Application of Stable Isotopes in Aquaculture Nutrition Research

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Abstract

The carbon and nitrogen contained in fish tissue originate from the diet of the animal. The ratio of the stable isotopes ($^{13}C/^{12}C$, $^{15}N/^{14}N$) of the diet is transferred (with a small, but important modification) to the tissue of the fish. Therefore, the analysis of stable isotopes in fish tissues provides information on the isotopic composition of the fish's diet. This analysis is of special interest in semi-intensive aquaculture systems, in which the growth depends on natural food as well as on compound feed, and in which alternative methods for the study of diet composition are difficult to apply. Other fields for application of this method are the tracing of individual components from a balanced diet in the metabolism of fish, e.g. the tracing of the metabolic fate of different protein (fish meal, plant protein) in fish metabolism.

Key words: Aquaculture; Nutrient flows; Stable isotopes; $\delta^{13}C$; $\delta^{15}N$; Live food; Fish meal substitution

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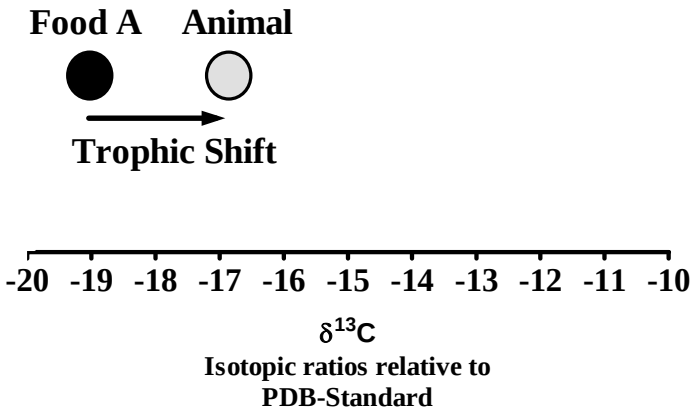
Nutrient flows are intrinsic characteristics of aquaculture systems. They occur as flows into the system via feeds and/or fertilizers, flows within the system and flows from the system into the environment. The relative importance of the different flows depends mainly on the aquaculture system, in intensive aquaculture systems, in which the growth of fish depends entirely on the nutrients provided with the feed, feed input is the biggest individual flow, internal flows are virtually absent, and outbound flows are essential in order to eliminate feed residues, feces and metabolites like ammonia from the system. In semi-intensive aquaculture systems, in which the growth of fish depends on natural food, which may be enhanced by fertilization or supplemented with compound feed, internal flows such as the conversion of inorganic nitrogen from fertilizers or ammonia excreted by the fish into feed items for the fish via primary and secondary production, maybe in the same order or even higher than inflows, and the outflow of nutrients is reduced to the minimum in order to enhance fish production. In all aquaculture systems, knowledge on nutrient flows is essential for the proper management and improvement of efficiency, but conventional methods such as feeding or fertilization trials provide only information on the external flows and not on the internal flows, which is an obstacle especially for the development of semi-intensive systems in which these flows are most important for cost efficient fish production.

In 1983, G. Schroeder published several papers (Schroeder 1983a, b) introducing a new method for analyzing nutrient flows into aquaculture science, the analysis of stable isotope ratios in inputs, feed items and tissues of the cultured organisms. The method is based on the observation that organic materials frequently have small, but measurable differences in the ratios of the stable isotopes of carbon (^{12}C, ^{13}C). By convention, the ratios of stable isotopes are not reported in absolute values, but given in "δ" units in relation to a standard, in case of carbon a biogenic rock called "Pee Dee Belemnite", in short "PDB". This mineral is very rich in ^{13}C, therefore negative "δ" values result for most biological materials. The $\delta^{13}C$ of plant materials as primary organic matter depends on the $\delta^{13}C$ of the carbonate fixed, the availability of carbonate and the physiological pathways for carbon fixation. In case of terrestrial plants, the source is rather similar and constant, the metabolic pathways for carbon fixation (C_3-plants vs. C_4-plants) result in the biggest differences e.g. wheat $\delta^{13}C$= -26‰ vs. corn $\delta^{13}C$= -16‰. In aquatic primary

producers, the source is more variable both in its isotopic composition as well as in its abundance, typical values are in the range from –24 to -18‰.



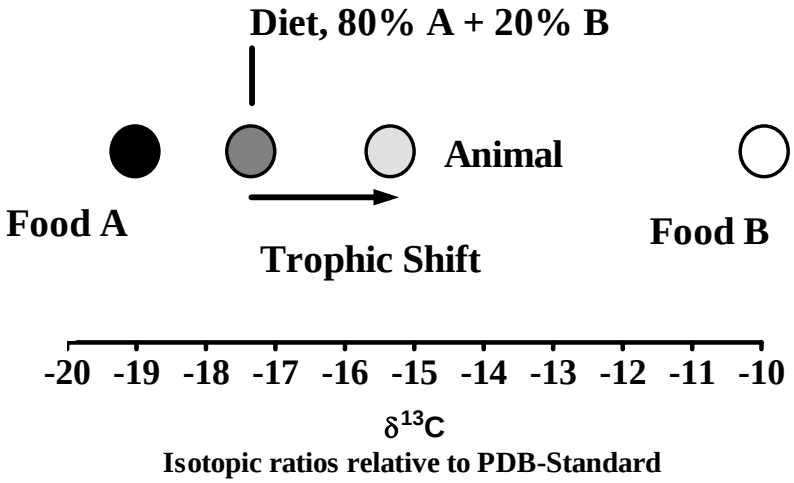


Figure 1: a) Transfer of isotopic signature in the food chain Typically, the animal is slightly enriched in ^{13}C, i.e. has less negative $\delta^{13}C$-values, in relation to its diet. b) In case of two isotopically distinct food sources A and B, their relative contribution to the diet can be calculated by subtracting the trophic shift from the $\delta^{13}C$-value of the animal and then calculating which mixture of A and B will result in this value.

In the food chain, this “carbon signature” of organic materials is passed on the next level with a small modification, called “trophic shift”. Assuming a certain value for the tropic shift it is possible to calculate the contributions of different food sources to the growth of fish or shrimps (Fig. 1a, b). Although the method appears rather elegant, its application in aquaculture research has remained limited, and some of the published results contradict other observations.

One of the semi-intensive aquaculture systems our group at the University of Hohenheim has studied extensively is the milkfish (*Chanos chanos*) culture in the Philippines. Milkfish culture in the Philippines is one of the few examples in which a coastal finfish aquaculture is producing predominantly for local and national markets, most other systems like culture of sea bass, sea bream and shrimp produce predominantly for urban and international markets.

Shallow brackish-water ponds are fertilized and stocked with milkfish fingerlings, which feed on mats of filamentous algae as well as on supplemental feeds and reach commercial size (~250 – 350 g) in 4-6 months. The relative proportions of natural food and supplemental feed in the daily ration of the fish as well as their contribution to fish growth are unknown. Due to the low quality of some feed ingredients, e.g. rice bran, it has been assumed that the some of the supplemental feed is utilized by the fish only after microbial transformations or even only after complete mineralization and therefore acts as fertilizer rather than as feed (Figure 2).

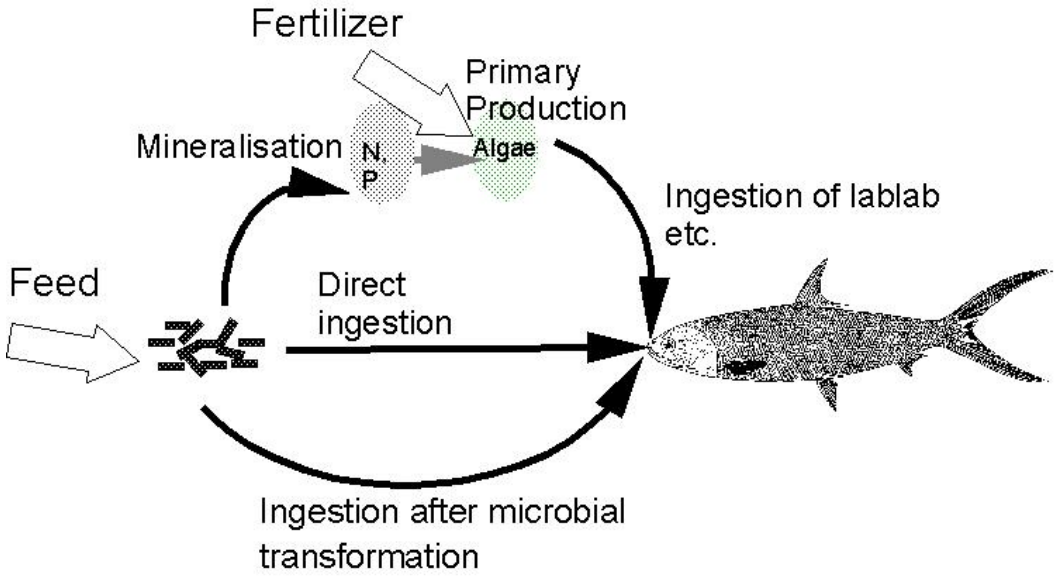


Figure 2: Main pathways from the inputs (Feed, Fertilizer) to fish growth in the semi-intensive aquaculture system: Fertilizer enhances primary production, feed may either be directly ingested, ingested after microbial transformation or become mineralized and then act as fertilizer.

In order to elucidate the food web, I have analyzed the $\delta^{13}C$ of milkfish tissue through the grow-out period as well as that of natural food and supplemental feed (Figure 3a). Interpretation of these data according to Schroeder (1983a) would be that the contribution of natural food is initially high (60-70% in the first 2 months), but is reduced in the later month of the culture period (40%). However, taking into consideration that the isotopic signature of lipids and lipid-free fraction (mainly protein) within the same individual differs substantially (Focken & Becker 1998) and that the lipid content may be increasing from fingerling to table-size fish (> 250 g), we have analyzed $\delta^{13}C$ in lipid and lipid-free fraction separately (Figure 3b), and it becomes obvious that the contribution of natural food and supplemental feed is almost constant over the grow-out period. The exact quantification of the contribution of natural food and supplemental feed is not possible on the base of these data, as this will require knowledge on the trophic shift specifically for the species and the feeds in question.

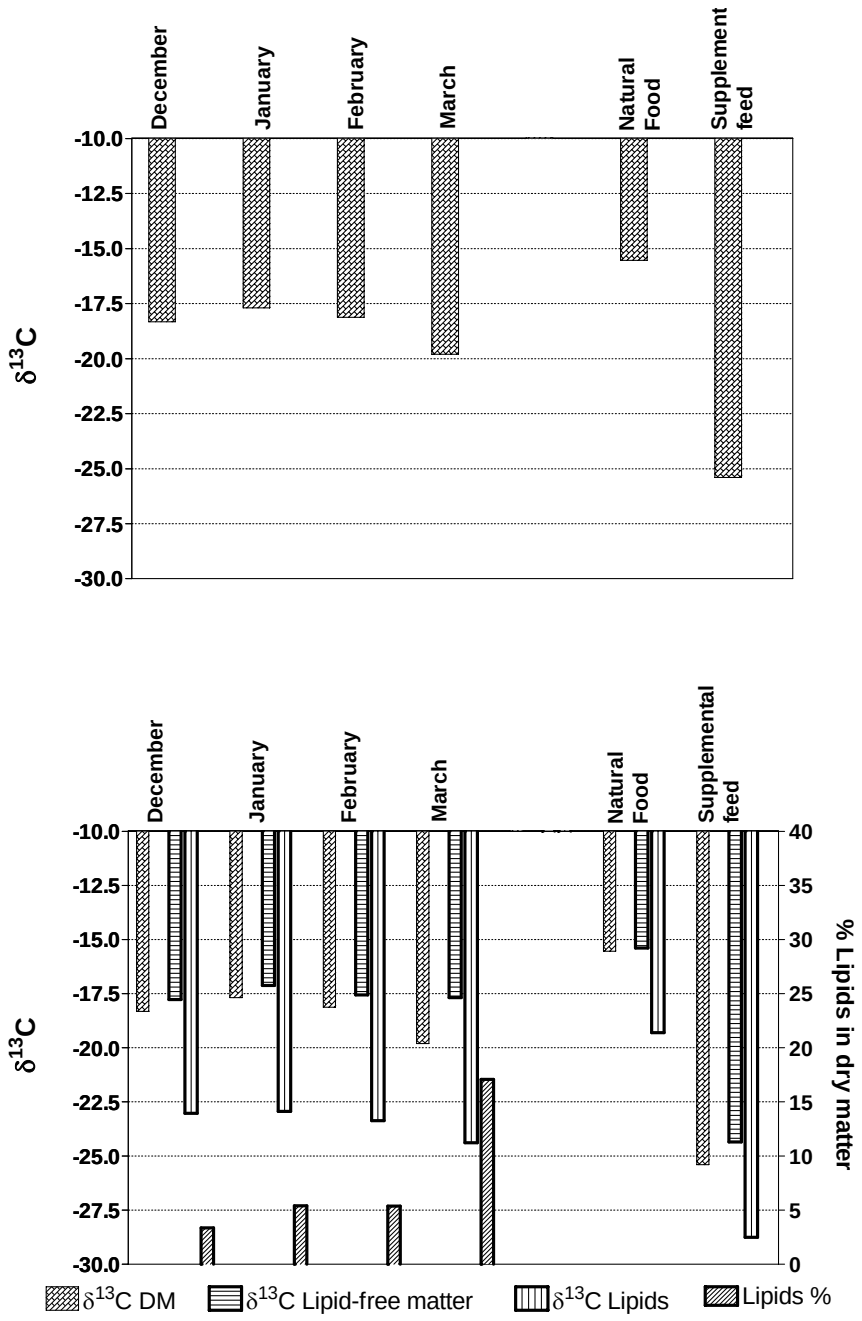


Figure 3: Isotopic signatures of milkfish tissues in the course of the growing season and of natural food and supplemental feed: a) In total dry matter b) in lipid-free and lipid fraction. Lipid content in dry matter on the right Y-axis (Focken 2007).

Simultaneous studies on the tilapia reared in fertilized ponds and receiving supplemental feed have shown that the trophic shift for natural food and compound feed is not the same (Focken *et al.* 1999), and an improved model for the back-calculation has been suggested (Figure 4).

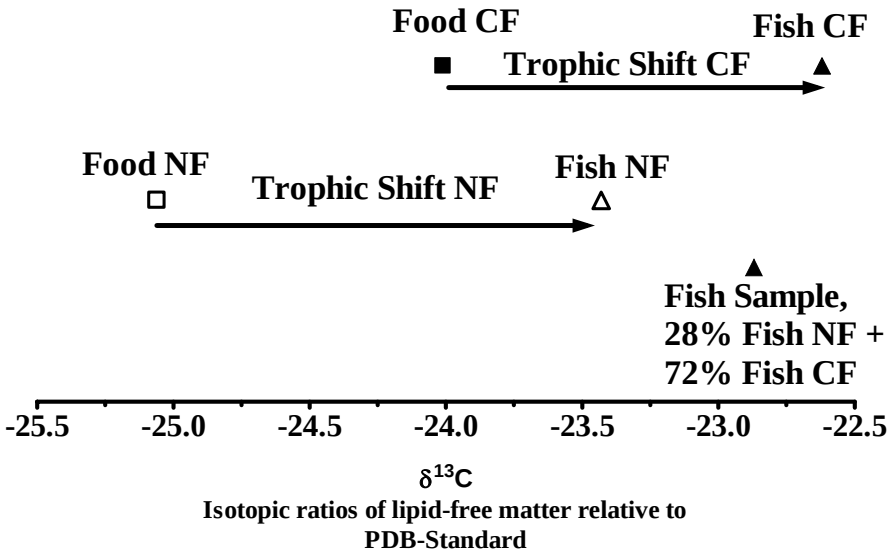


Figure 4: Modified system for back calculation of food sources from isotopic data: Fat-free matter base, 2 food sources having different values for trophic shift. Calculation is entirely based on fish reared exclusively on natural food (Fish NF) or compound feed (Fish CF) (Focken *et al.* 1999)

In addition to diet quality and fish species (Gaye-Siessegger *et al.* 2003), the trophic shift also depends on the daily ration and is thus linked to the growth rate (Focken 2001, Gaye-Siessegger *et al.* 2004a). Meanwhile, we have developed methods for the estimation of the trophic shift in fish based on the simultaneous analysis of enzyme activities from the fish liver (Gaye-Siessegger *et al.* 2004b, 2005).

Knowledge on the contribution of different pathways to fish production in semi-intensive aquaculture systems, e.g. in carp production, will open new perspectives for the systematic improvement of the respective management schemes and thereby contribute to increased ecological as well as economic efficiency of these systems.

Other applications of stable isotope techniques in aquaculture include studies on the incorporation of carbon from live food or compound larval diets in fish or crustacean larvae at the onset of exogenous nutrition (Schlechtriem *et al.* 2004, 2005).

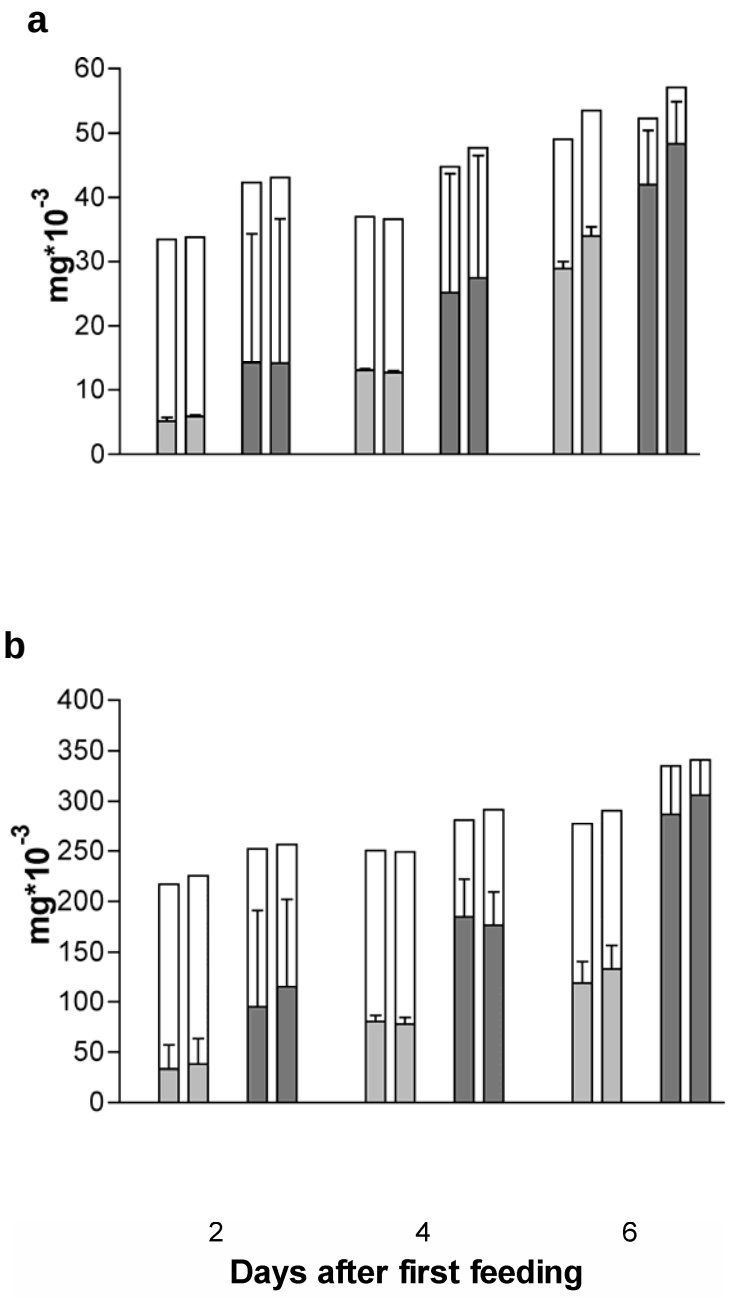


Figure 5: Estimated proportion of egg-borne (white) and assimilated carbon in lipids and lipid-free matter of *Cyprinus. carpio* fed nematodes cultured on corn (light grey) and wheat (dark grey) based medium. Mean values, n = 2; bars indicate upper 95% confidence intervals. (TS = 0; Schlechtriem *et al.* 2004)

In order to monitor the incorporation the assimilation of live food from the diet in first-feeding carp (*Cyprinus carpio*) larvae, we cultivated nematodes (*Panagrellus redivivus*) on either wheat or corn-based media and thus obtained live food with clearly distinct carbon isotope signatures. These nematodes were offered to first-feeding carp larvae. At days 2, 4 and 6, samples of the fish larvae were collected, defatted and analyzed for $\delta^{13}C$ in lipids and lipid-free matter. In Figure 5, the amount of egg-born and assimilated carbon is given as calculated by a linear two-source mixing model (Schlechtriem *et al.* 2004). It is clearly visible that bothe carbon in lipids as well as in lipid-free matter increase over time, with the increase (= growth being higher for the larvae fed nematodes cultured on wheat-based medium. Both the proportion and the absolute amount of egg-born material are significantly reduced during the first 6 days of external feeding, indicating the turn-over of tissues.

In a similar experiment we used whitefish (*Coregonus lavaretus*) larvae and nematodes produced on corn-based medium (Figure 6, Schlechtriem *et al.* 2005).

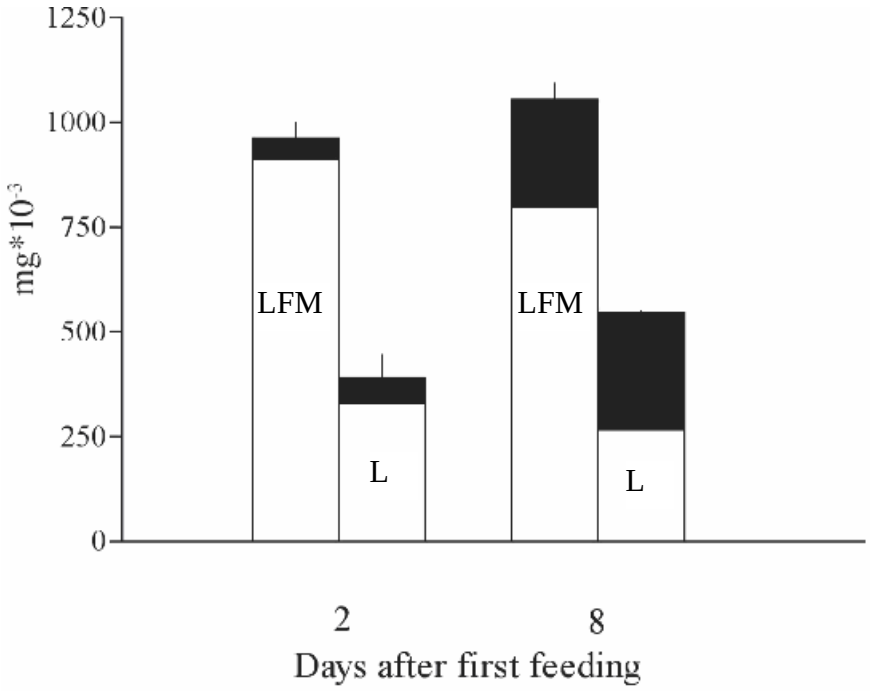


Figure 6: Estimated proportion of initial (white part of columns) and assimilated material (black part of columns) in lipid-free matter (LFM) and lipids (L) of *Coregonus lavaretus* larvae fed nematodes. (Mean values, N = 2; bars indicate upper 95% confidence intervals). (Schlechtriem *et al.* 2005).

In comparison to the experiment with carp, the incorporation of carbon from the live food is much lower during the experimental period. This can be explained by the lower temperature (13°C vs. 23°C) and the fact that whitefish larvae start exogenous feeding before the yolk sack is completely resorbed.

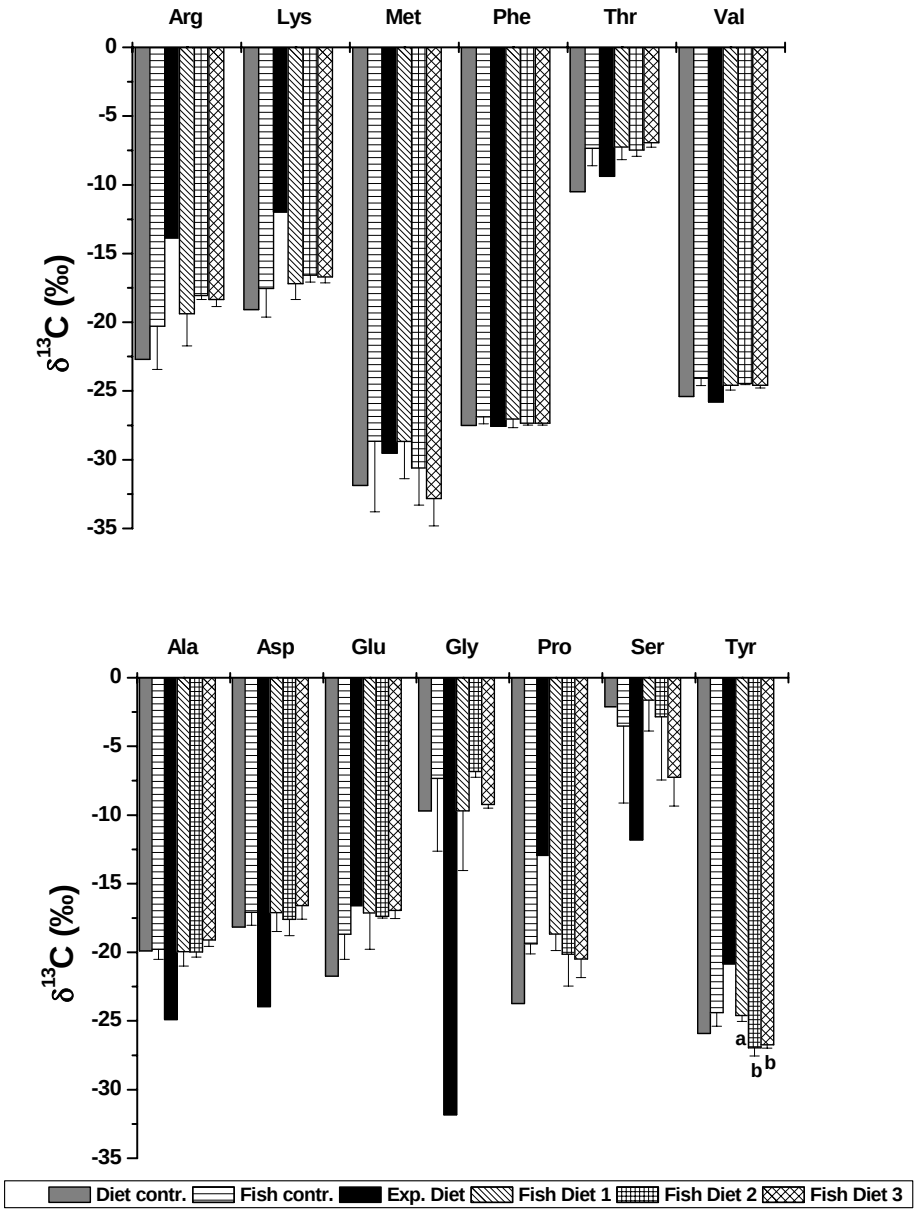


Figure 7. $\delta^{13}C$ of selected essential (top) and non-essential / conditionally essential amino acids (down) from diets and tissues of *Oncorhynchus mykiss* fed diets containing full and reduced spectrum of non-essential amino acids (n=3-4; HSD Tukey, $p<0.05$). (McCullagh *et al.* 2008)

A further application of stable isotopes in aquaculture nutrition is tracing the metabolic fate of specific compounds such as individual amino acids. As an example, in Figure 7 data on the $\delta^{13}C$ of individual amino acids from an experiment with rainbow trout (*Oncorhynchus mykiss*) are given. All diets did not contain any protein, just amino acids: Diet 1 resembles the amino acid composition of fish meal, in Diet 2, some non-essential amino acids are replaced by their respective metabolic precursors and in Diet 3, all non-essentials are replaced by the respective amount of glutamate. It can be clearly seen that the composition of the diet is reflected in the isotopic signature of individual amino acids (McCullagh *et al.* 2008) which offers potential to trace e.g. the fate of different protein sources such as fish meal and plant protein from a compound diet.

Although the application of stable isotope methods as tools in aquaculture research has already been suggested more than two decades ago, it must be concluded that it is an emerging technology, showing a large potential in several important fields of aquaculture research.

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