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The effect of dietary protein quality on nitrogen isotope discrimination in mammals and birds

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Abstract We tested the competing hypotheses that (1) nitrogen discrimination in mammals and birds increases with dietary nitrogen concentration or decreasing C:N ratios and, therefore, discrimination will increase with trophic level as carnivores ingest more protein than herbivores and omnivores or (2) nitrogen discrimination increases as dietary protein quality decreases and, therefore, discrimination will decrease with trophic level as carnivores ingest higher quality protein than do herbivores. Discrimination factors were summarized for five major diet groupings and 21 different species of birds and mammals. Discrimination did not differ between mammals and birds and decreased as protein quality (expressed as biological value) increased with trophic level (i.e., herbivores to carnivores). Relationships between discrimination factors and dietary nitrogen concentration or C:N ratios were either the opposite of what was hypothesized or non-significant. Dietary protein quality accounted for 72% of the variation in discrimination factors across diet groupings. We concluded that protein quality established the baseline for discrimination between dietary groupings, while other variables, such as dietary protein intake relative to animal requirements, created within-group variation. We caution about the care needed in developing studies to understand variation in discrimination and subsequently applying those discrimination factors to estimate assimilated diets of wild animals.

Keywords Assimilated diet · Discrimination · Nitrogen · Stable isotopes · Trophic level

Introduction

The estimation of assimilated diet from stable isotopes depends on accurate estimates of the enrichment occurring between the diet and tissue of the consumer being sampled (i.e., discrimination; Pearson et al. 2003). However, relatively few avian and mammalian ecologists using stable isotopes to estimate assimilated diets of free-ranging animals collect and feed the natural diet to create study-specific discrimination factors (Haramis et al. 2001). In the absence of such values, one must either use published, interspecific means (e.g., $3.4 \pm 1.1\%$ for ^{15}N ; Minagawa and Wada 1984), use the $\delta^{15}\text{N}$ signature measured in other species (e.g., use an area-specific ungulate signature as the herbivorous end-point for the omnivorous grizzly bear; Jacoby et al. 1999), or develop predictive regressions by feeding numerous, often artificial diets from which unknown discrimination factors can be predicted (Hilderbrand et al. 1996; Felicetti et al. 2003b; Hobson and Bairlein 2003).

Although the latter approaches can be useful and may be accurate, they are of concern as they do not necessarily represent an understanding of the causative factors producing variation in discrimination. Currently, there are several competing hypotheses to explain variation in nitrogen discrimination (Post 2002; McCutchan et al. 2003; Vanderklift and Ponsard 2003). These hypotheses include: (1) nitrogen discrimination will increase with dietary nitrogen concentration or decreasing C:N ratio and, therefore, discrimination factors will increase with trophic level as carnivores ingest more protein than herbivores or omnivores (i.e., quantity hypothesis; Pearson et al. 2003) or (2) nitrogen discrimination will increase as dietary protein quality decreases and, therefore, discrimination factors will decrease with trophic level as carnivores ingest higher

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quality protein than herbivores do (i.e., quality hypothesis; Roth and Hobson 2000). Both hypotheses are based on the idea that as nitrogen metabolism and excretion increases, selective renal retention of ^{15}N will elevate the animal's isotope signature and therefore, discrimination. However, these hypotheses and resulting conclusions are opposites and both cannot be correct. If either hypothesis is correct (i.e., variation is real and predictable), a much greater understanding of the link between diet, animal metabolism, and discrimination is necessary if we are to use stable isotopes to estimate assimilated diet (Fantle et al. 1999). In this paper, we test these hypotheses by comparing nitrogen discrimination across a wide range of diets consumed by mammals and birds.

Methods

Discrimination factors were found in the literature for five major diet groupings and either serum, plasma or whole blood of 21 different species of birds and mammals. Blood isotope signatures were chosen because (1) there are no significant differences between the blood constituents when equilibrated with the diet (Hilderbrand et al. 1996) and (2) they are most numerous in the literature and, therefore, provide the largest sample sizes. Diets in which protein came from a single ingredient were sought as protein quantity and quality could be identified. Thus, diet groupings included (1) milk, (2) fish, (3) other animals including mammals, birds and insects, (4) hays and silage fed to ruminants, and (5) fruit. Data for commercial diets or mixed diets having several protein sources were not used because their unknown ingredients or composition prevented an estimate of protein quality (see "Discussion").

Animal species included black bears (*Ursus americanus*), black-tailed deer (*Odocoileus hemionus*), coyotes (*Canis latrans*), crows (*Corvus brachyrhynchos*), domestic cows (*Bos taurus*), domestic goats (*Capra hircus*), domestic pigs (*Sus scrofa*), domestic sheep (*Ovis aries*), dunlins (*Calidris alpina pacifica*), gray seals (*Halichoerus grypus*), great skuas (*Catharacta skua*), grizzly bears (*Ursus arctos horribilis*), harp seals (*Pagophilus groenlandicus*), harbour seals (*Phoca vitulina*), mink (*Mustela vison*), moose (*Alces alces*), peregrine falcons (*Falco peregrinus*), polar bears (*Ursus maritimus*), ringed seals (*Phoca hispida*), ring-billed gulls (*Larus delawarensis*), and yellow-rumped warblers (*Dendroica coronata*).

All studies except for wild polar bears consuming "almost exclusively" ringed seals (Hobson and Welch 1992) utilized captive animals. Feeding levels for the captive animals were either maternally controlled (i.e., nursing offspring; Jenkins et al. 2001); ad libitum (Hobson and Clark 1992; Hilderbrand et al. 1996; Jenkins et al. 2001; Lesage et al. 2002; Felicetti et al. 2003b; Pearson et al. 2003; Sponheimer et al. 2003a; Ogden et al. 2004); a constant measured amount (Bearhop et al. 2002); or unstated and frequently not controlled by the

investigator (e.g., seals held in public aquariums; Steele and Daniel 1978; Hobson et al. 1996; Hobson and Bairlein 2003). However, none of the investigators restricted feed intake below maintenance levels. With the exception of the fruit diets that did not contain enough nitrogen for the animals to be in positive nitrogen balance (Hilderbrand et al. 1996; Felicetti et al. 2003a, b), all other diets should have met all energy and nutrient requirements.

While several discrimination studies that met the diet criteria (i.e., single protein source) reported the carbon and nitrogen composition of the diet, many did not. When this occurred, carbon and nitrogen contents were extrapolated from other studies that analyzed the gross composition of the same foods. Nitrogen contents were estimated as 16% of the crude protein content. Carbon contents were estimated using the constants of 52% carbon in protein, 75% in fats, and 44% in carbohydrates (Robbins 1993). Small errors in these estimates should be meaningless as nitrogen content and C:N ratios can vary widely across trophic levels.

Several measures of protein quality exist in the animal nutrition literature. Biological value is the most common measure and is defined as the percentage of absorbed protein that is retained (Robbins 1993). Classically, biological values were measured in growing or producing animals at relatively low dietary protein concentrations that maximized protein utilization (Mitchell 1924; Mitchell et al. 1936). When done in this manner, the most important variable is the extent to which the essential amino acid spectrum of the absorbed proteins matches the animal's requirement. When biological values are measured in conditions outside of the classical experimental criteria, they can vary widely. For example, the biological value of any protein will be zero if measured on an adult animal that is not growing or producing and therefore has no net nitrogen retention. Similarly, as the dietary protein concentration increases above the animal's requirement for growth or production, biological values must decrease as the excess nitrogen is excreted (Robbins 1993).

Thus, the biological values for the diets used in this study are those meeting the classical criteria in which protein is a limiting nutrient. The specific values are 96% for milk (McDonald et al. 1973), 92% for fish (Lewis and Morris 1983), 78% (average for the meat of domestic animals) for mammals, birds, and insects (Lewis and Morris 1983; Kellems and Church 2002), and 71% for plant diets fed to ruminants (ARC 1965). Because biological values for fruits could not be found, an average for commercial grains (corn, oats, wheat and barley) fed to monogastrics was used (55%; Lewis and Morris 1983; Kellems and Church 2002) as fruits are generally very low in protein and much of the nitrogen is not in the form of amino acids (Izhaki 1993). This range in biological values presumably reflects (1) the selective pressures on maternal milk production to minimize protein transfer by maximizing the efficiency of protein utilization in the developing offspring and (2) the more

Table 1 Diets, isotope signatures, carbon and nitrogen concentration, and isotopic discrimination for various diets consumed by mammals and birds. Carbon and nitrogen concentrations are reported on a 100% dry matter basis. The listed diet is either the only or virtually the only dietary protein source (see footnote). Discrimination means are significantly different if followed by a different letter

| Species | Diet | | Diet | | | | Animal | | | | Source |
|---------------------------------|----------------------------|---------------------------|----------|------|------------------|--------|---------------------------|----------------------------------|---------------------------|--|--------|
| | Diet | $\delta^{15}\text{N}$ (‰) | %N | %C | C:N | Sample | $\delta^{15}\text{N}$ (‰) | $\Delta \delta^{15}\text{N}$ (‰) | | | |
| Black-tailed deer | Milk | 5.3 | 4.4 | 59 | 13.3 | Plasma | 7.2 | 1.9 | Jenkins et al. 2001 | | |
| Coyotes | Milk | 10.4 | 5.3 | 56 | 9.5 | Plasma | 11.8 | 1.4 | | | |
| Domestic pigs | Milk | 3.7 | 4.6 | 56 | 8.2 | Plasma | 5.5 | 1.8 | | | |
| Domestic sheep | Milk | 5.6 | 5.0 | 43 | 11.7 | Plasma | 8.6 | 3.0 | | | |
| Grizzly bears | Milk | 10.0 | 3.5 | 60 | 5.9 | Plasma | 11.8 | 1.8 | | | |
| Moose | Milk | 5.0 | 6.2 | 61 | 10.1 | Plasma | 6.5 | 1.5 | | | |
| | Milk | | 5.3±0.8 | 56±7 | 9.8±2.6 | | | 1.9±0.6 ^a | | | |
| Black bears | Salmon | 11.1 | 12.0 | 55 | 4.6 ¹ | Plasma | 13.4 | 2.3 | Hilderbrand et al. 1996 | | |
| Crows | Perch | 14.2 | 11.8 | - | - | Blood | 16.0 | 1.8 | Hobson and Clark 1992 | | |
| Dunlins | Fish meal ² | 13.7 | - | - | - | Blood | 16.9 | 3.2 | Ogden et al. 2004 | | |
| Gray seals | Herring | 13.3 | - | - | - | Serum | 16.4 | 3.1 | Lesage et al. 2002 | | |
| Great skuas | Sprat | 11.3 | - | - | - | Blood | 13.9 | 2.6 | Bearhop et al. 2002 | | |
| Grizzly bears | Salmon | 11.2 | 10.8 | 57 | 5.2 | Plasma | 14.8 | 3.6 | Felicetti et al. 2003b | | |
| Harbor seals | Herring | 13.1 | - | - | - | Serum | 16.1 | 3.0 | Lesage et al. 2002 | | |
| Mink | Salmon | 12.3 | 12.0 | 55 | 4.6 | Plasma | 14.6 | 2.2 | Ben-David and Schell 2001 | | |
| Ring-billed gulls | Perch | 14.2 | 11.8 | - | - | Blood | 17.3 | 3.1 | Hobson and Clark 1992 | | |
| Harp, ringed, and harbour seals | Herring | 13.0 | 11.3 | 55 | 4.9 | Blood | 14.7 | 1.7 | Hobson et al. 1996 | | |
| | Fish | | 11.5±0.5 | 56±1 | 4.9±0.3 | | | 2.7±0.6 ^{a, b} | | | |
| Black bears | Deer | 6.4 | 7.2 | 63 | 8.8 | Plasma | 10.5 | 4.1 | Hilderbrand et al. 1996 | | |
| Great skuas | Beef | 7.6 | - | - | - | Blood | 11.6 | 4.0 | Bearhop et al. 2002 | | |
| Mink | Beef | 6.2 | 6.5 | 64 | 9.8 | Plasma | 10.2 | 4.0 | Ben-David and Schell 2001 | | |
| Peregrine falcons | Quail | 6.5 | - | - | - | Blood | 9.8 | 3.3 | Hobson and Clark 1992 | | |
| Polar bears | Ringed seals | 17.3 | 5.1 | 62 | 12.1 | Plasma | 21.1 | 3.8 | Hobson and Welch 1992 | | |
| Yellow-rumped warblers | Mealworms ³ | 6.1 | 8.4 | 54 | 6.4 | Plasma | 9.1 | 3.0 | Pearson et al. 2003 | | |
| Domestic cows | Mammals, birds, or insects | 0.6 | 6.8±1.4 | 61±5 | 9.3±2.4 | Blood | 4.8 | 3.7±0.4 ^{b, c} | Steele and Daniel 1978 | | |
| | Silage | 0.1 | 3.4 | 44 | 12.9 | Blood | 4.1 | 4.0 | Sponheimer et al. 2003a; | | |
| | Alfalfa | | | | | | | | Sponheimer, unpublished | | |
| Domestic goats | Alfalfa | 0.3 | 2.9 | 45 | 15.6 | Plasma | 5.0 | 4.7 | Sponheimer et al. 2003a; | | |
| | | | | | | | | | Sponheimer unpublished | | |
| Domestic sheep | Hay | 1.5 | 2.9 | - | - | Plasma | 6.6 | 5.1 | Jenkins et al. 2001 | | |
| | Ruminant diets | | 3.1±0.3 | 44 | 14.3 | | | 4.5±0.5 ^{c, d} | | | |
| Black bears | Apples | 2.3 | 0.4 | 44 | 110 | Plasma | 6.5 | 4.2 | Hilderbrand et al. 1996 | | |
| Grizzly bears | Apples | 0.7 | 0.6 | 44 | 73 | Plasma | 6.5 | 5.8 | Felicetti et al. 2003b | | |
| | Fruit | | 0.5 | 44 | 92 | | | 5.0 ^d | | | |

¹Salmon composition and carbon content from Phillips and Koch (2002); deer composition from Pritchard and Robbins (1990); seal composition from Stirling and McEwan (1975); and herring meal with minimal gelatin from NAS (1971)

²Fish meal with minimal gelatin as a binder

³97.1% mealworms and 2.3% bananas

similar amino acid profile between consumer requirements and whole animal proteins (i.e., excluding purified animal proteins like gelatin that has a biological value of 0–15%) relative to mixtures of plant proteins.

Simple linear regressions were used to graph and test the slope of the relationships between discrimination factors and dietary nitrogen concentration, C:N ratio, and protein quality. While fruit diets were shown in all graphic comparisons, they were not used to compute regressions or statistical tests because the animals in those trials were in negative nitrogen balance (Felicetti et al. 2003a). Therefore, blood protein synthesis in the fruit studies (Hilderbrand et al. 1996; Felicetti et al. 2003b) was most likely from some combination of dietary nitrogen and internal catabolic processes rather than the primarily anabolic processes occurring in animals consuming all other diets. ANOVA was used to test for differences in discrimination factors between diet groups and between birds and mammals consuming fish and other animals (SAS 1998). Means are reported $\pm 1SD$.

Results

Mean discrimination factors varied across the five dietary groupings from 1.9‰ to 5.0‰, nitrogen content from 0.5% to 11.5%, and C:N ratios from 4.9 to 92.0 (Table 1). Discrimination factors for birds ($2.7 \pm 0.6‰$; $n=4$) and mammals ($2.7 \pm 0.7‰$; $n=6$) consuming fish did not differ ($F=0.190$, $P=0.68$). The single discrimination factor available for birds consuming mammals (4.0‰; Bearhop et al. 2002) was identical to the mean for mammals consuming mammals ($4.0 \pm 0.2‰$; $n=3$; Table 1). The single discrimination factors reported for birds consuming birds (3.3‰; Hobson and Clark 1992) and birds consuming invertebrates (3.0‰; Pearson et al. 2003) were lower, but certainly insufficient to determine a pattern.

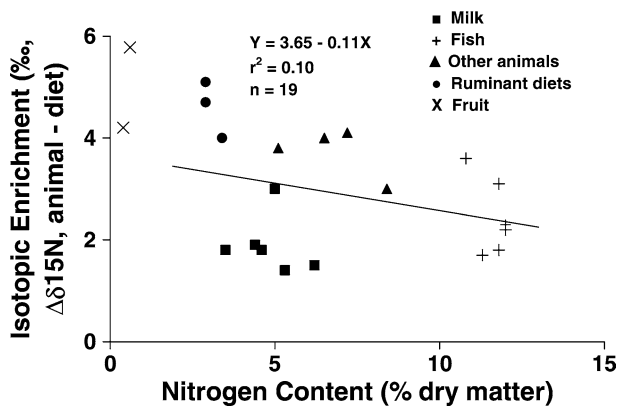


Fig. 1 The relationship between nitrogen isotope discrimination in mammals and birds and dietary nitrogen content in diets composed of a single protein source. Values from Table 1. The fruit values, although plotted, were not used in calculating the regression because the animals were in negative nitrogen balance

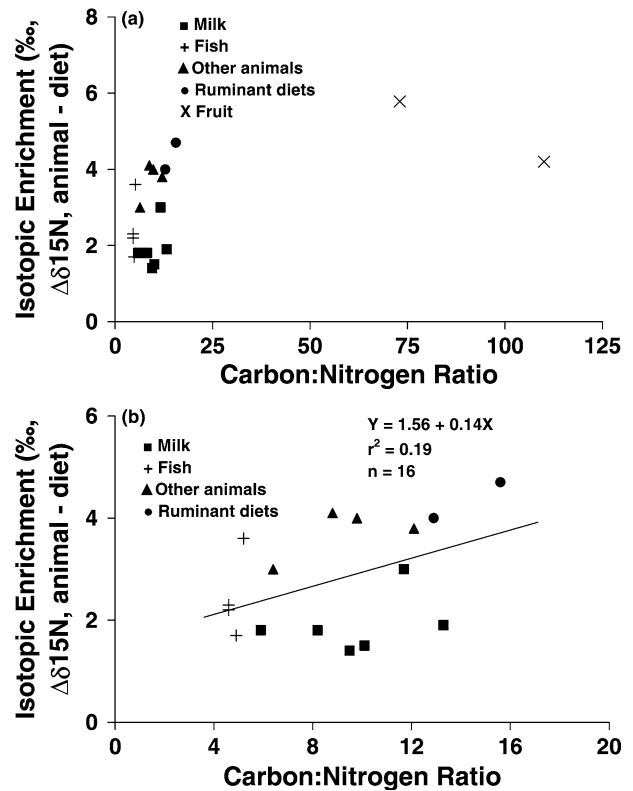


Fig. 2 The relationship between nitrogen isotope discrimination in mammals and birds and C:N ratio in diets composed of a single protein source. Values from Table 1. No regression was calculated for **a** because data are not normally distributed. **b** uses all data from **a** except the two fruit points

Discrimination factors did not vary significantly with either dietary nitrogen content (Fig. 1; $F=1.935$, $P=0.18$) or C:N ratio (Fig. 2b; $F=1.040$, $P=0.20$). Discrimination factors decreased as dietary protein quality increased (Fig. 3; $F=56.07$, $P<0.0001$). Protein quality (biological value) accounted for 72% of the variation between the four dietary groupings.

Discussion

Pearson et al. (2003) initially hypothesized that nitrogen discrimination would increase with dietary nitrogen concentration and, therefore, across trophic levels based on the results of a study in which yellow-rumped warblers were fed mixtures of mealworms and bananas. However, Hobson and Bairlein (2003), using garden warblers (*Sylvia borin*), found no such relationship between discrimination and nitrogen concentration or C:N ratios. Similarly, the current compilation yields the opposite trend suggested by Pearson et al. (2003) with high protein, low C:N ratio fish and meat diets producing lower discrimination factors than plant diets (Fig. 1). Neither of the regressions between discrimination and dietary nitrogen content or C:N ratio generated from the current, tightly focused dataset nor a much

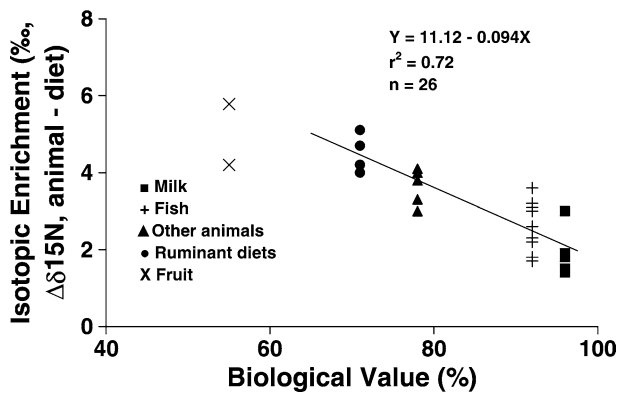


Fig. 3 The relationship between nitrogen isotope discrimination in mammals and birds and dietary protein quality (biological value) in diets composed of a single protein source. Values from Table 1. Biological values used were 96% (milk), 92% (fish), 78% (other animals), 71% (ruminant diets), and 55% (fruit). The regression does not include the two fruit values

broader dataset (Vanderklift and Ponsard 2003) provide any predictive capability. Although exclusion of the milk data from the discrimination-nitrogen content regression would provide a much better relationship across trophic levels (Fig. 1), the slope of the regression would be opposite of that needed to support the “quantity hypothesis”. Similarly, variation within the entire dataset suggests that such a relationship would not be cause-effect.

The Roth and Hobson (2000) quality hypothesis of decreasing discrimination with increasing protein quality or trophic level is supported by the current analysis. Additional studies of discrimination by non-ruminant herbivores and granivores would be helpful to determine if the ruminant data is characteristic of herbivores in general or driven in part by the greater nitrogen recycling capability of ruminants. Similarly, the relatively large, unexplained variation within each dietary grouping should be the basis for extensive studies to determine its cause (Ponsard and Averbuch 1999). Contrasting results and hypotheses in Hobson and Bairlein (2003) and Pearson et al. (2003) indicate how complex study design must be to differentiate between competing hypotheses when dietary protein quality, concentration, and intake vary simultaneously across diets. Similarly, animal age, growth, productivity, and, therefore, protein requirements frequently were not controlled in previous feeding studies.

The mean discrimination factors reported in this summary might be useful in studies of carnivores if study-specific values cannot be generated. Because fish, mammals and other animals generally have very consistent amino acid profiles dictated by structural and metabolic requirements (Robbins 1993; Kellems and Church 2002), one would not expect large variation in discrimination within carnivores consuming specific types of animals due to differing dietary amino acid profiles. While it is tempting to suggest that nitrogen discrimination in ruminants might be similarly buffered

by rumen microbe metabolism (Van Soest 1994), large differences in discrimination factors for hair were observed between alpacas (*Lama pacos*), domestic cattle, and domestic goats consuming high- and low-protein diets (Sponheimer et al. 2003a). Discrimination was from 1.5‰ to 2.8‰ greater when a high-protein (21%) alfalfa (*Medicago sativa*) was consumed (biological value = 30% for goats) relative to a low-protein (10%) coastal bermudagrass (*Cynodon dactylon*; biological value = 46%; Robinson et al. 2005). This increase in discrimination with increasing dietary nitrogen content supports Pearson et al.’s (2003) hypothesis, but at a more basic level reflects a decreasing biological value as nitrogen excretion increased when dietary protein intake exceeded animal requirements (Robinson et al. 2005). While the Pearson et al. (2003) quantity hypothesis is currently not supported across trophic levels, it may help explain variation within trophic levels if discrimination increases as dietary protein intake exceeds animal requirements. Thus, we hypothesize that both dietary protein quality and quantity will ultimately be important in understanding discrimination.

Moreover, many wild animals are either omnivores or consume complex mixtures of foods within any specific diet grouping. For these animals, one cannot assume that a discrimination factor can be predicted from the weighted average of all major dietary components (dietary content of each item times its protein content times the biological value). Biological values and, therefore, presumably discrimination factors are not additive. For example, the objective of commercial feed manufacturers is to take relatively cheap protein sources with imbalanced amino acid spectrums and blend them into a final diet with a balanced amino acid spectrum and, therefore, higher biological value and potentially lower discrimination factor than any of the individual ingredients (Kellems and Church 2002). Commercial rodent and poultry diets that are cereal grain-based have a mean discrimination factor of 2.7 ± 0.6 ‰ (DeNiro and Epstein 1981; Minagawa and Wada 1984; Hobson and Clark 1992; Haramis et al. 2001), which is similar to the very best protein in fish (Table 1) and almost half what would be expected for individual cereal grains as they have relatively low biological values (45–65) due to specific amino acid deficiencies when fed to monogastrics (Lewis and Morris 1983; Peoples et al. 1994; Kellems and Church 2002).

One could hypothesize that non-ruminant wild granivores and omnivores are doing the same dietary blending to produce similarly low discrimination factors. If this is true, then a single, low, and constant discrimination factor developed from commercial diets could be used to estimate the assimilated diets of many wild animals (e.g., 2.7 ± 0.6 ‰). However, commercial diets or ones formulated by investigators to match the gross composition of the seasonal or annual diets of wild animals that produce a balanced amino acid spectrum in each bite may not yield appropriate discrimination factors if foods are being eaten and metabolized in distinct,

temporally isolated bouts. If the latter is occurring, then the time-specific discrimination factors may be higher than that measured for the blended diet. However, animals do have a limited capability to temporarily store amino acids and, thereby, balance the amino acid profile of successive meals with imbalanced amino acid spectrums. Thus, high discrimination factors would occur only if foods with imbalanced amino acids were consumed many hours to perhaps a day or more apart (Hands 2000).

In summary, understanding and estimating discrimination factors will be much more complex than previously appreciated, and certainly far beyond simple ideas regarding nitrogen concentration or C:N ratios (Robbins et al. 2002; Gaye-Siessegger et al. 2004). Animal ecologists and nutritionists must work together to develop better controlled studies using captive animals and, thereby, a much greater understanding of the internal processes determining variation in discrimination before we can have much faith in assimilated diet estimates for wild animals (Robbins et al. 2002; Sponheimer et al. 2003b).

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