



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. Furthermore, 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 5 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). Furthermore, 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; Del 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 indirectly 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 5 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 *O. torridus* were collected from field sites near Animas, Hidalgo Co., New Mexico (31.813436, -108.813772); *P. leucopus* near Murray, Calloway Co., Kentucky (36.686582, -88.221204); and *M. 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

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

Species	Total length	Mean body mass	Distribution and habitat	Diet	References
<i>Onychomys torridus</i>	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)
<i>Peromyscus leucopus</i>	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)
<i>Microtus montanus</i>	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)

(see [Supplementary Data SD1](#)). 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 leftover 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 four to eight 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 calipers that measured to two significant digits. The average length and width were calculated for each individual using the 16 largest (20%) pellets. We then carried out an analysis of covariance (ANCOVA) for each species, with fecal width as the dependent variable, fiber and protein as independent variables, and either body mass (g) or body length (measured as nose-to-anus, in mm) as covariates. We compare least-square mean values of fecal dimensions across treatment groups to evaluate the effect size of dietary treatments. We define statistical significance as $P \leq 0.05$. We undertook similar analyses for fecal length.

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

Finally, we carried out a path analysis, a form of structural equation modeling that allows the identification of potential and existing relationships among measured variables. The

lavaan package (version 0.5–6) in RStudio (version 1.2.5001) was used to estimate and predict relationships between our observed variables ([Rosseel 2012](#)). We developed one full path model that then was compared and examined with numerous a priori proposed models, each unique and missing particular variables (see [Supplementary Data SD2](#)). We ranked models using the Akaike information criterion and used it together with R^2 , root mean square error of approximation, and standardized root mean residual 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 was 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 [Supplementary Data SD3](#).

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 \times 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

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

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 indirectly 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 degu (*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 2 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

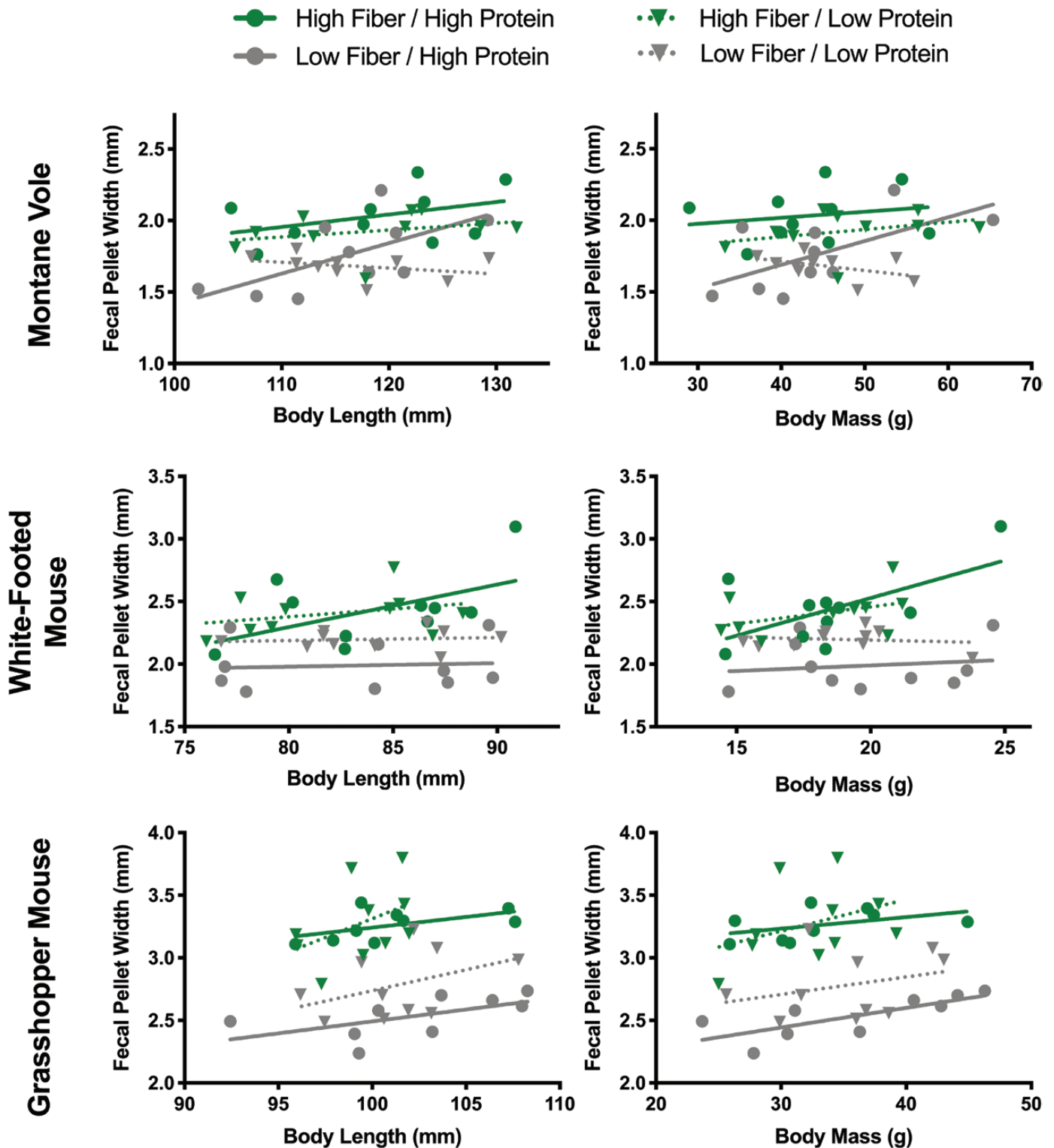


Fig. 1.—Effects of diet and body size on fecal pellet widths produced by three rodent species. Each point represents an individual animal. Lines depict linear regressions, which were conducted independently for each group. $N = 8-10$ animals per group. Results from statistical analyses can be found in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

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

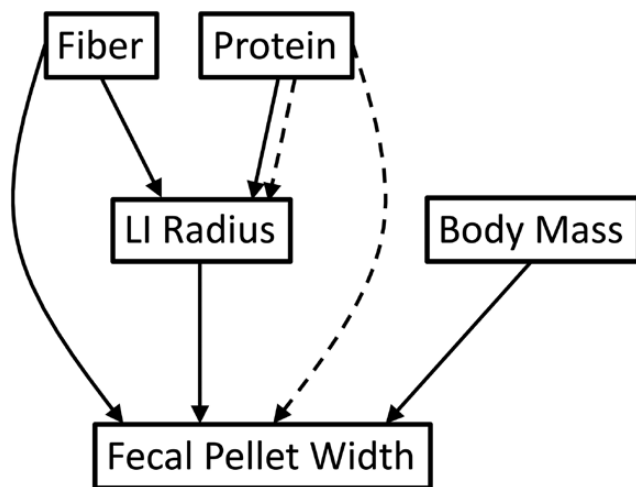


Fig. 2.—Diet directly and indirectly alters fecal pellet widths. All three rodent species independently exhibited the same structural equation model as being best supported based on Akaike information criterion, R^2 , root mean square error of approximation, and standardized root mean residual. See Table 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).

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 \times 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 2 days and did not observe any increases in fecal pellet width (Hallett and Wigand 2001). However, given the results of our structural equation modeling, it appears that fiber may alter fecal pellet width by altering the dimensions of the large intestine, which may take longer than 2 days to respond. Lastly, it should be noted that previous studies have found higher error rates for smaller juvenile animals (woodrats below 80 g; 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

Table 3.—Statistics and standardized coefficients (Std.all) for diet, large intestine radius, and body mass in determining average fecal pellet widths

	Montane vole		White-footed mouse		Grasshopper mouse	
	P-value	Std.all	P-value	Std.all	P-value	Std.all
Fecal pellet width						
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

Standardized coefficients allow the relative effects of variables to be compared. Models and statistics were determined separately for each rodent species.

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

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

This is not to say that the use of fecal pellet dimensions is invalid for scientific studies. Fecal pellet analyses offer interesting and validated opportunities, such as understanding the paleoecological evolution of body size (Smith et al. 1995) or the ability to ascertain demographic information of mammalian populations (MacCracken and Ballenberge 1987). Our consistent 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 MATERIALS

Supplementary data are available at *Journal of Mammalogy* online.

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.

LITERATURE CITED

- BEAUMONT M., ANDRIAMIHAJA M., ARMAND L., GRAUSO M., JAFFRÉZIC F., LALOË D., MOROLDO M., DAVILA A-M., TOMÉ D., BLACHIER F., ET AL. 2017. Epithelial response to a high-protein diet in rat colon. *BMC Genomics*. 18:116.
- BERG N.D., GESE E.M. 2010. Relationship between fecal pellet counts and snowshoe hare density in western Wyoming. *Journal of Wildlife Management*. 74:1745–1751.
- BOZINOVIC F. 1995. Nutritional energetics and digestive responses of an herbivorous rodent (*Octodon degus*) to different levels of dietary fiber. *Journal of Mammalogy*. 76:627–637.
- CAMPBELL K.L., MACARTHUR R.A. 1996. Seasonal changes in gut mass, forage digestibility, and nutrient selection of wild muskrats (*Ondatra zibethicus*). *Physiological Zoology*. 69:1215–1231.
- COLLINS W.B., URNESS P.J. 1981. Habitat preferences of mule deer as rated by pellet-group distributions. *Journal of Wildlife Management*. 45:969–972.
- DELIBES-MATEOS M., ROUCO C., VILLAFUERTE R. 2009. Can adult and juvenile European rabbits be differentiated by their pellet sizes? *Acta Oecologica*. 35:250–252.
- EYZAGUIRRE-VELÁSQUEZ J., OLAVARRÍA-RAMÍREZ L., GONZÁLEZ-ARANCIBIA C., DÍAZ-MERINO C., ARIZ R., LÓPEZ S., QUIROZ W., BELTRÁN C.J., BRAVO J.A., MARCELA JULIO-PIEPER M. 2017. Protein malnutrition during juvenile age increases ileal and colonic permeability in rats. *Journal of Pediatric Gastroenterology and Nutrition*. 64:707–712.
- FLEMING T., RAUSCHER R. 1978. On the evolution of litter size in *Peromyscus leucopus*. *Evolution*. 32:45–55.
- FRANCO C.L.D.M., SANT'ANA D.D.M.G., ARAÚJO E.J.D.A. 2010. Intestinal wall atrophy and increase of sulphomucin secretion in the jejunal epithelium of rats submitted to severe protein malnutrition. *International Journal of Morphology*. 28:497–502.
- GREEN D.A., MILLAR J.S. 1987. Changes in gut dimensions and capacity of *Peromyscus maniculatus* relative to diet quality and energy needs. *Canadian Journal of Zoology*. 65:2159–2162.
- GROSS J.E., WANG Z., WUNDER B.A. 1985. Effects of food quality and energy needs: changes in gut morphology and capacity of *Microtus ochrogaster*. *Journal of Mammalogy*. 66:661–667.
- HALLETT J.C., WIGAND P.E. 2001. The role of dietary fiber in dung size of bushy-tailed woodrats, *Neotoma cinerea*: its potential application to paleoclimatic interpretation. *Western North American Naturalist*. 61:57–63.
- HAMMOND K.A., WUNDER B.A. 1995. Effect of cold temperatures on the morphology of gastrointestinal tracts of two microtine rodents. *Journal of Mammalogy*. 76:232–239.
- HERREL A., HUYGHE K., VANHOYDONCK B., BACKELJAU T., BREUGELMANS K., GRBAC I., VAN DAMME R., IRSCHICK D.J. 2008. Rapid large-scale evolutionary divergence in morphology and performance associated with exploitation of a different dietary resource. *Proceedings of the National Academy of Sciences of the United States of America*. 105:4792–4795.

- JUSTICE K.E., SMITH F.A. 1992. A model of dietary fiber utilization by small mammalian herbivores, with empirical results for *Neotoma*. *The American Naturalist*. 139:398–416.
- KUCERA T.E. 1997. Fecal indicators, diet, and population parameters in mule deer. *Journal of Wildlife Management*. 61:550–560.
- LACKEY J.A., HUCKABY D.G., ORMISTON B.G. 1985. *Peromyscus leucopus*. *Mammalian Species*. 247:1–10.
- MACCRACKEN, J.G., BALLEBERGE V. 1987. Age and sex-related differences in fecal pellet dimensions of moose. *Journal of Wildlife Management*. 51:360–364.
- MCCARTY R. 1975. *Onychomys torridus*. *Mammalian Species*. 59:1–5.
- MU C., YANG Y., LUO Z., GUAN L., ZHU W. 2016. The colonic microbiome and epithelial transcriptome are altered in rats fed a high-protein diet compared with a normal-protein diet. *The Journal of Nutrition*. 146:474–483.
- PEI Y.X., WANG D.H., HUME I.D. 2001a. Effects of dietary fibre on digesta passage, nutrient digestibility, and gastrointestinal tract morphology in the granivorous Mongolian gerbil (*Meriones unguiculatus*). *Physiological and Biochemical Zoology: Ecological and Evolutionary Approaches*. 74:742–749.
- PEI Y.X., WANG D.H., HUME I.D. 2001b. Selective digesta retention and coprophagy in Brandt's vole (*Microtus brandti*). *Journal of Comparative Physiology, B: Biochemical, Systemic, and Environmental Physiology*. 171:457–464.
- POST D.M. 1993. Detection of differences in nutrient concentrations by eastern woodrats (*Neotoma floridana*). *Journal of Mammalogy*. 74:493–497.
- PRICE S.A., HOPKINS S.S., SMITH K.K., ROTH V.L. 2012. Tempo of trophic evolution and its impact on mammalian diversification. *Proceedings of the National Academy of Sciences of the United States of America*. 109:7008–7012.
- REILLY J. 2002. Growth in the Sumatran elephant (*Elephas maximus sumatranus*) and age estimation based on dung diameter. *Journal of Zoology*. 258:205–213.
- ROSSEEL Y. 2012. lavaan: an R package for structural equation modeling. *Journal of Statistical Software*. 48:1–36.
- ROUCO C., STARKLOFF A., DELIBES-MATEOS M., SCHUBERT M., RÖDEL H.G. 2012. Differentiation of animals from different age classes by means of pellet size: assessment of a field method in European rabbits. *Mammalian Biology*. 77:451–454.
- SABAT P., BOZINOVIC F. 2000. Digestive plasticity and the cost of acclimation to dietary chemistry in the omnivorous leaf-eared mouse, *Phyllotis darwini*. *Journal of Comparative Physiology B: Biochemical, Systemic, and Environmental Physiology*. 170:411–417.
- SANCHEZ-ROJAS G., GALLINA S., EQUIHUA M. 2004. Pellet morphometry as a tool to distinguish age and sex in the mule deer. *Zoo Biology*. 23:139–146.
- ŞENSOY E., ÖZNURLU Y. 2019. Determination of the changes on the small intestine of pregnant mice by histological, enzyme histochemical, and immunohistochemical methods. *Turkish Journal of Gastroenterology*. 30:917–924.
- SERA W.E., EARLY C.N. 2003. *Microtus montanus*. *Mammalian Species*. 716:1–10.
- SIKES R.S.; ANIMAL CARE AND USE COMMITTEE OF THE AMERICAN SOCIETY OF MAMMALOGISTS. 2016. 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy*. 97:663–688.
- SMITH, F.A., BETANCOURT J.L., BROWN J.H. 1995. Evolution of body size in the woodrat over the past 25,000 years of climate change. *Science*. 270:2012–2014.
- SONG, Z.G., WANG D.H. 2006. Basal metabolic rate and organ size in Brandt's voles (*Lasiopodomys brandtii*): Effects of photoperiod, temperature and diet quality. *Physiology and Behavior*. 89:704–710.
- SOUTHGATE R. 2005. Age classes of the greater bilby (*Macrotis lagotis*) based on track and faecal pellet size. *Wildlife Research*. 32:625–630.
- STAPP P. 1999. Size and habitat characteristics of home ranges of northern grasshopper mice (*Onychomys leucogaster*). *The Southwestern Naturalist*. 44:101–105.
- STARK A., NYSKA A., MADAR Z. 1996. Metabolic and morphometric changes in small and large intestine in rats fed high-fiber diets. *Toxicologic Pathology*. 24:166–171.
- DEL VALLE J.C., BUSCH C., LÓPEZ MAÑANES A.A. 2006. Phenotypic plasticity in response to low quality diet in the South American omnivorous rodent *Akodon azarae* (Rodentia: Sigmodontinae). *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*. 145:397–405.
- VELOSO C., BOZINOVIC F. 1993. Dietary and digestive constraints on basal energy metabolism in a small herbivorous rodent. *Ecology*. 74:2003–2010.
- WANG L., CAVIEDES-VIDAL E., KARASOV W.H. 2019. Diet composition modulates intestinal hydrolytic enzymes in white-footed mice (*Peromyscus leucopus*). *Journal of Mammalogy*. 5:1512–1521.
- WANG B., WAN-LONG Z., LIAN X., WANG Z.K. 2009. Seasonal variations of the digestive tract morphology in *Apodemus chevrieri* distributed in Hengduan mountains region. *Acta Ecologica Sinica*. 29:1719–1724.
- WOODRUFF, S.P., JOHNSON T.R., WAITS L.P. 2016. Examining the use of fecal pellet morphometry to differentiate age classes in *Sonoran pronghorn*. *Wildlife Biology*. 22:217–227, 211.
- YANG P.J., LEE A.B., CHAN M., KOWALSKI M., QIU K., WAID C., CERVANTES G., MAGONDU B., BIAGIONI M., VOGELNEST L., ET AL. 2021. Intestines of non-uniform stiffness mold the corners of wombat feces. *Soft Matter*. 17:475–488.
- YANG, S.D., LIOU C.H., LIU F.G. 2002. Effects of dietary protein level on growth performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus bidyanus*). *Aquaculture*. 213:363–372.

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

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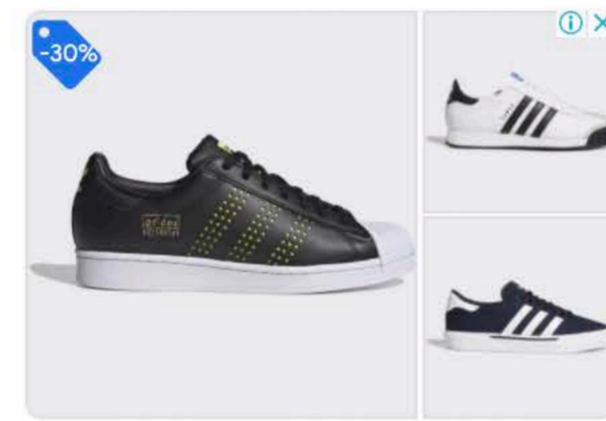
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Abstract

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. Furthermore, 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 5 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.

Keywords: fecal dimensions, fiber, paleoecology, noninvasive measures

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Diet alters rodent fecal pellet size

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

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15 Key words: Fecal Dimensions, Fiber, Paleoecology, Noninvasive Measures

16 Measurements of fecal pellet size can provide important information about wild

17 mammals such as body size and demographic information. Previous studies have not rigorously

18 tested whether diet can confound these measurements. Further, it is unknown whether diet might

19 alter fecal dimensions directly or through changes in animal physiology. Here, we studied three

20 closely related rodent species that differ in natural feeding strategies. Individuals were fed diets

21 that varied in protein and fiber content for five weeks. We then measured body size, fecal widths

22 and lengths, and the radius of the large intestine. Diet composition significantly changed fecal

23 widths in all species. High fiber content significantly increased fecal widths and would cause

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24 overestimations of body size if applied to wild feces. Using path analysis, we found that fiber can
25 increase fecal widths both directly and indirectly through increasing the large intestine radius.
26 Protein affected each species differently, suggesting that protein effects vary by species feeding
27 strategy and existing physiology. Overall, diet and large intestine morphology can alter fecal
28 pellet measurements. Therefore, studies using fecal measurements must consider these effects in
29 their conclusions.

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For Review Only

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

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

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70 Several mechanisms could yield diet-related changes in fecal size. First, fiber material is
71 difficult to digest, and thus a large portion of ingested fiber ends up being defecated by the
72 animal undigested, resulting in increased fecal output (Bozinovic 1995). Many species prioritize
73 protein in their diets, so protein levels can dictate total food intake (Post 1993) and possibly total
74 fecal production. Additionally, it may make intuitive sense that the size of fecal pellets could be
75 partially dictated by the size of digestive organs, such as the large intestine. The gut is a highly
76 dynamic organ, and various gut regions can physiologically respond to dietary variation by
77 changing in size and dimension. For example, rodent guts can increase in size and mass to
78 accommodate high fiber diets (Gross et al. 1985; Green & Millar 1987; Valle et al. 2006). High
79 protein diets could also affect animal physiology. While previous studies in rodents have not
80 demonstrated changes in intestinal morphology as a result of dietary protein (Sabat & Bozinovic
81 2000; Wang et al. 2019), low protein has been shown to increase gut length in fish (Yang et al.
82 2002). Overall, it is possible that diet composition could affect the dimensions of fecal pellets
83 both directly and through the effects on gut morphology.

84 In this study, we tested whether diet composition alters the dimensions of fecal pellets.
85 We focused on three rodent species with different natural feeding strategies: herbivorous
86 montane voles (*Microtus montanus*), omnivorous white-footed mice (*Peromyscus leucopus*), and
87 insectivorous southern grasshopper mice (*Onychomys torridus*). See Table 1 for information
88 about each species, including natural diet composition and habitat. Individuals of each species
89 were fed diets varying in protein and fiber content for five weeks. At the end of the trial, we
90 measured body size and fecal pellet width and length for each individual. Animals were then
91 dissected, and we measured the dimensions of the large intestine. We hypothesized that dietary
92 composition would alter the relationship between body mass and fecal pellet width, perhaps

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93 through altering the radius of the large intestine. We use structural equation modeling to
94 understand the relationships between these variables and compare the relative effects of diet,
95 large intestine morphology, and body size in determining fecal pellet widths.

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97

MATERIALS AND METHODS

98 Wild *Onychomys torridus* were collected from field sites near Animas, Hidalgo Co., NM
99 (31.813436, -108.813772), *Peromyscus leucopus* near Murray, Calloway Co., KY (36.686582, -
100 88.221204), and *Microtus montanus* near Timpie Springs Waterfowl Management Area, Dugway,
101 Tooele Co., UT (40.753708, -112.639903). Forty individuals of each species were collected using
102 baited Sherman live traps under the following state permits: *O. torridus* (New Mexico Department
103 of Game and Fish, #3562), *P. leucopus* (KY Dept. of Fish and Wildlife, SC1911097), and *M.*
104 *montanus* (UT Division of Wildlife Resources, 1COLL5194-2). Animals were singly housed in
105 captivity and randomly assigned to one of four possible isocaloric diet treatments that varied in
106 protein and carbohydrate content (see supplemental data). Animals were maintained on
107 experimental diets for a period of 5 weeks prior to dissections under Institutional Animal Care and
108 Use Committee (IACUC) protocols registered at Northern Arizona University (#15-014 and #16-
109 001 to B. Pasch), Murray State University (2018-026 to T. Derting), and the University of Utah
110 (16-02011 to M.D. Dearing). All research protocols followed the American Society for
111 Mammalogists guidelines (Sikes et al. 2016). We used ground diets to prevent animals from
112 sorting and selective foraging. However, we did not analyze left over food and therefore cannot
113 fully exclude the fact that animals may have still been able to do some selective foraging, and thus
114 may have consumed slightly different diets than what were offered (Justice & Smith 1992).

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

121 During the feeding trial, cages and bedding were changed weekly, and so the fecal pellets
122 present at the end of the experiment were excreted during the last week of the trial. Fecal pellets
123 were collected and dried overnight at 40 °C. We randomly chose 80 fecal pellets to be measured
124 per individual. The length and width of the fecal pellets were determined using electronic
125 calipers that measured to the hundredths place. The average length and width were calculated for
126 each individual using the 16 largest (20%) pellets. We then conducted analysis of covariance
127 (ANCOVA) for each species, with fecal width as the dependent variable, fiber and protein as
128 independent variables, and either body mass (g) or body length (measured nose-to-anus; mm) as
129 covariates. We compare least-square mean values of fecal dimensions across treatment groups to
130 evaluate the effect size of dietary treatments. We define statistical significance as $P \leq 0.05$. We
131 conducted similar analyses for fecal length.

132 Next, we predicted the error that dietary fiber could introduce to estimates of body size.
133 We used the regression lines between body size and fecal width for these purposes, as this
134 measurement is most widely used in the literature. First, we calculated average body mass for a
135 given species and used the regression line to calculate the fecal width measurement for animals if
136 they were feeding on the high protein / high fiber diet as expected for a typical diet in nature. .
137 This fecal width measurement was then used to solve for “Body Mass” using the regression line

138 determined for the high protein / low fiber diet group as expected for a laboratory-based diet.
139 The difference in predicted body mass was then calculated for each species by comparing these
140 two regression lines.

141 Finally, we conducted path analysis, one form of structural equation modeling (SEM) that
142 allows the identification of potential and existing relationships among measured variables. The
143 lavaan package (version 0.5-6) in RStudio (version 1.2.5001) was used to estimate and predict
144 relationships between our observed variables (Rosseel 2012). We developed one full path model
145 that was then compared and examined with numerous *a priori* proposed models, each unique and
146 missing particular variables (see Supplementary Data S2). We ranked the Akaike Information
147 Criterion (AIC) and used it together with R^2 , root mean square error of approximation (RMSEA),
148 and standardized root mean residual (SRMR) values to determine the strongest model supported
149 by our data. We used standardized coefficients, which standardize the variation of each variable
150 to equal 1, to then compare the relative effects of each variable (body size, diet, etc.)

151

152 RESULTS

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

159 First, we tested whether fecal dimensions (pellet length and width) correlated with
160 aspects of body size (body mass and body length). Measurements of fecal pellet width increased

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161 with increasing body length, though this relationship was not statistically significant for all
162 species (Table 2, Fig. 1). In all species, fecal pellet width increased significantly with increasing
163 body mass (Table 2, Fig. 1). However, fecal pellet length was not as informative for aspects of
164 animal body mass and length. In montane voles, fecal pellet length was significantly correlated
165 with body length ($P = 0.025$) and body mass ($P = 0.01$), but there were no significant correlations
166 of fecal pellet length and body size in white-footed mice or grasshopper mice. These results are
167 consistent with previous studies that report fecal pellet width as a better predictor of body size
168 than fecal pellet length (Smith et al. 1995). Therefore, we focus on fecal pellet width data in the
169 main text, and data regarding fecal pellet length can be found in the Supplementary Data SD1.

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

180 Next, we calculated examples of errors that dietary fiber might introduce into predicting
181 animal body size. For example, in montane voles, the average experimental body mass (45.18 g)
182 would be predicted to produce feces with a width of 2.039 mm when on the high protein / high
183 fiber diet. However, if researchers used the regression line developed for the high protein / low

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184 fiber diet, animals producing feces with a width of 2.039 mm would be predicted to have a body
185 mass of 61.06 g, or 1.35× larger. When this same method was applied to white-footed mice and
186 grasshopper mice, the predicted body masses were 3.88× and 2.43× larger, respectively. Using
187 similar methods, we estimate that if researchers were to use regression equations based on
188 animals feeding on low fiber diets, but the actual feces were collected from animals feeding on
189 high fiber diets, the body length of animals would be overestimated by factors of 1.09, 2.78, and
190 1.39 for montane voles, white-footed mice, and grasshopper mice, respectively.

191 Last, while it might seem intuitive that the size of fecal pellets could be partially dictated
192 by the size of digestive organs, such as the large intestine radius, this has never been
193 demonstrated. Using path analysis, we found a consistent best-supported model across all three
194 rodent species (See Supplementary Data SD2 for results of all models compared). In the best-
195 supported model, factors of diet composition (high/low fiber and protein combinations) were
196 included as exogenous binary variables, large intestine radius was a mediator variable, and body
197 mass was a secondary exogenous variable. Body mass, the variable largely estimated using fecal
198 pellet widths in paleoecology studies, exhibited a statistically significant relationship with fecal
199 width in the grasshopper mouse, but not the other rodent species (Figure 2, Table 3). Rather, we
200 found that fiber had the largest standardized effects in driving fecal width across all species.
201 Fiber intake can directly lead to changes in fecal pellet width and at the same time, it can
202 indirectly affect fecal pellet width through altering the radius of the large intestine (Figure 2,
203 Table 3). The large intestine radius showed strong and significant associations with fecal pellet
204 widths in herbivorous montane voles and insectivorous grasshopper mice, though this
205 relationship was not statistically significant in omnivorous white-footed mice (Table 3). Diets
206 with high protein content generally resulted in smaller fecal pellet widths, though this result was

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207 only statistically significant in the white-footed mouse (Table 3). Overall, results from our path
208 analysis suggest that diet composition can alter animals' fecal pellet width both directly and
209 through increases in large intestine radius, and that these effects are stronger than the effect of
210 body mass.

211

212

DISCUSSION

213 Here, we tested whether dietary variation influences the size of rodent fecal pellets, as such
214 effects could have numerous implications for studies on wild mammals. Overall, we found that
215 fecal sizes predicted animal body size, as both body lengths and masses were typically positively
216 and significantly correlated with fecal width. However, diet also had a significant impact on
217 these relationships. We found that high fiber diets yielded significantly wider feces across all
218 three rodent species studied. High protein diets affected each species differently, such that they
219 increased fecal widths of montane voles, but decreased fecal widths in grasshopper mice.
220 Results from structural equation modeling reveal that diet strongly affects fecal width, both
221 directly and indirectly through modifications to morphology of the large intestine. It should be
222 noted that in the wild *O. torridus* are unlikely to eat the high amounts of fiber used in our
223 experimental diets, as they primarily consume animal material. However, *P. leucopus* and *M.*
224 *montanus* are more likely to encounter high amounts of fiber in their natural diets of seeds and
225 vegetation, so the experimental diets are more ecologically relevant for them (Table 1).
226 Nonetheless, our results remained quite consistent across all species. Below, we discuss potential
227 mechanisms of these changes and the implications our results have for studies that estimate
228 mammalian body size for paleoecological or demographic studies.

229 Dietary fiber had the largest impact on fecal dimensions. Fiber may directly increase
230 fecal matter size by changing diet digestibility and the total amount of feces produced. For

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231 example, herbivorous common degus (*Octodon degus*) fed high fiber diets for 27 weeks
232 produced significantly more feces and had lower apparent digestibility of dry matter and protein
233 (Veloso & Bozinovic 1993). Likewise, gerbils fed high fiber diets for two weeks had lower
234 apparent digestibility of dry matter and fiber (Pei et al. 2001a). Thus, the relationship between
235 diet and fecal width could be explained by decreased digestibility causing increased fecal output
236 (Bozinovic 1995). Dietary fiber can also indirectly alter fecal dimensions by affecting large
237 intestine morphology. In our study, fiber significantly increased large intestine radii of all three
238 rodent species. These results are somewhat consistent with previous research on laboratory rats
239 and wild caught Brandt's voles. While large intestinal radii or circumferences were not
240 measured, rats fed high-fiber pectin diets for 4 weeks exhibited significant lengthening of the
241 small and large intestines, and rats fed high cellulose diets exhibited significant lengthening of
242 the colon (Stark et al. 1996). Likewise, Brandt's voles fed high-fiber diets for 14 days showed
243 significant increases in the total length and mass of the gut, specifically in the cecum, proximal
244 colon, and distal colon (Pei et al. 2001b). While we were unable to track changes in fecal size
245 over time, it would be useful in future work to differentiate the immediate and direct effects of
246 fiber from the indirect effects of large intestine morphology on fecal size, which may develop
247 over time. Nonetheless, the best-supported path analysis from our study suggests that an increase
248 in large intestine radius could lead to an increase in fecal width. Overall, dietary fiber modifies
249 diet digestibility, fecal production, and large intestine morphology, thus resulting in changes in
250 fecal dimensions.

251 The effects of protein on fecal production and gut morphology were not as strong or as
252 consistent as the effects of fiber. Our data suggest that protein can also change fecal widths both
253 directly and indirectly by altering the radius of the large intestine. However, rodent species

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254 responded to protein levels differently, such that high protein increased fecal widths of montane
255 voles, decreased fecal widths of grasshopper mice, and there was a significant protein x fiber
256 interaction in white-footed mice. Therefore, the effects of protein may vary by species-specific
257 feeding strategy and existing physiology. Low protein diets have been shown to cause
258 histomorphological changes in the intestines of lab rats, such as shortened colonic crypts and
259 wall atrophy of the jejunum (Franco et al. 2010; Eyzaguirre-Velásquez et al. 2017). Furthermore,
260 high protein levels affect large intestinal gene expression of rats (Mu et al. 2016; Beaumont et al.
261 2017). Thus, it is reasonable that a high protein diet would also induce changes in gut
262 morphology, with resultant changes in fecal dimensions. Notably, lipid content was held constant
263 in our experimental diets, so we could not test the potential effects of dietary lipids on fecal
264 dimensions. However, recent work demonstrated that white-footed mice fed a high lipid diet did
265 not exhibit significant changes in body mass or small intestine mass and length (Wang et al.
266 2019). Future studies could investigate whether dietary lipids alter the dimensions of fecal
267 pellets.

268 Our results counter previous studies that conclude minimal impacts of diet on fecal pellet
269 widths. One study concluded that diet did not impact fecal width by collecting feces from several
270 woodrat species (*Neotoma* spp.) from a variety of habitats across seasons and comparing
271 predicted body masses to actual body masses. This technique revealed a prediction error of 21%,
272 and concluded that diet did not influence the roughly 20-50% changes in body mass estimated to
273 occur between the last glacial maximum and the mid-Holocene (Smith et al. 1995). Another
274 experiment fed woodrats high fiber diets for a period of two days, and did not observe any
275 increases in fecal pellet width (Hallett & Wigand 2001). However, given the results of our SEM,
276 it seems that fiber may alter fecal pellet width through alterations to dimensions of the large

Diet alters rodent fecal pellet size

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277 intestine, which may take longer than two days to respond. Lastly, it should be noted that
278 previous studies have found higher error rates for smaller juvenile animals (woodrats below 80g)
279 (Smith et al. 1995) and the rodent species in our study are all considerably smaller than
280 woodrats. Given the rather consistent results of our path analysis across three species with
281 distinct feeding strategies, we predict that these alterations to fecal dimensions would also occur
282 in larger species, though this remains to be tested. Overall, our study shows that diet, especially
283 fiber content, can alter fecal pellet widths, and perhaps lead to drastic overestimations in body
284 size. It is notable that diet treatments in our study resulted in prediction errors of 35-300%
285 depending on the species, which are larger than prediction errors reported in previous studies
286 (Smith et al. 1995).

287 While our experiments were conducted under controlled laboratory conditions, natural
288 variation in both habitats and animal physiology could affect fecal pellet dimensions. For
289 instance, if diet composition significantly changes by season, fecal pellets could greatly change
290 in size. Seasonal changes in diet and environment can significantly affect gut size and
291 digestibility in muskrats and field mice (Campbell & MacArthur 1996; Wang et al. 2009).
292 Further, measures of fecal nitrogen from deer varied seasonally and annually (Kucera 1997), and
293 may translate to changes in fecal dimensions. Additionally, factors such as pregnancy (Şensoy &
294 Öznurlu 2019) and temperature (Hammond & Wunder 1995) can impact the dimensions of the
295 gastrointestinal tract. Cold temperatures caused voles to increase food intake, which could cause
296 downstream effects on fecal production (Song & Wang 2006). Additionally, across mammalian
297 phylogeny dietary strategies can evolve and transition, with herbivory most commonly
298 transitioning to omnivory (Price et al. 2012). Further, the rapid evolution of dietary strategies are
299 often with associated evolutionary changes in morphology (Herrel et al. 2008). These

Diet alters rodent fecal pellet size

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300 physiological and evolved adaptations might also change the size and total amount of feces
301 produced, and so may need to be considered in studies that rely on fecal analyses.

302 This is not to say that the use of fecal pellet dimensions are invalid for scientific studies.

303 Fecal pellet analyses offer interesting and validated opportunities, such as understanding the
304 paleoecological evolution of body size (Smith et al. 1995) or the ability to ascertain demographic
305 information of mammalian populations (MacCracken & Ballenberge 1987). Our consistent
306 findings across rodent species with distinct feeding ecologies suggest that diet and large
307 intestinal morphology do have the potential to alter fecal dimensions to the extent that body size
308 could be significantly over or under estimated depending on the directionality of the dietary shift.
309 Thus, studies using fecal dimensions to garner information about animals should interpret their
310 data with caution in light of the potentially confounding effects of diet.

311

312

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316 *leucopus*.

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For Review Only

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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.

For Review Only

LITERATURE CITED

- 336
337
338 AGUILAR, S. 2011. *Peromyscus leucopus*. Animal Diversity Web.
339 https://animaldiversity.org/accounts/Peromyscus_leucopus/. Accessed 18 February 2021.
340 BEAUMONT, M., et al. 2017. Epithelial response to a high-protein diet in rat colon. *BMC*
341 *Genomics* 18:116.
342 BERG, N. D. AND E. M. GESE. 2010. Relationship Between Fecal Pellet Counts and Snowshoe
343 Hare Density in Western Wyoming. *The Journal of Wildlife Management* 74:1745-1751.
344 BOZINOVIC, F. 1995. Nutritional Energetics and Digestive Responses of an Herbivorous
345 Rodent (*Octodon degus*) to Different Levels of Dietary Fiber. *Journal of Mammalogy* 76:627-
346 637.
347 CAMPBELL, K. L. AND R. A. MACARTHUR. 1996. Seasonal Changes in Gut Mass, Forage
348 Digestibility, and Nutrient Selection of Wild Muskrats (*Ondatra zibethicus*). *Physiological*
349 *Zoology* 69:1215-1231.
350 COLLINS, W. B. AND P. J. URNESS. 1981. Habitat preferences of mule deer as rated by pellet-
351 group distributions. *The Journal of Wildlife Management* 45:969-972.
352 DELIBES-MATEOS, M., C. ROUCO, AND R. VILLAFUERTE. 2009. Can adult and juvenile European
353 rabbits be differentiated by their pellet sizes? *Acta Oecologica* 35:250-252.
354 EYZAGUIRRE-VELÁSQUEZ, J., et al. 2017. Protein Malnutrition During Juvenile Age Increases
355 Ileal and Colonic Permeability in Rats. *Journal of Pediatric Gastroenterology and Nutrition* 64.
356 FRANCO, C. L. D. M., D. D. M. G. SANT'ANA, AND E. J. D. A. ARAÚJO. 2010. Intestinal Wall
357 Atrophy and Increase of Sulphomucin Secretion in the Jejunal Epithelium of Rats Submitted to
358 Severe Protein Malnutrition. *International Journal of Morphology* 28:497-502.
359 GREEN, D. A. AND J. S. MILLAR. 1987. Changes in gut dimensions and capacity of *Peromyscus*
360 *maniculatus* relative to diet quality and energy needs. *Canadian Journal of Zoology* 65:2159-
361 2162.
362 GROSS, J. E., Z. WANG, AND B. A. WUNDER. 1985. Effects of Food Quality and Energy Needs:
363 Changes in Gut Morphology and Capacity of *Microtus ochrogaster*. *Journal of Mammalogy*
364 66:661-667.
365 HALLETT, J. C. AND P. E. WIGAND. 2001. The role of dietary fiber in dung size of bushy-tailed
366 woodrats, *Neotoma cinerea*: its potential application to paleoclimatic interpretation. *Western*
367 *North American Naturalist* 61:57-63.
368 HAMMOND, K. A. AND B. A. WUNDER. 1995. Effect of Cold Temperatures on the Morphology
369 of Gastrointestinal Tracts of Two Microtine Rodents. *Journal of Mammalogy* 76:232-239.
370 HERREL, A., et al. 2008. Rapid large-scale evolutionary divergence in morphology and
371 performance associated with exploitation of a different dietary resource. *Proceedings of the*
372 *National Academy of Sciences* 105:4792-4795.
373 JUSTICE, K. E. AND F. A. SMITH. 1992. A model of dietary fiber utilization by small
374 mammalian herbivores, with empirical results for *Neotoma*. *The American Naturalist* 139:398-
375 416.
376 KESTER, D. 1999. *Onychomys torridus*. Animal Diversity Web.
377 https://animaldiversity.org/accounts/Onychomys_torridus/. Accessed 18 February 2021.
378 KUCERA, T. E. 1997. Fecal Indicators, Diet, and Population Parameters in Mule Deer. *The*
379 *Journal of Wildlife Management* 61:550-560.

Diet alters rodent fecal pellet size

18

- 380 LACKEY, J. A., D. G. HUCKABY, AND B. G. ORMISTON. 1985. *Peromyscus leucopus*. Mammalian
381 Species:1-10.
- 382 MACCRACKEN, J. G. AND V. BALLEBERGE. 1987. Age and Sex-Related Differences in Fecal
383 Pellet Dimensions of Moose. *The Journal of Wildlife Management* 51:360-364.
- 384 MCCARTY, R. 1975. *Onychomys torridus*. Mammalian Species:1-5.
- 385 MU, C., Y. YANG, Z. LUO, L. GUAN, AND W. ZHU. 2016. The Colonic Microbiome and Epithelial
386 Transcriptome Are Altered in Rats Fed a High-Protein Diet Compared with a Normal-Protein
387 Diet. *The Journal of Nutrition* 146:474-483.
- 388 PEI, Y.-X., D.-H. WANG, AND I. D. HUME. 2001a. Effects of Dietary Fibre on Digesta Passage,
389 Nutrient Digestibility, and Gastrointestinal Tract Morphology in the Granivorous Mongolian
390 Gerbil (*Meriones unguiculatus*). *Physiological and Biochemical Zoology: Ecological
391 and Evolutionary Approaches* 74:742-749.
- 392 PEI, Y. X., D. H. WANG, AND I. D. HUME. 2001b. Selective digesta retention and coprophagy
393 in Brandt's vole (*Microtus brandti*). *Journal of comparative physiology B, Biochemical, systemic,
394 and environmental physiology* 171:457-464.
- 395 POST, D. M. 1993. Detection of Differences in Nutrient Concentrations by Eastern
396 Woodrats (*Neotoma floridana*). *Journal of Mammalogy* 74:493-497.
- 397 PRICE, S. A., S. S. B. HOPKINS, K. K. SMITH, AND V. L. ROTH. 2012. Tempo of trophic evolution
398 and its impact on mammalian diversification. *Proceedings of the National Academy of Sciences*
399 109:7008-7012.
- 400 REILLY, J. 2002. Growth in the Sumatran elephant (*Elephas maximus sumatranus*) and
401 age estimation based on dung diameter. *Journal of Zoology* 258:205-213.
- 402 ROSSEEL, Y. 2012. lavaan: An R Package for Structural Equation Modeling. *Journal of
403 Statistical Software* 48:1-36.
- 404 ROUCO, C., A. STARKLOFF, M. DELIBES-MATEOS, M. SCHUBERT, AND H. G. RÖDEL. 2012.
405 Differentiation of animals from different age classes by means of pellet size: Assessment of a
406 field method in European rabbits. *Mammalian Biology* 77:451-454.
- 407 SABAT, P. AND F. BOZINOVIC. 2000. Digestive plasticity and the cost of acclimation to dietary
408 chemistry in the omnivorous leaf-eared mouse, *Phyllotis darwini*. *Journal of Comparative
409 Physiology B* 170:411-417.
- 410 SANCHEZ-ROJAS, G., S. GALLINA, AND M. EQUIHUA. 2004. Pellet morphometry as a tool to
411 distinguish age and sex in the mule deer. *Zoo Biology* 23:139-146.
- 412 ŞENSOY, E. AND Y. ÖZNURLU. 2019. Determination of the changes on the small intestine of
413 pregnant mice by histological, enzyme histochemical, and immunohistochemical methods. *Turk
414 J Gastroenterol* 30:917-924.
- 415 SERA, W. E. AND C. N. EARLY. 2003. *Microtus montanus*. Mammalian Species:1-10.
- 416 SIKES, R. S., T. A. CARE, AND U. C. O. T. A. S. O. MAMMALOGISTS. 2016. 2016 Guidelines of the
417 American Society of Mammalogists for the use of wild mammals in research and education.
418 *Journal of Mammalogy* 97:663-688.
- 419 SMITH, F. A., J. L. BETANCOURT, AND J. H. BROWN. 1995. Evolution of Body Size in the Woodrat
420 over the Past 25,000 Years of Climate change. *Science* 270.
- 421 SONG, Z. G. AND D. H. WANG. 2006. Basal metabolic rate and organ size in Brandt's voles
422 (*Lasiopodomys brandtii*): Effects of photoperiod, temperature and diet quality. *Physiology &
423 behavior* 89:704-710.

- 424 SOUTHGATE, R. 2005. Age classes of the greater bilby (*Macrotis lagotis*) based on track
425 and faecal pellet size. *Wildlife Research - WILDLIFE RES* 32.
- 426 STARK, A., A. NYSKA, AND Z. MADAR. 1996. Metabolic and Morphometric Changes in Small
427 and Large Intestine in Rats Fed High-Fiber Diets. *Toxicologic Pathology* 24:166-171.
- 428 STIDHAM, C. 2020. *Microtus montanus*. Animal Diversity Web.
429 https://animaldiversity.org/accounts/Microtus_montanus/. Accessed 18 February 2021.
- 430 VALLE, J. C. D., C. BUSCH, AND A. A. L. MAÑANES. 2006. Phenotypic plasticity in response to
431 low quality diet in the South American omnivorous rodent *Akodon azarae* (Rodentia:
432 Sigmodontinae). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative*
433 *Physiology* 145:397-405.
- 434 VELOSO, C. AND F. BOZINOVIC. 1993. Dietary and Digestive Constraints on Basal Energy
435 Metabolism in a Small Herbivorous Rodent. *Ecology* 74:2003-2010.
- 436 WANG, B., Z. WAN-LONG, X. LIAN, AND Z. K. WANG. 2009. Seasonal variations of the digestive
437 tract morphology in *Apodemus chevrieri* distributed in Hengduan mountains region. *Shengtai*
438 *Xuebao/ Acta Ecologica Sinica* 29:1719-1724.
- 439 WANG, L., E. CAVIEDES-VIDAL, AND W. H. KARASOV. 2019. Diet composition modulates
440 intestinal hydrolytic enzymes in white-footed mice (*Peromyscus leucopus*). *Journal of*
441 *Mammalogy*.
- 442 WOODRUFF, S. P., T. R. JOHNSON, AND L. P. WAITS. 2016. Examining the use of fecal pellet
443 morphometry to differentiate age classes in Sonoran pronghorn. *Wildlife Biology* 22:217-227,
444 211.
- 445 YANG, S.-D., C.-H. LIOU, AND F.-G. LIU. 2002. Effects of dietary protein level on growth
446 performance, carcass composition and ammonia excretion in juvenile silver perch (*Bidyanus*
447 *bidyanus*). *Aquaculture* 213:363-372.
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FIGURE LEGENDS

Figure 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.

Figure 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).

Diet alters rodent fecal pellet size

465 Table 1. Information about rodent species used in the study.

466

Species	Body length	Mean body mass	Distribution and habitat	Diet	References
<i>Onychomys torridus</i>	120-163 mm; tail usually more than half of body length	22 g	Hot, low, arid, scrub vegetation of in the Lower Sonoran Desert	Feed almost exclusively on animal material, primarily arthropods (scorpions, othopterans).	(McCarty 1975; Kester 1999)
<i>Peromyscus leucopus</i>	130-205 mm; tail usually 45-100 mm	Range from 15.0-25.0 g. Mean is 23 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; Aguilar 2011)
<i>Microtus montanus</i>	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 & Early 2003; Stidham 2020)

Diet alters rodent fecal pellet size

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

	Montane vole			White-footed mouse			Grasshopper mouse		
	<i>F</i>	<i>d.f.</i>	<i>P</i>	<i>F</i>	<i>d.f.</i>	<i>P</i>	<i>F</i>	<i>d.f.</i>	<i>P</i>
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

Diet alters rodent fecal pellet size

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469
 470 Table 3. Statistics and standardized coefficients (Std.all) for diet, large intestine radius, and body
 471 mass in determining average fecal pellet widths. Standardized coefficients allow the relative
 472 effects of variables to be compared. Models and statistics were determined separately for each
 473 rodent species.

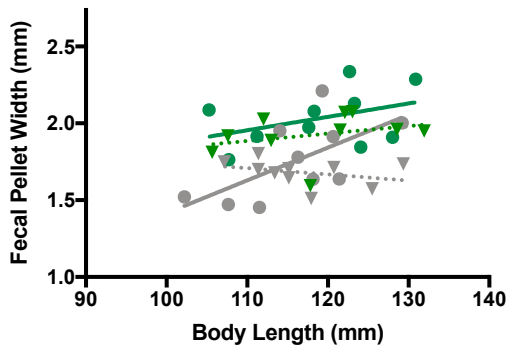
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	Montane vole		White-footed mouse		Grasshopper mouse	
Fecal pellet width ~	P-value	Std.all	P-value	Std.all	P-value	Std.all
Fiber	0.016	0.230	<0.001	0.567	<0.001	0.766
Protein	0.196	-0.102	0.035	-0.250	0.080	-0.146
LI radius	<0.001	0.719	0.512	0.099	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

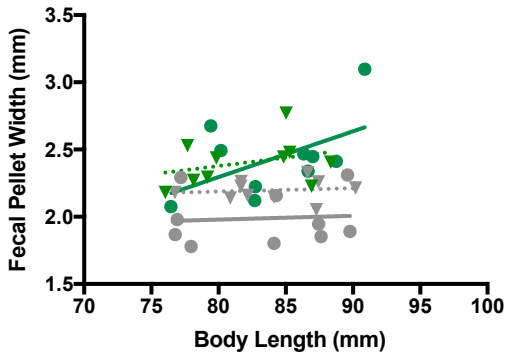
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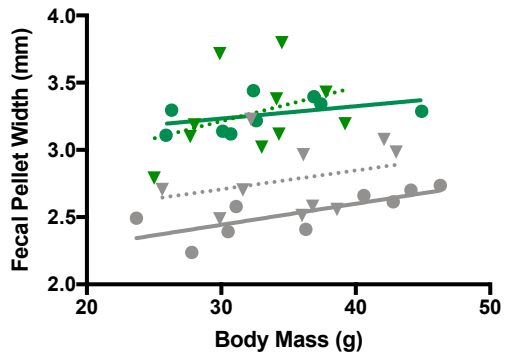
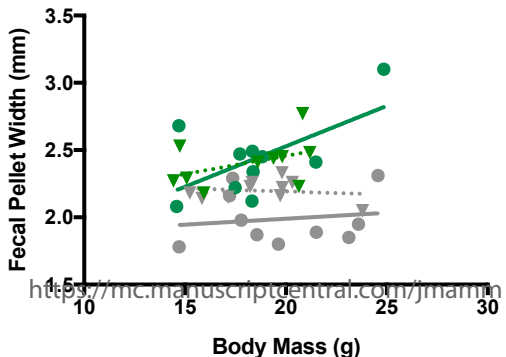
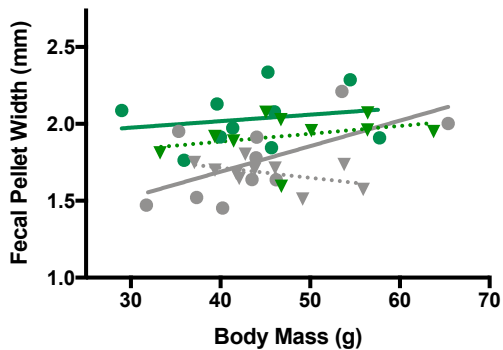
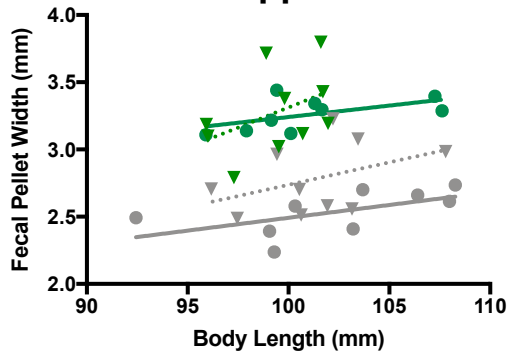
Montane Vole

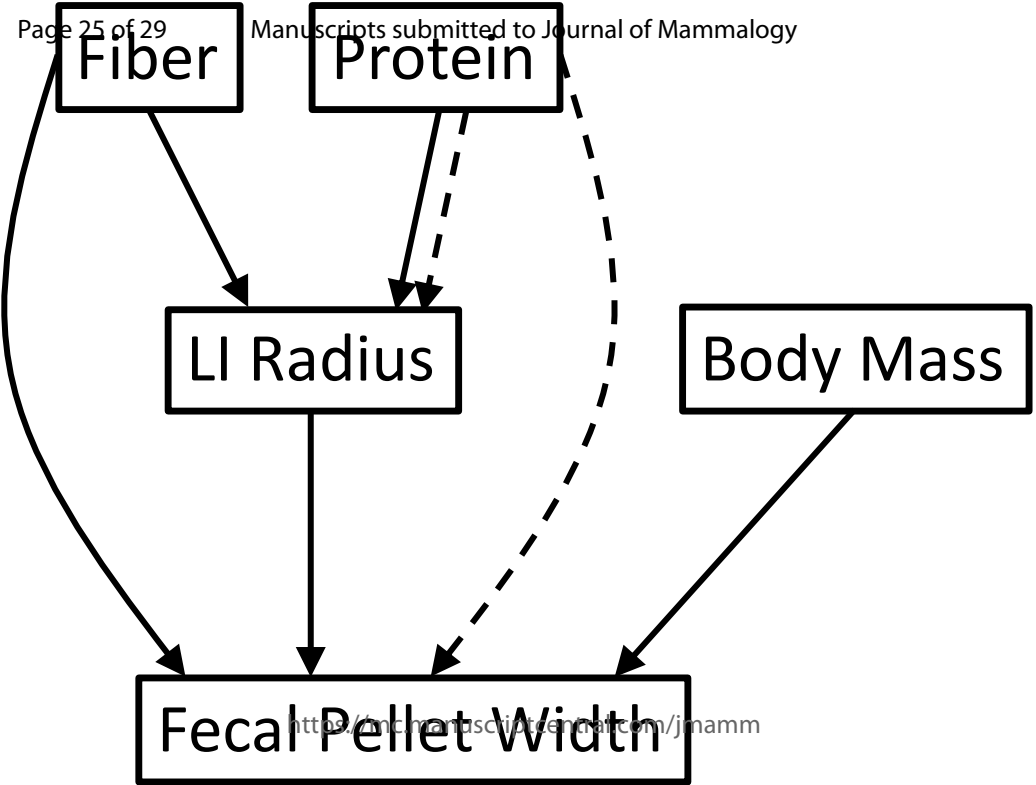


White-Footed Mouse



Grasshopper Mouse





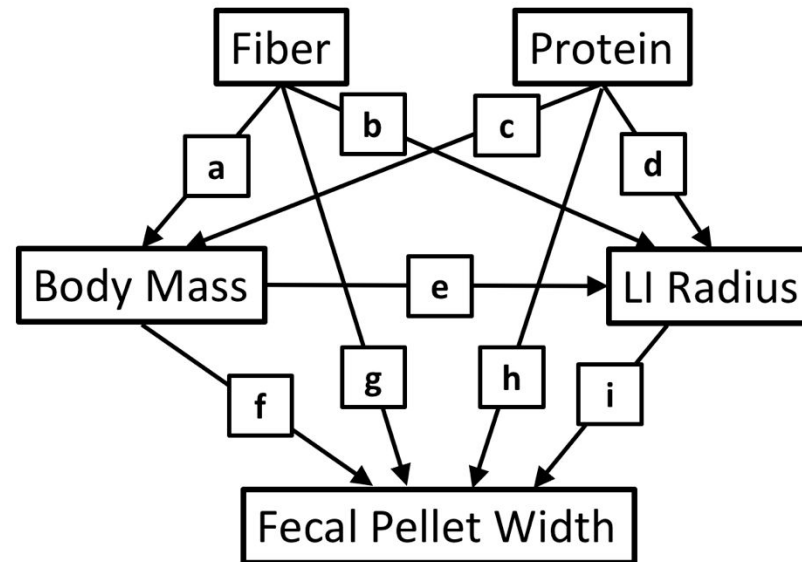
Fecal Pellet Width

Supplementary Data SD1 – 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

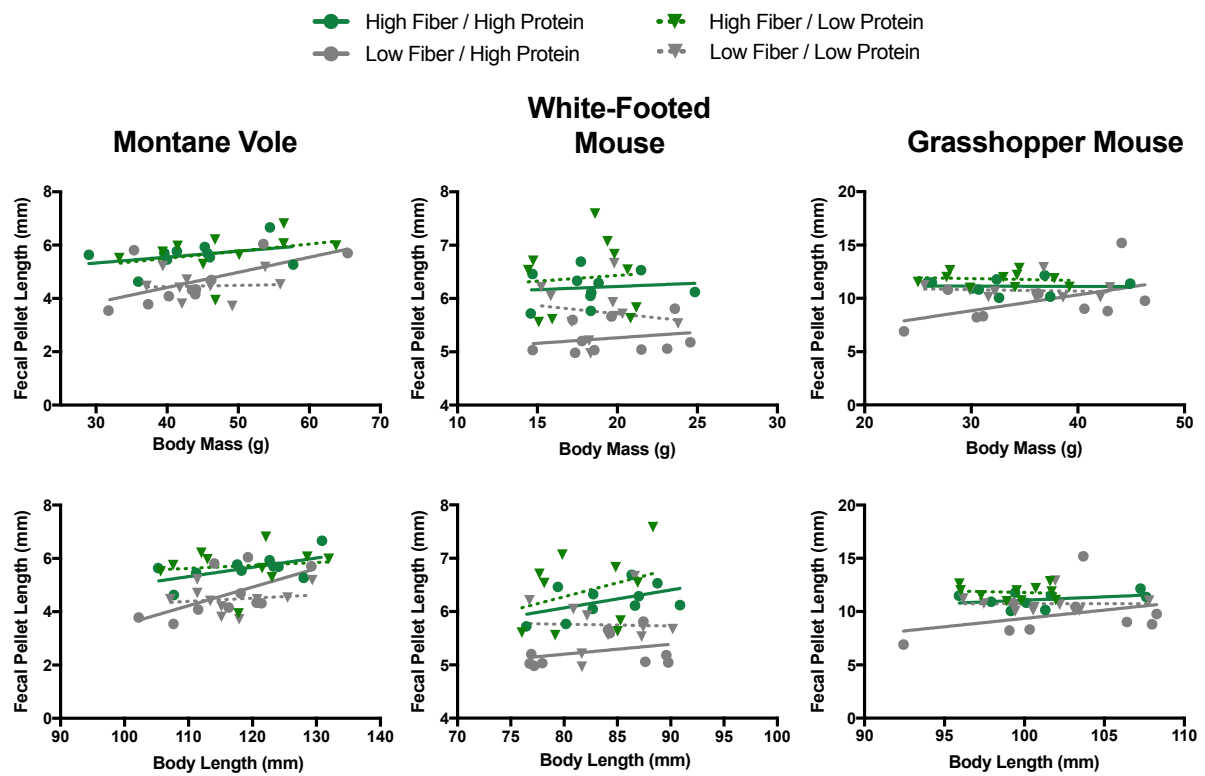
	Low Protein, High Fiber	Low Protein, Low Fiber	High Protein, High Fiber	High Protein, Low Fiber
Protein, g/Kg	140.1948	140.39185	277.2178	277.43864
CHO, g/Kg	264.4677	526.227	124.1082	392.2296
Fat, g/Kg	49.8245	50.28413	49.64	49.7454
Fiber, g/Kg	149.16	32.89575	156.1153	40.6536
NDF, g/Kg	365.0764	120.08963	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 Data SD2 – Models compared using structural equation modeling (SEM). Fit statistics for each model and each species are presented in the table.



Model	Variables	Montane vole					White-footed mouse					Grasshopper mouse				
		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 Data SD3 – Data and statistics comparing fecal pellet lengths across diet treatments for three species of rodents.



	<u>Montane vole</u>			<u>White-footed mouse</u>			<u>Grasshopper mouse</u>		
	<i>F</i>	<i>d.f.</i>	<i>P</i>	<i>F</i>	<i>d.f.</i>	<i>P</i>	<i>F</i>	<i>d.f.</i>	<i>P</i>
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