Shifting to an energy-poor diet for nitrogen? Not the case for wintering herbivorous Lesser White-fronted Geese in China

Xin Wang · Anthony D. Fox · Xuliang Zhuang ·
Lei Cao · Fanjuan Meng · Peihao Cong

Received: 27 August 2013 / Revised: 26 January 2014 / Accepted: 17 February 2014 / Published online: 9 April 2014
© Dt. Ornithologen-Gesellschaft e.V. 2014

Abstract Geese often forage on mid-winter foods that fail to satisfy daily energy needs, but they may do so to acquire other nutrients, such as nitrogen. We tested this hypothesis by evaluating nitrogen budgets, namely the balance of nitrogen income against expenditure, of wintering Lesser White-fronted Geese *Anser erythropus* feeding at two sites within East Dongting Lake, China, where they could and could not balance daily energy budgets. Geese could balance nitrogen budgets in energy-rich habitats but were less able to do so in habitats where they failed to balance energy budgets. This study presents the first full nitrogen budget for a wintering goose species, and suggests that, rather than acting as a source of nitrogen, use of energy-poor but undisturbed habitats may represent a refuge from human disturbance at other habitats.

Keywords East Dongting Lake · Energy budget · Foraging decisions · Nitrogen budget · Recessional grasslands · Uric acid

Zusammenfassung

Wechseln zu energieärmer Nahrung zugunsten der Stickstoffaufnahme? Bei überwinternden Zwerggänsen in China ist das nicht der Fall

Zur Mitte des Winters ernähren sich Gänse häufig von Futterpflanzen, mit denen sie zwar nicht ihren täglichen Energiebedarf decken können, die ihnen aber zur Versorgung mit anderen Nährstoffen wie zum Beispiel Stickstoff dienen. Wir überprüften diese Hypothese, indem wir die Stickstoffbilanz in Form des Verhältnisses zwischen Stickstoffaufnahme und Stickstoffverbrauch bei überwinternden Zwerggänsen *Anser erythropus* untersuchten, die auf zwei Flächen am östlichen Dongting-See in China Nahrung suchten, wobei sie auf einer von diesen ihre Energiebilanz ausgleichen konnten, auf der anderen dagegen nicht. Die Gänse konnten ihren Stickstoffhaushalt in energiereichen Habitaten ausgleichen; dies gelang ihnen aber weniger gut in Lebensräumen, in welchen sie keine ausgewogene Energiebilanz erreichten. Diese Studie stellt die erste vollständige Stickstoffbilanz für eine überwinternde Gänseart dar, und die Ergebnisse sprechen eher dafür, dass energiearme aber störungsfreie Habitate als Rückzugsgebiete von Störungen durch Menschen an anderen Orten genutzt werden, als dass sie eine Rolle als Stickstoffquelle spielen.

X. Wang · X. Zhuang · L. Cao (✉) · F. Meng
Key Laboratory of Environmental Biotechnology, Research
Center for Eco-Environmental Sciences, Chinese Academy of
Sciences, Beijing 100085, China
e-mail: leicao@rcees.ac.cn

A. D. Fox
Department of Biosciences, Aarhus University,
Kalø, Grenåvej 14, 8410 Rønde, Denmark

L. Cao · P. Cong
School of Life Sciences, University of Science and Technology
of China, Hefei 230026, Anhui, China

Introduction

Animals need to regulate their intake of a variety of nutrients that are critical to survival (e.g. Mayntz et al. 2005). In situations with a choice of diets differing in two

nutrients, typically carbon (energy) and nitrogen in terrestrial endotherms (White 1993; Klasing 1998), organisms will concentrate on one food type until need induces a temporary switch to supplement the scarcer nutrient (Houston et al. 2011). This mechanism may explain the unexpected shifts in distribution of free-living animals, when food is heterogeneously distributed and varies in nutrient profitability (e.g. Ely and Raveling 2011).

The globally threatened Lesser White-fronted Goose *Anser erythropus* is designated as ‘Vulnerable’, as a result of its continuous range contraction and declines in abundance (BirdLife International 2010). Unlike most other wintering Anatidae species, the wintering Lesser White-fronted Goose is highly restricted within the Yangtze River floodplain, and specifically at East Dongting Lake, which supports more than 90 % of the Eastern Palearctic wintering population (Wang et al. 2012). The extraordinarily concentrated distribution implies that this species is vulnerable to winter habitat loss and degradation, emphasizing the urgent requirement to understand what determines its localized distribution.

Recent studies of Lesser White-fronted Goose at East Dongting Lake suggest that its unique biogeographical distribution is shaped by its dietary reliance on heterogeneous grasslands, and specifically, on *Alopecurus* spp., *Eleocharis* spp. and *Cynodon dactylon* (Wang et al. 2013). At Caisang Lake, a regular grazing site within East Dongting Lake (see Fig. 1), geese mainly feeding on *Alopecurus*, *Cynodon* and *Eleocharis* could achieve positive energy budgets, while at Daxi Lake, another site within East Dongting Lake, geese grazing *Carex* spp., which is widespread in the Yangtze River floodplain, failed to provide a profitable source of energy (see Fig. 5 of Wang et al. 2013). This finding explains the restricted distribution of the Lesser White-fronted Goose at East Dongting Lake, but begs a new question: since disturbance and inter- and intra-specific competition at both sites was negligible (Wang, in preparation), why did the geese exploit *Carex* at Daxi Lake?

In order to address this question, we propose a new hypothesis, namely that geese fed on energy-poor *Carex* at Daxi Lake to fulfil nutritional needs other than energy, and

Fig. 1 The location of the study area within China, showing the position of East Dongting Lake (inset right) and the study sites, Caisang and Daxi Lakes; the open circle indicates the monitoring and management station of the nature reserve (lower inset)

speculate specifically that this could be nitrogen, because of its crucial role in protein synthesis. Although herbivorous endotherms should generally select food according to digestible carbon (i.e. energy) rather than nitrogen (Klaassen and Nolet 2008), studies suggest that nitrogen content may play a role (e.g. Bucher et al. 2003; Sedinger 1997), perhaps because (1) avian migrants on wintering grounds have elevated nitrogen or protein requirements at certain stages of the lifecycle, e.g. in autumn when birds need to rebuild organs reduced in size during migration (Piersma and Lindstrom 1997), and spring when premigratory muscle hypertrophy occurs (Evans et al. 1992); and (2), even outside these periods of heightened nitrogen demand, migratory avian herbivores may also need to select for high nitrogen foods in order to meet their requirement for self-maintenance if many of the available foods have low levels of nitrogen.

According to this hypothesis, we formulated the predictions that: (1) *Carex* was higher in nitrogen than *Alopecurus*, *Cynodon* and *Eleocharis*, or that the nitrogen assimilation efficiency of the *Carex*-dominant diet at Daxi Lake was higher than that at Caisang Lake, where geese mainly fed on *Alopecurus*, *Cynodon* and *Eleocharis* (Wang et al. 2013); (2) the metabolisable dietary nitrogen intake at Daxi Lake was higher than at Caisang Lake; (3) the nitrogen budget, i.e. the balance of nitrogen income against expenditure, at Daxi was positive and higher than at Caisang Lake; and (4) daily uric acid excretion at Daxi was higher than at Caisang Lake, because the uric acid excretion generally increases as the nitrogen budget becomes more positive (Allen and Hume 2001). To examine these predictions, we estimated, for the first time in wild geese, monthly nitrogen budgets of wintering Lesser White-fronted Geese in two distinct habitat types.

Methods

Study sites and duration

The study was conducted at Caisang and Daxi Lakes in the northwest of East Dongting Lake (Hunan Province, China, 29.32°N, 112.98°E; Fig. 1) which support considerable numbers of Lesser White-fronted Geese (Cong et al. 2012; Wang et al. 2012). Separated by a dyke, Daxi and Caisang Lakes experience different water regimes which affect the vegetation present: Caisang Lake is controlled by a sluice, while Daxi Lake is directly connected to the Yangtze River. *Carex* was dominant at Daxi Lake, whereas heterogeneous vegetation, including *Alopecurus*, *Eleocharis* and *Carex*, predominated at Caisang Lake. These vegetation differences were reflected in contrasting dietary composition and energy budgets of geese exploiting the two sites

(Wang et al. 2013). Both lakes are adjacent to a management station of the East Dongting Lake Nature Reserve, which prevents hunting and other human disturbance. We conducted this study from early November 2009 to late March 2010, the entire Lesser White-fronted Goose wintering period at East Dongting Lake.

Nitrogen budget estimation

We estimated goose nitrogen budgets by comparing the content in food and goose droppings corrected for uptake, using an indigestible marker, acid detergent fibre (ADF), following the detailed methods in Wang et al. (2013). We calculated the mass of daily ingested food (m_F) using this simplified equation:

$$m_F = \frac{ADF_d}{ADF_p} \times m_D$$

where ADF_d and ADF_p are the ADF percentage in droppings and plants respectively, m_D is the daily output of dropping mass (estimated from daily active time and dropping intervals), and average mass of droppings. Total nitrogen content of edible plant leaves and droppings was determined by an Elementar Rapid N Cube (Elementar Analysensysteme, Hanau, Frankfurt, Germany), from which we calculated the daily total ingested nitrogen (N_I) and daily total dropping nitrogen, and calculated their difference as the net nitrogen budget (N_B). The total dropping nitrogen is comprised of egested dietary (undigested) nitrogen (N_E) and the products of dissimilated (endogenous) nitrogen excretion, mainly in the form of uric acid excretion (Fox and Kahlert 1999). We measured the uric acid content in droppings following the method of Marquardt (1983), and then calculated the uric acid nitrogen excretion (N_U) by multiplying by the coefficient 0.333 (the nitrogen proportion in uric acid molecular). Based on the assumption that the endogenous nitrogen came only from uric acid in goose species, we calculated the daily dietary egested (undigested) nitrogen (N_E) by subtracting N_U from daily total dropping nitrogen. Furthermore, we calculated the metabolizable dietary nitrogen intake (N_M) (the difference between N_I and N_E), and the nitrogen assimilation efficiency (N_M divided by N_I). We accept that some small amounts of volatile ammonia are likely lost from faeces after ejection, but consider this trivial in amount and unlikely to contribute to differences between habitats in this analysis. All mass values were presented on an ash-free dry mass basis.

Sample collection and treatment

Sample collection and treatment follow Wang et al. (2013). Forty fresh and intact goose droppings were collected at

weekly or fortnightly intervals ('sampling period' as below), according to goose exploitation of the study sites. We assessed diet composition at the two sites by faecal analysis (Wang et al. 2013), and collected edible parts of major dietary plants every month according to goose diet. All samples were oven-dried, weighed and analysed for nitrogen and ADF as described above. All measurements are presented in ash-free dry mass values.

To estimate nitrogen budget from daily dropping mass, we obtained at least 20 goose dropping intervals for each sampling period by direct observations, and assumed that the geese defecated at even intervals throughout the entire period of the daytime when geese were active, which is defined as the time interval between when the geese reached the studied feeding sites and when they returned to the roost. The active daytime was determined according to the daylong activity budget observations as presented in Wang et al. (2013).

Statistical analyses

Statistical analyses were done using R 2.12.2 (R Development Core Team 2011). Two-tailed two independent sample *t* tests were used to test differences between nitrogen and ADF content in plants. To examine the effect

of 'foraging site' on the nitrogen assimilation efficiency, metabolizable dietary nitrogen, net nitrogen budget and uric acid, we applied linear mixed-effect models with 'month' as the random factor and 'foraging site' as the fixed factor, by using the 'lme' function in the 'nlme' package (Giraudeau 2012). The effect or difference was considered significant if $P < 0.05$.

Results

The results of food quality analyses failed to support our hypothesis regarding the nitrogen profitability at the energy-poor site due to the small sample size. The nitrogen content of *Carex* (3.69 ± 0.26 % SE, $n = 6$) was only non-significantly lower than in *Alopecurus* (4.67 ± 0.45 % SE, $n = 7$, $t_{11} = -1.807$, $P = 0.098$), *Eleocharis* (4.99 ± 0.59 % SE, $n = 5$, $t_9 = -2.135$, $P = 0.062$) and *Cynodon* (5.00 ± 1.39 % SE, $n = 2$, $t_6 = -1.611$, $P = 0.158$). The ADF content of *Carex* (26.82 ± 0.92 % SE, $n = 6$) was significantly higher than in *Alopecurus* (20.31 ± 1.56 % SE, $n = 7$, $t_{11} = 3.430$, $P = 0.006$), but was not significantly greater than in *Eleocharis* (23.96 ± 1.87 % SE, $n = 5$, $t_9 = 1.454$, $P = 0.180$) and *Cynodon* (23.73 ± 1.94 % SE, $n = 2$, $t_6 = 1.616$, $P = 0.158$).

Fig. 2 Monthly **a** nitrogen assimilation efficiency, **b** metabolizable nitrogen intake (g/day), **c** nitrogen budget (g/day) and **d** nitrogen excreted as uric acid (g/day) ($\pm$ SE) at Caisang (closed circle) and Daxi (open square) Lakes. Dashed line in (c) indicates a nitrogen budget balance of zero

All the nitrogen parameters suggest higher nitrogen budgets at Caisang Lake than at Daxi Lake. The nitrogen assimilation efficiency at Daxi Lake was significantly lower than at Caisang Lake ($F_{1,23} = 22.56$, $P < 0.001$; Fig. 2a); the metabolizable nitrogen intake at Daxi Lake was significantly lower than at Caisang Lake ($F_{1,23} = 27.01$, $P < 0.001$; Fig. 2b); the overall nitrogen budget was negative at Daxi (-0.42 ± 0.27 SE g/day) but positive at Caisang (0.54 ± 0.50 SE g/day; Fig. 2c), although the effect of ‘foraging site’ on the nitrogen budget was not significant ($F_{1,23} = 2.96$, $P = 0.10$); significantly less nitrogen was excreted as uric acid at Daxi Lake than at Caisang Lake ($F_{1,23} = 4.90$, $P = 0.04$; Fig. 2d).

Discussion

To our knowledge, this is the first attempt to study changes in nitrogen budgets in free-living geese throughout an entire winter. At Caisang Lake, geese maintained a similar level of nitrogen assimilation efficiency and nitrogen budget throughout the winter (Fig. 2a, c), although metabolizable nitrogen intake and uric acid excretion increased in spring (Fig. 2b, d), probably the result of improved food availability and longer daylength. Elevated nitrogen metabolism in spring also probably reflects changes in goose physiology prior to migration: geese may rebuild muscles and other organs for migration, which requires protein (Piersma and Lindstrom 1997).

Our results failed to support our hypothesis about the nutritional determinants of distribution: wintering Lesser White-fronted Geese failed to gain more nitrogen by foraging on an energy-poor *Carex* diet at Daxi Lake. In general, *Carex* was lower in nitrogen but higher in ADF content than *Alopecurus*, *Eleocharis* and *Cynodon*, implying that extracting nitrogen compounds was more difficult and slower for geese feeding on *Carex*, whose cell walls were more fibrous and tougher, confirmed by estimates of nitrogen assimilation efficiency, metabolizable dietary nitrogen intake, nitrogen budget and uric acid excretion. All these results show that the *Carex* diet at Daxi Lake was inferior in balancing energy and nitrogen budgets, compared to the diet at Caisang Lake, consisting mainly of *Alopecurus*, *Eleocharis* and *Cynodon*.

Our results shed light on the debate about the effects of restricted food resources on endothermic herbivores, at least in the wintering period, because this study and another energetics study (Wang et al. 2013) provide the first simultaneous estimates of metabolizable energy and nitrogen intake in free-living wintering herbivorous birds. It is often stated that the limited availability of protein compared with other nutrients and energy in plants makes nitrogen the limiting resource and the driving force behind

much foraging behaviour (Mattson 1980; White 1993). However, this statement remains elusive because most, if not all, herbivorous bird studies claiming selection for nitrogen-rich food (e.g. Bucher et al. 2003; Sedinger 1997) failed to measure metabolisable energy content or intake simultaneously with the nitrogen budget. Based on biological stoichiometry principles, Klaassen and Nolet (2008) argue that the protein requirements of endotherms can be satisfied by eating most plants. Our nitrogen budget estimates, complementing the energy budgets of wintering Lesser White-fronted Geese at the same sites in the same year (Wang et al. 2013), show that the nitrogen-rich diet is also energy-rich, necessitating corresponding measures of energy and nitrogen intake before concluding about the limiting resources of the diet.

The question of why the Lesser White-fronted Geese exploited Daxi *Carex* meadows remains unresolved, since our results rejected the two hypotheses that the geese foraged on *Carex* for reasons of nitrogen or energy intake or profitability. Although animals may be constrained by elements and nutrients other than nitrogen (e.g. Hohman et al. 1992), such constraints have rarely been demonstrated, and the habitat choices of wintering geese are usually considered only in relation to energy and nitrogen demands. Wang et al. (2013) suggested that disturbance in foraging sites south of Daxi Lake, e.g. fishing, buffalo grazing and sand-extraction, may have caused geese to use the less disturbed Daxi Lake. Disturbance at foraging sites induces extra energy-costly behaviours, like flight and vigilance, reduces foraging opportunity and reallocates geese to less profitable feeding areas. As a result, geese exploiting other sites within East Dongting Lake might graze *Carex* temporarily at Daxi Lake, despite the poor foraging profitability of grazing *Carex* at Daxi Lake for nitrogen and energy. In order to examine this possibility, data on the habitat use and activity budget of the geese in relation to different levels of disturbance need to be collected at favourable and unfavourable sites in future studies. However, we cannot reject the hypothesis that *Carex* has a more attractive amino acid balance, especially in mid-winter, than the other dietary species (for example because of its higher content of essential or limiting amino acids such as methionine and lysine, Hohman et al. 1992, which vary between plant species, Felton 1996). Such a situation could contribute to an explanation for the lower uric acid excretion amongst geese consuming *Carex*, despite their similar overall nitrogen balance compared to those consuming the higher protein plants throughout the winter, except March. This possibility needs thorough investigations of plant chemical composition and goose metabolism in future studies. Finally, these studies were undertaken at the ‘population’ level without any insight into the behaviour of the individual. The use of individual

marking and telemetry would greatly enhance our understanding of how individuals move between these different feeding opportunities, both in regard to their exploitation of habitats and dietary items but also in response to disturbances to feeding associated with these areas.

Acknowledgments We thank East Dongting Lake National Nature Reserve for permission to work in the nature reserve and assistance with field work, and Liu Jing, Yang Xiuli, Yang Wei and Yu Xiaoxi for assistance. We are grateful to Eileen Rees and one anonymous referee for constructive and insightful comments on an earlier draft of the manuscript. The study was supported by the National Natural Science Foundation of China (Grant No. 31071941).

Conflict of interest The authors declare that they have no conflict of interest.

References

- Allen LR, Hume ID (2001) The maintenance nitrogen requirement of the zebra finch *Taeniopygia guttata*. *Physiol Biochem Zool* 74:366–375
- BirdLife International (2010) Lesser White-fronted Goose *Anser erythropus*. BirdLife International species factsheet. Available at <http://www.birdlife.org/datazone/speciesfactsheet.php?id=377>. Accessed 14 December 2011
- Bucher EH, Tamburini D, Abril A, Torres P (2003) Folivory in the white-tipped plantcutter *Phytotoma rutila*: seasonal variations in diet composition and quality. *J Avian Biol* 34:211–216
- Cong P, Wang X, Cao L, Fox AD (2012) Within-winter shifts in Lesser White-fronted Goose *Anser erythropus* distribution at East Dongting Lake, China. *Ardea* 100:5–11
- Ely CR, Raveling DG (2011) Seasonal variation in nutritional characteristics of the diet of greater white-fronted geese. *J Wildl Manag* 75:78–91
- Evans PR, Davidson NC, Uttley JD, Evans RD (1992) Premigratory hypertrophy of flight muscles: an untrastructural study. *Ornis Scand* 23:238–243
- Felton GW (1996) Nutritive quality of plant protein: sources of variation and insect herbivore responses. *Arch Insect Biochem Physiol* 32:107–130
- Fox AD, Kahlert J (1999) Adjustments to nitrogen metabolism during wing moult in Greylag Geese, *Anser anser*. *Funct Ecol* 13:661–669
- Giraudoux P (2012) pgirmess: data analysis in ecology
- Hohman WL, Jefferies RL, Gordon DH (1992) Ecology and management of post-breeding waterfowl. In: Batt BDJ, Afton AD, Anderson MG et al (eds) Ecology and management of breeding waterfowl. University of Minnesota Press, Minneapolis, pp 128–189
- Houston AI, Higginson AD, McNamara JM (2011) Optimal foraging for multiple nutrients in an unpredictable environment. *Ecol Lett* 14:1101–1107
- Klaassen M, Nolet BA (2008) Stoichiometry of endothermy: shifting the quest from nitrogen to carbon. *Ecol Lett* 11:785–792
- Klasing KC (1998) Comparative avian nutrition. CAB International, London
- Marquardt RR (1983) A simple spectrophotometric method for the direct determination of uric acid in avian excreta. *Poult Sci* 62:2106–2108
- Mattson WJ (1980) Herbivory in relation to plant nitrogen-content. *Annu Rev Ecol Syst* 11:119–161
- Mayntz D, Raubenheimer D, Salomon M, Toft S, Simpson SJ (2005) Nutrient-specific foraging in invertebrate predators. *Science* 307:111–113
- Piersma T, Lindstrom A (1997) Rapid reversible changes in organ size as a component of adaptive behaviour. *Trends Ecol Evol* 12:134–138
- R Development Core Team (2011) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna
- Robbins CT (1993) Wildlife feeding and nutrition, 2nd edn. Academic, New York
- Sedinger JS (1997) Adaptations to and consequences of an herbivorous diet in grouse and waterfowl. *Condor* 99:314–326
- Wang X, Fox AD, Cong P, Barter M, Cao L (2012) Changes in the distribution and abundance of wintering lesser white-fronted geese *Anser erythropus* in eastern China. *Bird Conserv Int* 22:128–134
- Wang X, Fox AD, Cong P, Cao L (2013) Food constraints explain distribution of wintering Lesser White-fronted Geese *Anser erythropus* in China. *Ibis* 155:576–592
- White TCR (1993) The inadequate environment. Springer, Berlin