Feeding ragworm (*Nereis virens*) or mussel (*Mytilus edulis*) to common sole *(Solea solea)* alleviates their nutritional anaemia KJ2

August 2016, Jeroen Kals

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Diapositiva 1

KJ2 abstract number 22866

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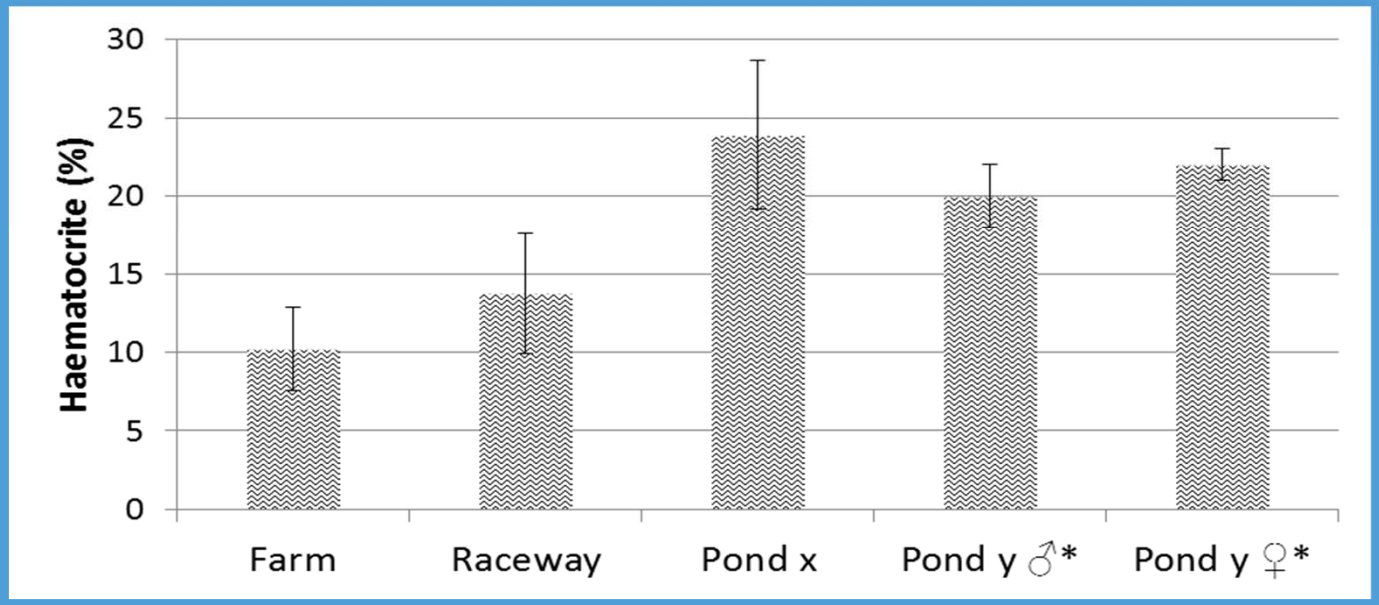
Feeding ragworm or mussel to common sole alleviates their nutritional anaemia

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

Introduction

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



Farm: Solea B.V., fish fed commercial pellets; Raceway: Imares, fish fed commercial pellets, Pond x & y: ZT, pond stocked with ragworm

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*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

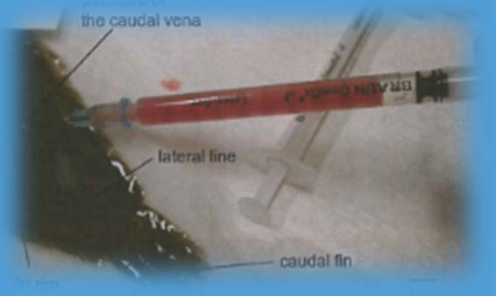
- test if a change in diet from commercial pellets (CPEL) to RW or MU increases Hct & Hb levels in sole
- determine rate of increase & time needed to develop a new steady state of Hct & Hb
- is it feeding RW per se or a higher feed intake that alleviates anaemia & stimulates growth
- determine if the 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 raised on CPEL & anaemic at start
- Treatments: RW & CPEL
- Fish fed to satiation

- Sampling
  - RW, sampled every 2-3 days to day 26
  - CPEL, only sampled at day 26
  - Fish experimental unit (n=10)



Material and Methods

- Trial 2: Does MU has a comparable effect as RW?

- Sole raised on CPEL and anaemic at start
- Dietary treatments:
  - CPEL
  - MU
  - RW

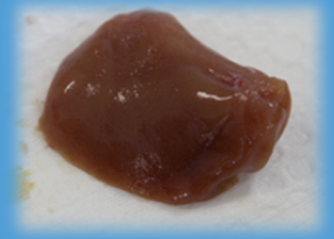
- Feeding: restricted, equal feeding levels for all diets
- Duration 23 days, sampling at start & day 23
- Tank experimental unit (n=3, 10 fish.tank^{-1})

Material and Methods

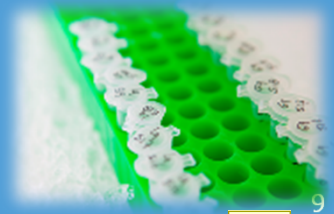
- Trial 3:
  - Is it feeding RW per se or a higher FI that alleviates anaemia & stimulates growth
  - Determine if anaemia is caused by an inflammatory response or nutritional deficiency
- Dietary treatments:
  - CPEL with FS,
  - CPEL with ragworm extract,
  - RW
- Fish fed to satiation for 57 days
- 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



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KJ3

Iron transporter transferrin,
Iron storage protein ferritin
Iron absorption regulator hepcidin
casp3 indicator of apoptosis
hsp70 is indicator of cell stress

Hepcidin: master regulator of iron homeostasis via its ability to block ferroportin, the only known iron export protein.

Hepcidin expression is induced independently by the accumulation of iron or inflammation and is suppressed when iron stores are depleted, by anaemia, hypoxemia and accelerated erythropoiesis

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Results

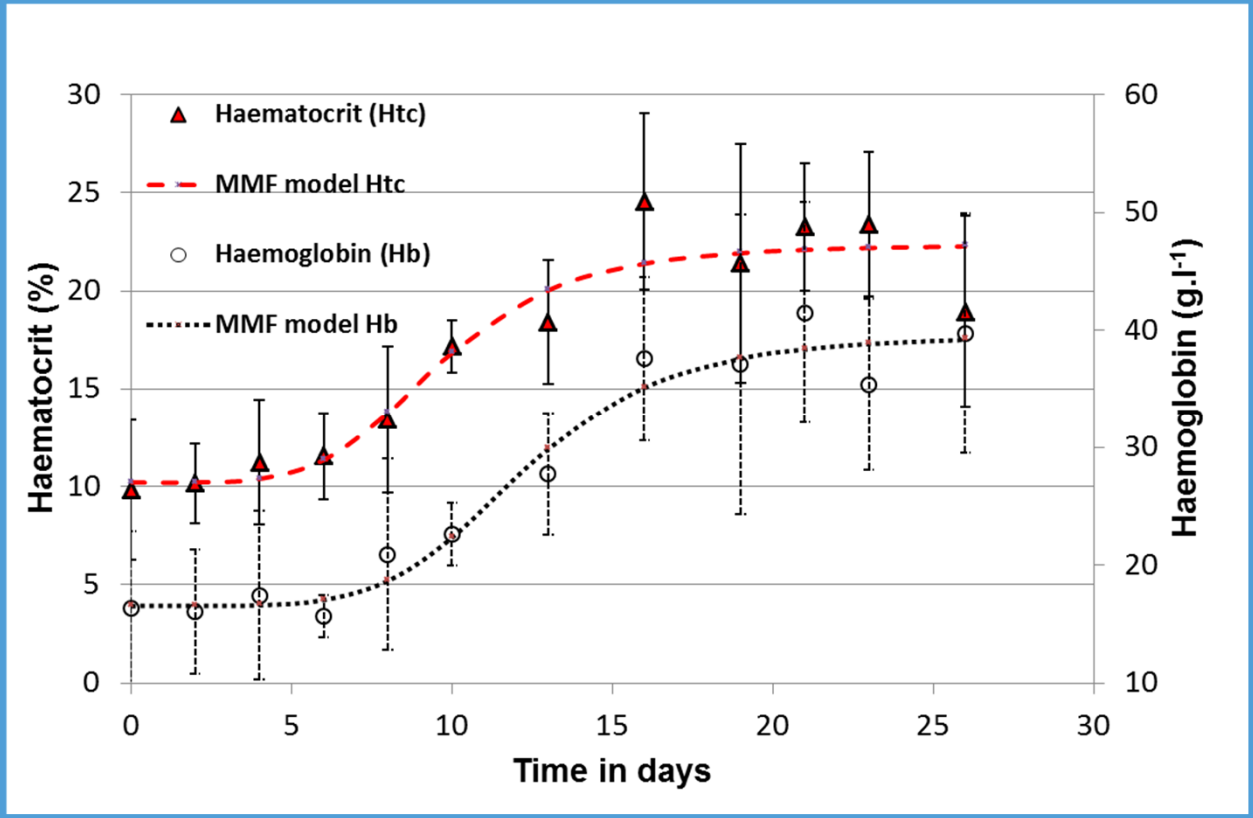
- Trial 1: Effect of a dietary change from CPEL to RW on Hct & Hb in sole

Diet	Day	N	Hct(%)	Hb (g l^{-1})
---[a]	0	24	9.8[a] ± 3.6	16.3[a] ± 6.6
CPEL	26	10	8.8[a] ± 3.3	11.7[a] ± 5.5
RW	26	10	19.0[b] ± 4.9	39.7[b] ± 10.2

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

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*. 2015[a]

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KJ1 MMF => Morgan-Mercer-Flodin growth model

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Results

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

Diet	Day	N	Hct (%)	Hb (g l^{-1})
----	0	20	12.5 ± 3.17[a]	19.6 ± 8.10[a]
CPEL	23	3	13.1 ± 1.16[a]	18.9 ± 3.28[a]
MU	23	3	17.4 ± 1.95[b]	26.7 ± 4.38[b]
RW	23	3	19.0± 1.42[b]	34.1 ± 5.13[b]
p-value	--		<0.01	0.01

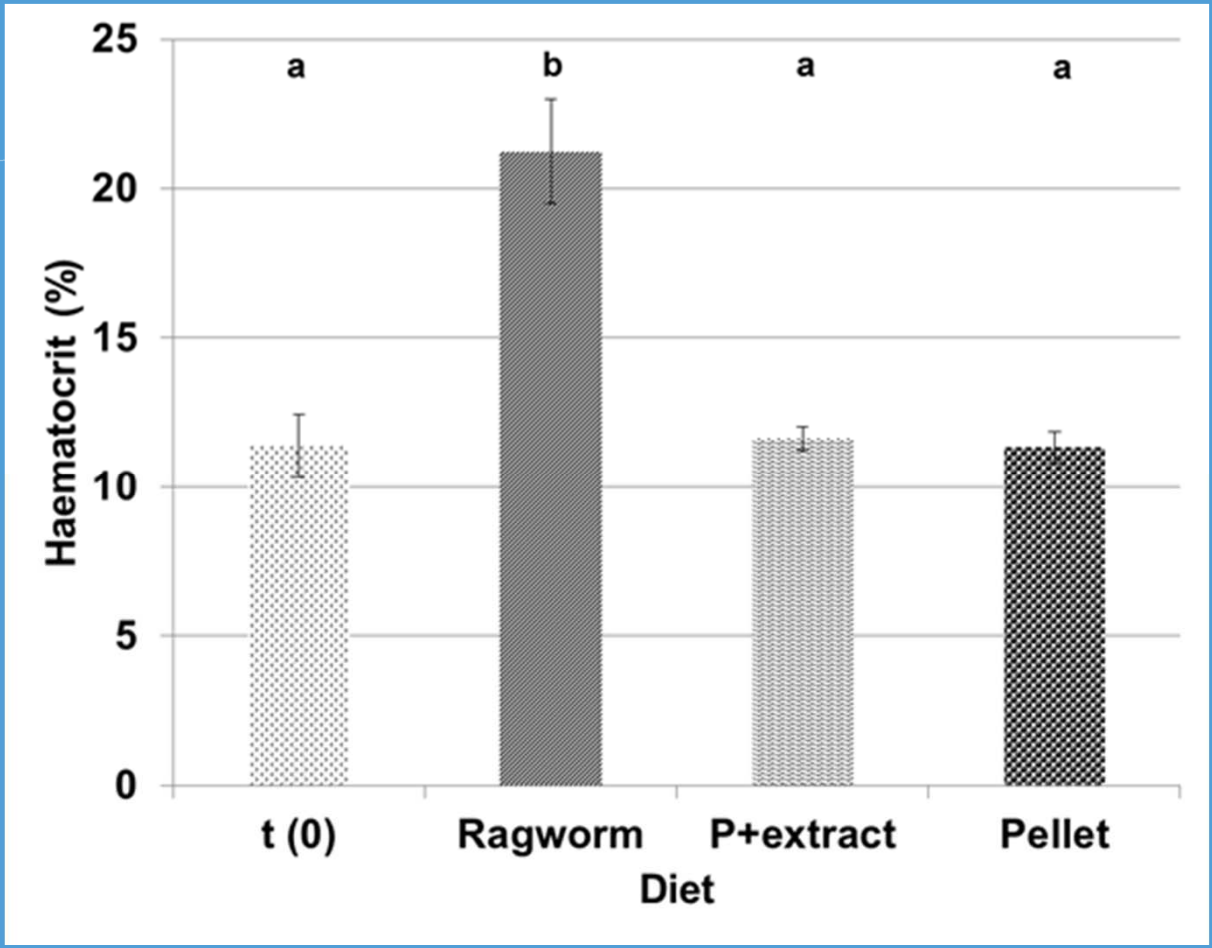
[ab]Means within columns with a common superscript are not significantly different using the one or two sided Fisher LSD post hoc test depending on the hypothesis (Kals *et al.* 2015[b]).

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.* 2015[c]).

Results

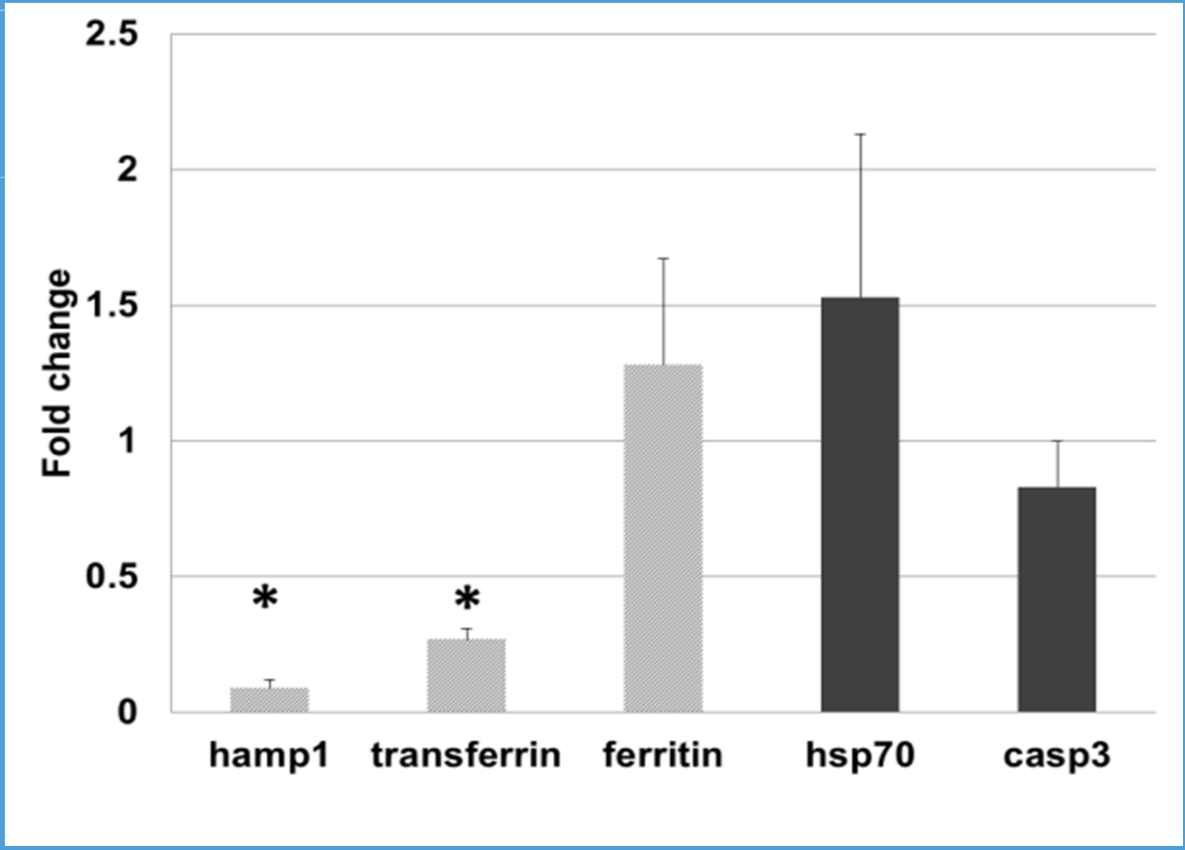
- Feed intake (FI), feed conversion (FCR) & growth

Diet	Ragworm	Pellet + extract	Pellet	P-value
BW_{start} (g)	78.95±3.73	78.04±2.56	75.22±2.35	0.34
BW_{end} (g)	118.8±6.85^{a}	109.9±6.25^{b}	98.8±2.66^{c}	**0.01**
FI (g dm fish d^{-1})	0.69±0.04^{a}	0.68±0.03^{a}	0.56±0.06^{b}	**0.01**
Growth (g d^{-1})	0.70±0.08^{a}	0.56±0.08^{b}	0.41±0.09^{c}	**0.02**
FCR_{DM}	1.02±0.03	1.25±0.17	1.34±0.20	0.09

- 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

Liver 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 expr. of *beta actin* in liver shown as fold change of sole fed CPEL+extract relative to sole fed RW set to 1

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Results

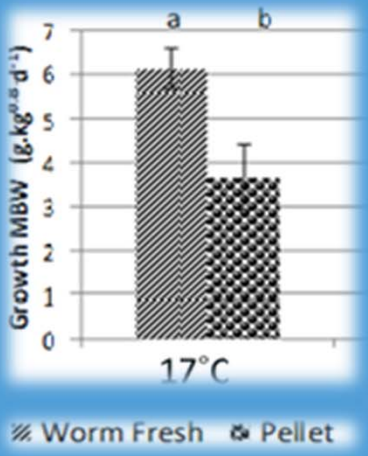
Gene	Expression[1]	Indication
Hepcidin	Down regulated	anaemia without immune response
Transferrin	Down regulated	an acute phase response, yet no iron deficiency
Ferritin	similar	no oxidative stress, iron overload, iron deficiency, inflammatory conditions and/or major intracellular changes
Casp3	similar	no apoptosis
Hsp70	similar	no cell stress

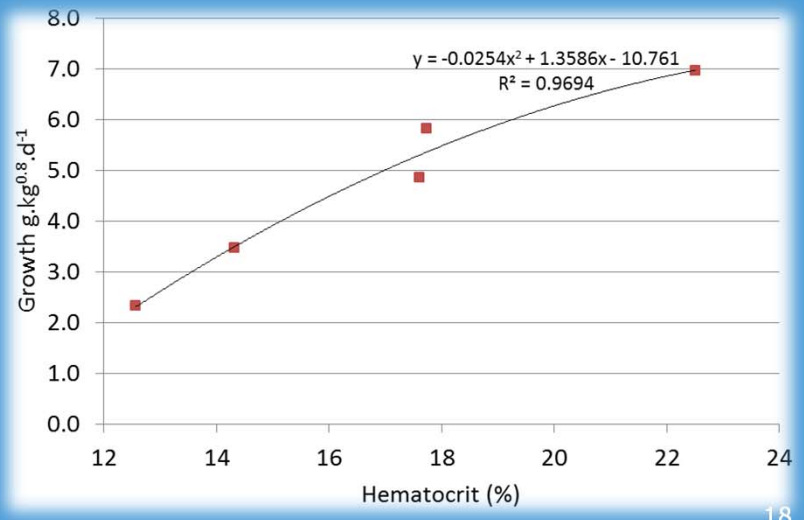
Results indicates a nutritional anaemia, but not necessarily an iron deficiency anaemia, in sole fed treated pellets

[1] Sole fed treated pellets vs. ragworm

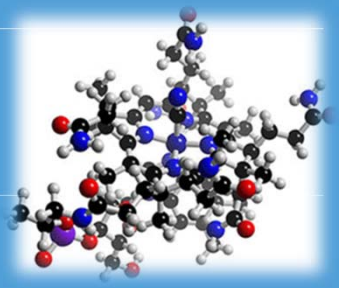
Discussion and conclusions

- 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



- PA, iron & B12 content of diets and requirements

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

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

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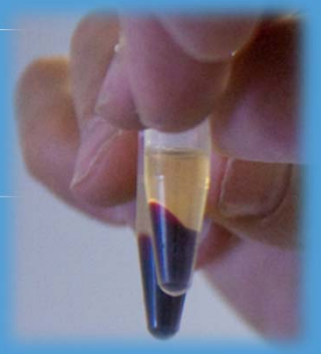
Discussion

- Why?
  - alkaline character of sole's intestine
  - low pepsin like activity in stomach
- Iron absorption
  - non heme requires reduction of Fe, hence a low pH
  - heme has its own pathway and is independent of pH
- B12
  - active uptake needs low pH & pepsin like activity
  - sole depends on diffusion
  - sole may depend on high levels of dietary B_{12}

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
  - has a limited effect on growth
  - does not improve Hct & Hb

- 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

References

- Palstra A.P., Blok M.C., Kals J., Blom E., Tuinhof-Koelma N., Dirks R.P., Forlenza M. & Blonk R.J.W. (2015) In- and outdoor reproduction of first generation common sole Solea solea under a natural photothermal regime: temporal progression of sexual maturation assessed by monitoring plasma steroids and gonadotropin mRNA expression. General and Comparative Endocrinology SI: 10ISRPF. http://dx.doi.org/10.1016/j.ygcen.2014.12.004

- Kals J., Blonk R.J.W., Mheen H.W. van der, Schrama J.W. & Verreth J.A.J. (2015)[a]. Feeding ragworm (*Nereis virens* Sars) increases haematocrit and haemoglobin levels in common sole (*Solea solea* L.). *Aquaculture Research*, DOI: 10.1111/are.12767.

- Kals J., Blonk R.J.W., Mheen H.W. van der, Schrama J.W. & Verreth J.A.J. (2015)[b]. Mussel (*Mytilus edulis L.*) and ragworm (*Nereis virens, Sars*) both alleviate anaemia in common sole (Solea, solea L.) *Aquaculture Research*, DOI 10.1111/are,12871.

- Kals J., Blonk R.J.W., Palstra A.P, Sobotta T.K., Mongile, F., Schneider O, Planas J.V, Schrama J.W. & Verreth J.A.J.(accepted)[c]. Feeding ragworm (*Nereis virens* Sars) to common sole (*Solea, solea* L.) alleviates nutritional anaemia and stimulates growth.

- van Kampen E.J. & Zijlstra W.G. (1961). Standardization of hemoglobinometry II. The hemiglobincyanide method. *Clinica Chimica Acta* **6**, 538–544.

Thank you!

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*Co-authors of articles

*Animal caretakers

Questions?

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

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