

In vivo

Zebra-Fish-Proteomics

Diet

for

SILAC-Zebra-Fish-Lys(6)

SILAC-Zebra-Fish-Feed

Introduction:

Silantes has developed a series of **SILAC feeds** for different model organisms, such as

- Mouse
- Yeast (*Saccharomyces cerevisiae*)
- Fly (*Drosophila melanogaster*)
- Worm (*Caenorhabditis elegans*)

Silantes' most recent development is **SILAC Zebra Fish Feed** (*Danio rerio*).

Based on the SILAC food chain concept shown in Fig. 2, the feed for **SILAC Zebra Fish Feed** (patent pending) has been developed in cooperation with Prof. Marcus Krüger. This feed allows specific labelling of zebra fish with ¹³C-lysine (95%-98% of all lysine carbons in the zebra fish are exchanged).

Applications:

Tissue of the labeled Zebra Fish can be used as "spike in" for quantitative proteomics (Konzer *et al.*, J. Proteome Res. 2014, 13, 2162–2174; Konzer *et al.*, 2013, Molecular & Cellular Proteomics 12, 1502–1512)

The **SILAC-Zebra-Fish-Feed- KIT** is delivered as a dry powder and consists of three different components:

- | | | |
|----|--|--------|
| a) | Juvenile fish feed (ZFJu), ¹³ C -lysine labeled:
The ZFJu is administered over a period of 6 weeks at
Week 1: 4mg/day, at Week 2: 6mg/day, at Week 3-6: 8mg/day,
as indicated in the feeding scheme (Fig.1). | ~350mg |
| b) | Juvenile fish feed (ZFUn), unlabeled:
The ZFUn is administered over a period of 6 weeks,
once a week on day 7 (4mg/day 7),
as indicated in the feeding scheme (Fig.1, #). | ~25mg |
| c) | Adult fish feed (ZFAd), ¹³ C-lysine labeled:
ZFAd is administered over a period of 6 weeks at
Week 7–Week12: 19mg/day,
as indicated in the feeding scheme (Fig.1, #). | ~900mg |

The **SILAC Zebra Fish Feed** consists of a mixture of SILAC labeled organisms shown in Fig. 2. The formulation of the feed is adapted to juvenile and adult fish with respect to consistency and portion from the different SILAC organisms shown in Fig. 2.

The workflow for feeding in Fig. 1 shows the amounts for one fish and a feeding period of 12 weeks. Multiples of the **SILAC Zebra Fish Feed KIT** can be delivered on request.

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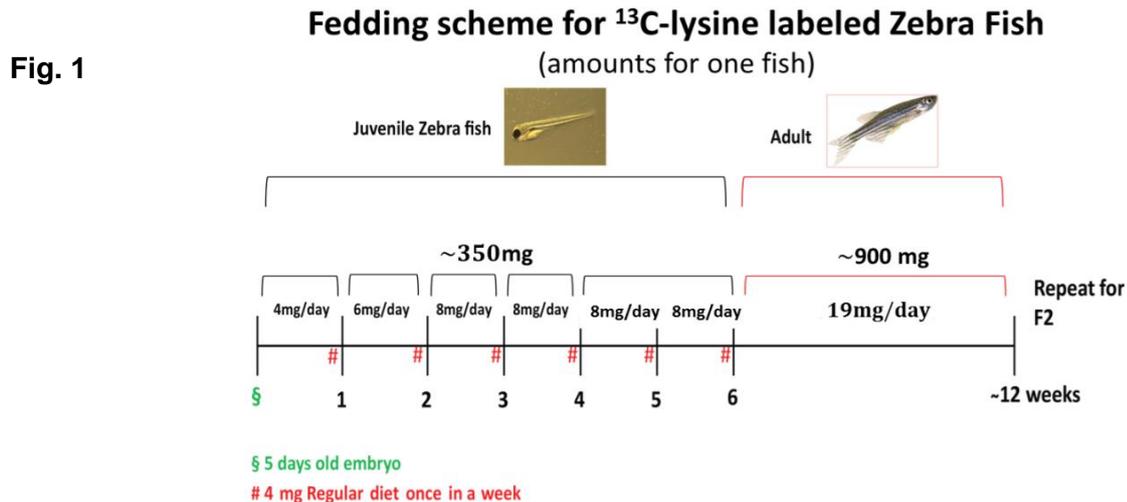


Fig.1 shows Workflow of the feeding procedure for 12 weeks. Data for one fish.

Preparation of the feed:

Since it is difficult to prepare an artificial diet, as can be done in the case for mice, Silantes has developed a **SILAC Zebra Fish Feed** based on the concept of a “virtual” feeding chain as pointed out in Fig. 2.

Fig.2 „Virtual“ SILAC-food chain for preparing zebra fish feed

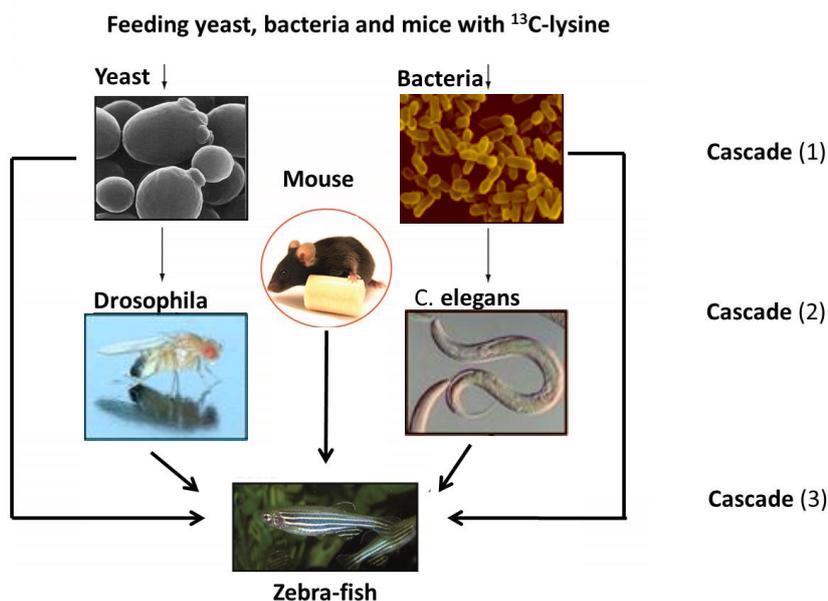


Fig. 2: The SILAC-food-chain concept

The SILAC label is transferred through a cascade of ^{13}C -lysine labeled organisms which serve as food components for the following cascade level. The isotopic enrichment of the organism in ^{13}C -lysine is determined at each cascade up to the cascade zebra fish. A slight decrease in the enrichment (of up to 1%) is observed from one cascade to the next.

Fig. 3 Comparison of the survival rates on regular and SILAC feed

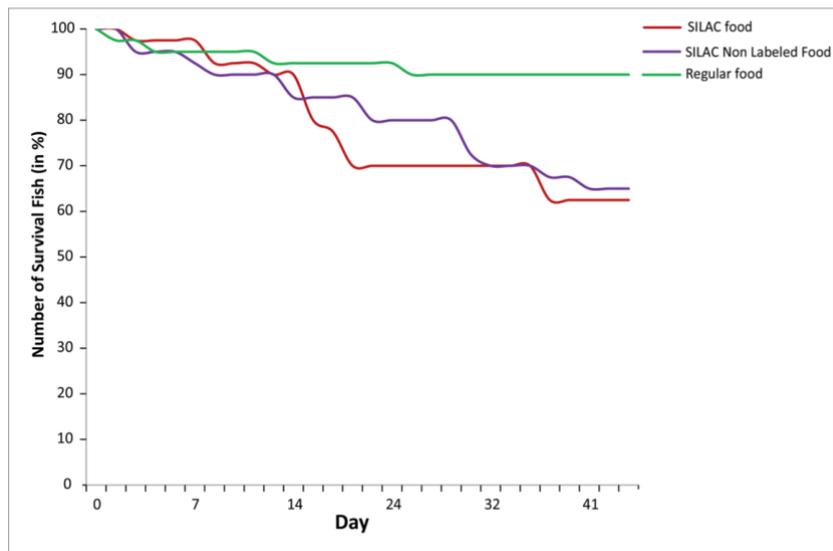


Fig.3: Survival rate on different feeds

Survival rate of zebra fish on regular feed (green), unlabeled SILAC feed (purple) and ¹³C-lysine labeled SILAC feed (red): the data show that the survival rate of zebra fish is 90% on regular feed. It decreases by another 25% on the SILAC zebra fish feed after 6 weeks (starting with 100 fish).

Average ¹³C-lysine enrichment in Zebra fish

Fig. 4

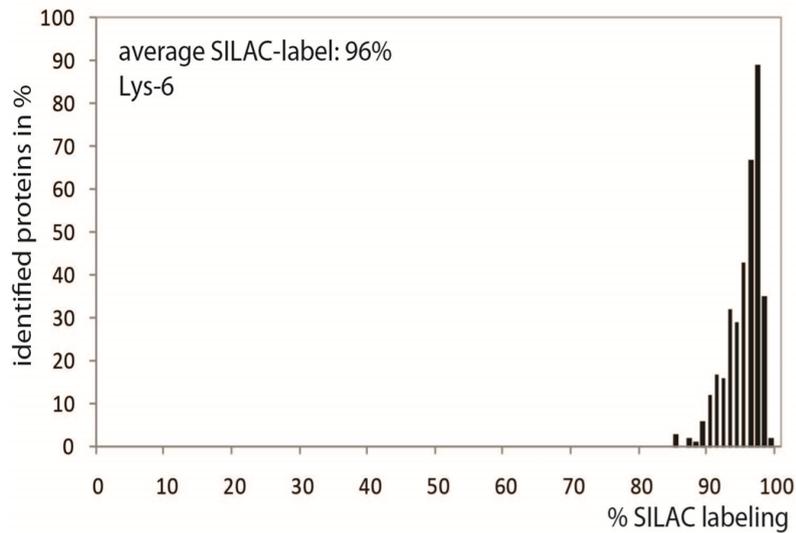


Fig.4: ¹³C-lysine average enrichment in zebra fish after 12 weeks feeding is 96%