



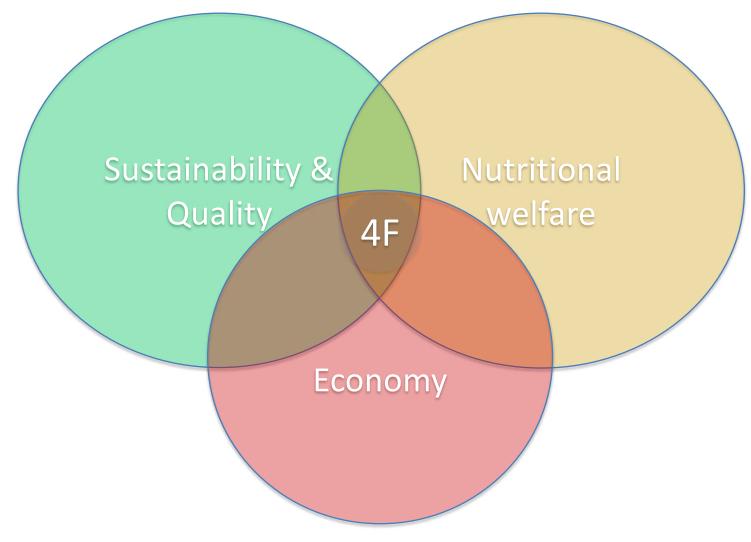


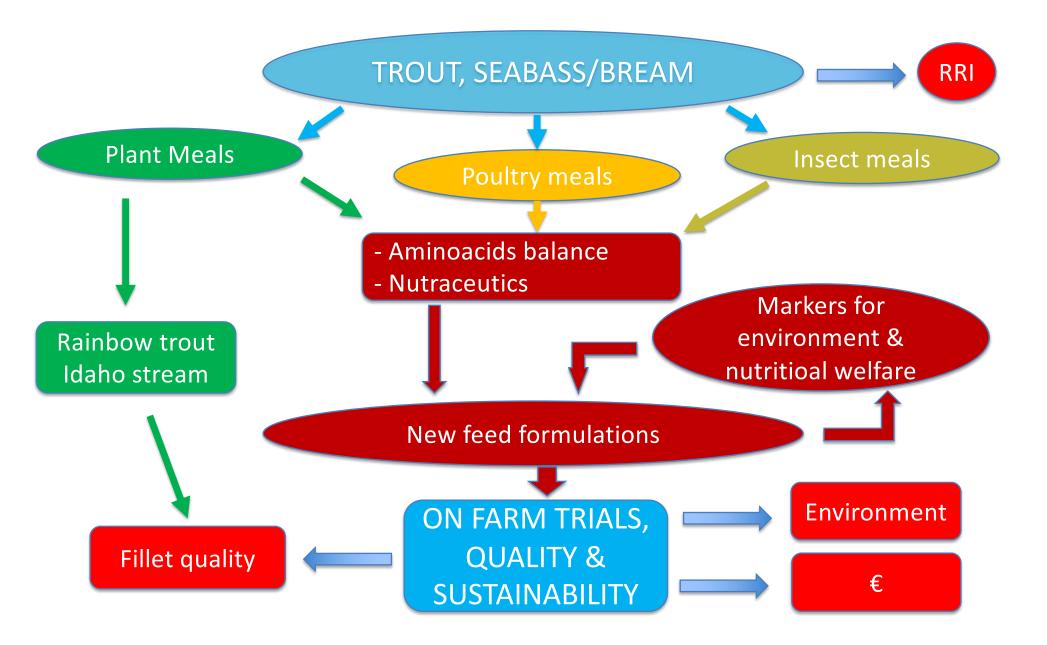
AGER 4F: Balancement of the carnivorous fish diet, for the minimization of FM inclusion

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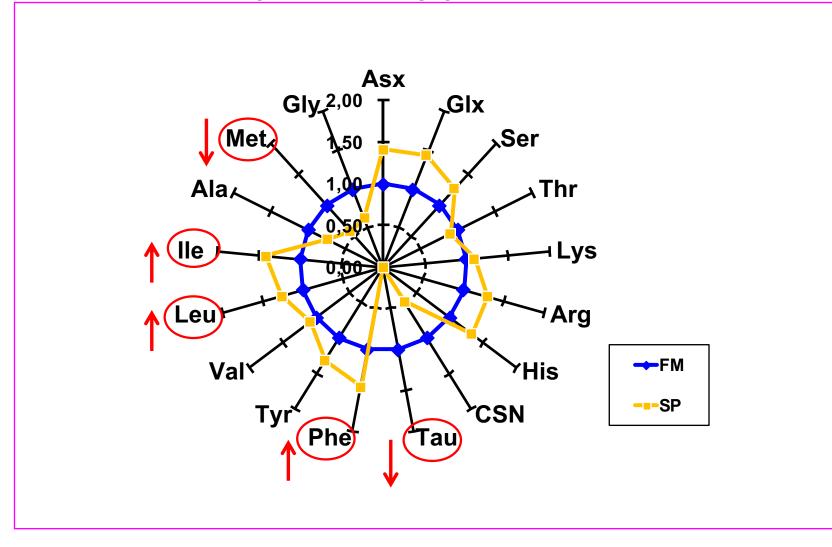
Fish meal substitution



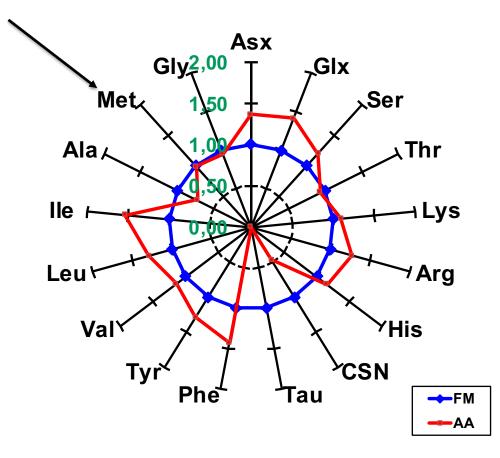


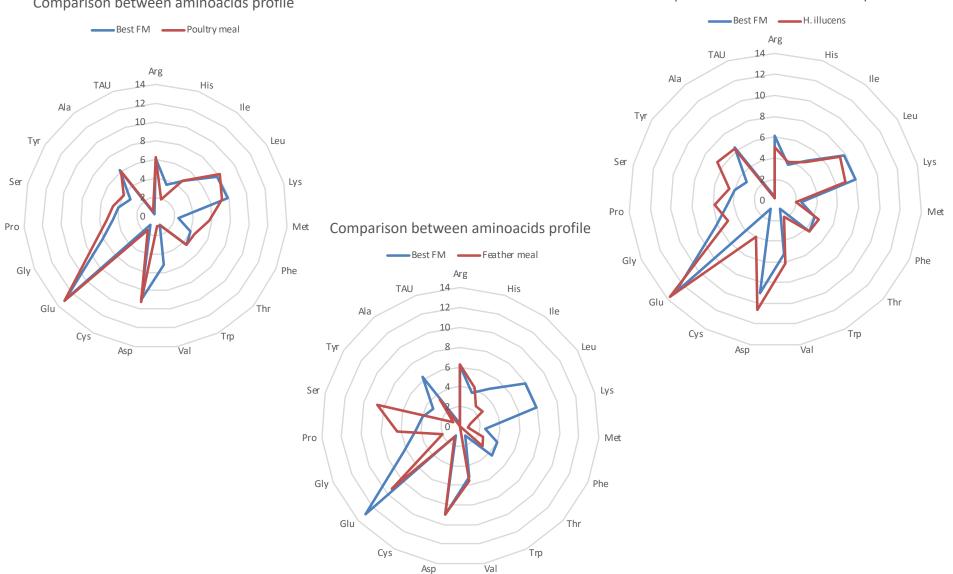
AMINOACIDS BALANCE: THE EXAMPLES OF METHIONINE AND TAURINE

Amino acid profile soy protein and fishmeal



Supplement with Methionine



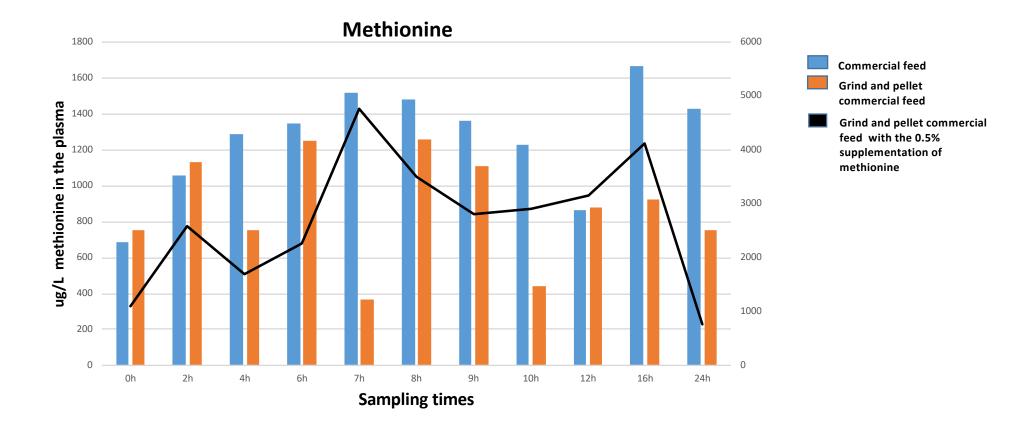


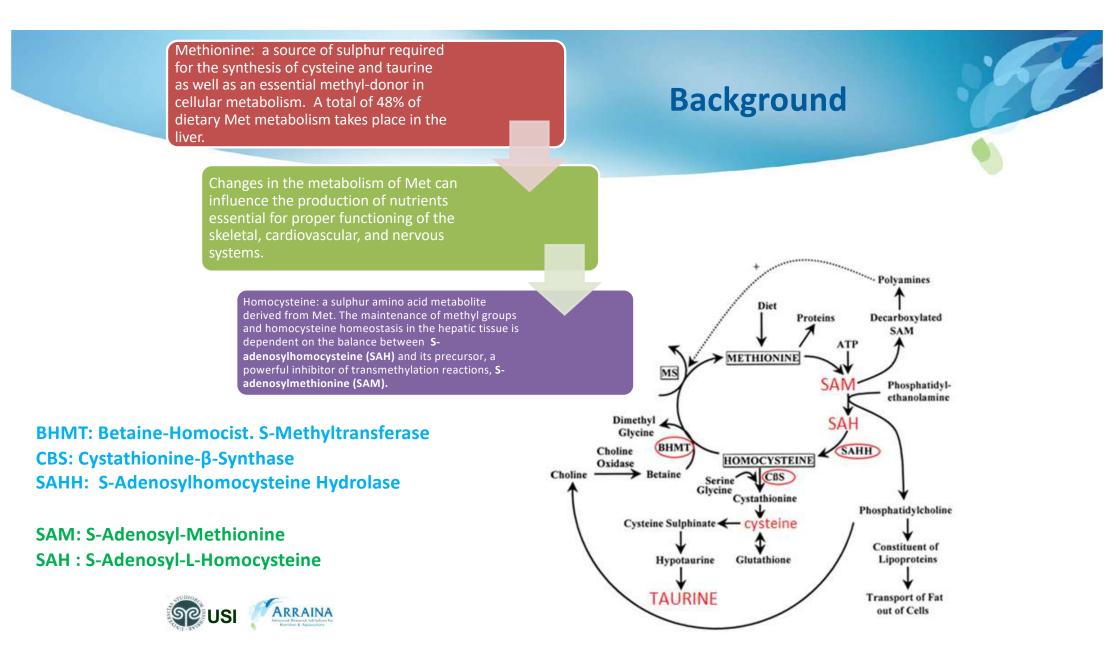
Comparison between aminoacids profile

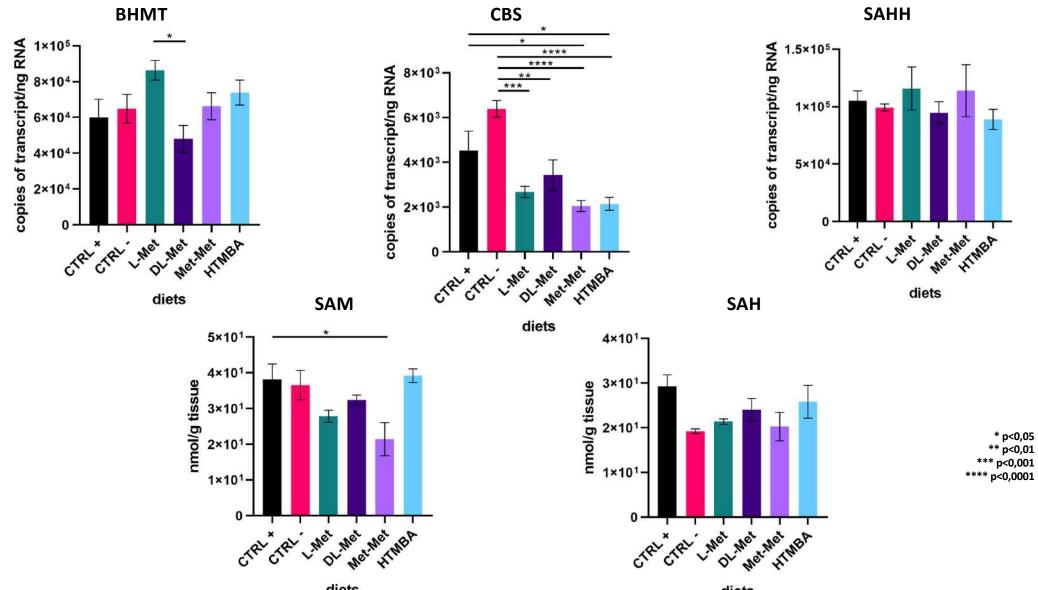
Comparison between aminoacids profile

Trial with 3 kind of feeds:

commercial feed, grind and pellet commercial feed, grind and pellet commercial feed with the 0.5% supplementation of methionine.







diets

diets

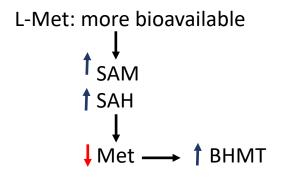
	CTRL +	CTRL -	L-Met	DL-Met	Met-Met	НТМВА
FCR BIO	1.33 ± 0.04	1.42 ± 0.06	1.35 ± 0.01	1.32 ± 0.01	1.32 ± 0.03	1.35 ± 0.05
FCR ECO	1.34 ± 0.04	1.43 ± 0.06	1.36 ± 0.01	1.33 ± 0.01	1.33 ± 0.03	1.36 ± 0.04
SGR	0.83 ± 0.02	0.79 ± 0.02	0.83 ± 0.01	0.82 ± 0.01	0.82 ± 0.01	0.81 ± 0.04
Pm iniz. (g)	46.38±0.69	46.25 ± 0.47	46.26 ± 0.47	46.74 ± 0.41	47.67 ± 0.68	46.77 ± 1.21
Pm fin. (g)	103.32 ± 1.19	99.38 ± 2.09	103.21±0.34	103.62 ± 1.54	105.69 ± 0.57	102.26 ± 1.22
Accr. (%)	122.82 ± 5.05	114.88±4.48	123.11 ± 1.51	121.69 ± 1.76	121.76 ± 2.22	118.77 ± 8.02
Mortalità (%)	0.62 ± 0.54	0.94 ± 0.01	0.62 ± 0.54	0.94 ± 0.94	0.61 ± 0.52	0.92 ± 0.92
Feed Intake	75.83 ± 1.52	67.23 ± 13.26	68.88 ± 13.88	67.17±14.60	68.35 ± 12.90	67.03 ± 14.21

FCR BIO:

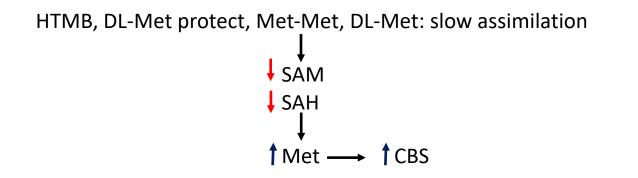
CTRL- vs HTMBA p<0.05

FCR ECO: CTRL- vs DL-Met p<0.05 CTRL- vs HTMBA p<0.05

Hypothesis:



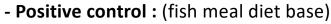
L-met can be directly used to synthesize SAM or can be degraded through pathways such as transamination. SAM can undergo the transmethylation pathway to synthetize homocysteine or the transsulfuration pathway, with the products cysteine, glutathione, taurine.



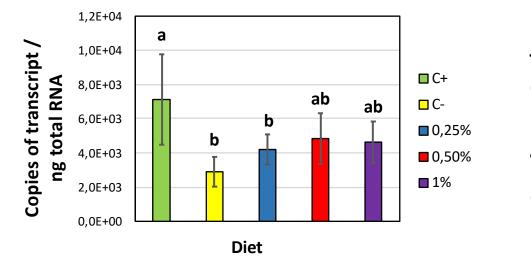
It is a temporal issue

Taurine need in the diet for Rainbow trout

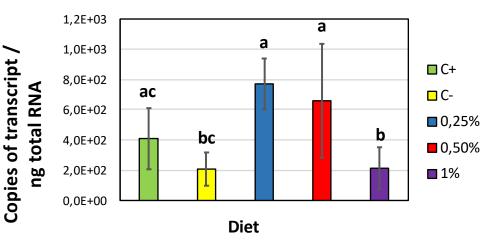
TauT expression in kidney



- Negative control: (vegetable meal diet poor in Tau)
- **0,25%** = (negative control + 0,25% Tau)
- 0,50% = (negative control + 0,50% Tau)
- **1,0%** = (negative control + 1,0% Tau)



TauT expression in liver



Taurine



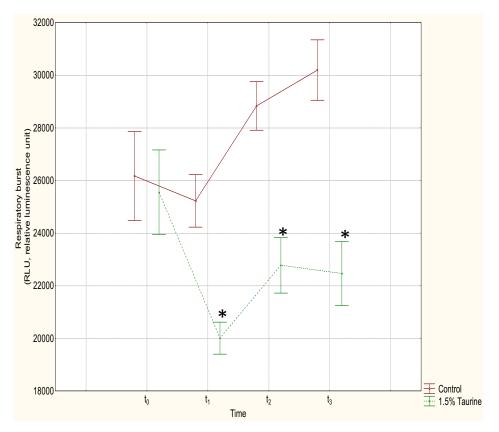
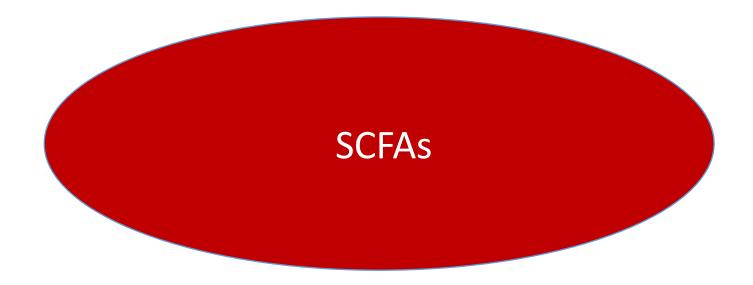
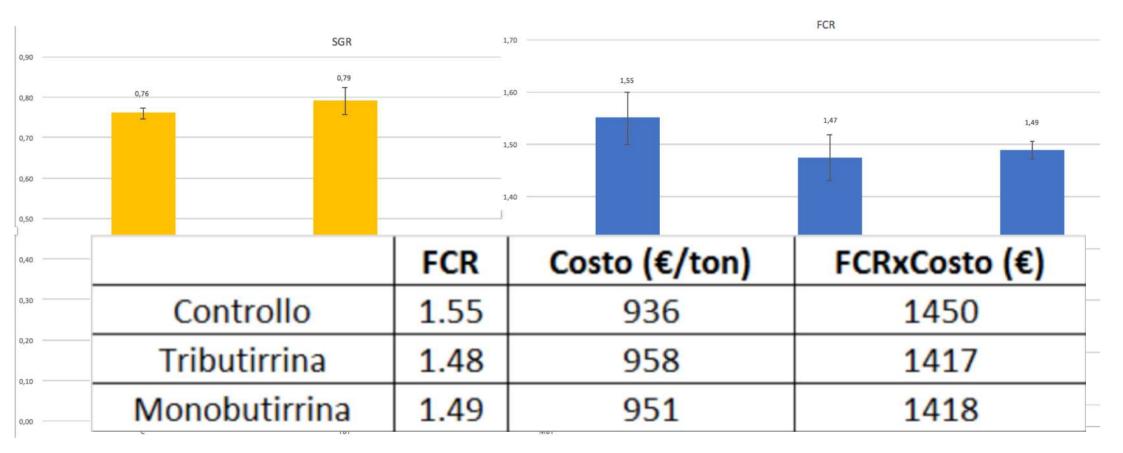


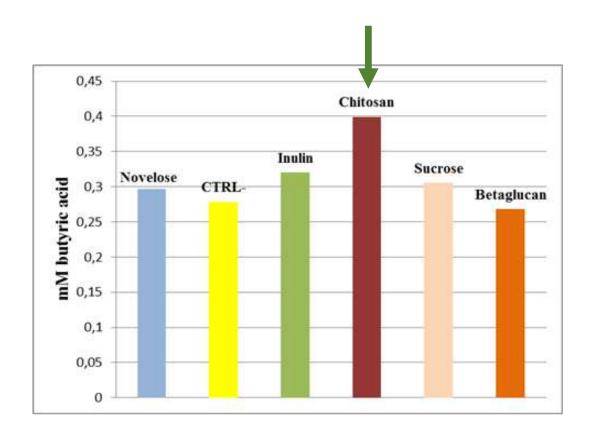
Table 3. The RBA values after PMA stimulation in Ctrl and 1.5%Tau group. (*) means significant differences between groups for
the same t (p<0.05).</td>



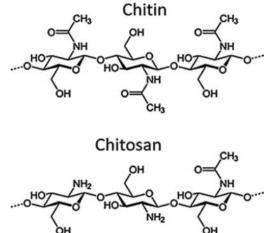
Effects of Butyrate on SGR and FCR



Preliminary *in vitro* test for butyric acid quantification



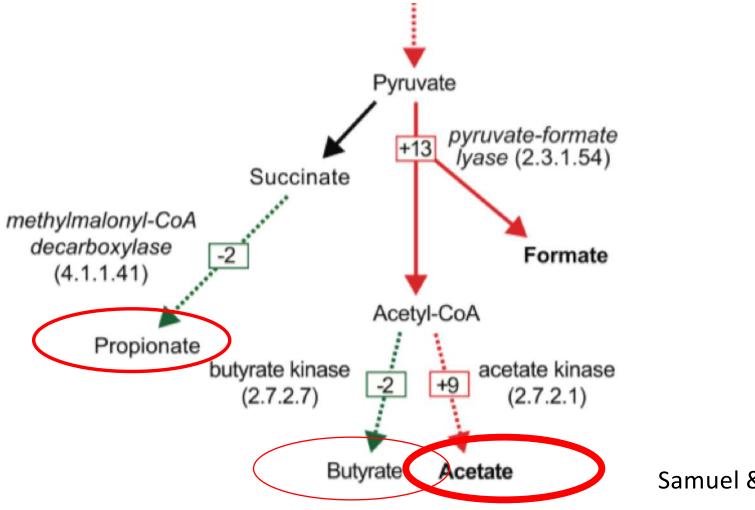
C. Ceccotti, 2017, PhD Thesis



- Collecting feces from seabass fed with a control diet (Bulk collection);
- Anaerobic growth of feces in medium with prebiotics addition;
- Extraction of butyric acid following De Baere *et al.* (2013) protocol;
- Quantification by HPLC-UV;
- Among all prebiotics, chitosan resulted the substrate giving the highest level of butyric acid.

Rainbow trout fecal content

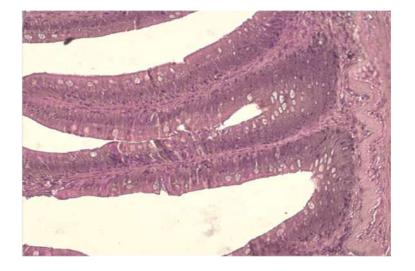
	Acetate (mM/l)	Propionate (mM/l)	Butyrate (mM/l)
Time T ₀	7.05	Traces	Traces
Control	6.38	Traces	0.51
Mono-Butyrate	12.88	1.97	1.48
Insect-chitin	12.26	1.22	2.06
Shrimp meal	13.74	0.74	0.30



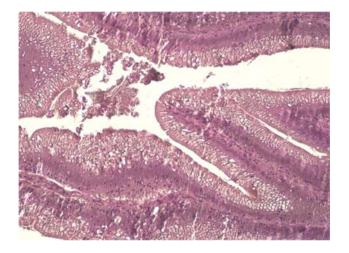
Samuel & Gordon, 2006, modif.

PROXIMAL INTESTINE

DISTAL INTESTINE



CTRL



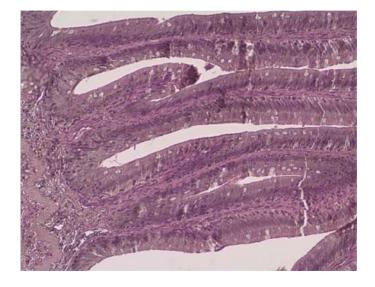
10 X

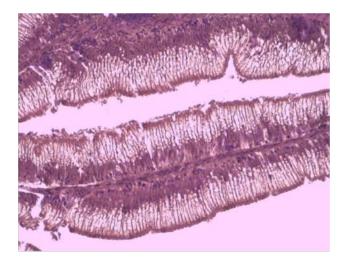
10 X

CTRL + CHITIN

PROXIMAL INTESTINE

DISTAL INTESTINE





10 X

10 X

WATER AND GUT MICROBIOTA AS MARKER OF ENVIRONMENTAL CONDITIONS AND NUTRITIONAL WELFARE - WATER MICROBIOLOGICAL GARDEN A peculiar microbic ecosystem has been observed caractherizing the farming systems, where **Proteobacteria** and **Bacteroidetes** were the main colonizing phyla, indicating different risks for bacterial diseases;

- No significant differences were observed among water samples collected within different formulation tests, but among samples collected at different time, indicating a continuous evolution of the water microbiota.

MUDEGLI STUDI MILANO RICOCCA

INS F

Substitution plant oil with insect oil 66%

Growth performances

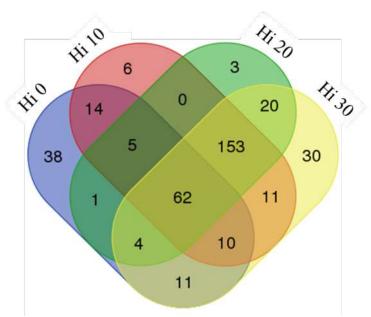
	Hi 0	Hi 10	Hi 20	Hi 30
BW _i (g)	67.01 ± 1.71	66.38 ± 2.51	65.63 ± 0.42	66.95 ± 2.31
$BW_{f}(g)$	223.20 ± 23.67	220.34 ± 29.60	216.97 ± 26.16	221.74 ± 22.25
WG (g)	156.86 ± 4.33	154.20 ± 6.04	146.89 ± 8.03	152.30 ± 10.18
SGR	1.42 ± 0.01	1.40 ± 0.06	1.38 ± 0.04	1.38 ± 0.04
FCR	0.90 ± 0.02	0.93 ± 0.04	0.95 ± 0.03	0.93 ± 0.04

BWi initial body weight, BWf final body weight, WG weight gain, SGR specific growth rate, FCR feed conversion ratio

All fish had tripled their initial body weight, and growth performance parameters (WG and SGR) likewise FCR were not negatively affected by the diet composition.

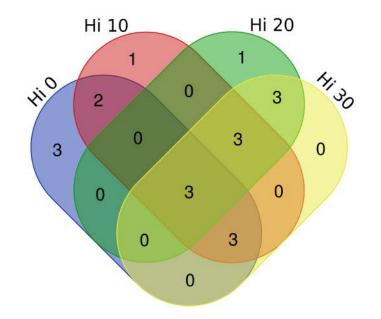
Analysis of gut microbiota structure

FECES



The "core microbiota" was constituted by 62 OTUs (shared by 80% of samples), **23 OTUs** were common to 100% of samples with a dominance of bacteria assigned to *Firmicutes* phylum.

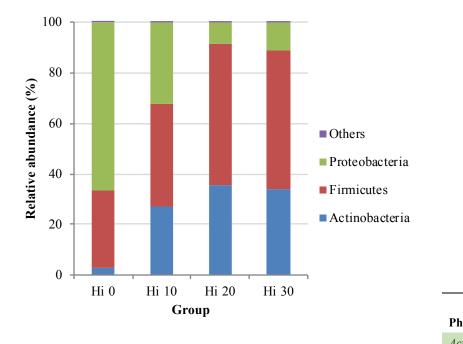
MUCOSA



The "core microbiota" was constituted by only **3 OTUs** assigned to *Propiobacterinae*, *Shewanella*, and *Mycoplasma* genera.

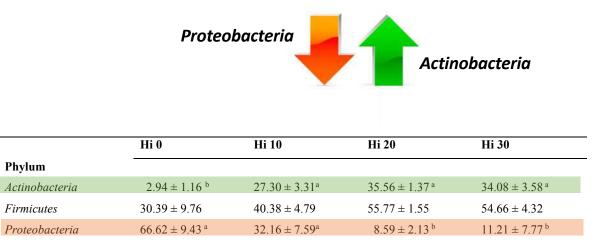
Effects of *H. illucens* supplemented diets on intestinal microbial communities

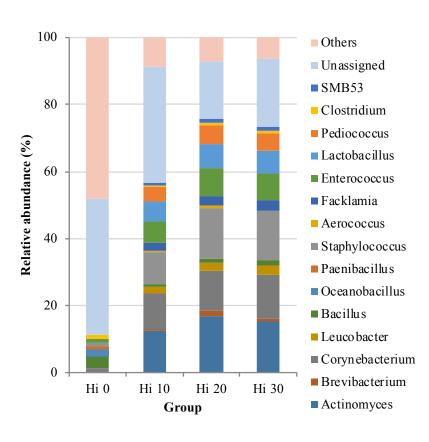
The whole microbial community profile of fecal samples was mainly comprised of 7 phyla, 12 classes, 26 orders, 68 families, 98 genera, and 55 species



At phylum level

The amounts of **Actinobacteria** and **Proteobacteria** were significantly influenced (p<0.05) by including insect meal in the diet





At genus level

	Hi 0	Hi 10	Hi 20	Hi 30
Genus				
Actinomyces	0.25 ± 0.07^{b}	12.29 ± 1.30^{a}	16.86 ± 0.44 a	15.08 ± 1.51^{a}
Brevibacterium	0.10 ± 0.03 ^b	0.65 ± 0.04^{ab}	1.38 ± 0.16^{a}	$1.12\pm0.32^{\text{ a}}$
Corynebacterium	$1.23 \pm 0.42^{\ b}$	10.74 ± 1.78 ^a	12.33 ± 0.94 ^a	$13.04\pm1.40^{\text{ a}}$
Leucobacter	$0.14 \pm 0.08^{\; b}$	1.76 ± 0.16^{a}	$2.27\pm0.25^{\text{ a}}$	$2.83\pm0.48^{\text{ a}}$
Bacillus	3.60 ± 2.00	0.76 ± 0.07	1.10 ± 0.18	1.66 ± 0.75
Oceanobacillus	1.96 ± 0.75 a	$0.24\pm0.04^{\;ab}$	$0.19\pm0.03^{\ ab}$	$0.10\pm0.01^{\ b}$
Paenibacillus	0.99 ± 0.52	0.12 ± 0.03	0.23 ± 0.05	0.27 ± 0.06
Staphylococcus	$0\;.97 \pm 0.34^{\;b}$	9.69 ± 1.54^{a}	15.13 ± 1.11 ^a	14.66 ± 1.89^{a}
Aerococcus	n. d. ^b	0.61 ± 0.13 ^a	0.68 ± 0.04^{a}	0.42 ± 0.07 a
Facklamia	$0.07 \pm 0.02^{\;b}$	$2.34\pm0.46^{\ a}$	2.84 ± 0.20^{a}	$3.22\pm0.57^{\text{ a}}$
Enterococcus	$1.32\pm0.48^{\;b}$	$6.36\pm0.77~^{a}$	8.35 ± 0.27^{a}	$7.93\pm0.87^{\text{ a}}$
Lactobacillus	$0.32 \pm 0.08^{\; b}$	$5.98\pm1.00^{\text{ a}}$	7.33 ± 0.59^{a}	6.75 ± 0.43 $^{\rm a}$
Pediococcus	$0.23 \pm 0.14^{\; b}$	$4.28\pm0.68^{\ a}$	$5.45\pm0.29^{\text{ a}}$	5.03 ± 0.53 ^a
Clostridium	1.05 ± 0.43	0.49 ± 0.18	0.66 ± 0.18	0.89 ± 0.49
SMB53	$0.04 \pm 0.02^{\;b}$	$0.77\pm0.12^{\ ab}$	$1.30\pm0.23~^{a}$	$1.32\pm0.11~^{a}$



Actinomyces, Brevibacterium, Coryneb Leucobacter, and Staphylococcus LAB: Aerococcus, Facklamia, Ente Lactobacillus, and Pediococcus

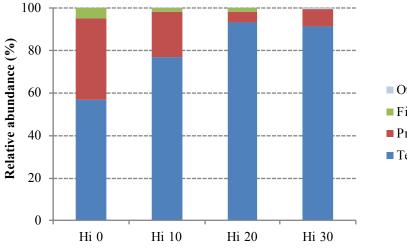
Corynebacterium,

Enterococcus,

Beneficial bacterial species positively correlated to insect-meal diets: *Corynebacterium variabile, Lactobacillus paraplantarum, Lactobacillus zeae, Weisella cibaria, Clostridium butyricum,* and *Clostridium fimentarium*

Dietary modulation of autochthonous intestinal microbiota

The bacterial OTUs found in fish mucosa samples were mainly comprised of 6 phyla, 7 classes, 10 orders, 15 families, and 22 genera



- OthersFirmicutesProteobacteria
- Tenericutes

At phylum level

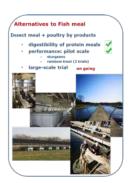
Tenericutes and *Proteobacteria* were significantly influenced by insect meal inclusion (20% and 30%) in the diet.



Phylum	Hi 0	Hi 10	Hi 20	Hi 30
Tenericutes	56.48 ± 21.74^{b}	77.06 ± 17.44^{ab}	93.03 ± 10.12^{a}	91.52 ± 12.68^{a}
Proteobacteria	38.36 ± 18.26^{a}	21.07 ± 16.85^{ab}	5.03 ± 7.51^{b}	7.69 ± 12.82^{b}
Firmicutes	5.05 ± 8.37	1.84 ± 4.00	1.86 ± 4.34	$0.49 ~\pm~ 0.96$

Insect meal: not only growth but beneficial effects on the microbiota garden

- \checkmark Insect meal was well accepted by fish up to 30% inclusion.
- ✓ Our results confirmed that bacterial communities adhering to mucosa differ from transient microflora in fish intestine: Proteobacteria and Firmicutes dominated the allochthonous gut microbiota while Tenericutes and Proteobacteria phyla harboured trout intestinal mucosa.
- ✓ Dietary Hi meal positively modulated gut microbiota (autochthonous and allochthonous) increasing its richness and diversity: effect of chitin and lauric acid (C12:0) contained in Hi prepupae.
- ✓ OTUs attributable to LAB (beneficial genera) were only found in high amount in the gut content samples of trout fed insect meal, but were practically absent in gut mucosa of the same fish. *The increasing number of LAB could be promoted by chitin, which acts as a prebiotic.*
- ✓ An increased number of bacteria belonging to *Mycoplasma* genus was found in the mucosa of trout fed Hi 20 and Hi 30 diets. They are obligate commensal microorganisms of the gut ecosystem that produce lactic acid and acetic acid as their major metabolites. *Mycoplasma could have a beneficial action on host health.*
- ✓ Hi meal caused a significant reduction of Proteobacteria (Gammaproteobacteria) both in the gut digesta and mucosa. Interestingly all genera adversely affected by insect-meal diets were Gram-negative bacteria that include potential pathogen species.



Rainbow trout on-farm trial feeding "AGER 4F Feeds"

3 diets (4 replicates)

- Contr Commercial feed (Plant based)
 PBP (Poultry meal based feed)
- 3) HI (Insect meal based feed)

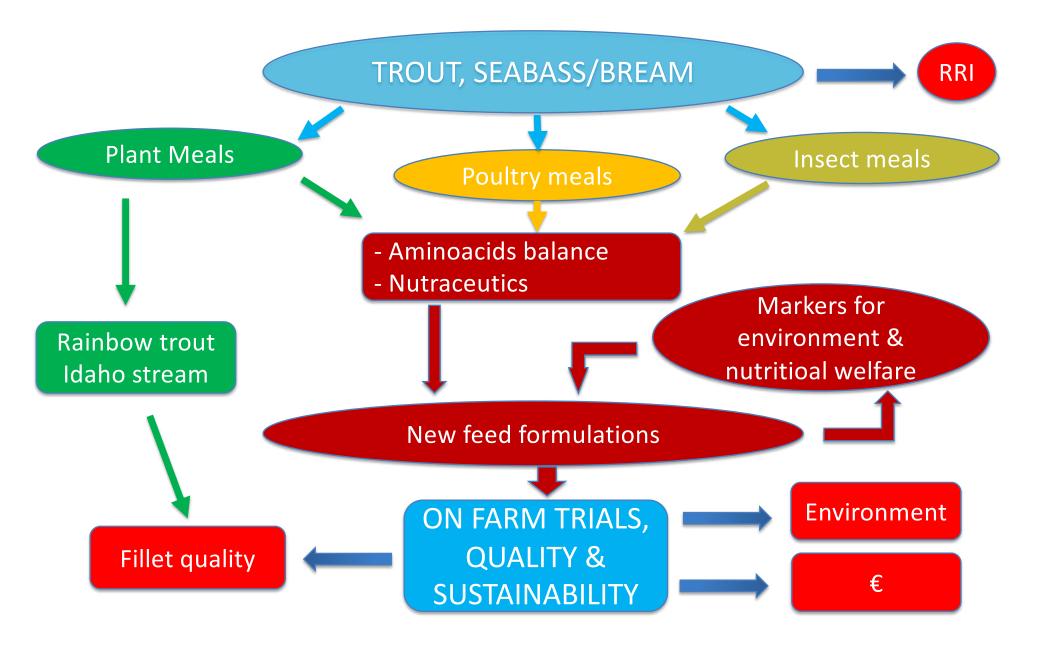


		CTRL	PBP	ні
·/	PI (kg)	50,20	50,25	50,13
	Peso al 3/02/2020 (kg)	77,05	77,01	76,27





A challenging on-farm trial, feeding Sea bream with Zero FM, will start next month



DISSEMINATION AND RESPONSIBLE RESEARCH & INNOVATION (RRI)

Acquacoltura & Sostenibilità

Comunicazione e disseminazione dei risultati a cura di Consorzio Italbiotec

L'attività di comunicazione condotta dal progetto 4F – FineFeedForFish mira alla diffusione di nuove buone pratiche e soluzioni ecosostenibili al comporto ittico e ad incrementare la consapevolezza dei cittadini circa il contributo dell'acquacoltura nell'affrontare la sfida al cambiamento climatico. Fin dall'avvio del progetto, 4F si è impegnato nella progettazione e sviluppo di prodotti di comunicazione in grado di coinvolgere gli stakeholder, qui di seguito si riportano i più significativi:

- 35 articoli di divulgazione pubblicati sul sito AGER e condivisi su testate online + 16.000 lettori raggiunti
- 14 articoli scientifici pubblicati su riviste peer review
- 1 servizio televisivo su rete nazionale
- 28 presentazioni orali dei risultati scientifici presso congressi nazionali ed internazionali
- 12 video divulgativi dedicati alla valorizzazione degli obiettivi e delle strategie +500 visualizzazioni
- 1 Corso e-learning (8 video lezioni) dedicato all'acquacoltura sostenibile e al benessere animale
- 1 Summer School «Acquacoltura & Sostenibilità» con 30 studenti, ricercatori e professionisti
- 1 pubblicazione «ACQUACOLTURA E COMUNICAZIONE: strumenti e metodi di debunking»
- Poster, roll-ups, leaflets prodotti e distribuiti in forma digitale e cartacea + 2500 lettori raggiunti
- 1 Community online «Acquacoltura& Sostenibilità» + 250 utenti attivi

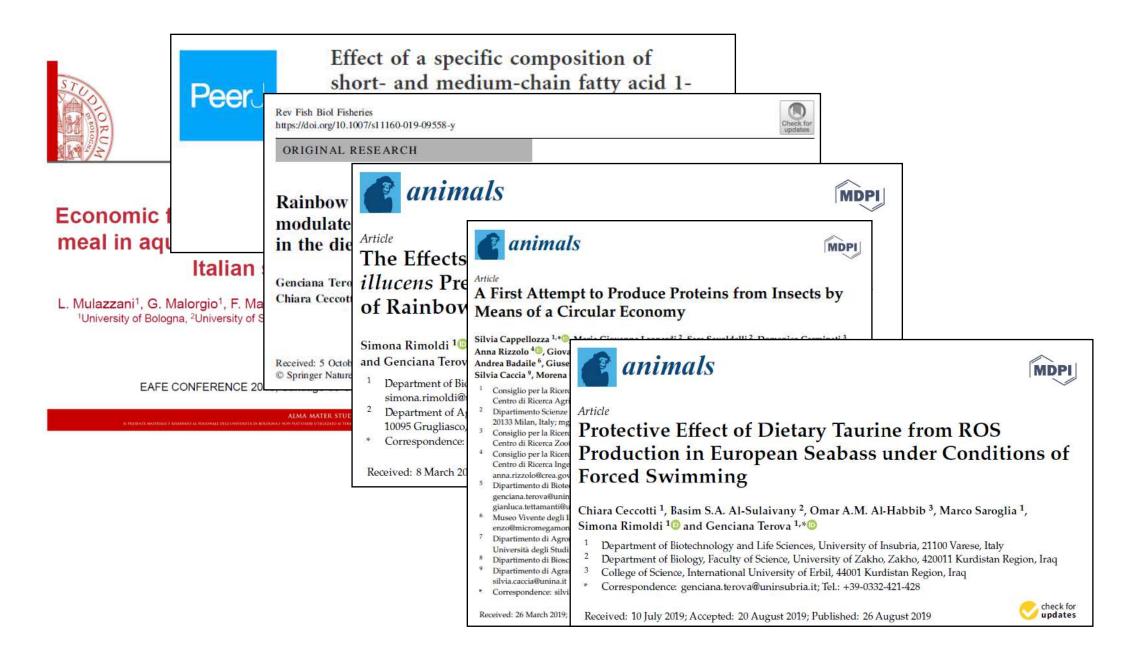


ACQUACOLTURA E COMUNICAZIONE: strumenti e metodi di debunking

Acquacoltura Summer School 2019 - Alghero











THE PRINCIPAL MEMBERS OF THE "4F" TEAM: Marco Saroglia¹, Genciana Terova¹, Laura Gasco², Pietro Pulina³, Sergio Uzzau⁴, Massimo Labra⁵, Ilaria Re⁷, Ronald Hardy⁸, Roberto Anedda⁴, Marino Prearo⁹, Ivo Zoccarato²,

(and coll.)

















https://acquacoltura.progettoager.it/index.php/acquacolturaper-la-ricerca/itemlist/user/51-4f