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

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

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LESAFFRE ANIMAL CARE

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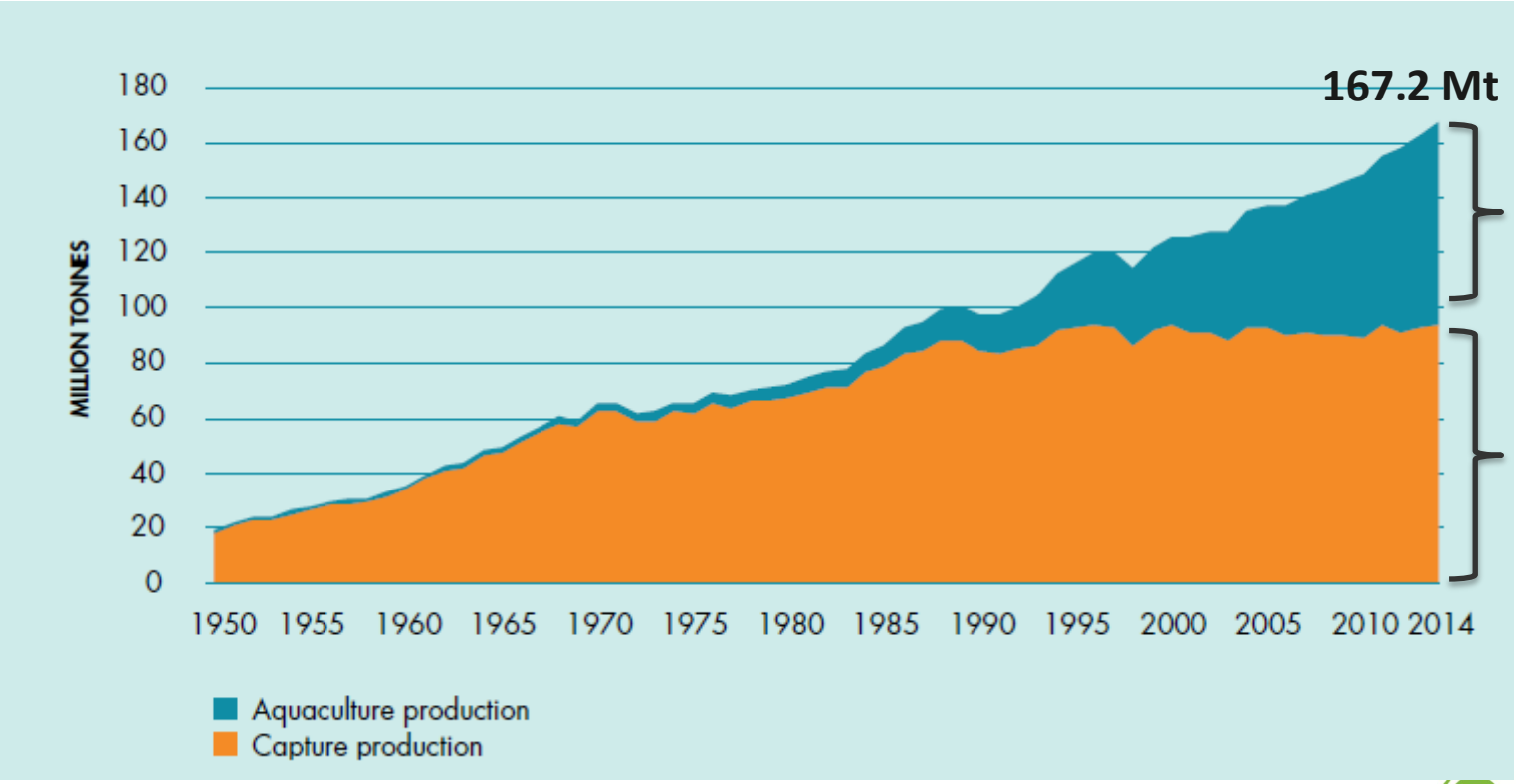
Expansion of Aquaculture

- Best growth rate in animal food production sector

- World capture fisheries and aquaculture production (FAO, 2016): Fish and seafood production



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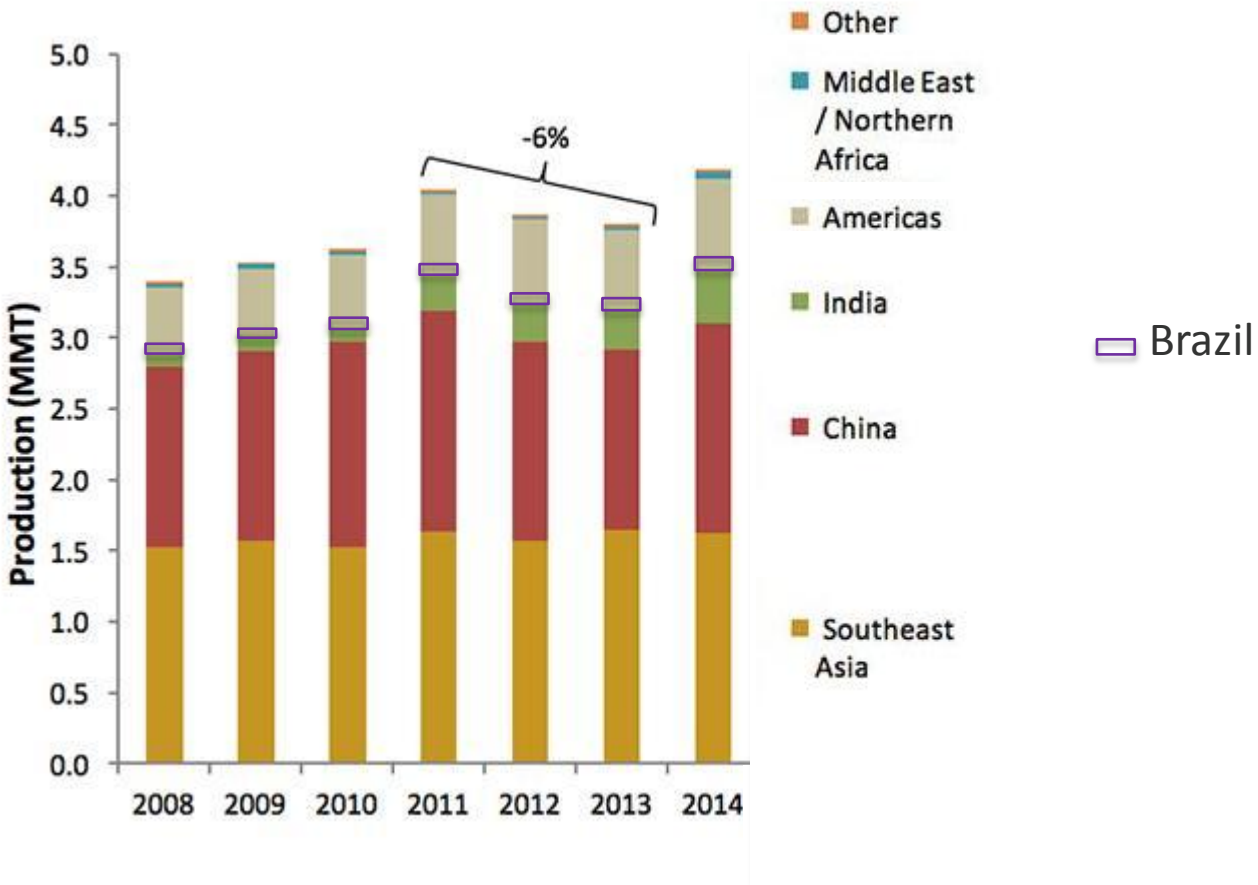
Global farmed shrimp production



Adapted from Anderson, 2016.

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Shrimp farming in Brazil

- Changes in the production system:
  implementation of a 3 phases production system

Phase Nursery

Phase Raceways

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Shrimp farming in Brazil

Phase Ponds

- Main brake of further development: uncontrollable disease outbreak

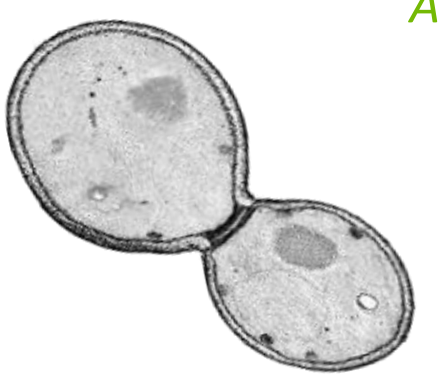
Current tools and trends

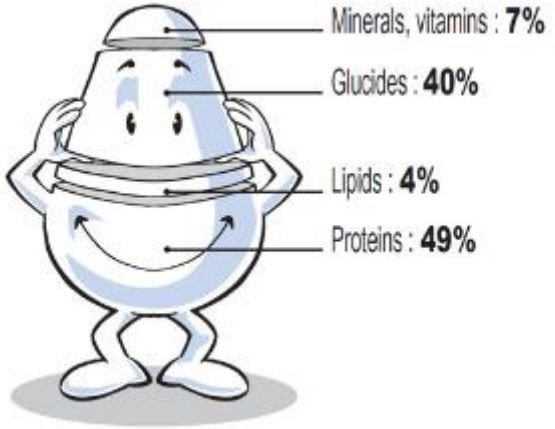
- Implementation of the Best Aquaculture Practices and Biosecurity Measures
- Better control of physico-chemical parameters *(°C, pH, alkalinity, etc…)*
- Water ionic balance *(maintain a ratio among the main ions)*
- Use of greenhouse *(or similar structures, for temperature control)*
- Use of specific aquaculture fertilizers
- Use of organic minerals
- Use of organic acids *(not yet common in Brazil)*
- Use of phytobiotics
- Use of prebiotics
- Use of probiotics
- Functional food

Yeasts

- Eukaryotic unicellular microorganisms (5x10 µm)

About 100 times bigger than a bacteria (volume)





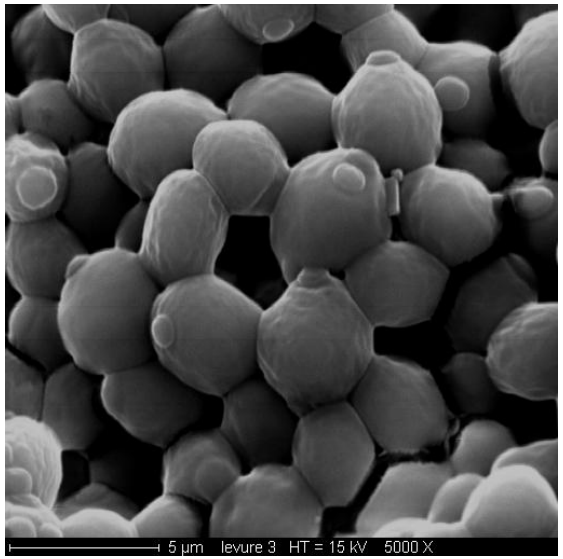
- Nutritional interests:

  - rich in proteins
  - rich in vitamins (B1, B2, B5, B6, B7, B9,…)
  - rich in minerals (Se, Cr,…)

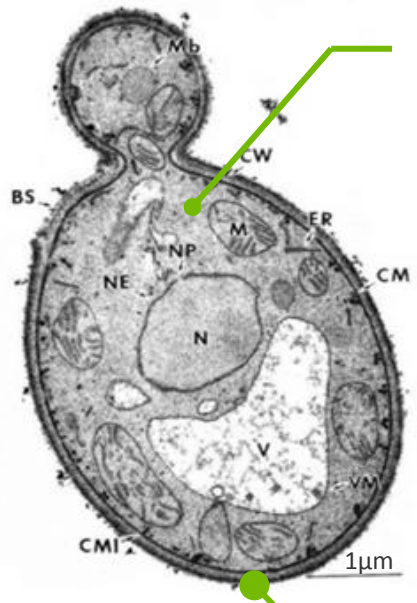
- Very common in the environment (natural gut microbiota, on the skin of fruits and vegetables, in the soil, etc…)

Yeast *Saccharomyces cerevisiae*

Bakery's yeast
Brewery's yeast



MEB picture (x5000)



Cellular content:
Cytoplasm + nucleus + organelles

Rich in proteins, peptides, nucleic acids, vitamins

Parietal fraction:
Plasma membrane + Cellular wall

Rich in mannans, beta-glutan

Represents 15-30% cell dry weight
25-50% cell volume
100-200 nm

Development of yeast product solutions for Aquaculture at Phileo:

- Yeast parietal fractions

- Yeast enriched in selenium

- Yeast rich in proteins

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Yeast parietal fractions
&
Benefits in shrimp health management

SafMannan

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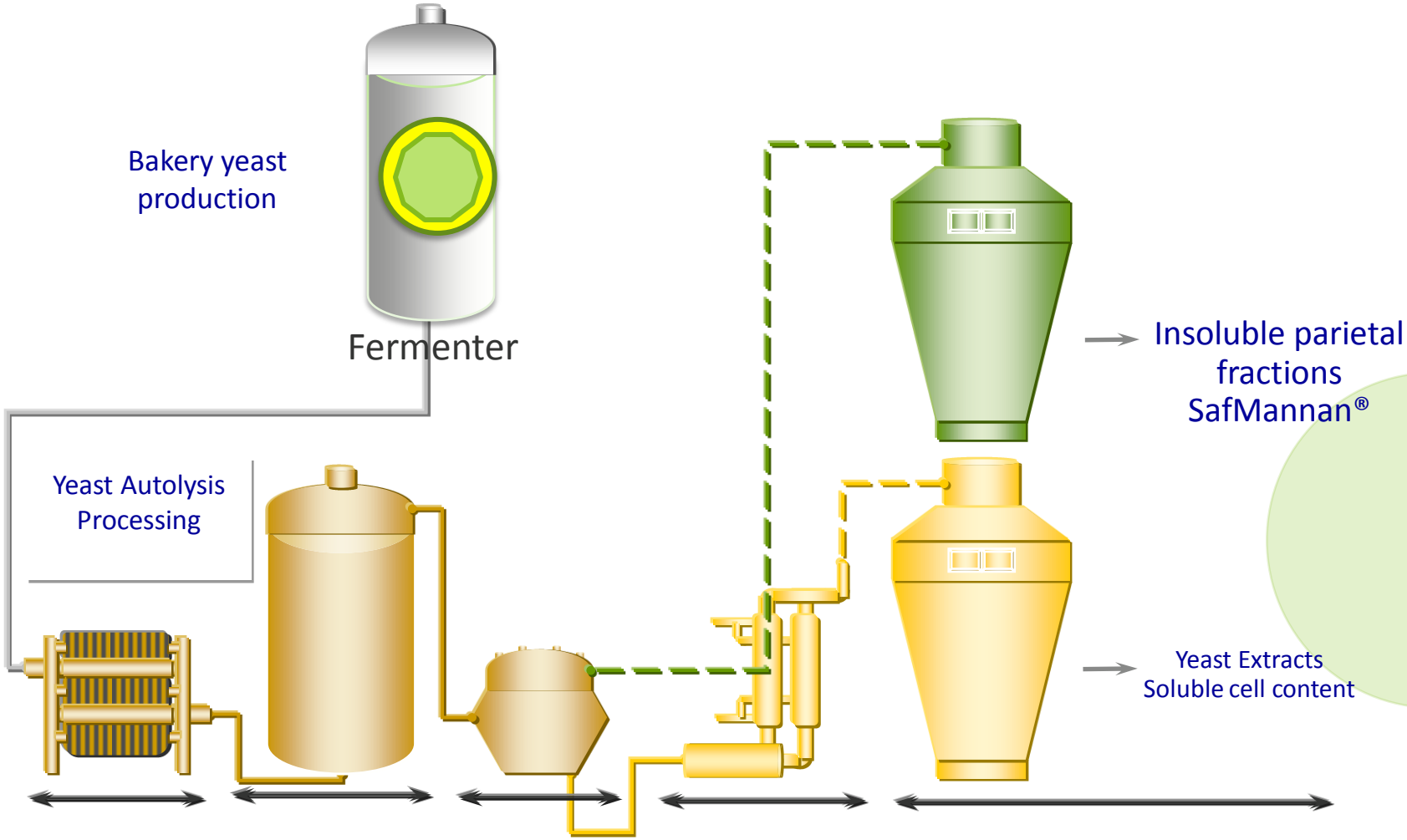
Parietal fractions production process



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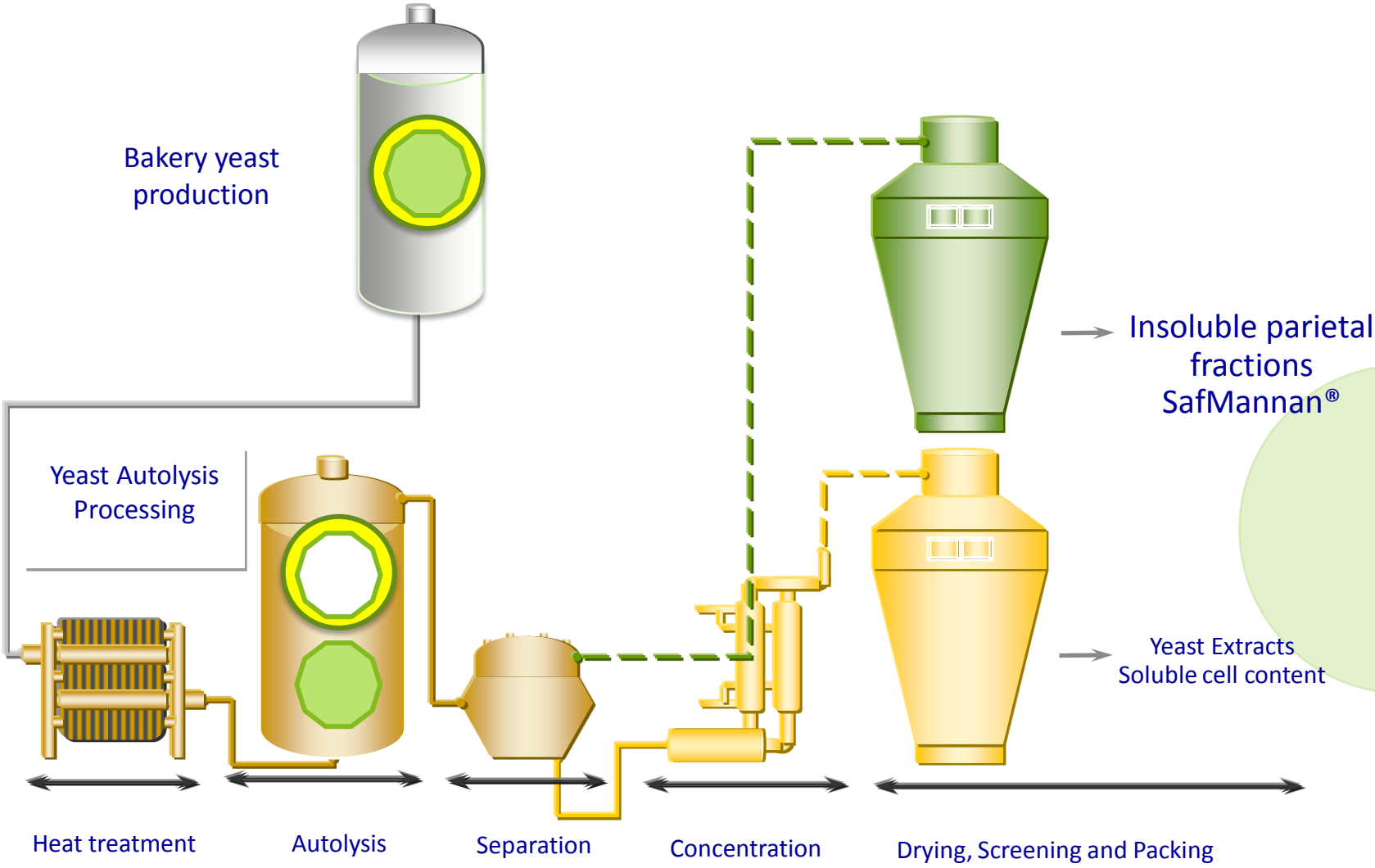
Parietal fractions production process



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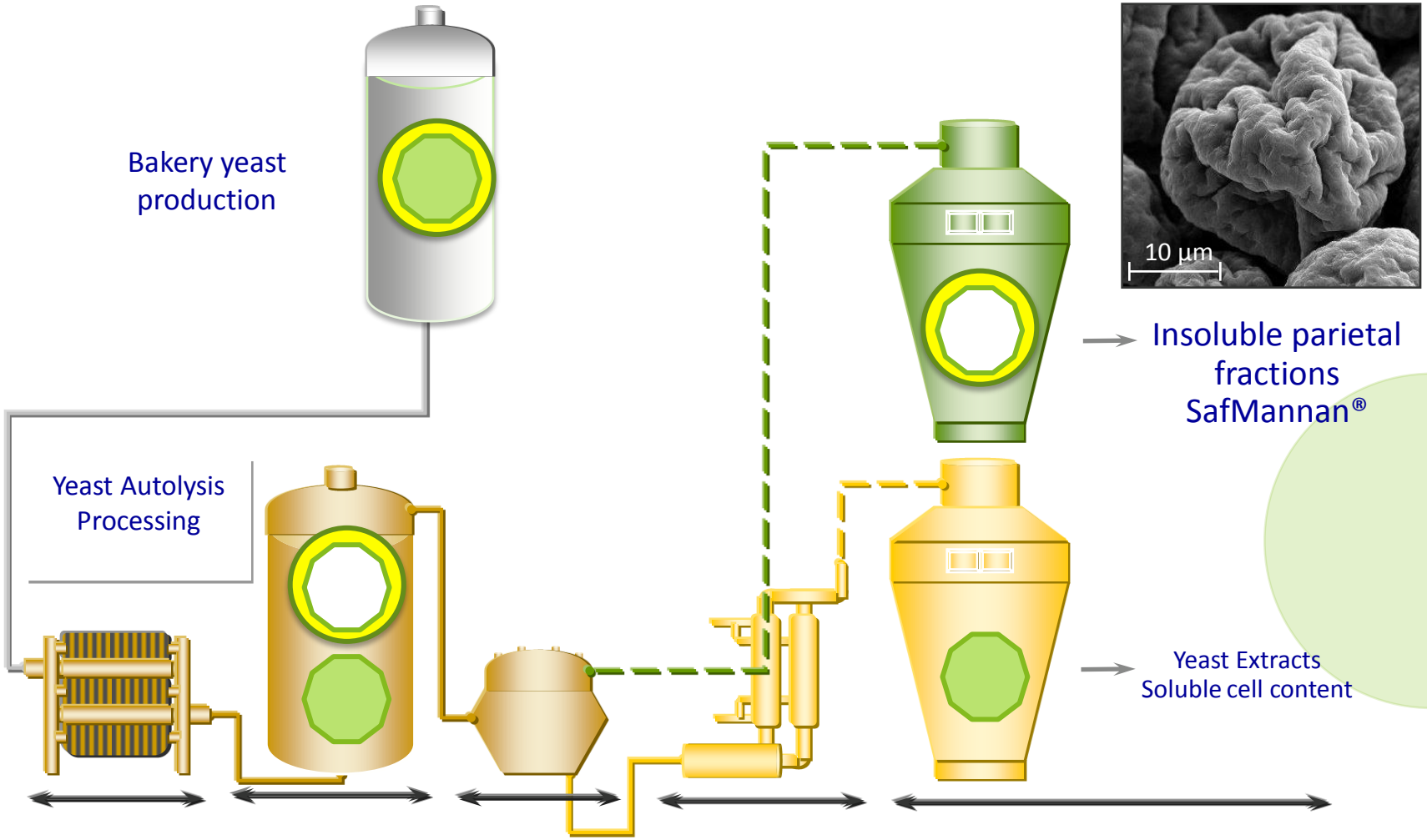

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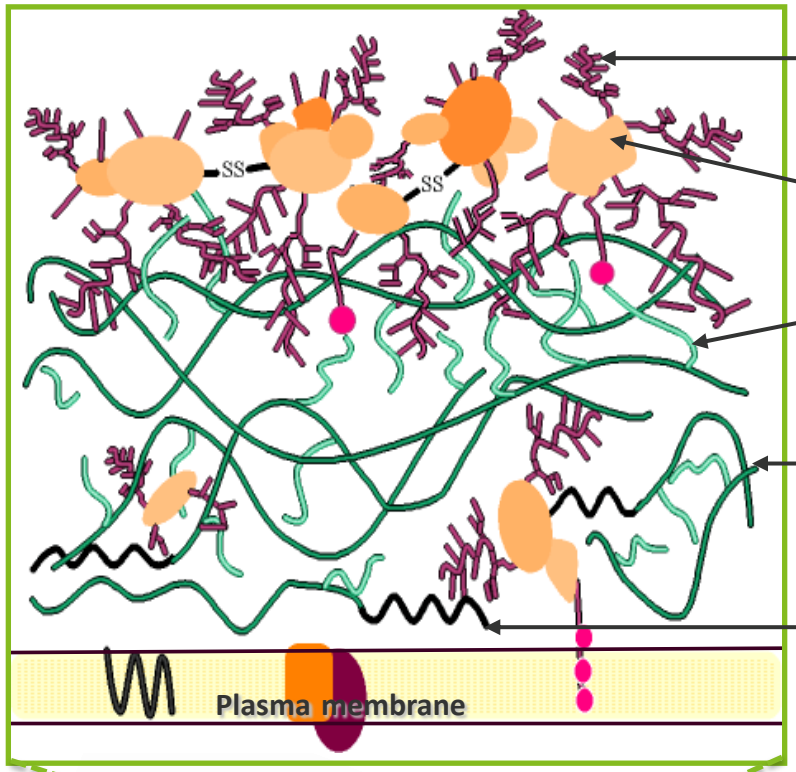
Yeast parietal fraction components

Outer layer

Inner layer

200nm



Mannose
-Soluble

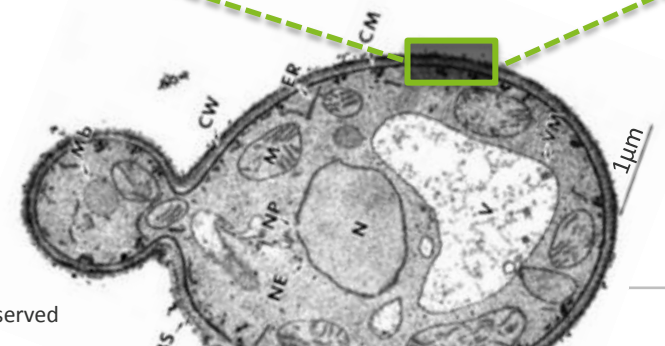
Protein

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)

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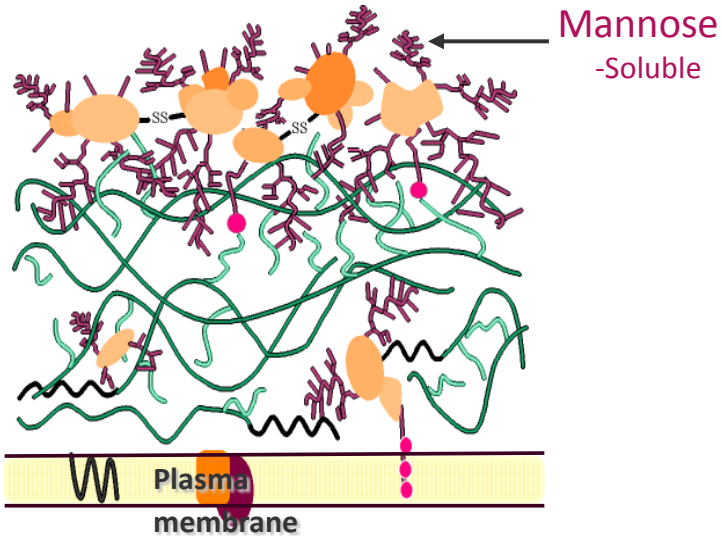
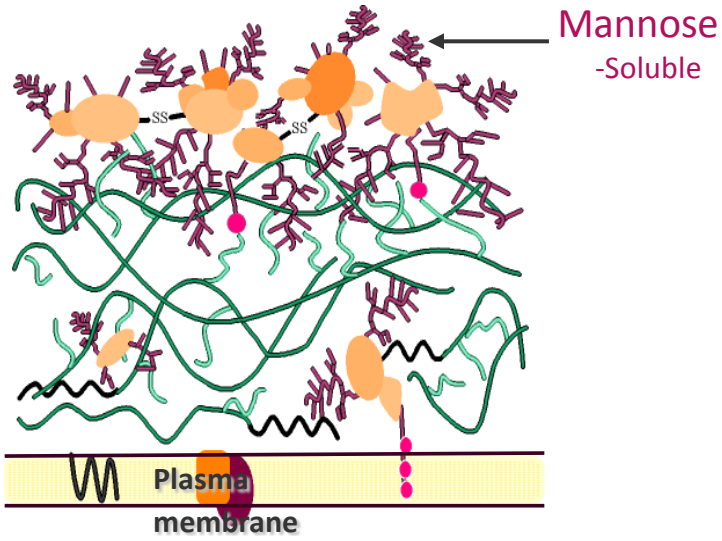
Mannan oligosaccharides



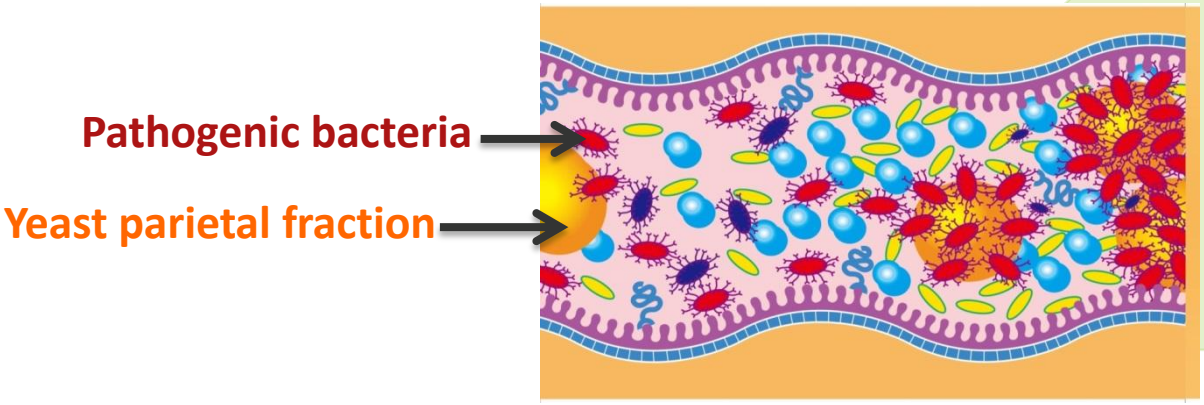
- **Prebiotics** : nutrient source used by beneficial bacteria in intestinal tract for growth

Mannan oligosaccharides

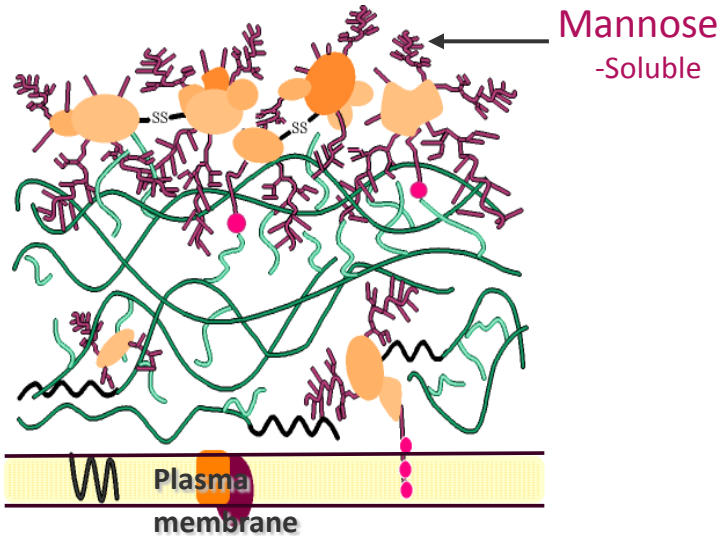


- **Prebiotics** : nutrient source used by beneficial bacteria in intestinal tract for growth

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



Mannan oligosaccharides



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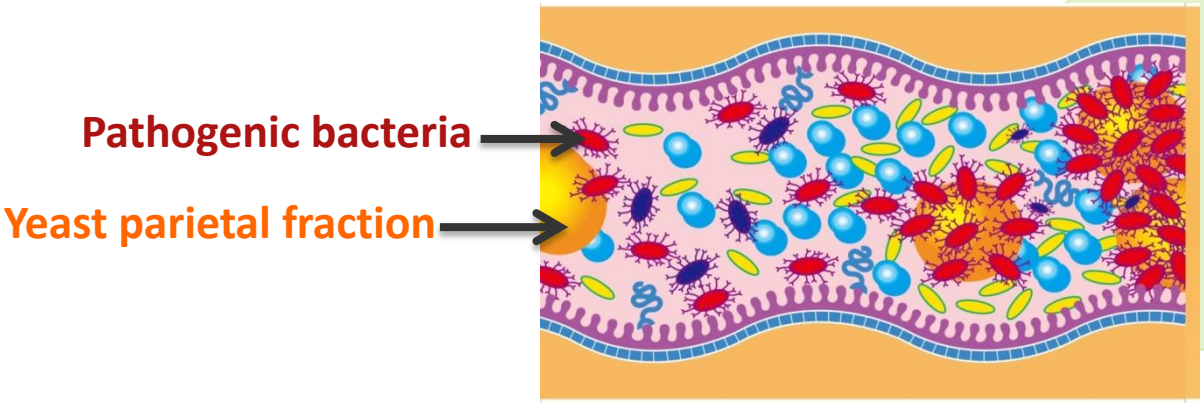
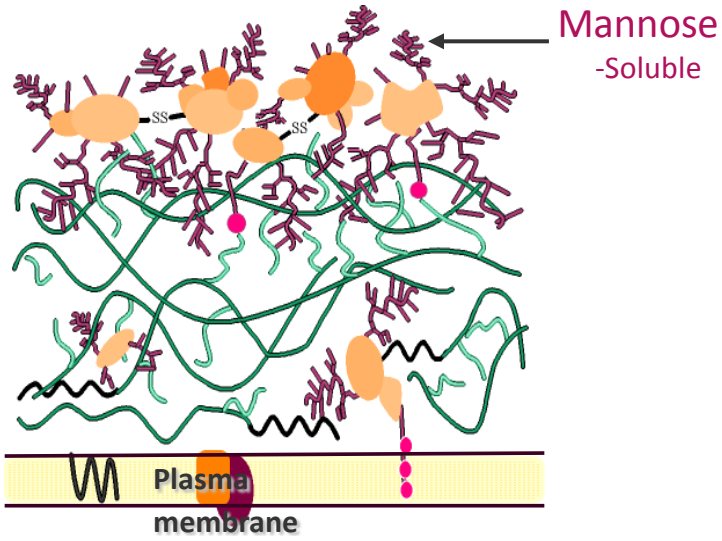


Prevention of pathogen colonisation in intestine

Mannan oligosaccharides



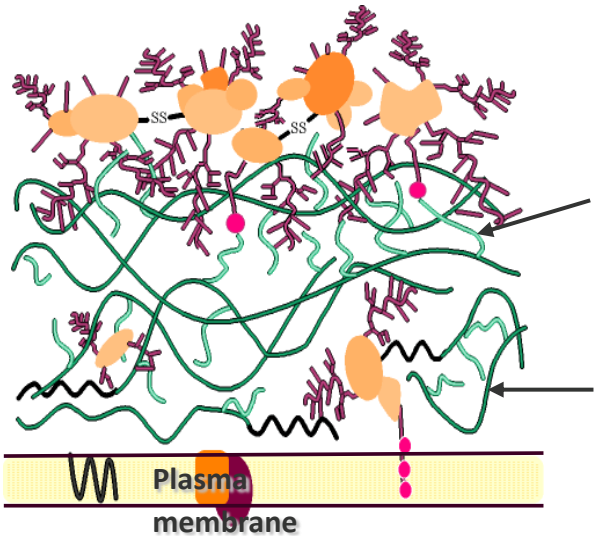
- **Prebiotics** : nutrient source used by beneficial bacteria in intestinal tract for growth

- **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**
Via TLR2

B-glucans



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

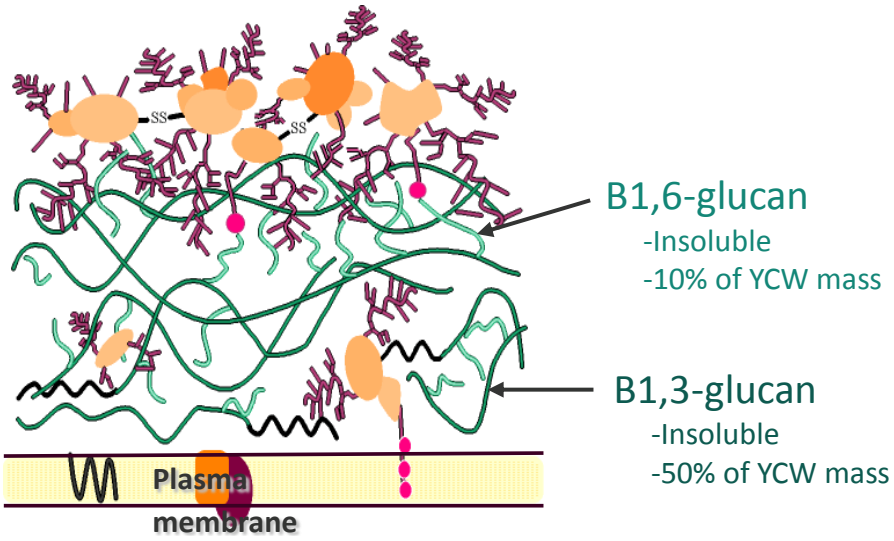
B1,3-glucan
-Insoluble
-50% of YCW mass

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

- Insoluble part of parietal fraction

- **Stimulation of immune system**
Via Dectin1

B-glucans



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

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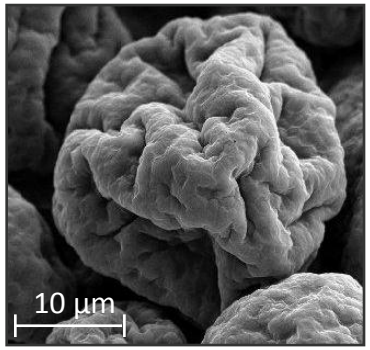
- **Stimulation of immune system** Via Dectin1

SafMannan composition:

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



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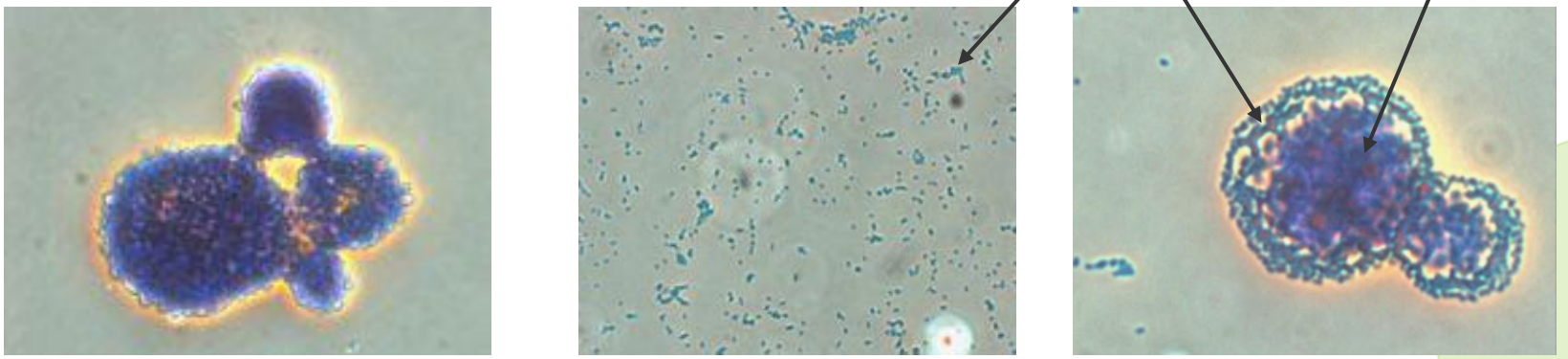
Yeast parietal fractions bind to pathogens

Parietal fractions can bind *Vibrio campbelii*, a virulent bacteria in shrimp

Safmannan® is put in contact with bacteria for 30 mins before fixation and staining



SafMannan alone

Vibrio alone

SafMannan + vibrio

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

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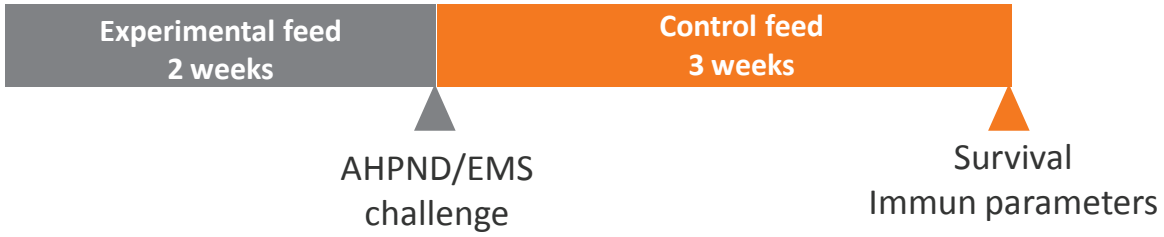
Whiteleg shrimp (*Litopenaeus vannamei*) Trial

- Vietnam, University of Can Tho
- Juveniles, 2-3 g
- 20 shrimp per aquaria
- 3 replicates per treatment
- **Diets: - CTRL: commercial feed**

  - SafMannan at 0.5, 2, 5 kg/T *(SafMannan top-dressed on CTRL feed)*

  2 weeks feeding



- **Challenge with *Vibrio parahaemolitycus* (AHPND/EMS)**

(immersion for 15 mins at 10^8 cfu ml^{-1}, *then addition of sea water to reduce density to* 10^6 cell ml^{-1}*)*

- 3 weeks after challenge: Measurement of immun parameters (before & after)
  Survival monitoring

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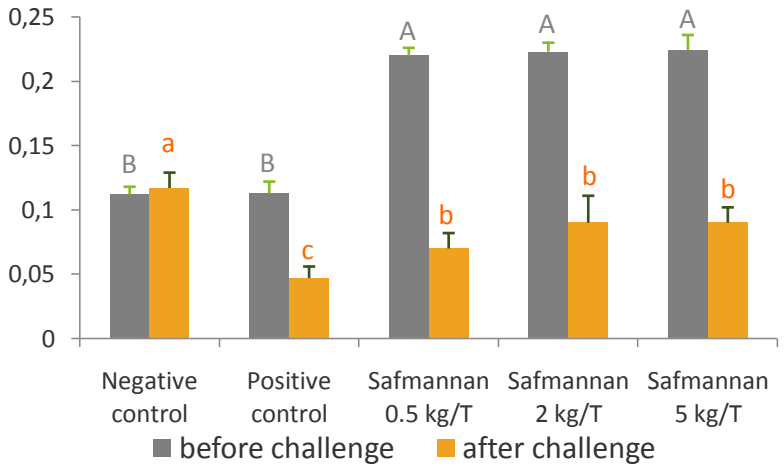
Whiteleg shrimp (*Litopenaeus vannamei*) Trial

- **Prophenoloxidase activity in hemolymph**

(units/min/mg protein):



➢ Part of Innate humoral immune response

➢ Clear the bacteria from the circulation after infection

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Whiteleg shrimp (*Litopenaeus vannamei*) Trial

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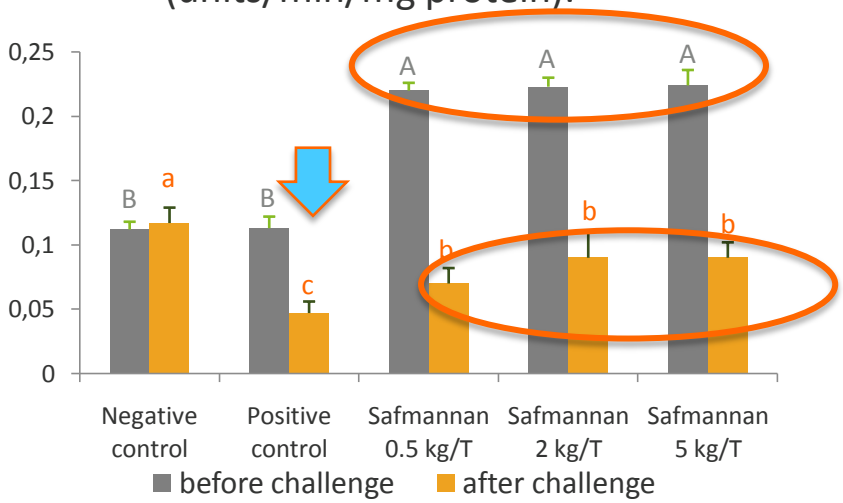

➢ Part of Innate humoral immune response
➢ Clear the bacteria from the circulation after infection

- Before the challenge, parietal fractions **increase prophenoloxidase** activity at all concentrations and they maintain it at a high level after challenge.

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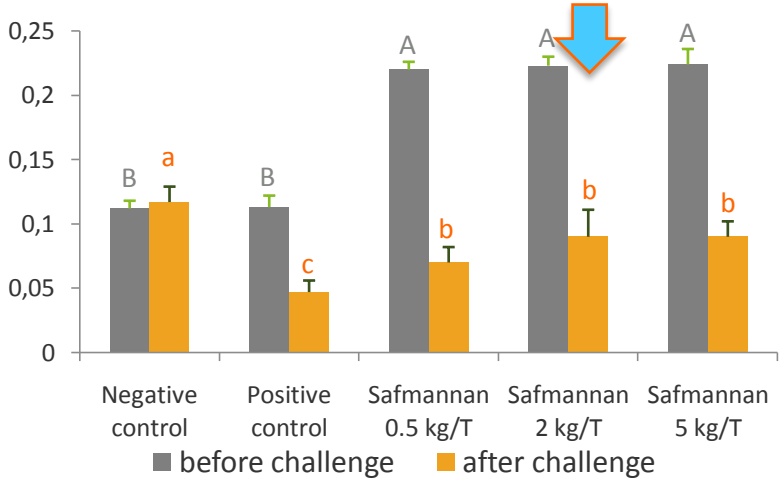
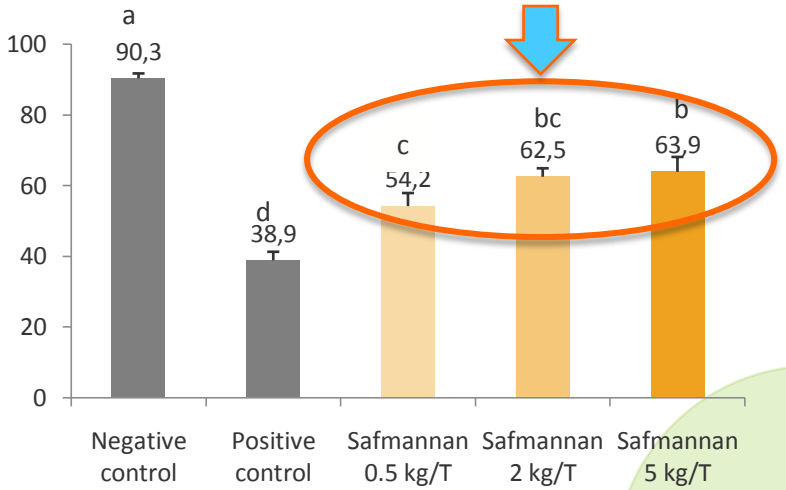
Whiteleg shrimp (*Litopenaeus vannamei*) Trial

- **Prophenoloxidase activity in hemolymph**

(units/min/mg protein):



- **Survival after 3 weeks of challenge (%):**



➢ Part of Innate humoral immune response

➢ Clear the bacteria from the circulation after infection

- Before the challenge, parietal fractions **increase prophenoloxidase** activity at all concentrations and they maintain it at a high level after challenge.
- Parietal fractions **improve survival** after challenge (39% -> 62%).
- **2 kg/T is the best concentration (2 weeks).**

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Shrimp- recommendations

Hatchery/ Nursery

- Boost the immune system in post larvae: **1 to 2 kg/T, all time**
- Before a stress (transportation) : **1 to 2kg/T - 2 to 4 weeks.**

Grow out ponds

- Help in the prevention of bacterial diseases : **1 kg/T** all time

Selenium-enriched Yeast
&
benefits in anti-oxidant status

SelSaf

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Oxidative stress

- Farming practices & environmental parameters changes
  - *Handling*
  - *Transportation*
  - *High density*
  - *T°c, O_2, pH, salinity…*
  - *Infections*
  - *Nutrient deficiency*
  - *…*

→ Stress in shrimp →

↑ Production of ROS (reactive oxigen species) in the cells

Oxidative stress

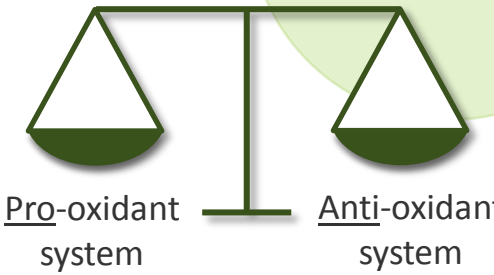
- Farming practices & environmental parameters changes → Stress in shrimp → ↑ Production of ROS (reactive oxigen species) in the cells

↓

ROS neutralised by cellular antioxidant defense system

↓

Oxidative status balance maintained



Healthy animal

Oxidative stress

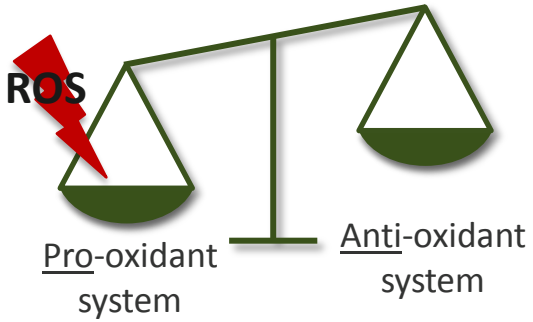
- Farming practices & environmental parameters changes

→ Stress in shrimp

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

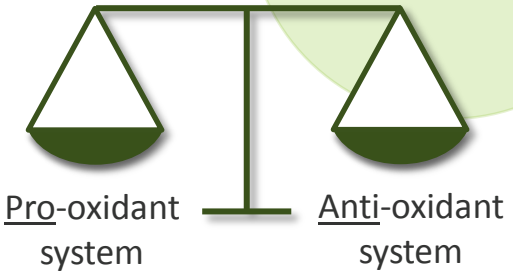
↓ Cellular antioxidant defense capacity overwhelmed

↓ **Imbalanced oxidative status**



↓ ROS neutralised by cellular antioxidant defense system

↓ **Oxidative status balance maintained**



Healthy animal

Oxidative stress

- Farming practices & environmental parameters changes

→ Stress in shrimp

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

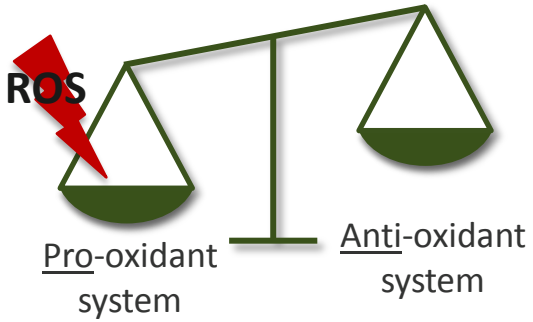
→ Cellular antioxidant defense capacity overwhelmed

→ Imbalanced oxidative status

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

→ Disruption of cell membrane integrity

→ **Oxidative stress**



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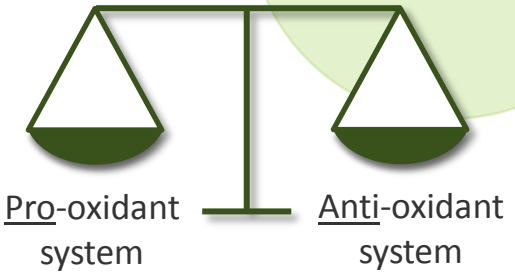


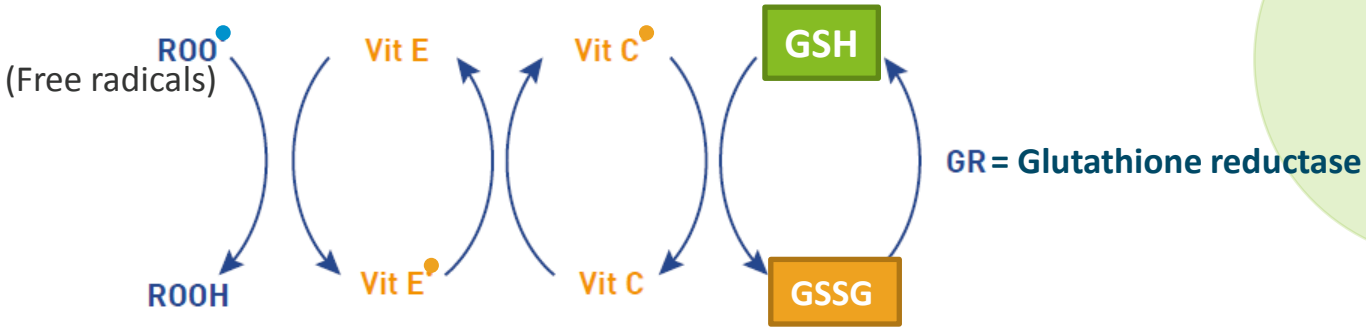
Healthy animal

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

Antioxidant defences

- **Non-enzymatic antioxidants compounds of the cell**:
  - Vitamin E
  - Vitamin C
  - Glutathione **GSH**
    - Tripeptide
    - Required to return oxidized antioxidants to their active forms (Vit. C)
    - Acts also as a cofactor for the antioxidant enzyme **GPX**

Oxidized glutathione is formed during both processes, and is then reduced by GR



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

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Antioxidant defences

- **Antioxidants enzymes:**

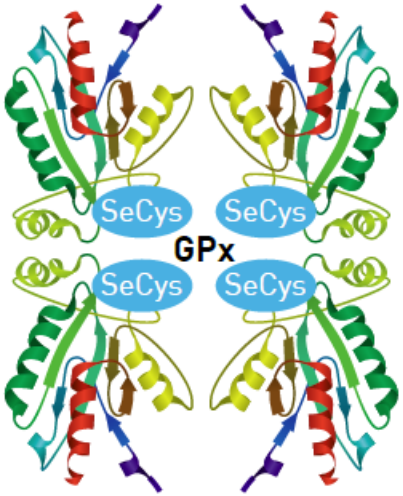
  - Superoxide dismutase (**SOD**)

    $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$ *O_2^- : superoxide anions*

  - Catalase (**CAT**)

    $2\ H_2O_2 \rightarrow 2H_2O + O_2$ *H_2O_2 : hydrogenperoxide*

  - Glutathione peroxidase (**GPX**): selenoenzyme



$H_2O_2 + 2$ **GSH** $\rightarrow$ **GSSG** $+ 2H_2O$

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

ROOH: organic hydroperoxide (ex: lipid hydroperoxide)

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

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Selenium

- Essential micronutrient for animal health

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

Abnormal swimming, nerve cord pathologies & liver pathologies

- Fishmeal is an important source of Se

	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

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	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

Substitution of FM by plant protein sources in aquafeed requires Se supplementation to meet shrimp Se requirements

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Selenium

- Two forms of Se can be added in feed:

Inorganic forms : Sodium selenite (SS) salts

Sodium selenate salts

Organic forms: Selenomethionine

Selenized yeast (SelSaf)

Selenium

- Two forms of Se can be added in feed:

Inorganic forms : Sodium selenite (SS) salts

Sodium selenate salts

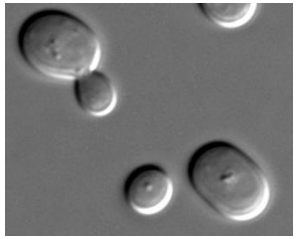
Organic forms: Selenomethionine

Selenized yeast (SelSaf)

Production process of Selsaf

Yeast

S. cerevisiae



Inorganic Selenium (SS)

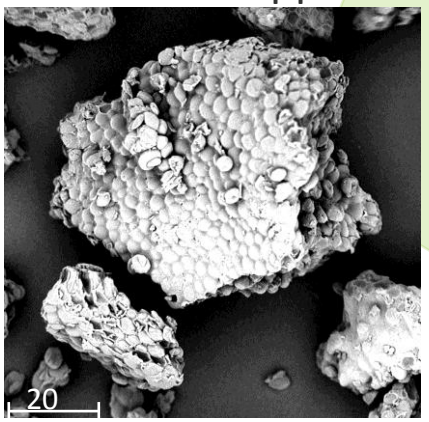
Bakery yeast production

Fermenter

Drying

Inactivated and dry selenized yeast

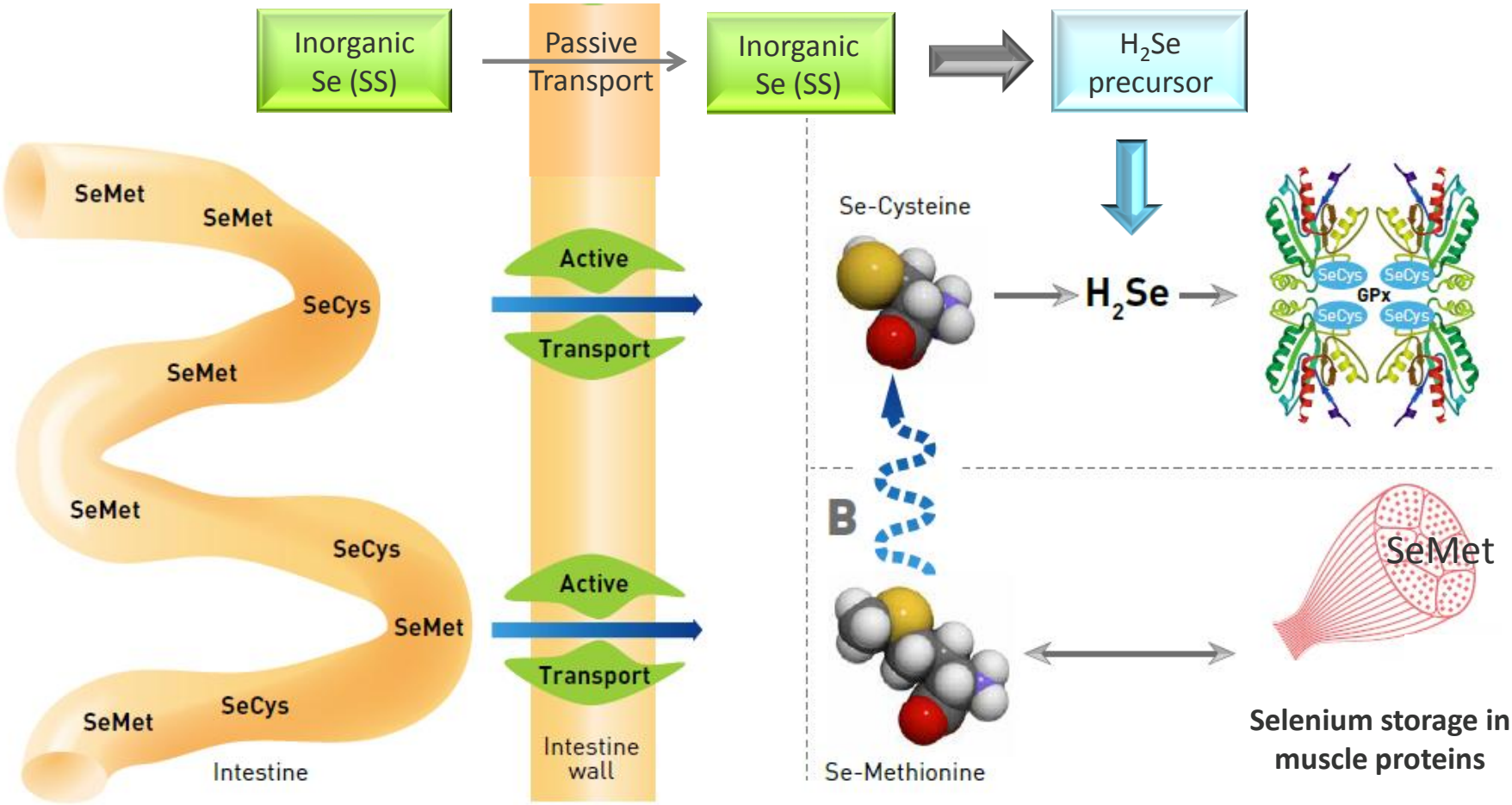
2000 or 3000 ppm Se



Se: 67% SeMet

33% SeCys and other organic Se compounds

Selenium absorption, assimilation and antioxidant defenses



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Rainbow trout (*Oncorhynchus mykiss*) Trial

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

Se concentration in diets (mg/kg)	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	**1.6**
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 in fish body**

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

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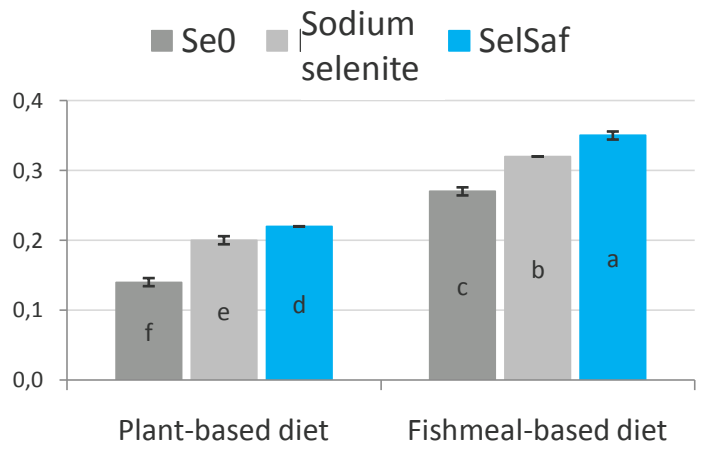
Rainbow trout (*Oncorhynchus mykiss*) Trial

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



- All 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 in both plant-based and FM-based diets.**

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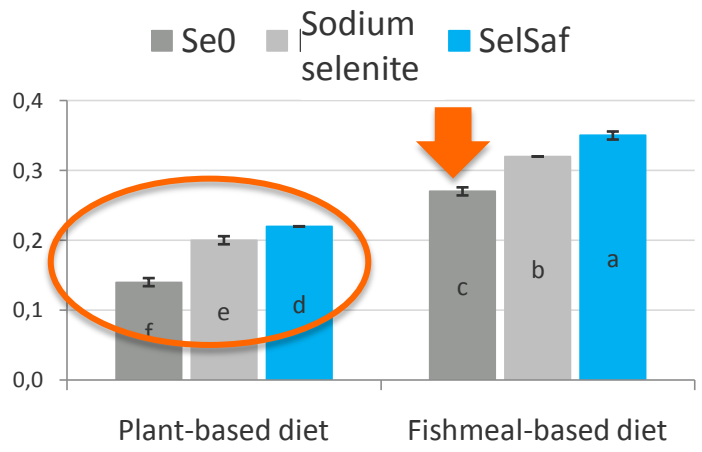

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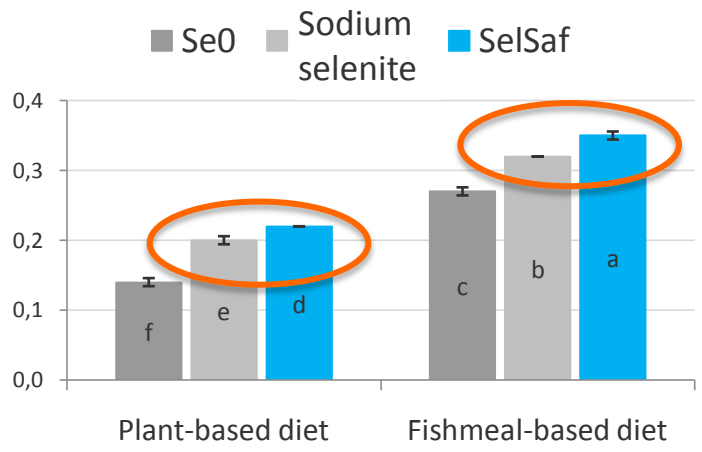

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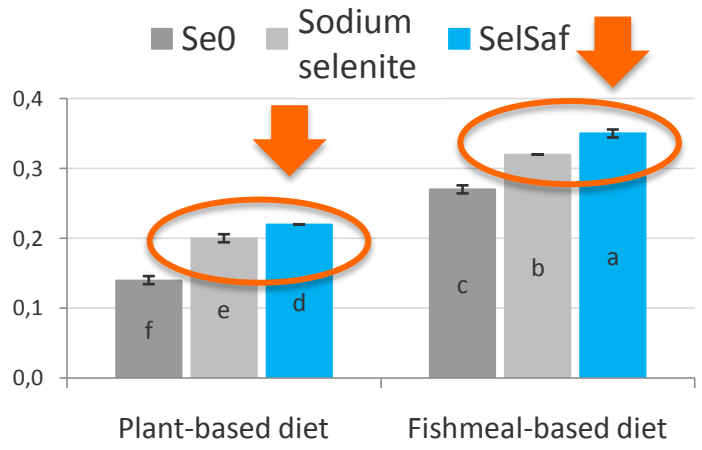

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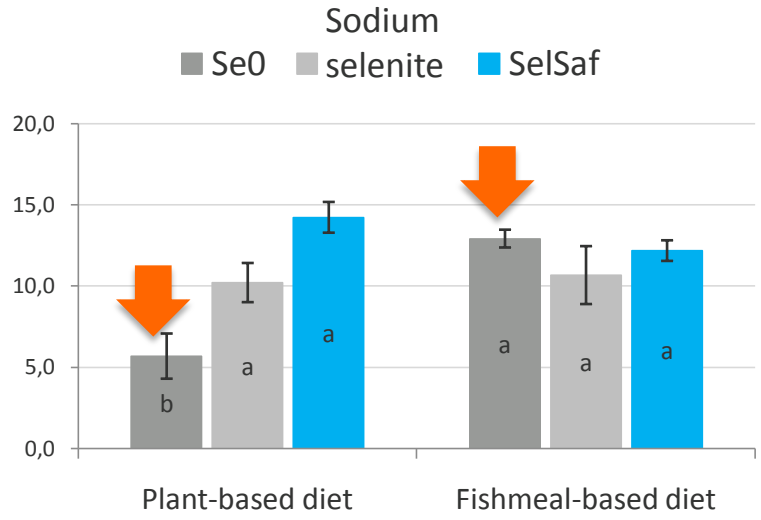
Rainbow trout (*Oncorhynchus mykiss*) Trial

- **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.**

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Rainbow trout (*Oncorhynchus mykiss*) Trial

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

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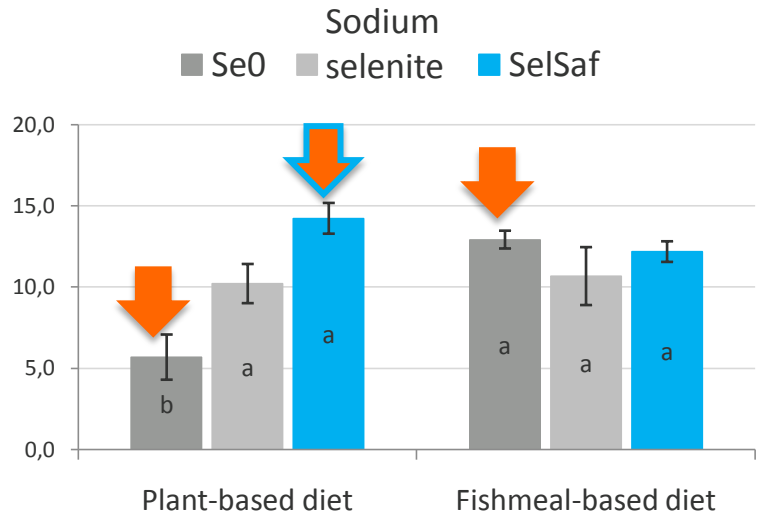

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Use of Selsaf

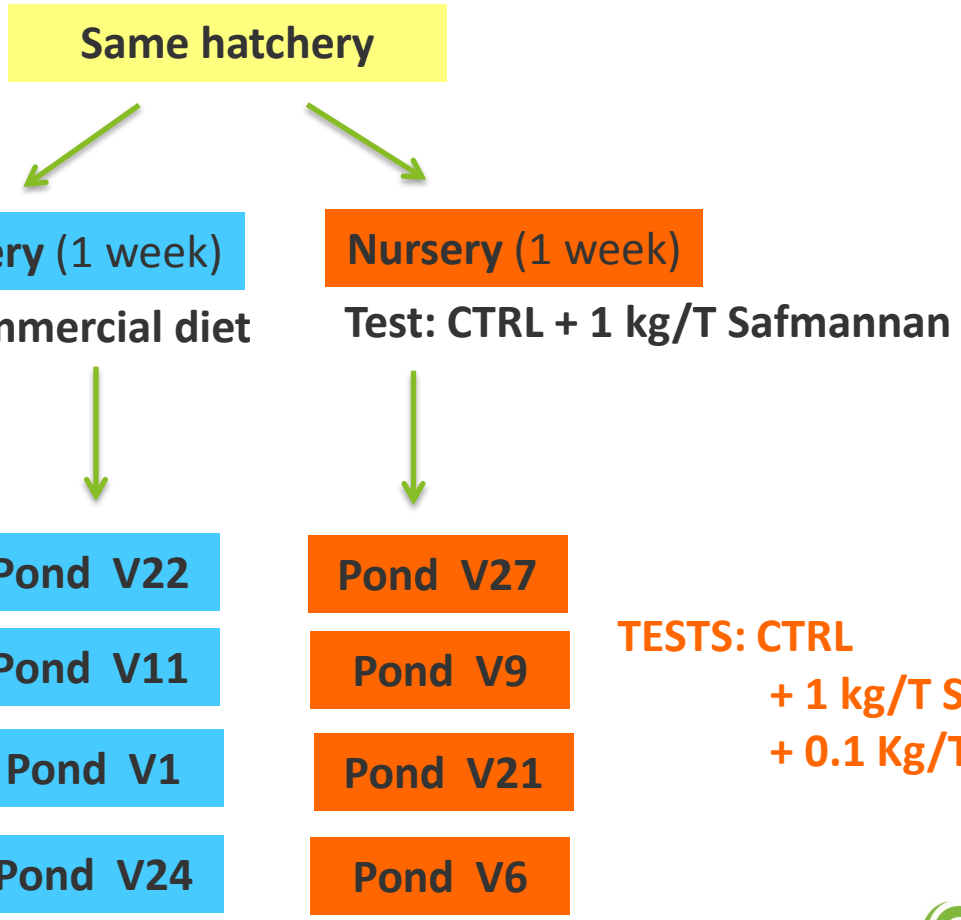
Targeted applications are

- PLs, juveniles, whose antioxidant defences are not optimum.
- Broodstock to improve quality of gametes
- Shrimp under high stress, for example before transportation, in situation of water quality changing (O_2, pH, salinity), to help the recovery from stress.
- Improvement of meat quality and meat shelf-life

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

Ongoing field exemple
Interaction Safmannan® & Selsaf ® supplementation

- Fazenda camarave, São Bente do Norte/Rio Grande do Norte, Brazil
- Treatments:



CTRL: commercial diet

TESTS: CTRL
+ 1 kg/T Safmannan
+ 0.1 Kg/T Selsaf

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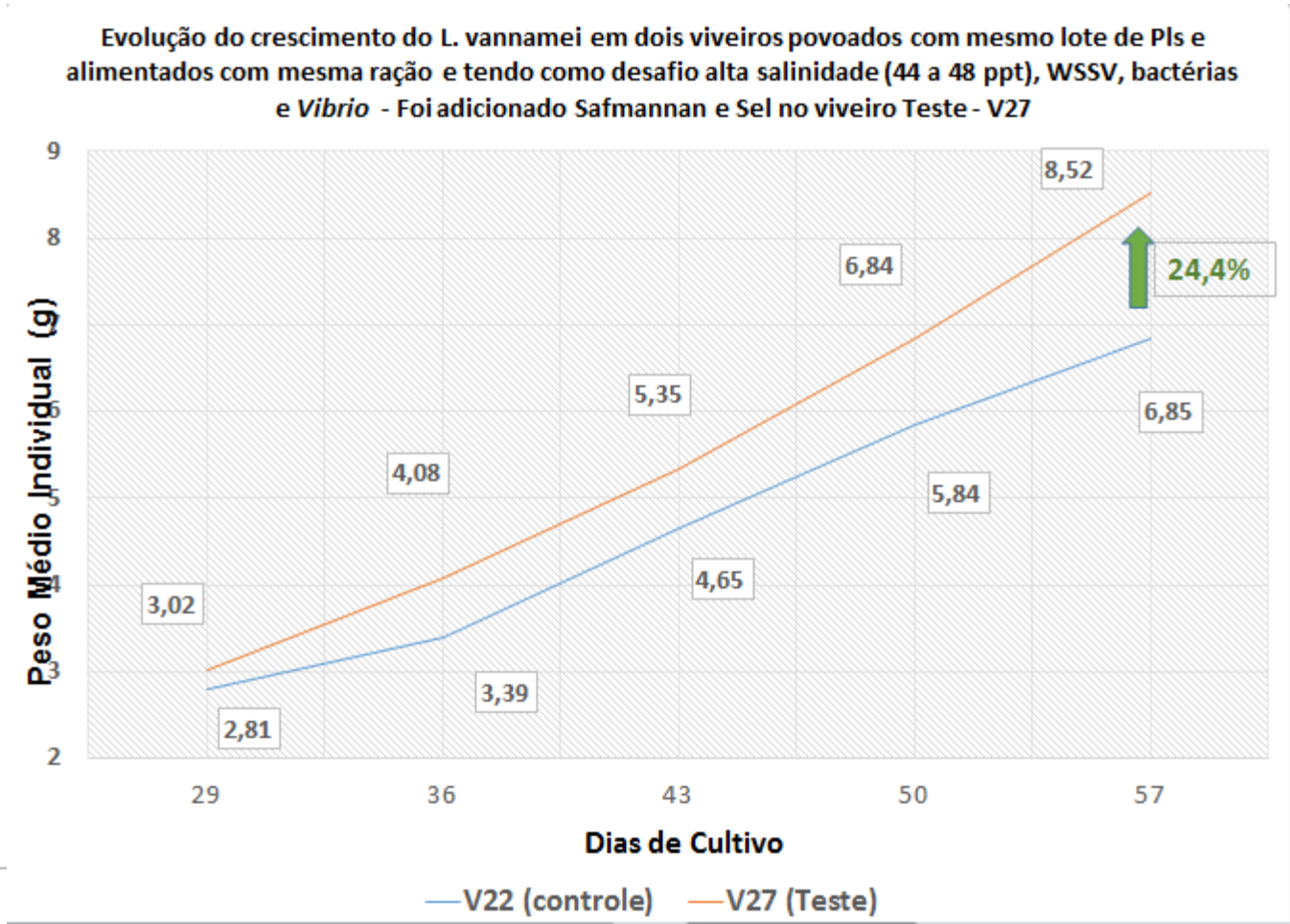
Ongoing field exemple

Interaction Safmannan® & Selsaf ® supplementation

- **Growth Performances**



Ongoing field exemple

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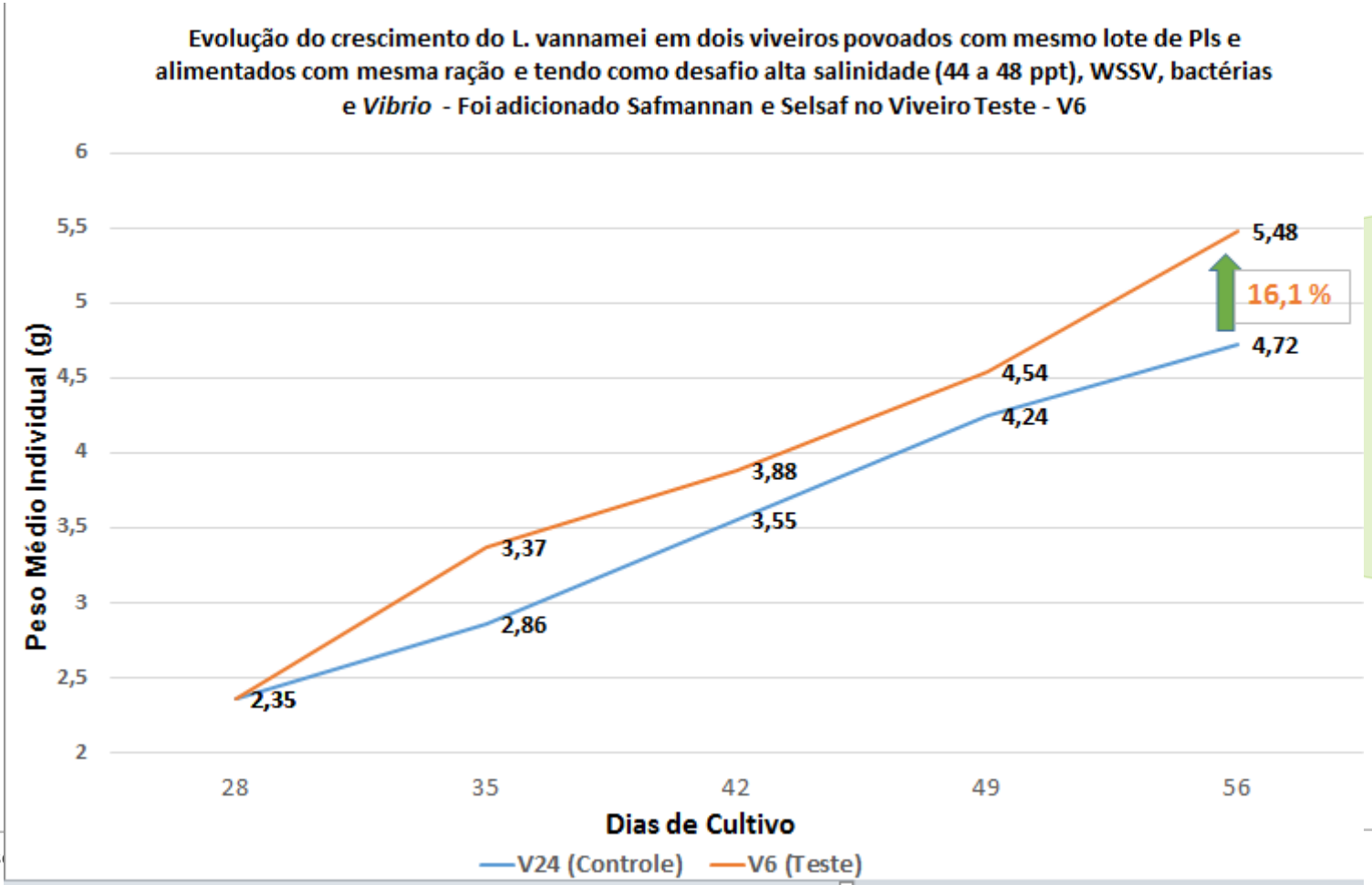

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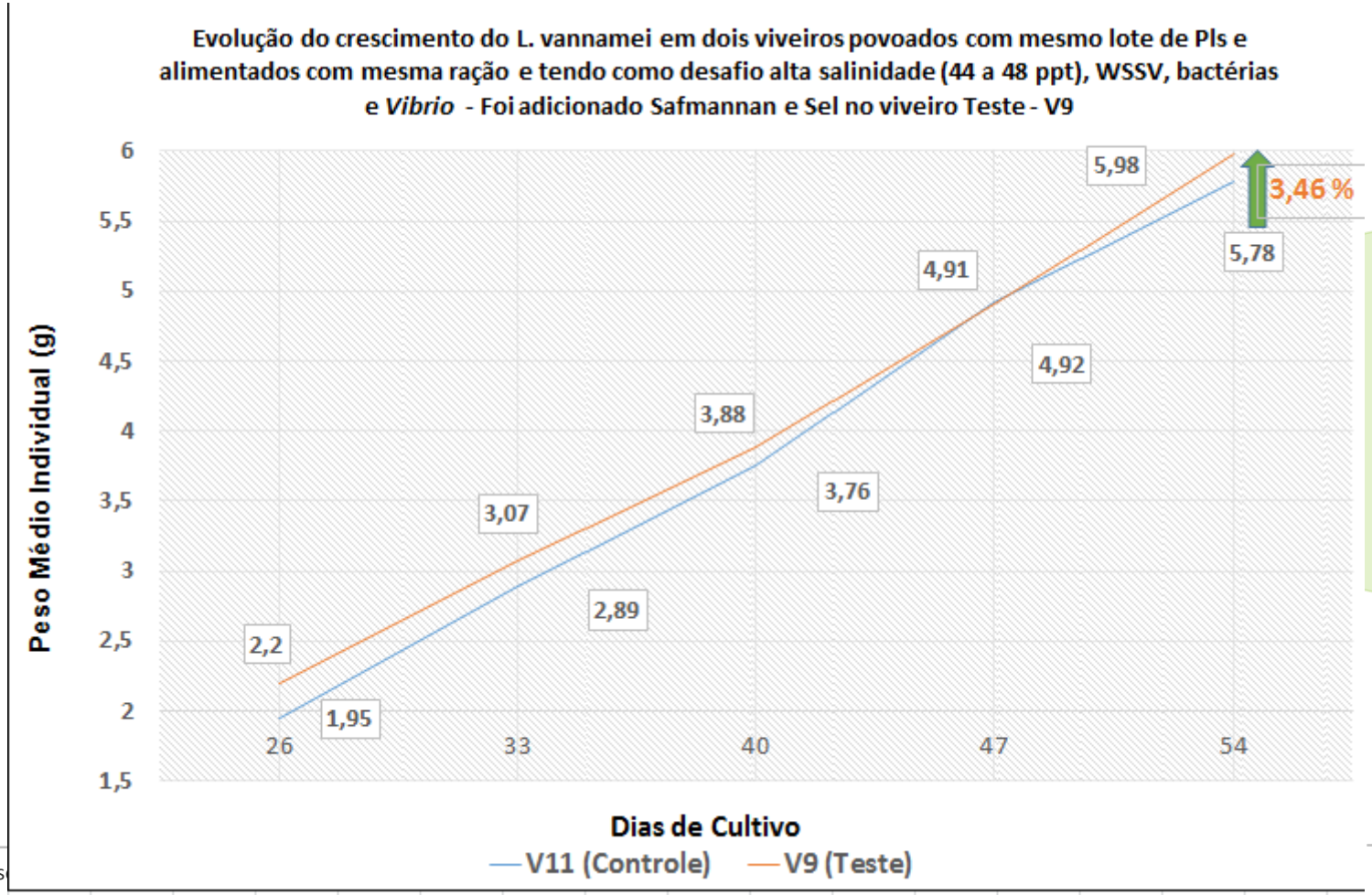

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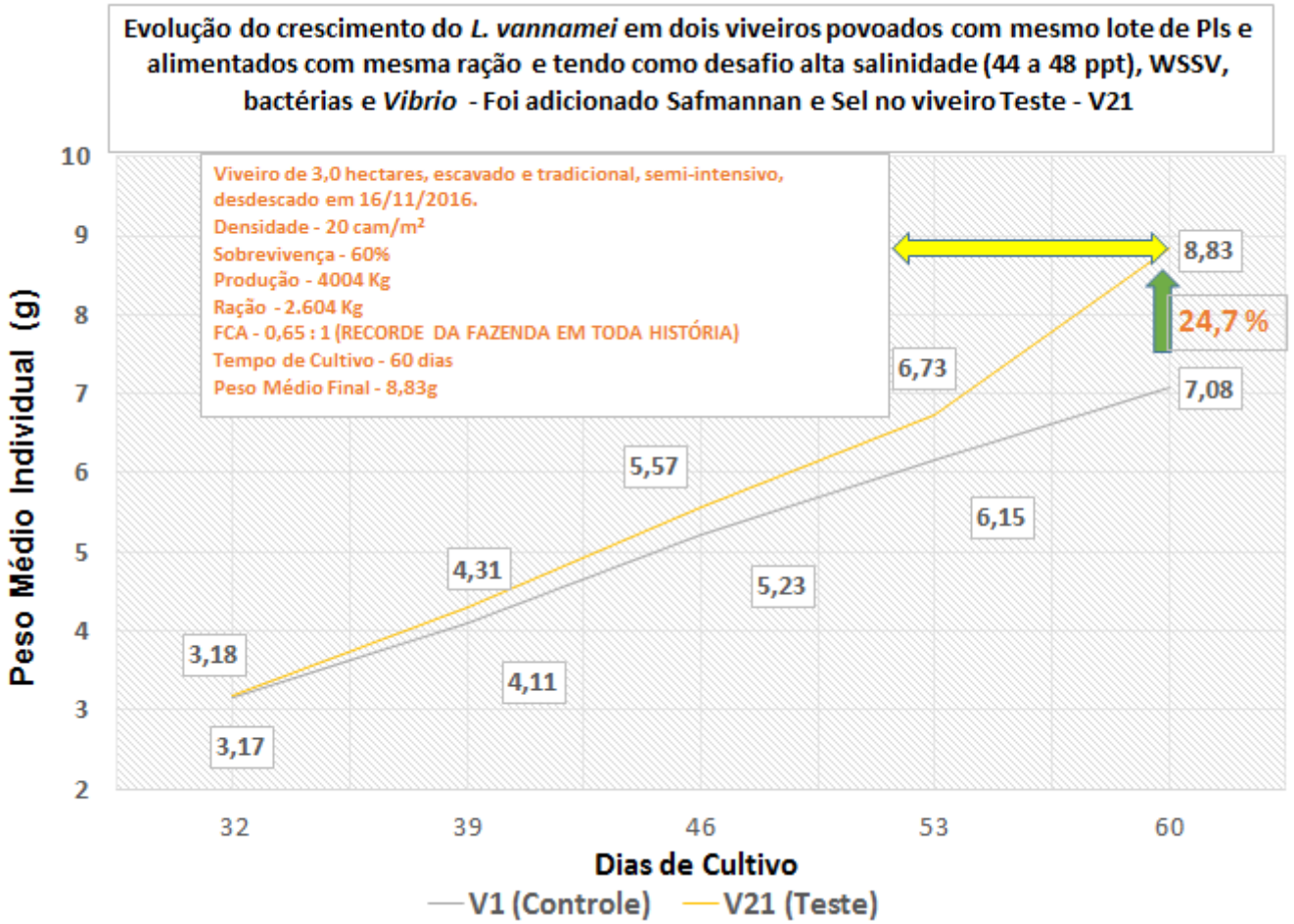

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FRE ANIMAL CARE

Inactivated yeast rich in protein as alternative source of protein

NutriSaf

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Towards sustainable aquafeed

- Aquafeed traditional ingredients: Fishmeal (FM) & Fish oil (FO)

- Decreasing availability of FM & FO: **Need to find alternative ingredients**

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- 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…

Soybean

Rapeseed

Wheat

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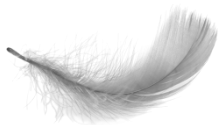
  - Animal by-products (blood, feather, meat & bone) and insects:


Blood meal


Feather


Meat & bone


Insect

→ Not well perceived by consumers

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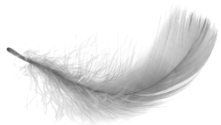

Feather

Meat & bone

Insect

→ Not well perceived by consumers

  - Unicellular microorganisms:

Microalgae

Yeast

Bacteria

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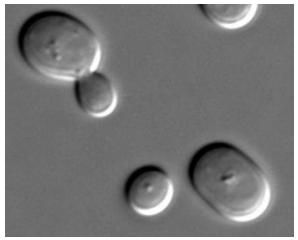
Production process

Yeast
S. cerevisiae



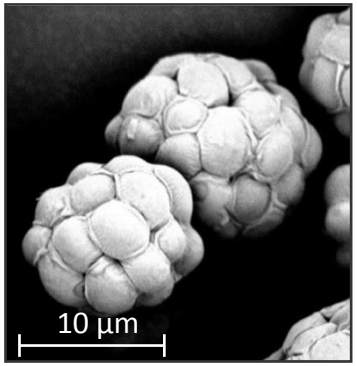
Bakery yeast production

Fermenter

Drying

Inactivated dry yeast

NutriSaf



Crude protein ≥ 50%

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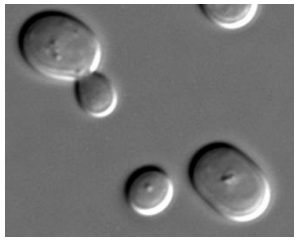
Production process

Yeast
S. cerevisiae



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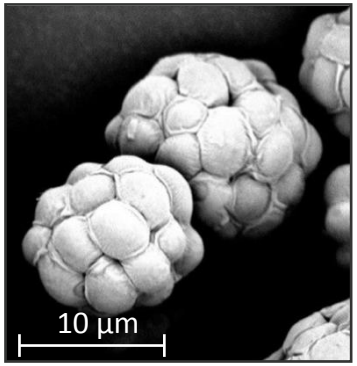

NutriSaf

Crude protein ≥ 50%

Amino acid composition (% total AA):

	Nutrisaf	Fishmeal	Soybean meal 48
Trp	**0.7**	**0.7**	0.5
Cys	**0.6**	**0.65**	**0.6**
His	**1.4**	**1.55**	1.0
Thr	**2.7**	**2.9**	1.6
Phe	**2.4**	**2.65**	2.0
Val	**2.8**	**3.65**	1.9
Ile	**2.3**	**3.1**	1.8
Met	**0.8**	**1.85**	0.6
Lys	**4.1**	**5.1**	2.4
Leu	**3.9**	**5.1**	3.0
Arg	2.2	**3.8**	**3.0**

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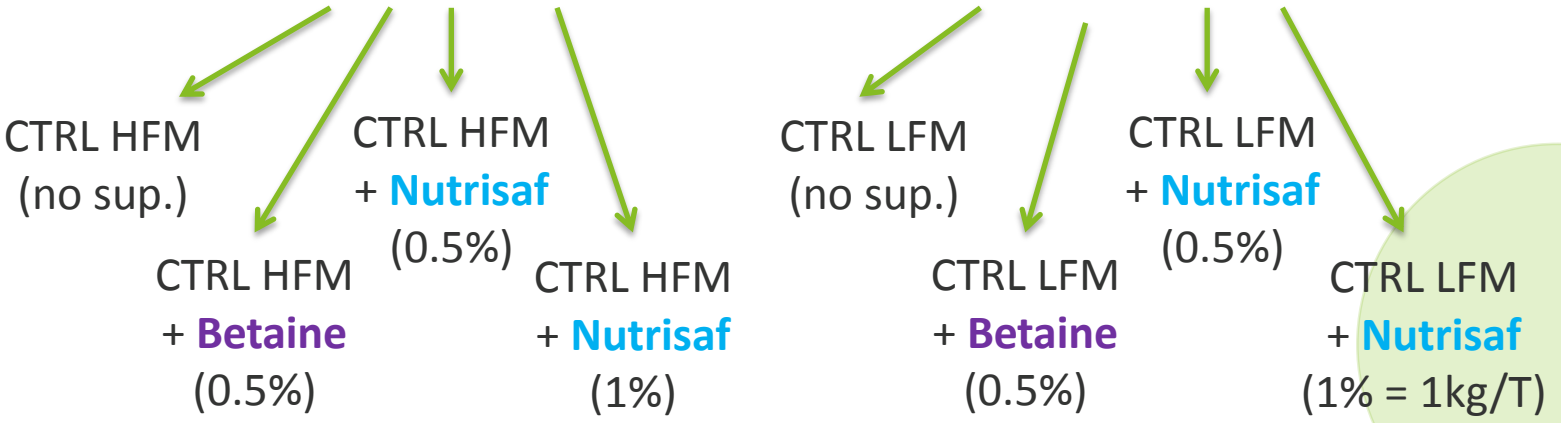
Attractant property – Carp gibel Trial

- China, Feed Research Institute, CAAS
- Gibel carp (*Carassius auratus gibelio*) juveniles, 113 g
- Treatments: Diet High fishmeal (12%) or Diet Low Fishmeal (6%)



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).

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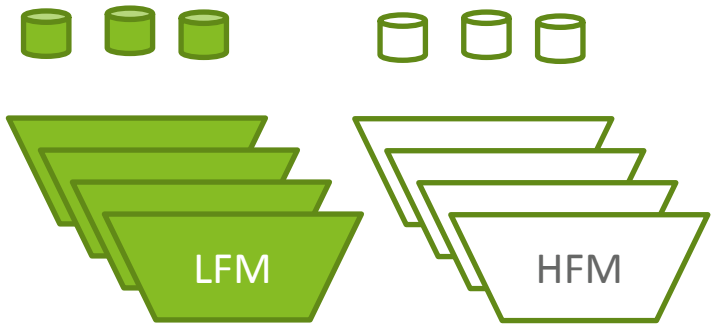
Attractant property – Carp gibel Trial

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

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

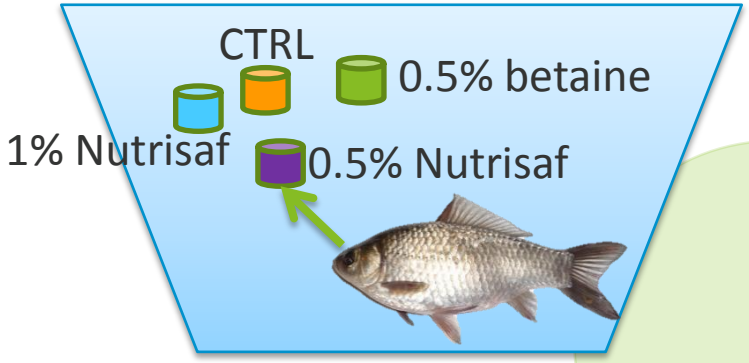
7 days control diets



4 tanks
30 fish/tank

4 tanks
30 fish/tank

7 days experimental diets



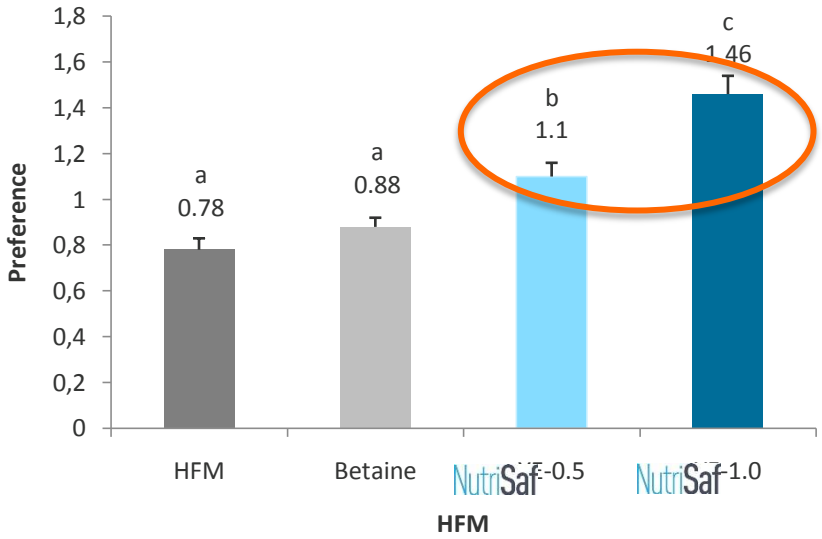
Feces collection
on the 3 last days

Analysis of rare earth oxide in feces

Determination of diet preference

Attractant property – Carp gibel Trial

- **Diets High Fishmeal:**



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

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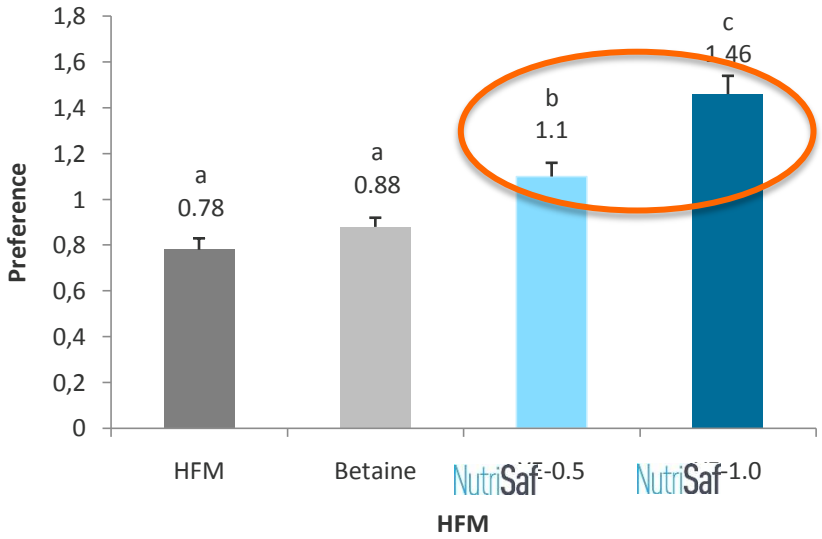
Attractant property – Carp gibel Trial

- **Diets High Fishmeal:**



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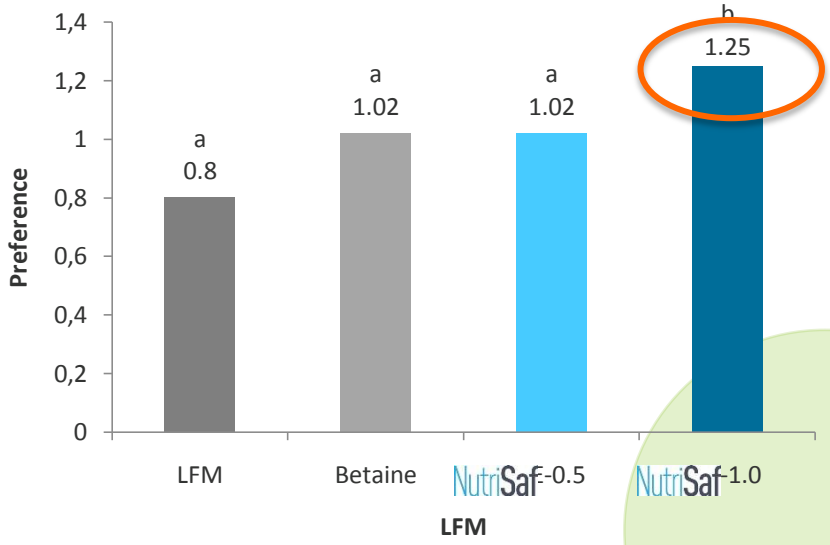

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- Nutrisaf at 0.5 and 1% has a **better palatability than betaine** in HFM diets
- Nutrisaf at 1% has a **better palatability than betaine** in LFM diets

- **Nutrisaf brings attractivity to a LFM or HFM diet, higher than betaine.**
- **A bigger quantity of Nutrisaf is needed in a LFM compared to a HFM diet.**

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General Conclusions

To improve health & performances management:

- Best Aquaculture Practices and Biosecurity Measures
- Meet nutritional requirements of the species & stages

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

Booth n° 43

Thanks for your attention!

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Websites: www.lesaffre.com

www.phileo-lesaffre.com

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