GAIN

Green Aquaculture Intensification

SUMMER SCHOOL

FROM AUGUST 30TH
TO SEPTEMBER 3RD

This project receives funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 773330 (GAIN)

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Aquafeeds Revolution

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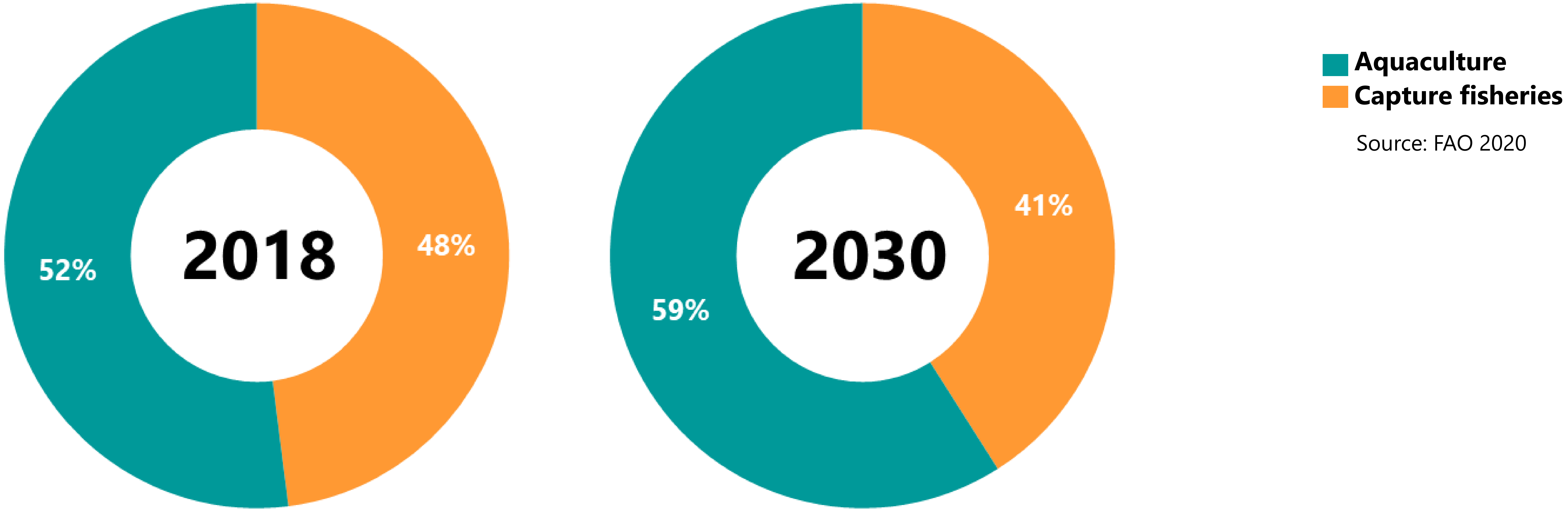
Contribution of aquaculture to seafood supply

Aquaculture is expected to grow by +32% by 2030!!!



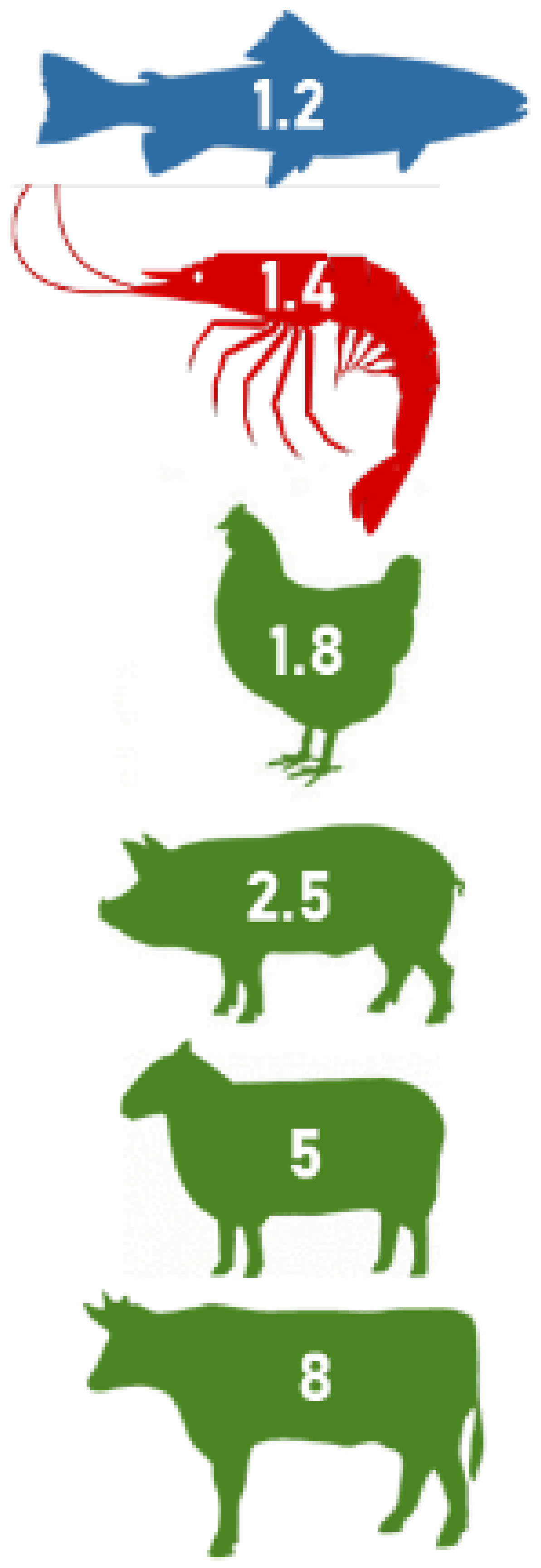
Source: FAO 2020

But we need to ensure that it grows...SUSTAINABLY

Green Aquaculture Intensification

Aquaculture will be part of the solution

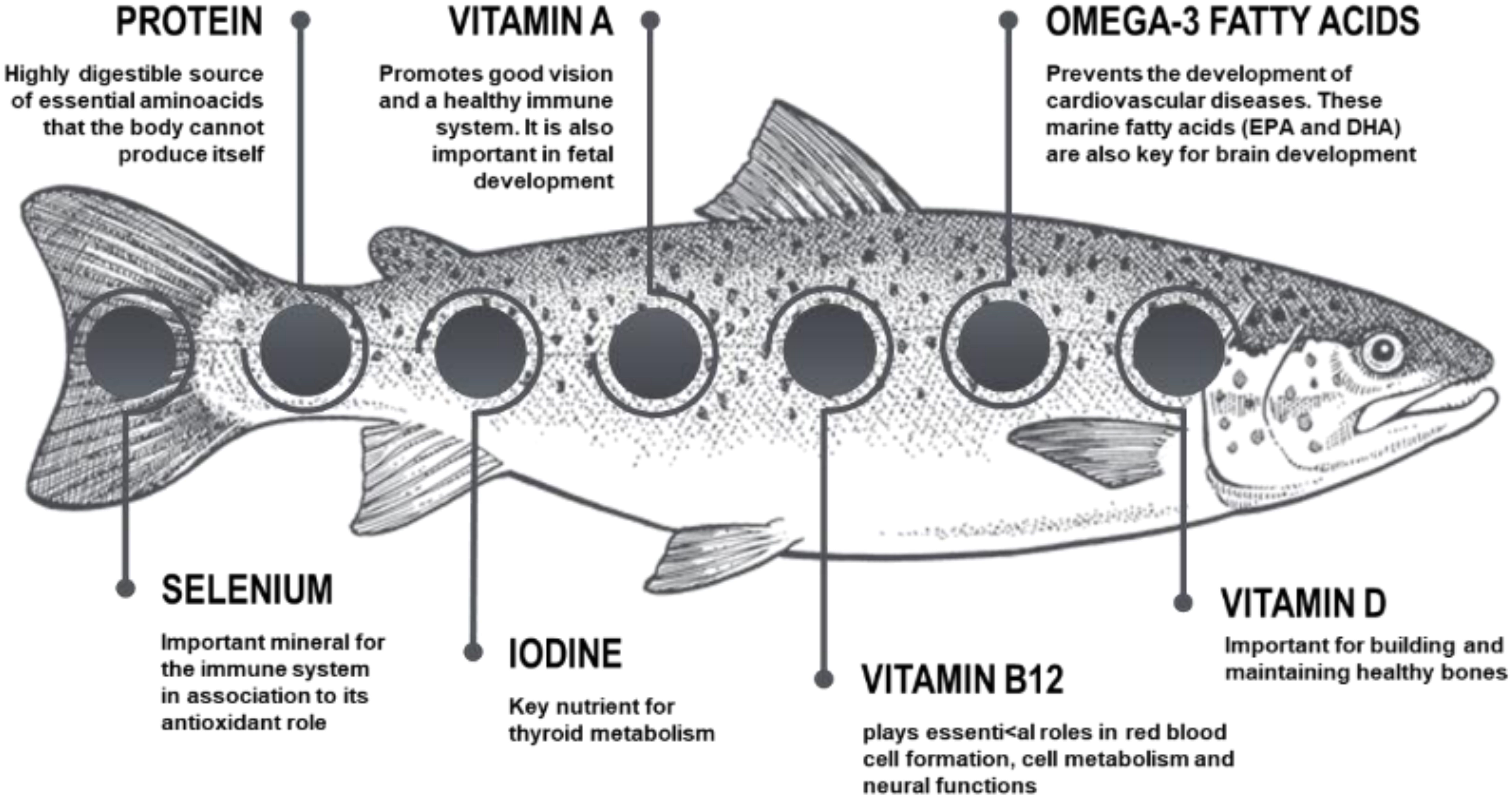
Fish are among the most efficient farm animals in converting feed nutrients into edible meat



Kg of feed needed to generate 1 kg of edible meat

In a resource-constrained world, aquaculture is an attractive option to cater future protein needs

Fish are a rich source of…

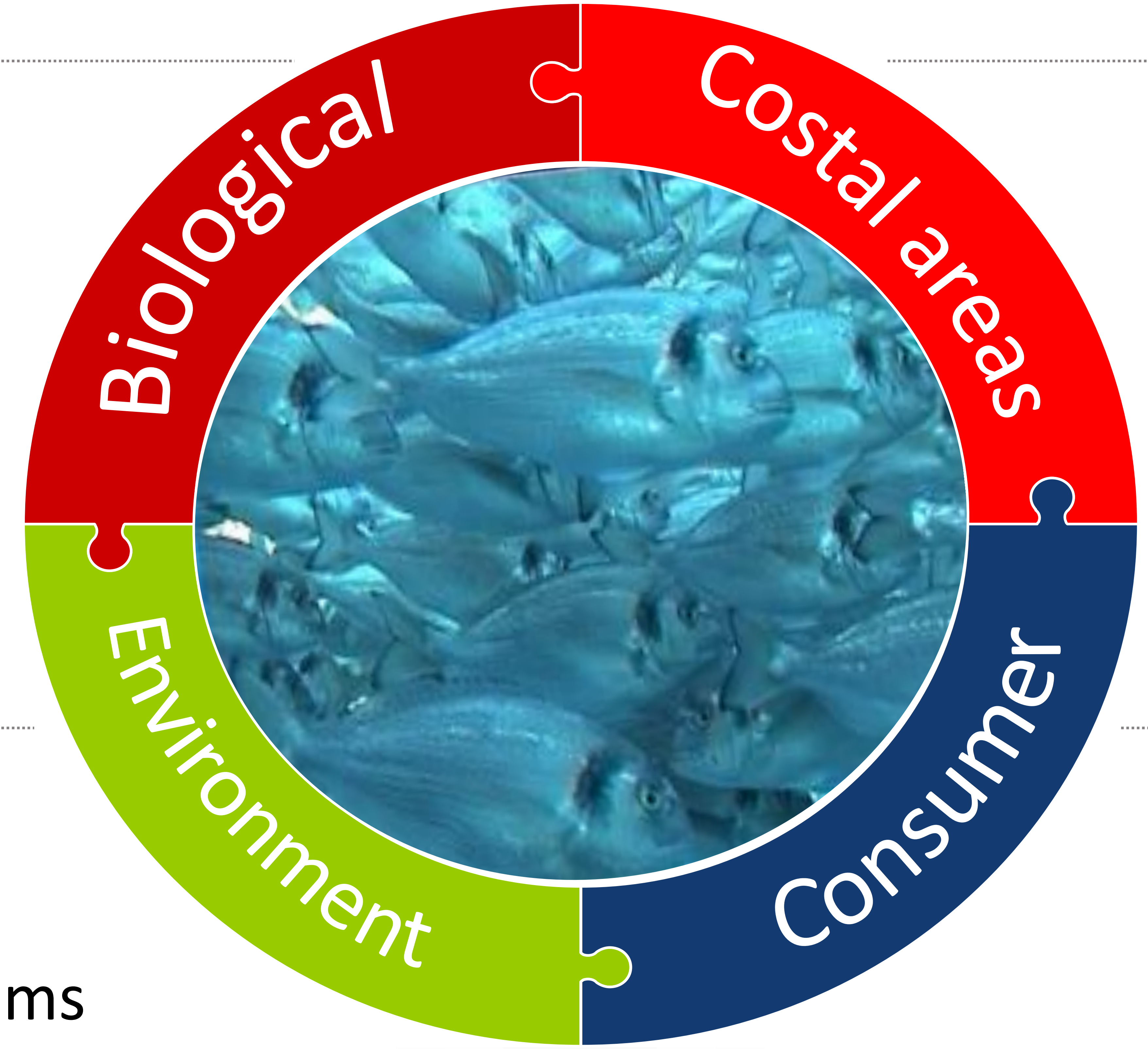


Aquaculture sustainability challenges

Biological

Animal performance
Welfare & health
Biodiversity

- *Alien /exotic species*
- *Escapees*



Ocupation of Coastal areas

Production systems
(RAS, IMTA, off-shore)

Environmental

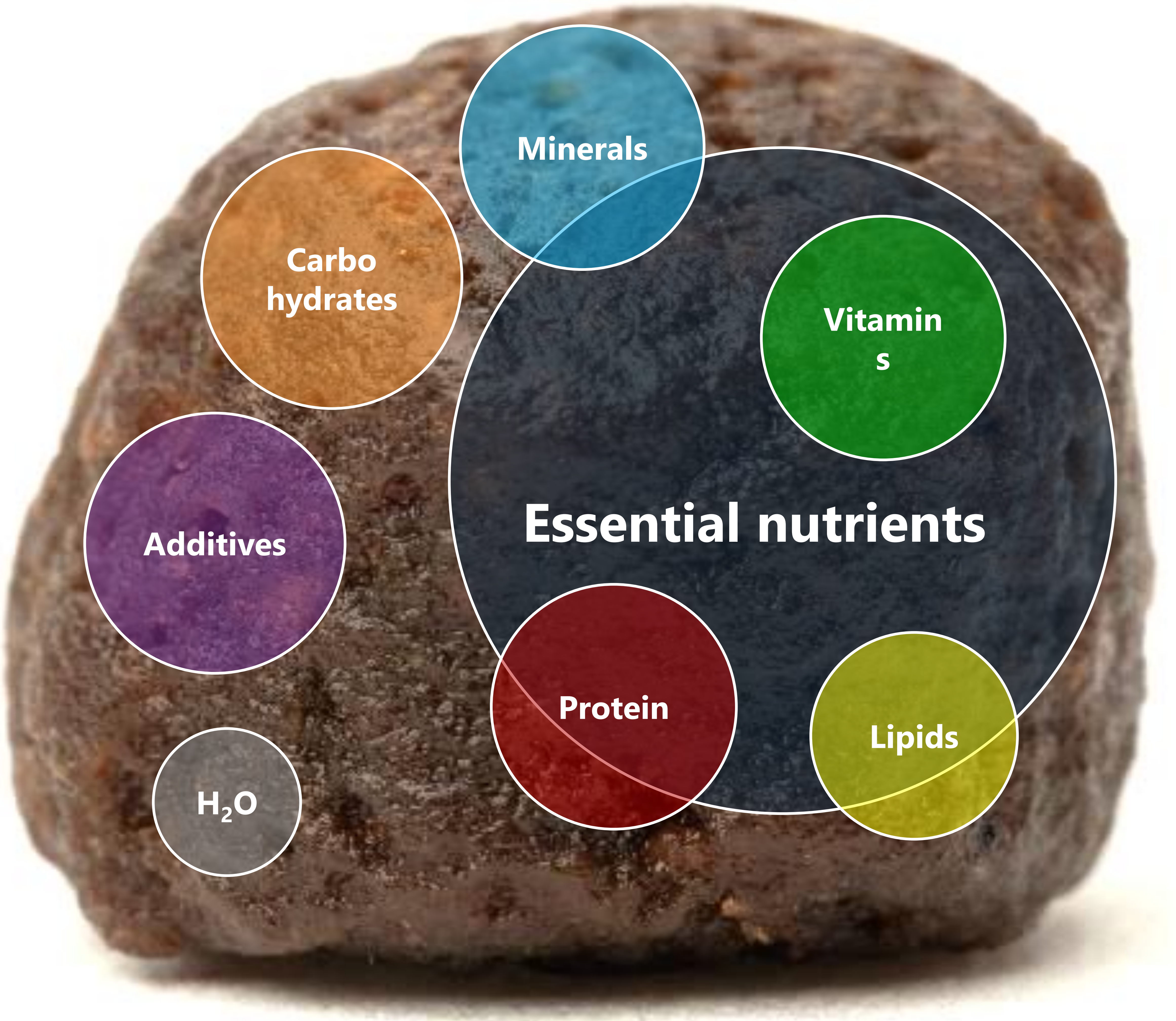
Sustainable feed resources
Low pollution charges
Integrated production systems

Consumers

Food safety
Food quality & health value
Ethical farming standards

Optimal nutrition is key to several of these challenges

When formulating feeds for fish and shrimp…

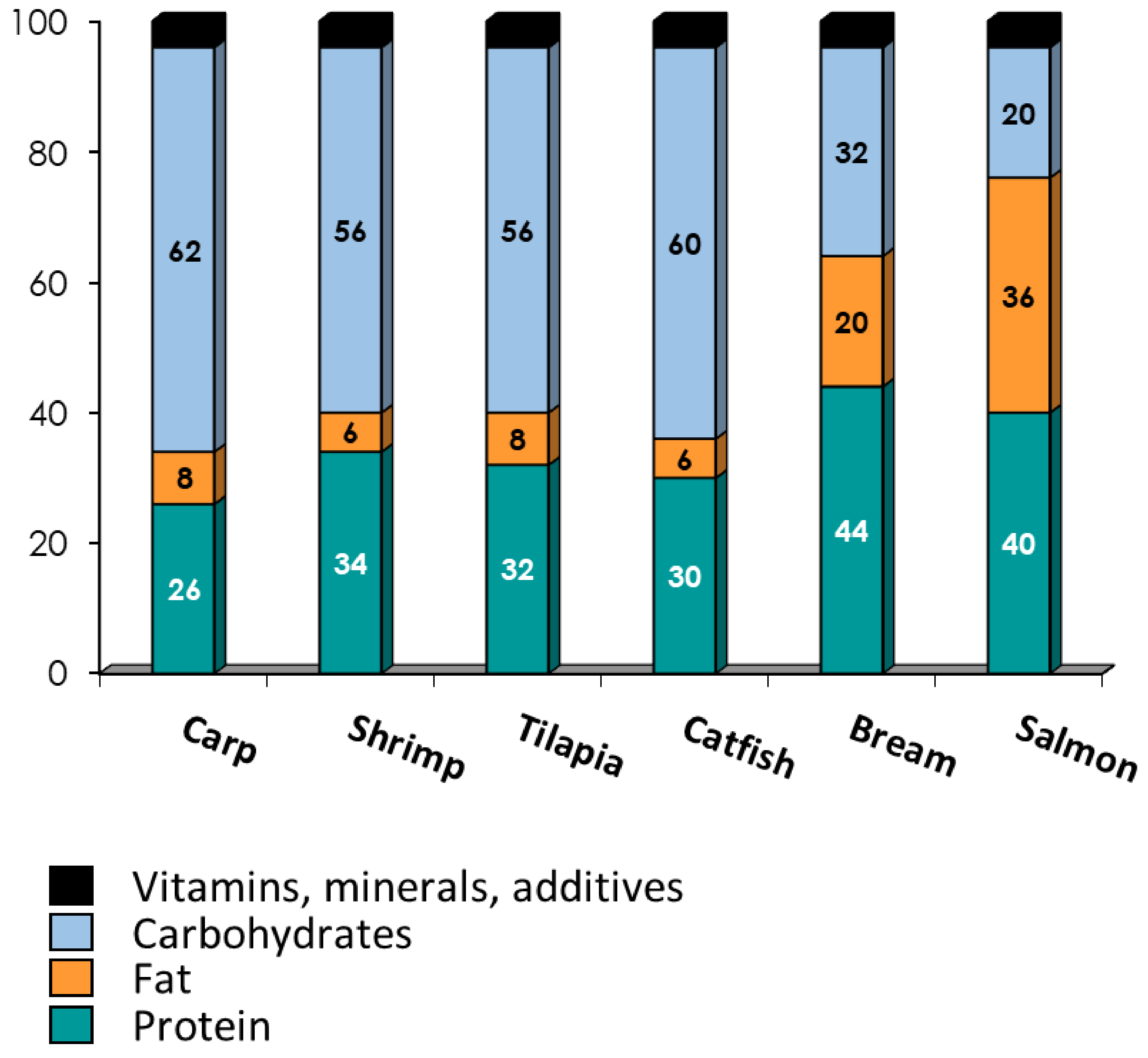


We have to cover the nutritional requirements (needs) in terms of all essential nutrients

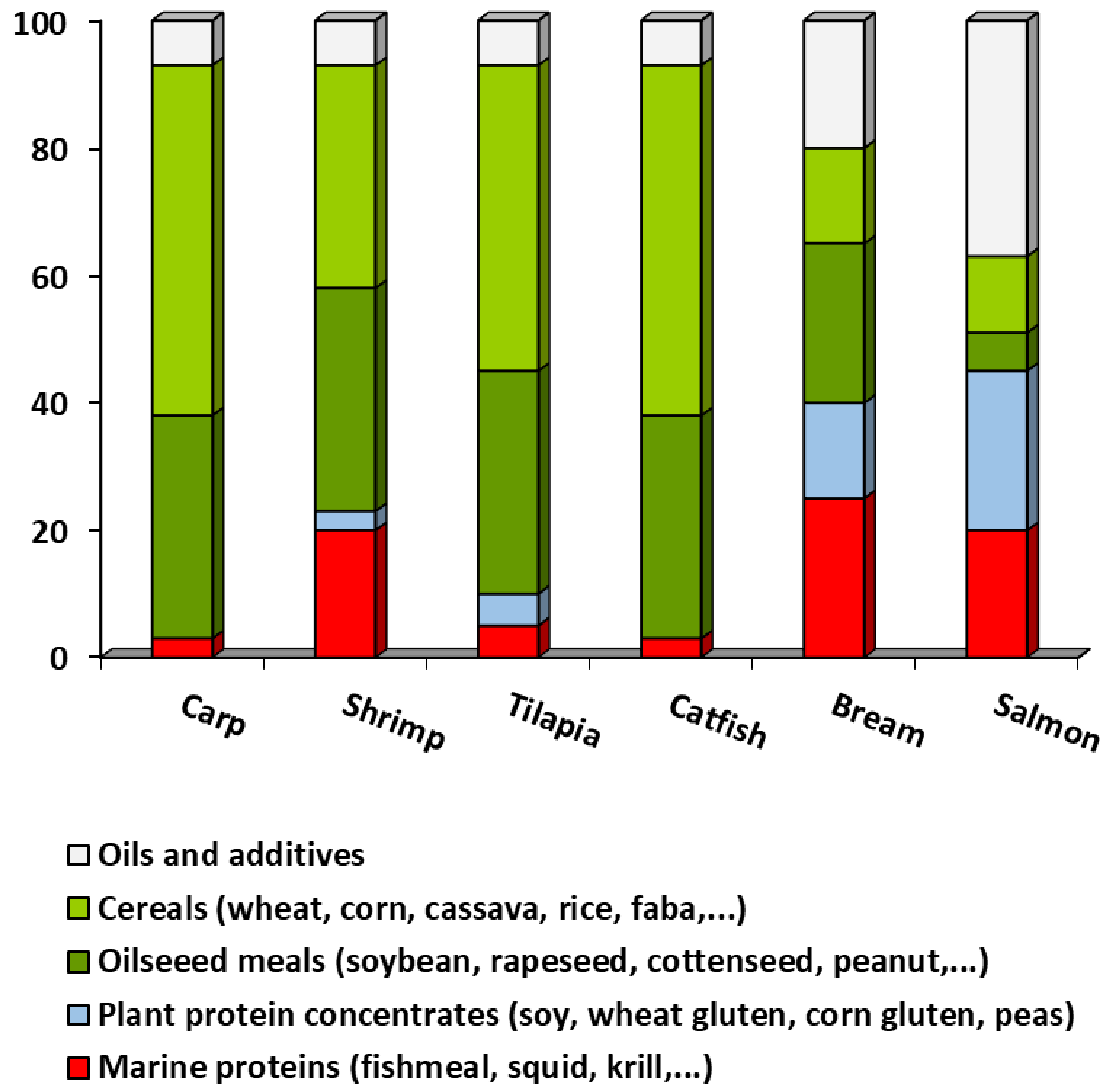
But also guarantee that we promote the well-being of the animals

But we face a wide range of scenarios…

Quantitative requirements change with species



Raw materials in formula vary with species



The work of a fish nutritionist

Crude nutrients (100%)

→ Fecal losses (non digested)

Digestible Nutrients

→ Metabolic losses I

Metabolizable Nutrients

→ Metabolic losses II

Net Nutrients

→ Metabolic losses III

Retained Nutrients for Growth & Reproduction

REDUCE LOSSES!!!

When formulating an optimal feed our objective is to minimize nutrient losses (or maximize the nutrients that are retained by the fish)

Overcovering the requirements will lead to high losses

We need to reach the highest possible digestibility to avoid nutrient losses to the aquatic environment

We need to achieve a delicate balance on the quantitative and qualitative supply of nutrients to promote optimal metabolism and minimize metabolic losses

Ingredients traditionally used in aquafeeds

Protein sources

- **Marine origin**
  - Fishmeal (~70% harvested and ~30% from by-products)
  - Marine protein concentrates and hydrolysates from by-products
  - Krill meal, squid meal, shrimp meal (by-product of aquaculture and fisheries: shrimp cephalothorax)

- **Processed animal protein meals from by-products**
  - Poultry meal, hydrolyzed feather meal, blood meal
- **Vegetable origin**
  - Protein concentrates from soy, pea, potato, lupin, wheat gluten, corn gluten
  - Defatted oilseed meals from soybean, rapeseed, sunflower seed

Lipid sources

- Fish oils, plant oils, terrestrial animal fats

Carbohydrate sources

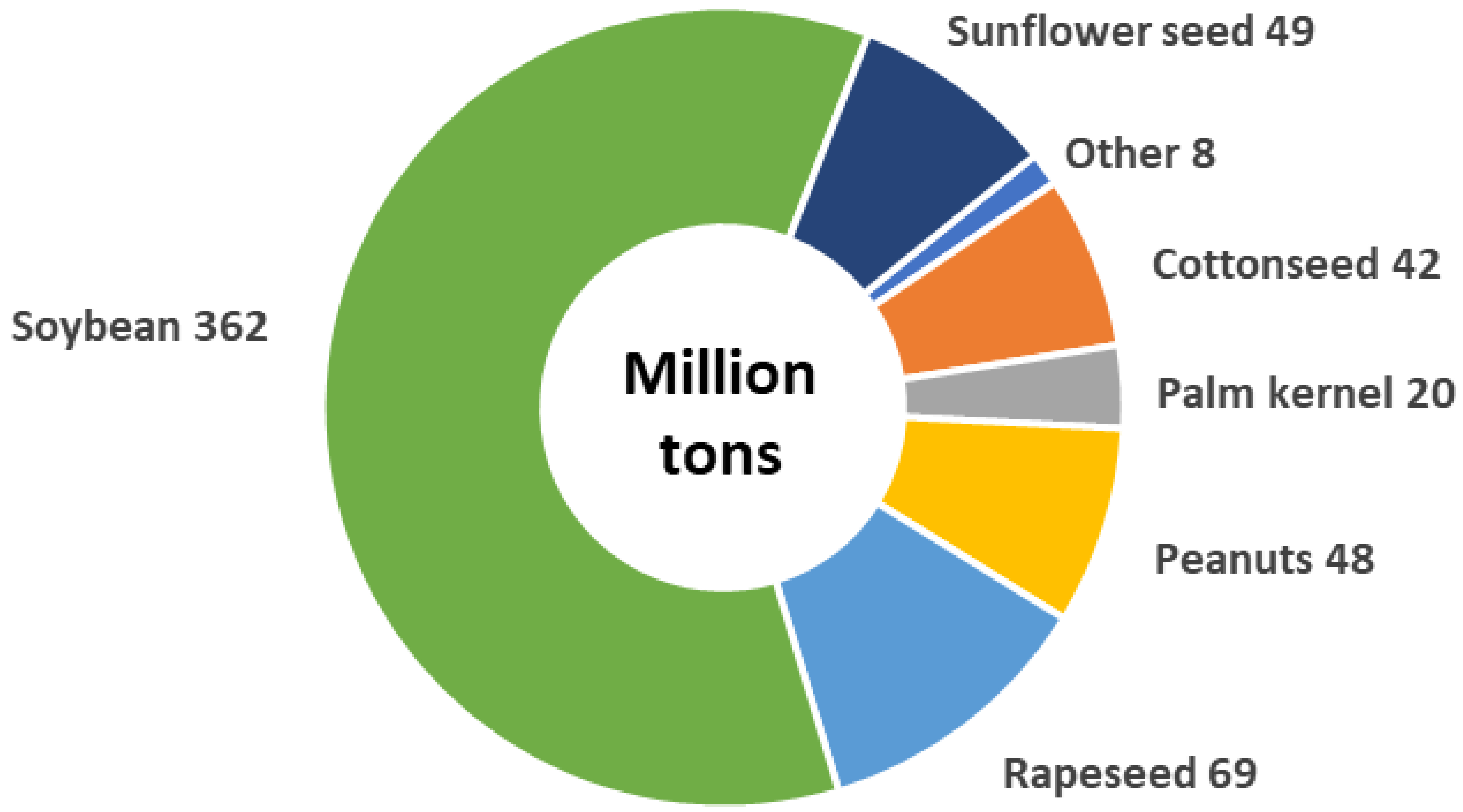
- Whole cereals and co-products (e.g. wheat, corn, rice), legumes (e.g peas, faba), technical starches

Premix of vitamin and minerals, other feed additives

Vegetable proteins

Plant proteins have a high market availability = lower price

World production of oilseeds 2020



Most common plant protein sources used in aquafeeds

- Protein concentrates from soy, pea, potato, lupin, wheat gluten, corn gluten
- Defatted oilseed meals from soybean, rapeseed, sunflower seed, cottonseed, etc.
- Their composition can vary depending on the processing (dehulling, oil extraction,…)

	Moisture	Protein	Lipids	Fiber	Ash
Dehulled Solvent extracted SBM	8	48	1.2	3.3	4.0
Solvent extracted SBM	11	44	1.2	6.5	6.1
Full-fat SBM	10	38	17	7	4.1
Soy protein concentrate	8	61	<1	0.1	3.5

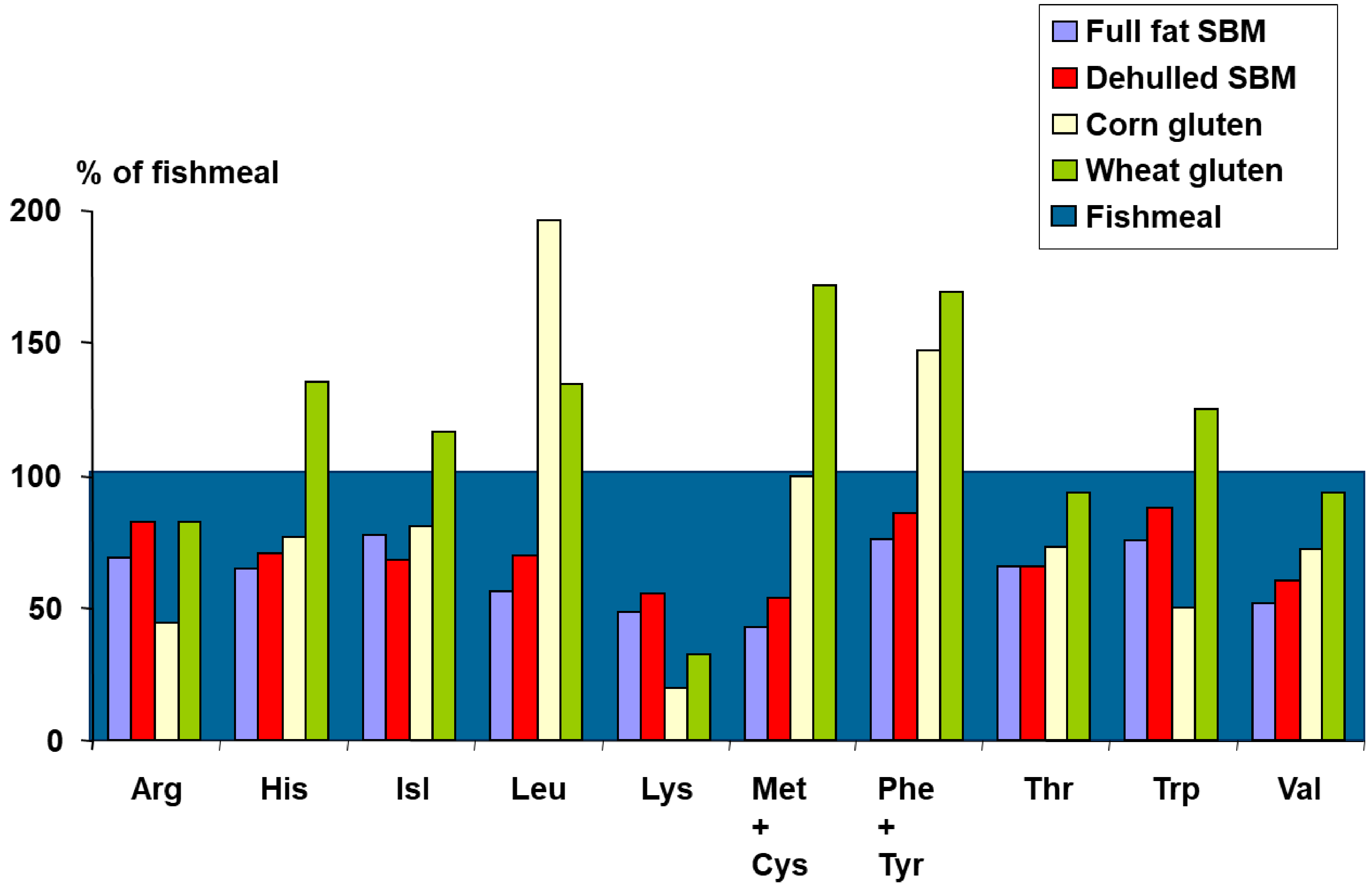
Vegetable proteins

While plant protein concentrates are well utilised by fish and shrimp
Oilseed meals tend to have a lower nutritional value (specially to carnivorous species)

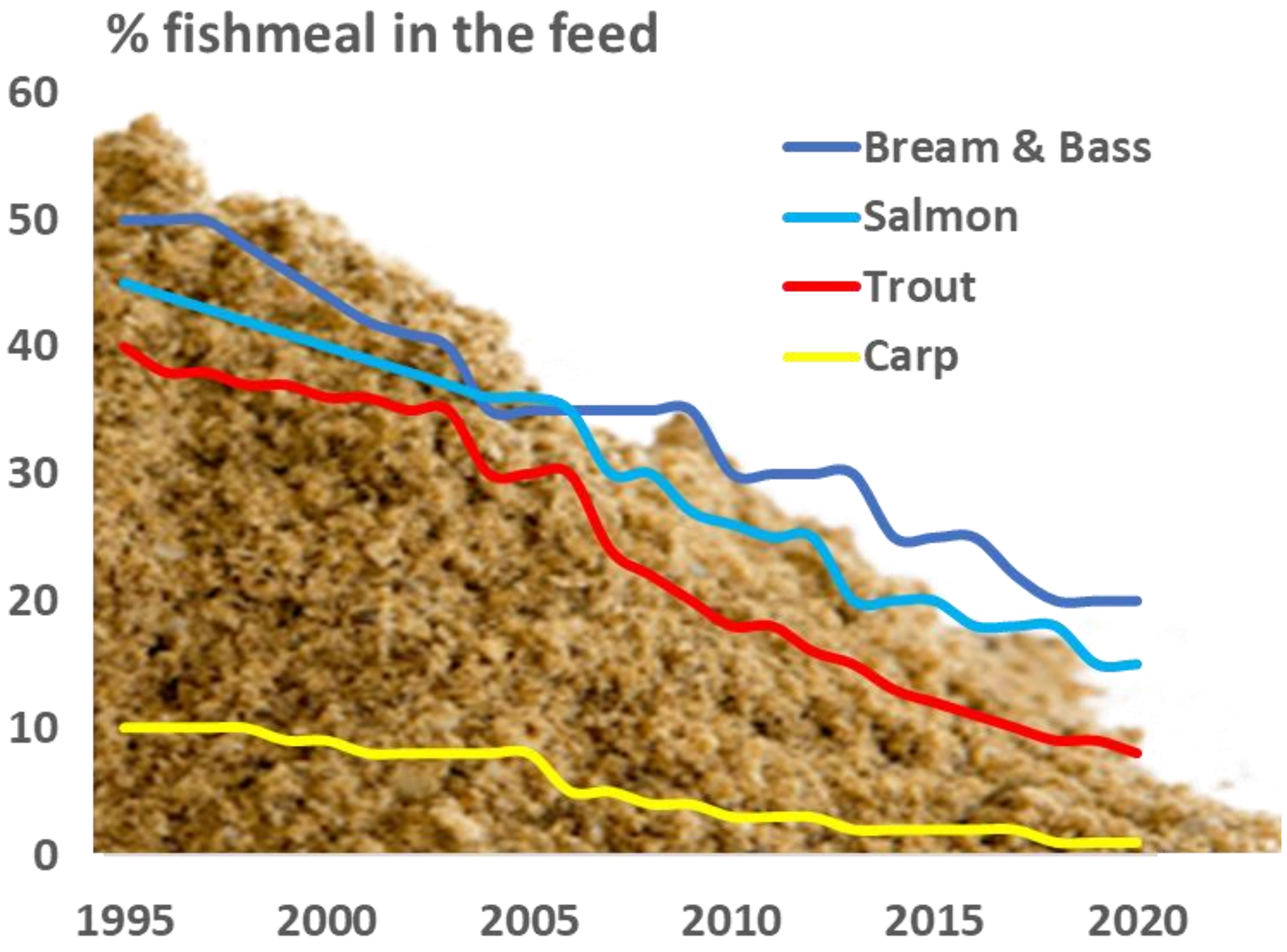
This lower nutritional value is often due to:

- Unbalanced amino acid profile
- High levels of fiber (undigestible to some fish)
- Lower energy density
- Presence of some antinutritional factors (ANFs) that may compromise growth and immune status of fish

ANFs are natural compounds present in plants that reduce the availability of one or more nutrients. A few examples are saponins, tannins, phytic acid, gossypol, lectins, protease inhibitors.



The gradual replacement of fishmeal…



Over the last decades we witnessed a trend towards a general reduction of fishmeal levels in aquafeeds

Achieved by a gradual replacement of fishmeal by

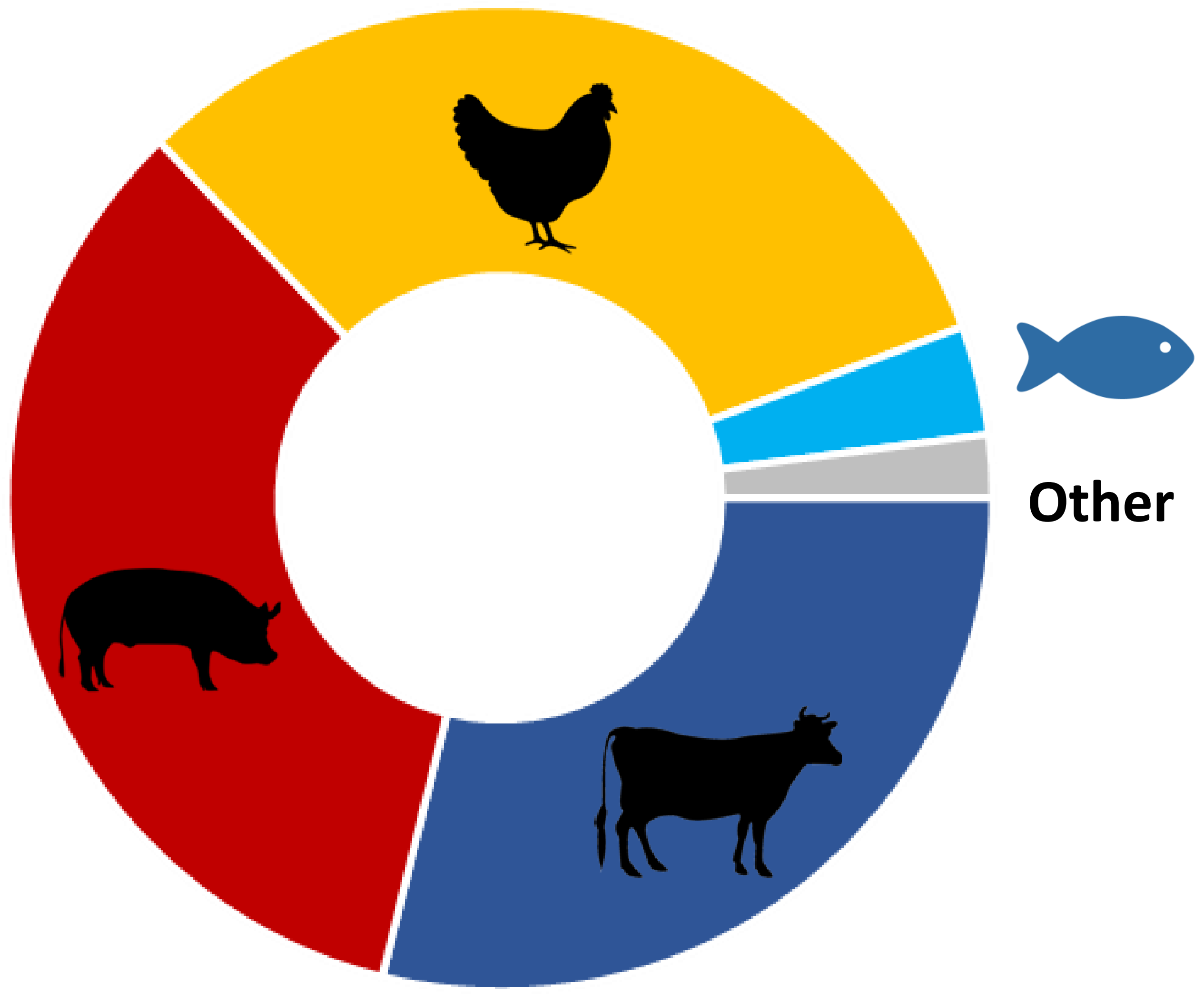
- **Vegetable proteins**
- **Processed animal by-products**

Protein deficit for EU animal feeds

EU27 + UK in 2019 : Animal compound feeds = 165 Million Ton

AQUAFEED production in 2016 = 5.9 Million Ton

2019/2020	EU total use in feed (Million tons/year)	Feed use EU origin (Million tons/year)	EU self-sufficiency (%)
Soybean meal	29.6	1	3
Sunflower meal	8	4.2	53
Rapeseed meal	12.5	9	72
Cereals (wheat, corn,…)	176.6	156.3	89
Pulses (peas, beans,…)	3.6	3.1	86
Fishmeal	0.6	0.5	83
PAPs	1.9	1.9	100



EU animal feed market shows an important protein deficit

Further growth is dependent on the arrival of novel protein sources

Alternative protein sources on the rise

Single cell proteins

Produced in large quantities from micro-organisms such as microalgae, bacteria, fungi and yeast

Very efficient protein production systems due to fast growth, high protein content and the ability to grow on a variety of substrates (e.g. hydrocarbons, natural gas, agro-industrial by-products)

Microbial

Yeast

Microalgae

- Protein content can vary depending on products, but can reach > 65%
- The profile of essential amino acids shows a good adequacy to fish requirements
- The nutritional profile can also be manipulated or enhanced by modifying the culture media, growth conditions, and post-harvest treatments
- Some biomasses can bring along some other "functionalities" that have been associated to a reinforcement of the immune system of fish and shrimp

All with a strong focus on aspects related to valorisation of by-products (circular economy), CO_2 mitigation and low environmental footprint

Alternative protein sources on the rise

Insect meals

Farmed industrially under highly standardised conditions

The use of insect meals in aquafeeds is approved (Regulation EU 2017/893)

Black Soldier Fly *Hermetia illucens*	**Larvae composition (Dry basis)**	**Yellow Mealworm** *Tenebrio molitor*
35-45	Protein	48-56
21-37	Fat	18-25
4-20	Ash	1-5
0.5-2-0	Chitin	0.2-1.0

Musca domestica, Acheta domesticus, and other species are also produced for feed applications

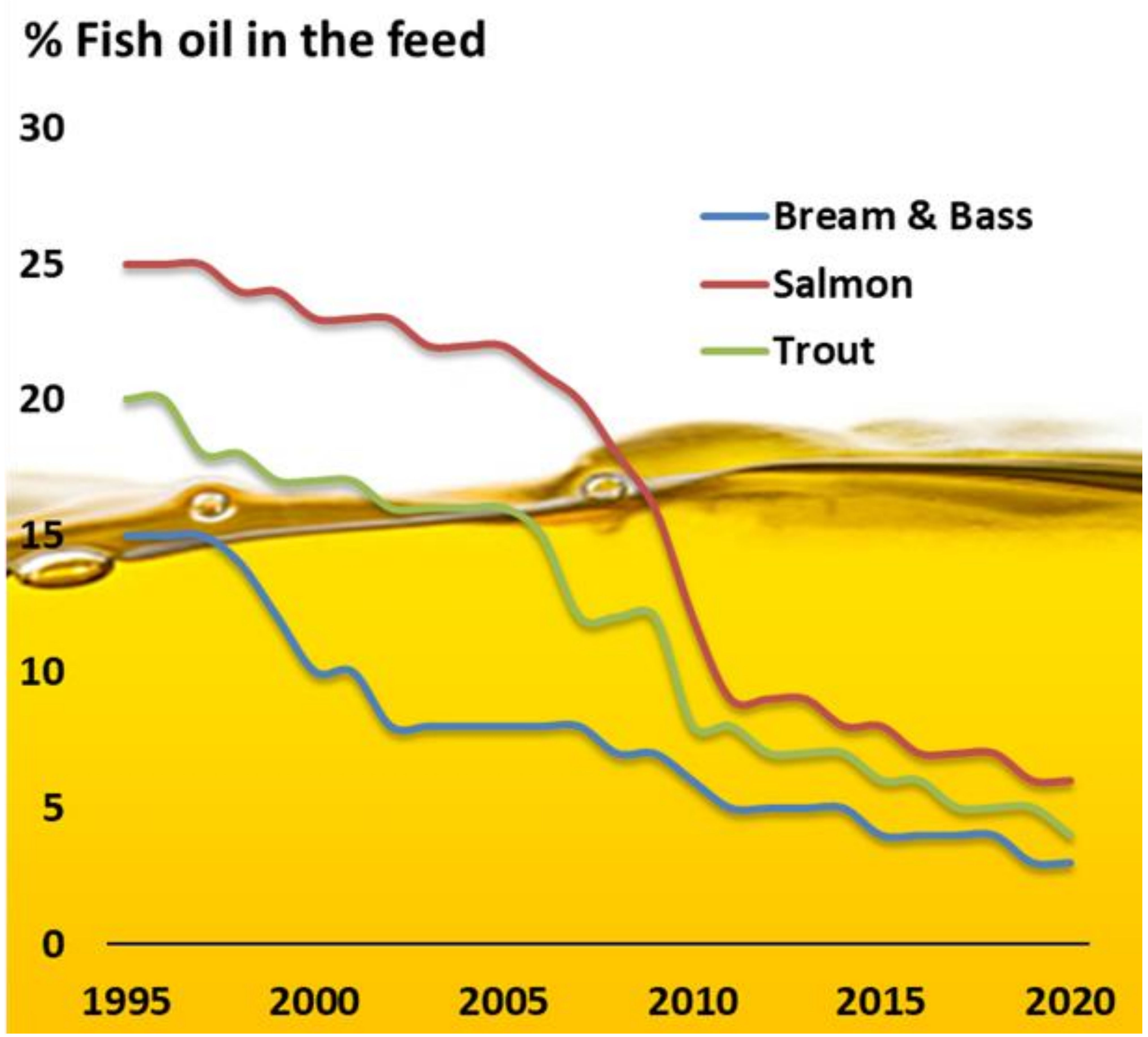
Consumer acceptance is still an issue… Further information on the sustainability benefits associated to the use of insect meals as a protein source is key

Most insect meals are sold as defatted products

- Protein levels are high (55-70%)
- The essential amino acid profile is similar to fishmeal
- High digestibility

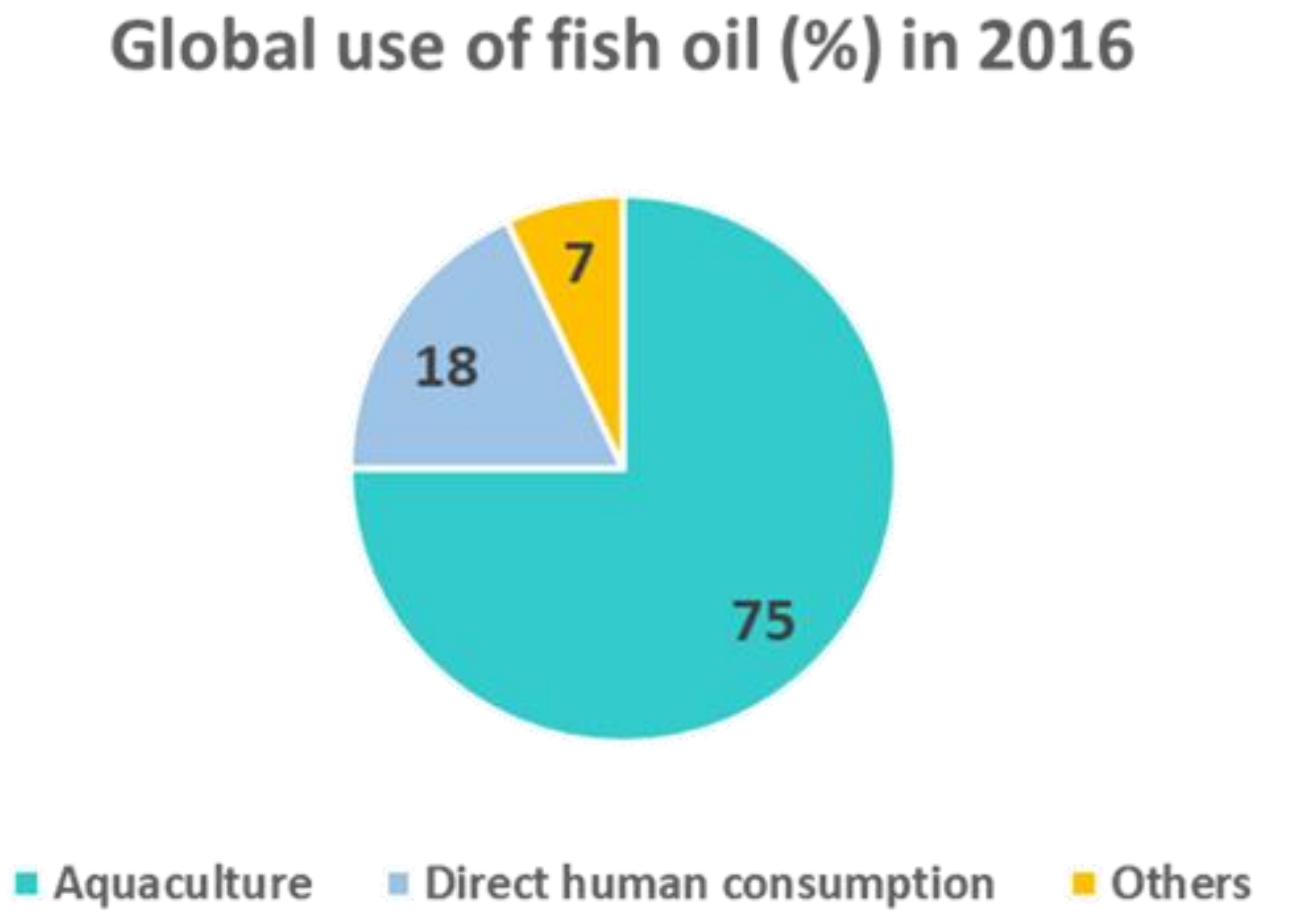
Remember:
Insects are part of the eating habits of several fish in the wild!!!

The gradual replacement of fish oil…



Over the last decades a general reduction of fish oil levels was achieved by a gradual replacement of fish oil by **vegetable oils**

But we still have a high dependency of fish oil



Source: International Fishmeal and Fish Oil organization (IFFO)

Critical to further growth of marine aquaculture

We urgently need alternatives to fish oil

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EPA and DHA are ESSENTIAL FATTY ACIDS to marine species

Oil sources	Saturated	Mono-unsaturated	Linoleic (18:2n6)	Arachidonic (20:4n6)	Linolenic (18:3n3)	EPA (20:5n-3)	DHA (22:6n3)
Marine							
Anchovy	28.8	24.9	1.2	0.1	0.8	**17.0**	**8.8**
Capelin	20.0	61.7	1.7	0.1	0.4	**4.6**	**3.0**
Menhaden	30.5	24.8	1.3	0.2	0.3	**11.0**	**9.1**
Herring	20.0	56.4	1.1	0.3	0.6	**8.4**	**4.9**
Vegetable							
Palm	**48..8**	37.0	9.1		0.2		
Soy	14.2	23.2	**51.0**		6.8		
Canola	4.6	**62.3**	20.2		12.0		
Sunflower	10.4	19.5	**65.7**				
Corn	12.7	24.2	**58.0**		0.7		
Linseed	9.4	20.2	12.7		**53.3**		
Animal fats							
Poultry fat	**28.5**	**43.1**	19.5		1.0		

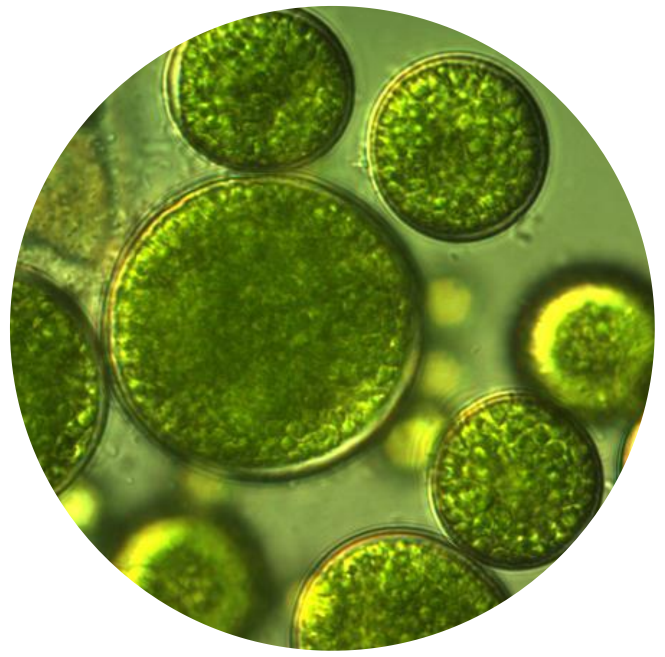
EPA and DHA only exist in marine-derived oils

We need novel sources of EPA and DHA

Other alternative sources of EPA and DHA

Thraustochytrids of the genera *Schizochytrium* and *Aurantiochytrium* accumulate DHA-rich oils (made by fermentation)

EPA & DHA-rich *Schizochytrium* oil (made by fermentation)



Still a “bit” expensive to fully replace fish oil

Its sensitive use allows a cost-effective reduction of FO usage

But algae oils are gaining industrial relevancy and scale

Strong research effort on:

- Algae species with capacity to acummulate high lipids (>60%)
- Cultivation conditions to enhance EPA and DHA levels
- Green technologies for oil extraction
- Algae oils seem to contain specific lipids (e.g. glycolipids, oxylipins) with a bioactive effect

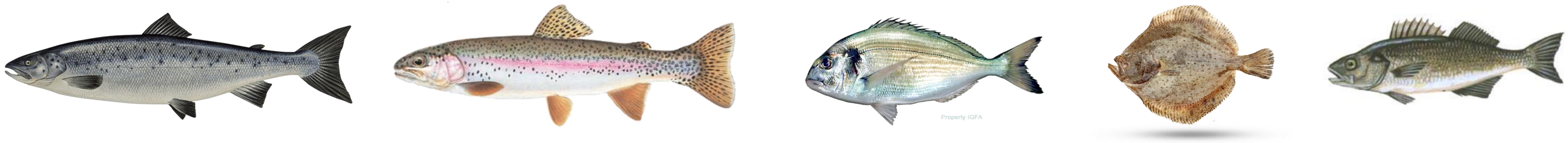
The GAIN approach & results

Objective: formulation of eco-efficient fish feeds

=> new “generation” of sustainable fish feeds

- facilitate aquaculture eco-intensification
- increased circularity and resource utilisation
- Test candidate ingredients

Target species



Formulation concepts

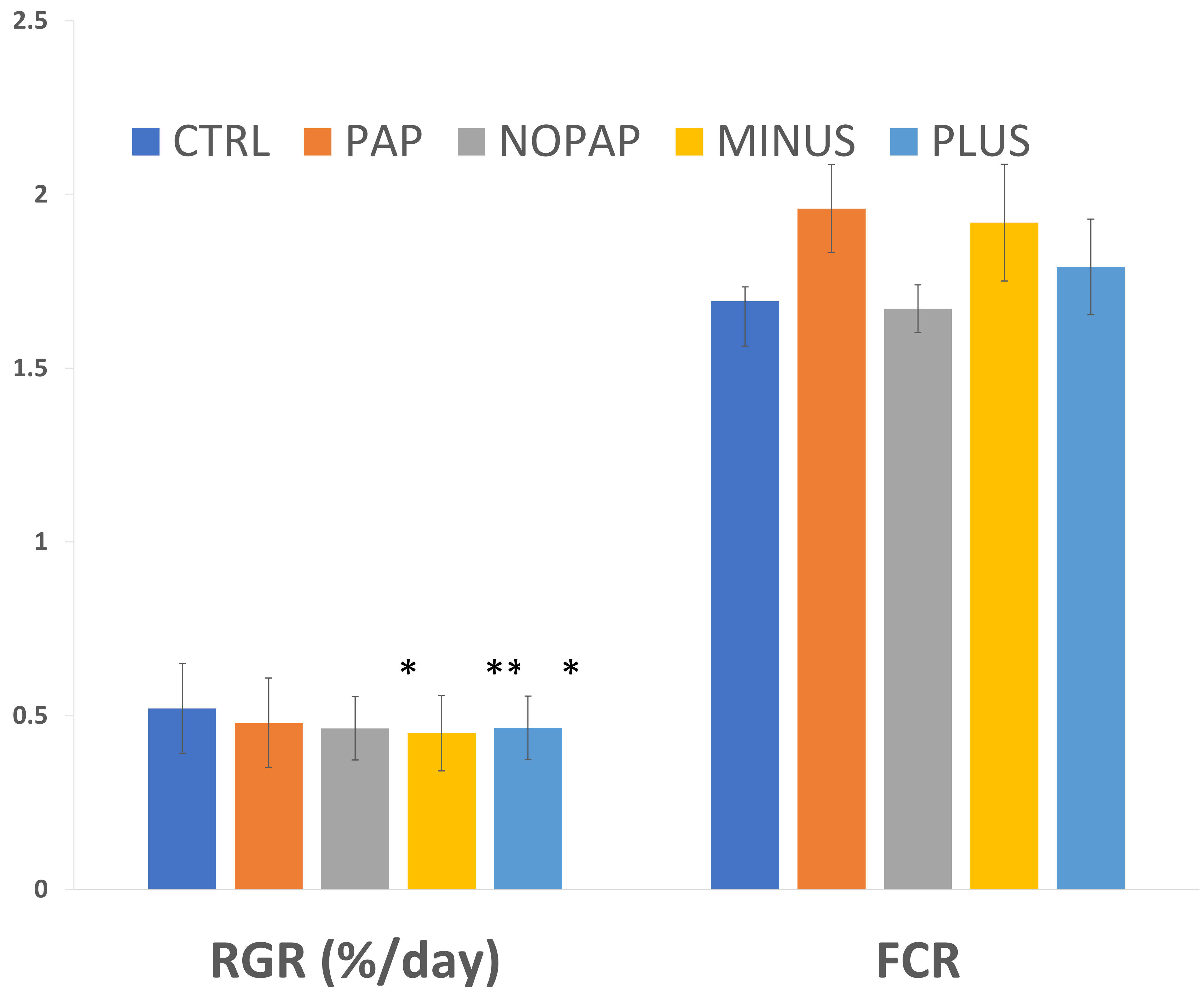
CTRL	PAP	NoPAP	Mixed
• Current standard feed • Moderate levels of fish -meal and -oil	• Rich in processed land animal proteins(**PAPs**) • VPCs	• Reduction FM and no PAPs • VPCs, Insect & microbial biomasses	• reduction FM • PAPs, VPCs, Insect & microbial biomasses

VPCs – vegetable protein concentrates (preferably EU)

GAIN ingredients:
- fish protein hydrolisates
- Micro- and macro-algae rich in Se and other minerals

Commercialy available ingredients:
- Fish meal from fisheries by-products
- Salmon oil (aquaculture by-product)
- Algal (n-3 HUFA rich) oils and vegetable oils

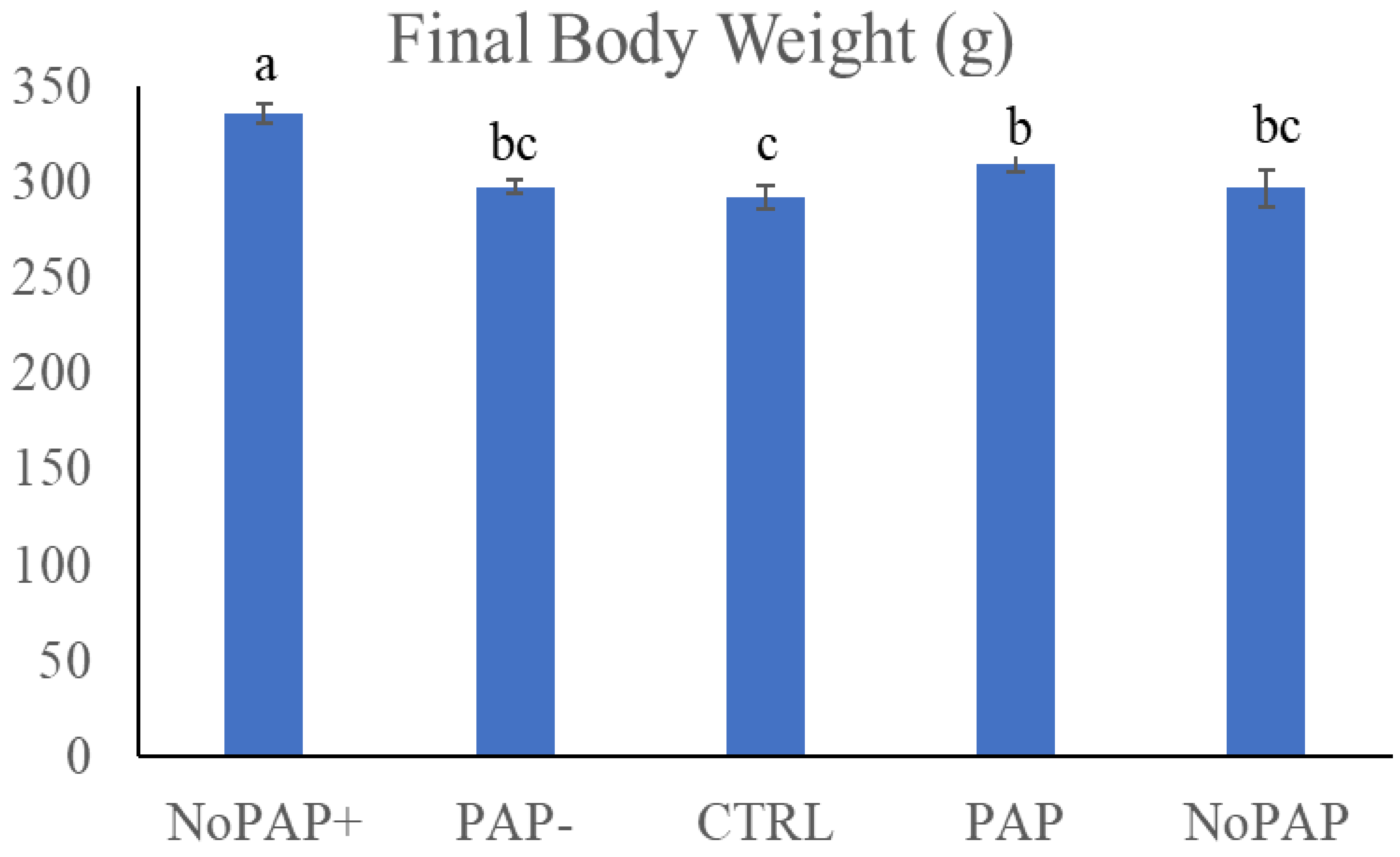
Growth, feed and sensory performance of large seabass



- CTRL and PAP : similar in RGR and FCR
    - NOPAP, MINUS and PLUS: sign. Reduced growth, similar FCR
- PLUS sign. decreased total protein content in plasma compared to CTRL, PAP and NOPAP.
- No differences in sensory analysis

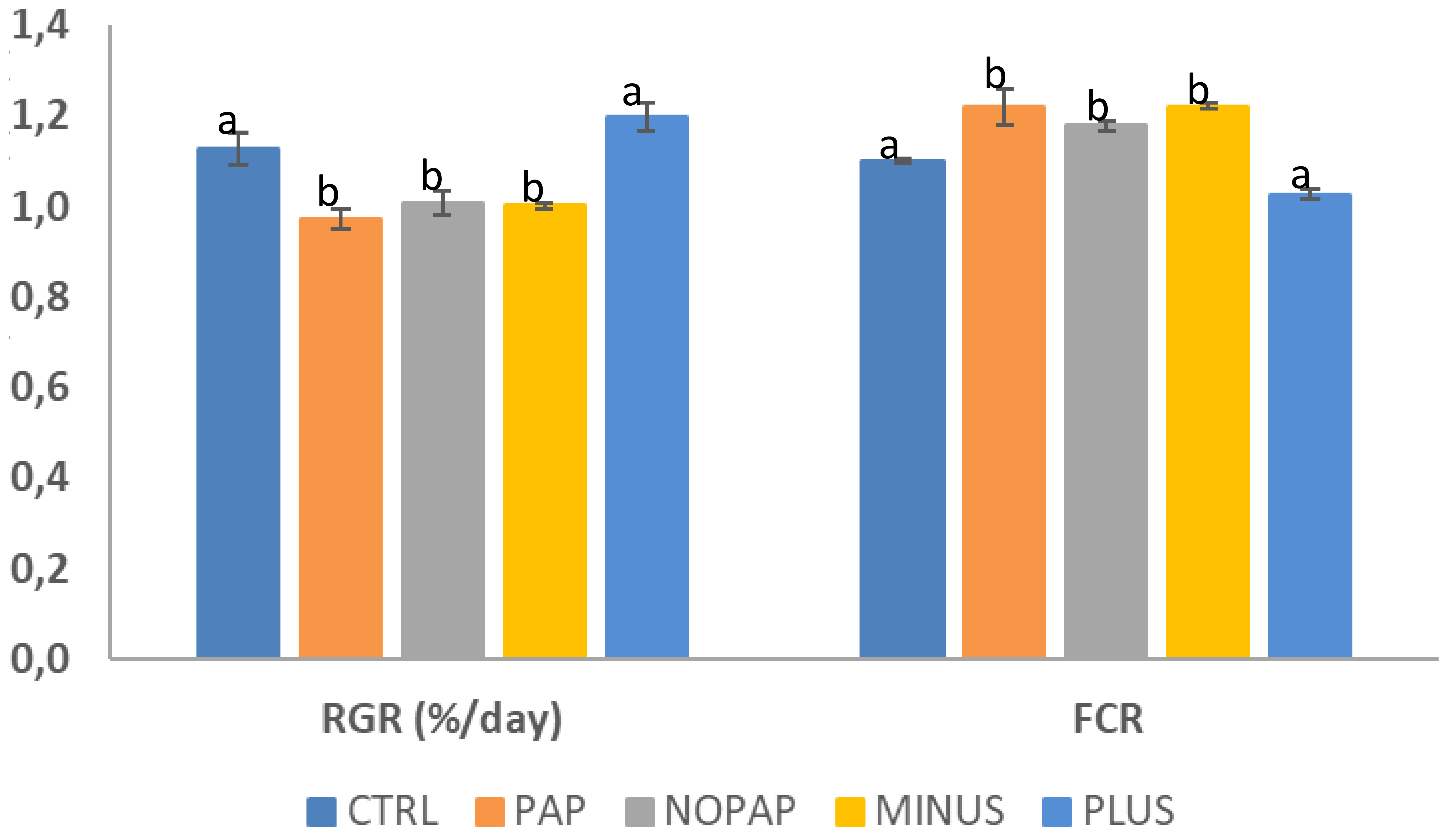
  (taste, smell, juice- and fat-separation)

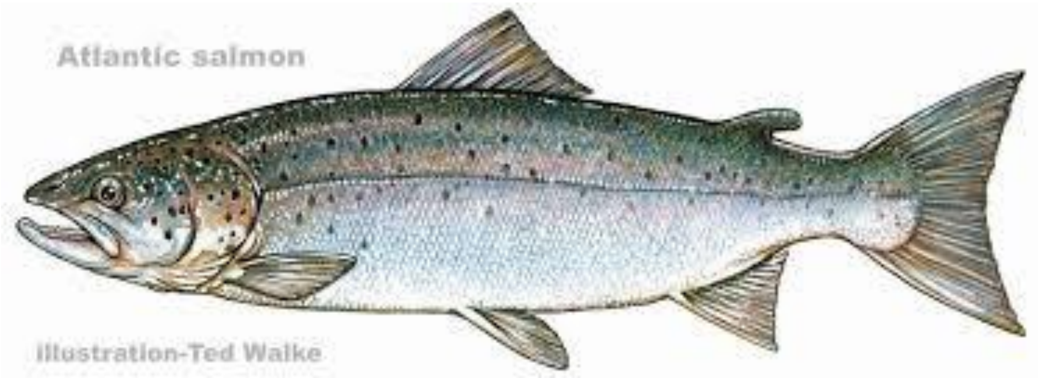
Growth, feed and sensory performance of Trout



- All diets performed very well:
  high growth, low FCR
- **PAP outperformed CTRL** in growth, but similar in FCR, and **PLUS** had the best performance
- Sensory analysis (100 consumers): slightly better texture in **NOPAP** than **CTRL** and **PAP**, no differences for taste, appearence and odour

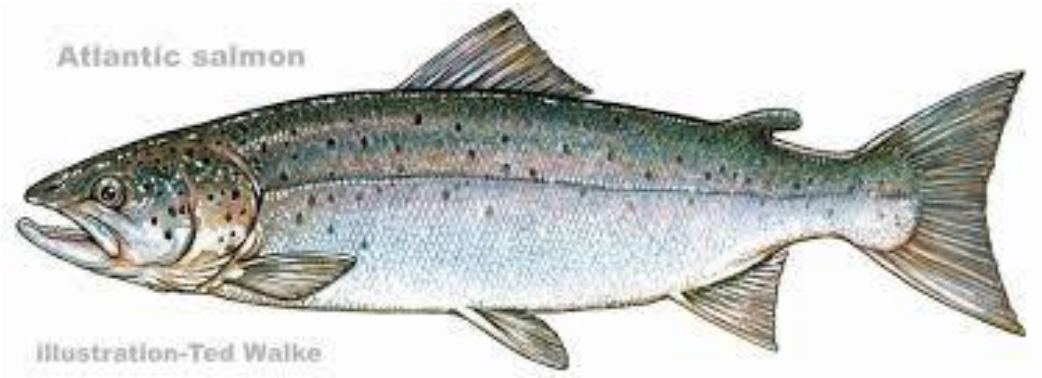
Growth, feed and sensory performance of Salmon

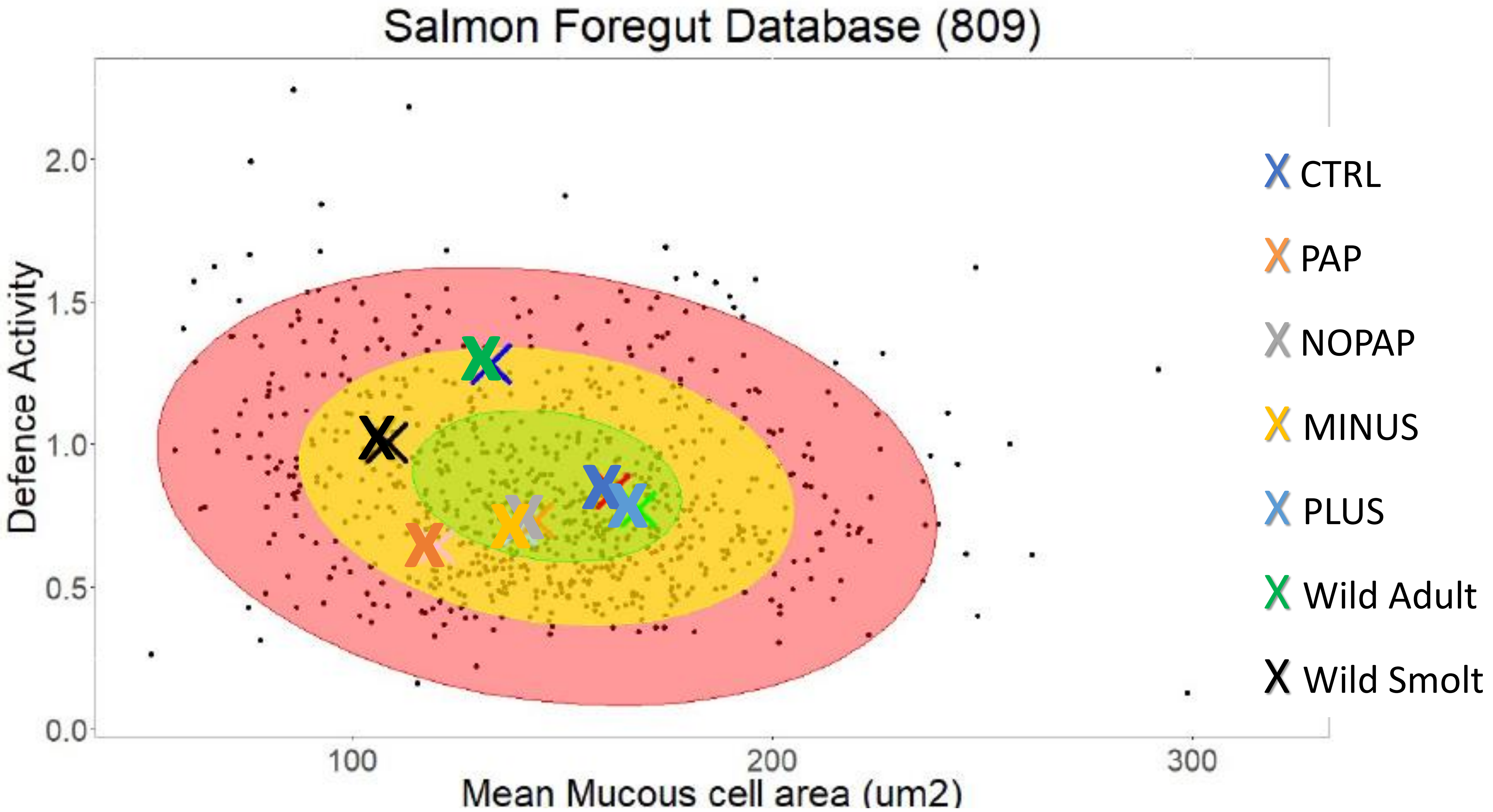




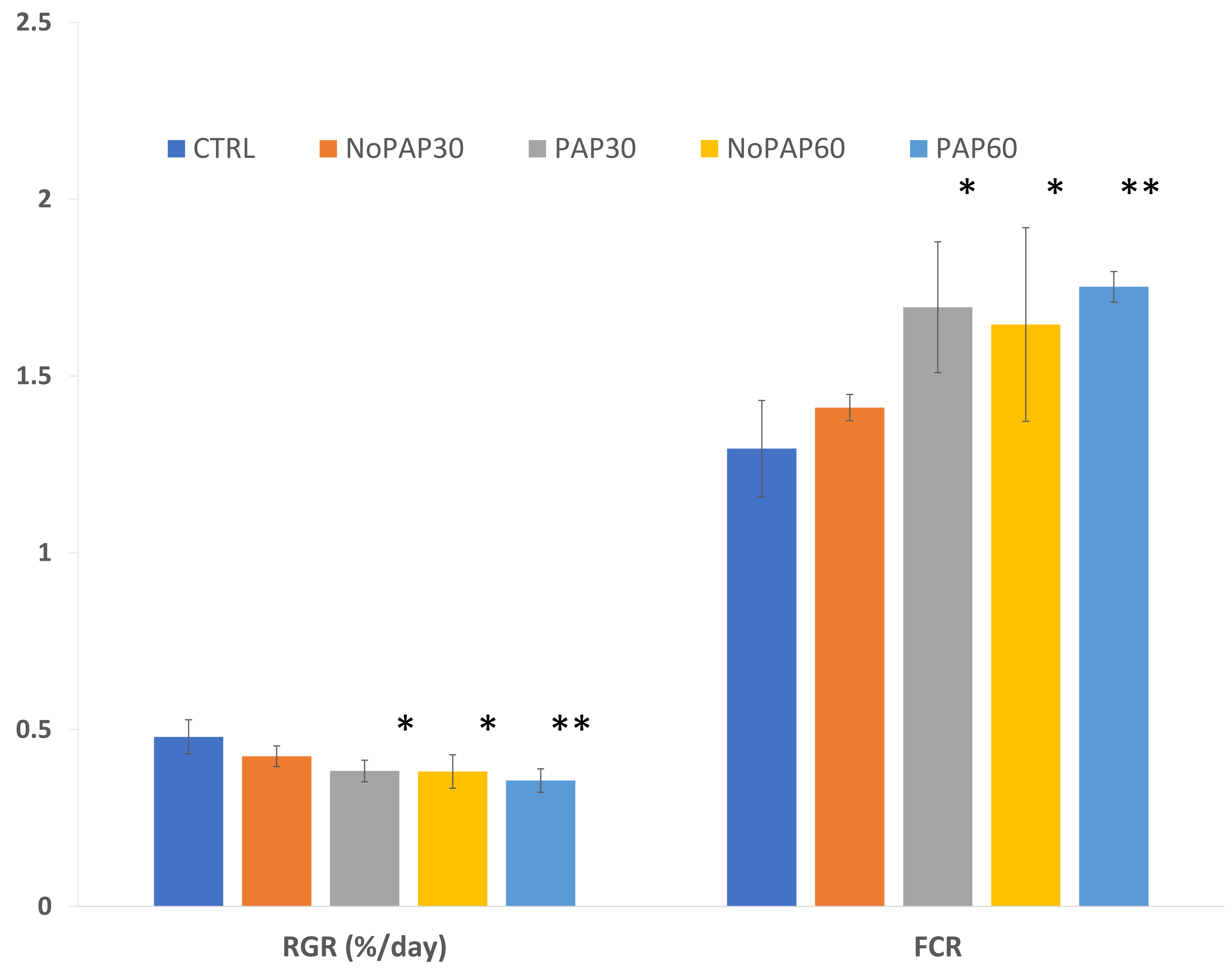
- CTRL and PLUS: similar in RGR and FCR
  - PAP, NOPAP and MINUS : Small reduction in growth, and higher FCR & PER
- Tendency for lower protein digestibility, energy retention and barrier status in PAP. Still all within reference values.
- Sensory analysis (100 consumers): slightly better Odour in NOPAP than CTRL and PAP, no differences for Taste, appearence and texture

Gut barrier status (mucosal mapping) in Salmon





Growth and feed performance of large turbot

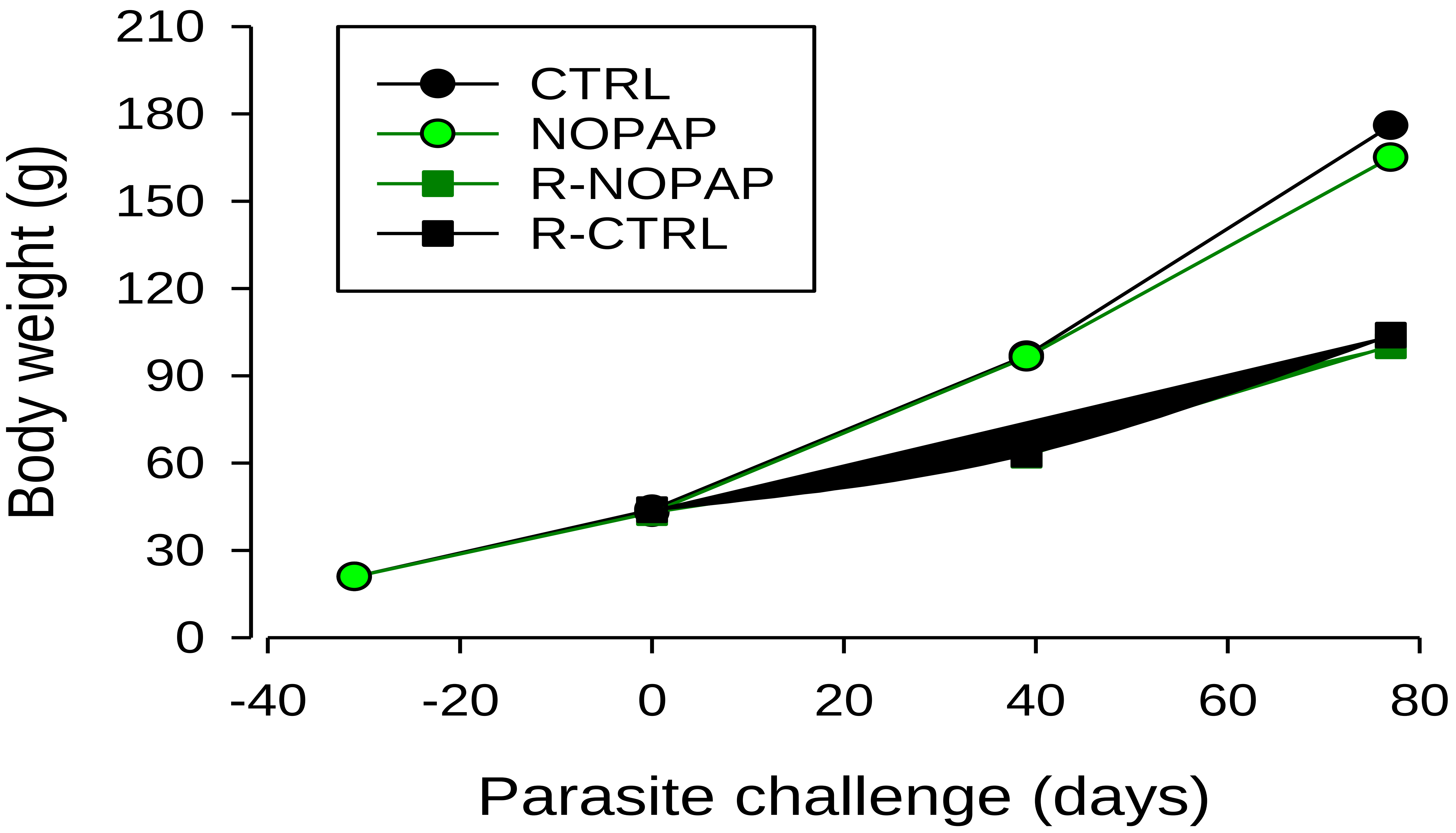


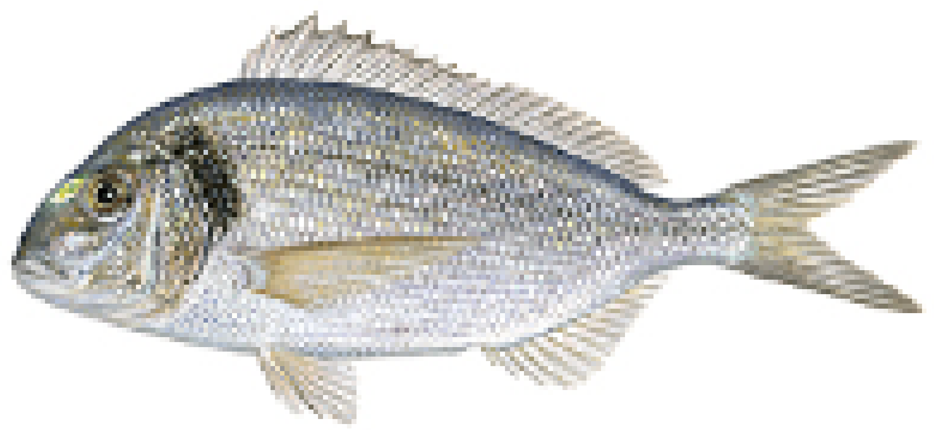
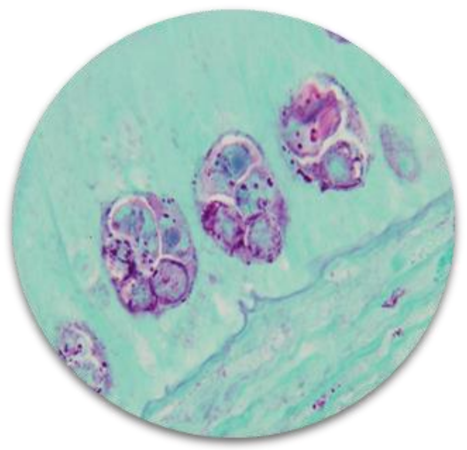
- CTRL and NoPAP30: similar performance
- PAP30, NoPAP60 and PAP60: decreased growth and FCR performance

NOPAP > PAP and **30 > 60**

The GAIN approach & results

Parasite challenge with gilthead seabream



enteritis ← *Enteromyxum leei*

Pre–challenge:

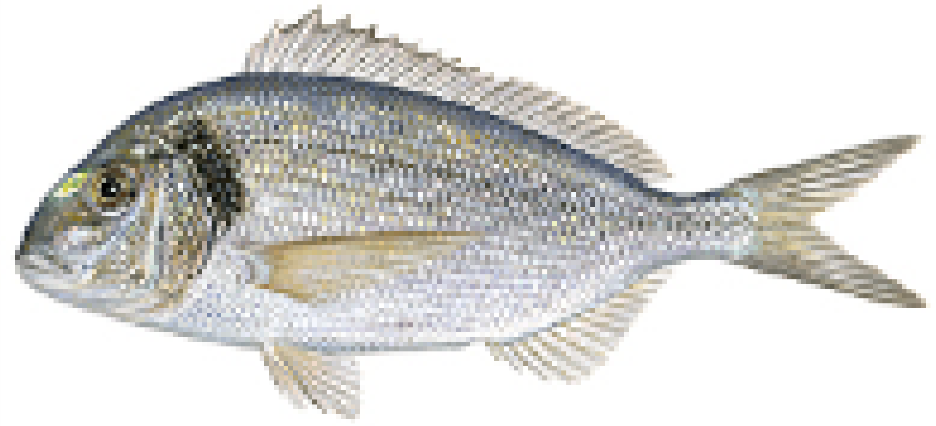
- CTRL and NOPAP-SANA: similar performance

Post –challenge:

- CTRL and NOPAP-SANA: similar performance, but poorer SGR and FCR in infected fish
- Intensity and prevalence are similar in both dietary treatments
- Early infected fish recover in NOPAP SANA fed fish but not in fish fed CTRL diet

Parasite challenge with gilthead seabream

- Transcriptomic analysis before challenge (Customized PCR-arrays)
- Target tissues: Liver, Head Kidney, Posterior Intestine



Liver, 16 DE genes (16/44)

elov6, fads2, scd1a, hl, pla2g6, cyp7a1, pparb, pparg, h-fabp, nd5, cox2, ucp1, gpx4, prdx5, sod1, sod2

Reduced lipogenesis

Reduced mitochondrial activity & risk of oxidative stress

HK 2 DE genes (2/29)

Il8, tlr2

Anti-inflammatory profile

PI 4 DE genes (4/44)

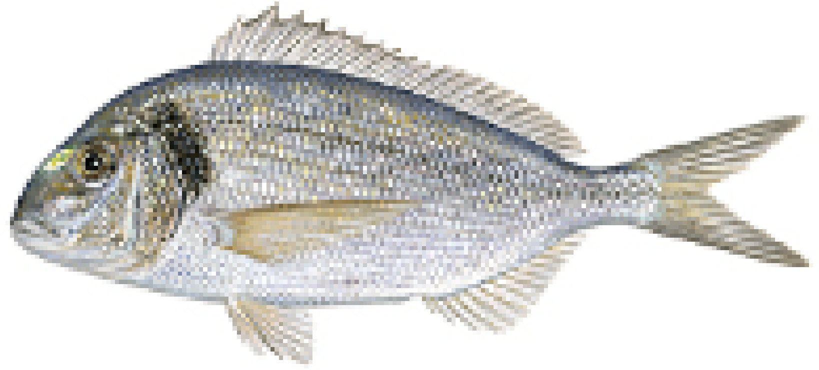
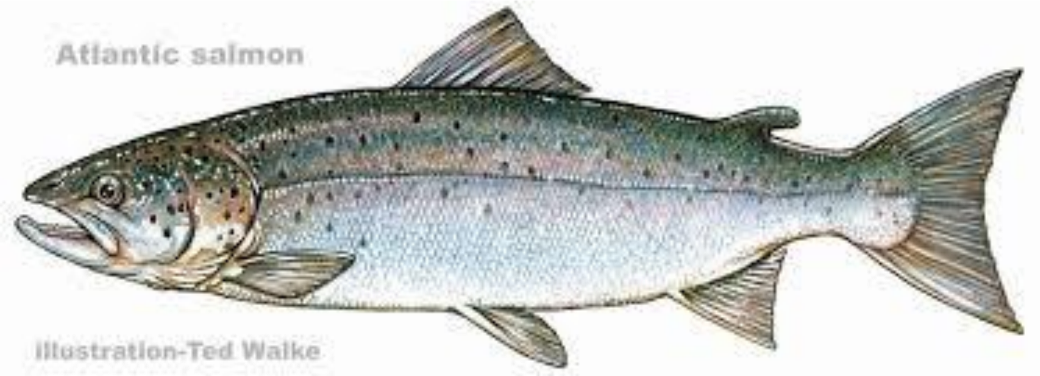
cd17, muc2, il12b, cd8b

Anti-inflammatory profile

The GAIN approach & results

GAIN Green Aquaculture Intensification

Summary

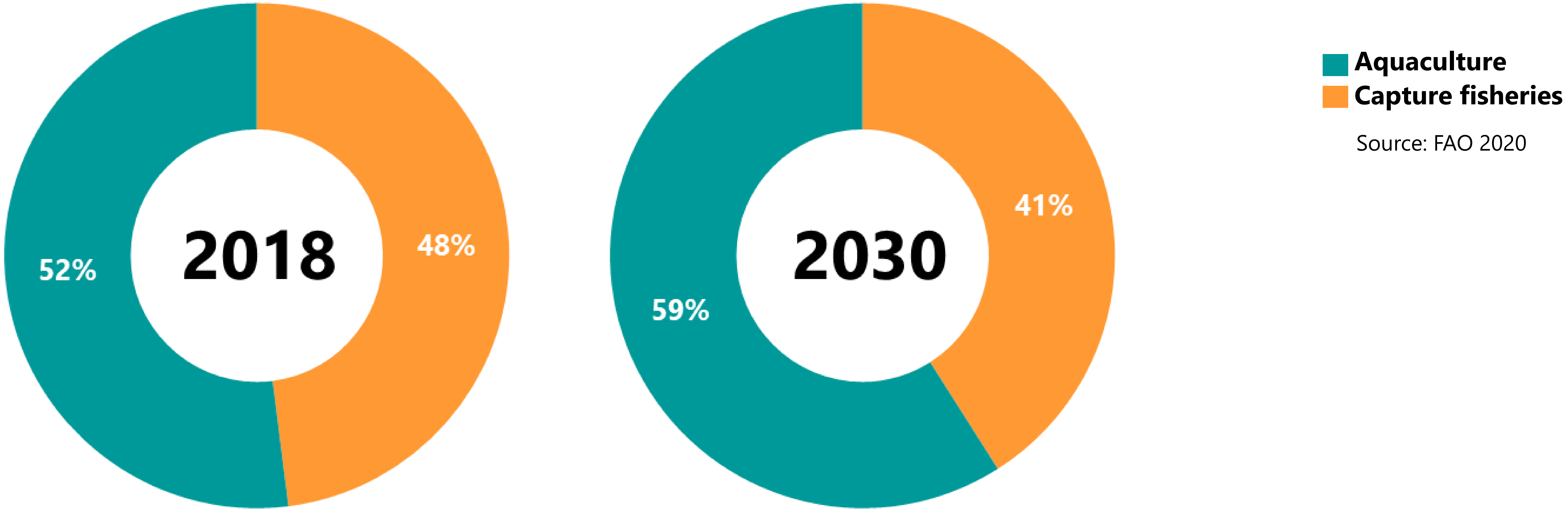


- The growth performances and feed conversions in all GAIN trials were very good (salmon, seabream, seabass, trout and turbot) for most feed formulations tested;
- Results support that the new formulations tested are viable options for the species tested; KPIs other than growth and FCR suggest that the novel formulations affect fish physiology, likely looking for a new allostatic balance, but no clear detrimental effects to fish health could be identified.

=> GAIN feed formulations are viable options for Eco-efficient European fish farming

Contribution of aquaculture to seafood supply

Remember...
Aquaculture is expected to grow by +32% by 2030!!!



Source: FAO 2020

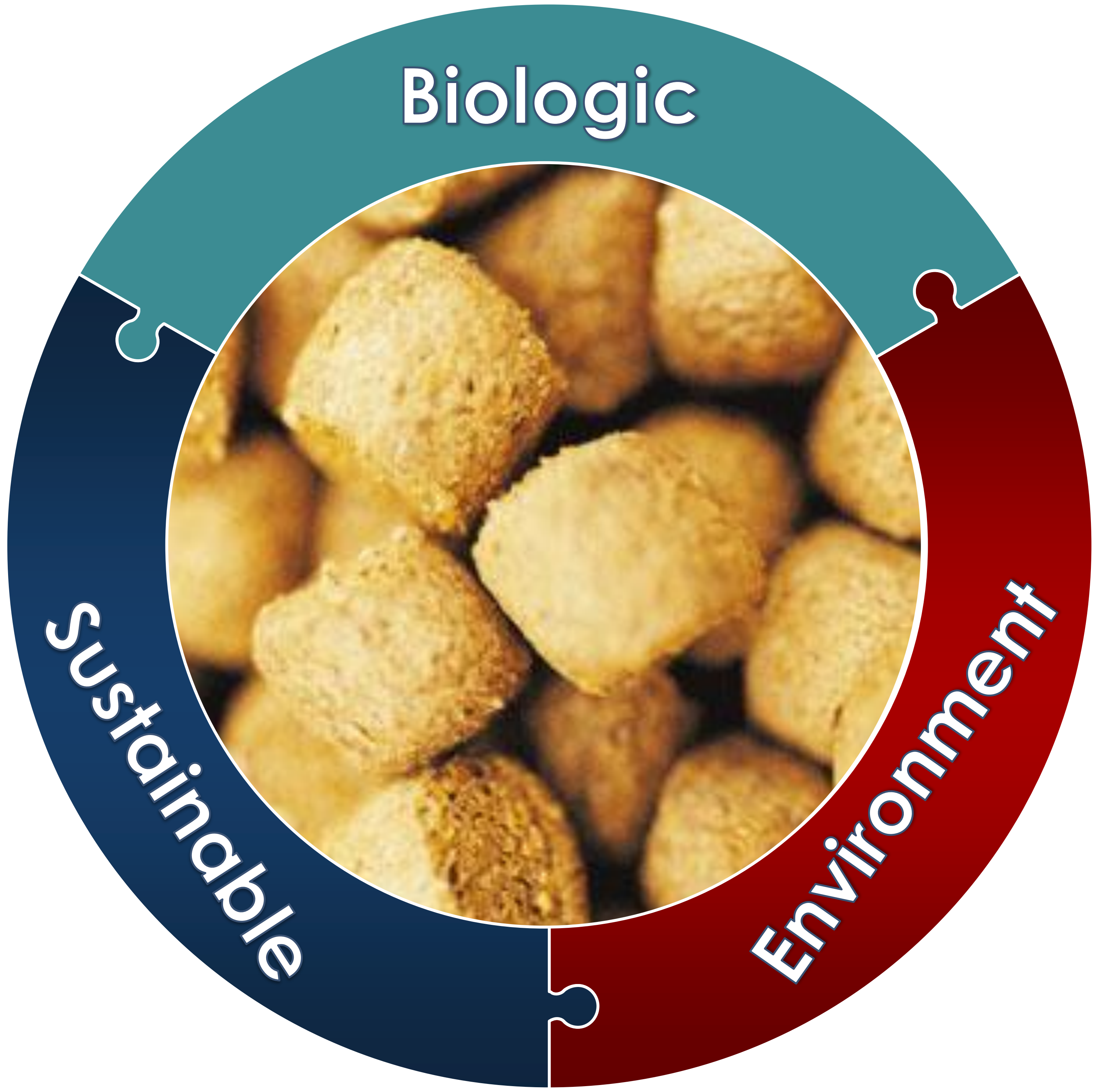
We need to ensure that it grows...SUSTAINABLY

Sustainable aquafeeds

Key element to allow a sustainable and eco-efficient growth of aquaculture

- Cover the nutritional requirements
- Promote well-being and optimal functionalities (e.g. immune status, stress/disease resistance,...)

- Low fishmeal and fish oil
- Alternative/Emergent raw materials with a lower environmental footprint (e.g. circular economy, geographical proximity, etc.)



- Guarantee that fish/shrimp continue to be a rich source of healthy nutrients to consumers

- Low pollution feeds

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