Development of Functional Feed

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DSM Nutritional products
Bangkok, Thailand
Concept: Aquaculture functional feeds

Functional feeds include specific (micro) ingredients targeting specific functions or product characteristics, thus bringing solutions to recurrent problems in animal production cycles rather than only focusing on growth.

- **Product quality**
  - Flesh/skin pigmentation
  - Shelf life of flesh color (tuna)
  - PUFA content

- **Reproduction**
  - Fertilization
  - Embryo development
  - Larval quality

- **Sustainability**
  - Fish oil replacement
  - Fish meal replacement
  - Feed cost control

- **Health**
  - Stress mitigation
  - Nutritional prophylaxis
Target: product quality

Ranking criteria in purchasing dynamics
Base: Total Phase II, n = 630

1. Color
2. Retailer
3. Eyes
4. Texture
Functional feeds for product quality
ω-3 PUFAs, DHA and EPA improve attention and may reduce ADHD in children.

<table>
<thead>
<tr>
<th>Duration of Supplementation (Days)</th>
<th>Control</th>
<th>Treatment</th>
<th>Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Days of illness During Supplementation</td>
<td>6.03</td>
<td>3.38</td>
<td>1.78</td>
</tr>
<tr>
<td>Days of illness / Episode of illness</td>
<td>5.78±3.24</td>
<td>4.47 ± 2.71</td>
<td>1.29</td>
</tr>
</tbody>
</table>

Supplementation with DHA and EPA reduced the number of days of absence from school due to illnesses.
Functional feed with high EPA & DHA levels at the end of the production cycle

Gilthead seabream. Seven-month feeding with high levels of vegetable oil followed by feeding FO

Izquierdo 2005
Target: reproduction

<table>
<thead>
<tr>
<th>Function</th>
<th>Micronutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fecundity in gilthead seabream</td>
<td>n-3 HUFA (1.6%) and vitamin E</td>
</tr>
<tr>
<td>Fecundity in tilapia</td>
<td>n-6 FA</td>
</tr>
<tr>
<td>Fertilization</td>
<td>EPA, Arachidonic Acid, Vitamin C</td>
</tr>
<tr>
<td>Embryo development</td>
<td>Antioxidants: E, C, astaxanthin</td>
</tr>
<tr>
<td>Larval quality</td>
<td>DHA, Vitamin E</td>
</tr>
</tbody>
</table>

Izquierdo et al. 2001

![Graph showing viable eggs, fertilized eggs, and larval survival](image)

Dietary E/n-3 HUFA ppm/%d.w.

Fernandez-Palacios et al. 2001
## Broodstock feeds

### Traditionally:
- Fresh marine by-products
- Fresh marine by-products + formulated feeds
- Disease propagation
- Environmental impact
- Nutrient levels inadequate

### Improved broodstock diets:
- Increased n-3 HUFA
- Increased Vitamin E
- Specific raw materials (squid meal)
- Increased cost
- Species specific
**Target: sustainability**

Replacement of all or part of the fish meal with renewable plant protein raw materials

Effect of SBM on intestinal epithelium of Atl. salmon

Effect phytic acid on vertebral structure of Atl. salmon

Burrell et al. 2001

Helland et al. 2006
Functional feeds for fish meal replacement

Hydrolyze/inactivate

- (Micro)nutrients
- FM
- ANF
- Plant

ADD
- AA
- ENZ
- Se
- Nuc
Dietary phytase degrades phytates / releases available phosphorus
Dietary xylanase to degrade arabino-xylans and release nutrients from plant cell walls

Diaz and Verlhac 2011

<table>
<thead>
<tr>
<th>12 weeks</th>
<th>FM effect</th>
<th>Enz. effect</th>
<th>Interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBW (g)</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>FCR</td>
<td>S</td>
<td>S</td>
<td>NS</td>
</tr>
</tbody>
</table>
Xylanase increases protein digestibility in Pangasiuss

ADC Proteins %

Xylanase FXU/kg

Hung 2010
Dietary nucleotides maintain the integrity of the intestinal epithelium

Standard commercial diet

Standard commercial diet supplemented with nucleotides

Three weeks after beginning of feeding,
- mean fold height
- lateral branching (BF) increased
- total gut surface
Functional blends or premixes contribute to a smooth switch from High fish meal diets to high plant meals diets.
Target: resistance to infectious diseases

Antibiotics in Norwegian Salmon farming 1980-2005

Directorate of Fisheries and Norwegian Food Safety Authority
Functional feeds at work in Norway

EWOS boosterfeed

Protec and Response Xtra diets
http://www.skretting.co.uk/Internet/SkrettingUKIreland/webInternet.nsf/wprlid/0A16BEA26F47028980257408003873A8!OpenDocument

Smart feed
http://www.biomar.com/en/Species--Products/
Requirements and priorities of the immune system for nutrients  

Humphrey, Koustos and Klasing 2002

- Immune system must obtain necessary nutrients at right times in right amounts to combat infection.
- Infection increases nutrient demand.
- Functional feeds must be formulated to meet nutrient demand when immune system is challenged.
Immune cells in the intestinal epithelium of fish

Intraepithelial lymphocytes (→) in distal intestine of carp.
Uran et al. 2008

Macrophages (→) in intestinal epithelium of striped catfish
Rodriguez et al. 2010
The defense mechanisms of fish
Verlhac Trichet 2009

Innate immunity

1. Phagocyte
   - Phagosome
   - Lysosome
   - Phagolysosome
   - Enzymes
   - Microbes
   - Extracellular
   - ROS
   - NO

2. Time since infection

Adaptive immunity

3. B lymphocyte
   - Proliferation
   - Plasma cells
   - Antibody production
   - T lymphocyte
   - Antigen lysis
   - Help
   - Antibody + Complement + Antigen

DSM
BRIGHT SCIENCE. BRIGHTER LIVING.
Functional (micro)ingredients for health

Nutrients
- Vitamins (C, E, D₃)
- Carotenoids
- Minerals (Se)
- Amino acids (Arginine)
- Fatty acids (n-3 PUFAs)
- Nucleotides

Non-nutritive immunostimulants
- β-glucans
- Carageenans
- Chitosan
- Peptidoglycan
- Alginates
- Plant extracts
- Lactoferrin

Others
- Prebiotics (MOS, FOS)
- Probiotics
- Organic acids
- Essential oils
Vitamin C concentration affects functions of immune cells

**Diagram:**

- **Lymphocytes** and **Phagocytes**
  - **AA nmole/10^8 cells**
  - **Dietary Vit. C (ppm)**
  - **Phagocyte Vit C nmoles/10^8 cells**

- **Pinocytosis**
  - **Phagocytosis**

**Graphs:**

- Bar graph showing the relationship between dietary Vitamin C concentration and AA nmole/10^8 cells for both Lymphocytes and Phagocytes.
- Bar graph showing the relationship between dietary Vitamin C concentration and Phagocyte Vit C nmoles/10^8 cells.
Vitamin C enhances disease resistance in Tilapia fry
Suwanmanee et al. 2011

- Nile tilapia during the 21 day sex-reversal period
- IBW 0.25-0.32 g
- Basal diet; commercial shrimp powder 40% CP
- Asc. ac. supplementation: 1, 2, 3 g/kg diet
- Feeding duration: 7, 14, 21d
- Challenge by immersion with *Streptococcus agalactiae* for 7d
- Survival determined 7d post challenge
Vitamin E enhances phagocyte functions
Vitamin E affects leukocyte apoptosis

Apoptosis: capacity of cells to induce a death program when they are in bad condition or infected.

<table>
<thead>
<tr>
<th>Stressor</th>
<th>DNA fragmentation</th>
<th>Disrupted membrane</th>
<th>Cell shrinking</th>
<th>Apoptotic bodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apoptosis Index</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- E150 Lipid 14%
- E150 Lipid 26%
- E1200 Lipid 14%
- E1200 Lipid 26%


**REVIEW**

β-glucans as conductors of immune symphonies

Roy A. Dalmo*, Jarl Bøgwald**

*Department of Marine Biotechnology, University of Tromsø, N-9037 Tromsø, Norway

Received 17 December 2007; revised 17 April 2008; accepted 18 April 2008

Available online 25 April 2008.
**β-Glucan: in vitro activation of macrophages**

- Polysaccharides
- Several types: origin, molecular weight
- Glucose molecules linked in β 1-3 and β 1-6 found in cell walls of yeasts and mycelial fungi
- β-glucan receptor on macrophage mb

**Activate phagocyte functions**

**Graph:**

Activation of macrophages with graded doses of glucan

Oxidative burst

- RLU*10^6
- Dose in mg per 10^6 cells

*Dose in mg per 10^6 cells*
Dietary nucleotides

- Save energy since *de novo* synthesis requires high energy P groups (7-8)
- Lymphocyte proliferation
  - Signaling through TLR

Not in intestinal epithelium and immune cells

**HEALTH & PERFORMANCE**
Toll like receptors (TLR) are present in fish and shrimp

<table>
<thead>
<tr>
<th>Species</th>
<th>Authors, Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. vannamei</td>
<td>Yang et al., 2007</td>
</tr>
<tr>
<td>P. monodon</td>
<td>Arts et al., 2007</td>
</tr>
<tr>
<td>F. chinensis</td>
<td>Yang et al., 2008</td>
</tr>
<tr>
<td>P. japonicus</td>
<td>Megata et al., 2008</td>
</tr>
<tr>
<td>D. rerio</td>
<td>Jault et al., 2004</td>
</tr>
<tr>
<td>S. aurata</td>
<td>Franch et al., 2006</td>
</tr>
<tr>
<td>P. olivaceus</td>
<td>Takano et al., 2007</td>
</tr>
<tr>
<td>C. semilaevi</td>
<td>Yu et al., 2009</td>
</tr>
</tbody>
</table>
# Dietary nucleotides in fish

<table>
<thead>
<tr>
<th>Species</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tilapia</td>
<td>Growth, survival</td>
<td>Ramadan &amp; Atef, 1991</td>
</tr>
<tr>
<td>Tilapia</td>
<td>Ab titer/vacc., lymphocyte prolif.</td>
<td>Ramadan et al. 1994</td>
</tr>
<tr>
<td>RBT</td>
<td>Growth</td>
<td>Adamek et al., 1996</td>
</tr>
<tr>
<td>RBT</td>
<td>Survival [V. anguillarum challenge]</td>
<td>Burrels et al. 2001a</td>
</tr>
<tr>
<td>RBT</td>
<td>Survival [ISA virus challenge]</td>
<td>Burrels et al. 2001a</td>
</tr>
<tr>
<td>Coho salmon</td>
<td>Survival [P. salmonis challenge]</td>
<td>Burrels et al. 2001a</td>
</tr>
<tr>
<td>Atl. Salmon</td>
<td>Sea lice infection</td>
<td>Burrels et al. 2001a</td>
</tr>
<tr>
<td>Atl. Salmon</td>
<td>Ab titer/vacc., survival</td>
<td>Burrels et al. 2001b</td>
</tr>
<tr>
<td>Atl. Salmon</td>
<td>Intestinal fold</td>
<td>Burrels et al. 2001b</td>
</tr>
</tbody>
</table>

Adapted from Li and Gatlin, 2006
# Dietary nucleotides in fish

<table>
<thead>
<tr>
<th>Species</th>
<th>Effects</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common carp</td>
<td>Phagocytosis, respiratory burst ➤ complement, lysozyme ➤ <em>A. hydrophila</em> infection ➣</td>
<td>Sakai et al., 2001</td>
</tr>
<tr>
<td>RBT (all)&lt;)</td>
<td>B lymphocytes, resistance to IPN ➤ Plasma cortisol ➣</td>
<td>Leonardi et al., 2003</td>
</tr>
<tr>
<td>Turbot</td>
<td>Altered immunogen expression</td>
<td>Low et al., 2003</td>
</tr>
<tr>
<td>Hybrid stripped bass</td>
<td>Neutrophil oxidative radical prod. ➤ Survival [{<em>S. iniae</em> challenge}] ➤</td>
<td>Li et al., 2004</td>
</tr>
<tr>
<td>Common carp</td>
<td>Survival [{<em>V. anguillarum</em> challenge}] ➤</td>
<td>Huttenhuis et al., 2006</td>
</tr>
<tr>
<td>Grouper</td>
<td>Growth, O²⁻ production ratio ➤</td>
<td>Lin et al., 2009</td>
</tr>
</tbody>
</table>

Partly adapted from Li and Gatlin, 2006
Dietary nucleotides in aquatic animals enhance:

- Lymphocyte proliferation
- Integrity of intestinal epithelium
- Antibody titer after vaccination
- Respiratory burst
- Complement
- Lysozyme

Survival

Growth
Effect of dietary nucleotide on Tilapia immune response and resistance to a bacterial challenge
Shiau (unpublished)

Leukocyte proliferation & survival after challenge with *S. iniae*

**Stimulation Index**

<table>
<thead>
<tr>
<th>Exogenous nucleotides (ppm)</th>
<th>ConA</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>120</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>240</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>360</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>480</td>
<td>b</td>
<td>b</td>
</tr>
<tr>
<td>600</td>
<td>b</td>
<td>b</td>
</tr>
</tbody>
</table>

Survival %

Dietary nucleotide on Tilapia immune response and resistance to a bacterial challenge.
Effect of Nucleotides on tilapia survival after experimental infection - Survival

Survival after challenged with *S. agalactiae*

Areechon et al. 2012

![Graph showing survival rates](image-url)
Effects of dietary nucleotides on immune responses in *L. vannamei*

Shi-Yen Shiau 2012

**Respiratory burst (superoxide anion)**

- Control
- NX 75
- NX 150
- NX 300

**Prophenoloxidase activity of hemocytes**

- Control
- NX 75
- NX 150
- NX 300
# Effects of dietary nucleotides on growth & immune response in *L. vannamei*

<table>
<thead>
<tr>
<th></th>
<th>Minimum NX dose for a significant enhancement</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth</strong></td>
<td>75 ppm</td>
</tr>
<tr>
<td>Feed efficiency</td>
<td>75 ppm</td>
</tr>
<tr>
<td>Tot. hemocyte count (10^5/ml)</td>
<td>75 ppm</td>
</tr>
<tr>
<td>Hemi-granular cell count(10^5/ml)</td>
<td>75 ppm</td>
</tr>
<tr>
<td>Granular cell count(10^5/ml)</td>
<td>75 ppm</td>
</tr>
<tr>
<td>Respiratory burst (superoxide anion)</td>
<td>75-150 ppm</td>
</tr>
<tr>
<td>Prophenoloxidase activity of hemocytes</td>
<td>75-150 ppm</td>
</tr>
<tr>
<td><strong>Growth</strong></td>
<td>150-300 ppm</td>
</tr>
<tr>
<td>Tot. hemocyte count (10^5/ml)</td>
<td>150 ppm</td>
</tr>
<tr>
<td>Phagocytic activity</td>
<td>75 ppm</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td>150 ppm</td>
</tr>
<tr>
<td>Lysozyme activity</td>
<td>300 ppm</td>
</tr>
</tbody>
</table>
Effects of nucleotides on disease resistance and immunity of *L. vannamei*

Nontawith Areechon, 2012

Survival rate (%) during 14 d after challenge with *V. harveyi*

- Control
- 75 ppm
- 150 ppm
- 300 ppm

Survival rate %

0.00 10.00 20.00 30.00 40.00 50.00 60.00 70.00 80.00 90.00 100.00

Days

1 3 5 7 9 11 13

63.7% b
63.3% b
56.7% ab
36.7% a
Dietary nucleotides in aquatic animals

**Functions**
- Support cell proliferation
- Enhance innate & adaptive immune response
- Enhance resistance to diseases
- Reduce effects of stressors
- Enhance performance

**Strengths**
- All species
- No residues
- No resistance
- Very stable through feed processing/storage
Why we use functional feeds?

Like any other animal fish is subject to stress. It can be due to environment (eg. temperature changes), handling (eg. grading, transport), diseases, etc. In these situations the immune system needs support to secure good growth. It is recommended to use ALLER VITAMAX feeds before the predicted stress conditions appear to strengthen the immune system of the fish.
Conclusion

- Research and practice shows that functional feeds containing selected additives have well defined and strong effects on given physiological functions.

- The use of these feed must be part of a nutritional strategy contributing to the sustainability of aquaculture, higher productivity and enhanced resistance to infectious diseases.

- Understanding the interactions of functional components in the feeds and the biochemical and physiological functions of the animal is key for the further development of functional feeds.
Thank you for your kind attention