Feeding ragworm (*Nereis virens*) or mussel (*Mytilus edulis*) to common sole (*Solea solea*) alleviates their nutritional anaemia

22 October, Jeroen Kals
Feeding ragworm or mussel to common sole alleviates their nutritional anaemia

- Introduction
- Hypotheses
- Aims
- Material & methods
- Results
- Discussion
- Conclusions
Introduction

- Common sole, in intensive production conditions have lower haematocrit (Hct) than in ponds feeding on natural food

Farm: Solea BV, fish fed commercial pellets; Raceway: IMARES, fish fed commercial pellets, Pond x & y: Zeeuwse Tong, pond stocked with ragworm

*Palstra et al. (2015)*
Hypothesis

- Based on the feeding ecology of sole and our observations:

  Feeding ragworm (RW) or mussel (MU) to sole will increase Hct and haemoglobin (Hb) levels and alleviates anaemia
Aims of the studies

- test if a change in diet from commercial pellets (CPEL) to RW or MU increases Hct & Hb levels in sole,

- determine rate of increase & time needed to develop a new steady state of Hct & Hb,

- is it feeding RW per se or a higher feed intake that alleviates anaemia & stimulates growth,

- determine if the anaemia is caused by an inflammatory response to infection or a nutritional deficiency,
Material and Methods

- **Trial 1**: test if a change in diet from CPEL to RW increases Hct & Hb in sole and determine the rate of increase

- Sole were raised on CPEL and anaemic at start

- Treatments: RW & CPEL

- Fish fed to satiation

- **Sampling**
  - RW, sampled every 2-3 days until day 26
  - CPEL, only sampled at day 26
  - Fish experimental unit (n=10)
Material and Methods

- Trial 2: Does MU have a comparable effect as RW?
- Sole were raised on CPEL and anaemic at start
- Dietary treatments:
  - CPEL
  - MU
  - RW
- Feeding: restricted, equal feeding levels for all diets
- Duration 23 days, sampling at start & day 23
- Tank experimental unit (n=3, 10 fish.tank⁻¹)
Material and Methods

▪ Trial 3:
  ● Is it feeding RW per se or a higher FI that alleviates anaemia & stimulates growth
  ● Determine if anaemia is caused by an inflammatory response or nutritional deficiency

▪ Dietary treatments:
  ● CPEL with FS,
  ● CPEL with ragworm extract (1.4 g dm kg⁻¹ feed),
  ● RW

▪ Fish fed to satiation for 57 days

▪ Tank experimental unit (n=3, 15 fish.tank⁻¹)
Analyses

- **Hct**: centrifuging blood (5 min, 5000g)
- **Hb**: colorimetric (van Kampen & Zijlstra 1961)
- **Weight**
- **Feed intake (g.dm.d⁻¹)**: feed given – feed recovered
- **Real-time Q-PCR liver marker genes related to iron homeostasis and/or inflammatory response to infection**
  - Hepcidin
  - Ferritin
  - Transferrin
  - Cysteine-aspartic acid peptidase 3 - casp3
  - Heat shock protein 70 - hsp 70
## Results

- **Trial 1: Effect of a dietary change from CPEL to RW on Hct & Hb in sole**

<table>
<thead>
<tr>
<th>Diet</th>
<th>Day</th>
<th>N</th>
<th>Hct(%)</th>
<th>Hb (g l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>---a</td>
<td>0</td>
<td>24</td>
<td>9.8ᵃ ± 3.6</td>
<td>16.3ᵃ ± 6.6</td>
</tr>
<tr>
<td>CPEL</td>
<td>26</td>
<td>10</td>
<td>8.8ᵃ ± 3.3</td>
<td>11.7ᵃ ± 5.5</td>
</tr>
<tr>
<td>RW</td>
<td>26</td>
<td>10</td>
<td>19.0ᵇ ± 4.9</td>
<td>39.7ᵇ ± 10.2</td>
</tr>
</tbody>
</table>

ᵃᵇ Means within columns with a common superscript are not significantly different using the Tukey post hoc test (P<0.05). Kals et al. 2015ᵃ.
Results

- Trial 1: rate of increase and time needed to develop a new steady state of Hct & Hb

Recovery pattern of sole fed ragworm

Kals et al. 2015a
Results

- Trial 2: Does MU has an equal effect as RW?

<table>
<thead>
<tr>
<th>Diet</th>
<th>Day</th>
<th>N</th>
<th>Hct (%)</th>
<th>Hb (g l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>----</td>
<td>0</td>
<td>20</td>
<td>12.5 ± 3.17ᵃ</td>
<td>19.6 ± 8.10ᵃ</td>
</tr>
<tr>
<td>CPEL</td>
<td>23</td>
<td>3</td>
<td>13.1 ± 1.16ᵃ</td>
<td>18.9 ± 3.28ᵃ</td>
</tr>
<tr>
<td>MU</td>
<td>23</td>
<td>3</td>
<td>17.4 ± 1.95ᵇ</td>
<td>26.7 ± 4.38ᵇ</td>
</tr>
<tr>
<td>RW</td>
<td>23</td>
<td>3</td>
<td>19.0 ± 1.42ᵇ</td>
<td>34.1 ± 5.13ᵇ</td>
</tr>
<tr>
<td>p-value</td>
<td></td>
<td>&lt;0.01</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

ᵃᵇMeans within columns with a common superscript are not significantly different using the one or two sided Fisher LSD post hoc test depending on the hypothesis (Kals et al. 2015ᵇ).
Results

▪ Trial 3
  ● is it feeding RW per se or a higher feed intake that alleviates the anaemia and stimulates growth,
  ● is the anaemia caused by an inflammatory response or by a nutritional deficiency?

▪ Hct
  ● Hct at start was low and remained low for fish fed treated or untreated pellets
  ● Hct of sole fed RW increased with ±85% up to 21.2%
Results

P<0.00

- **t(0):** values at start
- **Ragworm:** chopped ragworm
- **P+extract:** pellet treated with ragworm extract
- **Pellet:** untreated pellet

(Kals et al. 2015c).
Results

- Feed intake (FI), feed conversion (FCR) & growth

<table>
<thead>
<tr>
<th>Diet</th>
<th>Ragworm</th>
<th>Pellet+extract</th>
<th>Pellet</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW_{start} (g)</td>
<td>78.95±3.73</td>
<td>78.04±2.56</td>
<td>75.22±2.35</td>
<td>0.34</td>
</tr>
<tr>
<td>BW_{end} (g)</td>
<td>118.8±6.85&lt;sup&gt;a&lt;/sup&gt;</td>
<td>109.9±6.25&lt;sup&gt;b&lt;/sup&gt;</td>
<td>98.8±2.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>FI (g dm fish d&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.69±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.68±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.01</td>
</tr>
<tr>
<td>Growth (g d&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.70±0.08&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.56±0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.41±0.09&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.02</td>
</tr>
<tr>
<td>FCR_{DM}</td>
<td>1.02±0.03</td>
<td>1.25±0.17</td>
<td>1.34±0.20</td>
<td>0.09</td>
</tr>
</tbody>
</table>

- FI sole fed RW or treated pellets was equal
- Despite equal FI, sole fed RW grew 25% faster
- Sole fed CPEL had 25% lower FI and 71% slower growth compared to sole fed RW
Results

Marker genes:

Iron homeostasis and or Immune response

Hepcidin – hamp1, Transferrin, Ferritin, Heat shock protein 70 - hsp 70, Cysteine-aspartic acid peptidase 3 - casp3,

Expression normalized for expression of beta actin in liver shown as fold change of sole fed pellet+extract relative to sole fed RW, set to 1

(Kals et al. accepted).
Results

Results indicates a nutritional anaemia, but not necessarily an iron deficiency anaemia, in sole fed treated pellets

<table>
<thead>
<tr>
<th>Gene</th>
<th>Expression¹</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepcidin</td>
<td>Down regulated</td>
<td>anaemia without immune response</td>
</tr>
<tr>
<td>Transferrin</td>
<td>Down regulated</td>
<td>an acute phase response, yet no iron deficiency</td>
</tr>
<tr>
<td>Ferritin</td>
<td>similar</td>
<td>no oxidative stress, iron overload, iron deficiency, inflammatory conditions and/or major intracellular changes</td>
</tr>
<tr>
<td>Casp3</td>
<td>similar</td>
<td>No difference in apoptosis</td>
</tr>
<tr>
<td>Hsp70</td>
<td>similar</td>
<td>no difference in cell stress</td>
</tr>
</tbody>
</table>

¹ Sole fed treated pellets vs. ragworm
Discussion and conclusions

- Clear effect of RW or MU on Hct and Hb in sole

- Feeding RW or MU alleviates nutritional anaemia in sole

- The slow growth of sole fed CPEL might be a consequence of low Hct, which hampers oxygen uptake (OCC) and lowers metabolic scope for growth
# Discussion and conclusions

- PA, iron & B12 content of diets and requirements

<table>
<thead>
<tr>
<th>Nutrients/diets</th>
<th>Unit</th>
<th>RW</th>
<th>MU</th>
<th>CPEL</th>
<th>NRC (2011)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry matter</td>
<td>g kg(^{-1})</td>
<td>181</td>
<td>252</td>
<td>894</td>
<td>NA</td>
</tr>
<tr>
<td>Crude protein</td>
<td>g kg(^{-1}) dm</td>
<td>685</td>
<td>575</td>
<td>670</td>
<td>NA</td>
</tr>
<tr>
<td>Ether extract</td>
<td>g kg(^{-1}) dm</td>
<td>138</td>
<td>103</td>
<td>177</td>
<td>NA</td>
</tr>
<tr>
<td>Fe</td>
<td>mg kg(^{-1}) dm</td>
<td>352</td>
<td>372</td>
<td>277</td>
<td>30-150*</td>
</tr>
<tr>
<td>B(_{12})</td>
<td>µg kg(^{-1}) dm</td>
<td>1602</td>
<td>1671</td>
<td>338</td>
<td>20-50*</td>
</tr>
</tbody>
</table>

*Minimum and maximum values of different species as no values of sole are given. NA is not applicable.

We suggest the rise of Hct & Hb in sole fed MU or RW can be a combined effect of heme & high B12 levels.
Conclusions

- Pellet-fed sole suffer from a nutritional anaemia
- Feeding RW or MU alleviates this nutritional anaemia
- Addition of RW extract to CPEL levels long term feed intake with sole fed RW, yet
  - does not improve Hct & Hb
  - has a limited effect on growth
- The rise of Hct & Hb in sole fed MU or RW can be a combined effect of heme & high B12
- yet we cannot exclude that other factors in MU & RW could affect Hct, Hb and growth of sole
Thank you!

Thanks to everybody who helped me with the presented work, yet especially,

*Co-authors of the recent articles
*Animal caretakers

Questions?

A patent application covering the use of Annelida and Mollusca in fish feed has been filed