XIII FENACAM, 21-24 November 2016, Fortaleza, Brazil

Yeast product solutions for a better management of fish health and performances

Nadège Richard[1], Philippe Tacon[1], Eric Auclair[1], Marcelo Borba[2]

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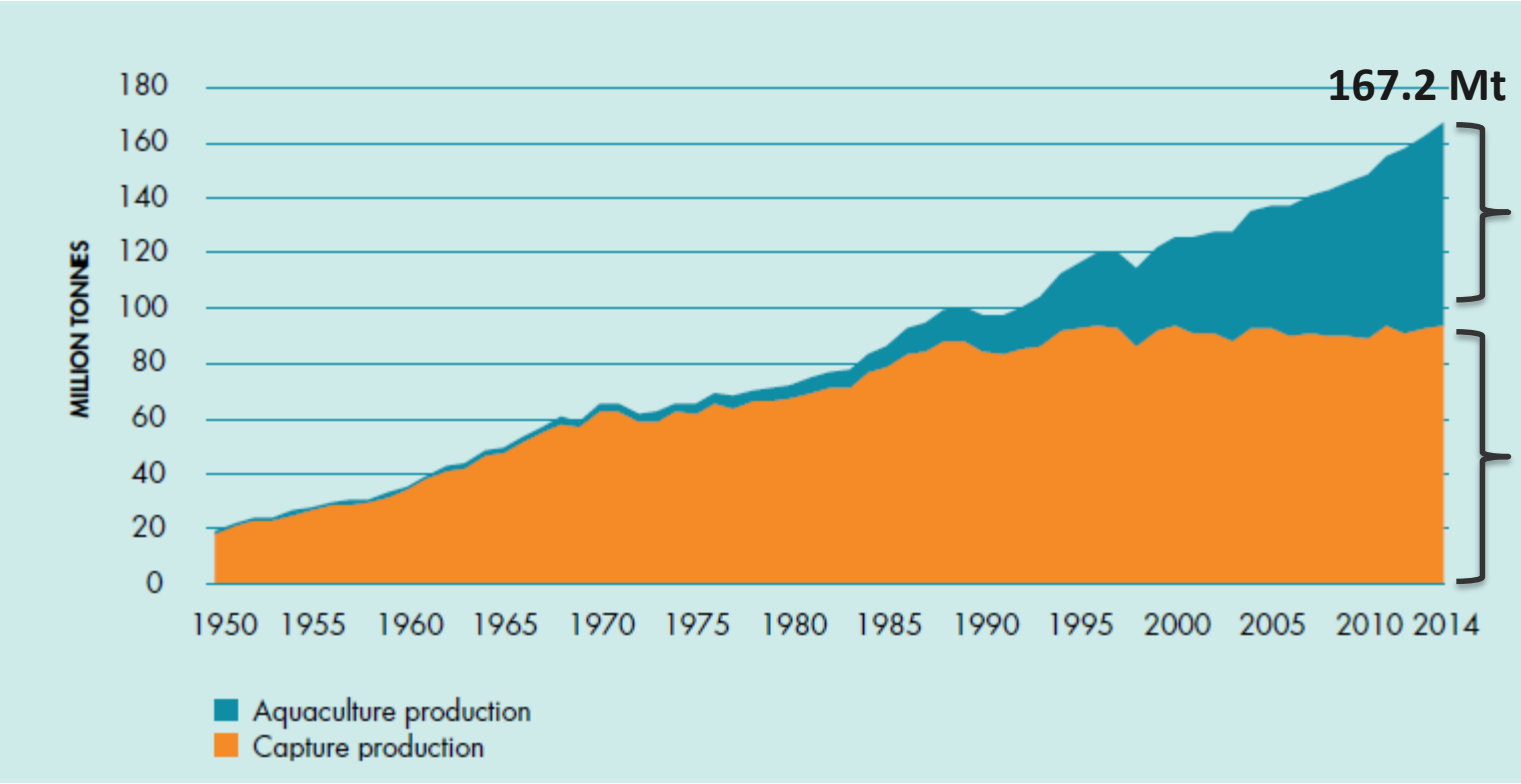
Expansion of Aquaculture

- Best growth rate in animal food production sector
- World capture fisheries and aquaculture production (FAO, 2016):



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Expectatives

- Growing population: Human population is estimated to reach 9 billion people by 2050

- Growing demand for food production, and in particular for seafood

- Aquaculture is expected to play a very important role as food supplier (protein supplier) to feed the planet

Prediction 2050: 70% of fish will be farmed

Need to increase the fish production capacity, in a sustainable way.

Challenges limiting aquaculture growth

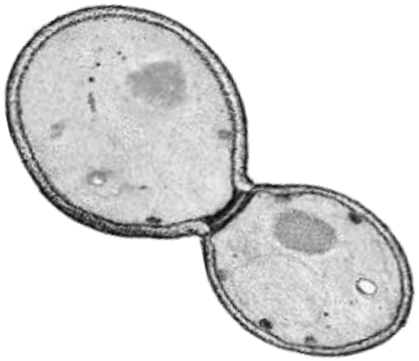
Global Aquaculture Alliance's GOAL conference (September, China) survey:

- What is the most important challenge limiting aquaculture?
  - Health & disease management **53%**
  - Environmental/social issues 21%
  - Leadership/investment/marketplace 11%
  - Education 9%
  - Feed 6%

Current tools and future trends

- Control of physico-chimical parameters & sanitary precautions
- Use of organic minerals
- Use of fertilizers
- Use of organic acids
- Use of phytobiotics
- Use of prebiotics
- Use of probiotics
- Recirculation aquaculture systems (RAS)

Yeasts



- Eukariotic single cell microorganisms (5x10 µm)

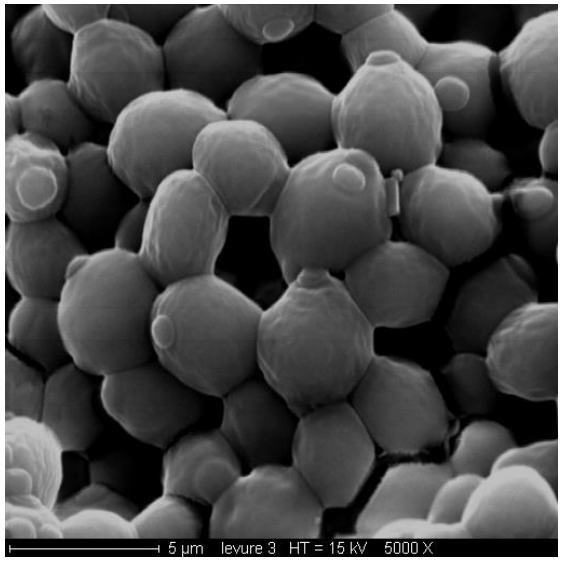
  100 times bigger than a bacteria

- Nutritional interests:

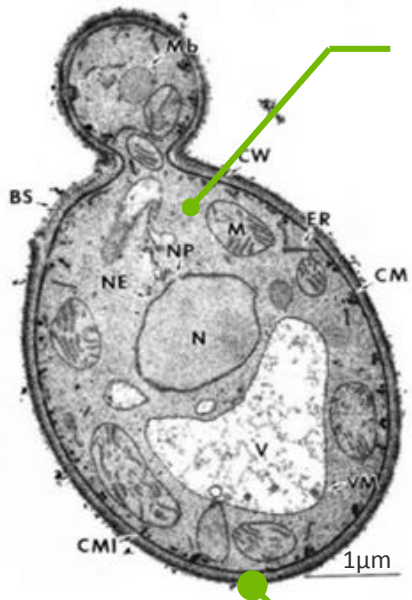
  - rich in proteins
  - rich in minerals
  - rich is vitamins (B1, B2,…)

- Uses in aquaculture

Yeast *Saccharomyces cerevisiae*



MEB picture (x5000)



Cellular content:
Cytoplasm + nucleus + organelles

Rich in proteins, peptides, nucleic acids, vitamins

Parietal fraction:
Cellular wall + plasma membrane

Rich in mannans, beta-glutan

Represents 15-30% DW cell
25-50% cell volume.
100-200 nm

Yeast parietal fractions
&
Benefits in fish health management

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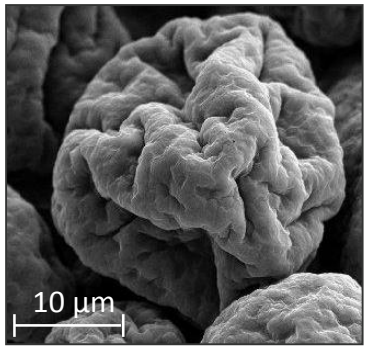
Parietal fractions production process



Bakery yeast production

Insoluble parietal fractions SafMannan®

Yeast Autolysis Processing

Yeast Extracts
Soluble cell content

Heat treatment

Autolysis

Separation

Concentration

Drying, Screening and Packing

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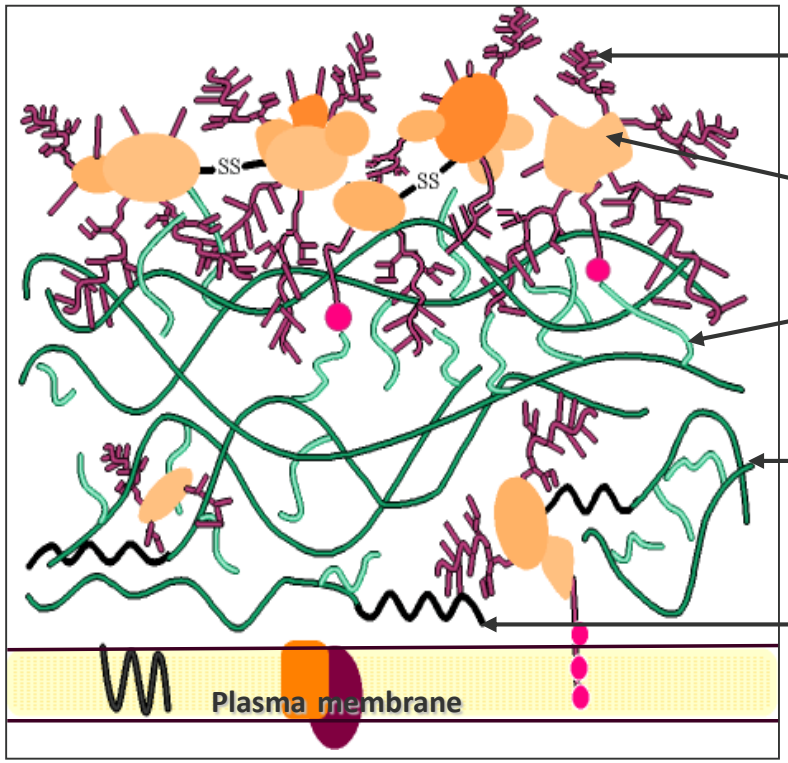
Yeast parietal fraction components



Mannose
-Soluble

Protein

Mannoprotein
-40% of YCW mass

B1,6-glucan
-Insoluble
-10% of YCW mass

B1,3-glucan
-Insoluble
-50% of YCW mass

Chitin
-Insoluble
-1-3% of YCW mass

(Lipke et Ovalle, 1998)
(Cabib et Arroyo, 2013)
(Netea et al 2008)

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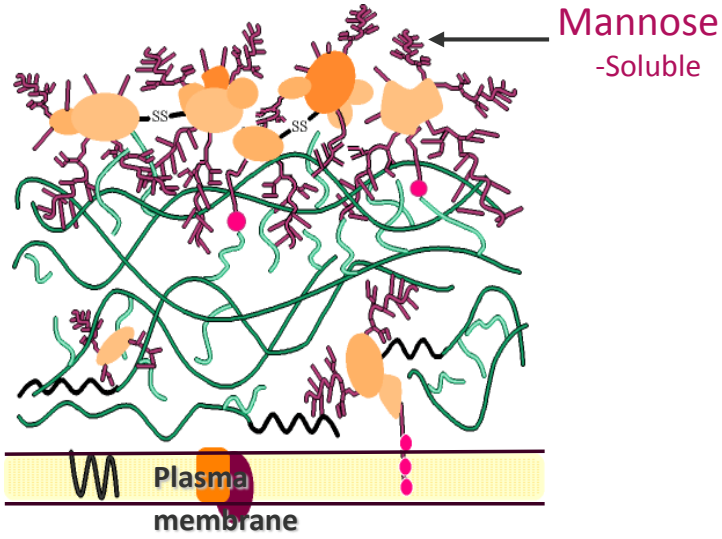
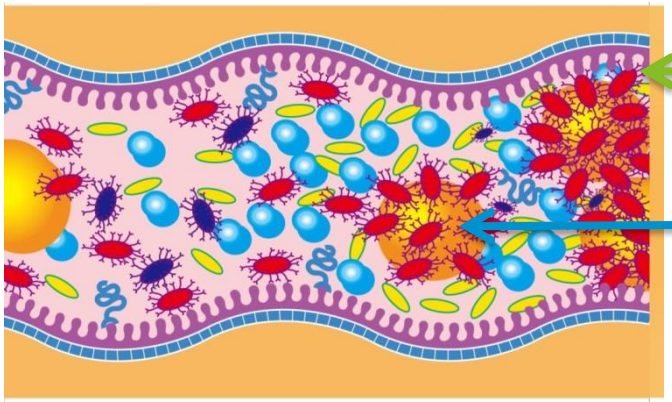
Mannan oligosaccharides



- **Prebiotics** : nutrient source used by beneficial bacteria in intestinal tract for growth

- **Pathogen binding capacity**: able to bind pathogenic bacteria presenting fimbriae on their surface.



Prevention of pathogen colonisation in intestine

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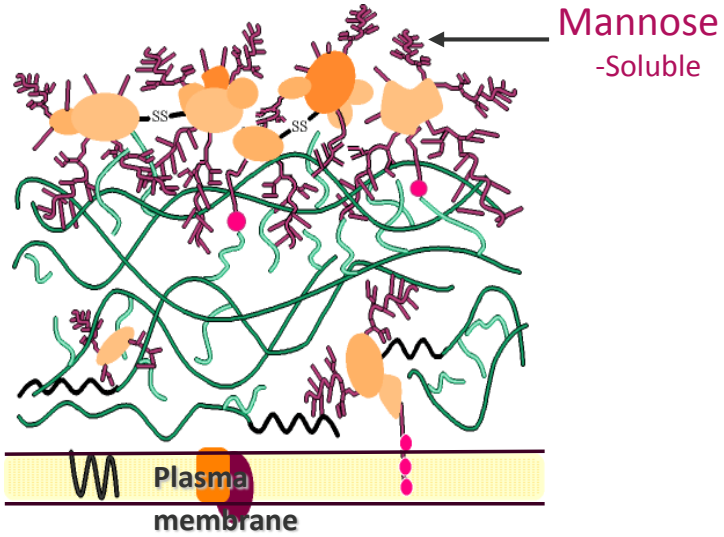
Mannan oligosaccharides



- **Prebiotics** : nutrient source used by beneficial bacteria in intestinal tract for growth

- **Pathogen binding capacity**: able to bind pathogenic bacteria presenting fimbriae on their surface.

- **Improvement of gut architecture :** increased microvilli height and density

- **Stimulation of immune response**

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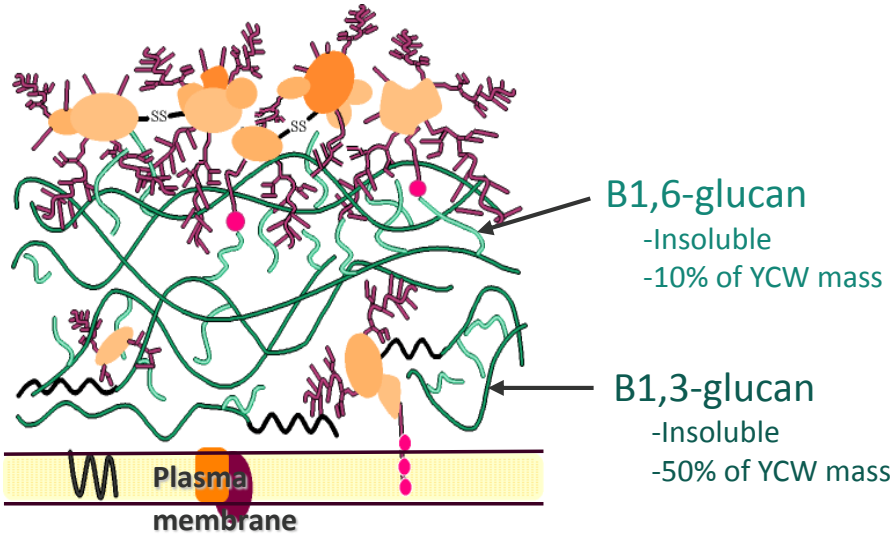
B-glucans



- Glucose units linked by β1-3 and β1-6 bonds

- Insoluble part of parietal fraction

- **Stimulation of immune system**

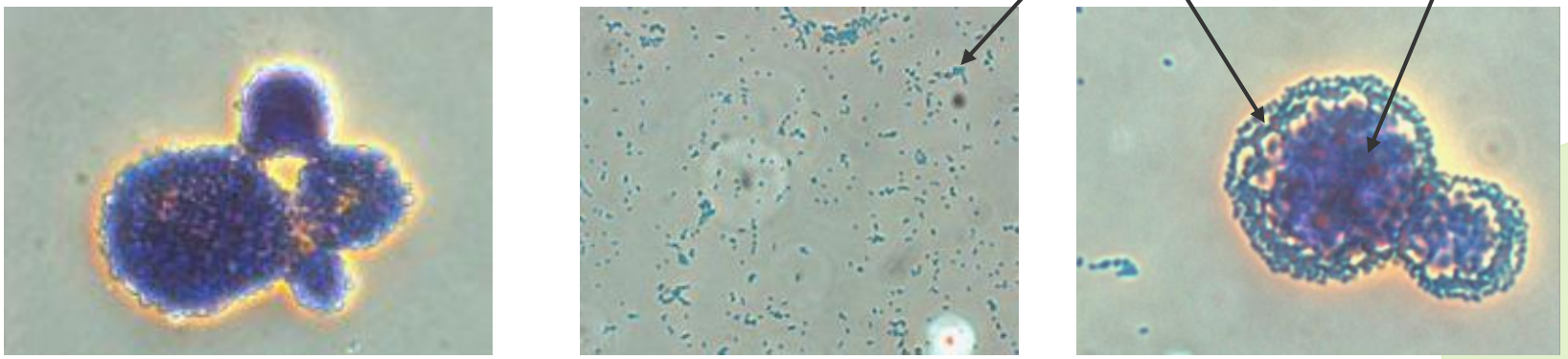
SafMannan composition:

- > 20% β-glucans
- > 20% Mannan-oligosaccharides
- 10-25% proteins

Yeast parietal fractions bind to pathogens

Safmannan® can bind *Vibrio campbelii*, a virulent bacteria in shrimp hatcheries

Safmannan® is put in contact with bacteria for 30 mins



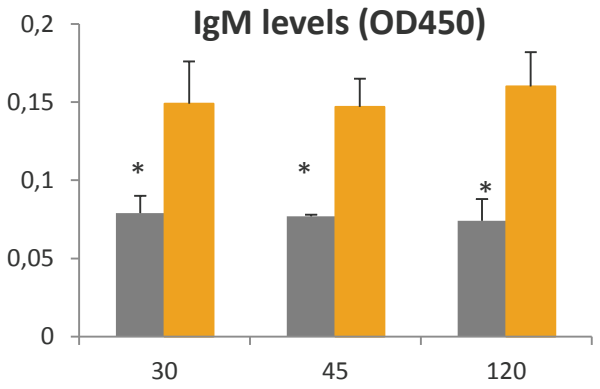
SafMannan alone | Vibrio alone | SafMannan +vibrio

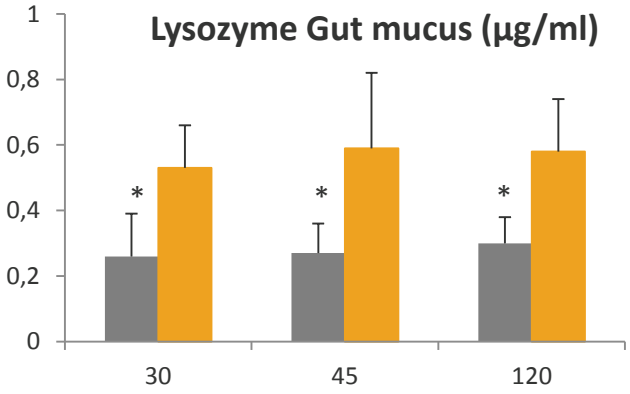
Trial performed at IMAqua laboratory, Univ. of Ghent, Belgium

Nile tilapia Trial 1 (*Oreochromis niloticus*)

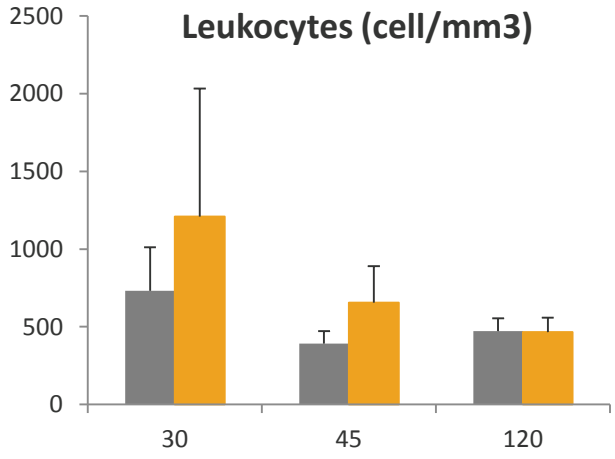
- Mexico
- Juveniles, 6 g
- Diets:
  - CTRL: commercial formula
  - SafMannan: CTRL + 3 kg/T
- 8 m^3 cages, triplicates
- 3600 fish per cage
- Duration: 60 days
- At days 30, 45, 60: - Measurement of immun parameters
  - Challenge with Aeromonas hydrophilia (10 fish/cage)

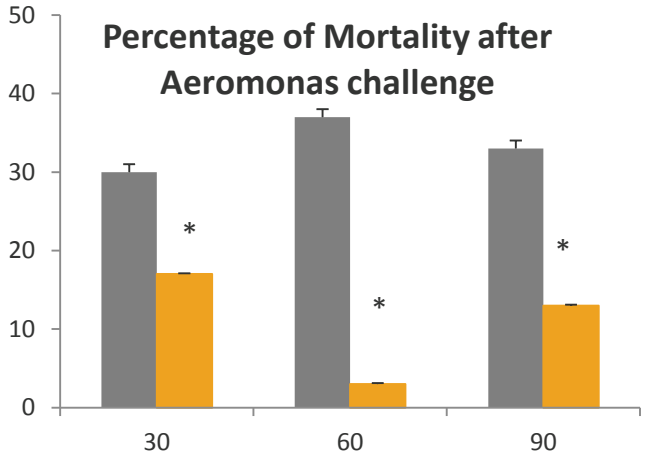
Nile tilapia Trial 1 (*Oreochromis niloticus*)





Yeast parietal fraction enhances innate immunity indicators





Yeast parietal fraction improves survival after *Aeromonas* challenge

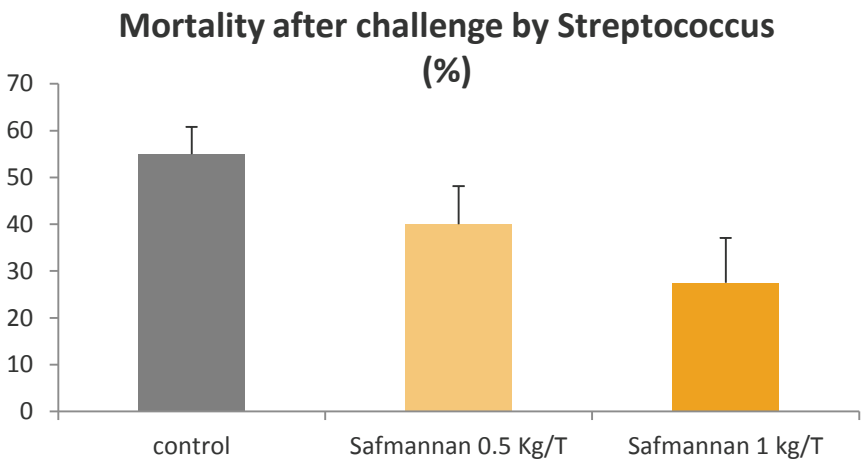
Control Safmannan

Nile tilapia Trial 2 (*Oreochromis niloticus*)

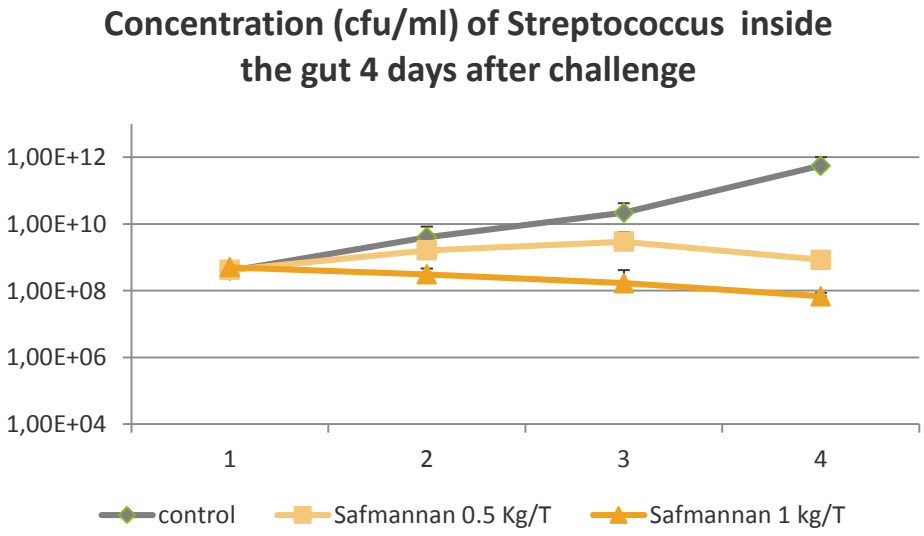
- Thailand
- Juveniles, 30 g
- Diets:
  - CTRL: commercial formula
  - SafMannan at 0.5 kg/T
  - SafMannan at 1 kg/T
- Cages (2mx2mx1m), triplicates
- 100 fish per cage
- Duration: 3 months
- **Challenge with *Streptococcus agalactiae* (20 fish/cage)**

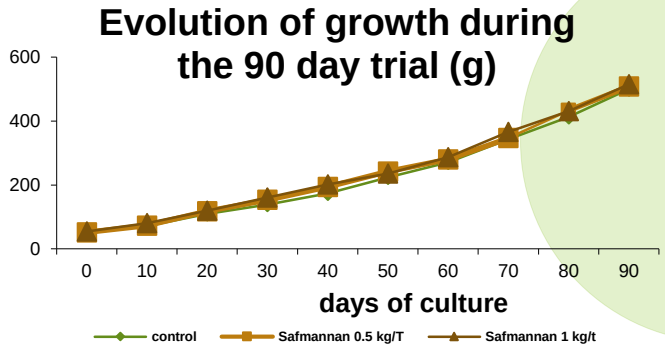
Composition	first month	second and third month
Fish meal 55%	25.0	**22.0**
Fish meal 64%	10.0	**9.0**
Corn meal	28.0	**23.0**
Casava meal	10.0	-
Soy bean meal	12.0	-
Peanut bean meal	8.0	**34.0**
Squid meal powder	5.0	**4.0**
Vitamins and minerals	2.0	**2.0**
Soybean sauce by product	-	**6.0**
Safmannan®0.5 kg/T	50	**50**
Safmannan®1 kg/T	**100**	**100**

Nile tilapia Trial 1 (*Oreochromis niloticus*)



- Reduction of mortality in treated groups
- Concomitant reduction of pathogenic bacteria in the gut of treated fish
- This immune stimulation did **not have negative effect** on growth in near pond conditions (cages in pond)





Yeast parietal fractions improves survival during *Streptococcus agalactiae* challenge and maintains growth performances

Freshwater fish - recommendations

A strong effect in stress situation and on pathogen prevention

Hatchery/Nursery.

- During masculinization as it is a stressful process and tilapia can be subjected to pathogen challenge – **1 to 2 kg/T** (not more than 4 weeks).
- Before transportation to nursery and to grow out farm. Preparation against stress – **1 to 2 kg/T for 2 to 4 weeks.**

Grow out farms

- After transportation, starter feeds. **1kg/T for 1 month.**
- Prevention of pathogens such as Streptococcus **0.5 kg/T** all the time. Increase to **1 kg/T** around the pathogen risk period (hot temperatures for example).

Japanese seabass Trial (*Lateolabrax japonicus*)

- Feed Research Institute, Beijing, China
- Juveniles, 18 g
- All feeds with 25% fish meal- 20% Soybean meal, but a positive control with 38,5 FM – 0% SBM

  4 Safmannan®dosages : 0.25 ; 0.5 ; 1; 2 kg/T on SBM diets
- 6 tanks per treatment
- 30 fish per tank
- Duration: 10 weeks
- **Challenge with *Aeromonas veronii* (6-7 fish/tank)**

Ingredients	FM	SBM	Saf 0.25
Fish meal	38.5	25	25
Soybean protein concentrate	20	20	20
Soybean meal	0	21	21
Wheat flour	21	21	21
Fish oil	6	6.4	6.4
Monocalcium phosphate($Ca(H_2PO_4)_2$)	1	2.1	2.1
Microcrystalline cellulose	10.1	1	0.975
Phospholipid (93%)	2	2	2
Choline chloride (50%)	0.4	0.4	0.4
Vitamin and mineral Premix	1	1	1
Methionine hydroxy analog-Ca (98%)	0	0.1	0.1
Safmannan® (mg/kg)	0	0	250

Yu et al. 2014 Aquaculture 432:217-224

SafMannan

Japanese seabass Trial (*Lateolabrax japonicus*)

Aquaculture 432 (2014) 217–224

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journal homepage: www.elsevier.com/locate/aqua-online

Efficacy and tolerance of yeast cell wall as an immunostimulant in the diet of Japanese seabass (*Lateolabrax japonicus*)

H.H. Yu [a], F. Han [a], M. Xue [a,b,*], J. Wang [a], P. Tacon [c], Y.H. Zheng [a], X.F. Wu [a], Y.J. Zhang [d]

[a] National Aquafeed Safety Assessment Station, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[b] Key Laboratory of Feed Biotechnology of Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China
[c] Société Industrielle Lesaffre, Lesaffre Additive Division, Marcq en Baroeul, France
[d] The Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, 5 Yushan Road, Qingdao, Shandong 266003, China

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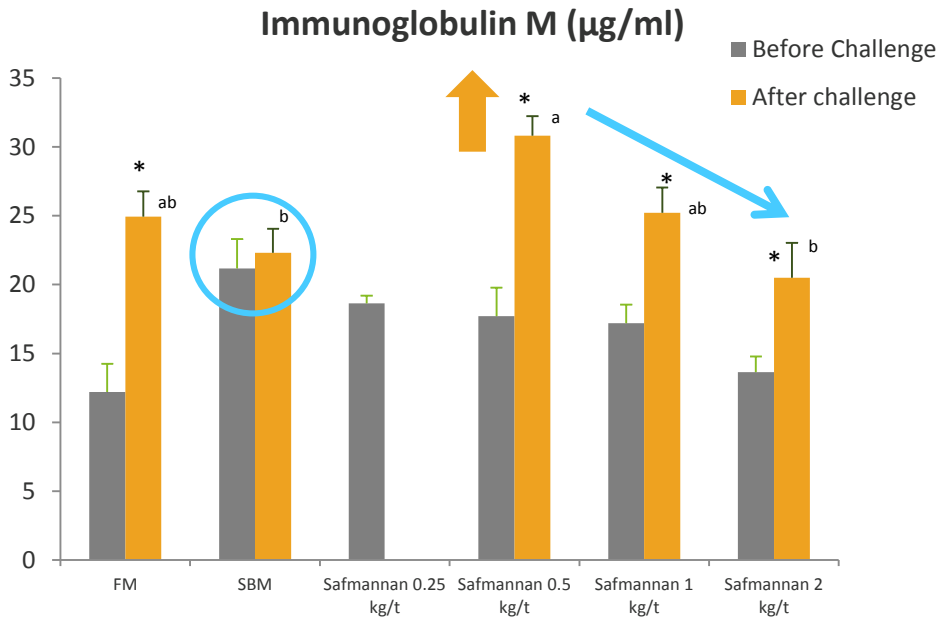
Japanese seabass Trial (*Lateolabrax japonicus*)

- **Immun parameter: Ig M concentration in plasma, before (BC) and after (AC) challenge**



- BC: IgM levels already elevated for SBM group – **enteritis effect**
- AC: Significant increase in IgM levels with parietal fractions **0.5 kg/T** (compared to SBM)
- AC: With increase parietal fraction dose, decrease in IgM levels

- **SBM control has a pathological issue (confirmed by histology)**
- **Safmannan® at 0.5 kg/T is the most adequate dose to stimulate IgM production during a pathogene challenge**

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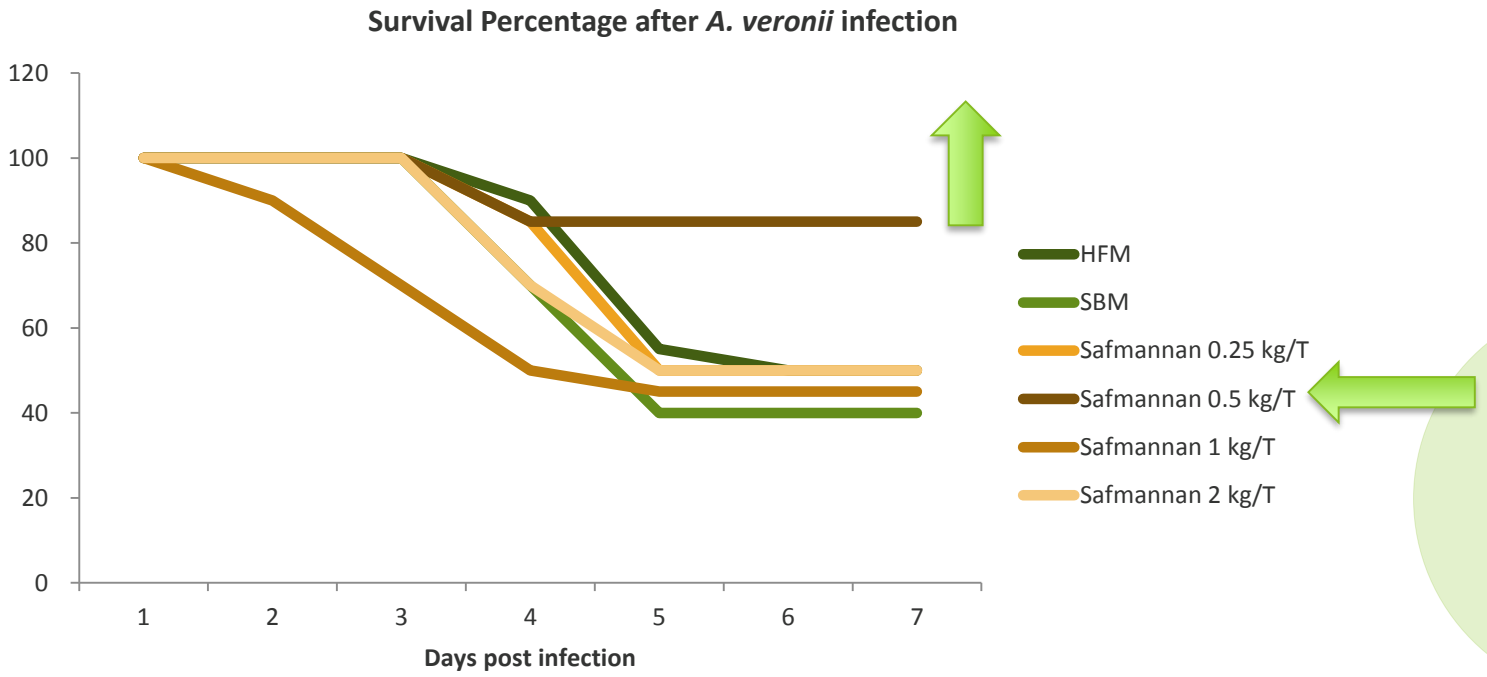
Japanese seabass Trial (*Lateolabrax japonicus*)

- **Survival rate during *Aeromonas veronii* challenge:**



Best Immune stimulation obtained with Safmannan® at 0.5 kg/T

Enhanced production of IgM and better resistance to pathogene challenge

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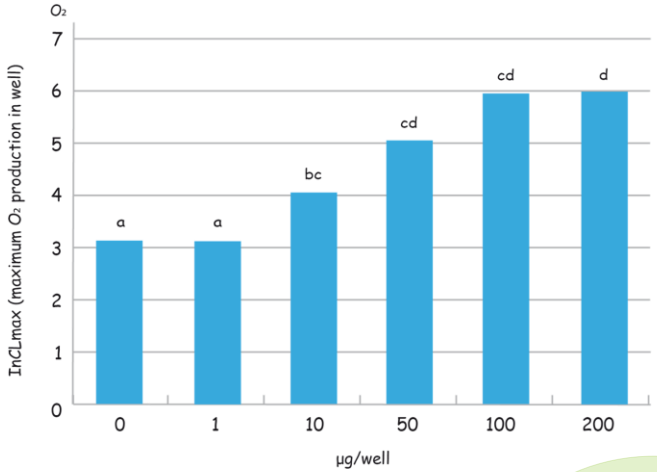
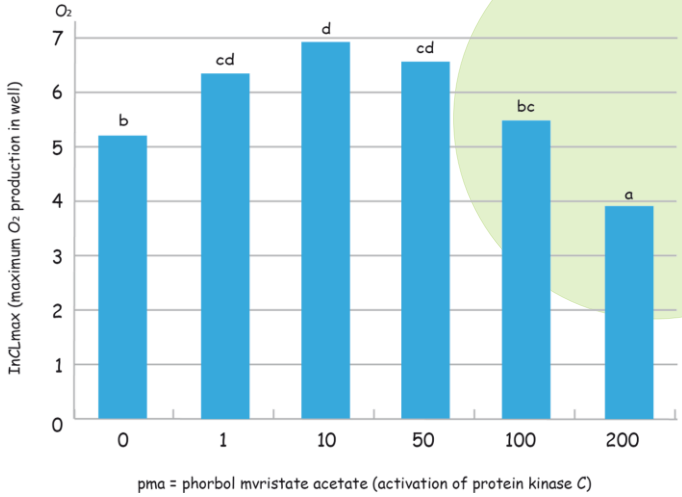
European seabass (*Dicentrarchus labrax*)

- Greece (HCMR)
- Juveniles, 100 g
- Blood collection: white blood cells
- Blood cell in contact with yeast material

  Direct stimulation with yeast fractions

  Stimulation with yeast fractions after prestimulation with pma





YCW: Be careful of overstimulation!

Strong or prolonged stimulation with high doses of parietal fractions can lead to an over stimulation of the immune system

Marine Fish- recommendations

Hatchery/ Nursery

- Boost the immune system in larvae and fry: **0.5 kg/T, all time**
- Adaptation to stress before transportation : **0.5 to 1 kg/T - 2 to 4 weeks.**

Grow out cages

- Prevention of bacterial diseases : **0.5 kg/T** all time.
- Can help fight against parasites as parietal fractions can increase the production of skin mucus.

Selenium-eriched Yeast & benefits in fish anti-oxidant status

SelSaf

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Oxidative stress

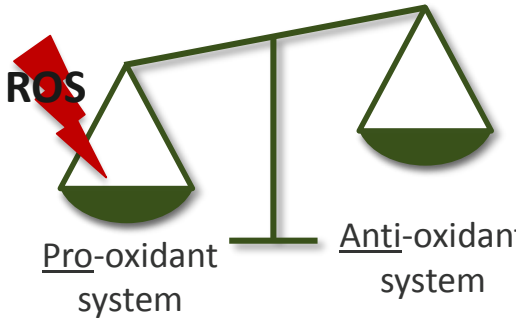
- Aquaculture practices & environmental parameters changes
  - Handling
  - Transportation
  - T°c, O_2, …
  - Infections
  - High density
  - Nutrient deficiency
  - …

→ Stress in fish → Production of ROS (reactive oxigen species) in the cells

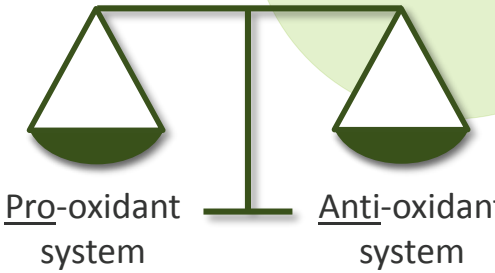
Cellular antioxidant defense capacity overwhelmed

ROS neutralised by cellular antioxidant defense system

Imbalanced oxidative status



Oxidative status balance maintained



Damages to tissue proteins, unsaturated fatty acids , DNA, …

Disruption of cell membrane integrity

Disruption of physiological processes, reduction of animal performances, appearance of muscle degeneration, decreased resistance to infections and diseases

Healthy animal

Antioxidant defences

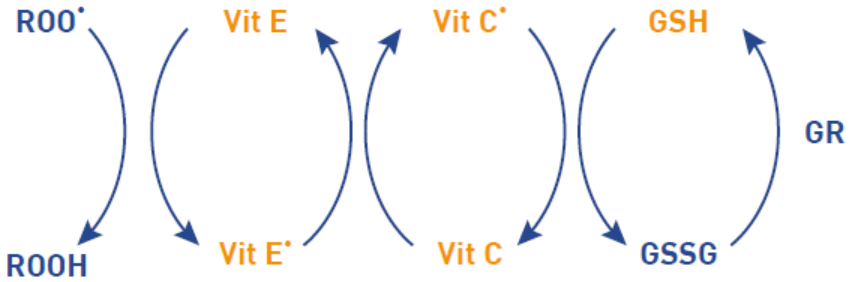
- Non-enzymatic antioxidants compounds:

  - Vitamin E

  - Vitamin C

  - Glutathione (GSH): tripeptide required to return oxdized antioxidants to their active forms and which acts also as a cofactor for the antioxidant enzyme GPX. Oxidized glutathione is formed during both processes, and is then reduced by GR (Glutathione reductase)

Figure 2 : Non-enzymatic antioxidant mode of action

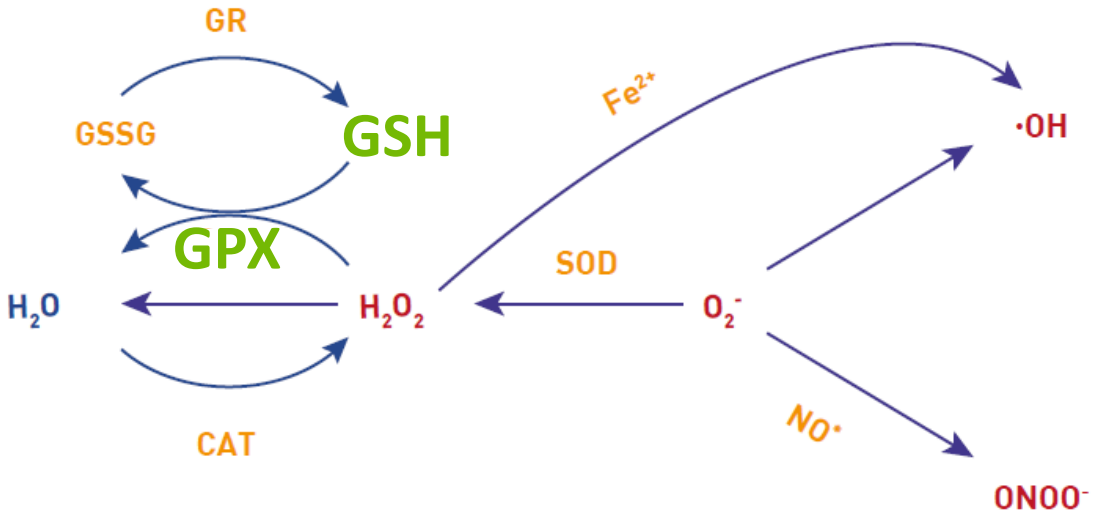


Supplementation of antioxidant compounds in aquafeed to counteract/prevent oxidative stress and its detrimental effects of animal health and meat quality

Antioxidant defences

- Antioxidants enzymes:
  - Superoxide dismutase (SOD) $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$
  - Catalases (CAT) $2\ H_2O_2 \rightarrow 2H_2O + O_2$
  - Glutathione peroxidase (GPX): selenoenzyme $H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O$

$ROOH + 2GSH \rightarrow GSSG + H_2O + ROH$



GPX is an essential enzyme of antioxidant defence system and needs selenium in its active sites to function properly

Selenium

- Essential micronutrient for animal health

Se deficiency in fish: Reduction of growth performances & increased mortality rate

Abnormal swimming, nerve cord & liver pathologies

- Fishmeal is an important source of Se for fish

	Selenium content (mg/kg)
Fishmeal LT 70	1.6
Fishmeal 60	1.2
Fish protein concentrate	1.9
Krill meal	12.0
Squid meal	0.5

	Selenium content (mg/kg)
Soyprotein concentrate	0.1
Soybean meal 48	0.3
Rapeseed meal	1.1
Sunflower meal	0.5
Wheat gluten	0.4
Corn gluten meal	0.8
Pea protein concentrate	0.1

Substituion of FM by plant protein sources in aquafeed requires Se supplementation to meet fish Se requirements

Selenium

- Two forms of Se can be added in feed:

Inorganic forms : Sodium selenite salt

Sodium selenate salts

Organic forms: Selenomethionine

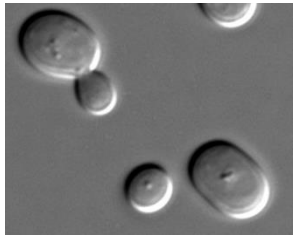
Selenocysteine

Selenized yeast (SelSaf)

Production process of Selsaf

Yeast

S. cerevisiae



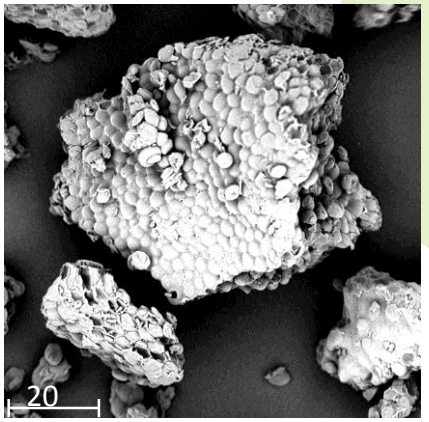
Inorganic Selenium

Bakery yeast production

Drying

Inactivated and dry selenized yeast

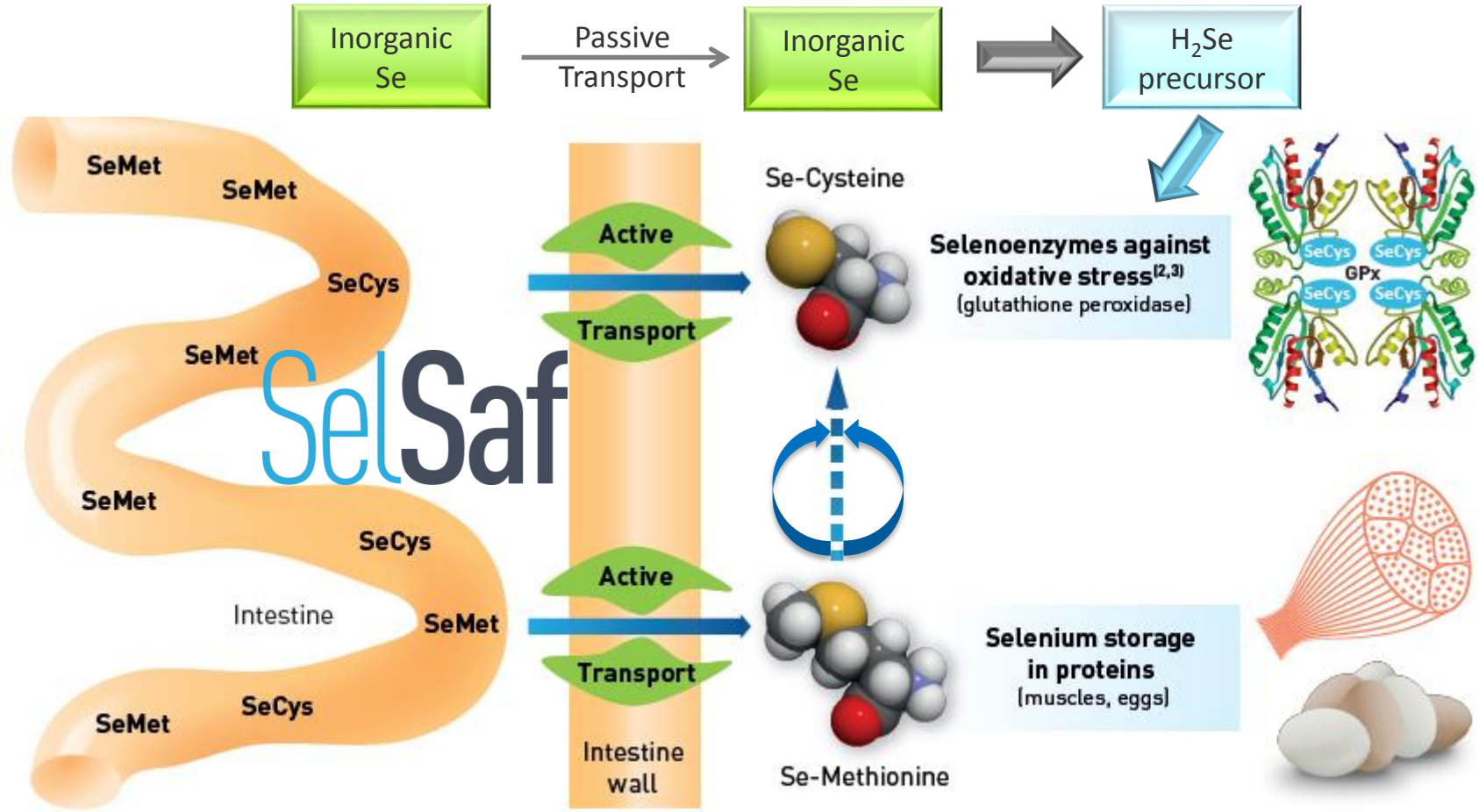
2000 or 3000 ppm Se



Se: 67% SeMet

33% SeCys and other Se compounds

Selenium absorption, assimilation and antioxidant defenses



Rainbow trout (*Oncorhynchus mykiss*) Trial 1

- National Institute for Agricultural Research (INRA), France
- Fry, 91 mg
- 200 trouts per tank
- 3 replicates per treatment
- Treatments:

Se concentration in diets (mg/T)	No Se suppl.	Sodium selenite (eq. 300 ppm Se)	Selenized yeast (Selsaf) (eq. 300 ppm Se)
3 FM&FO-based diets	1.2	1.6	1.7
3 Plant-based diets	0.5	0.9	0.9

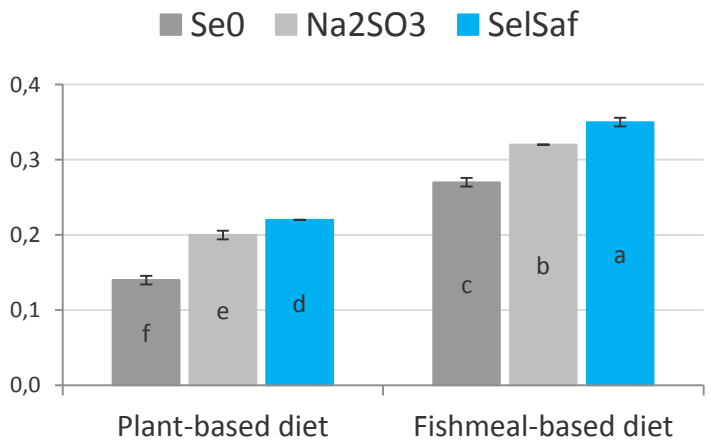
FM&FO-based diets: 62% FM, 12% FO
Plant-based diets: 75% plant meals, 5% FO, 10.5% plant oils

- Duration: 12 weeks of feeding from first-feeding
- End of trial: Measurement of Se concentration if fish body

  Measurement of antioxidant status indicators: GPX activity in whole body.

Rainbow trout (*Oncorhynchus mykiss*) Trial 1

- Whole-body Se content (µg/g of fish):

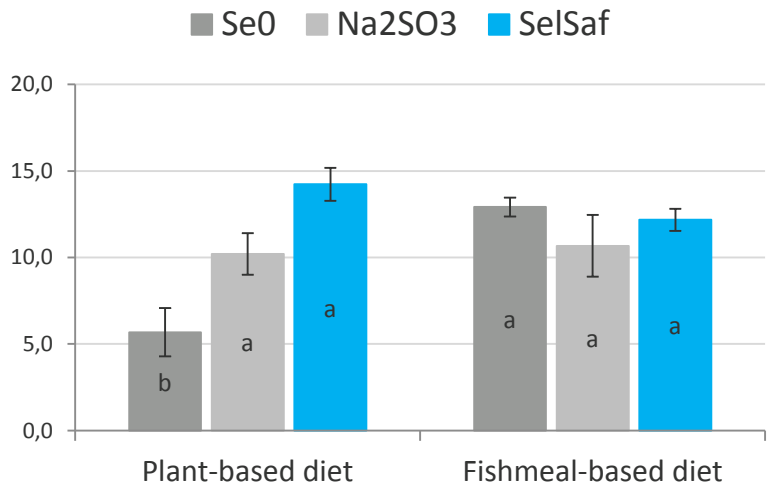


- Plant-based diets induced a decrease in body Se content.
- Supplementation with Se lead to an increase in body Se content in both plant-based and FM-based diets.
- **Supplying Se as selenized-yeast allowed a higher retention of Se in the body of trout compared to the inorganic form of Se.**

Rainbow trout (*Oncorhynchus mykiss*) Trial 1

- Activity of Se-dependent GPX in whole-body of trout:

(pmol NADPH oxidised/min per mg protein)



- Plant-based diet, when not supplemented in Se, induced a decrease in GPX activity compared to FM-based diets.
- **Supplying Se as selenized-yeast allowed a restoration of GPX activity, comparable to that measured in fish fed FM-based diets.**

Rainbow trout (*Oncorhynchus mykiss*) Trial 1

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Influence of the forms and levels of dietary selenium on antioxidant status and oxidative stress-related parameters in rainbow trout (*Oncorhynchus mykiss*) fry

Stéphanie Fontagné-Dicharry[1]*, Simon Godin[2], Haokun Liu[1,3], Philip Antony Jesu Prabhu[1], Brice Bouyssière[2], Maïté Bueno[2], Philippe Tacon[4], Françoise Médale[1] and Sadasivam J. Kaushik[1]

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Article

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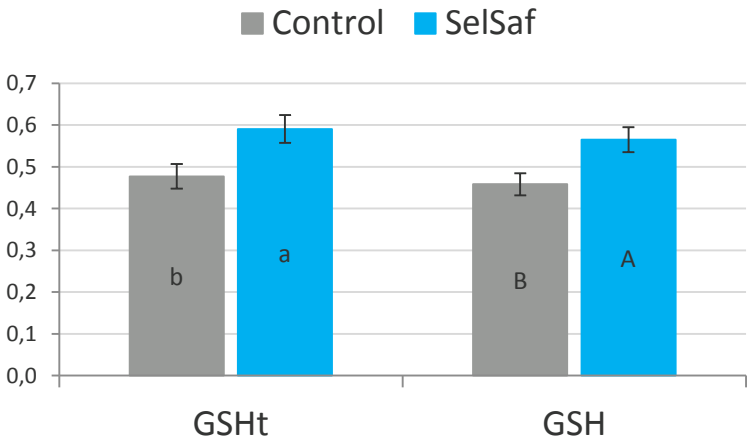
Influence of Dietary Selenium Species on Selenoamino Acid Levels in Rainbow Trout

Simon Godin,*[,†] Stéphanie Fontagné-Dicharry,[§] Maïté Bueno,[†] Philippe Tacon,[#] Philip Antony Jesu Prabhu,[§] Sachi Kaushik,[§] Françoise Médale,[§] and Brice Bouyssiere[†]

Rainbow trout (*Oncorhynchus mykiss*) Trial 2

- National Institute for Agricultural Research (INRA), France
- Juveniles, 43 g
- 25 trouts per tank, 3 replicates per treatment
- Treatments: - Diet CTRL: plant-based formulation
  - Diet Selsaf: CTRL + 0.15 g/kg of Selenized yeast (eq. 300 ppm Se)
- Duration: 12 weeks of feeding
- End of trial:

Total glutathione (GSHt) and reduced glutathione (GSH) levels in plasma (µmol/mL):



- Addition of selenized yeasts in the diet increased the concentration of GSHt in plasma of the fish.
- This increase in GSHt was related with a higher concentration of the reduced form GSH, indicating a **better oxidative status** in those fish.

Use of yeast enriched in selenium

The recommended dosages need to follow the level of Selenium present in the feed

Targeted applications are

- Larvae, fry, whose antioxidant defences are not optimum.
- Broodstock to improve quality of gametes
- Fish under high stress, for example before transportation, to help the recovery from stress.
- Improvement of meat quality

Inactivated yeast rich in protein as alternative dietary protein source

NutriSaf

Phileo
LESAFFRE ANIMAL CARE

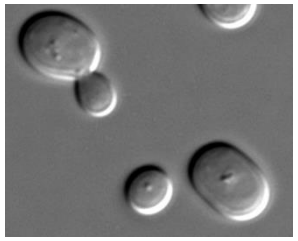
Towards sustainable aquafeed

- Feeding: can represent up to 50% of operational costs of fish farming
- Aquafeed traditional ingredients: Fishmeal (FM) & Fish oil (FO)
- Decreasing availability of FM & FO: **Need to find alternative ingredients**
- Search for alternative ingredients:

  - Plant meals & plant oils → Wide range of uses → Not that sustainable
    - human feeding
    - cattle feeding
    - biofuel production…
  - Animal by-products (blood, feather, meat & bone) and insects: → Not well perceived by consumers
  - Unicellular microorganisms: - Microalgae
    - **Yeast**
    - Bacteria

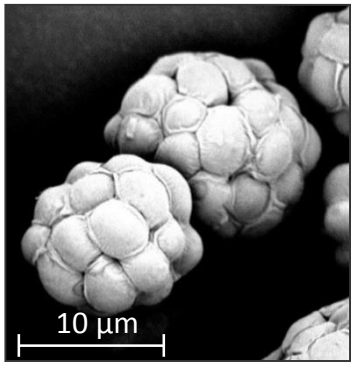
Production process

Bakery yeast production



Drying

Inactivated dry yeast



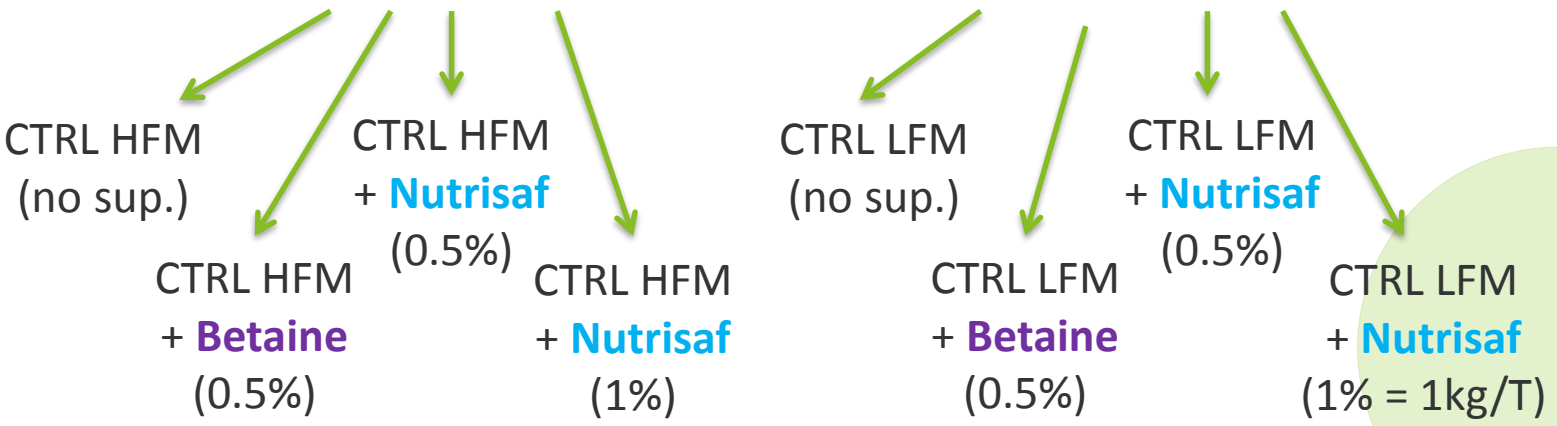
Crude protein ≥ 50%

Amino acid composition (% total AA):

	Nutrisaf	Fishmeal
Trp	0.7	0.7
Met	0.8	1.85
Cys	0.6	0.65
Thr	2.7	2.9
Lys	4.1	5.1
Val	2.8	3.65
Phe	2.4	2.65
Ile	2.3	3.1
Arg	2.2	3.8
Leu	3.9	5.1
His	1.4	1.55

Attractant property – Carp gibel Trial

- China, Feed Research Institute, CAAS
- Gibel carp (*Carassius auratus gibelio*) juveniles, 113 g
- Treatments:



The 8 experimental diets were iso-nitrogenous and iso-energetic.

Each diet contained a unique rare earth oxide as inert marker (Y_2O_3, Yb_2O_3, La_2O_3, or Nd_2O_3).

Attractant property – Carp gibel Trial

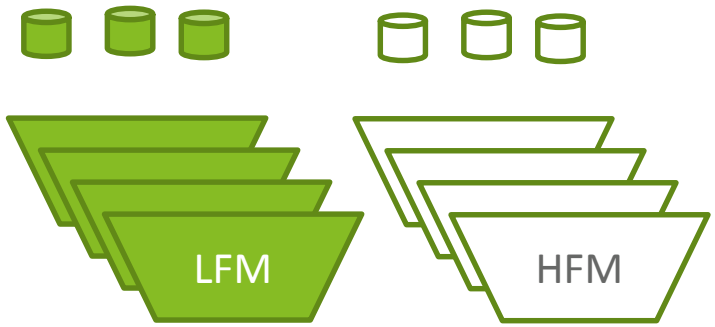
	HFM	Betaine	Nutrisaf-0.5	Nutrisaf-1.0	LFM	betaine	Nutrisaf-0.5	Nutrisaf-1.0
Ingredients								
fishmeal,Peru 65	12	12	12	12	6	6	6	6
Cottonseed meal50	15	15	15	15	15	15	15	15
Wheat short	6	6	6	6	0	0	0	0
Wheat flour	25.5	25.5	25	24.5	26.8	26.8	26.3	25.8
Soybean meal	20	20	20	20	30	30	30	30
Rapeseed meal	13.4	13.4	13.4	13.4	13.4	13.4	13.4	13.4
CaH_2PO_3	1.8	1.8	1.8	1.8	2	2	2	2
Choline chloride	0.2	0.15	0.2	0.2	0.2	0.15	0.2	0.2
Soybean oil	3	3	3	3	3.5	3.5	3.5	3.5
Soy lecithin	2	2	2	2	2	2	2	2
Vitamin and mineral premix	1	1	1	1	1	1	1	1
Betaine		0.5				0.5		
Nutrisaf			0.5	1			0.5	1
Y_2O_3	0.1				0.1			
Yb_2O_3		0.1				0.1		
La_2O_3			0.1				0.1	
Nd_2O_3				0.1				0.1
Analysed chemical compositions (% as it)								
Crude protein	35.04	35.25	35.49	35.55	34.84	34.61	34.24	34.31
Crude liquid	6.61	6.21	6.68	6.10	6.17	6.37	6.23	6.65
Crude ash	9.27	8.01	8.06	7.98	9.06	7.99	7.88	8.92

Attractant property – Carp gibel Trial

Gibel carp (*Carassius auratus gibelio*), 113 g.

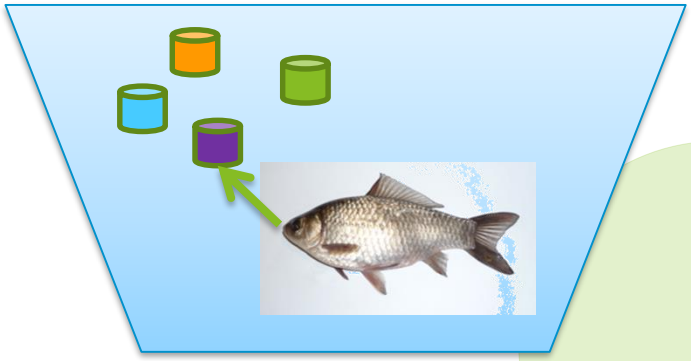
All diets given at the same time
Fish choose the most palatant one

7 days control diets → **7 days experimental diets**



4 tanks
30 fish/tank

4 tanks
30 fish/tank



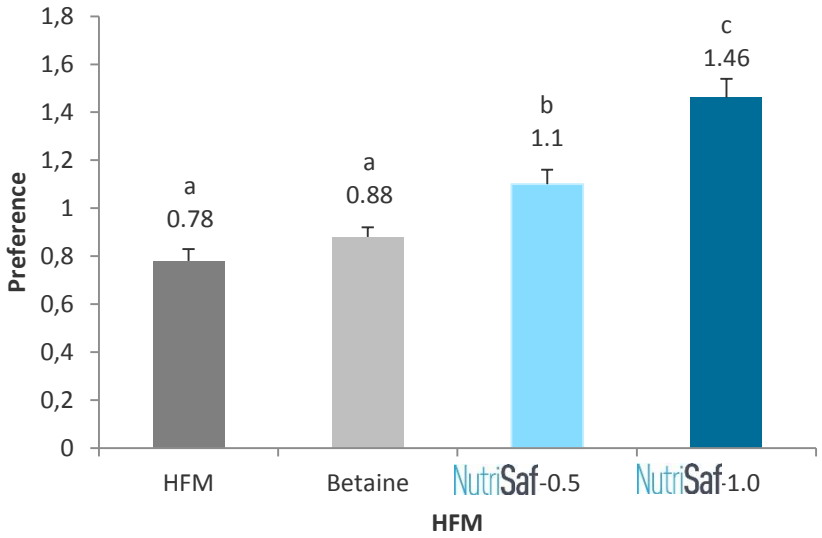
Feces collection
on the 3 last days

Analysis of rare earth oxide in feces

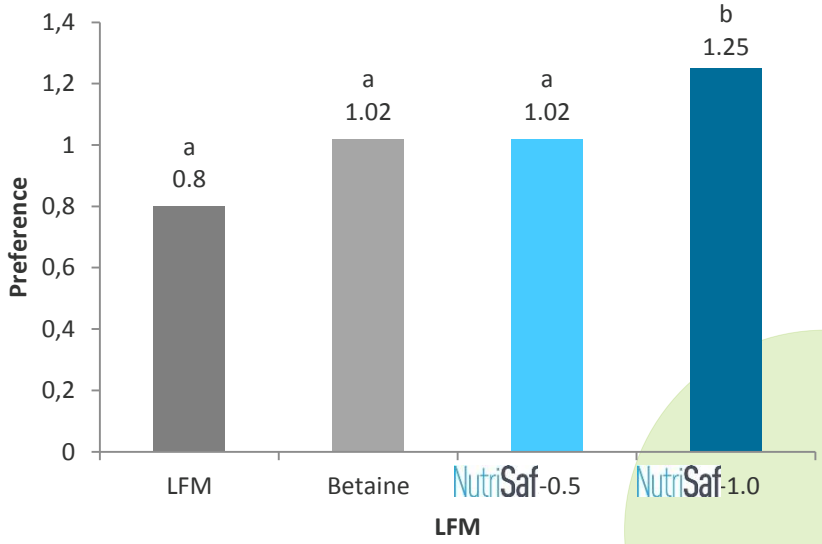
Determination of diet preference

Attractant property – Carp gibel Trial

- **Diets High Fishmeal:**



- **Diets Low Fishmeal:**



Preference of gibel carp when fed diets with HFM diet. Preference is expressed as the ratio of concentration in faeces of the marker in a diet to the total concentration of markers. Error bars represent mean ±S.E.M, and different superscript in each row are significantly different ($P<0.05$).

- Nutrisaf at 0.5 and 1% has a **better palatability than betaine** in HFM diets
- Nutrisaf at 1% has a **better palatability than betaine** in LFM diets

- **Nutrisaf is more efficient as attractant in a high FM diet. There is a synergy between FM and Nutrisaf.**

Conclusions

To improve health & performances management:

- Good control of physico-chemical parameters (O_2, Temperature, pH)
- Meet nutritional requirements of the species & stages

- **Yeast solutions:**
  - **Yeast parietal fractions**
    To counteract pathogen pressure
  - **Yeast enriched in selenium**
    To reduce oxidative stress
  - **Inactivated yeast rich in protein**
    Attractant alternative dietary protein source