Development of Zn and Mn enrichment method in live feed and nutritional importance in marine fish larvae

By

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Introduction

Growth and survival of hatchery-raised finfish are affected by the nutritional value of live feeds such as rotifers and *Artemia*.

Manipulation of nutrient profiles of live feeds has been improved growth, survival and quality of hatchery-raised fingerlings (example: enrichment of live feeds with HUFAs, vitamin A, taurine....)

So far, little effort has been directed toward the enrichment of live foods with minerals
Zn and Mn are important minerals for growth and normal skeletal development.

**Zn**
- Stimulates bone mineralization and bone protein synthesis
- Deficiency causes dwarfism and disturbance of skeletal formation

**Mn**
- Constitutes of enzymes and involves in carbohydrate, lipid and protein metabolism
- Deficiency causes impaired growth and abnormal skeleton
Vertebral deformity in hatchery-raised red sea bream Hattori et al., 2003

- **1999**: 91% Normal, 8% Deformed, 7% Others
- **2000**: 81% Normal, 18% Deformed
- **2001**: 93% Normal, 7% Others
Objectives

- Develop new method to enrich Zn and Mn in rotifers and *Artemia* nauplii to the level of these minerals in natural zooplanktons.
- To investigate the effects of Zn and Mn supplementation in *Artemia* on early growth, body compositions and skeletal deformity of red sea bream larvae.
Experiment 1: Examination direct absorption of Zn by rotifers

Can rotifer directly absorb Zn from enrichment media?
Experimental conditions

• Zinc source: ZnSO₄ (0, 5, 10 ppb)
• Organism: rotifer (*Brachionus plicatilis*) (L size)
• Stocking Density:
  \[
  \begin{align*}
  \text{rotifer: } & 500\text{ind/ml} \\
  \text{chlorella: } & 3 \times 10^6 \text{cell/mL}
  \end{align*}
  \]
• Sampling: Rotifer, Culture media at 0 h, 12 hrs, 24 hrs
• Tank: 30 L round-shaped tank
• Water temperature: 27°C
• Replication: Duplicate
The rotifers could not absorb and retain zinc in their body from enrichment media effectively.
Zinc concentration in *Chlorella*

*Chlorella* could absorb water-bound zinc more effective than the rotifer.
It is possible to enrich *Chlorella* with zinc as a first step of enrichment. The *Chlorella* thus produced contained high levels of zinc and can be fed to rotifers.
Experiment 2: Zn absorption by *Chlorella*

**Aims**
- To examine the zinc accumulation ability of the *Chlorella*
- To determine the suitable duration for *Chlorella* enrichment with Zn
Experimental conditions

- Zinc source: ZnSO$_4$ (0, 4, 8 and 16 mg/10 g of condensed *Chlorella regularis*
- Sampling: 0h, 1h, 3h, 6h, 12h, 24h
- Replication: duplicate
- Temperature: 27°C
Zn accumulation in the *Chlorella* (DM)

*Chlorella* could absorb water-bound zinc effectively and quickly within the first 1 h of enrichment.
Experiment 3: Enrichment of rotifer with Zn-enriched *Chlorella*

- **Objective:**
  - To investigate incorporation ability of Zn in rotifer by feeding with Zn enriched *Chlorella*

- **Experimental conditions**
  - Condensed freshwater *Chlorella vulgaris*
  - Supplemented with 0.0; 0.8 and 1.6 mg Zn g$^{-1}$
  - Incubation duration was 12 hours at 25ºC
  - Organism: rotifer (*Brachionus plicatilis*) (L size); 300 ind/mL
  - *Chlorella* density 1.8x10$^6$ cell/mL
Zn content in enriched rotifer
Summary experiment 3

• Zn in rotifer was successfully improved by enrichment with *Chlorella* that pre-accumulated with Zn.

• Zn content in the rotifer was elevated with Zn supplementation in the Chlorella.

• The Zn content in the 1.6 mg Zn group after 24 h enrichment was 640.3 μg g⁻¹, almost equivalent to Zn content in natural *Acartia clausi* which contains 700 μg g⁻¹ zinc (DM).
Exp 4: Enrichment *Artemia* with Zn and Mn

**Objectives**

- To determine the incubation duration of Marine ω A® with Zn and Mn
- To investigate incorporation ability of Zn and Mn in *Artemia*
Materials

Marine ω A ®: A commercial enrichment material made by microalgae that had been removed the cell-wall (Nisshin Marine Tech Co., Ltd., Japan)

Artemia: Newly-hatched nauplii
Artemia franciscana (E.G. grade, INVE, Belgium)

Minerals: Zn and Mn
Methods

• Incubation of marine ω A®.
  - Zn levels: 0, 1.6, 3.2 mg/10g marine ω A®
  - Mn levels: 30 and 60 µg/g marine ω A®
  - Incubation duration: 0, 1, 3 and 6 hours

• Artemia enrichment
  - Zn levels: 0, 0.4, 0.8 and 1.6 mg/g marine ω A®
  - Mn levels: 0.12, 0.24 mg/g marine ω A®
  - Incubation duration: 2 hours
  - Enrichment duration: 24 hours
Incorporation of Zn and Mn in Marine $\omega^\text{®}$

Incubation 1-2 hrs was enough for Marine $\omega^\text{A®}$ to incorporate Zn and Mn
Zn and Mn content in *Artemia* at 24 hrs enrichment (DW)

Enrichment *Artemia* with Marine ω A® supplemented with Zn and Mn could increase Zn and Mn contents in the nauplii.
Nutritional important of Zn and Mn in marine fish larvae

Experiment 5: Effect of Zn and Mn enrichment in *Artemia* on growth and vertebral deformity in red sea bream larvae
Objective

To investigate the effects of Zn and Mn supplementation in *Artemia* on growth, body compositions and skeletal deformity of red sea bream larvae
Feeding scheme & experimental conditions

- Rotifer L strain 5 ind mL\(^{-1}\) (twice daily)
- Artemia (thrice daily)
- Artificial diet (once / 2 h)

Culture duration (day)

- Tank volume: 1000 L, triplicates
- Density: 20,000 larvae per tank
- Water T\(^\circ\)C: 20-23 \(^\circ\)C
- Photoperiod: 12 h dark : 12 h light
- Culture duration: 30 days
Artemia enrichment with Zn (0.1 mg/g) and Mn (2.4 mg/g)

Marine ω A® incubation 2 hours

Artemia enrichment

Harvesting

Feeding to larvae

Control Z M ZM

150 nauplii/mL
26 °C
4 g marine ω®/L

24, 29, 33 hours
Zn and Mn contents in enriched Artemia (dry-matter basis)
Growth of red sea bream at 30 dph

- Control
- Z
- M
- ZM
Survival of red sea bream during 15-30 dph

The graph shows the survival (%) of red sea bream during 15-30 days post-hatch (dph) in different treatments.

- Control
- Z
- M
- ZM

The graph indicates that the survival rate is highest in the Control group and decreases in the Z, M, and ZM groups, with no significant differences between them. The survival rates are marked with 'a'.
Zn and Mn content in whole body of red sea bream at 30 dph (dry-matter basis)
Typical vertebral deformities in red sea bream at 30 dph

Dentary

Pugheadness

Vertebral fusion

Lordosis

Kyphosis

Rib deformity

Caudal vertebrae

Fin support
Average percentage of fish with at least 1 skeletal deformity at 30 dph (%)

![Bar chart showing the percentage of fish with at least 1 skeletal deformity for different treatments: Control, Z, M, ZM. The chart indicates that the Control group has the highest percentage of deformities, with the Z, M, and ZM groups having lower percentages.](chart.png)
Percentage of major skeletal deformities of red sea bream at 30 dph (n=600)

- Jaw
- Vertebral column
- Neural spine
- Hemal spine
- Dorsal fin ray

Categories: Control, Z, M, ZM
Occurrence of vertebral deformity in red sea bream at 30 dph (n=600)
Summary experiment 5

• Mn supplementation in *Artemia* 12-42.8 µg g\(^{-1}\) (dry-matter) improves growth performance of the red sea bream larvae

• Zn and Mn supplementation in *Artemia* promotes normal skeletal development of red sea bream larvae

• Zn and Mn contents in whole body of red sea bream were affected by Zn and Mn content in *Artemia*

• Zn and Mn should be supplemented together to prevent decline of Mn in enriched *Artemia*
Conclusion

• The Zn concentration in rotifers was significantly increased indirectly by feeding zinc-enriched *Chlorella* for 6 to 12 h.

• Zn and Mn contents in *Artemia* were significantly improved indirectly by feeding Zn and Mn enriched marine ω A®

• Supplementation of Zn and Mn in *Artemia* improves growth performance and promotes normal skeletal development of red sea bream larvae
Further readings

Examination of a practical method for zinc enrichment of euryhaline rotifers (Brachionus plicatilis)

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Effect of zinc and manganese supplementation in Artemia on growth and vertebral deformity in red sea bream (Pagrus major) larvae

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THANK YOU VERY MUCH FOR YOUR KIND ATTENTION