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SHORT COMMUNICATION

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Individual protein balance strongly influences δ^{15}N and δ^{13}C values in Nile tilapia, *Oreochromis niloticus*

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Abstract Although stable isotope ratios in animals have often been used as indicators of the trophic level and for the back-calculation of diets, few experiments have been done under standardized laboratory conditions to investigate factors influencing δ^{15}N and δ^{13}C values. An experiment using Nile tilapia [*Oreochromis niloticus* (L.)] was therefore carried out to test the effect of different dietary protein contents (35.4, 42.3, and 50.9%) on δ^{15}N and δ^{13}C values of the whole tilapia. The fish were fed the isoenergetic and isolipidic semi-synthetic diets at a relatively low level. δ^{15}N and δ^{13}C values of the lipid-free body did not differ between the fish fed the diets with different protein contents, but the trophic shift for N and C isotopes decreased with increasing protein accretion in the individual fish, for N from 6.5‰ to 4‰ and for C in the lipid-free body from 4‰ to 2.5‰. This is the first study showing the strong influence of the individual protein balance to the degree to which the isotopic signature of dietary protein was modified in tissue protein of fish. The extrapolation of the trophic level or the reconstruction of the diet of an animal from stable isotope ratios without knowledge of the individual physiological condition and the feeding rate may lead to erroneous results.

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Introduction

The use of stable isotopes in ecological research has increased tremendously over the last decade. Stable isotope ratios are used, for example, to reconstruct the diets of animals (Szepanski et al. 1999), to determine the fate of assimilated nutrients (Ambrose and Norr 1993), or to investigate the trophic position of animals in ecological systems (Welch and Parsons 1993). Animals are usually enriched in the heavier isotope compared with their diet (DeNiro and Epstein 1978, 1981). For N isotopes an enrichment of 1.3–5.3‰ has been observed (Minagawa and Wada 1984), but in practice a constant diet–tissue discrimination factor (trophic shift) of 3.5‰ is often used for the back-calculation of diets (Herrera et al. 2002). Feed quality seems to be important for the absolute value of the trophic shift. Hobson and Clark (1992) observed lower trophic shifts for N in crows (*Corvus brachyrhynchos*) raised on a perch-based diet compared with that in crows raised on a plant-based diet. Gaye-Siessegger et al. (2003) found significantly higher values for the trophic shift of C and N isotopes in Nile tilapia fed a semi-synthetic wheat-based diet compared with those fed a fish-meal-based diet. Adams and Sterner (2000) fed water fleas (*Daphnia magna*) with green algae (*Scenedesmus acutus*) which differed in their C:N ratio (7.3–24.8). The δ^{15}N values of the daphnids and the trophic shifts were inversely related to the nitrogen content of the algae. Pearson et al. (2003) fed songbirds (*Dendroica coronata*) with diets differing in their relative proportions of fruit and insects, and determined δ^{15}N and δ^{13}C values of blood and feathers. In contrast to the previously mentioned results, they found higher discrimination factors for carbon and nitrogen isotopes when the carbon and nitrogen concentrations of the diets increased. It should be noted that in this experiment, the birds did not grow.

Although δ^{15}N values serve as an indicator for the trophic level of an animal in an ecological system, little information is available on factors influencing these values. Gannes et al. (1997) demanded more laboratory research to interpret data gathered in the field and called

for a better understanding of the processes that lead to fractionation in different body components. The aim of this work was therefore to investigate the influence of different protein/energy ratios in the diet on the $\delta^{15}N$ and $\delta^{13}C$ values of the whole body of Nile tilapia, *Oreochromis niloticus* (L.), under controlled laboratory conditions.

Materials and methods

The semi-synthetic isoenergetic and isolipidic diets used here were made from wheat gluten, wheat starch, and wheat germ oil with three different protein/energy ratios. Synthetic amino acids (arginine, lysine, threonine, tryptophan, isoleucine, methionine) were added to meet the tilapia's nutritional requirements (National Research Council 1993). Additional ingredients of the diets were vitamin- and mineral-premixes and cellulose. The proximate composition, the metabolizable energy, and $\delta^{13}C$ and $\delta^{15}N$ values of the three semi-synthetic diets are given in Table 1. The diets were offered in the form of 2-mm moist pellets and were stored frozen until required.

The experimental fish were obtained from the University of Göttingen and were all from the same batch. Genetically male fish were used, since Nile tilapia are mouthbrooding and females do not feed while they are carrying eggs. Thirty-two fish were reared individually with the diet P42 for 11 weeks to ensure a constant individual feed intake and a dynamic equilibration with the semi-synthetic diet. At the beginning of the main experiment, the fish had an average body mass of 22.3±4.0 g. Seven tilapia were sacrificed to estimate the initial body composition. The remaining fish were randomly assigned to three groups and were fed individually on the semi-synthetic diets at 5 g per $kg^{0.8}$ per day, which is slightly above the maintenance level of tilapia calculated for a diet with the same proximate composition (Richter et al. 2002). The experiment lasted for 8 weeks.

Table 1 Proximate composition, metabolizable energy and $\delta^{13}C$ and $\delta^{15}N$ values of the semi-synthetic diets. DM = Dry matter

	P35	P42	P51
Proximate composition			
Crude protein (% of DM)	35.4	42.3	50.9
Crude lipids (% of DM)	10.5	9.8	9.7
Crude ash (% of DM)	5.0	4.6	5.0
Metabolizable energy (kJ/g DM)[a]	14.9	14.5	14.4
$\delta^{13}C$ and $\delta^{15}N$ values[b]			
$\delta^{13}C$ lipid-free (‰)	−26.1	−26.3	−26.4
$\delta^{13}C$ lipids (‰)	−28.0	−28.1	−28.2
$\delta^{15}N$ (‰)	2.2	2.3	2.2

[a] Calculated from Brett and Groves (1979) using 33.5 kJ/g for lipids, 18.8 kJ/g for protein and 13.8 kJ/g for carbohydrates
[b] Differences between duplicate determinations for $\delta^{13}C$ <0.2‰ and for $\delta^{15}N$ <0.3‰

At weekly intervals, the fish were weighed to adjust the feeding ration. The water temperature was maintained at 27°C (±0.1°C). Fish were kept under standardized conditions. At the end of the experiment, all fish were fasted for 48 h before they were sacrificed.

Analytical methods

The samples were prepared as described by Gaye-Siessegger et al. (2003). The $\delta^{13}C$ values were analyzed separately in the lipid-free matter and the lipids (Focken and Becker 1998). Mass spectrometry was carried out at the Forest Ecosystems Research Center of the University of Göttingen (Germany) using an isotope ratio mass spectrometer (Finnigan-MAT 251) coupled to an element analyzer (EA 1108 Fisons Instruments). $\delta^{13}C$ data are expressed in the differential notation relative to the PDB standard, $\delta^{15}N$ data relative to air. The standard deviations for $\delta^{13}C$ and $\delta^{15}N$ values of the external standard (acetanilide) were below 0.1‰.

Table 2 Initial composition of tilapia and initial and final body mass, body mass gain, feed conversion, proximate composition, fat, protein, and energy utilization parameters in tilapia fed the three semi-synthetic diets

	Initial	P35	P42	P51
Experimental details				
Number of experimental animals (*n*)	7	9	8	8
Initial body mass (g)	21.5 (±2.07)	20.8 (±4.04)	24.7 (±4.64)	22.6 (±3.93)
Final body mass (g)		26.6 (±5.87)	32.0 (±7.75)	28.8 (±6.29)
Body mass gain (g)		5.7 (±3.11)	7.2 (±4.08)	6.2 (±3.55)
FCR (g/g)		3.2 (±2.81)	2.3 (±0.94)	2.6 (±1.11)
Chemical composition of fish				
Dry matter (% of FM)	25.7 (±1.58)	24.6 (±1.41)	24.8 (±1.79)	23.0 (±1.97)
Crude protein (% of DM)	59.3 (±3.52)	59.6^{b} (±2.19)	60.1^{b} (±2.69)	64.2^{a} (±4.82)
Crude lipids (% of DM)	24.4 (±4.42)	23.6^{a} (±4.48)	24.0^{a} (±4.42)	16.8^{b} (±5.88)
Crude ash (% of DM)	13.9 (±1.26)	15.2^{ab} (±1.94)	13.7^{b} (±1.74)	16.2^{a} (±1.39)
Gross energy (kJ/g DM)	23.6 (±1.08)	23.1^{ab} (±1.44)	23.6^{a} (±1.38)	21.7^{b} (±1.85)
Efficiencies				
Lipid gain (g)		0.3^{a} (±0.46)	0.4^{a} (±0.73)	-0.3^{b} (±0.42)
ALC (%)		20.9^{a} (±34.74)	29.0^{a} (±45.69)	-23.0^{b} (±37.15)
Protein gain (g)		0.7 (±0.52)	1.0 (±0.82)	0.8 (±0.63)
PPV (%)		16.6 (±10.51)	16.8 (±10.26)	11.9 (±8.66)
Energy gain (kJ)		28.1 (±28.62)	41.4 (±48.89)	8.7 (±25.74)
Energy retention k_{tot} (%)		9.9 (±10.35)	12.3 (±12.66)	2.5 (±9.85)

DM = Dry matter, FM = Fresh matter
FCR = dry matter feed intake (g) / body mass gain (g)
ALC = 100 × [final fish body lipid (g) − initial fish body lipid (g)]/crude lipid intake (g)
PPV = 100 × [final fish body protein (g) – initial fish body protein (g)]/crude protein intake (g)
Data are the average of 8–9 fish and standard deviation. For tilapia fed the semi-synthetic diets, values within one line not sharing the same letter code differ significantly (DMRT, $P<0.05$)

Statistics

A multivariate analysis of variance (MANOVA) was used, followed by ANOVAs for each dependent variable. Statistical comparisons between treatments were made using Duncan's multiple range test (DMRT). The significance level was set at $P<0.05$. The software used was STATISTICA 5.1. The linear regressions were calculated using GraphPad InStat 3.05.

Results

Multivariate analysis showed a significant effect of dietary protein content on the dependent variables (Rao's $R=7.21$; $df=30, 16$; $P<0.0001$). The three experimental groups did not differ significantly from each other with respect to initial ($F=1.82$; $df=2, 22$; $P=0.1849$) and final body mass ($F=1.39$; $df=2, 22$; $P=0.2712$), body mass gain ($F=0.37$; $df=2, 22$; $P=0.6971$) or feed conversion ($F=0.53$; $df=2, 22$; $P=0.5958$), nor from the initial group with respect to body mass ($F=1.62$; $df=3, 28$; $P=0.2074$) as shown in Table 2. The fish fed diet P51 had significantly higher crude protein values ($F=4.51$; $df=2, 22$; $P=0.0228$) and lower crude lipid values ($F=5.43$; $df=2, 22$; $P=0.0120$) than the fish fed the diets P35 and P42. The lipid gain was significantly higher in fish fed the diets P35 and P42 compared with that of fish fed diet P51 ($F=3.85$; $df=2, 22$; $P=0.0369$).

There were no significant differences in $\delta^{13}C$ values of the lipids ($F=1.10$; $df=2, 22$; $P=0.3511$; data not shown) and the lipid-free material ($F=0.88$; $df=2, 22$; $P=0.4299$) and in $\delta^{15}N$ values ($F=0.10$; $df=2, 22$; $P=0.9026$) between the averages of the three feeding groups, but regardless of the experimental diet, in the individual fish the trophic shift declined significantly for both N and C isotopes with increasing protein efficiency, for N from 6.5‰ to 4‰ and for C of the lipid-free material from 4‰ to 2.5‰ (for N: $y=-0.0538x+5.9793$, $r^2=0.78$, $P<0.001$, $n=25$; for C: $y=-0.0354x+3.7396$, $r^2=0.69$, $P<0.001$, $n=25$; see Fig. 1).

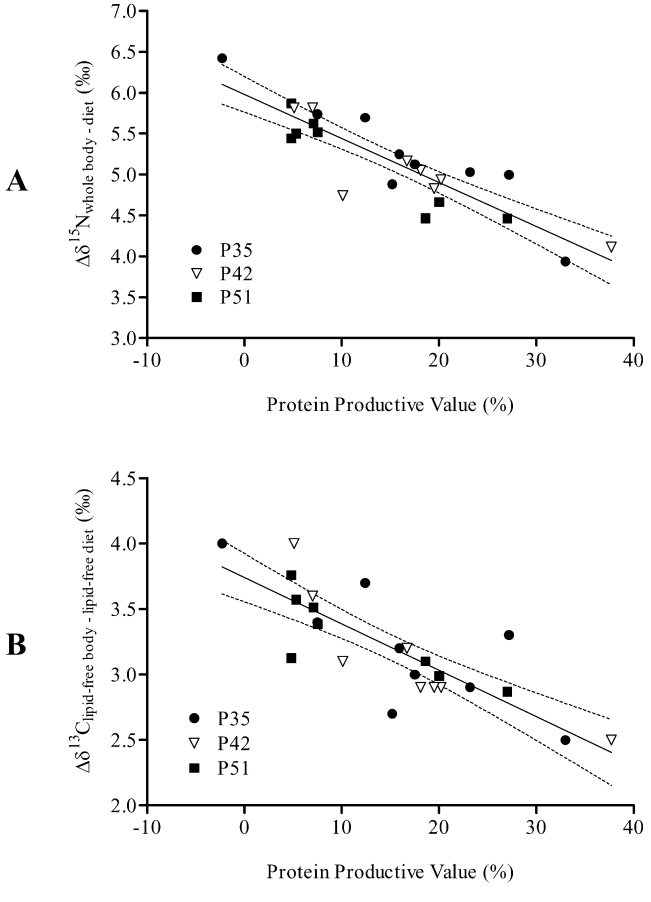

Fig. 1 $\Delta\delta^{15}N_{\text{whole body—diet}}$ (**A**) and $\Delta\delta^{13}C_{\text{lipid-free body—lipid-free diet}}$ (**B**) decreased significantly with increasing protein accretion in the individual fish, showing a considerable overlap between groups. Note different scale of *y*-axis

Discussion

All fish were fed at the same level to avoid an influence of the feeding level on $\delta^{15}N$ and $\delta^{13}C$ values (Gaye-Siessegger et al. 2003, 2004).

The optimum protein level in the diet of fish depends on the digestibility and amino acid composition of the protein, the water temperature and salinity, the level of non-protein energy, the feeding level, and the culture system. The requirement also varies between species and life stages. On the basis of growth, survival, and feed conversion, Santiago et al. (1982) stated a dietary crude protein requirement of 35% for tilapia fry. This is equivalent to the protein content of diet P35 used here, whereas diets P42 and P51 had markedly higher protein contents.

Some of the results, for instance differences between average body masses, might become significant at a greater sample size. Although no significant differences could be observed for average $\delta^{15}N$ and $\delta^{13}C$ values in the different feeding groups of this experiment, the trophic shift for N and C isotopes decreased with increasing protein retention in the individual fish, because more amino acids from the feed were used directly for the synthesis of proteins and therefore the $\delta^{15}N$ and $\delta^{13}C$ values of the fish approached the $\delta^{15}N$ and $\delta^{13}C$ values of the feed. For N, the difference between the highest and lowest trophic shift observed here was 2.5‰, which corresponds to almost one trophic level (Fry and Sherr 1984). Gannes et al. (1998) stated that the body protein of animals fed a high-protein diet appears to resemble the isotopic values of dietary protein and not of the bulk diet. The excess protein is deaminated and the carbon skeleton burned to provide energy. In contrast, animals feeding on protein-deficient diets recycle the amino-groups from degraded tissue protein to synthesize new amino acids, using the carbon skeletons derived from dietary carbohydrates and lipids (Fisler et al. 1982). Higher deamination and transamination rates result in a higher enrichment in ^{15}N (Macko et al. 1986; Schimerlik et al. 1975).

Vanderklift and Ponsard (2003) conducted a literature review with 134 estimates from controlled studies of consumer-diet ^{15}N enrichment. They suggested that the

most important source of variation in ^{15}N enrichment is the major biochemical pathway for excretion of nitrogenous metabolites. In their meta-analysis, ammonotelic organisms show lower ^{15}N enrichment than ureotelic or uricotelic organisms. Focken and Becker (1993) observed that in carp (*Cyprinus carpio*) fed high-protein diets, the sum of the body constituents protein (Kjeldahl-nitrogen × 6.25), lipids and ash exceeded 100%, and they attributed this to the presence of non-protein nitrogen, most likely urea. Da Silva (1991) reported that urea excretion can contribute up to 45% of total nitrogen excretion in the cichlid fish (*Sarotherodon galilaeus*), depending on the environmental conditions. According to Vanderklift and Ponsard (2003), in such a situation, a higher ^{15}N enrichment in the tissue can be expected compared to situations when the fish excrete only ammonia. McCutchan et al. (2003) expected high values for the trophic shift for N isotopes when dietary N either exceeds or is well below requirements for optimal growth. The trophic shift for N should be highest at highest rates of N excretion relative to assimilation of N. This is in agreement with the results of the present study.

Gannes et al. (1997) predicted that the protein balance has an important influence on the degree to which the isotopic signature of dietary protein is conserved in a consumer's tissue. This experiment demonstrates the high influence of the individual protein balance on the $\delta^{13}C$ and $\delta^{15}N$ values in fish fed at relatively low intensity. In ecological studies, neither the individual physiological condition, nor the protein intake, nor the feeding rate of an animal are known. All these factors increase the uncertainty of the trophic shift and subsequent interpretations of the trophic level and back-calculated diets, and should therefore be considered in the light of the results from stable isotope analysis.

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