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Fecal nitrogen as an index of dietary nitrogen in two sika deer *Cervus nippon* populations

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We tested the reliability of fecal nitrogen (FN) to predict dietary nitrogen (DN) in two sika deer *Cervus nippon* Heude, 1884 populations with greatly differing habitats. One was near the village of Nishiokoppe (area A) and the other was on Nakanoshima Island (area B) in Hokkaido, Japan. To estimate FN, we washed feces through a sieve, and diet was identified based on rumen-content analysis. The diet in area A was mostly composed of grass and legumes of agricultural origin, with browse being only a minor component. In contrast, browse such as deciduous foliage was the main component of the diet in area B. Dietary nitrogen was significantly regressed by FN within specific areas. On the other hand, the DN-FN-relationship had a similar slope but significantly different intercepts between areas. DN-FN-relationships differed between diets with and without an agricultural component, irrespective of browse. Thus, the difference in the DN-FN-relationship between areas was explained by differences in dietary composition. We therefore conclude that FN may be useful in predicting DN in diverse dietary situations regardless of the ratio of browse in the diet of free-ranging ungulates, but that dependence on agricultural pastures may hinder the utility of FN as an index of DN.

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Introduction

Determination of diet quality is essential to effectively monitor the nutritional status of wild ruminants. Previous studies have shown that dietary improvement positively influences body mass (Kay 1985), pregnancy rates (Robinson

1996, Cook *et al.* 2001), and viability of newborns (Sæther and Heim 1993, Robinson 1996). However, it is difficult to estimate dietary quality. One way of estimating quality is via a nutritional analysis of rumen contents, and another is by plant collection based on direct observations of feeding behaviour. Collecting rumen contents requires killing the study animal, and

close observation of free-ranging individuals can be difficult (Asada and Ochiai 1999). Therefore, our purpose was to identify an index by which dietary quality can be easily estimated, without sacrificing animals.

Fecal nitrogen (FN) has been regarded as equivalent to dietary nitrogen (DN), which in turn has been regarded as equivalent to crude protein ($DN \times 6.25$), one of the best indices of diet quality (Mubanga *et al.* 1985, Hodgman *et al.* 1996). FN has shown a positive simple linear relationship with DN, for example, when domestic ruminants (Raymond 1948, Holechek *et al.* 1982, Wehausen 1995) and wild ruminants (eg buffalo *Syncerus caffer*, gazelle *Gazella thomsonii*; Arman *et al.* 1975) were fed with grass under controlled conditions.

However, when diets are composed of grass and browse, some studies have questioned the utility of FN. Browse contains phenolics that can bind to dietary protein in the rumen. These complexes are not digestible at rumen pH and are passed out in the feces, thereby elevating FN estimates and artificially inflating estimates of DN (Osborn and Ginnett 2001). Feeding experiments using captive elk *Cervus elaphus* or white-tailed deer *Odocoileus virginianus* have demonstrated that a diet containing phenolics causes FN to overestimate DN (Mould and Robbins 1981, Osborn and Ginnett 2001). Nunez-Hernandez *et al.* (1992) reported significant relationships between FN and DN in cattle *Bos taurus* and mountain goats *Oreamnos americanus* fed grass alone and a combination of forbs and shrubs, respectively; however, FN was not strongly correlated with percent dietary crude protein when all diets were combined.

Despite the suggestions of these experimental studies, the simple linear relationship between FN and DN has been verified in cattle fed both grass and browse in captivity (Holechek *et al.* 1982), as well as in captive black-tailed deer *Odocoileus hemionus columbianus* and mule deer *O. h. hemionus* fed a mixture of grass and browse (Mubanga *et al.* 1985, Hodgman *et al.* 1996) and in North American elk, black-tailed deer, and sika deer *Cervus nippon* Heude, 1884 fed both grass and browse in the field (Leslie and Starkey 1985, 1987, Watanabe and Takatsuki 1993, but

see Hobbs 1987). FN reflected the nutritional status of free-ranging deer on winter ranges, where browsing was common (Hodgman and Bowyer 1986). Therefore, the binding effect of the phenolics may be mitigated to some extent, which makes FN a valid proxy for DN within certain areas.

Most studies, however, have concentrated on analyzing the relationship between FN and DN for a particular area within a species. This approach simply verifies the usefulness of FN only as a local indicator of DN. To generally use FN, we must examine whether the relationship between DN and FN is the same among areas with different diet composition within species. Only three studies have examined the difference in this regression relationship: two for livestock, and the other for wild ruminants in the field. Experimental studies with cattle and sheep showed different regression line slopes between grass and legumes (Wehausen 1995). Regression lines for elk are likely to be different in slope between diets with and without browse, although this was not statistically demonstrated (Leslie and Starkey 1985). Conversely, no difference was found in linear regression equations between grassland and forest areas for cattle (Holechek *et al.* 1982). Therefore, more data are needed on regression lines between DN and FN for free-ranging ruminants that consume grass and browse to investigate the usefulness of FN in areas with different proportions of browse in the diet.

Sika deer in Japan have adapted to a wide range of vegetation, from semitropical habitats to boreal forests. Their dependence on browse is low in northern Japan and high in southern Japan (Takatsuki 1991). Diets of sika deer in Hokkaido, in the northern part of Japan, vary seasonally. Deer consume graminoids and browse in winter, forbs and agricultural crops in spring and summer, and all of these plant foods in autumn (Yokoyama *et al.* 2000). In contrast, in central Japan, graminoids and woody plants are the primary foods throughout the year, and in winter the use of evergreen broad leaves increases (Asada and Ochiai 1996). These findings indicate that sika deer may use different percentages of browse in heterogeneous environments. There-

fore, the sika deer is a suitable species with which to test the reliability of using FN as an indicator of DN in diverse dietary situations.

Our objective was to test the reliability of FN as a predictor of DN under diverse dietary conditions. We hypothesized that FN is a reliable indicator of DN in natural populations. First, we tested the significance of the regression relationship between DN and FN within specific areas. Next, we compared DN-FN relationships between two areas that greatly differed in habitat, with the prediction that the regression lines would not differ. Third, we compared DN-FN-relationships among three distinctive diet categories to explore the effect of dietary differences on the relationships between FN and DN.

Material and methods

Study areas

Study area A, in the village of Nishiokoppe (308 km²), was located in north-eastern Hokkaido (44°12'N, 142°48'E), Japan. Forests cover about 90% of the total area, while corn fields, pastures, farms, and residential areas cover the remaining area (Nishiokoppe Village Office 2002). Forests consist of deciduous broad-leaved trees, including *Betula platyphylla* var. *japonica* and *Quercus mongolica* var. *grosseserrata*, and conifers such as *Abies sachalinensis* and *Picea jezoensis*. The forest floor is covered by forbs or dwarf bamboos (*Sasa senanensis*, *S. kurilensis*). The sika deer population in area A has been increasing for the last 10 years (Hokkaido Institute of Environmental Sciences, unpubl.). Because no deer-proof fences have been erected between the cultivated land and the forest, deer can forage freely on the cultivated areas.

Study area B was on Nakanoshima Island (5 km²), which is located in a caldera lake, Toya, in the Shikotsu

Toya National Park wildlife reserve (42°36'N, 140°51'E) in south-western Hokkaido. The vegetation is divided into three types; deciduous broad-leaved forest (91.8%) dominated by *Acer mono* and *Tilia japonica*, coniferous plantation (6.3%), and grassland (1.6%). The rest of the island (0.3%) is inaccessible to deer (Miyaki and Kaji 2004).

Three sika deer were introduced into area B between 1957 and 1965. The population size had increased to 57.5 deer/km² by 1983 but drastically decreased to 26.3 deer/km² in 1984 because of malnutrition (Kaji *et al.* 1988). After the population crash, the deer lost about 20% of their body mass and showed delayed age at primiparity (Kaji 2001). Because food availability is low at this site, deer forage on fallen deciduous foliage (Takahashi and Kaji 2001, Miyaki and Kaji 2004).

Diet composition and nitrogen analysis

In area A, we shot 22 males and 17 females between May 2003 and May 2004. For area B, we shot 7 males and 12 females between May 2004 and January 2005. Shooting was performed in accordance with the "Guiding Principles for the Care and Use of Mammals" of the Mammalogical Society of Japan. The samples were classified by season according to the plant phenology definition with regard to seasons in Hokkaido: spring (April–May), summer (June–August), autumn (September–November), and winter (December–March). The sample sizes by season are listed in Tables 1 and 2.

After shooting the deer, we sampled 500 ml of rumen contents and 20 fecal pellets from the rectum of each individual. Samples were stored frozen at –20°C until processing. Feces and rumen contents were washed using sieves with 0.5-mm and 2-mm mesh sizes, respectively, to remove metabolic excretions including microbial matter from fragmented plant tissue. Samples were then uniformly mixed.

The diet composition was determined by the point-frame method (Leader-Williams *et al.* 1981). This method provides a way of estimating percentage composition of surface area and is easier than weighing methods (Asada and Ochiai 1996). We randomly picked subsamples and spread them over a dish with a 5-mm grid to identify plant fragments on the intersection points. We identified approximately 30 plant fragments per subsample, repeating

Table 1. Diet composition (%) by vegetation class for sika deer at area A.

Diet composition	Spring (n = 3)		Summer (n = 5)		Autumn (n = 28)		Winter (n = 3)		Total (n = 39)	
	\bar{x} (%)	SD	\bar{x} (%)	SD	\bar{x} (%)	SD	\bar{x} (%)	SD	\bar{x} (%)	SD
Agricultural grass and legumes	72.8	0.21	75.1	0.10	44.3	0.36	0	0	47.0	0.36
Agricultural crops	0	0	0.0	0	1.8	0.08	0	0	1.3	0.07
Graminoids	0.5	0.01	0.3	0.01	8.1	0.16	66.3	0.37	11.0	0.23
Forbs	15.9	0.12	23.0	0.10	24.1	0.17	13.2	0.09	22.6	0.15
Deciduous broad leaves	1.9	0.03	0.8	0.01	1.9	0.06	0	0	1.6	0.05
Coniferous leaves	0	0	0	0	0.5	0.03	0	0	0.4	0.02
Twigs and bark	6.0	0.10	0	0	0.9	0.03	0	0	1.1	0.04
Others	3.0	0.05	0.8	0.01	18.4	0.26	20.5	0.35	15.1	0.24

Table 2. Diet composition (%) by vegetation class for sika deer at area B.

Diet composition	Spring (<i>n</i> = 6)		Summer (<i>n</i> = 4)		Autumn (<i>n</i> = 4)		Winter (<i>n</i> = 5)		Total (<i>n</i> = 19)	
	\bar{x} (%)	SD								
Agricultural grass and legumes	0	0	0	0	0	0	0	0	0	0
Agricultural crops	0	0	0	0	0	0	0	0	0	0
Graminoids	2.4	0.01	1.9	0.02	0.8	0.01	0	0	1.3	0.02
Forbs	9.0	0.04	5.7	0.07	0	0	26.1	0.30	10.9	0.18
Deciduous broad leaves	71.9	0.20	84.7	0.11	68.0	0.21	27.4	0.19	62.1	0.28
Coniferous leaves	0.1	0	0.2	0	4.8	0.05	2.4	0.04	1.7	0.03
Twigs and bark	15.7	0.19	7.3	0.05	2.6	0.04	43.7	0.33	18.5	0.25
Others	0.9	0.01	0.2	0	23.8	0.14	0.4	0.01	5.4	0.11

the process until the total number of intersection points accumulated was over 400 for each rumen sample. Plant fragments were categorized as (1) grass and legumes of agricultural origin, (2) agricultural crops, (3) graminoids, (4) forbs, (5) deciduous broad leaves, (6) coniferous leaves, (7) twigs and bark, and (8) other (seeds, fruits, ferns, mushrooms, mosses, and unidentified materials). Diet composition was expressed as the percentage of occurrence of all items.

To measure nitrogen concentration (DN and FN), the washed rumen contents and the washed feces were oven-dried at 70°C for 48 hours and ground with a mill to an average size of 2 mm (Asada and Ochiai 1999). Nitrogen concentration per dry matter was measured using an NC analyzer (NC-900, Sumika-Shimadzu) (Asada and Ochiai 1999).

We estimated diet composition using the washed rumen contents following the methods of previous studies (Segelquist *et al.* 1972, Beier 1987, Massey *et al.* 1994, Asada and Ochiai 1996, Yokoyama *et al.* 2000). Diet composition analysis based on feeding behaviour or bite marks would be biased to plants for which observation is easy, if herbivores eat each plant part selectively in different amounts. Therefore, we believe that rumen content analysis provides a more accurate method for assessing deer diets.

We assumed that the nitrogen concentration per dry matter of washed rumen contents (washed RN) represented the nitrogen concentration per dry matter of the diet (DN) overall. This assumption was supported with comparisons of nitrogen content of the washed rumen samples to samples of forage available as food (Klein 1962). Washed RN has been used as a proxy for DN in previous studies (Klein 1968, Klein and Schönheyder 1970, Takatsuki and Ikeda 1993, Asada and Ochiai 1999, Latham *et al.* 1999). In addition, nitrogen concentration determined without first washing the rumen contents (unwashed RN) has also been used to approximate DN (Holechek *et al.* 1982, Waid and Warren 1984, Takatsuki and Ikeda 1993, Watanabe and Takatsuki 1993, Soper *et al.* 1993). Because washed RN is strongly correlated with unwashed RN (Takatsuki and Ikeda 1993), we consider that both methods could reflect DN.

Fecal nitrogen has previously been estimated using whole feces (eg Asada and Ochiai 1999). In this study, however, we washed and sieved feces to estimate FN. This tech-

nique has been used for botanical analysis with feces (eg Van Sæst 1982, McCullough 1985, Campos-Arceiz and Takatsuki 2005) and has been applied to FN analysis (Padmalal and Takatsuki 1994, Padmalal *et al.* 2003, Takatsuki 2003). Feces contain two basic fractions: undigested forage (UF), composed almost entirely of dietary cell wall constituents (Van Sæst 1967), and metabolic fecal products (MFP), consisting of digestive tract cells, digestive secretions, and digestive system microbes (Van Sæst 1982). By washing feces through a 0.5-mm sieve, UF is likely to be retained. Because nitrogen estimation with washed feces has a clearer relationship with DN than nitrogen estimation with whole feces (Appendix), we used the nitrogen estimation based on washed feces to represent FN in this study.

Statistical analyses

First, we used a simple linear regression to examine the relationships between DN and FN within each area, treating DN as the dependent variable and FN as the independent variable. Secondly, we used an analysis of covariance (ANCOVA) to examine the difference in slopes and intercepts of the regression lines between the two areas, treating FN as a covariate. Third, we used ANCOVA to examine the difference in slopes and intercepts of the regression lines among three distinctive diet categories, treating FN as a covariate. We divided individual data of FN and DN based on their diet features. Fecal nitrogen was enhanced by browse when the proportion of browse (deciduous broad leaves, twigs and bark, and coniferous leaves) exceeded 33% (Mould and Robbins 1981). In addition, grass and legumes of agricultural origin are generally digested better than wild forage (eg Robbins 1993). Therefore, we divided individual data of FN and DN into three categories: diet composed of > 33% browse without plants of agricultural origin ('browse diet'); < 33% browse and without plants of agricultural origin ('grass and forb diet'); and < 33% browse and with plants of agricultural origin ('agriculture diet'). There were no individual data that could have been categorized into a high browse diet (> 33% browse) with plants of agricultural origin (see Table 3). We performed all statistical procedures using SPSS 10.0.

Table 3. Diet composition (%) by vegetation class in three diet categories.

Diet composition	Study area A				Study area B	
	Forb and grass diet ($n = 12$)		Agriculture diet ($n = 27$)		Browse diet ($n = 19$)	
	\bar{x} (%)	SD	\bar{x} (%)	SD	\bar{x} (%)	SD
Agricultural grass and legumes	0	0	67.9	0.20	0	0
Agricultural crops	0	0	1.8	0.08	0	0
Graminoids	32.1	0.32	1.7	0.07	1.3	0.02
Forbs	29.3	0.20	19.5	0.12	10.9	0.18
Deciduous broad leaves	4.4	0.09	0.4	0.01	62.1	0.28
Coniferous leaves	1.2	0.04	0	0	1.7	0.03
Twigs and bark	1.7	0.04	0.8	0.03	18.5	0.25
Others	31.3	0.32	7.9	0.16	5.4	0.11

Results

Diet composition

In area A, the most common foods in the diets of deer were grasses and legumes of agricultural origin, including timothy grass *Phleum pratense* and alfalfa *Medicago sativa*, and forbs were the second most common food except during winter (Table 1). Wild graminoids like dwarf bamboos were the primary winter food. The proportion of browse such as deciduous broad leaves, twigs and bark, and coniferous leaves was 3.1% throughout the year (Table 1).

Conversely, in area B, deciduous broad leaves were the main constituents of deer diets (Table 2), with a high proportion in spring, summer, and autumn. The second-most dominant food items were twigs and bark, which were consumed in winter. Graminoids were a minor food source in area B throughout the year (Table 2).

Relationships between DN and FN in two areas

Dietary nitrogen was significantly positively regressed by FN in both area A and area B (Fig. 1: area A: $Y = 1.023X + 0.675$, $R^2 = 0.58$, $n = 39$, $p < 0.001$; area B: $Y = 1.027X + 0.217$, $R^2 = 0.61$,

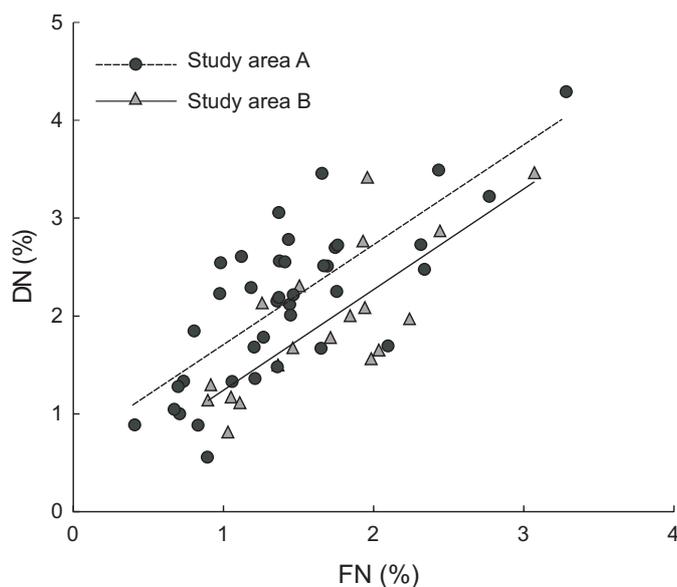


Fig. 1. Relationships between fecal nitrogen (FN) and dietary nitrogen (DN). The regression lines were fitted for area A (solid line) and for area B (dotted line).

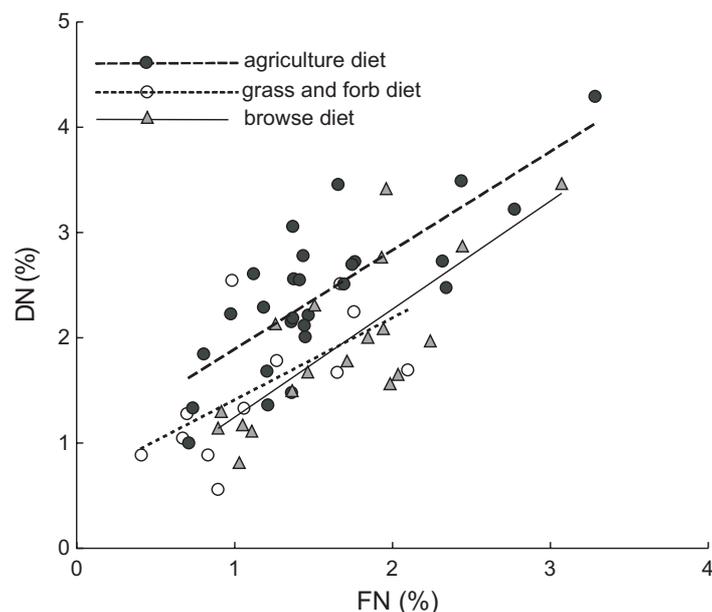


Fig. 2. Relationships between fecal nitrogen (FN) and dietary nitrogen (DN) in three diet categories; agriculture diet, grass and forbs diet, and browse diet.

$n = 19$, $p < 0.001$). The DN-FN-relationship in area A had a similar slope as in area B (Fig. 1; $df = 1, 54$, $F = 0.000$, $p = 0.988$), but a higher intercept than area B (Fig. 1; $df = 1, 55$, $F = 9.648$, $p = 0.003$).

DN-FN-relationships in the three diet categories

Diet compositions in the three diet categories are shown in Table 3. In the grass and forb diet and the agriculture diet, the proportions of browse including deciduous broad leaves, coniferous leaves, and twigs and bark were as low as 7.3 and 1.2% respectively, whereas the proportion of browse was as high as 62.1% in the browse diet. Because the two study areas were highly contrasting in their habitats, all individual data from area A were categorized into the grass and forb diet or the agriculture diet, and all data from area B were categorized into the browse diet.

DN-FN-relationships were not significantly different between the browse diet and the grass and forb diet (Fig. 2; $p = 1.000$). The DN-FN-relationship of the agriculture diet had a similar slope (Fig. 2; $df = 2, 52$, $F = 0.258$, $p = 0.774$) but a higher intercept than the grass and forb diet ($p = 0.014$) and the browse diet ($p < 0.001$).

Discussion

Our results demonstrate that FN cannot always be used as a reliable indicator to predict DN. Although simple linear relationships of DN to FN were confirmed within areas, the DN-FN-relationships had different intercepts between the study areas, which indicates that FN is useful as a local indicator but is not suitable as a general indicator.

We detected no significant effect of browse on the regression lines in this study. The regression line of DN to FN within the browse diet was not significantly different from those of DN to FN within the grass and forb diet. Holechek *et al.* (1982) also showed no difference in the FN to DN relationships between grassland and forest, although browse was only a minor diet component for cattle in both of these vegetation types in their study. Despite the high dependence on browse in our study, where the average proportion of browse throughout the year was high, we found no effect of browse on the regression lines.

Browse contains much higher levels of phenolics than forbs and grasses (Robbins *et al.* 1987), and phenolics reduce protein digestibility (Shipley and Felicetti 2002), causing artificially elevated FN relative to DN (Mould and Robbins 1981). However, these secondary compounds

may not markedly bias the relationship of FN to DN (Nicholson *et al.* 2006). Apparent protein-binding digestibility is reduced much less in animals that secrete tannin-binding salivary proteins, such as mule deer, black bear *Ursus americanus*, and laboratory rats *Rattus rattus*, than in those that do not, eg, prairie voles *Microtus ochrogaster* or domestic sheep *Ovis aries* (Robbins *et al.* 1991). The magnitude of the reduction varies depending on the animal's ability to minimize or neutralize the protein-binding capacity of the tannins (Robbins 1993). Unfortunately, the secretion of tannin-binding salivary proteins has not been investigated in sika deer. However, tannin-tolerant bacteria that degrade hydrolyzable tannin have been isolated from rumen microflora of sika deer (Sawabe 2006) as well as bush duiker *Sylvicapra grimmia*, giraffe *Giraffa camelopardalis*, Grant's gazelle *Gazella granti*, sheep, and goats (Odenyo *et al.* 2001). Although the efficiency of tannin-tolerant bacteria has not been quantified, it is possible that the effect of phenolics on the regression lines of DN to FN may be mitigated in the diet of sika deer.

Another explanation for the insignificant effect of browse on DN-FN-relationships may be attributed to our method. By washing feces, we removed MFP and estimated the nitrogen concentration of UF. Because phenolics in browse that bind protein are in MFP, the elevation in FN caused by phenolics could be reflected in the nitrogen concentration of MFP. Because we did not measure MFP, we could not have found a significant effect of browse on DN-FN-relationships.

On the other hand, the intercept of the regression line was significantly higher with the agriculture diet than the grass and forb diet and the browse diet. By washing feces, we estimated only plant-fiber-associated nitrogen. Generally, grass and legumes from agricultural origin are digested better than wild forage (eg Robbins 1993). Therefore it is clear that for agricultural forage, a large part of the fiber-bound nitrogen fraction can be digested, and hence we found relatively lower FN. Thus, the difference in the intercept might reflect the difference in protein digestibility. Therefore, the difference in the regression lines of DN to FN was explained by de-

pendence on agricultural forage regardless of the ratio of browse in the diets.

In our study, rumen contents were used to estimate DN. We did not detect the most digestible species in the rumen contents, because they would have been digested faster than other species ingested at the same time. The DN-FN-relationships with the agriculture diet might show such a bias more strongly, because grass or legumes of agricultural origin are more highly digestible than natural forage. However, such a bias would make our results conservative. Because such a bias would increase the slope or intercept of regression lines of the agriculture diet relative to estimated values, the position of the regression lines of the two areas or three diet categories would not change.

Considering that it takes time for rumen contents to be defecated as feces, it might be questionable to use rumen contents and feces collected at the same time to examine the DN-FN-relationships. A feeding trial using timothy hay stained with crystal violet in sika deer revealed that the excretion rate peaks after 2.5 days and is complete by about the fifth day after ingestion (Kunishige and Togari 2003). Contents of feces at time (t) would be mostly representative of rumen contents at (t - 2.5 days), whereas the excretion of rumen contents at (t - 2.5 days) would not be completed by (t). Dietary material remains in the ruminoreticulum for 2.5 days after ingestion (Kunishige and Togari 2003). Therefore, rumen contents at (t - 2.5 days) would still remain in the rumen at (t). Thus, rumen contents at (t) would overlap with contents of feces at (t). Therefore, examining the regression relationship of DN to FN collected at the same time is valid.

Considering seasonal variation in deer diets, DN-FN-relationships may differ among seasons. However, we could not examine the seasonal effect on DN-FN-relationships because our sample sizes were too small, with the exception of autumn in area A. By examining the DN-FN-relationship across seasons, future studies could identify the season(s) during which the DN-FN-relationship is the same between areas.

We did not consider a potential effect of sex when calculating regression lines. However, sex

may influence diet selection (eg Mysterud 2000). Deer in area B feed on the fallen leaves of deciduous trees throughout the year (Takahashi and Kaji 2001), and molar wear rates were not different between the sexes (Takahashi *et al.* 1999), suggesting that sex differences in diet selection were small. In area A, no sexual difference in diet selection is known. Because habitat greatly differs between the two areas, the diet differences between areas likely mask any possible sexual differences within the two areas.

The difference in the DN-FN-relationship between the two areas was explained by the dependence on grass and legumes of agricultural origin in the diet rather than browse. Because individual data from area A were categorized into the agriculture diet or the grass and forb diet, and all data from area B were categorized into the browse diet, the difference in DN-FN-relationships between areas was explained by the difference in diet composition within area A, and not by diet differences between areas A and B.

Therefore, FN would not be suitable for use in areas where artificial forage is available. Although FN was useful for predicting DN even within area A, where deer utilize agricultural food, the accuracy of DN would have been lower with combined FN data that include grass and legumes from agricultural origin. On the other hand, FN might be useful irrespective of dependence on browse. Therefore, FN would be expected to work even in herbivores utilizing both browse and grass in natural populations.

We conclude that FN may be useful for comparing DN among areas regardless of the ratio of browse in diverse diets of free-ranging ruminants, but that dependence on agricultural pastures could hinder the utility of FN.

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Appendix. Comparison between nitrogen estimation with washed feces and whole feces.

Fecal nitrogen has been estimated using whole feces, whereas the method of washing feces has been used to examine the botanical composition of feces. By washing feces through a fine sieve, metabolic fecal products (MFP) is removed and only plant material is retained. Therefore, nitrogen estimations from washed feces may be more reflective of DN than whole feces. We compared nitrogen estimation with washed feces and whole feces to examine which is more predictive of DN.

Nitrogen estimation with washed feces and DN (washed RN) data are cited from the main text. We estimated nitrogen concentration of whole feces as well as the washed feces described in the main text. Because there were two individuals in area A from which we could not collect enough feces to perform both estimations, the sample sizes from areas A and B were 37 and 19, respectively. We used simple linear regression to examine the relationships between DN and nitrogen with washed feces, and DN and fecal nitrogen with whole feces within each area.

Nitrogen estimation from whole feces was higher than from washed feces with respect to DN, possibly because MFP, which is retained in whole feces, elevated the nitrogen concentration. Nevertheless, DN was significantly regressed by nitrogen of whole feces as well as nitrogen of washed feces for both areas (area A: whole feces: $Y = 0.305X + 1.038$, $df = 1, 35$, $F = 4.99$, $p = 0.03$; washed feces: $Y = 0.946X + 0.745$, $df = 1, 35$, $F = 35.87$, $p < 0.01$; area B: whole feces: $Y = 0.669X + 0.176$, $df = 1, 17$, $F = 6.28$, $p = 0.02$; washed feces: $Y = 1.027X + 0.217$, $df = 1, 17$, $F = 26.56$, $p < 0.01$). However, the coefficient of determination (R^2) was higher with washed feces than whole feces (area A: washed feces; $R^2 = 0.51$; whole feces; $R^2 = 0.12$; area B: washed feces; $R^2 = 0.61$; whole feces; $R^2 = 0.27$), which indicates that nitrogen estimation from washed feces is more predictive of DN than nitrogen estimation from whole feces.

Metabolic fecal products originate from plant-derived protein but contain animal-derived microorganisms. Because the nitrogen estimation of whole feces includes different trophic levels, it may have a weaker relationship with DN than the nitrogen estimation of washed feces. Therefore, we adopted nitrogen estimation of washed feces to represent FN in the main text.

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