PROGETTO Ager
AGROALIMENTARE E RICERCA

CHI (RI)CERCA TROVA

UN CICLO DI **WEBINAR** PER CONOSCERE
I RISULTATI DEI PROGETTI AGER

30 Aprile 2021

Qualità e sostenibilità economica del pesce allevato

AGER 2 4F

Fine Feed For Fish *è un progetto sostenuto da* ***AGER - AGroalimentare E Ricerca****, Grant 2016-0101*

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Ordine dei Medici Veterinari della provincia di Milano

PortoConte Ricerche

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AGER 2 4F

Lo studio del microbiota intestinale, per la formulazione dei mangimi: manipolazione delle comunità microbiche intestinali del pesce in allevamento intensivo

Simona Rimoldi, Genciana Terova

simona.rimoldi@uninsubria.it

Università degli Studi dell’Insubria

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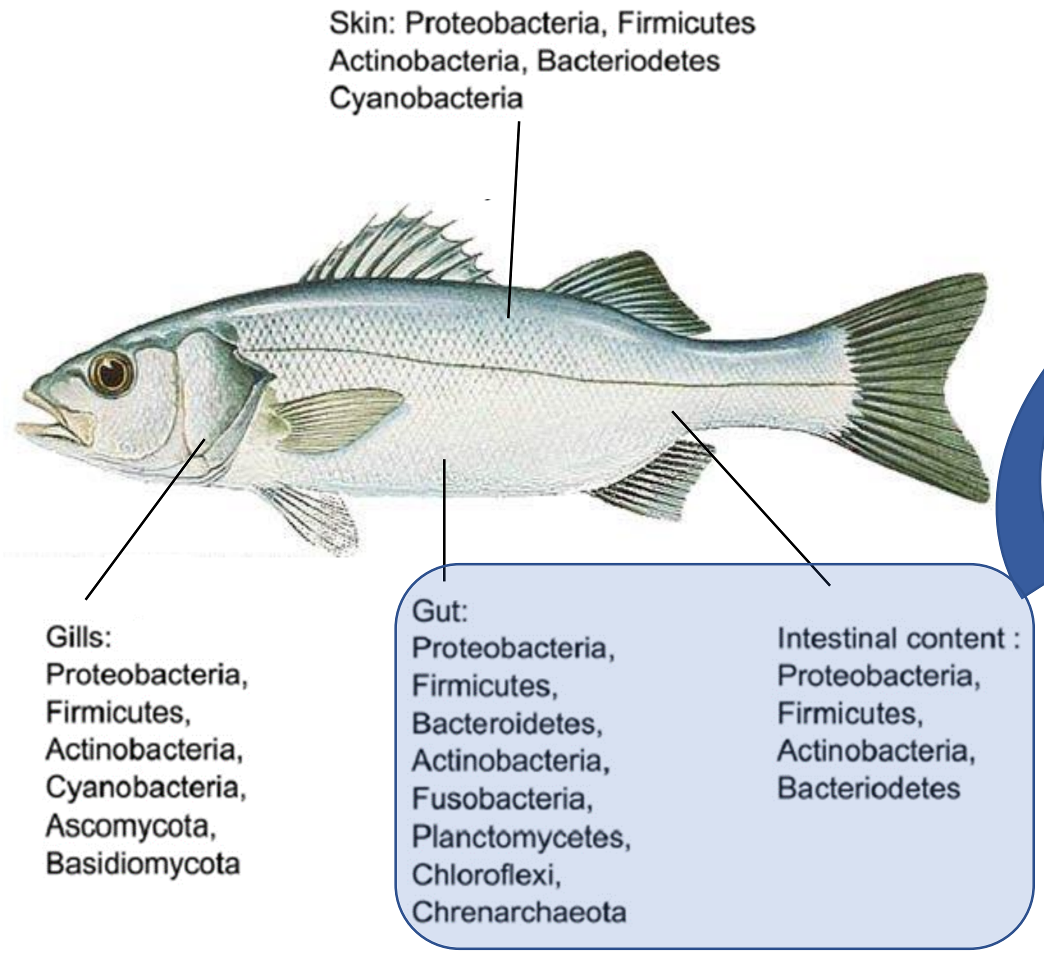
UNIVERSITAS STUDIORUM INSUBRIAE · UNIVERSITÀ DEGLI STUDI DI MILANO BICOCCA · PortoConte Ricerche · Consorzio Italbiotec

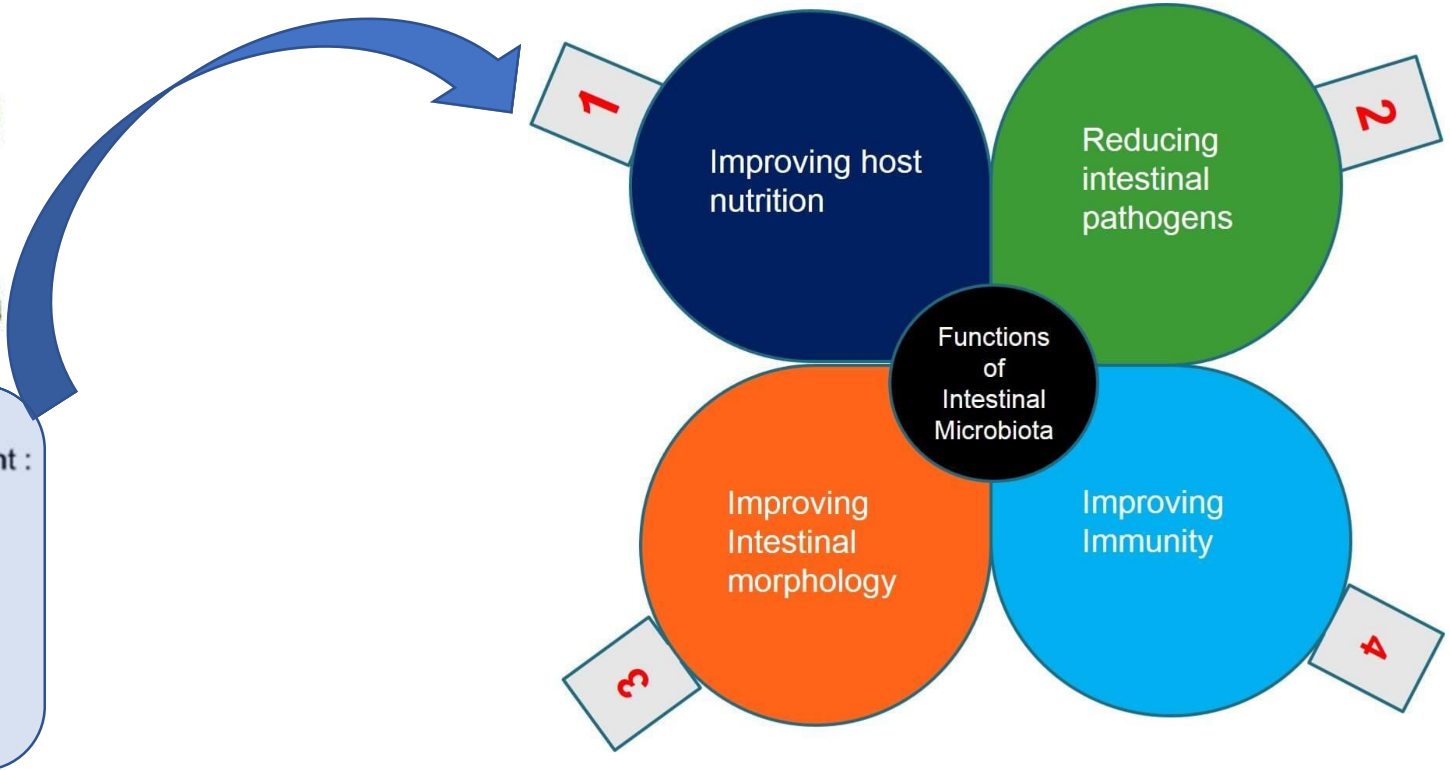
Fish microbiota overview

Fish microbiota is a complex community comprising bacteria, fungi, and viruses, inhabits the skin, gills, and gastrointestinal tract.



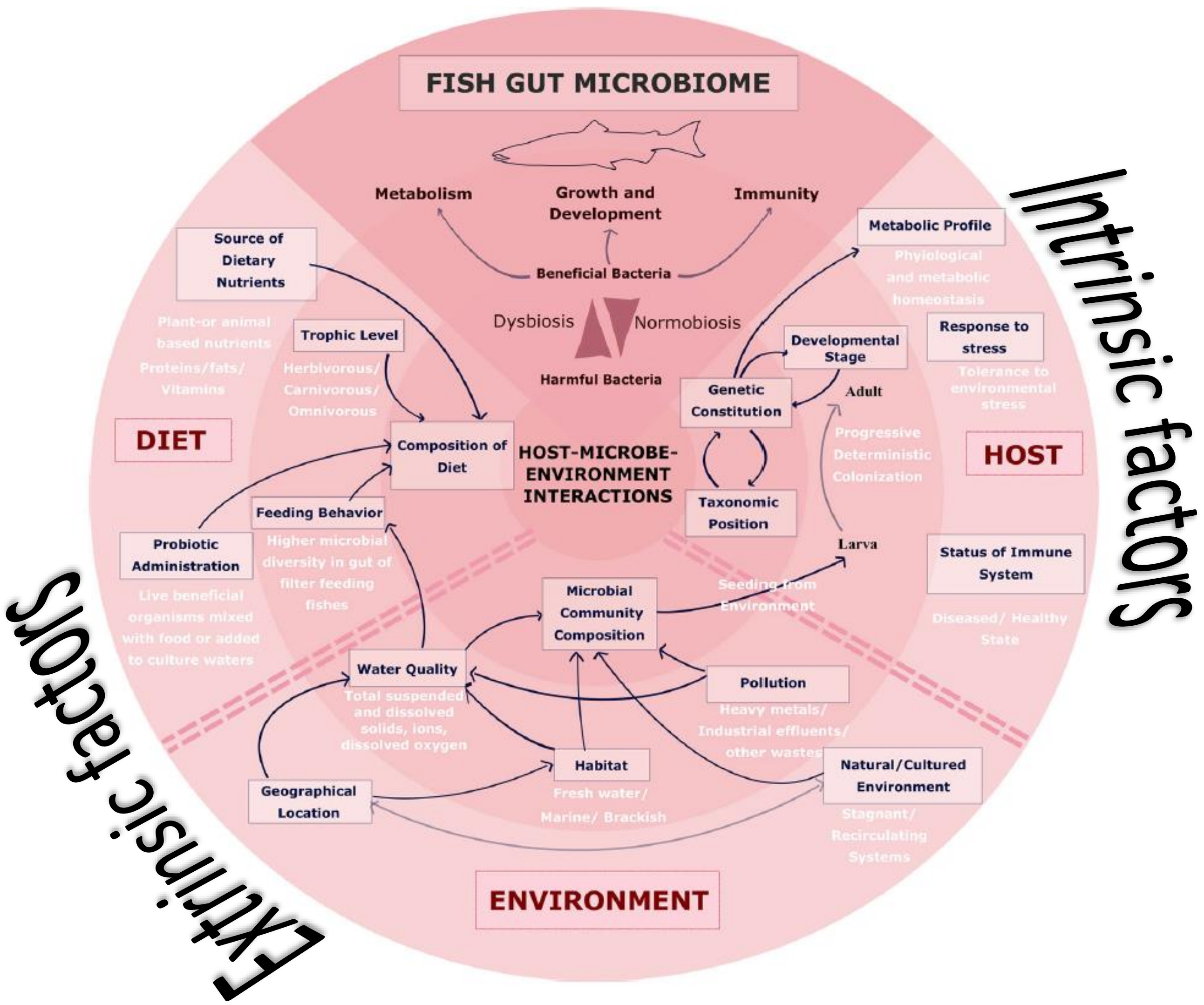
Gut microbiota balance is essential to maintain overall health in fish

(growth performances, feed efficiency, energy uptake, feed conversion rate and resistance to diseases)



Llewellyn et al. (2014). Frontiers in Microbiology Volume 5, Article 207

Factors influencing the diversity and function of the gut microbiota of fish.



The imbalance between beneficial and harmful microorganisms (**Dysbiosis**) has deleterious impacts to the gut, including impaired digestion, inflammation in the intestinal wall and lack of local intestinal immunity.

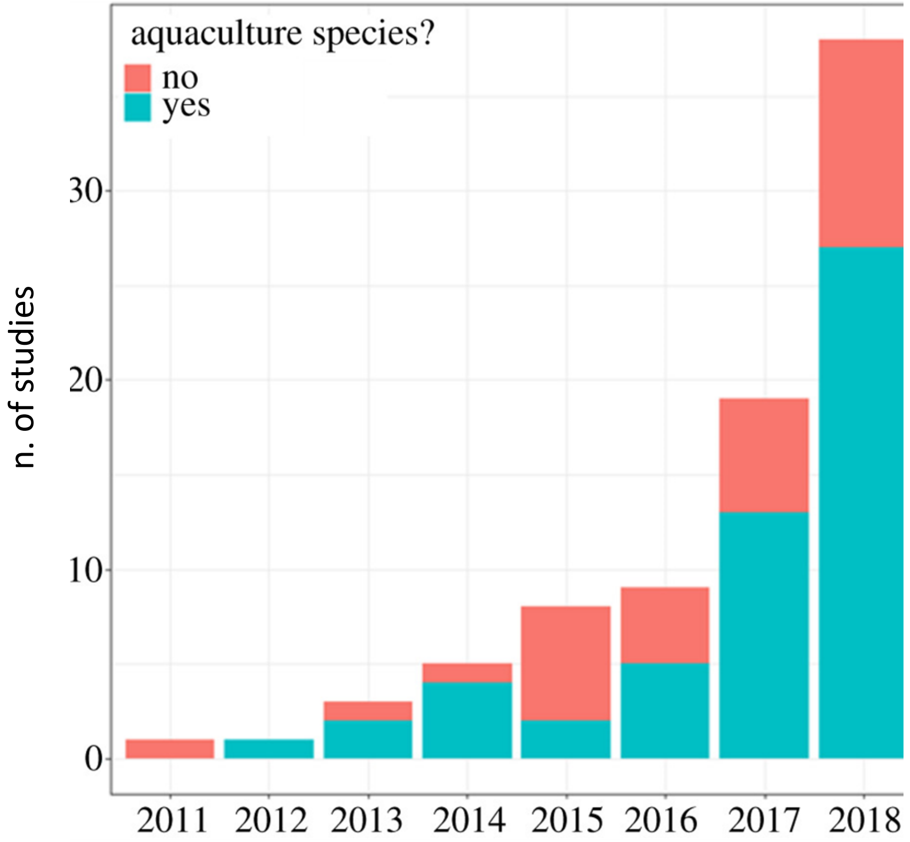
Indian J Microbiol (2018) 58(4):397–414

The aquaculture industry is interested in the **manipulation of gut microbiota to improve welfare and nutrition of cultured fish**.

DIET is a major factor driving the composition and metabolism of the gut microbiota.

Different components of the diet can shape the gut bacterial communities

- **PROTEIN AND LIPID SOURCES**
- **PROBIOTICS**
- **PREBIOTICS**
- **BIOACTIVE COMPOUNDS** (organic acids, phytogenics, etc…..)



Perry WB, et al. (2020).Proc. R. Soc. B 287: 20200184

Our research in the project

Evaluate the benefits of novel and more sustainable feed formulations on gut microbiota of cultured fish (European sea bass, gilthead sea bream, and rainbow trout)

Rev Fish Biol Fisheries (2019) 29:465–486
https://doi.org/10.1007/s11160-019-09558-y

ORIGINAL RESEARCH

Rainbow trout (*Oncorhynchus mykiss*) gut microbiota is modulated by insect meal from *Hermetia illucens* prepupae in the diet

Genciana Terova · Simona Rimoldi · Chiara Ascione · Elisabetta Gini · Chiara Ceccotti · Laura Gasco

Rimoldi et al. BMC Veterinary Research (2020) 16:118
https://doi.org/10.1186/s12917-020-02335-1
BMC Veterinary Research

RESEARCH ARTICLE Open Access

Effects of hydrolyzed fish protein and autolyzed yeast as substitutes of fishmeal in the gilthead sea bream (*Sparus aurata*) diet, on fish intestinal microbiome

S. Rimoldi[1], E. Gini[1], J. F. A. Koch[2], F. Iannini[1], F. Brambilla[3] and G. Terova[1*]

Fish Physiol Biochem
https://doi.org/10.1007/s10695-020-00918-1

Intestinal microbial communities of rainbow trout (*Oncorhynchus mykiss*) may be improved by feeding a *Hermetia illucens* meal/low-fishmeal diet

Simona Rimoldi · Micaela Antonini · Laura Gasco · Federico Moroni · Genciana Terova

PLOS ONE

RESEARCH ARTICLE

Assessment of dietary supplementation with galactomannan oligosaccharides and phytogenics on gut microbiota of European sea bass (*Dicentrarchus Labrax*) fed low fishmeal and fish oil based diet

Simona Rimoldi[1], Silvia Torrecillas[2], Daniel Montero[2], Elisabetta Gini[1], Alex Makol[3], Victoria Valdenegro V.[4], Marisol Izquierdo[2], Genciana Terova[1*]

animals MDPI

Article

The Effects of Dietary Insect Meal from *Hermetia illucens* Prepupae on Autochthonous Gut Microbiota of Rainbow Trout (*Oncorhynchus mykiss*)

Simona Rimoldi [1], Elisabetta Gini [1], Federica Iannini [1], Laura Gasco [2] and Genciana Terova [1,*]

PeerJ

Effect of a specific composition of short- and medium-chain fatty acid 1-Monoglycerides on growth performances and gut microbiota of gilthead sea bream (*Sparus aurata*)

Simona Rimoldi[1], Emi Gliozheni[1], Chiara Ascione[1], Elisabetta Gini[1] and Genciana Terova[1,2]

frontiers in Marine Science
ORIGINAL RESEARCH
published: 12 April 2021
doi: 10.3389/fmars.2021.659519

The Effects of Nisin-Producing *Lactococcus lactis* Strain Used as Probiotic on Gilthead Sea Bream (*Sparus aurata*) Growth, Gut Microbiota, and Transcriptional Response

Federico Moroni[1†], Fernando Naya-Català[2†], M. Carla Piazzon[3], Simona Rimoldi[1], Josep Calduch-Giner[2], Alberto Giardini[4], Inés Martínez[5], Fabio Brambilla[6], Jaume Pérez-Sánchez[2] and Genciana Terova[1*]

OPEN ACCESS

Edited by:
Fotini Kokou,
Wageningen University and Research,

Journal of Animal Science and Biotechnology

RESEARCH Open Access

Effects of full replacement of dietary fishmeal with insect meal from *Tenebrio molitor* on rainbow trout gut and skin microbiota

Genciana Terova[1*], Elisabetta Gini[1], Laura Gasco[2], Federico Moroni[1], Micaela Antonini[1] and Simona Rimoldi[1]

PROTEIN

INSECT
POULTRY
VEGETABLE
SCP

LIPID

VEGETABLE
OILS

ADDITIVES

SCFA
ESSENTIAL OILS

PREBIOTICS

GMOS
CHITIN

PROBIOTICS

LACTIC ACID
BACTERIA

INSECT MEAL

Black Soldier Fly
(*Hermetia illucens*)

Rainbow trout (*Oncorhynchus mykiss*)

Gilthead sea bream (*Sparus aurata*)

Yellow Mealworm
(*Tenebrio molitor*)

Rainbow trout (*Oncorhynchus mykiss*)

INSECT MEAL

Hermetia illucens larva meal was provided by MUTATEC (Caumont-sur-Durance, France)

H. illucens Meal	DIET			
	Hi 0	Hi 10	Hi 20	Hi 30
Ingredients (% as it)				
Fishmeal [a]	60	54	48	42
Hermetia illucens meal	0	10	20	30
Fish oil	7	7	7	7
Soybean oil	5	4	3	2
Wheat bran	10	7	4	1
Wheat meal	4	4	4	4
Starch gelatinized D500	11	11	11	11
Vitamin premix [b]	1.5	1.5	1.5	1.5
Mineral premix [c]	1.5	1.5	1.5	1.5

Ingredients (g Kg^{-1})	Ctrl	Hi15
Fishmeal[a]	200.0	100.0
Hermetia illucens larva meal[b]	0.0	150.0
Wheat gluten	130.0	130.0
Soybean meal	200.0	200.0
Porcine haemoglobin	92.0	82.0
Wheat starch	233.9	193.9
Fish oil	69.8	69.8
Soybean oil	69.8	69.8
Minerals[c]	2.5	2.5
Vitamins[d]	2.0	2.0

FISH	INSECT	DIET	RESULTS

Rainbow trout

Hermetia illucens

0, 10, 20, 30% Inclusion

15% inclusion
(50% FM replacement)

- SGR and WG were comparable among the treatments. FCR was < 1 in all groups
- Hi meal inclusion (≥ 15%) in the diet positively modulated both allochthonous and autochthonous gut microbiota, increasing species richness and diversity
- Several bacteria taxa were discriminatory between diets

Allochthonous microbiota:

- *the amounts of **Actinobacteria** and **Firmicutes** were positively modulated by Hi. Proteobacteria decreased with Hi dietary inclusion*
- *fish fed with Hi meal had **higher percentage of Actinomyces, LAB** (Facklamia, Enterococcus, Lactobacillus, and Pediococcus), **and Bacillus**. **Many beneficial bacterial species were correlated to Hi, some of them were butyrate producers** (Corynebacterium variabile, Lactobacillus paraplantarum, Lactobacillus zeae, Weisella cibaria, Clostridium butyricum, and Clostridium fimentarium)*

Autochthonous microbiota:

- *Tenericutes and Proteobacteria were significantly influenced by insect meal inclusion (20% and 30%) in the diet*
- *trout fed Hi 20 and Hi 30 diets were enriched in Mycoplasma genus*
- *Hi meal caused a significant reduction of Proteobacteria both in the gut digesta and mucosa. **All genera adversely affected by insect-meal diets (Shewanella, Aeromonas, Citrobacter) were Gram-negative bacteria that include potential pathogen species.***

- Dietary Hi meal upregulated those pathways involved in starch and sugar metabolism

Terova et al. (2019). Rev Fish Biol Fisheries 29:465–486

Rimoldi et al. (2019).Animals 9, 143

Rimoldi et al. (2021) Fish Physiol Biochem 47(2):365-380

INSECT MEAL

	DIET	
	Hi 0	Hi 10
Fishmeal	15.0	5.0
Processed animal protein from poultry	10.7	10.7
SCP	3.9	3.9
Hermetia illucens meal *	0	10.0
Vegetable meals	34	34
Maize gluten meal,	18.9	18.9
Fish oil	3.9	5.0
Rapeseed oil	9.1	7.8
Vitamin & mineral premix	1.15	1.15

*Insect meal was provided by HIPROMINE (Poland)

SPAROS LDA
Ÿnsect (Evry, France)

Item	TM 0	TM 100
Ingredients, %		
Fishmeal 65 (Peruvian)	20	-
Tenebrio molitor larvae meal	-	20
Soy protein concentrate	18	18
Wheat gluten	7.75	7.06
Corn gluten	8	8
Soybean meal (48%)	7	7
Wheat meal	14.23	13.8
Sardine oil	4.3	4.1
Soybean oil	8.6	8.2
Rapeseed oil	8.6	8.2
Soy lecithin	0.5	0.5
Vitamin[a] and mineral premix[b]	1	1

FISH	INSECT	DIET	RESULTS
 **Gilthead sea bream**	*Hermetia illucens* 	0, 10% inclusion	• Hi inclusion improved the growth performances and FCR • No differences in alpha- and beta- diversity • Gut autochthonous microbial communities were not affected by 10% inclusion of Hi meal
 **Rainbow trout**	 *Tenebrio molitor*	0, 20% Inclusion (FM substitution of 0, 100%)	• The growth parameters were comparable between two feeding groups. • Skin microbiota had higher species richness and biodiversity than intestinal ones. • No evident sign of dysbiosis, but only slight microbiota changes: **Gut mucosa**: reduction of Proteobacteria, Neisseriaceae and Ruminococcaceae families and *Citrobacter* genus **Skin mucosa:** reduction of Neisseriaceae (g_*Deefgea*) and increase of Clostridiaceae

Terova et al. Journal of Animal Science and Biotechnology (2021) 12:30

POULTRY MEAL & VEGETABLE MEAL

❖ Since June 2013, the European Union has re-authorized the use of non-ruminant animal by-product meal in aquafeeds.

FISH	DIET	RESULTS
 **Rainbow trout**	Seven diets: 25-69% Inclusion of alternative animal proteins CTRL: diet E	• Diet D gave the best SGR and FCR (0.96), while A and B were the worst. • No overall effect on bacterial richness and diversity in response to FM substitution. • Beta diversity analysis revealed a relationship between diet type and gut microbiota. • The inclusion of at least 25% of plant proteins in the diet favored the presence Firmicutes • Highest percentage of PBM of commercial F and G diets had an adverse effect on the gut microbial community by reducing LAB. • *Corynebacterium* and *Fusobacterium* genera were enriched in the intestine of D group with the best performances

Table 2. Formul

Fish meal
Dried swine hem
Dried swine plas
Poultry by-produ
Fish oil
Rapeseed meal
Soybean meal
Guar germ meal
Wheat flour
Corn gluten
Vital wheat glute
Peas
Soy protein conc
Soybean oil
DL- methionine
Lisin
Taurin
Antioxidants pre
Vitamin and mir
Stay C 35%

	A	B	C	D	E	F	G
FP/TP	20.6	20.9	10.6	11.2	37.3	20.0	11.0
TAP/TP	68.0	68.0	64.0	56.0	62.0	75.0	80.0
AP (TAP-FP)	47.4	47.4	53.4	44.8	24.7	55.0	69.0
VP/TP	32.0	32.0	36.0	44.0	38.0	25.0	20.0

➢ ***bacteria belonging to Fusobacteria phylum can produce butyrate and synthesize vitamins***

Rimoldi S, (2018). PLoS ONE 13(3): e0193652.

VEGETABLE MEAL

FISH	DIET	RESULTS
 **Rainbow trout** **ITA**: non-selected **USA**: selected for growth on a all plant-based diet (University of Idaho)	• **A**: COMPLETE FEED FOR TROUT (commercial diet, Naturalleva) containing ANIMAL PROTEINS (poultry, fish, and shrimp meal) • **B**: ALL PLANT PROTEIN DIET	• USA strain fed with diet A showed higher final weight and better FCR than ITA strain • Neither diet nor genotype affected gut bacterial α-diversity • Gut microbial communities were mainly affected by genotype in term of absence/presence of specific genera: ***Cetobacterium* 10 times more abundant in USA strain** ***This genus is capable of producing vitamin B-12 and antimicrobial metabolites***

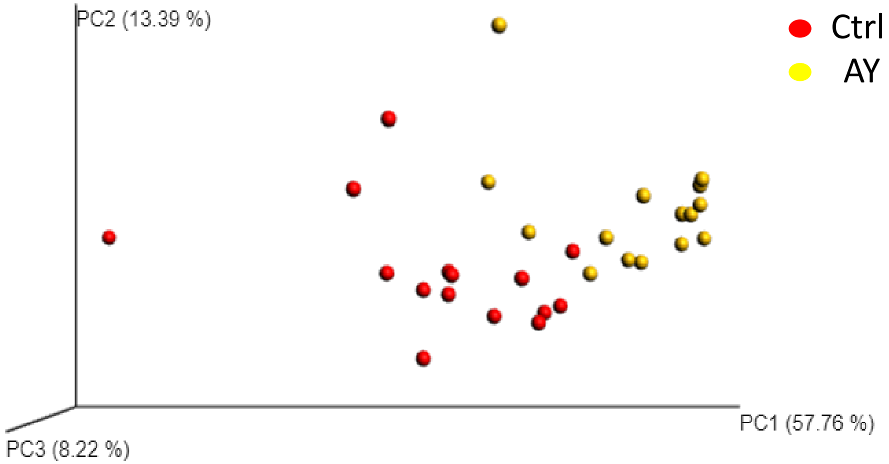
POULTRY, VEGETABLE & INSECT MEAL

Large scale trial

FISH	DIET	RESULTS
 **Rainbow trout** Fattoria del Pesce (Cerano, NO)	**CTRL:** 27% FM **PM:** 12% FM, 15% PM **Hi:** 12% FM, 10% Hi *Hermetia illucens* meal was provided by MUTATEC (Caumont-sur-Durance, France)	• Trout fed insect diet showed higher final weight and SGR than other two groups. • No changes in alpha- and beta-diversity • Diet did not affect gut microbial composition
 **Gilthead sea bream** Croatia	A: 20% PM + 10% FM B: 20% PM + 10% VM C: commercial diet	• Commercial diet gave the best SGR, while economic FCR was improved by diet A • No changes in alpha- and beta-diversity. • Diet did not affect gut microbial composition.

SCP – yeast protein

FISH	DIET	RESULTS
 Gilthead sea bream	• AY: 17.8% FM and 4.6% autolysed yeast • Ctrl: 22% FM	• Dietary inclusion of 5% AY did not affect intestinal bacterial species richness • Changes in beta-diversity, were found both in type and abundance of specific taxa.



- Indigestible carbohydrate degrading and SCFA producing bacteria, were positively affected by AY
  - ❖ only sea bream fed with AY harbored members of genera *Prevotella* and *Megasphera*
- AY dietary inclusion promoted the growth of *Bacillus* genus.

Rimoldi et al. BMC Veterinary Research (2020) 16:118

	DIETS	
	Ctrl	AY
Ingredients (% as it is):		
Fishmeal	22.25	17.80
Corn gluten meal	17.80	17.71
Guar germ meal	15.13	17.68
Soybean meal	10.70	10.68
Soy Protein Concentrate	9.38	10.04
Wheat middling	7.45	7.12
Fish oil 92	6.93	6.93
Fish protein hydrolysed		
HiCell® – autolysed yeast		4.60
Pea meal	4.45	1.53
Cameline oil	2.42	2.42
Mineral/Vitamin supplement	2.00	2.00
Rapeseed oil	1.49	1.49

**HiCell (Biorigin) is an autolysed dry yeast obtained from the fermentation of a strain of Saccharomyces cerevisiae (OGM free)*

CAMELINA OIL

Feed ingredients (%)	Diets 1 (ctrl)	2	3	4
Fish meal	19.4	19.4	19.4	19.4
Soybean meal	10.1	10.1	10.1	10.1
Guar germ meal	12.7	12.7	12.7	12.7
Wheat	14.5	14.5	14.5	14.5
Corn gluten	18.6	18.6	18.6	18.6
Soy protein concentrate	3.8	3.8	3.8	3.8
Fish oil	15.3	12.4	9.3	6.0
Camelina oil	0.0	2.9	6.0	9.3
Vitamins and minerals[a,b]	5.6	5.6	5.6	5.6

FISH	DIET	RESULTS
Gilthead sea bream Parque Científico-Tecnológico Marino (PCTM) of the University of Las Palmas de Gran Canaria	Diet 1, ctrl diet (100% FO) Diet 2 (20% CO, 80% FO) Diet 3 (40% CO, 60% FO) Diet 4 (60% CO, 40% FO)	• FCR and SGR were not affected by diet, final weight was significantly lower for fish fed the 60% CO diet. • α and β-diversity of gut bacteria in both feces and mucosa were not affected by diet • In gut mucosa *Corynebacterium* and Rhodospirillales (*Acetobacter*, *Enhydrobacter*), were associated with the 60% CO diet ***higher abundance of Corynebacterium in the mucosa of fish fed 60% CO diet increased the KEGG pathway of fatty acid synthesis*** • In feces, genera of *Peptostreptococcus, Vagococcus* and *Massilia* increased in fish fed diets with 60% replacement with CO • Lowest abundance of LAB, specifically *Lactobacillus*, in the feces of fish fed the 60% CO • **This study demonstrated that up to 40% of FO can be replaced with CO without negative effects on growth performance, and gut microbiota of gilthead sea bream**

Huyben et al., (2020). PeerJ 8:e10430

PREBIOTICS - CHITIN

FISH	RESULTS

Rainbow trout

- No differences were found between groups for SGR and FCR (1.09 – 1.13)
- Dietary chitin supplementation (1.6%) increased intestinal bacterial species richness and diversity
- Gut microbial community profiles qualitatively differed:

  ✓ Firmicutes and Actinobacteria were enriched by chitin in the diet
  ✓ At genus level relative amount of *Corynebacterium* was higher in chitin feeding group – inflammation-inhibiting bacteria genus involved in fatty-acid synthesis

- Specific bacteria genera were found only in trout receiving CHITIN diet:
  f__ Brevibacteriaceae; g__Brevibacterium
  f__ Staphylococcaceae; g__Jeotgalicoccus
  f__ Bacillaceae; __g_Bacillus
  f__ Bacillaceae; __g_Oceanobacillus
  f__ Actinomycetaceae; g__Actinomyces
  f__ Aerococcaceae; g__Atopostipes

- Dietary CHITIN enhanced SCFA production

Fecal content of SCFA (mM)

Fecal pool	Acetic acid	Propionic acid	Butyric acid
Chitin	16.26	1.22	2.06
Ctrl	6.38	Trace	0.51

GMOS & PHYTOGENICS

Ingredients	Diets (%) CONTROL	GMOS	PHYTO	GMOSPHYTO
Fish meal[1]	10	10	10	10
Soya protein concentrate	18.9	18.9	18.9	18.9
Soya meal	12.0	12.0	12.0	12.0
Corn gluten meal	25.0	25.0	25.0	25.0
Wheat	8.7	8.2	8.7	8.2
Wheat gluten	2.0	2.0	2.0	2.0
Guar meal	8.0	8.0	8.0	8.0
Rapeseed extracted	3.0	3.0	3.0	3.0
Fish oil[2]	6.7	6.7	6.7	6.7
Rapeseed oil[3]	5.4	5.4	5.4	5.4
Vitamin and mineral premix[4]	3.7	3.7	3.7	3.7
Antioxidant [5]	0.06	0.06	0.06	0.06
Galactomannan oligosaccharides[6]	0	0.5	0	0.5
Phytogenic[7]	0	0	0.02	0.02

FISH	DIET	RESULTS
 **European sea bass**	CONTROL: plant-based formulation with 10% FM and 6% FO GMOS: 0.5% PHYTO: 0.02% of a mixture of garlic and labiatae-plants oils GMOSPHYTO: a combination of two 0.52%	• The experimental diets did not induce differences in fish growth or diet utilization. • The dietary inclusion of GMOS (0.5%) and PHYTO (0.02%) induced changes in bacterial community composition of European sea bass. • Allochthonous intestinal microbiota of sea bass fed GMOS, but not GMOSPHYTO diet, showed reduced number of Vibrionales. • the Clostridiales order was more abundant in intestinal content of fish fed PHYTO diet, as well as, in mucosa-associated microbiota of fish receiving GMOSPHYTO diet. ***the Clostridiales order includes several butyrate producers. Ruminococcus genus, found only in the intestinal lumen of fish fed PHYTO diet, plays an important role in the degradation of indigestible carbohydrate, thus contributing to the more efficient energy utilization of feed.*** • *Escherichia* genus was practically undetectable in allochthonous microbiota of fish fed PHYTO and GMOSPHYTO diets. • Only GMOSPHYTO diet increased the abundance of Bacteroidales, Lactobacillales, and Clostridiales resident bacterial orders

Rimoldi et al. (2020). PLoS ONE 15(4): e0231494.

SCFA

*__*SILOhealth 108Z (SILO SpA)__ Is composed of a specific combination of 1-monoglycerides of short- and medium-chain fatty acids (from C3 to C12), in which 1-monobutyrin represents 65% of total blend*

FISH	DIET	RESULTS
 **Gilthead sea bream** Rimoldi et al. (2018). PeerJ 6:e5355	**CTRL:** commercial plant-based diet **Sh108:** CTRL + supplemented with 0.5% of SILOhealth 108Z* (SILO SpA)	• SGR did not reveal any significant differences between control and SILOhealth 108Z-supplemented dietary group. Economic FCR was improved by diet Sh108. • The relative abundance of Firmicutes was significantly higher in fish fed with diet Sh108 than in controls • the number of Proteobacteria, in particular Gammaproteobacteria, was negatively affected by SILOhealth 108Z • The number of bacteria assigned to the *Lactobacillus* genus was significantly higher in Sh108 samples *L. agilis, was only found in fish receiving Sh108 diet* ***SILOhealth 108Z positively modulated the fish intestinal microbiota by increasing the relative abundance of beneficial lactic acid bacteria***
 **Rainbow trout**	**CTRL+**: fish-based diet **CTRL-:** plant-based diet CTRL- supplemented with: **0.5%, 1%,** or **3%** of **SILOhealth 108Z*** (SILO SpA)	• Alpha and beta diversity analysis did not show any difference • Fish fed diets supplemented with monobutyrin were enriched in Enterobacteriales respect to controls, and showed a decrease in bacteria assigned to *Vibrio* genus • LAB abundance was not improved by monobutyrin inclusion

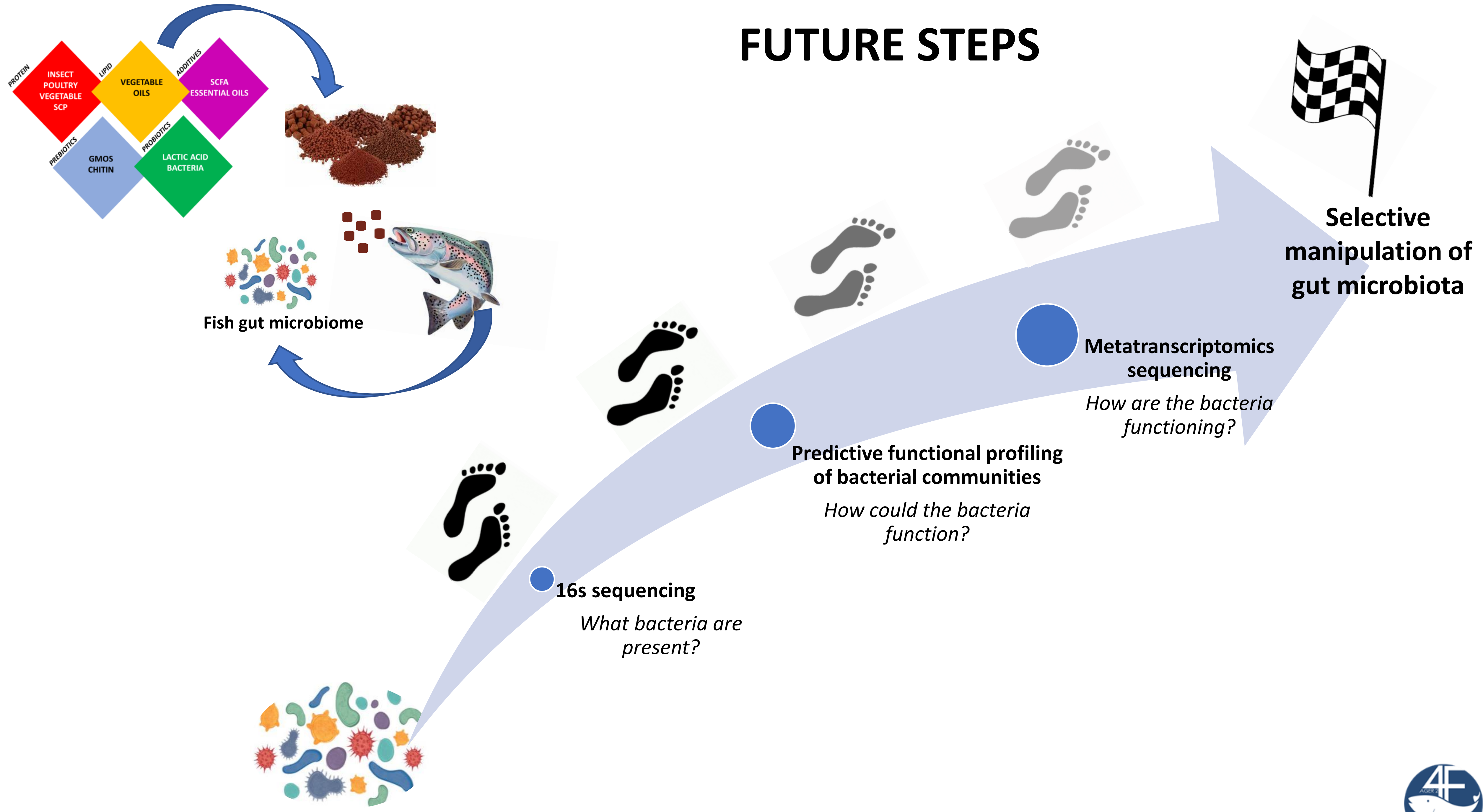
PROBIOTICS – *Lactococcus lactis* SL242

FISH	DIET	RESULTS
 **Gilthead sea bream** Experimental facilities of IATS-CSIC (Castellón, Spain)	Diet A: **Ctrl** Diet B: supplemented with 2.5g / 100kg of *L. lactis subsp. lactis* (2 x 10^9 CFU/kg, **low dose**) Diet C: supplemented with 6.2g / 100Kg of *L. lactis subsp. lactis* (5 x 10^9 CFU/Kg, **high dose**)	• There were no significant differences between groups for the FCR and SGR, but final weight of fish fed diet C was higher than control • **Lack of colonization of the probiotic in the host's intestinal mucosa**. • **Probiotic did modulate the fish gut microbiota:** – bacterial species richness and diversity of group C was lower than control – fish fed diet B and C were enriched with Actinomycetales as compared to control. – fish fed diets B and C showed a higher Firmicutes/Bacteroidetes ratio than control fish and this could be correlated to better growth performances. – group C was characterized by a Proteobacteria/Firmicutes ratio five times higher than in the other groups and higher amount of Spirochetes phylum ❖ *changes in Proteobacteria/Firmicutes ratio were due to antibiotic nisin A produced by L. lactis SL242* – pathways related to protein digestion and absorption were over-represented in fish fed with probiotic diets

Moroni et al. (2021). Front. Mar. Sci. 8:659519

Ingredients	Diet A
Fishmeal	10.1
Corn gluten	24.3
Guar germ meal	10.0
Soybean meal	13.1
Soya protein concentrate	13.6
Wheat	10.8
Fish oil	7.5
Rapeseed oil	3.5
Camelina oil	3.5
Lactic bacteria	0.0
Lysine	0.9
DL-methionine	0.4
Monoammonium phosphate	1.2
Taurine	0.4
Vitamins[a] and Minerals[b]	0.7

FUTURE STEPS



www.dbsm.uninsubria.it/acquacoltura/

Prof. Genciana Terova
Prof. Marco Saroglia

Dr. Chiara Ceccotti
Dr. Federico Moroni
Dr. Federica Iannini
Dr. Micaela Antonini
Dr. Simona Rimoldi

thanks