

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

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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 modeling 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 ongoing 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.

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

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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, Kremer et al. 2013, Kerr et al. 2014a, b). 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 (Kremer et al. 2013, Kerr et al. 2014a) 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 (e.g., 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 the ferret's diet comprises prairie dogs (Sheets et al. 1972, Campbell et al. 1987, Brickner et al. 2014, Biggins and Eads 2017). However, between the late 1800s 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 ongoing 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 1980s 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 additionally 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 as 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, Colorado, USA).

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 cutoff, 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 labeled plastic bags with 5 mL of 25% sodium ascorbate solution for vitamin analysis; any residual stomach contents were stored in a separate, labeled 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 was placed into labeled 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 labeled 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, and 20 g subsamples taken and placed into labeled 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 months before overnight shipment to the Nutrition Laboratory, Wildlife Health Center at the New York Zoological Society (Bronx, New York, USA).

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 h, 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 et al. (1994). Data analyzed as $\mu\text{g}/\text{mL}$ were converted to vitamin activity using conversion factors of $0.3 \mu\text{g} = 1 \text{ IU}$ vitamin A (Olson 1984), and $1 \text{ mg } \alpha\text{-tocopherol} = 1.49 \text{ IU}$ 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 and calculated as $\text{total N} \times 6.25$, 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, Michigan, USA). 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 Appendix S1: Table S1.

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. Afterward, multivariate linear models were used to investigate the impact of species, sex, age, season, and 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 Appendix S1: Table S2, and sample size for each analysis is found in Appendix S1: Table S3 (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 (\pm 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 (Appendix S1: Table S3). Ranges for carcass vitamin E measured were similar between species (black-tailed prairie dogs 44–76 IU/kg DM, white-tailed 39–79 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 1–4).

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 (Fig. 1).

Sex differences in vitamin A concentrations were not detected in any of the tissues analyzed for either species (Tables 1, 3). 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 3). 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 (Fig. 2).

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 1, 3). 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 (Fig. 3, Tables 2, 4), 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.

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 5). Among the BT sampling sites (PG, RMA, WEL), carcass ash and stomach ash were both highest at RMA, and 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.

Table 1. Black-tailed prairie dog (*Cynomys ludovicianus*) composition according to body component, sex, and age.

Variable	Sex				Age			
	Female		Male		Adult		Juvenile	
	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: All values expressed on a dry matter (DM) basis except weight and DM, which are fresh wet basis. Abbreviations are DM, dry matter; VitA, vitamin A; VitE, Vitamin E.

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

Mineral composition

Distribution of tissues sampled for mineral analysis is outlined in Appendix S1: Table S4. 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 subset of prairie dogs' carcasses and stomachs are provided in Table 6. 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, while Pb was 3–9 mg/kg at two sites, but 25–53 mg/kg at the remaining 4 sites (data not shown).

Inter-species differences were detected for carcass K and Na and stomach P, Na, Cu, Fe, Mn, and Zn (Appendix S1: Table S5). The only sex difference detected was in Fe concentration of stomach contents (male > female), while age influenced carcass K and stomach K, Mg, Na, and P, as well as Fe and Mn (Appendix S1: Table S6). Season had a significant effect on most minerals and body components measured, with

Table 2. Black-tailed prairie dog (*Cynomys ludovicianus*) composition according to body component and season.

	Spring		Summer		Fall		Winter		
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE	<i>P</i>
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: All values expressed on a dry matter (DM) basis except weight and DM, which are fresh weight basis. Abbreviations as in Table 1; different letters for means within rows differ significantly.

the exception of stomach K and Zn (Table 7). 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 8).

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-based, 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.

Table 3. White-tailed prairie dog (*Cynomys leucurus*) nutrient composition according to body component, sex, and age.

Variable	Sex				Age			
	Female		Male		Adult		Juvenile	
	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: All values expressed on a dry matter (DM) basis except weight and DM, which are fresh weight basis. Abbreviations as in Table 1.

Distinct differences in lipid metabolism and feeding behaviors, previously described for the two species of prairie dogs (Thompson et al. 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

Table 4. White-tailed prairie dog (*Cynomys leucurus*) nutrient composition according to body component and season.

	Spring		Summer		Fall		
Variable	Mean	SE	Mean	SE	Mean	SE	<i>P</i>
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: All values expressed on a dry matter (DM) basis except weight and DM, which are fresh weight basis. Abbreviations as in Table 1; different letters for means within rows differ significantly.

composition in spring compared 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 behavior 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 et al. 2012) and digging intensity by ferrets increases in the winter, suggesting that hibernating WT

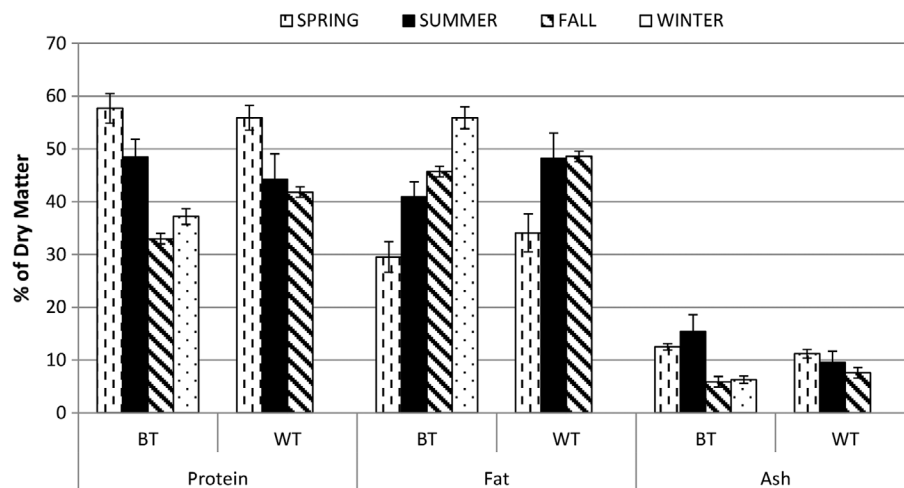


Fig. 1. Seasonal changes in carcass nutrient analyses in black-tailed (BT) and white-tailed (WT) prairie dogs as prey of black-footed ferrets. (Samples of winter WT not available.).

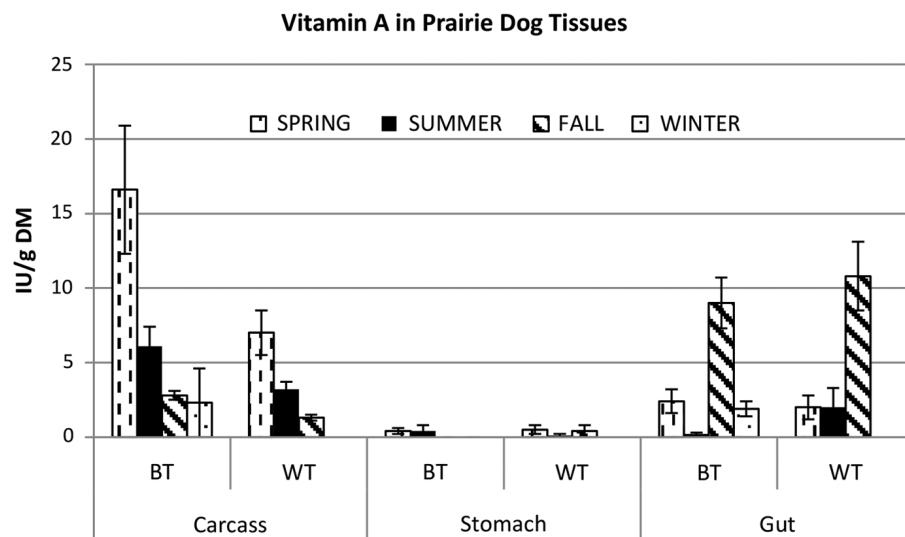


Fig. 2. Vitamin A (IU/g dry matter, measured as retinol), in black-tailed (BT) and white-tailed (WT) prairie dog tissues, as prey eaten by black-footed ferrets. No WT samples were available in winter.

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 prebreeding 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

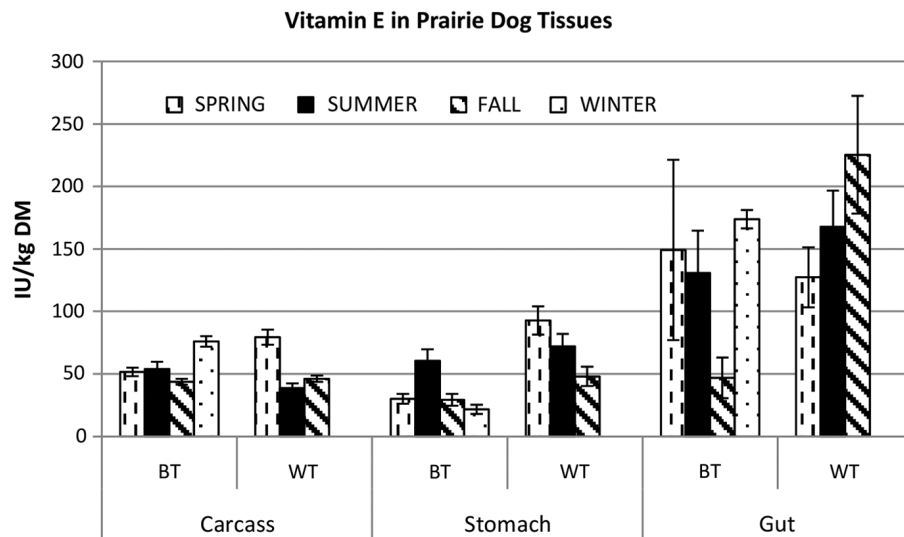


Fig. 3. Vitamin E (IU/kg dry matter, measured as α -tocopherol), in black-tailed (BT) and white-tailed (WT) prairie dog tissues, as prey eaten by black-footed ferrets. No WT samples were available in winter.

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 data) 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

Table 5. Effect of sampling site on prairie dog parameters and nutrient concentrations.

Variable	PG (BT)	RMA (BT)	WAV (BT)	WEL (BT)	LAR (WT)	MB (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: Data are reported as mean (\pm SE). Different letters for means within rows differ significantly. All nutrient data are reported on a dry matter (DM) basis; weights and DM are fresh weight basis. 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. Abbreviations are BT, black-tailed prairie dog; WT, white-tailed prairie dog; DM, dry matter; VitA, vitamin A; VitE, vitamin E.

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

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 are 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

Table 6. Mineral composition of prairie dog carcasses and stomachs, regardless of age, sex, site or season.

Variable	Carcass		Stomach	
	Mean (\pm SD)	Median (min, max)	Mean (\pm 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: All data are reported on a dry matter basis. Abbreviations are Ca, calcium; K, potassium; Mg, magnesium; Na, sodium; P, phosphorus; Cu, copper; Fe, iron; Mn, manganese; Zn, zinc; Al, aluminum; Ba, barium; Pb, lead.

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

Table 7. Effect of season on mineral levels in prairie dog carcasses and stomachs.

	Spring		Summer		Fall		Winter		
Variable	Mean	SE	Mean	SE	Mean	SE	Mean	SE	P
Carcass									
Macrominerals (mg/kg)									
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 (mg/kg)									
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									
Macrominerals (mg/kg)									
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: All values expressed on a dry matter basis. Abbreviations as in Table 6; different letters for means within rows differ significantly.

*** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$.

(Thompson et al. 1993), where modulating PUFA intakes and subsequent lipid peroxidation systems are critical for successful hibernation (Frank et al. 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, while 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

Table 8. Effect of sampling site on mineral concentrations in prairie dog carcasses and stomachs.

Variable	PG (BT)		RMA (BT)		WAV (BT)		WEL (BT)		LAR (WT)		MB (WT)		P
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
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: All values expressed on a dry matter basis. Abbreviations as in Tables 5, 6; different letters for means within rows differ significantly.

** $P < 0.01$; * $P < 0.05$.

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 ongoing 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 0.3–4.3% 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 (7–23%) 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 data*). 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. While 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). 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 et al. 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.

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 predetermined 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 while 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 modeling, 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.

While 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.

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