STABLE-NITROGEN ISOTOPE ENRICHMENT IN AVIAN TISSUES DUE TO FASTING AND NUTRITIONAL STRESS: IMPLICATIONS FOR ISOTOPIC ANALYSES OF DIET

KEITH A. HOBSON, RAY T. ALISAUSKAS AND ROBERT G. CLARK
Department of Biology, University of Saskatchewan, Saskatoon, Saskatchewan S7N 0W0, Canada and Canadian Wildlife Service, 115 Perimeter Road, Saskatoon, Saskatchewan S7N 0X4, Canada

Abstract. Studies using measurements of δ15N to delineate diet or trophic level in natural ecosystems are based on the premise that δ15N values in consumer tissues can be reliably correlated with those in the diet. However, juvenile Japanese Quail (Coturnix japonica) fed a rationed diet designed to maintain, but not increase, body mass showed significantly enriched tissue δ15N values over a control group fed the same diet ad libitum. We tested the hypothesis that fasting or nutritional stress can also cause elevated δ15N values in tissues of wild birds by examining tissues of Arctic-nesting female Ross' Geese (Chen rossii) before and after their period of fasting during egg laying and incubation. Significant declines in body, pectoral muscle, liver and abdominal fat mass occurred from arrival through incubation. Post-incubating geese showed significantly higher pectoral muscle and liver δ15N values compared to geese taken before clutch initiation but δ14C values in these tissues were unchanged. We hypothesize a mechanism of tissue δ15N enrichment due to reduced nutrient intake and discuss the implications of these results to ecosystem studies using stable-nitrogen isotope analysis.

Key words: Fasting; nutritional stress; nitrogen-15; isotopic enrichment; natural variation; Coturnix japonica; Chen rossii.

INTRODUCTION

Measurement of the abundance of naturally-occurring stable isotopes of carbon (13C/12C) and nitrogen (15N/14N) are being used increasingly to delineate dietary patterns in wild animal populations (reviewed by Peterson and Fry 1987, Rundel et al. 1988). In particular, stable-nitrogen isotope ratios have been shown to exhibit stepwise enrichment through food chains and can thus provide trophic-level information of consumers in past and present ecosystems (e.g., Minagawa and Wada 1984, Ambrose and DeNiro 1986, Hobson and Montvecchi 1991, Hobson and Welch 1992). Specific applications of this technique to avian dietary studies were discussed recently by Hobson and Clark (1992).

An important assumption in the use of stable-nitrogen isotope analysis to provide source or trophic-level information is that the 15N/14N ratio of a consumer's tissue can be reliably associated with that of its diet (DeNiro and Epstein 1981, Tieszen et al. 1983). Recently, however, Ambrose and DeNiro (1987) cautioned that the 15N/14N ratios in consumer bone collagen may also be influenced by ecological and physiological processes such as water stress. Specifically, in their isotopic investigations of East African mammals, these authors found that drought-tolerant herbivores showed enriched 15N/14N ratios in their bone collagen compared to water-dependent species. These findings are particularly relevant because factors influencing variability in isotopic data are generally poorly understood (Owens 1987).

In their recent study using captive-raised individuals on known diets, Hobson and Clark (1992) suggested that nutritional stress during growth can influence 15N enrichment in tissues of juvenile birds. This was based on their observation that American Crows (Corvus brachyrhynchos) raised on a grain-based diet showed reduced mass gain and highly enriched 15N/14N ratios in their bone collagen compared to water-dependent species. These findings are particularly relevant because factors influencing variability in isotopic data are generally poorly understood (Owens 1987).

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nutritional stress can cause $^{15}$N enrichment in the tissues of growing birds. In addition, we investigated the broader relevance of our laboratory findings by determining also the extent of $^{15}$N enrichment in tissues of nesting Ross' Geese (Chen rossii) that fasted during incubation on their Arctic breeding grounds. Our findings have important ramifications to dietary studies using stable-nitrogen isotope analysis.

METHODS

CAPTIVE QUAIL

Ten 10-day old Japanese Quail (Coturnix japonica) were randomly selected from a quail colony at the University of Saskatchewan. Birds were raised from hatch on a single, homogenized batch of commercial turkey starter and this food was used throughout the experiment. The quail were then randomly split into control and experimental groups each containing five birds (one control bird that later became sick was excluded from analysis). Experimental birds were fed a ration designed to maintain but not increase their body mass whereas control birds were fed the same food ad libitum. Both groups were maintained under identical environmental conditions with ad libitum access to water. Quail were raised until age 28 days before tissue isotope ratios were determined. All statistical procedures were performed using the Statistical Analysis System (SAS Institute 1985).

WILD ROSS' GEESE

Temporal patterns of stable-carbon and nitrogen isotope ratios in tissues of female Ross' Geese were investigated during the breeding season, 1991, at Karrak Lake, Northwest Territories, the site of the largest known breeding colony of Ross' Geese (Ryder 1969). Geese arrived in early June with body reserves accumulated on wintering and migratory staging grounds. Clutches are initiated soon after arrival at Karrak Lake (Ryder 1972, pers. observ.). Incubation ranges 19–25 days, averaging 22 days (Ryder 1972). During this time, females remained on their territories where they essentially went without food until the young hatched and family groups left the immediate area (Ryder 1970, Ankney 1977, pers. observ.).

As part of an ongoing study of the migratory energetics of geese at this colony, five adult female Ross' Geese were collected on arrival at Karrak Lake, 7 June 1991. Birds were weighed and external measurements taken before dissection. The left pectoralis, abdominal fat deposits, liver, and reproductive organs were removed and weighed. Pectoralis and liver were subsampled and frozen for stable isotope analysis. All birds were adults about to commence breeding as evidenced by development of gonads. On 8 July, 1991, five female Ross' Geese that had just completed incubation were collected.

TISSUE PREPARATION AND ISOTOPIC ANALYSIS

Muscle, liver and blood samples were freeze-dried and then ground to a fine powder in an analytical mill. Feathers were cleaned of surface contaminants using ether, air dried, and then cut with stainless steel scissors into small fragments. Bone collagen was extracted from cleaned bones (see Longin 1971, Chisholm et al. 1983) and then freeze-dried. Lipids were removed from all except feather samples using a Soxhlet apparatus with chloroform solvent for 4–6 hr.

Samples for $^{13}$C analysis were loaded into Pyrex® tubes with 1 g CuO and silver wire, sealed under vacuum and then combusted at 550°C for 6 hr. After cryogenic distillation, the resultant CO$_2$ was analyzed using a VG-SIRA 12 mass spectrometer.

Nitrogen samples were first converted to ammonia using Kjeldahl digestion. Ammonia was then converted to N$_2$ gas using LiBrOH (Porter and O’Dean 1977). Nitrogen gas was analyzed using a dual inlet VG Micromass 602E mass spectrometer. Stable isotope ratios are expressed in δ notation as parts per thousand (‰) according to:

$$\delta X = \left( \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1,000$$

where $X = ^{13}$C or $^{15}$N and $R = ^{13}$C/$^{12}$C or $^{15}$N/$^{14}$N. Precision (+1 SD) of isotopic measurements, based on glycine (nitrogen) and collagen (carbon) standards, was ±0.1 and ±0.3‰, respectively.

Amino acid analyses of muscle tissues were performed using 6 N HCl for hydrolysis (22 hr at 100°C, with air excluded) and an ion exchange sodium-based column for derivitization. Analyses were performed on a Perkin-Elmer HPLC.

RESULTS

QUAIL SAMPLE

Quail fed a rationed diet maintained, but did not increase, body mass compared to the control
Experimental group (Fig. 1, mass change over 17 days: control, 78.1 ± 3.4 g, experimental, 2.0 ± 3.1 g; t-test, t = 37.2, df = 7, P < 0.001). With the exception of feathers, tissues of the experimental group showed 15N/14N values significantly enriched over those of the control group (Fig. 2). Amino acid profiles of the collagen from experimental and control groups resembled those of other modern animals with high concentrations of glycine, proline and alanine (Hare 1980; Table 1). Amino acids of bone collagen did not differ between control and experimental quail, except with glutamic acid (Table 1). The cumulative distributions of 16 amino acids found in bone collagen of the two groups did not differ (Kolmogorov-Smirnov test, K = 0.19, P = 0.94; the group mean value was used because no within-group heterogeneity was found).

TABLE 1. Results of amino acid assays of bone collagen from quail raised on rationed (experimental) and ad libitum (control) diets. Amino acid concentrations are expressed as mole percent (mean ± SD). Sample sizes given in parentheses.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Control (4)</th>
<th>Experimental (5)</th>
<th>t</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartate</td>
<td>6.4 ± 0.3</td>
<td>7.0 ± 0.2</td>
<td>10.7</td>
<td>NS</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.1 ± 0.1</td>
<td>2.4 ± 0.2</td>
<td>11.7</td>
<td>NS</td>
</tr>
<tr>
<td>Serine</td>
<td>2.4 ± 0.1</td>
<td>2.7 ± 0.3</td>
<td>4.9</td>
<td>NS</td>
</tr>
<tr>
<td>Glutamate</td>
<td>8.8 ± 0.5</td>
<td>10.0 ± 0.3</td>
<td>19.6</td>
<td>*</td>
</tr>
<tr>
<td>Proline</td>
<td>8.9 ± 1.0</td>
<td>8.1 ± 0.5</td>
<td>2.6</td>
<td>NS</td>
</tr>
<tr>
<td>Glycine</td>
<td>30.7 ± 0.7</td>
<td>29.1 ± 2.5</td>
<td>1.7</td>
<td>NS</td>
</tr>
<tr>
<td>Alanine</td>
<td>10.9 ± 1.3</td>
<td>10.4 ± 0.6</td>
<td>0.6</td>
<td>NS</td>
</tr>
<tr>
<td>Valine</td>
<td>2.1 ± 0.1</td>
<td>2.5 ± 0.2</td>
<td>13.4</td>
<td>NS</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.1</td>
<td>NS</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>1.7 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>9.5</td>
<td>NS</td>
</tr>
<tr>
<td>Leucine</td>
<td>3.1 ± 0.3</td>
<td>3.0 ± 0.7</td>
<td>0.01</td>
<td>NS</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.2 ± 3.3</td>
<td>0.7 ± 0.04</td>
<td>1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>1.9 ± 0.1</td>
<td>2.0 ± 0.1</td>
<td>1.9</td>
<td>NS</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.0 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>1.4</td>
<td>NS</td>
</tr>
<tr>
<td>Lysine</td>
<td>4.2 ± 0.7</td>
<td>4.7 ± 0.2</td>
<td>3.4</td>
<td>NS</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.0 ± 0.3</td>
<td>5.0 ± 0.2</td>
<td>0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

* The alpha criterion was adjusted to account for multiple comparisons.
ROSS' GOOSE SAMPLE

After fasting for approximately 4 weeks, female Ross' Geese lost significant amounts of somatic tissue mass including abdominal fat, pectoral muscle, and liver mass (Table 2). Females showed significant increases on the $\delta^{15}N$ ratios of both pectoral muscle (Wilcoxon rank-sum test, $Z = 2.38$, $n_1 = 4$, $n_2 = 5$, $P < 0.05$) and liver ($Z = 2.51$, $n_1 = 4$, $n_2 = 5$, $P < 0.05$, Fig. 3). However, $\delta^{13}C$ values for these tissues showed no change over the fasting period (muscle $\delta^{13}C$ before $= -24.3 \pm 1.2\%$, after $= -24.7 \pm 0.4\%$, $Z = 0.9$, $n_1 = 4$, $n_2 = 5$, $P > 0.4$; liver $\delta^{13}C$ before $= -25.8 \pm 0.7\%$, after $= -25.6 \pm 0.6\%$, $Z = 0.7$, $n_1 = 4$, $n_2 = 5$, $P > 0.5$). Tissue type did not significantly influence $\delta^{15}N$ values (ANOVA: $F = 2.19$, df $= 1$, $P = 0.16$) and there was no significant interaction between tissue and period of collection ($F = 3.19$, df $= 1$, $P = 0.09$).

DISCUSSION

POSSIBLE EXPLANATIONS FOR $^{15}N$ ENRICHMENT IN FASTING BIRDS

We have demonstrated that significant enrichment in $^{15}N$ occurs in the tissues of both nutritionally stressed captive and fasting wild birds during incubation. Ambrose and DeNiro (1986) similarly observed that $\delta^{15}N$ values of the bone collagen of drought-tolerant herbivores were higher than those of water-dependent species in East Africa and related this effect to physiological processes relating to mechanisms of water conservation.

These authors noted that herbivorous mammals on diets high in protein have the capacity to excrete highly concentrated urine under conditions of heat and water stress and that this is accompanied by often spectacular increases in urea output. Urea is the major form of excreted nitrogen in mammals and is significantly depleted in $^{15}N$ relative to the diet (Steele and Daniel 1978). The excretion of a more concentrated urine with a quantitative increase in the excretion of $^{14}N$-depleted urea in water-stressed mammals would result in higher $^{15}N/^{14}N$ ratios in the unexcreted nitrogen. Subsequent $^{15}N$ enrichment of the remaining body nitrogen incorporated into tissues is expected from considerations of isotopic mass balance (see also Ambrose and DeNiro 1987). We propose an analogous mechanism to account for apparent enrichment of $^{15}N$ observed in fasting birds.

Nitrogen available for the synthesis of body proteins can be recycled from metabolic amino acid pools with inputs from assimilated foods and through protein breakdown or catabolism. A primary source of nitrogen isotopic fractionation is believed to occur during processes of deamination and transamination of amino acids (Gaebler et al. 1966, Macko et al. 1982, Minagawa and Wada 1984). In this way, metabolized

<table>
<thead>
<tr>
<th>Variable</th>
<th>Arrival (n = 5)</th>
<th>Post incubation (n = 5)</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body$^1$</td>
<td>1,739.2 ± 42.5</td>
<td>954.0 ± 57.3</td>
<td>*</td>
</tr>
<tr>
<td>Pectoralis</td>
<td>147.2 ± 8.5</td>
<td>82.2 ± 9.5</td>
<td>*</td>
</tr>
<tr>
<td>Abdominal fat</td>
<td>61.4 ± 13.9</td>
<td>1.3 ± 0.5</td>
<td>*</td>
</tr>
<tr>
<td>Liver</td>
<td>39.4 ± 1.8</td>
<td>16.4 ± 1.7</td>
<td>*</td>
</tr>
</tbody>
</table>

$^1$ Body mass minus mass of reproductive organs and their contents.

$^*$ Results of Wilcoxon rank-sum test. $^* P < 0.05.$
amino acids are enriched during anabolism, and nitrogenous waste products depleted in $^{15}$N relative to diet (e.g., Steele and Daniel 1978). Under conditions of fasting and nutritional stress, a greater proportion of nitrogenous compounds available for protein synthesis are derived from catabolism and, since this source of nitrogen has already been enriched in $^{15}$N relative to diet, additional enrichment in the metabolic nitrogen pool must occur. A consequence of this process would be eventual enrichment in $^{15}$N of all body tissues relative to periods without stress (see also Swick and Benevenga 1977).

The extent of $\delta^{15}$N enrichment in tissues due to fasting and nutritional stress should be influenced by the isotopic turnover rates in those tissues. Metabolically active tissues (e.g., liver) are expected to more readily show the effects of enrichment due to stress over tissues with slower isotopic turnover (e.g., bone collagen in adult birds, Tieszen et al. 1983). Consistent with this suggestion is our finding that adult female Ross' Geese showed a greater enrichment in liver $\delta^{15}$N values compared with those of muscle (Fig. 3). Experimental quail showed high $\delta^{15}$N enrichment in both liver and bone collagen but these birds were still growing and bone isotope values were more dynamic than that expected for adult birds.

We found no evidence that changes in fractionation of tissue carbon occur as a result of fasting or nutritional stress (see Hobson and Clark 1992). In animals, carbon isotopic fractionation occurs primarily during respiration with $^{12}$C being preferentially lost as $^{12}$CO$_2$ during the oxidation of acetyl groups derived from the catabolism of lipids, proteins, and carbohydrates (reviewed by Galimov 1985). However, this fractionation effect is generally less pronounced than that described for nitrogen (DeNiro and Epstein 1978) and other studies have shown no such enrichment effect for carbon (Teeri and Schoeller 1979, Macko et al. 1982, Stepehenson et al. 1984). Amino acids differ in their $\delta^{15}$N values (Macko et al. 1983) and changes in the amino acid composition of tissues due to differential hydrolysis of amino acids during periods of stress (Felig 1975) might also account, in part, for $^{15}$N enrichment of body tissues. However, amino acid profiles of the bone collagen of control and experimental quail differed only slightly, suggesting that the main enrichment effect observed in the tissues of stressed birds was likely due to additional isotopic fractionation associated with catabolism, protein mobilization, or redeposition processes.

CONSEQUENCES FOR DIETARY INVESTIGATIONS USING $\delta^{15}$N ANALYSIS

Stable isotope analysis of the tissues of wild animals and their prey to delineate diet or trophic relationships within communities offers numerous advantages over conventional approaches (e.g., Ambrose and DeNiro 1986, Peterson and Fry 1987). However, the reliability of the isotope approach depends directly on our understanding of processes contributing to the abundance of stable isotopes in consumer tissues. Studies using $\delta^{15}$N analysis to infer diet or trophic position must take account of the nutritional history of the individuals whose tissues are being examined.

The effect of $^{15}$N enrichment associated with changes in body composition is particularly relevant to isotopic studies of birds, especially the many species who lose body mass during egg-laying and incubation. Among waterfowl, for example, it was previously felt that most of this mass loss reflected gonadal regression and depletion of fat reserves (e.g., Hanson 1962, Harris 1970). However, breast, leg and gizzard muscles are important protein reserves during incubation in Arctic-nesting Lesser Snow and Ross' Geese (this study, Ankney and MacInnes 1978). As noted by Ankney and MacInnes (1978), even in species in which the incubating female does not fast, considerable mass loss occurs (Weller 1957, Oring 1969, Anderson 1972). Penguins also fast during egg-laying (Richdale 1947) and several species of seabirds may undergo periods of food stress due to competition near colonies or crashes in prey stocks (e.g., Hunt et al. 1986, Erikstad 1990). In addition, nestling seabirds may experience protracted periods of growth due to intermittent food provisioning (e.g., Ricklefs et al. 1980). Although there is tremendous potential for the application of stable-isotope analysis to avian dietary studies, we caution researchers to consider possible $^{15}$N enrichment of tissues in some individuals or species due to physiological effects rather than diet per se.

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