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Stable carbon isotope analyses show dominance of C3 grasses in the feed selected by small herbivores at the alpine meadow at the Tibetan Plateau

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ABSTRACT

Muscle samples were collected from small herbivorous mammals (*Ochotona curzoniae*, *Microtus oecnomus*, *Myospalax fontanierii* and *Lepus oiostolus*) at the alpine meadow ecosystem at the Tibetan Plateau in order to address variability in stable carbon isotope composition. Stable carbon isotope values of muscles remain steady and show no significant variations (-25.72 to -27.04‰) among the four small mammal species. Based on the mass balance theory of stable isotopes, it is proposed that small herbivorous mammals mainly (or totally) rely on C3 grasses as food supply, and there is few or no distribution of C4 grasses at the ecosystem. The results reflect our previous study on the isotope patterns of plant species. Thus, stable carbon isotope analysis of muscles provides a method to address dietary selection and dietary variability in herbivores. In addition, stable carbon isotopic analyses can be used to address changes in vegetation distributions in ecosystem and paleovegetaion and paleoclimate.

KEY WORDS: stable carbon isotope, small mammal, C3/C4 plant dominance, vegetation composition, alpine meadow, Tibetan Plateau

INTRODUCTION

The analysis of stable isotopes in tissue samples will provide information about the average diet of individuals over a longer period (DeNiro and Epstein, 1978a),

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even a lifetime in a relatively short-lived animal. The stable isotope approach is based on the fact that naturally occurring stable isotope ratios in consumer tissues can be related to those in consumers' diets (DeNiro and Epstein, 1978a,b). Thus, isotope measurement of consumers' tissue can reveal information about their ingested foods and vegetation characteristics in ecosystems that are relatively simple and do not involve multiple isotopic inputs. In most herbivores, stable carbon isotope analyses of tissue samples provide information about recent dietary averages (DeNiro and Epstein, 1978b; Yi et al., 2004a,b). As the carbon isotopic composition of plants is correlated with that of animals, herbivorous muscle has the potential to provide an average isotopic signal for particular environments (Ehleringer and Cooperm, 1988; Korner et al., 1988; Cormie and Schwarcz, 1994.) due to its fast turnover rate. Terrestrial plants perform three distinct photosynthetic pathways (C3, C4 and CAM) that produce characteristic $\delta^{13}C$ tissue values (O'Leary, 1988). Plants using the C4 (Hatch-Slack) pathway have markedly higher $\delta^{13}C$ values (-17~-11‰) than plants employing the C3 pathway (-34~-25‰) (Bender, 1971; Smith and Epstein, 1971; Bender et al., 1972). Mean $\delta^{13}C$ values of -13.1±1.2‰ for C4 plants and -27.1±2.0‰ for C3 plants are reported for a large number of species grown under a variety of conditions (O'Leary, 1988). These distinct isotopic signatures in C3 and C4 plants are passed on to the tissues of consumers with a small change or fractionation related to biochemical processes within the animals. It was reported that changes in, or fractionation of, stable carbon isotope ratios ($^{13}C/^{12}C$) in muscle occur with trophic level and are of the order of 1 to 2‰ (DeNiro and Epstein, 1978a,b; Hobson and Clark, 1992; Hilderbrand et al., 1996). This paper addresses carbon isotope values of muscles of small herbivorous mammals, and tentatively reveals the distribution patterns of C3 and C4 grasses based on mass balance theory at the alpine meadow at the Tibetan Plateau.

MATERIAL AND METHODS

The study was carried out at the Haibei Alpine Meadow Ecosystem Research Station (HAMERS) of the Chinese Academe of Sciences (CAS), which was established in 1976 in order to understand the structure and function of alpine meadow ecosystem, type and development of biodiversity, the adaptive and strategies of species, and the impact of global changes on grassland ecosystem.

The HAMERS is located in the region of the Qinghai-Tibet Plateau, in a large valley oriented NW-SE surrounded on all sides by the Qilian Mountains with a range of 37°29' - 37°45'N and 101°12' - 101°33'E. The average altitude of the mountain area is 4000 m above sea level and 3200 m for the valley area.

The climate of the HAMERS is dominated by the southeast monsoon and the higher-pressure system of Siberia. It has a continental monsoon type climate, with severe and long winters and short cool summers. The average air temperature is -1.7°C with extreme maximum of 27.6°C and minimum -37.1°C. During winter months, the average temperature drops to -15 to -20°C in the highland area; during summer, the temperature in the warmest month (July) averages 14 - 22°C in the valleys and 4 - 10°C in the mountains. Average annual precipitation ranges from 426 to 860 mm, 80% of which falls in the short summer growing season from May to September. The annual average sunlight is 2462.7 h with 60.1% of total available sunshine. Vegetation was characterized by alpine shrub, alpine meadow, and swamp meadow. The research site was roughly confined in alpine meadow, with *Kobresia humilis* as dominant species and *Polygonum viviparum, Carex atro-fusca, Saussurea superba, Elymus nutans* and *Gentiana straminea* as sub-dominant species.

Back leg muscles of plateau pika *(Ochotona curzoniae),* root vole *(Microtus oecnomus),* plateau zokor *(Myospalax fontanierii)* and plateau hare *(Lepus oiostolus)* were collected in June and July 2003 and were air dried indoors to constant mass in an oven at 70°C for 48 h, ground finely, and dispatch to isotope ratio spectrometer under EAMS (element-analysis meter and spectrometer) condition. Interface between element-analysis meter and spectrometer is ConFlow III. Operation condition for the oxidizing furnace was a temperature of 900°C, reducing furnace at 680°C, pillar temperature at 40°C. The resulting CO_2, were purified in a vacuum line and injected in a Finnigan MAT DELTA^{PLUS}XL spectrometer (Finnigan Mat, Bremen, Germany) fitted with double inlet and collector systems. The results are expressed in $\delta^{13}C$ relative to the standards in the conventional δ per mil notation as follows:

$$^{13}C\ [\delta‰] = \frac{^{13}C{:}^{12}C_{sample} - {}^{13}C{:}^{12}C_{PDB}}{^{13}C{:}^{12}C_{PDB}} \times 1000$$

where $^{13}C{:}^{12}C$ are the isotopic ratios of sample and PDB (Peedee Belemnite formation from South Carolina, USA) standard. The overall (sample preparation plus analysis) analytical precision is ± 0.2‰.

C3 and C4 distribution pattern (PS) can be retrieved from stable carbon isotopic values of small mammals as shown in the following equation (Forsberg et al., 1993):

$$PS_{C4}\ (\%) = (\delta^{13}C_{animal} - E - \delta^{13}C_{C3}) / (\delta^{13}C_{C4} - \delta^{13}C_{C3}) \times 100$$

where PS_{C4} indicates relative percentage of C4 plants; $\delta^{13}C_{animal}$ refers to average stable isotopic value of small mammals muscle; $\delta^{13}C_{C3}$ and $\delta^{13}C_{C4}$ represent average values of C3 (-27.1‰) and C4 (-13.1‰) plants, respectively (O'Leary, 1988); E indicates the fractionation factor between diets and muscles, where1.05‰ is used to offset isotopic enrichment during carbon assimilation (Yi et al., 2004b).

RESULTS AND DISCUSSION

The results of this study show that $\delta^{13}C$ values in tissues of small mammals ranged from -24.67 to -25.99‰ and show minor variations (Table 1), which suggests that diets of small mammals predominantly are based on C3 plants. Based on the mass balance theory of stable isotopes, the distribution percentages of C3 and C4 plants are 94.68 and 5.32%, respectively, at the alpine meadow ecosystem.

Table 1. ^{13}C values of small mammals tissues and the calculated percentages of C3 and C4 vegetation composition by the herbivores

Species	^{13}C, mean δ‰	^{13}C, SD δ‰	C4 %	C3 %
Root vole (02.05)[1]	-25.48 (4)[3]	0.01	4.07	95.93
Root vole (02.07)[2]	-25.99 (3)	0.14	0.43	99.57
Root vole (02.07)	-25.92 (3)	0.44	0.93	99.07
Plateau zokor (02. 07)	-25.78 (4)	0.36	1.93	98.07
Plateau pika (02.05)	-25.07 (4)	0.55	7.00	93.00
Plateau pika (02.07)	-24.77 (3)	0.04	9.14	90.86
Plateau pika (02.07)[2]	-24.76 (3)	0.28	9.21	90.79
Plateau hare	-24.67 (2)	0.11	9.86	90.14
Average	-25.31	0.43	5.32	94.68

[1] data in parentheses represent collecting date; [2]refers to correspondent infants; [3]number in parentheses indicates sample size

The stability in muscle $\delta^{13}C$ values observed among the small mammals suggests that stable carbon isotope analyses can contribute to dietary information and plant distribution at the alpine meadow. Patterns of muscle $\delta^{13}C$ values can be attributed to inclusion of varying amounts of C3 and C4 grasses in animal diets. The more negative muscle $\delta^{13}C$ values reveal that animal rely more on C3 plants than C4 types. The results indicate that three small mammals mainly depend on C3 plants as food supply (Table 1), which firmly reflects our previous studies on stable carbon isotopic patterns of the plant populations (Yi et al., 2003a; Li et al., 2004a) and 0-5 cm layer soils (Yi, 2005a). Despite the isotopic composition of muscles truly reflecting community component of C3 and C4 plants, there is still a discrepancy between the previous and present studies. In the earlier study on 102 plant species (Yi et al., 2003b), there is no C4 type plant found at the study area, however, a percentage of 5.32% of C4 plants was obtained in this study. The observed differences in C4 plant distribution can be attributed to (i) 1.05‰ is used in the study as fractionation factor within the trophic levels to offset the isotopic enrichment during assimilation process. However, different fractionation values from plants to herbivore tissues are observed (DeNiro and Epstein, 1978a,b), which can result in some errors; (ii) there is a general variations in $\delta^{13}C$ values of C3 plants at the ecosystem (Yi et al., 2003b), the average values of -13.1‰ and -27.1‰ for C4 and C3 plants coarsely used may cause some differences; (iii) fractionation taking place in the metabolism of the nutrients in body and the different enrichment percentage in exhaled gases during process in body may show some impact. The stable isotope approach is based on the fact that naturally occurring stable isotope ratios in consumer tissues can be related to those in consumers' diets (DeNiro and Epstein, 1978a). Stable carbon isotopic patterns in muscles can reveal food base information of animals in given ecosystems. It seems that small mammals prefer to dicotyledon rather than monocotyledon in summer based on the stable isotopic values in this study and earlier data for plant species. The muscle stable carbon isotopic data suggest that it is reasonable to use small mammals to reveal the abundance and distribution of different photosynthetic pathway grasses within the ecosystem. Stable isotope analyses of plant and animal tissues are providing additional methods to study modern ecological systems (Rundel et al., 1989; Yi et al., 2003b; Li et al., 2004a; Yi, 2005a,b; Yi and Zhang, 2005) even paleoecology (Cormie and Schwarcz, 1994; Jahren et al., 1998; Boutton et al., 1999; Franz-Odendaal et al., 2002; Beazley et al., 2003).

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