Dietary Lysine Requirements for Juvenile and Adult Zebrafish

*Danio rerio*

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Nutrition vs Dietetic Research

• Dietetic Research
  – Compare common diets
    • Which one is best
  – Great for basic feeding strategies
  – Not ideal for defining daily nutrient requirements
    • Not well defined
    • Seasonal differences
    • Antinutritional factors, toxins, dyes, etc.

https://www.flickr.com/
http://www.petsmart.com/fish/food-cat-36-catid-300008
http://www.microscope-microscope.org/applications/pond-critters/animals/rotifers.htm
Nutrition vs Dietetic Research

- Nutrition Research
  - Determine individual nutrient requirements that provide optimal performance

- Calories
- Protein
- Fat
- Vitamins

Requirements not known for Zebrafish

Nutrition Facts

<table>
<thead>
<tr>
<th>Serving Size:</th>
<th>270 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serving per packet:</td>
<td>18</td>
</tr>
<tr>
<td>Amount per serving:</td>
<td></td>
</tr>
<tr>
<td>Calories:</td>
<td>517</td>
</tr>
<tr>
<td>% Daily value *</td>
<td></td>
</tr>
<tr>
<td>Total Fat</td>
<td>33.9 g</td>
</tr>
<tr>
<td>Saturated fat</td>
<td>4.1 g</td>
</tr>
<tr>
<td>Trans fat</td>
<td>&lt; 0.1 g</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>16.5 mg</td>
</tr>
<tr>
<td>Sodium</td>
<td>2939.1 mg</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>25.4 g</td>
</tr>
<tr>
<td>Dietary fiber</td>
<td>8.2 g</td>
</tr>
<tr>
<td>Sugars</td>
<td>9.0 g</td>
</tr>
<tr>
<td>Protein</td>
<td>27.6 g</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>0 %</td>
</tr>
<tr>
<td>Calcium</td>
<td>7 %</td>
</tr>
</tbody>
</table>

* Percent Daily Values are based on a 2,000 calories diet. Your daily values may be higher or lower depending on your daily needs.

http://scienceblog.com/54464/zebrafish-could-hold-the-key-to-understanding-psychiatric-disorders/#qrPY5P1jDBaYDuIL.97
Why Determine Nutrient Requirements?

• Standardized Zebrafish Diet
  – Rodents 30-40 years ago
    • Reduce variability in research
    – What is in commercial feeds??
      • Antinutritional factors and toxins in commercial diets
        – Want an open formula diet
        – Epigenetics
  • We want healthy fish
    – Too much fat
Nutrition Research – Where To Start?

Similarities:
- Freshwater fish
- Cyprinid family
- Warm water
- Omnivore
- Digestive tract

Common Carp (*Cyprinus carpio*)

Lysine

- 10 essential amino acids
- Lysine is needed in highest concentration in fish
- Deficiency
  - Reduced growth
  - Fin erosion
- Good place to start!

https://www.rpi.edu/dept/bcbp/molbiochem/MBWeb/mb2/part1/protease.htm
http://www.amazon.com/Spring-Valley-Dietary-Supplement-L-Lysine/dp/B0014077K0
Dose-Response

Formulate 7 diets
- Semi-purified
- Isonitrogenous
  - Casein
  - Gelatin
  - Crystalline AA’s
- Isocaloric
- Varying lysine

Measured Responses
- Growth
- Embryo Production
Dietary Treatments

- 7 diets with lysine ranging from 1.5-2.8% of diet
  - Carp lysine requirement 2.2%
- All other nutrients meet or exceed that of common carp

Juvenile Study

- WT zebrafish
  - 6 weeks old
- 10 fish per tank (2.8 L)
- 3 tanks/ treatment
- Fed diet treatment twice daily

- At 90 dpf
  - Length/Weight
  - Survival
Adult Study

- WT zebrafish
  - At least 90 dpf
  - Never been set-up to spawn
- 10 fish per tank (2.8 L)
  - 5 females and 5 males
- 3 tanks/treatment
- Fed diet treatment twice daily

Adult Study

• Set-up for breeding once per week for 6 weeks
  – Starting on day 28
  – Counted viable embryos
• Survival – weekly
• Plasma free amino acid analysis
Statistical Analysis

- Broken-line regression (R Statistical Computing)
- ANOVA
  - Tukey Post Hoc Analysis
- Significance p<0.05
Juvenile Survival

- At 90 dpf all treatments >80% survival
Juvenile Final Weight

[Graph showing the relationship between mean final weight (mg) and dietary lysine (% of diet).]
Juvenile Final Weight

![Graph showing the relationship between mean final weight (mg) and dietary lysine (% of diet). The graph has a linear trend line indicating a positive correlation.]
Juvenile Final Weight

![Chart showing the relationship between dietary lysine (% of diet) and mean final weight (mg). The chart has a positive linear trend for dietary lysine up to 2.4% and a negative trend above 2.4%. The data points are scattered, indicating variability at different lysine levels.]
Juvenile Final Weight

![Graph showing the relationship between mean final weight (mg) and dietary lysine (% of diet). The graph includes a scatter plot with data points and a trend line. The x-axis represents dietary lysine (% of diet) ranging from 1.4 to 3.0, and the y-axis represents mean final weight (mg) ranging from 0 to 60. A dashed line at 2.41 on the x-axis highlights a specific value.](Image)
Juvenile Final Weight

![Graph showing the relationship between dietary lysine (% of diet) and mean weight (mg) per treatment. The x-axis represents dietary lysine (% of diet) ranging from 1.4 to 3.0, and the y-axis represents mean weight (mg) per treatment ranging from 0 to 70. The graph includes error bars indicating variability.](image-url)
Juvenile Final Weight

![Graph showing the relationship between dietary lysine (% of diet) and mean weight (mg) per treatment. The graph indicates an increase in weight as dietary lysine increases up to a certain point, after which there is a decrease. The graph highlights a peak weight of 2.41 at a dietary lysine level of 2.4%.](image-url)
Adult Survival

- After 81 days all treatments >86% survival
Embryo Production

- Result of previous diet
- Diet not optimal
  - Calories?
  - Protein?
  - Digestibility?
- Poor growth in juveniles
Juvenile Final Weight

![Graph showing the relationship between dietary lysine and mean weight per treatment. The x-axis represents dietary lysine (% of diet) ranging from 1.4 to 3.0, and the y-axis represents mean weight (mg) per treatment ranging from 0 to 70. The data points are shown with error bars.]
Embryo Production

![Graph showing the relationship between dietary lysine and embryo production. The x-axis represents dietary lysine (% of diet) ranging from 1.4 to 3.0, while the y-axis represents embryos ranging from 0 to 600. The graph includes data points indicating a positive correlation between lysine intake and embryo production.]
Embryo Production

![Embryo Production Graph]

Embryos vs. Dietary Lysine (% of diet)

- X-axis: Dietary Lysine (% of diet)
- Y-axis: Embryos

Key point: 2.38

Legend:
- Green dots represent data points

Graph indicates a trend between dietary lysine and embryo production.
Preliminary Plasma Free Lysine Data

Plasma Free Lysine (μM) vs. Dietary Lysine (% of diet)
Conclusion

- Juvenile dietary lysine requirement
  - 2.41% of diet (6.3% of protein)
- Adult dietary lysine requirement
  - 2.38% of diet (6.2% of protein)
- Common Carp – 2.2% of diet (5.7% of protein)
- Channel Catfish – 1.2-1.5% of diet
- Atlantic Salmon – 2.4% of diet
Conclusion

• General poor growth and embryo production
  – Digestibility of ingredients
    • Not enough Calories
    • Crude protein content – 38%
    • Commercial feeds - 50% or more
  – Crude Lipid – 5%

More nutritional research = Better research diets
Zebrafish Nutritional Research

- First nutritional research for zebrafish
- Much more to be done:
  - Amino Acids
    - Lysine - 2.4% of diet (6.3% of protein)
    - Arginine – results in March 2015
    - 8 others
  - Calories
  - Crude Protein
  - Crude Lipids
  - Vitamins
  - Minerals
Acknowledgements

**U of M Zebrafish Core Facility**
- Brogen Lothert
- Ben Wilke
- Ellen Weiderhoeft
- Dana Rider
- Adam Seubert
- Elizabeth Duffy

**Schimmenti Lab**
- Dr. Lisa Schimmenti
- Stephanie Lerach

**Stern Lab**
- Isaac Salfer

**Hackett Lab**
- Bryan Hall

**Chen Lab**
- Dr. Chi Chen

**Lund Lab**
- Michelle Carter
Questions?

http://www.peds.umn.edu/genetics/zebrafish/
Diet Ingredients

- Casein
- Gelatin
- Dextrin
- Menhadden Oil
- Vitamin Mix
- Mineral Mix
- Carboxylmethyl Cellulose
- Crystalline Amino Acids
- Alpha Cellulose

<table>
<thead>
<tr>
<th>Feed Analysis</th>
<th>(%)</th>
</tr>
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<tbody>
<tr>
<td>Crude Protein</td>
<td>38</td>
</tr>
<tr>
<td>Moisture</td>
<td>6.7</td>
</tr>
<tr>
<td>Crude Fat</td>
<td>5.4</td>
</tr>
<tr>
<td>Crude Fiber</td>
<td>11.7</td>
</tr>
<tr>
<td>Ash</td>
<td>3.6</td>
</tr>
</tbody>
</table>
Embryo Production
Weeks 2-6

Embryos
Dietary Lysine (% of diet)

2.36
Length at 90 dpf

![Graph showing the relationship between mean length (mm) and dietary lysine (% of diet). The x-axis represents dietary lysine (% of diet) ranging from 1.4 to 3.0, and the y-axis represents mean length (mm) ranging from 0 to 20. The data points are marked with error bars indicating variability.](image-url)
Blood Draw
Reference Diet Embryo Production

Week

Total Embryo Production

0 500 1000 1500 2000 2500 3000

1 2 3 4 5 6