, %	20.2^{B}	1.3	14.5^{A}	1.2	21.6^{B}	0.4	***
Ash, %	27.4	4.9	21.3	1.5	16.8	0.5	
Protein, %	32.3	2.7	35.9	6.3	19.3	1.1	
VitA, IU/g	2.0	0.8	2.0	1.3	10.8	2.3	
VitE, IU/kg	127.3	24.0	167.8	28.9	225.4	47.1	
Face							
Weight, g	46.0	2.2	48.4	4.2	42.9	2.7	
Skin							
Weight, g	90.9^{B}	6.0	121.1^{A}	13.5	85.4^{A}	7.3	***
DM, %	41.5	2.7	37.8	4.5	31.5	1.9	
Ash, %	5.0	0.6	15.5	5.2	15.5	4.2	
Protein, %	89.2^{B}	4.7	60.2^{A}	9.0	65.2^{A}	8.9	**
Fat, %	11.6^{A}	3.6	35.8^{B}	4.7	30.7^{AB}	5.4	**
Feet							
Weight, g	20.3	0.8	18.3	0.9	16.1	0.6	

Notes Abbreviations as in Table 1a; different letters for means within rows differ significantly.

35 – Dierenfeld et al.

Table 3. Effect of sampling site on prairie dog parameters and nutrient concentrations. Data are reported as mean (±SE). All nutrient data are reported on a dry matter (DM) basis; weights and DM are fresh weight basis.

Body Component and variable measured	PG (BT†)	RMA (BT)	WAV (BT)	WEL (BT)	*p* (BT)	LAR (WT†)	MB (WT)	*p* (WT)
Carcass								
Weight, g	705.0 (47.7)	732.4 (25.4)	765.2 (47.6)	610.0 (32.5)		559.5 (31.9)	572.1 (42.5)	
DM, %	44.6^{AB} (1.9)	35.4^{A} (1.7)	40.7^{B} (1.7)	30.6^{AB} (1.7)	***	36.1 (1.8)	34.0 (1.7)	
Ash, %	5.6^{AB} (0.5)	13.3^{B} (2.0)	6.0^{A} (0.6)	12.0^{AB} (0.9)	***	7.5 (0.8)	11.9 (1.6)	**
Protein, %	33.7^{AB} (2.4)	47.4^{B} (2.8)	38.5^{A} (2.4)	53.3^{AB} (2.6)	***	45.3 (3.0)	49.0 (3.5)	
Fat, %	54.3^{AB} (2.3)	39.7^{A} (2.6)	48.6^{B} (2.2)	31.1^{AB} (4.1)	***	45.1 (3.5)	42.9 (3.5)	
VitA, IU/g	2.8 (0.5)	8.5 (2.4)	3.4 (0.5)	10.4 (2.0)	*	4.0 (0.9)	3.5 (0.8)	
VitE, IU/kg	80.1 (5.2)	57.5 (4.1)	43.7 (2.1)	50.7 (3.7)		54.3 (5.7)	52.0 (4.4)	
Intestinal tissue								
Weight, g	139.4 (9.8)	177.0 (8.3)	215.0 (9.6)	170.7 (11.2)		151.8 (8.6)	164.1 (11.5)	*
Stomach contents‡								
Weight, g	5.5 (0.5)	8.3 (0.3)	7.1 (0.4)	6.6 (0.4)		36.5 (2.3)	39.9 (2.9)	
DM, %	21.1^{A} (1.1)	17.1^{A} (1.2)	23.1^{B} (0.8)	19.0^{AB} (0.5)	***	20.4 (1.2)	19.9 (1.3)	
Ash, %	11.1^{AB} (2.3)	27.9^{B} (3.1)	21.5^{A} (0.9)	12.8^{AB} (2.8)	***	18.0 (1.2)	23.3 (2.0)	**
Protein, %	15.5^{A} (1.0)	22.9^{B}(2.1)	6.3^{A} (0.5)	23.3^{AB} (3.2)	***	12.3 (1.5)	13.5 (1.5)	
VitA, IU, g	0.0 (0.0)	0.3 (0.2)	0.0 (0.0)	0.3 (0.2)		0.2 (0.1)	0.4 (0.3)	
VitE, IU/kg	29.3^{AB} (4.5)	45.2^{B} (6.9)	31.0^{A} (5.0)	24.9^{AB} (4.2)	**	77.2 (9.8)	64.6 (7.4)	
Intestinal contents‡								
Weight, g	29.1 (2.6)	41.8 (2.8)	45.3 (2.8)	29.7 (2.2)		35.3 (3.4)	36.7 (3.1)	
DM, %	21.6 (1.2)	23.3 (1.5)	19.9 (0.6)	25.0 (1.5)		17.7 (1.0)	21.4 (0.8)	
Ash, %	23.0 (5.2)	24.7 (3.2)	18.0 (0.7)	38.5 (9.8)		20.8 (2.2)	23.7 (3.6)	
Protein, %	12.1 (1.8)	22.3 (2.2)	19.8 (1.0)	30.1 (5.5)		30.7 (3.4)	25.3 (2.7)	
VitA, IU/g	2.0 (0.6)	5.4 (1.5)	3.8 (1.1)	1.8 (0.7)		2.7 (0.9)	10.1 (2.6)	***
VitE, IU/kg	59.6 (8.3)	106.9 (32.6)	169.2 (22.4)	151.3 (107.3)		200.3 (30.3)	125.4 (16.1)	*
Face								
Weight, g	39.6 (2.9)	50.5 (2.5)	43.5 (2.8)	45.9 (1.9)		43.4 (1.8)	47.7 (3.1)	
Feet								
Weight, g	19.8 (1.1)	22.4 (0.6)	20.9 (0.8)	19.6 (0.9)		17.6 (0.7)	18.6 (0.7)	
Skin								
Weight, g	112.7 (11.6)	122.7 (4.5)	123.7 (7.3)	116.8 (14.5)		101.7 (7.8)	94.7 (8.3)	
DM, %	46.4 (3.3)	39.9 (2.5)	35.5 (3.0)	36.8 (3.4)		38.7 (2.2)	37.3 (4.8)	
Ash, %	2.2 (0.4)	12.5 (3.6)	13.3 (0.6)	4.3 (1.1)		10.3 (2.1)	13.7 (5.8)	

Protein, %	72.3 (8.5)	79.2 (3.3)	71.9 (5.5)	96.4 (1.3)		70.3 (9.6)	71.1 (8.6)	
Fat, %	19.0 (9.3)	20.2 (2.5)	26.0 (2.6)	10.9 (6.8)		26.8 (7.0)	27.0 (5.3)	

Notes: Sampling sites were: Pawnee Grassland (PG), Rocky Mountain Arsenal (RMA), Waverly (WA), Wellington (WE), in Colorado, USA and Laramie (LA) or Medicine Bow (MB) in Wyoming, USA.
†BT = black tailed prairie dog, WT = white-tailed prairie dog
DM = dry matter ; VitA = vitamin A; VitE = vitamin E
*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; different letters for means within rows differ significantly.

37 – Dierenfeld et al.

Table 4. Mineral composition of prairie dog carcasses and stomachs, regardless of age, sex, site or season. All data are reported on a dry matter basis.

Variable	Carcass		Stomach	
	Mean (±SD)	Median (min, max)	Mean (±SD)	Median (min, max)
Macrominerals, mg/kg				
Ca	16,676 (11,067)	14,900 (2,610-42,100)	4,220 (3,556)	2,975 (409- 12,200)
K	5,349 (1,618)	5,435 (3,060-8,940)	11,685 (5,475)	9,900 (3,850-29,400)
Mg	755 (310)	702 (286-1,480)	1,307 (1,195)	829 (108- 4,830)
Na	11,247 (17,070)	3,030 (1,560-57,200)	43,406 (35,854)	58,200 (1,560-93,000)
P	12,453 (6,509)	11,750 (3,730-26,700)	1,813 (1,366)	1,170 (321- 5,050)
Trace Minerals, mg/kg				
Cu	5.0 (2.5)	4.1 (1.6-12.9)	6.7 (16.7)	2.9 (0.7- 99.8)
Fe	238 (125)	217 (85.1-692)	978 (2081)	305 (65.0- 12,200)
Mn	3.8 (2.7)	2.9 (0.9-13.1)	34.0 (40.0)	18.4 (3.7- 209)
Zn	82.7 (34.6)	77.7 (34.1-157)	45.2 (46.0)	38.1 (7.5- 202)
Heavy Metals, mg/kg				
Al	90.7 (129.4)	62.1 (9.4-760)	1,324 (2,648)	471 (79- 15,500)
Ba	15.2 (10.4)	12.6 (2.7-40.4)	21.6 (20.1)	14.2 (2.4- 82.9)
Pb	36.7 (66.6)	8.4 (2.5-276)	1.9 (0.4)	2.0 (1.0- 3.1)

Notes: Ca = calcium, K = potassium, Mg = magnesium, Na = sodium, P = phosphorus; Cu = copper, Fe = iron, Mn = manganese, Zn = zinc; Al = aluminum, Ba = barium, Pb = lead.

Table 5. Effect of season on mineral levels in prairie dog carcasses and stomachs. All values expressed on a dry matter basis.

		Season								
		Spring		Summer		Fall		Winter		
Tissue	mg/kg	mean	SE	mean	SE	mean	SE	mean	SE	*p*
Carcass										
	Macro-minerals									
	Ca	29539.4^{B}	1.1	7989.2^{A}	1.3	12642.7^{AB}	1.2	11786.0^{AB}	1.3	**
	K	6230.3^{C}	1.1	5303.3^{B}	1.1	5132.3^{AB}	1.1	3639.4^{A}	1.1	***
	Mg	1126.7^{B}	1.1	517.1^{A}	1.1	785.6^{AB}	1.1	545.1^{A}	1.1	***
	P	20677.3^{B}	1.1	7562.7^{A}	1.2	10522.0^{A}	1.1	9636.3^{A}	1.2	***
	Na	3959.6^{A}	1.2	13006.6^{B}	1.5	2443.0^{A}	1.1	1890.8^{A}	1.1	***
	Trace minerals									
	Cu	5.7^{B}	1.2	3.7^{A}	1.2	4.1^{AB}	1.1	5.2^{AB}	1.2	*
	Fe	376.0^{B}	1.1	173.5^{A}	1.1	220.4^{A}	1.1	149.6^{A}	1.1	***
	Mn	5.0^{B}	1.2	2.5^{A}	1.2	4.1^{AB}	1.1	1.9^{A}	1.2	**
	Zn	130.0^{B}	1.1	57.2^{A}	1.1	73.9^{A}	1.1	68.1^{A}	1.1	***
Stomach contents										
	Macro-minerals									
	Ca	4917.0^{BC}	1.4	1442.4^{A}	1.2	1778.9^{AB}	1.4	8123.9^{C}	1.1	***
	K	12471.5	1.2	9741.1	1.1	8754.1	1.2	13444.3	1.1	
	Mg	2100.6^{B}	1.2	412.8^{A}	1.2	423.4^{A}	1.4	2378.9^{B}	1.2	***
	P	2751.6^{B}	1.3	913.5^{A}	1.1	684.5^{A}	1.1	3057.0^{B}	1.0	***
	Na	4064.3^{A}	1.2	62769.3^{B}	1.1	77014.6^{B}	1.0	3227.7^{A}	1.0	***
	Trace minerals									
	Cu	7.5^{B}	1.5	2.3^{A}	1.1	1.6^{A}	1.2	7.1^{B}	1.2	***
	Fe	1745.6^{B}	1.4	151.1^{A}	1.1	233.4^{A}	1.3	965.1^{B}	1.3	***
	Mn	50.3^{B}	1.3	9.9^{A}	1.1	10.6^{A}	1.2	66.1^{B}	1.1	***
	Zn	38.2	1.2	27.1	1.4	22.8	1.4	41.2	1.0	

Notes: Abbreviations as in Table 4; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$; different letters for means within rows differ significantly.

39 – Dierenfeld et al.

Table 6. Effect of sampling site on mineral concentrations in prairie dog carcasses and stomachs. All values expressed on a dry matter basis.

	PG (BT†)		RMA (BT)		WAV (BT)		WEL (BT)		LAR (WT†)		MB (WT)		
Tissue and Variable measured, mg/kg	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	**mean**	**SE**	***p***
Carcass													
Ca	9039.7	1.7	11812.8	1.3	13229.3	1.2	31555.0	1.1	17664.8	1.4	9854.6	1.3	
K	3694.6	1.1	4830.2	1.1	5055.2	1.1	6031.3	1.1	5192.7	1.2	6303.2	1.1	
Mg	484.5	1.2	685.7	1.2	663.4	1.1	1164.9	1.0	824.4	1.2	617.7	1.2	
Na	1886.2	1.1	5432.9	1.5	2202.1	1.1	5815.0	1.8	4622.4	1.5	11780.3	1.8	*
P	8110.5	1.4	10091.8	1.2	10799.4	1.1	21105.5	1.1	13748.1	1.3	8908.7	1.3	
Cu	4.3	1.2	5.5	1.2	3.2	1.1	5.7	1.5	5.4	1.2	3.3	1.2	
Fe	147.7	1.1	197.4	1.1	202.5	1.1	493.5	1.4	222.1	1.1	233.3	1.3	
Mn	2.0	1.3	2.8	1.2	3.1	1.3	9.8	1.3	3.9	1.3	2.8	1.3	
Zn	61.8	1.1	71.3	1.1	73.5	1.1	122.0	1.0	95.9	1.2	66.4	1.2	
Stomach contents													
Ca	9610.0^{AB}	1.2	3848.1^{B}	1.2	677.1^{A}	1.1	5049.0^{AB}	1.3	2005.2^{AB}	1.5	3566.5^{AB}	1.4	*
K	12621.4^{AB}	1.3	14395.4^{B}	1.1	6753.2^{A}	1.1	14132.5^{AB}	1.6	8950.9^{AB}	1.1	9649.8^{AB}	1.1	**
Mg	1903.9^{AB}	1.3	1289.4^{B}	1.3	209.7^{A}	1.2	2618.9^{AB}	1.8	571.8^{AB}	1.4	954.5^{B}	1.4	**
Na	3443.2	1.0	17814.7	1.6	86263.3	1.0	2773.2	1.8	26712.9	1.8	22909.6	1.6	
P	2790.4	1.0	1871.3	1.3	623.5	1.1	3156.9	1.4	891.6	1.3	1380.6	1.3	*
Cu	4.8^{AB}	1.1	4.8^{ABC}	1.2	1.2^{A}	1.1	25.3^{C}	3.9	2.6^{ABC}	1.2	2.7^{ABC}	1.2	**
Fe	1040.5	1.2	563.5	1.3	122.9	1.2	4058.3	3.0	218.9	1.5	434.9	1.6	**
Mn	50.9	1.2	30.4	1.3	7.0	1.1	106.8	2.0	13.5	1.4	16.8	1.4	**
Zn	40.2	1.1	35.4	1.2	48.8	1.8	50.0	1.3	19.4	1.4	16.4	1.3	

Notes: Abbreviations as in Tables 3 and 4; ** $p < 0.01$; * $p < 0.05$; different letters for means within rows differ significantly.

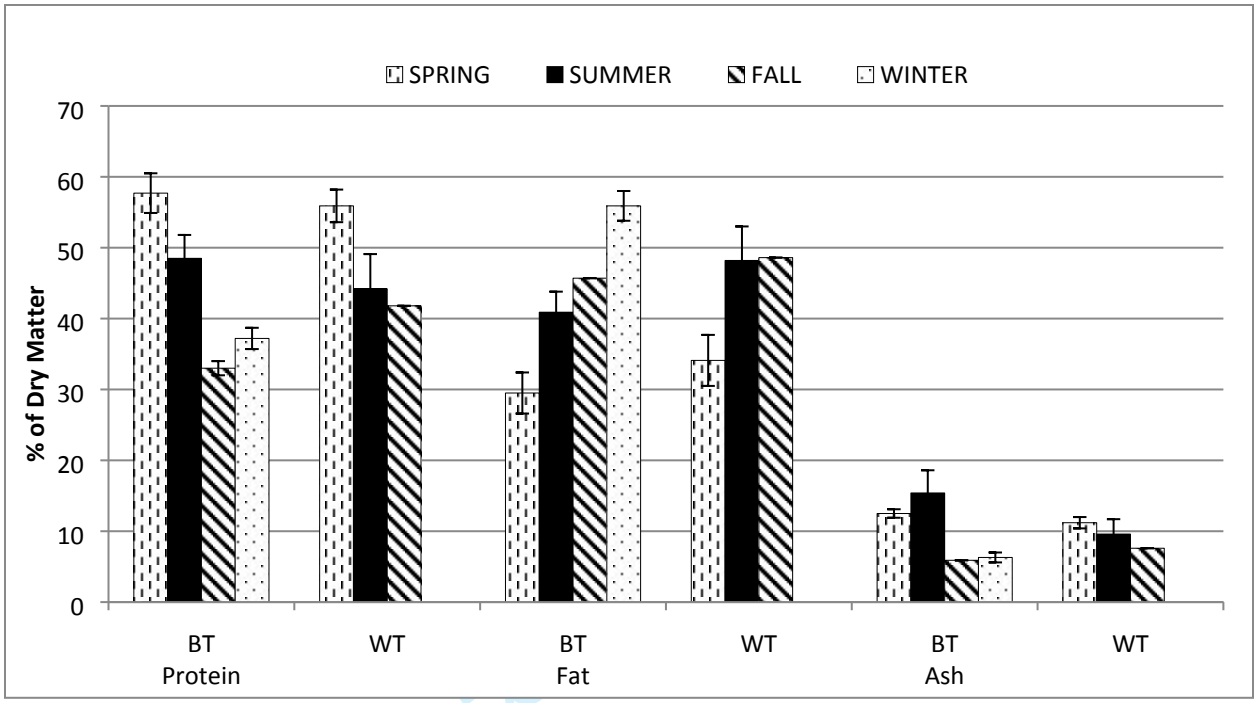

Figure 1. Seasonal changes in carcass nutrient analyses in black-tailed (BT) and white-tailed (WT) prairie dogs as prey of black-footed ferrets. (Samples of winter WT not available).

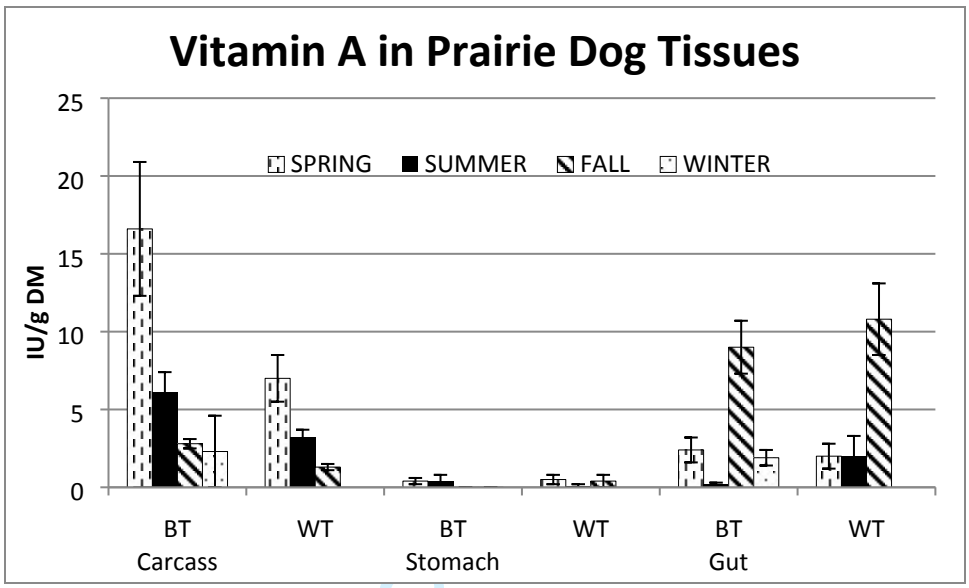

Figure 2. Vitamin A (IU/g dry matter, measured as retinol), in black tailed (BT) and white-tailed (WT) prairie dog tissues, as prey eaten by black-footed ferrets. No WT samples were available in winter.

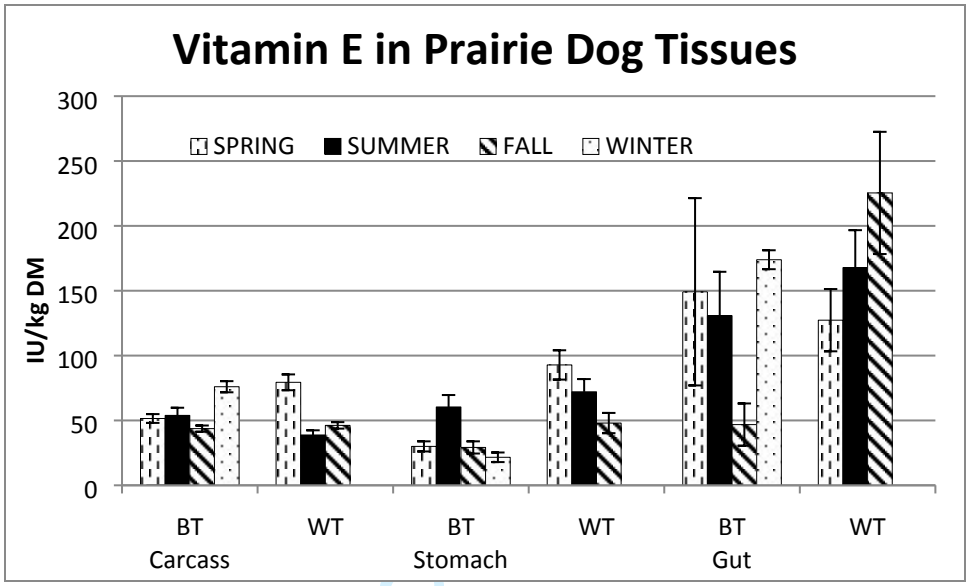

Figure 3. Vitamin E (IU/kg dry matter, measured as α-tocopherol), in black tailed (BT) and white-tailed (WT) prairie dog tissues, as prey eaten by black-footed ferrets. No WT samples were available in winter.

Table A1. Samples and nutritional analyses of prairie dogs used as food by black-footed ferrets (*Mustela nigripes*).

Sample	Component Description	Considered to be consumed	Analyses Conducted
Carcass	Meat, bones & stripped gastrointestinal (GI) tract endothelial tissues, excluding face and feet	Yes	Wt, DM, CP, Fat, Ash, VitA, VitE, Minerals
Gastrointestinal tissue	Stomach, small and large intestine tissue	Yes	Wt only, analyzed within carcass
Stomach contents	Vegetative stomach contents	Yes	Wt, DM, CP, Ash, VitA, VitE, Minerals
Intestinal contents	Vegetative intestinal tract contents (excluding stomach)	Yes	Wt, DM, CP, Ash, VitA, VitE
Face	Frontal part of skull, including nose & teeth	No	Wt only, not analyzed
Feet	All four feet	No	Wt only, not analyzed
Skin	Skin plus subcutaneous fat	Yes	Wt, DM, CP, Fat, Ash

Notes: Wt = weight, DM = dry matter, CP = crude protein, VitA = vitamin A (as retinol), Vit E = vitamin E (as α-tocopherol).

Table A2. Sampling distribution detail for black-footed ferret prey items by species.

Variable	Season	n	% of total
Black-tailed prairie dogs		81	
Season	Spring	18	22.2
	Summer	23	28.4
	Fall	22	27.2
	Winter	18	22.2
Age	A	64	79.0
	J	17	21.0
Sex	F	39	48.1
	M	64	51.9
Site	PG	10	12.3
	RMA	40	49.4
	WAV	21	25.9
	WEL	10	12.3
White-tailed prairie dogs		58	
Season	Spring	18	31.0
	Summer	18	31.0
	Fall	22	37.9
	Winter	N/A	N/A
Age	A	31	53.4
	J	27	46.6
Sex	F	26	45.6
	M	31	54.4
Site	LAR	29	50.0
	MB	29	50.0

Notes: A = adult, J = juvenile, F = female, M = male. Sampling sites were: Pawnee Grassland (PG), Rocky Mountain Arsenal (RMA), Waverly (WA), Wellington (WE), in Colorado, USA and Laramie (LA) or Medicine Bow (MB) in Wyoming, USA. N/A = not analyzed.

Table A3. Level of significant differences in tissue variables for species, sex, age, and season in prairie dogs eaten by black-footed ferrets.

Body component and variable measured		*p*-value			
Carcass	**N**	**Species**	**Sex**	**Age**	**Season**
Weight, g	139	***	***	***	***
DM, %	100	*		**	***
Ash, %	100				***
Protein, %	100				***
Fat, %	100				***
VitA, IU/g^	100	*			***
VitE, IU/kg	100				***
Gastrointestinal tract tissue					
Weight, g	139	***		**	***
Stomach contents					
DM, %	102			*	***
Ash, %	102				***
Protein, %	102	*		**	***
VitA, IU/g^	102				
Vit E, IU/kg	102	***		*	**
Intestinal contents					
Weight, g	138		**	**	***
DM, %	87	**			***
Ash, %	87			*	*
Protein, %	87	***		***	***
VitA, IU/g^	87			**	***
Vit E, IU/kg	87				*
Face					
Weight, g	137		**	***	*
Feet					
Weight, g	139	***	***	***	*
Skin					
Weight g	139	***	***	***	***
DM, %	34			**	**
Ash, %	34			*	***
Protein, %	34		*		***
Fat, %	33	*	*		***

Notes: DM = dry matter; VitA = vitamin A; VitE = vitamin E; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$

Table A4. Number of prairie dogs with samples available for mineral analyses.

Variable		n	% of total
Tissue	**Carcass**	34	50.0
	Stomach contents	34	50.0
Species	**BT**	22	64.7
	WT	12	35.3
Sex	**Female**	17	50.0
	Male	17	50.0
Age	**Juvenile**	10	29.4
	Adult	24	70.6
Season	**Spring**	8	23.5
	Summer	12	35.3
	Fall	8	23.5
	Winter	6	17.6
Site	**PG**	3	8.8
	RMA	11	32.4
	WAV	6	17.6
	WEL	2	5.9
	LAR	6	17.6
	MB	6	17.6

Notes: BT = black tailed prairie dog, WT = white-tailed prairie dog; sampling site abbreviations as in Table A2.

Table A5a. Effect of species on mineral levels in prairie dog carcasses and stomachs. All values expressed on a dry matter basis.

		Species				
		Black-tailed		White-tailed		
Tissue	mg/kg	mean	SE	mean	SE	*p*
Carcass						
	Macro-minerals					
	Ca	12844.4	1.2	13193.9	1.3	
	K	4811.2	1.1	5721.1	1.1	*
	Mg	680.1	1.1	713.6	1.2	
	P	10670.4	1.1	11067.0	1.2	
	Na	3699.1	1.3	7379.3	1.4	*
	Trace minerals					
	Cu	4.6	1.1	4.2	1.1	
	Fe	207.7	1.1	227.7	1.1	
	Mn	3.1	1.1	3.3	1.2	
	Zn	74.0	1.1	79.8	1.2	
Stomach contents						
	Macro-minerals					
	Ca	2782.0	1.3	2674.2	1.3	
	K	11483.1	1.1	9293.8	1.1	
	Mg	883.7	1.3	738.8	1.3	
	P	1535.6	1.2	1109.5	1.2	*
	Na	18485.7	1.4	24738.2	1.4	*
	Trace minerals					
	Cu	3.9	1.3	2.6	1.1	*
	Fe	484.0	1.3	308.5	1.4	*
	Mn	24.5	1.3	15.0	1.2	**
	Zn	40.6	1.2	17.8	1.2	*

Notes: Ca = calcium, K = potassium, Mg = magnesium, Na = sodium, P = phosphorus; Cu = copper, Co = cobalt, Fe = iron, Mn = manganese, Mo – molybdenum, Se = selenium, Zn = zinc; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.

Table A5b. Effect of sex and age on mineral levels in prairie dog carcasses and stomachs. All values expressed on a dry matter basis.

		Sex					Age				
		Female		Male			Juvenile		Adult		
Tissue	mg/kg	mean	SE	mean	SE	*p*	mean	SE	mean	SE	*p*
Carcass											
	Macro-minerals										
	Ca	13401.4	1.2	12592.1	1.2		10990.3	1.2	13891.6	1.2	
	K	4862.5	1.1	5349.5	1.1		6219.1	1.1	4714.3	1.1	**
	Mg	705.4	1.1	679.8	1.1		702.8	1.1	687.2	1.1	
	P	10829.1	1.1	10790.6	1.1		10116.3	1.1	11111.0	1.1	
	Na	4990.9	1.4	4491.8	1.3		5214.0	1.5	4528.4	1.3	
	Trace minerals										
	Cu	4.1	1.1	4.9	1.1		5.1	1.1	4.2	1.1	
	Fe	226.6	1.1	204.3	1.1		214.4	1.1	214.6	1.1	
	Mn	3.4	1.2	2.9	1.2		3.7	1.2	2.9	1.1	
	Zn	75.4	1.1	76.6	1.1		71.9	1.1	77.8	1.1	
Stomach contents											
	Macro-minerals										
	Ca	2633.4	1.3	2872.8	1.3		2016.7	1.4	3118.9	1.2	
	K	10875.9	1.1	10416.0	1.1		8467.5	1.1	11728.8	1.1	*
	Mg	780.1	1.3	888.8	1.3		566.6	1.4	972.3	1.2	*
	P	1420.3	1.2	1313.8	1.2		1018.2	1.2	1549.0	1.2	*
	Na	22415.2	1.4	18516.8	1.5		40426.7	1.5	15434.9	1.4	***
	Trace minerals										
	Cu	3.0	1.2	3.9	1.3		2.6	1.3	3.8	1.2	
	Fe	319.5	1.3	551.0	1.4	**	250.9	1.4	508.1	1.3	**
	Mn	18.3	1.3	23.5	1.3		15.3	1.3	23.3	1.2	*
	Zn	29.1	1.2	31.9	1.3		34.6	1.4	28.7	1.2	

Notes: Abbreviations as in Table A5a; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$.