Diet alters rodent fecal pellet size: implications for paleoecological and demographic studies using fecal dimensions

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Measurements of fecal pellet size can provide important information about wild mammals, such as body size and demographic information. Previous studies have not rigorously tested whether diet can confound these measurements. Further, it is unknown whether diet might alter fecal dimensions directly or through changes in animal physiology. Here, we studied three closely related rodent species that differ in natural feeding strategies. Individuals were fed diets

that varied in protein and fiber content for five weeks. We then measured body size, fecal widths and lengths, and the radius of the large intestine. Diet composition significantly changed fecal widths in all species. High fiber content significantly increased fecal widths and would cause overestimations of body size if applied to wild feces. Using path analysis, we found that fiber can increase fecal widths both directly and indirectly through increasing the large intestine radius. Protein affected each species differently, suggesting that protein effects vary by species feeding strategy and existing physiology. Overall, diet and large intestine morphology can alter fecal pellet measurements. Studies using fecal measurements therefore must consider these effects in their conclusions.

Key words: Fecal dimensions, fiber, paleoecology, noninvasive measures

The ability to ascertain information about mammals without direct observation is a powerful method in the field of mammalogy. For example, fecal pellets can provide a great deal of information about animal species and commonly are used to track population densities and habitat use (Collins and Urness 1981; Berg and Gese 2010). Similarly, fecal pellets can be used to identify age and sex classes in a diversity of species including lagomorphs, elephants, ruminants, and marsupials (MacCracken and Ballenberge 1987; Reilly 2002; Sanchez-Rojas et al. 2004; Southgate 2005; Delibes-Mateos et al. 2009; Rouco et al. 2012; Woodruff et al. 2016). Further, fecal dimensions have been used to estimate changes in rodent body size over geologic time (Smith et al. 1995). However, physiological and environmental factors might influence the size of fecal pellets that animals produce, which may limit our ability to use such measurements

to draw conclusions about the physiological state or size of the animal. Understanding how these factors influence fecal pellet size therefore contributes to validating their use for certain research purposes.

Diet is a somewhat unexplored factor in determining fecal pellet size. Prior research has considered diet as a confounding variable in using fecal measurements to predict body size, but the test used to determine dietary effects was limited in that diet was not actually modified (Smith et al. 1995). In addition, one study experimentally investigated whether high fiber diets can alter the fecal pellet sizes of rodents and found that high fiber increases the length of fecal pellets, but not the pellet width, which is a more commonly used measurement to estimate body size (Hallett and Wigand 2001). However, animals in that study only were fed different diets for 2 days, which may not have been long enough to induce changes in fecal widths. Moreover, other aspects of dietary composition, such as protein content, also could affect fecal pellet size. Overall, the relationship between diet and fecal pellet size still is not well understood.

Several mechanisms could yield diet-related changes in fecal size. First, fiber material is difficult to digest, and thus a large portion of ingested fiber ends up being defecated undigested by the animal, resulting in increased fecal output (Bozinovic 1995). Many species prioritize protein in their diets, so protein levels can dictate total food intake (Post 1993) and possibly total fecal production. In addition, it may make intuitive sense that the size of fecal pellets could partially be dictated by the size of digestive organs, such as the large intestine. The gut is a highly dynamic organ (Yang et al. 2021), and various gut regions can physiologically respond to dietary variation by changing in size and dimension. For example, rodent guts can increase in size and mass to accommodate high fiber diets (Gross et al. 1985; Green and Millar 1987; Valle et al. 2006). High protein diets also could affect animal physiology. While previous studies in

rodents have not demonstrated changes in intestinal morphology as a result of dietary protein (Sabat and Bozinovic 2000; Wang et al. 2019), low protein has been shown to increase gut length in fish (Yang et al. 2002). Overall, it is possible that diet composition could affect the dimensions of fecal pellets both directly and through the effects on gut morphology.

In this study, we tested whether diet composition alters the dimensions of fecal pellets. We focused on three rodent species with different natural feeding strategies: herbivorous montane voles (*Microtus montanus*), omnivorous white-footed mice (*Peromyscus leucopus*), and insectivorous southern grasshopper mice (*Onychomys torridus*); basic information about each species, including natural diet composition and habitat, is provided in Table 1. Individuals of each species were fed diets varying in protein and fiber content for five weeks. At the end of the trial, we measured body size and fecal pellet width and length for each individual. Animals then were dissected, and we measured the dimensions of the large intestine. We hypothesized that dietary composition would alter the relationship between body mass and fecal pellet width, perhaps by altering the radius of the large intestine. We use structural equation modeling to understand the relationships between these variables and compare the relative effects of diet, large intestine morphology, and body size, in determining fecal pellet widths.

MATERIALS AND METHODS

Wild *Onychomys torridus* were collected from field sites near Animas, Hidalgo Co., New Mexico (31.813436, -108.813772); *Peromyscus leucopus* near Murray, Calloway Co., Kentucky (36.686582, -88.221204); and *Microtus montanus* near Timpie Springs Waterfowl Management Area, Dugway, Tooele Co., Utah (40.753708, -112.639903). Forty individuals of each species were collected using baited Sherman live traps under the following state permits: *O. torridus*

(New Mexico Department of Game and Fish, #3562); *P. leucopus* (Kentucky Dept. of Fish and Wildlife, SC1911097); and *M. montanus* (UT Division of Wildlife Resources, 1COLL5194-2). Animals were housed in captivity singly and randomly assigned to one of four isocaloric diet treatments that varied in protein and carbohydrate content (see Supplemental Data SD2). Animals were maintained on experimental diets for a period of 5 weeks prior to dissections under Institutional Animal Care and Use Committee (IACUC) protocols registered at Northern Arizona University (#15-014 and #16-001 to B. Pasch), Murray State University (2018-026 to T. Derting), and the University of Utah (16-02011 to M. D. Dearing). All research protocols followed the guidelines of the American Society of Mammalogists (Sikes et al. 2016). We used ground diets to prevent animals from sorting and selective foraging. However, we did not analyze left over food and therefore cannot fully exclude the fact that animals may have still been able to do some selective foraging, and thus may have consumed slightly different diets than what were offered (Justice and Smith 1992).

After at least 5 weeks on experimental diets, animals were euthanized with an overdose of isoflurane. This feeding trial is part of a larger study to investigate phenotypic flexibility of digestive organs and the microbiome. During dissections, the large intestine was removed, cut open longitudinally and opened flat on a metal tray with ice underneath. We used digital calipers to take 4 – 8 measurements of the width of this tissue (essentially the circumference of the large intestine). These values were averaged and used to calculate the radius of the large intestine.

During the feeding trial, cages and bedding were changed weekly, thus fecal pellets present at the end of the experiment were excreted during the last week of the trial. Fecal pellets were collected and dried overnight at 40 °C. We randomly chose 80 fecal pellets to be measured per individual. The length and width of the fecal pellets were determined using electronic

116 calipers that measured to two significant digits. The average length and width were calculated for
117 each individual using the 16 largest (20%) pellets. We then carried out an analysis of covariance
118 (ANCOVA) for each species, with fecal width as the dependent variable, fiber and protein as
119 independent variables, and either body mass (g) or body length (measured as nose-to-anus, in
120 mm) as covariates. We compare least-square mean values of fecal dimensions across treatment
121 groups to evaluate the effect size of dietary treatments. We define statistical significance as P
122 ≤ 0.05. We undertook similar analyses for fecal length.

123 Next, we predicted the error that dietary fiber could introduce to estimates of body size.
124 We used the regression lines between body size and fecal width for these purposes, because this
125 measurement is used most widely in the literature. First, we calculated average body mass for a
126 given species and used the regression line to calculate the fecal width measurement for animals if
127 they were feeding on the high protein / high fiber diet as expected for a typical diet in nature.
128 This fecal width measurement then was used to solve for "Body Mass" using the regression line
129 determined for the high protein / low fiber diet group as expected for a laboratory-based diet.
130 The difference in predicted body mass then was calculated for each species by comparing these
131 two regression lines.

132 Finally, we carried out a path analysis, a form of structural equation modeling (SEM) that
133 allows the identification of potential and existing relationships among measured variables. The
134 lavaan package (version 0.5-6) in RStudio (version 1.2.5001) was used to estimate and predict
135 relationships between our observed variables (Rosseel 2012). We developed one full path model
136 that then was compared and examined with numerous *a priori* proposed models, each unique and
137 missing particular variables (see Supplementary Data S2). We ranked models using the Akaike
138 Information Criterion (AIC) and used it together with R^2, root mean square error of

approximation (RMSEA), and standardized root mean residual (SRMR) values to determine the strongest model supported by our data. We used standardized coefficients, which standardize the variation of each variable to equal 1, to then compare the relative effects of each variable (body size, diet, etc.)

RESULTS

For all species, the positive relationship between body length and body mass were statistically significant and strong ($R^2 > 0.6$ for all groups). In montane voles, we found a significant effect of protein on body mass: animals fed the high protein diets were 6.3% smaller than animals fed the low protein diets (using least-square means based on body length). When controlling for body length, neither fiber nor protein had significant effects on body masses of white-footed mice or grasshopper mice.

First, we tested whether fecal dimensions (pellet length and width) were correlated with aspects of body size (body mass and body length). Measurements of fecal pellet width increased with increasing body length, although this relationship was not statistically significant for all species (Table 2, Fig. 1). In all species, fecal pellet width increased significantly with increasing body mass (Table 2, Fig. 1). However, fecal pellet length was not as informative for aspects of animal body mass and length. In montane voles, fecal pellet length was significantly correlated with body length ($P = 0.025$) and body mass ($P = 0.01$), but there were no significant correlations of fecal pellet length and body size in white-footed mice or grasshopper mice. These results are consistent with previous studies that report fecal pellet width as a better predictor of body size than fecal pellet length (Smith et al. 1995). We therefore focus on fecal pellet width

data in the remaining text, and data regarding fecal pellet length can be found in the Supplementary Data SD1.

We found that diet composition significantly altered the measurements of fecal pellet width of all three species. Fiber had the largest effects on fecal pellet widths (Table 2; Fig. 1). Using least-square means to control for body mass, the high fiber diets yielded 14.6%, 17.0%, and 24.7% increases in fecal width in voles, white-footed mice, and grasshopper mice, respectively. Protein levels also influenced fecal measurements, although with contrasting effects across species (Table 2; Fig. 1). Voles fed high protein diets produced feces that were 6.2% wider than those fed low protein diets. In white-footed mice, there was a significant protein × fiber interaction for fecal width measurements, such that high protein diets decreased fecal width by 10.6% when animals were on low fiber diets but caused minimal change (< 1%) when animals were on high fiber diets. In grasshopper mice, high protein diets decreased fecal width by 4.6%.

Next, we calculated examples of errors that dietary fiber might introduce into predicting animal body size. For example, in montane voles, the average experimental body mass (45.18 g) would be predicted to produce feces with a width of 2.04 mm when on the high protein / high fiber diet. However, if researchers used the regression line developed for the high protein / low fiber diet, animals producing feces with a width of 2.04 mm would be predicted to have a body mass of 61.06 g, or 1.35× larger. When this same method was applied to white-footed mice and grasshopper mice, the predicted body masses were 3.88× and 2.43× larger, respectively. Using similar methods, we estimate that if researchers were to use regression equations based on animals feeding on low fiber diets, but the actual feces were collected from animals feeding on high fiber diets, the body length of animals would be overestimated by factors of 1.09, 2.78, and

1.39 for montane voles, white-footed mice, and grasshopper mice, respectively.

Finally, while it might seem intuitive that the size of fecal pellets could be partially dictated by the size of digestive organs, such as the large intestine radius, this never has been demonstrated. Using path analysis, we found a consistent best-supported model across all three rodent species (See Supplementary Data SD2 for results of all models compared). In the best-supported model, factors of diet composition (high/low fiber and protein combinations) were included as exogenous binary variables, large intestine radius was a mediator variable, and body mass was a secondary exogenous variable. Body mass, the variable largely estimated using fecal pellet widths in paleoecology studies, exhibited a statistically significant relationship with fecal width in the grasshopper mouse, but not the other rodent species (Fig. 2; Table 3). Rather, we found that fiber had the largest standardized effects in driving fecal width across all species. Fiber intake can lead directly to changes in fecal pellet width and at the same time, it can indirectly affect fecal pellet width by altering the radius of the large intestine (Fig. 2; Table 3). The large intestine radius showed strong and significant associations with fecal pellet widths in herbivorous montane voles and insectivorous grasshopper mice, although this relationship was not statistically significant in omnivorous white-footed mice (Table 3). Diets with high protein content generally resulted in smaller fecal pellet widths, although this result was only statistically significant in the white-footed mouse (Table 3). Overall, results from our path analysis suggest that diet composition can alter animals' fecal pellet width both directly and by increases in large intestine radius, and that these effects are stronger than the effect of body mass.

DISCUSSION

Here, we tested whether dietary variation influences the size of rodent fecal pellets, as such effects could have numerous implications for studies on wild mammals. Overall, we found that

fecal sizes predicted animal body size, because both body lengths and masses typically were positively and significantly correlated with fecal width. However, diet also had a significant impact on these relationships. We found that high fiber diets yielded significantly wider feces across all three rodent species studied. High protein diets affected each species differently, such that they increased fecal widths of montane voles, but decreased fecal widths in grasshopper mice. Results from structural equation modeling reveal that diet strongly affects fecal width, both directly and indirectly by modifications to morphology of the large intestine. It should be noted that in the wild, *O. torridus* are unlikely to eat the high amounts of fiber used in our experimental diets, because they primarily consume animal material. However, *P. leucopus* and *M. montanus* are more likely to encounter high amounts of fiber in their natural diets of seeds and vegetation, so the experimental diets are more ecologically relevant for them (Table 1). Nevertheless, our results remained consistent across all species. Below, we discuss potential mechanisms of these changes and the implications our results have for studies that estimate mammalian body size for paleoecological or demographic studies.

Dietary fiber had the largest impact on fecal dimensions. Fiber may directly increase fecal matter size by changing diet digestibility and the total amount of feces produced. For example, herbivorous common degus (*Octodon degus*) fed high fiber diets for 27 weeks produced significantly more feces and had lower apparent digestibility of dry matter and protein (Veloso and Bozinovic 1993). Likewise, gerbils fed high fiber diets for two weeks had lower apparent digestibility of dry matter and fiber (Pei et al. 2001a). Thus, the relationship between diet and fecal width could be explained by decreased digestibility causing increased fecal output (Bozinovic 1995). Dietary fiber also can indirectly alter fecal dimensions by affecting large intestine morphology. In our study, fiber significantly increased large intestine radii of all three

rodent species. These results are somewhat consistent with previous research on laboratory rats and wild caught Brandt's voles. While large intestinal radii or circumferences were not measured, rats fed high-fiber pectin diets for 4 weeks exhibited significant lengthening of the small and large intestines, and rats fed high cellulose diets exhibited significant lengthening of the colon (Stark et al. 1996). Likewise, Brandt's voles fed high-fiber diets for 14 days showed significant increases in the total length and mass of the gut, specifically in the cecum, proximal colon, and distal colon (Pei et al. 2001b). While we were unable to track changes in fecal size over time, it would be useful to differentiate the immediate and direct effects of fiber from the indirect effects of large intestine morphology on fecal size, which may develop over time. Nonetheless, the best-supported path analysis from our study suggests that an increase in large intestine radius could lead to an increase in fecal width. Overall, dietary fiber modifies diet digestibility, fecal production, and large intestine morphology, thus resulting in changes in fecal dimensions.

The effects of protein on fecal production and gut morphology were not as strong or consistent as the effects of fiber. Our data suggest that protein also can change fecal widths both directly and indirectly by altering the radius of the large intestine. However, rodent species responded to protein levels differently, such that high protein increased fecal widths of montane voles, decreased fecal widths of grasshopper mice, and there was a significant protein x fiber interaction in white-footed mice. The effects of protein therefore may vary by species-specific feeding strategy and physiology. Low protein diets have been shown to cause histomorphological changes in the intestines of lab rats, such as shortened colonic crypts and wall atrophy of the jejunum (Franco et al. 2010; Eyzaguirre-Velásquez et al. 2017). Furthermore, high protein levels affect large intestinal gene expression of rats (Mu et al. 2016; Beaumont et al.

2017). Thus, it is reasonable that a high protein diet also would induce changes in gut morphology, with resultant changes in fecal dimensions. Notably, lipid content was held constant in our experimental diets, so we could not test the potential effects of dietary lipids on fecal dimensions. However, recent work demonstrated that white-footed mice fed a high lipid diet did not exhibit significant changes in body mass or small intestine mass and length (Wang et al. 2019). Future studies could investigate whether dietary lipids alter the dimensions of fecal pellets.

Our results run counter to previous studies that conclude there are minimal impacts from diet on fecal pellet widths. One study concluded that diet did not impact fecal width by collecting feces from several woodrat species (*Neotoma* spp.) from a variety of habitats across seasons and comparing predicted body masses to actual body masses. This technique revealed a prediction error of 21%, and concluded that diet did not influence the ca. 20 – 50% changes in body mass estimated to occur between the last glacial maximum and the mid-Holocene (Smith et al. 1995). Another experiment fed woodrats high fiber diets for a period of two days, and did not observe any increases in fecal pellet width (Hallett and Wigand 2001). However, given the results of our structural equation modelling, it appears that fiber may alter fecal pellet width by altering the dimensions of the large intestine, which may take longer than two days to respond. Lastly, it should be noted that previous studies have found higher error rates for smaller juvenile animals (woodrats below 80g; Smith et al. 1995) and the rodent species in our study all are considerably smaller than woodrats. Given the rather consistent results of our path analysis across three species with distinct feeding strategies, we predict that these alterations to fecal dimensions also would occur in larger species, although this remains to be tested. Overall, our study shows that diet, especially fiber content, can alter fecal pellet widths, and perhaps lead to drastic

277 overestimations in body size. It is notable that diet treatments in our study resulted in prediction
278 errors of 35 – 300% depending on the species, which are larger than prediction errors reported in
279 previous studies (Smith et al. 1995).

280 While our experiments were carried out under controlled laboratory conditions, natural
281 variation both in habitat and physiology could affect fecal pellet dimensions. For instance, if diet
282 composition significantly changes by season, fecal pellets could greatly change in size. Seasonal
283 changes in diet and environment can significantly affect gut size and digestibility in muskrats
284 and field mice (Campbell and MacArthur 1996; Wang et al. 2009). Further, measures of fecal
285 nitrogen from deer varied seasonally and annually (Kucera 1997), and may translate to changes
286 in fecal dimensions. In addition, factors such as pregnancy (Şensoy and Öznurlu 2019) and
287 temperature (Hammond and Wunder 1995) can impact the dimensions of the gastrointestinal
288 tract. Cold temperatures caused voles to increase food intake, which could cause downstream
289 effects on fecal production (Song and Wang 2006). Furthermore, dietary strategies across
290 mammalian phylogeny can evolve and transition, with herbivory most commonly transitioning to
291 omnivory (Price et al. 2012). Moreover, the rapid evolution of dietary strategies often are
292 associated with evolutionary changes in morphology (Herrel et al. 2008). These physiological
293 and evolved adaptations also might change the size and total amount of feces produced, and so
294 may need to be considered in studies that rely on fecal size analyses.

295 This is not to say that the use of fecal pellet dimensions is invalid for scientific studies.
296 Fecal pellet analyses offer interesting and validated opportunities, such as understanding the
297 paleoecological evolution of body size (Smith et al. 1995) or the ability to ascertain demographic
298 information of mammalian populations (MacCracken and Ballenberge 1987). Our consistent
299 findings across rodent species with distinct feeding ecologies suggest that diet and large

intestinal morphology do have the potential to alter fecal dimensions to the extent that body size could be significantly over or under estimated depending on the directionality of the dietary shift. Studies using fecal dimensions to garner information about animals therefore should interpret their data with caution in light of the potentially confounding effects of diet.

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SUPPLEMENTARY DATA

Supplementary Data SD1.—Composition of experimental diets (g/kg)

Supplementary Data SD2.—Models compared using structural equation modeling (SEM). Fit statistics for each model and each species are presented in the table.

Supplementary Data SD3.—Data and statistics comparing fecal pellet lengths across diet treatments for three species of rodents.

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FIGURE LEGENDS

Fig. 1.—Effects of diet and body size on fecal pellet widths produced by three rodent species. Each point represents an individual animal. Lines depict linear regressions, which were conducted independently for each group. $N = 8 – 10$ animals per group. Results from statistical analyses can be found in Table 2.

Fig. 2.—Diet directly and indirectly alters fecal pellet widths. All three rodent species independently exhibited the same structural equation model (SEM) as being best supported based on Akaike information criterion, R^2, RMSEA and SRMR. See Table 2 for statistics. Solid lines depict relationships that are positive, while dashed lines depict relationships that are negative. Both solid and dashed lines are shown between Protein and LI Radius because this relationship is positive in montane voles, but negative in white-footed mice and grasshopper mice (see Table 3).

448 **Table 1.—**Information about rodent species used in the study.

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Species	Total length	Mean body mass	Distribution and habitat	Diet	References
Onychomys torridus	120-163 mm; tail usually more than half of body length	25 g	Hot, low, arid, scrub vegetation of in the Lower Sonoran Desert	Feed almost exclusively on animal material, primarily arthropods (scorpions, othopterans).	(McCarty 1975; Stapp 1999)
Peromyscus leucopus	130-205 mm; tail usually 45-100 mm	Range from 22-25 g.	Warm, dry forests and brushlands throughout most of the eastern United States	43% seeds 30% insects 25% vegetation 2% other	(Lackey et al. 1985; Fleming and Rauscher 1978)
Microtus montanus	140-220 mm; tail usually 24-69 mm	Range from 37.3-85.0 g.	Dry grasslands and agricultural lands in montane and intermontane areas of the western United States	85% leaves and forbs 9% grasses 6% other	(Sera and Early 2003)

Table 2.—Results of analysis of covariance (ANCOVA) of fecal pellet widths of three rodent species based on diet composition and body size.

	Montane vole			White-footed mouse			Grasshopper mouse		
	F	*d.f.*	*P*	*F*	*d.f.*	*P*	*F*	*d.f.*	*P*
Body Length									
Body Length	5.28	1,35	0.028	3.18	1,35	0.083	6.70	1,33	0.014
Protein	2.99	1,35	0.093	2.47	1,35	0.13	5.32	1,33	0.028
Fiber	20.05	1,35	<0.0001	28.82	1,35	<0.0001	82.49	1,33	<0.0001
Protein × Fiber	0.04	1,35	0.84	2.74	1,35	0.11	2.30	1,33	0.14
Body Mass									
Body Mass	4.47	1,35	0.041	4.85	1,35	0.034	6.29	1,33	0.017
Protein	4.16	1,35	0.049	3.03	1,35	0.09	3.90	1,33	0.056
Fiber	21.77	1,35	<0.0001	32.82	1,35	<0.0001	81.67	1,33	<0.0001
Protein × Fiber	0.25	1,35	0.62	4.41	1,35	0.043	2.76	1,33	0.11

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453 **Table 3**.—Statistics and standardized coefficients (Std.all) for diet, large intestine radius, and

454 body mass in determining average fecal pellet widths. Standardized coefficients allow the

455 relative effects of variables to be compared. Models and statistics were determined separately for

456 each rodent species.

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	Montane vole		White-footed mouse		Grasshopper mouse	
Fecal pellet width ~	*P*-value	Std.all	*P*-value	Std.all	*P*-value	Std.all
Fiber	0.016	0.230	<0.001	0.567	<0.001	0.766
Protein	0.196	-0.102	0.035	-0.250	0.080	-0.146
LI radius	<0.001	0.719	0.512	0.099	0.011	0.225
Body mass	0.078	0.135	0.938	0.009	0.004	0.244
LI radius ~						
Fiber	<0.001	0.594	<0.001	0.624	0.018	0.354
Protein	0.086	0.211	0.159	-0.170	0.293	-0.157

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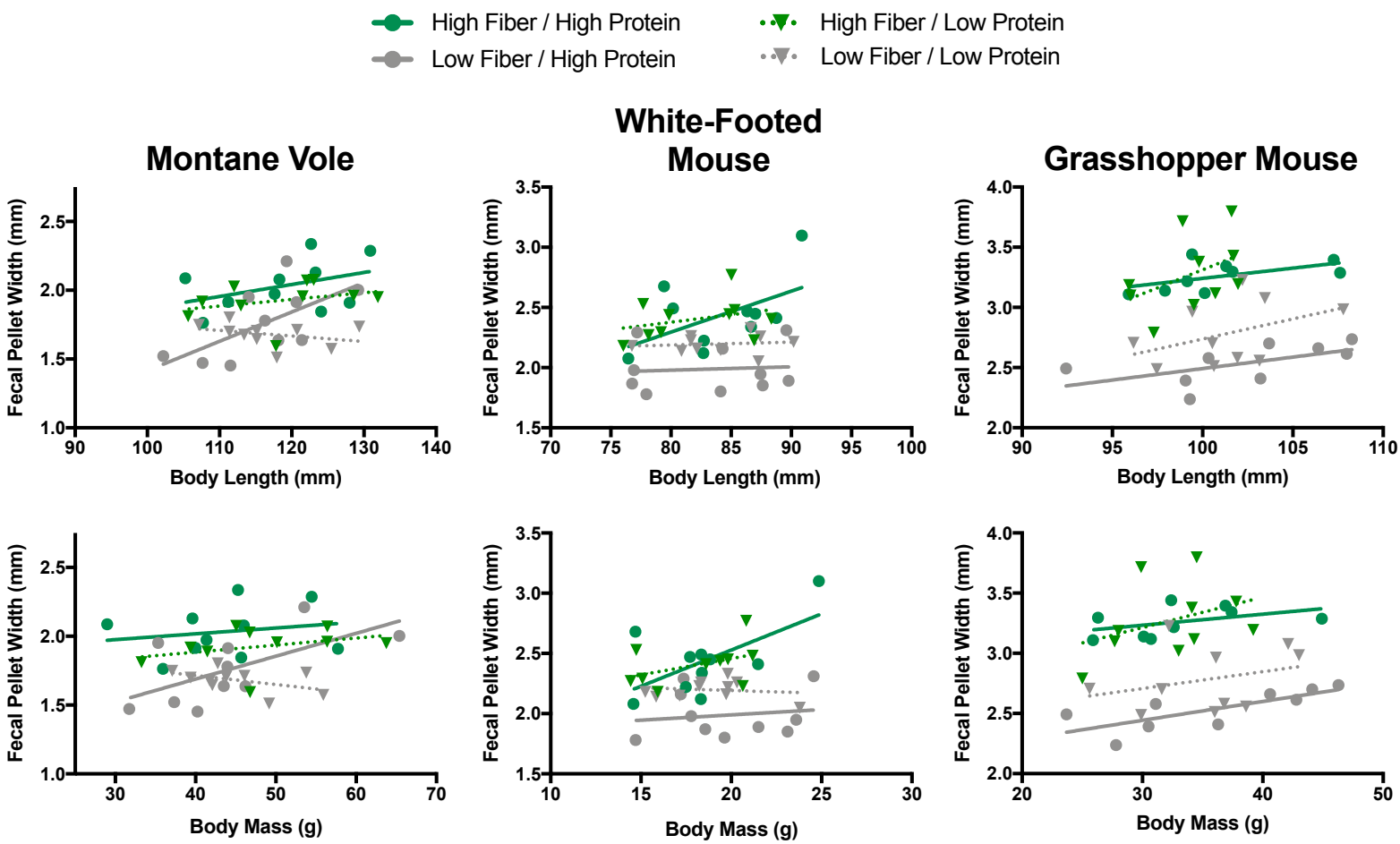
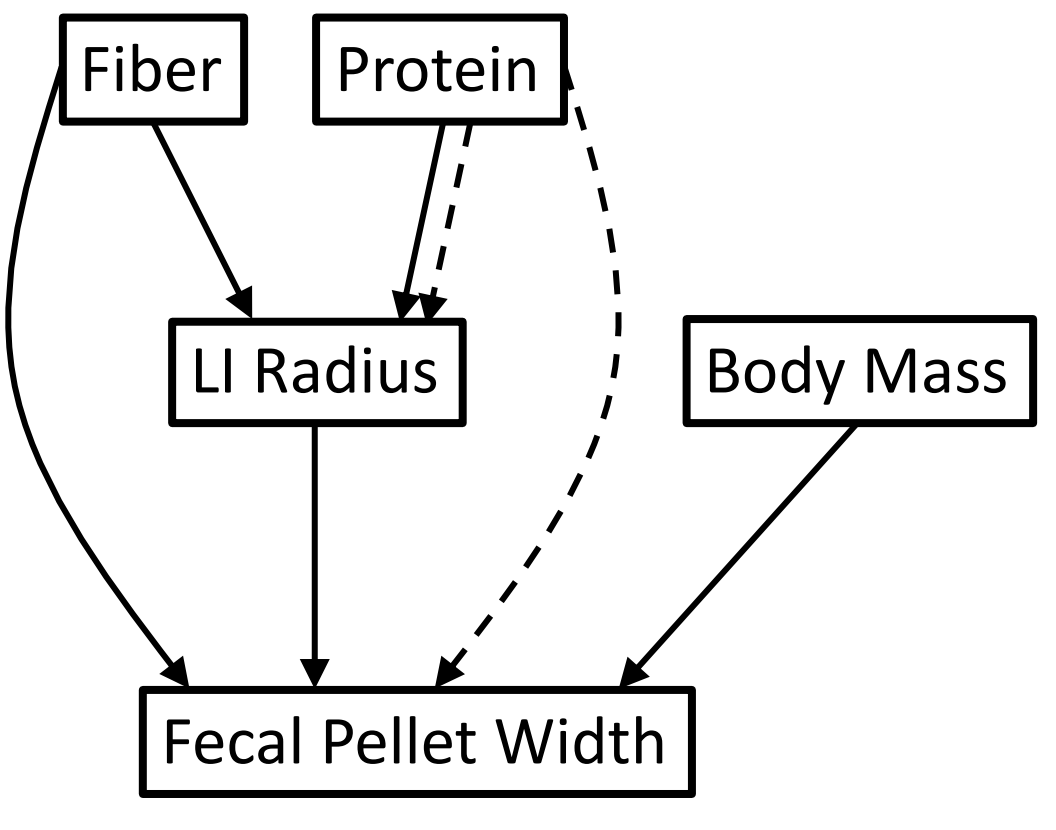

High Fiber / High Protein
Low Fiber / High Protein
High Fiber / Low Protein
Low Fiber / Low Protein
Montane Vole
White-Footed Mouse
Grasshopper Mouse
Fecal Pellet Width (mm)
Body Length (mm)
Body Mass (g)


Fiber
Protein
LI Radius
Body Mass
Fecal Pellet Width

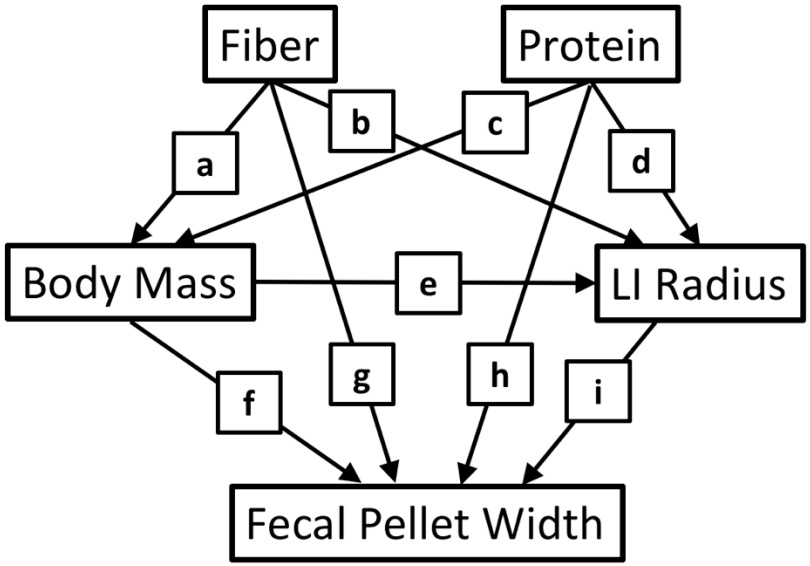
Supplementary Table – Composition of experimental diets (g/kg)

	Low Protein, High Fiber	Low Protein, Low Fiber	High Protein, High Fiber	High Protein, Low Fiber
Alfalfa Meal (17%), dehydrated	100	50	100	50
Casein	0	0	235	185
Corn	0	373.7	0	90
Corn Gluten Meal (60%)	52	90	0	55
Fish Meal	10	10	20	20
Oats	270.1	30	64.6	150
Oat Hulls	230	0	373	0
Wheat	0	370	0	376.8
Wheat Middlings	270	0	137	0
DL-Methionine, FG (99%)	1	1	0	0
L-Lysine HCl, FG (78%)	2	2	0	0
Soybean Oil	21	26	29	29
Vitamin Mix, Teklad (40060)	10	10	10	10
Mineral Mix, w/o Ca & P (98057)	13.4	13.4	13.4	13.4
Calcium Carbonate	13.2	14.4	16	18
Calcium Phosphate, dibasic	7.3	9.5	2	2.8

Supplementary Table – Nutritional content of experimental diets

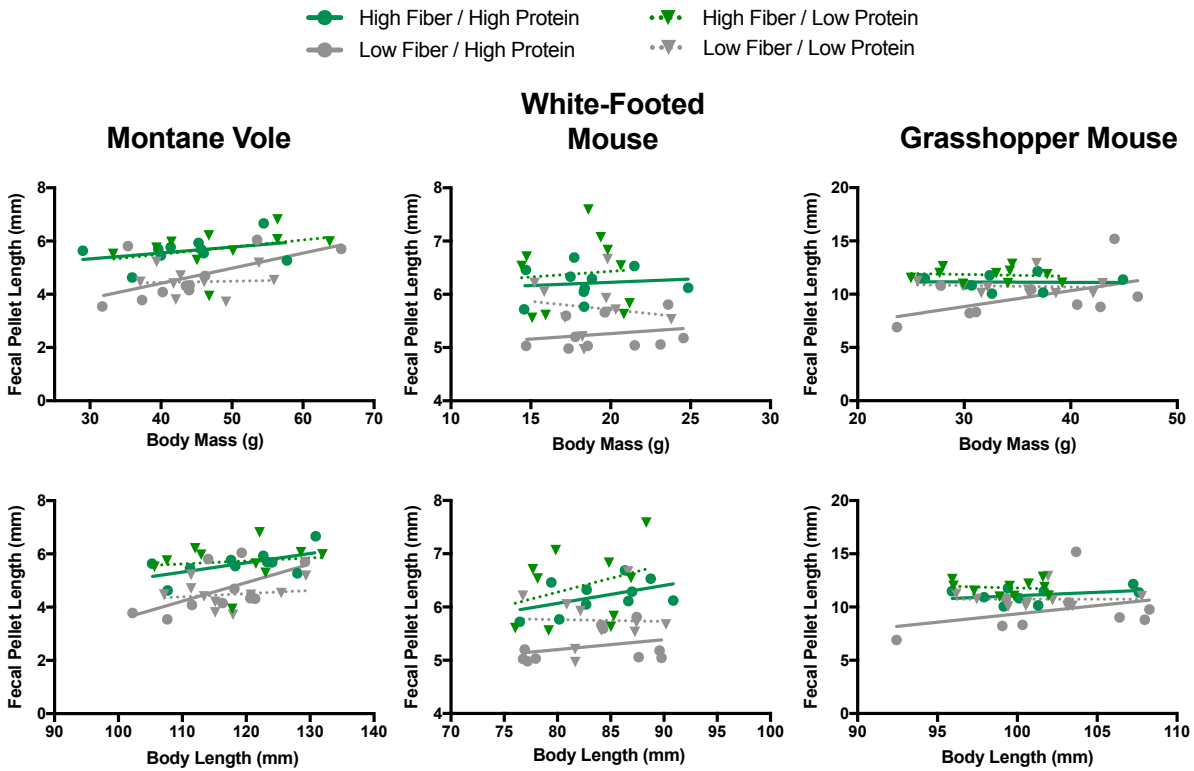
	Low Protein, High Fiber	Low Protein, Low Fiber	High Protein, High Fiber	High Protein, Low Fiber
Protein, g/Kg	140.1948	140.39185	277.2178	277.43864
CHO, g/Kg	264.4677	526.227	124.1082	392.2296
Fat, g/Kg	49.8245	50.28413	49.64	49.7454
Fiber, g/Kg	149.16	32.89575	156.1153	40.6536
NDF, g/Kg	365.0764	120.08963	365.2585	119.50556
Ca, g/Kg	9.98997	9.98924	9.93592	10.01272
Cl, g/Kg	4.05639	2.77124	3.12884	2.43576
K, g/Kg	10.46303	8.22585	9.56928	7.72402
Mg, g/Kg	2.22035	1.67166	1.6296	1.46622
Na, g/Kg	1.86505	1.36489	1.5319	1.41146
P, Avail, g/Kg	3.47656	3.47896	3.46456	3.46774
P, g/Kg	6.05633	5.6391	4.90928	5.10924
B-12, mg/Kg	0.0312	0.0312	0.0327	0.0327
B-6, mg/Kg	21.22605	21.64539	19.7735	20.46774
Biotin, mg/Kg	0.637508	0.55587	0.530188	0.55513
Folic Acid, mg/Kg	2.35264	2.35874	2.25154	2.35222
Niacin, mg/Kg	125.3524	136.3615	112.9096	129.7526
Pantothenate, mg/Kg	67.65978	68.42183	65.09838	67.71478
Riboflavin, mg/Kg	24.21815	23.43496	23.6544	23.37008
Thiamin, mg/Kg	25.00703	19.56229	21.19028	19.84392
Vit A, IU/Kg	19856	19856	19888	19888
Vit D, IU/Kg	2204.5	2204.5	2206.5	2206.5
Vit E, IU/Kg	153.7844	143.9244	142.0556	138.6888
Vit K, mg/Kg	50.591	50.06874	50.5112	50.012
Choline, mg/Kg	2115.184	2074.4582	1759.3898	2045.1088
Inositol, mg/Kg	963.648	1128.9175	314.268	1372.3182
PABA, mg/Kg	110.132	110.132	110.132	110.132
Vit C, mg/Kg	991.189	991.189	991.189	991.189

Supplementary File 2 – Models compared using structural equation modeling (SEM). Fit statistics for each model and each species are presented in the table.



		Montane Vole					White-footed Mouse					Grasshopper Mouse				
Model	Variables	**AIC**	**X^2**	**RMSEA**	**CFI**	**SRMR**	**AIC**	**X^2**	**RMSEA**	**CFI**	**SRMR**	**AIC**	**X^2**	**RMSEA**	**CFI**	**SRMR**
1	a-i	310.975	0	0.000	1	0.000	250.015	0	0.000	1	0.000	196.687	0.0	0.000	1	0.000
2	b,d,g,h,f,i	27.456	4.599*	0.300*	0.955	0.081	52.287	4.227*	0.284*	1	0.061	-51.938	0.050	0.000	1	0.009
3	b,d,e,g,h,f,i	24.857	0.000	0.000	1	0.000	50.060	0	0.000	1	0.000	-49.987	0.0	0.000	1	0.000
4	b,d,i	31.918	7.781*	0.269*	0.922	0.067	60.387	14.095*	0.389 *	0.712	0.116	-16.529	50.427*	0.648*	0.298	0.197
5	b,d,e,f,i	28.824	7.966*	0.273*	0.925	0.052	58.650	12.589*	0.364*	0.761	0.082	-12.938	41.049*	0.717*	0.234	0.165
6	b,g,f,i	27.866	3.013	0.224	0.973	0.084	54.334	3.251	0.237*	0.943	0.069	-51.899	0.079	0.000	1	0.014
7	b,e,g,f,i	26.852	0.000	0.000	1	0.000	53.083	0	0.000	1	0.000	-49.987	0	0	1	0
8	b,i	32.758	5.006*	0.316*	0.943	0.060	60.325	9.883*	0.471*	0.766	0.130	-17.438	31.856*	0.901*	0.303	0.251
9	b,e,f,i	31.248	6.396*	0.367*	0.928	0.050	59.564	8.481*	0.432*	0.809	0.087	-13.988	38.101*	0.988*	0.243	0.200

Supplementary File 3 – Data and statistics comparing fecal pellet lengths across diet treatments for three species of rodents.



	Montane Vole			White-footed Mouse			Grasshopper Mouse		
	F	*d.f.*	*P*	*F*	*d.f.*	*P*	*F*	*d.f.*	*P*
Body Length									
Body Length	5.50	1,35	**0.025**	2.27	1,35	0.14	1.84	1,33	0.18
Protein	0.05	1,35	0.82	5.55	1,35	**0.024**	4.92	1,33	**0.034**
Fiber	25.78	1,35	**<0.0001**	28.85	1,35	**<0.0001**	9.97	1,33	**0.003**
Protein × Fiber	0.56	1,35	0.46	0.62	1,35	0.43	0.11	1,33	0.74
Body Mass									
Body Mass	6.70	1,35	**0.01**	0.10	1,35	0.75	2.19	1,33	0.15
Protein	0.43	1,35	0.52	4.87	1,35	**0.034**	4.35	1,33	**0.045**
Fiber	28.89	1,35	**<0.0001**	26.14	1,35	**<0.0001**	10.37	1,33	**0.003**
Protein × Fiber	0.15	1,35	0.70	1.03	1,35	0.32	0.16	1,33	0.68