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

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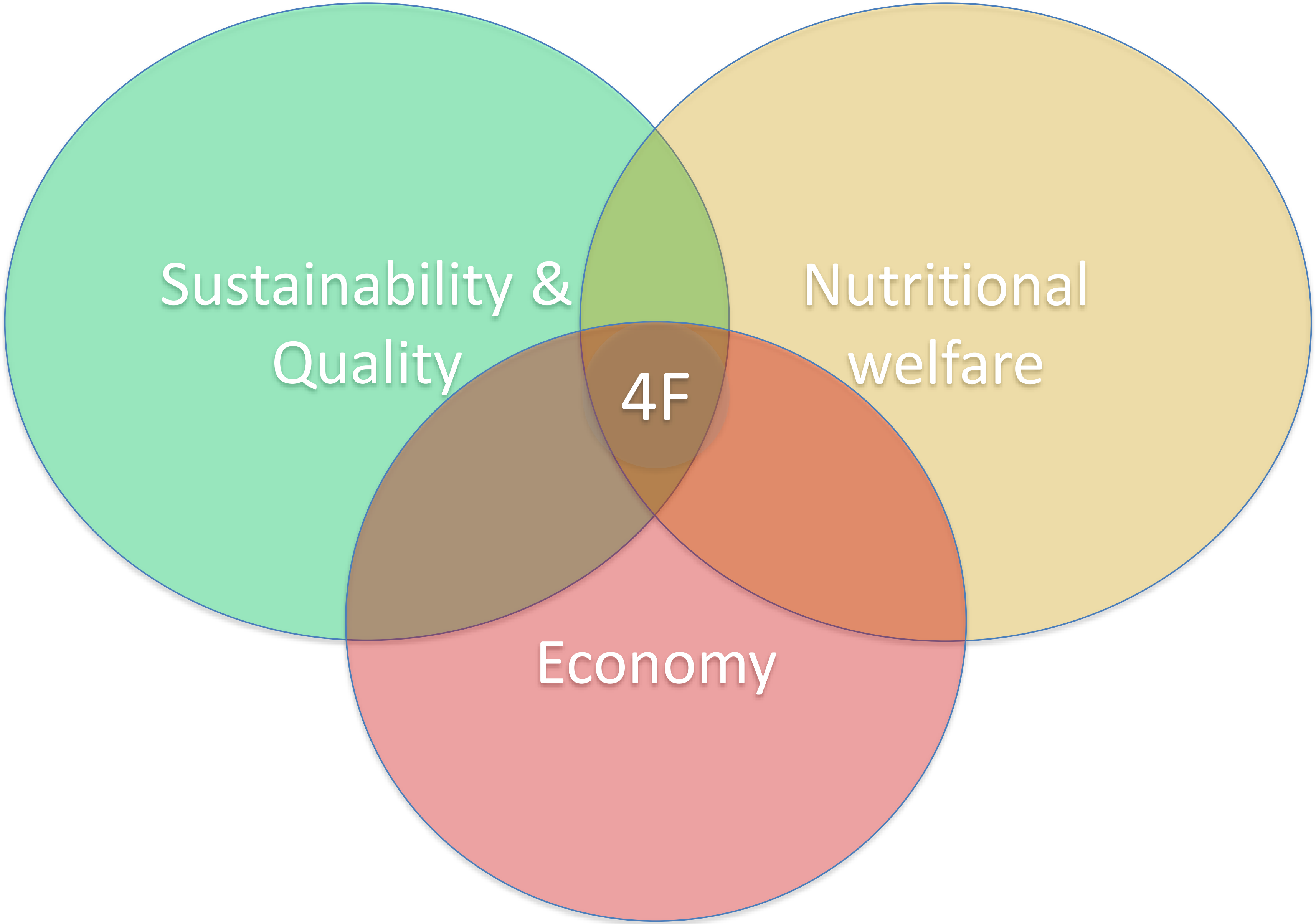
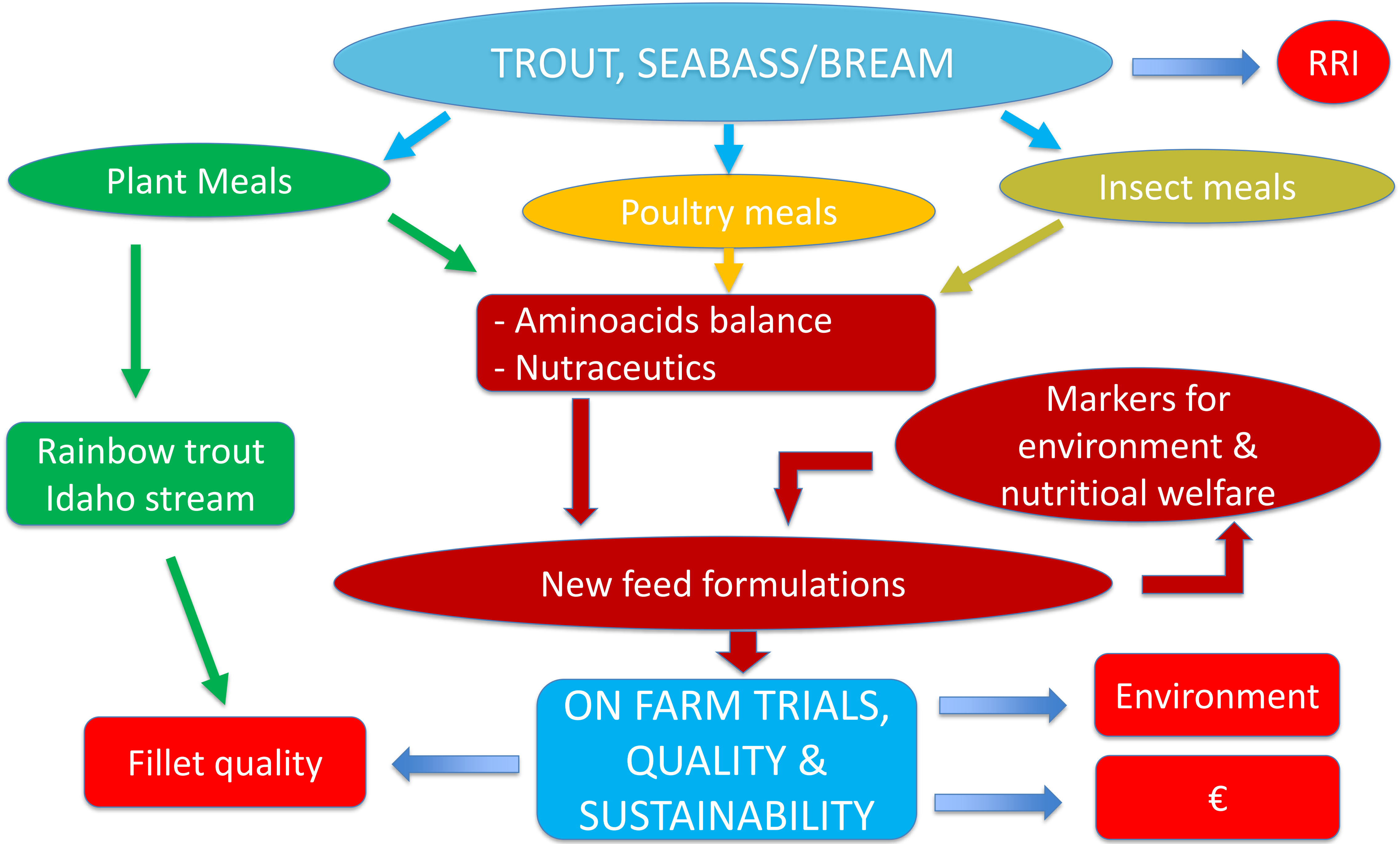
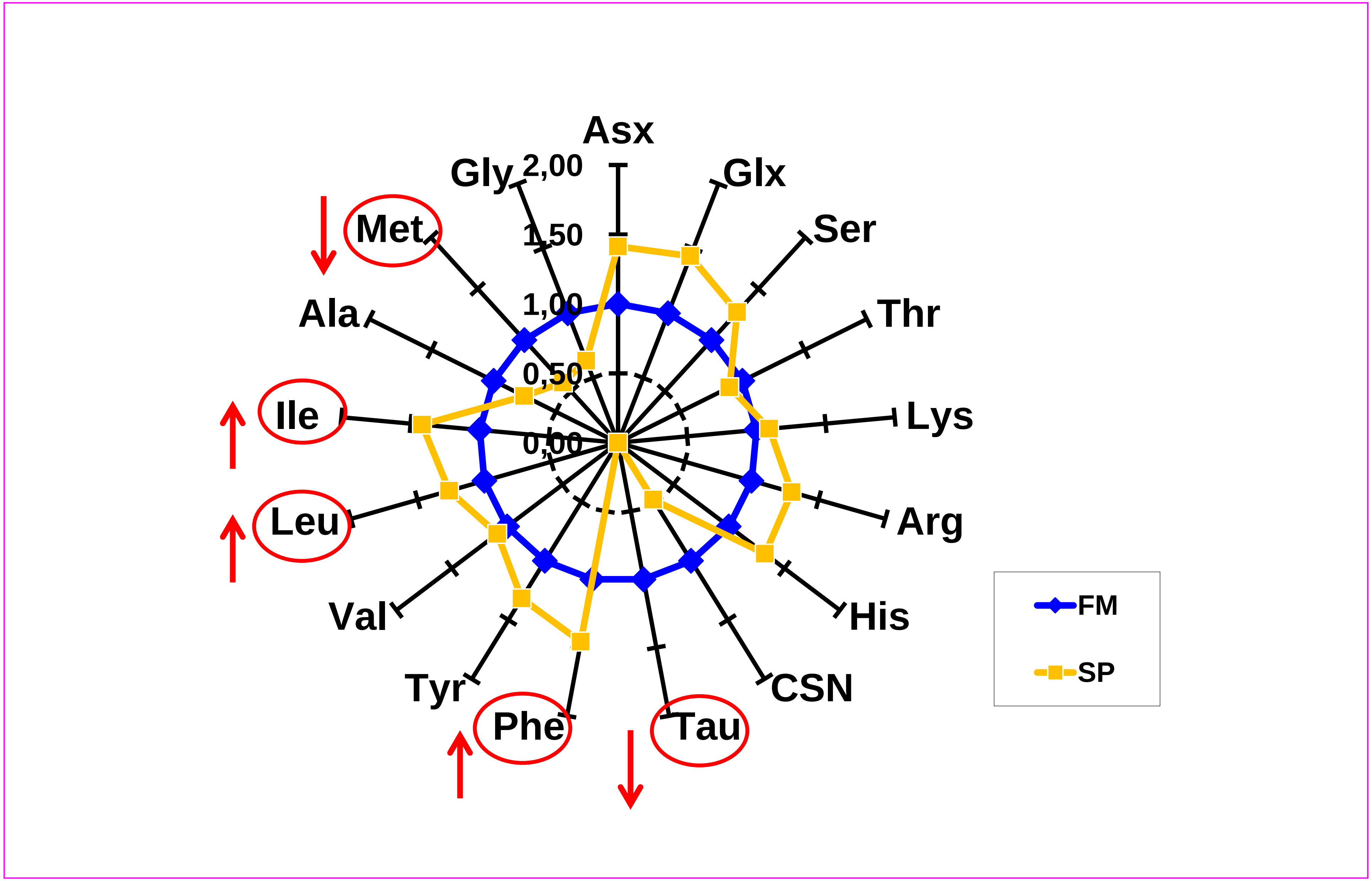
Fish meal substitution



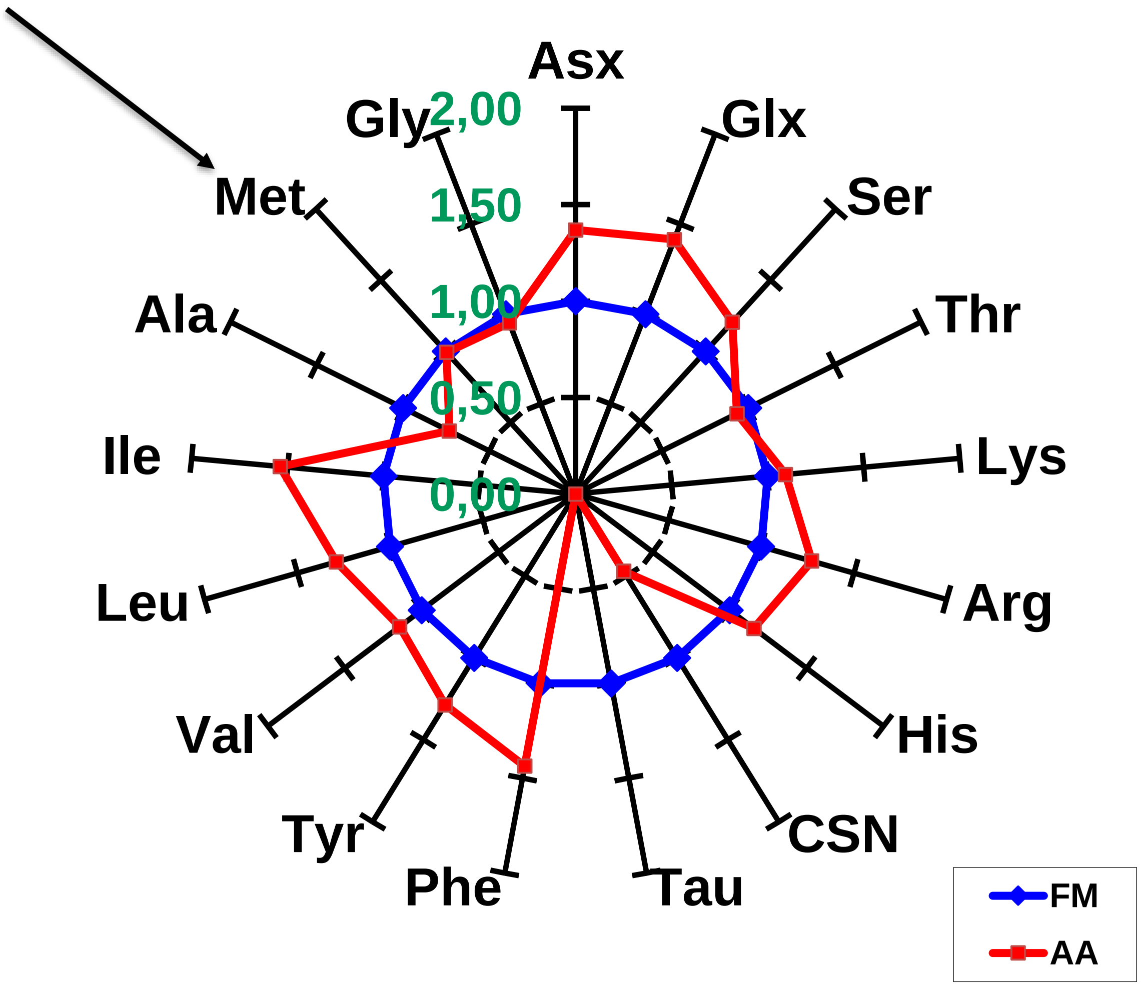

TROUT, SEABASS/BREAM
RRI
Plant Meals
Poultry meals
Insect meals
- Aminoacids balance
- Nutraceutics
Markers for environment & nutritioal welfare
Rainbow trout Idaho stream
New feed formulations
ON FARM TRIALS, QUALITY & SUSTAINABILITY
Environment
€
Fillet quality

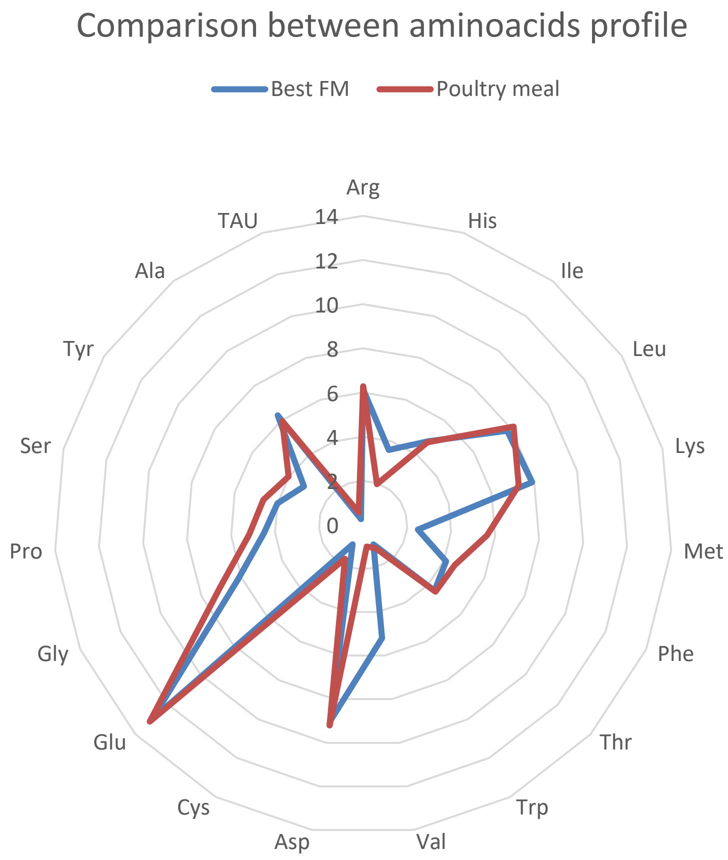
AMINOACIDS BALANCE: THE EXAMPLES OF METHIONINE AND TAURINE

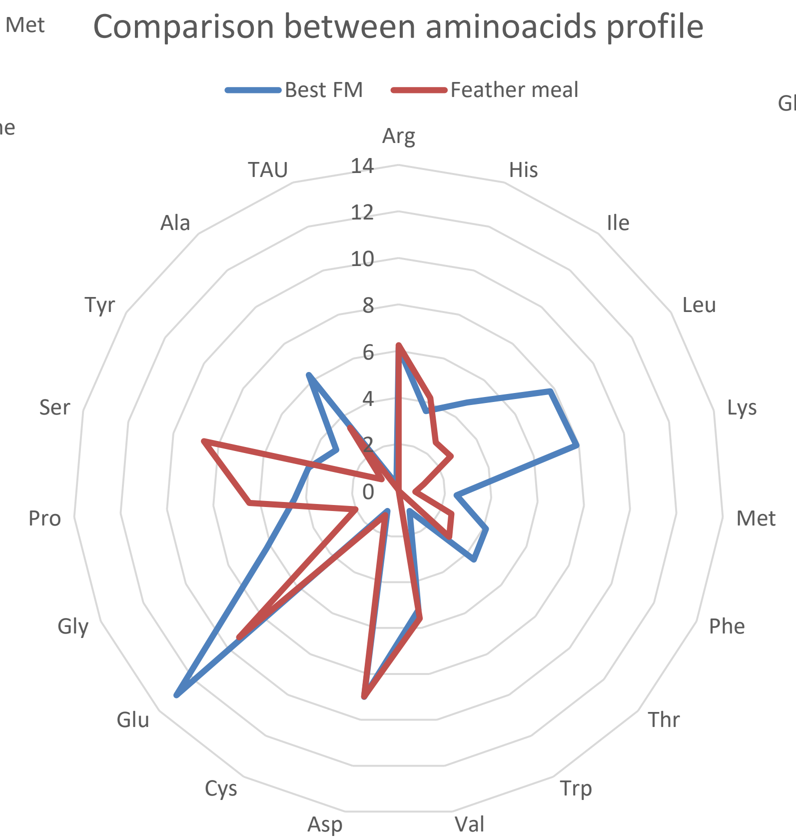
Amino acid profile soy protein and fishmeal



Supplement with Methionine




Comparison between aminoacids profile
Best FM
Poultry meal
Arg
His
Ile
Leu
Lys
Met
Phe
Thr
Trp
Val
Asp
Cys
Glu
Gly
Pro
Ser
Tyr
Ala
TAU
14
12
10
8
6
4
2
0


Comparison between aminoacids profile
Best FM
Feather meal
Arg
His
Ile
Leu
Lys
Met
Phe
Thr
Trp
Val
Asp
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Gly
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Ser
Tyr
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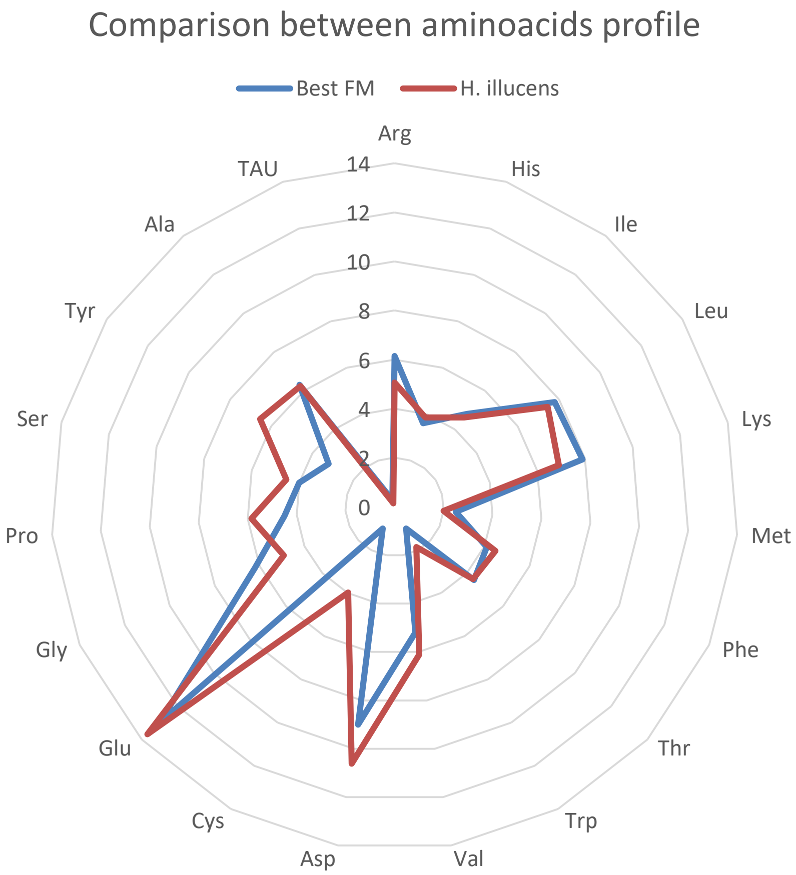
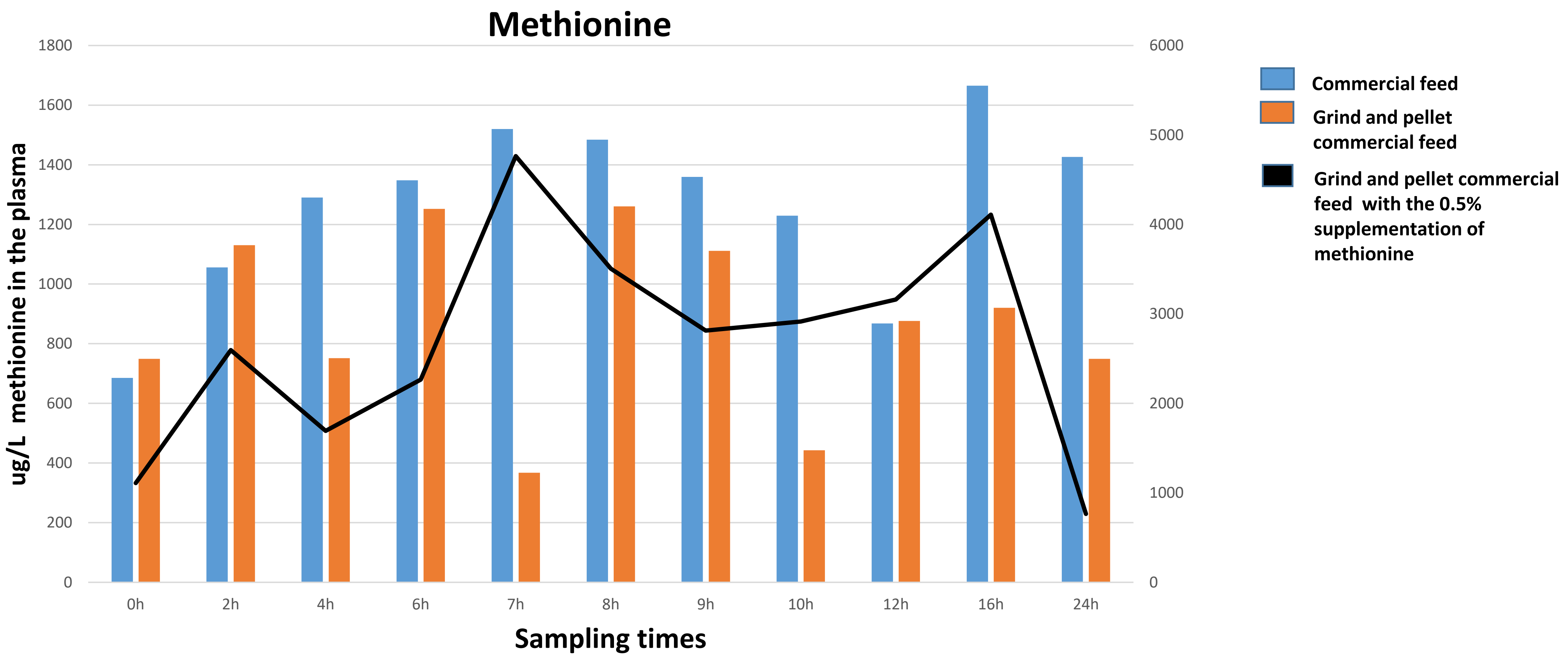

Comparison between aminoacids profile
Best FM
H. illucens
Arg
His
Ile
Leu
Lys
Met
Phe
Thr
Trp
Val
Asp
Cys
Glu
Gly
Pro
Ser
Tyr
Ala
TAU
14
12
10
8
6
4
2
0

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.

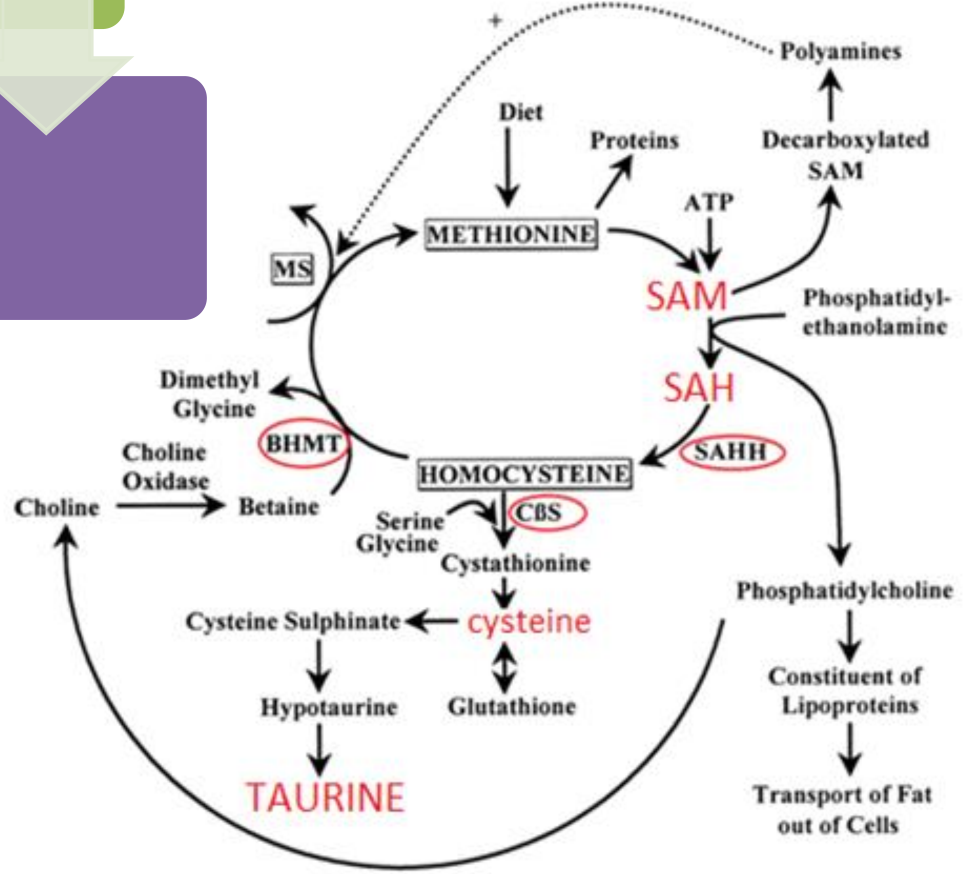


Background

Methionine: a source of sulphur required for the synthesis of cysteine and taurine as well as an essential methyl-donor in cellular metabolism. A total of 48% of dietary Met metabolism takes place in the liver.

Changes in the metabolism of Met can influence the production of nutrients essential for proper functioning of the skeletal, cardiovascular, and nervous systems.

Homocysteine: a sulphur amino acid metabolite derived from Met. The maintenance of methyl groups and homocysteine homeostasis in the hepatic tissue is dependent on the balance between **S-adenosylhomocysteine (SAH)** and its precursor, a powerful inhibitor of transmethylation reactions, **S-adenosylmethionine (SAM).**



BHMT: Betaine-Homocist. S-Methyltransferase
CBS: Cystathionine-β-Synthase
SAHH: S-Adenosylhomocysteine Hydrolase

SAM: S-Adenosyl-Methionine
SAH : S-Adenosyl-L-Homocysteine

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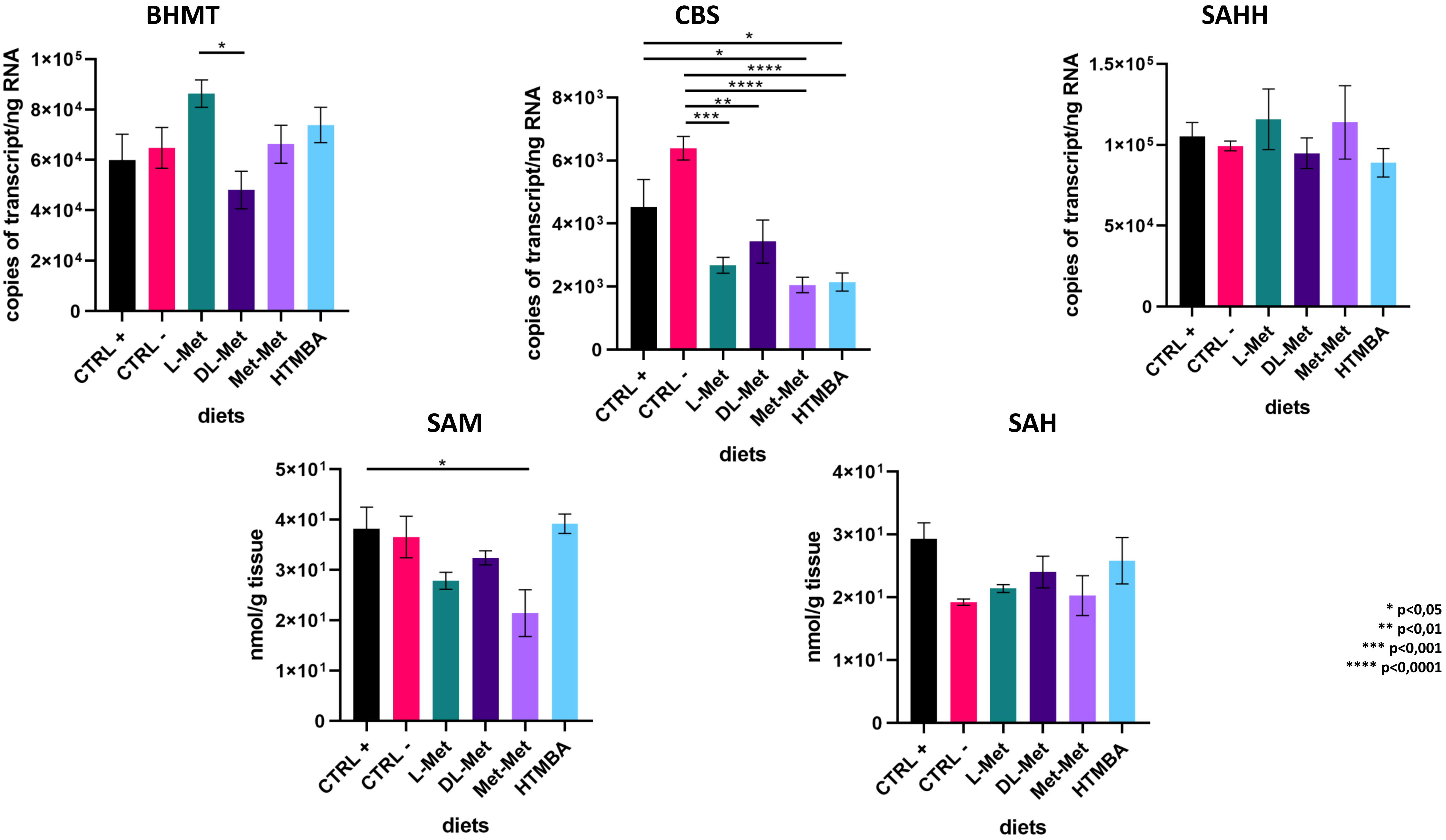

BHMT
copies of transcript/ng RNA
1×10^5
8×10^4
6×10^4
4×10^4
2×10^4
0
*
CTRL +
CTRL -
L-Met
DL-Met
Met-Met
HTMBA
diets
CBS
8×10^3
6×10^3
4×10^3
2×10^3
*
****
**
***
SAHH
1.5×10^5
1×10^5
5×10^4
SAM
nmol/g tissue
5×10^1
4×10^1
3×10^1
2×10^1
1×10^1
SAH
* p<0,05
** p<0,01
*** p<0,001
**** p<0,0001

	CTRL +	CTRL -	L-Met	DL-Met	Met-Met	HTMBA
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: more bioavailable
↓
↑ SAM
↑ SAH
↓
↓ Met → ↑ BHMT

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.

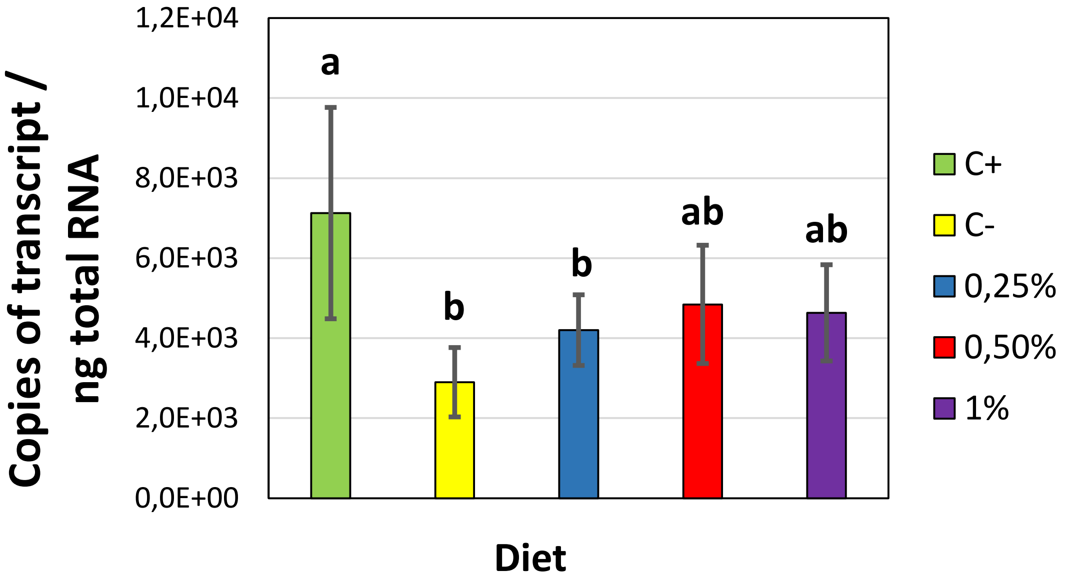
HTMB, DL-Met protect, Met-Met, DL-Met: slow assimilation
↓
↓ SAM
↓ SAH
↓
↑ Met → ↑ CBS

It is a temporal issue

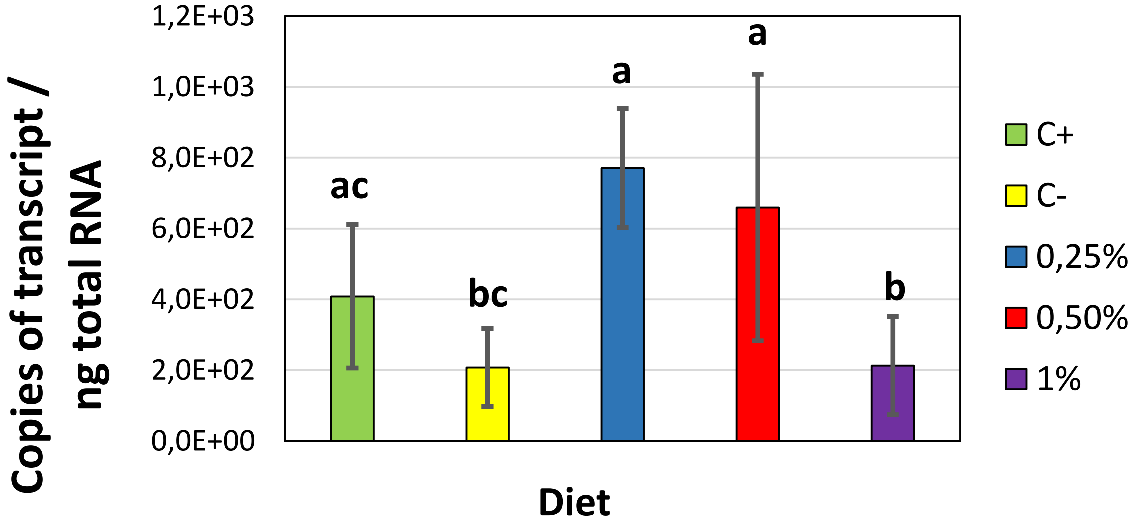
Taurine need in the diet for Rainbow trout

- **Positive control :** (fish meal diet base)
- **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)

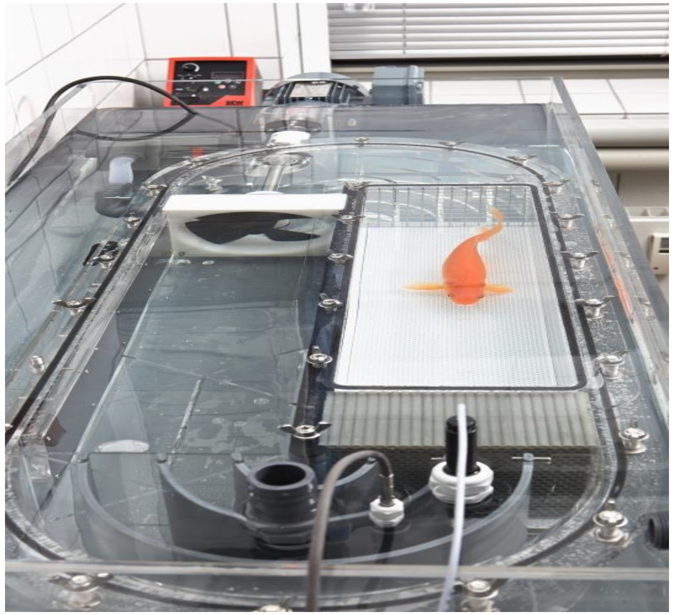
TauT expression in kidney



TauT expression in liver



Taurine



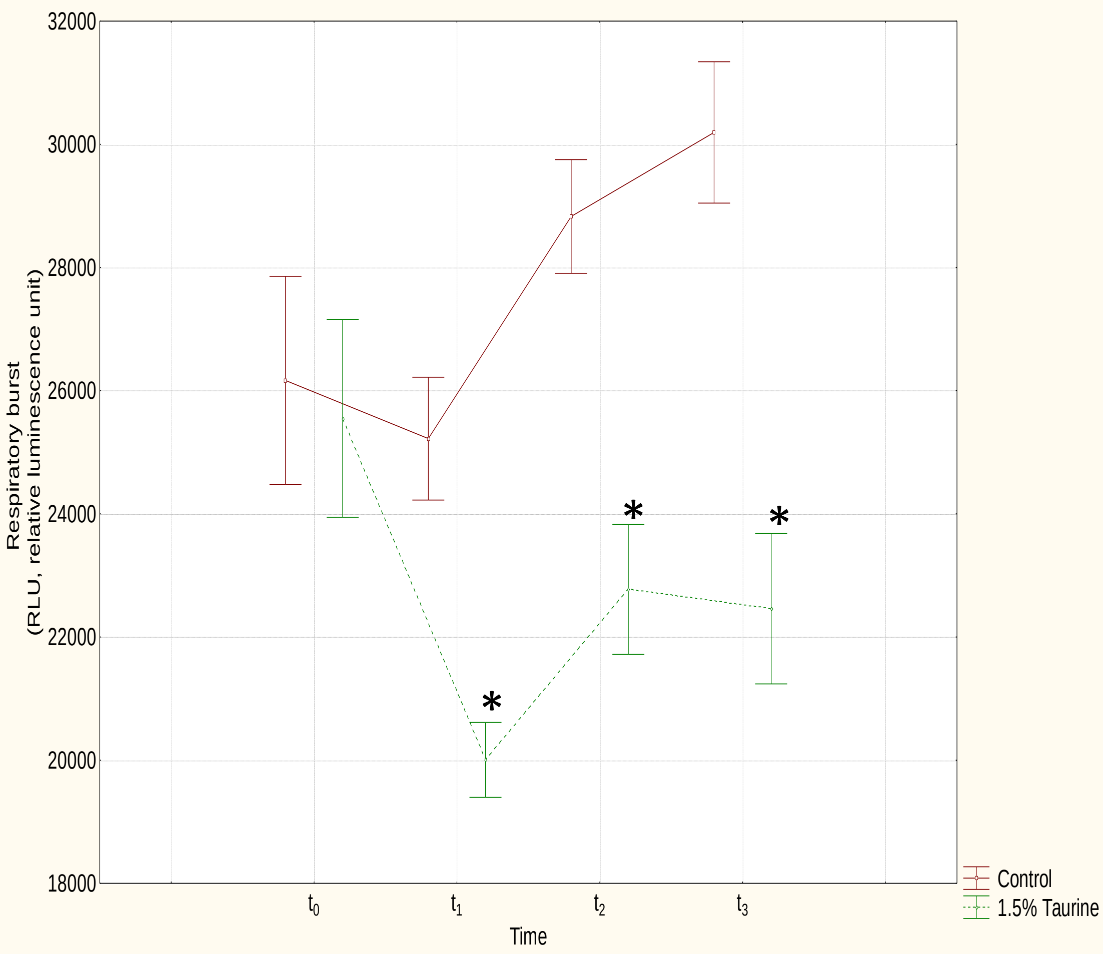
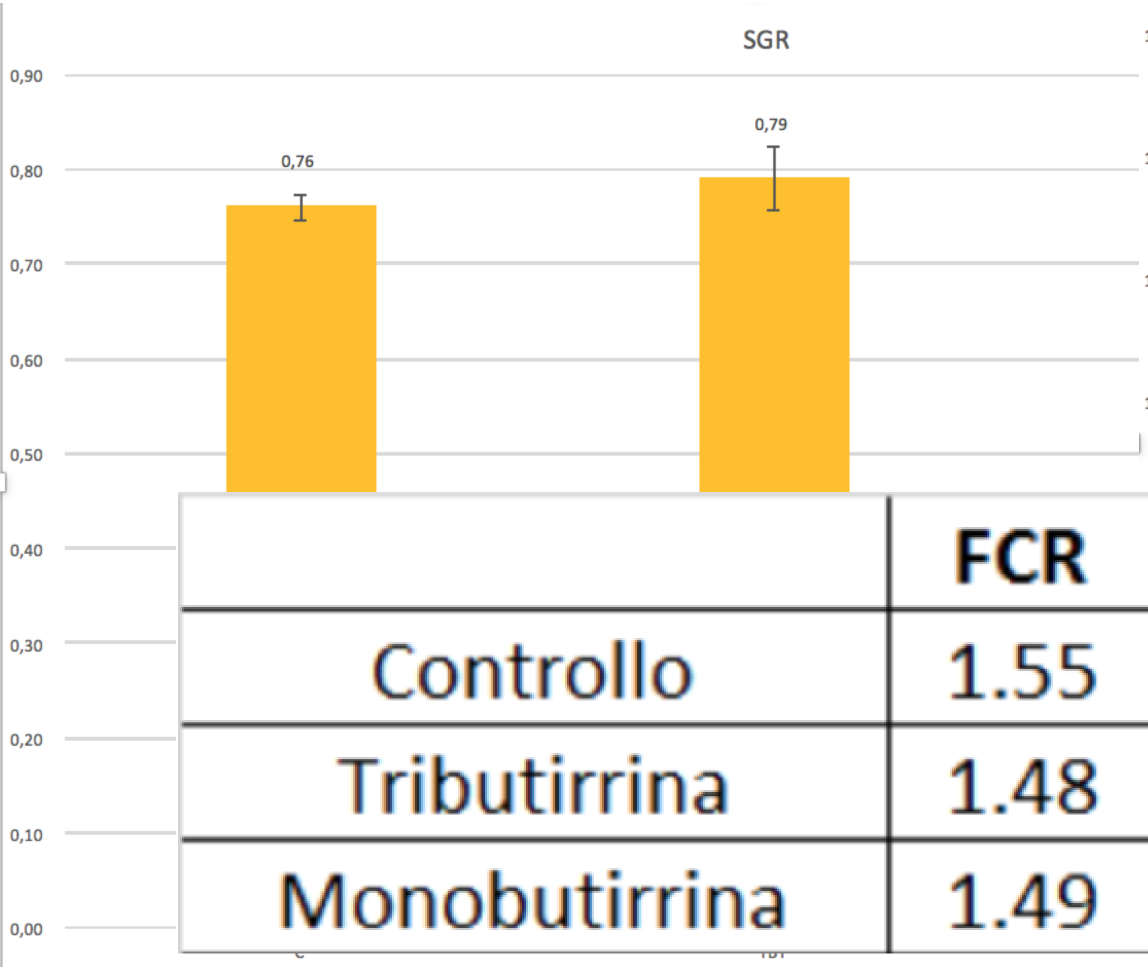
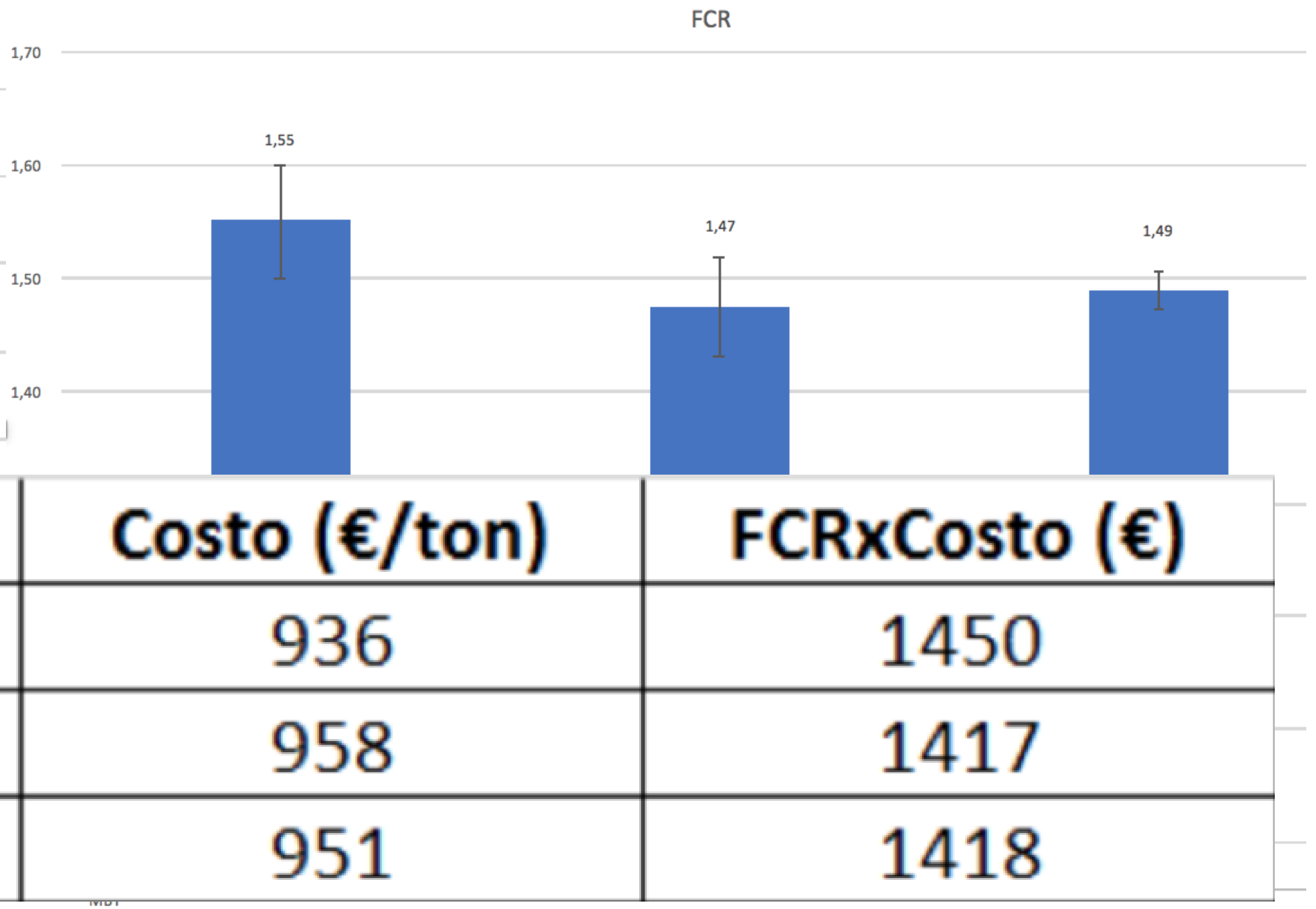


Table 3. The RBA values after PMA stimulation in Ctrl and 1.5% Tau group. (*) means significant differences between groups for the same t ($p<0.05$).

SCFAs

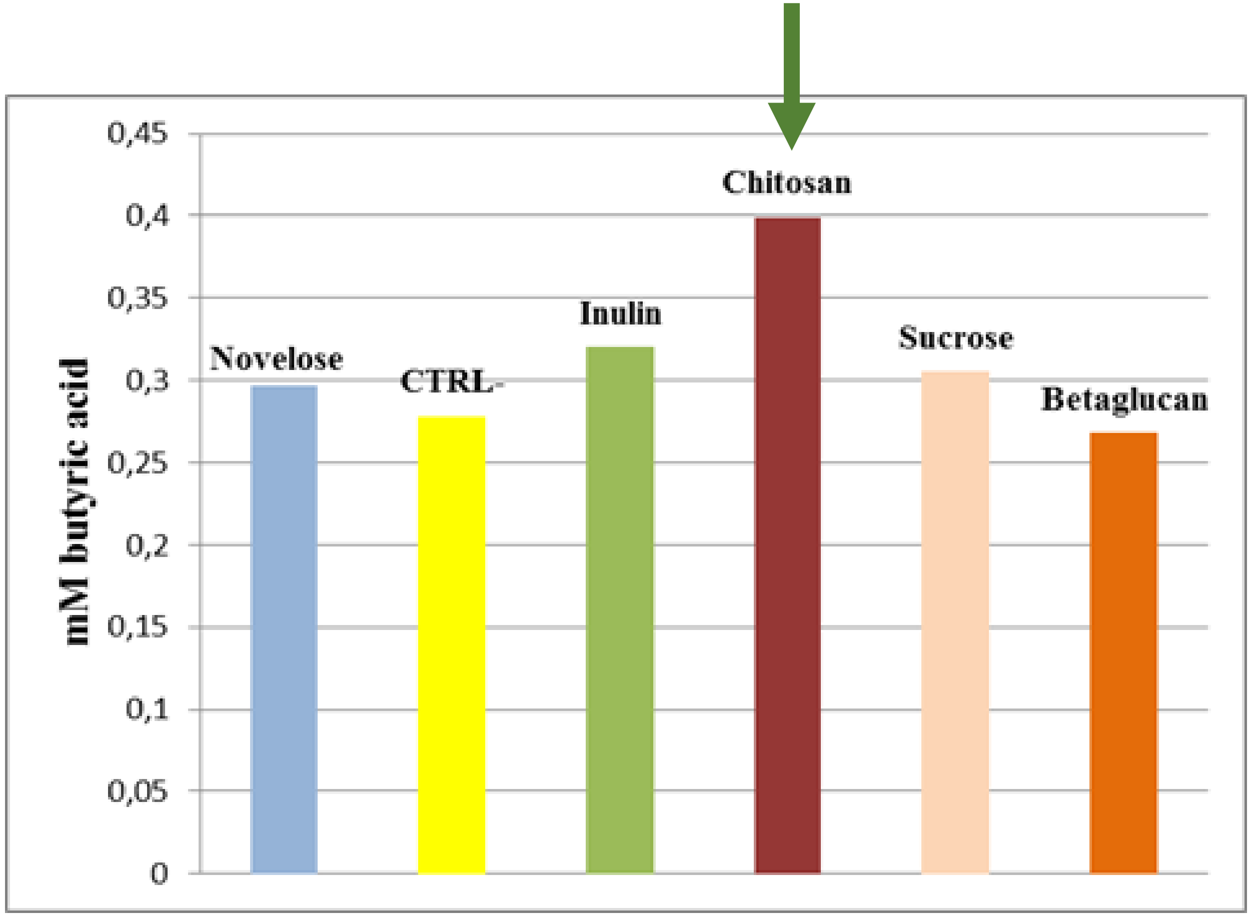
Effects of Butyrate on SGR and FCR

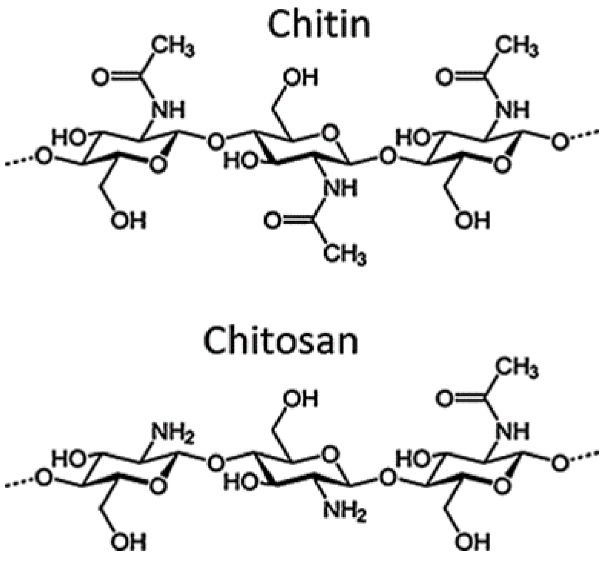




	FCR	Costo (€/ton)	FCRxCosto (€)
Controllo	1.55	936	1450
Tributirrina	1.48	958	1417
Monobutirrina	1.49	951	1418

Preliminary *in vitro* test for butyric acid quantification



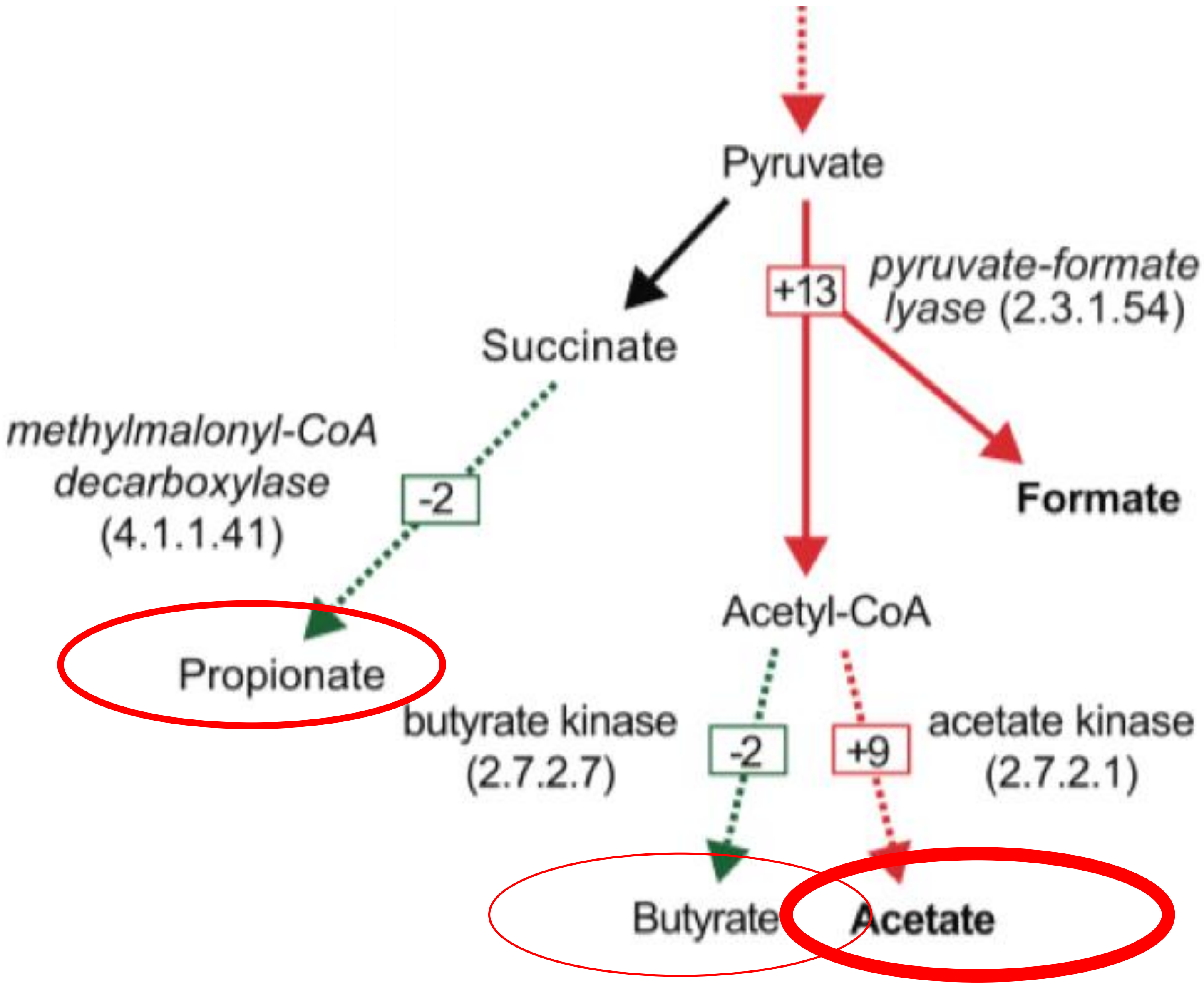


- Collecting feces from seabass fed with a control diet (Bulk collection);
- Anaerobic growth of feces in medium with prebiotics addition;
- Extraction of butyric acid following De Baere *et al*. (2013) protocol;
- Quantification by HPLC-UV;
- Among all prebiotics, chitosan resulted the substrate giving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

C. Ceccotti, 2017, PhD Thesis

Rainbow trout fecal content

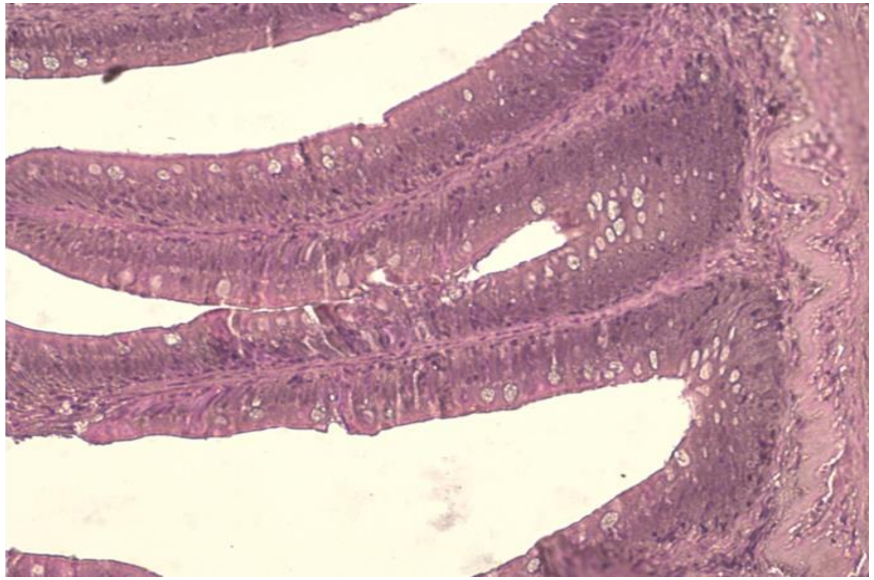
	Acetate (mM/l)	Propionate (mM/l)	Butyrate (mM/l)
Time T_0	7.05	Traces	Traces
Control	6.38	Traces	0.51
Mono-Butyrate	12.88	1.97	1.48
Insect-chitin	12.26	1.22	2.06
Shrimp meal	13.74	0.74	0.30



Samuel & Gordon, 2006, modif.

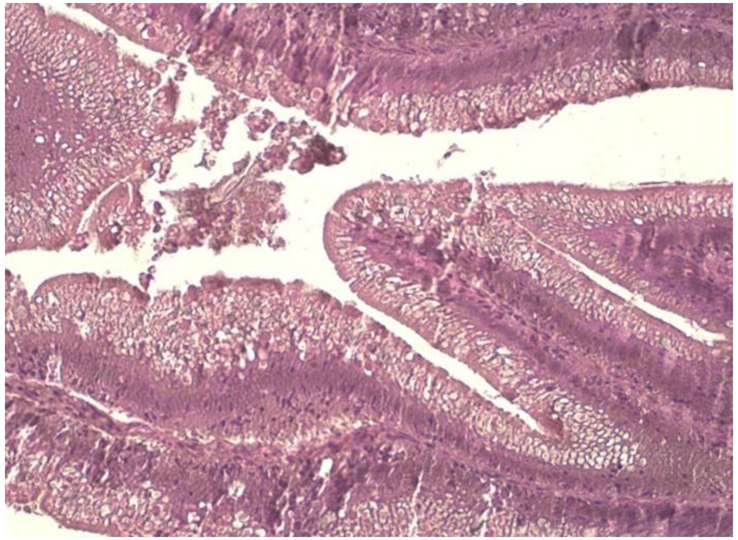
CTRL

PROXIMAL INTESTINE



10 X

DISTAL INTESTINE

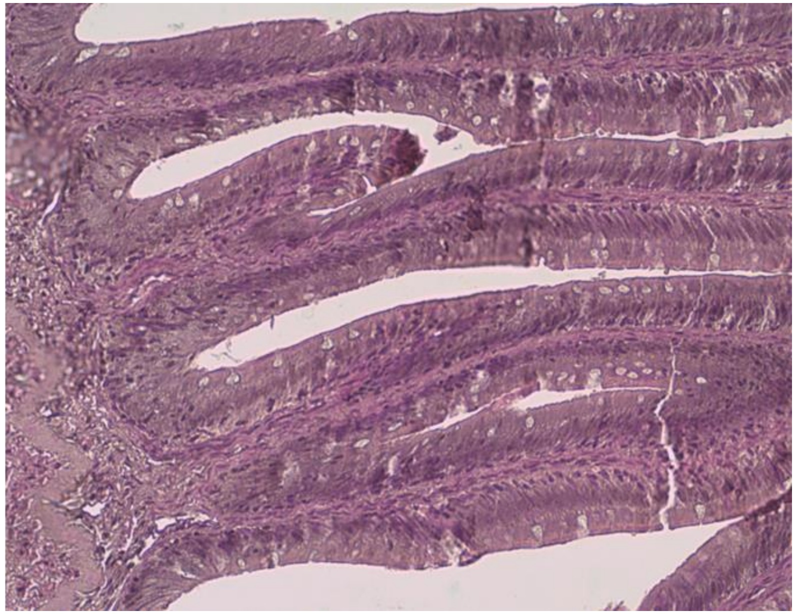


10 X

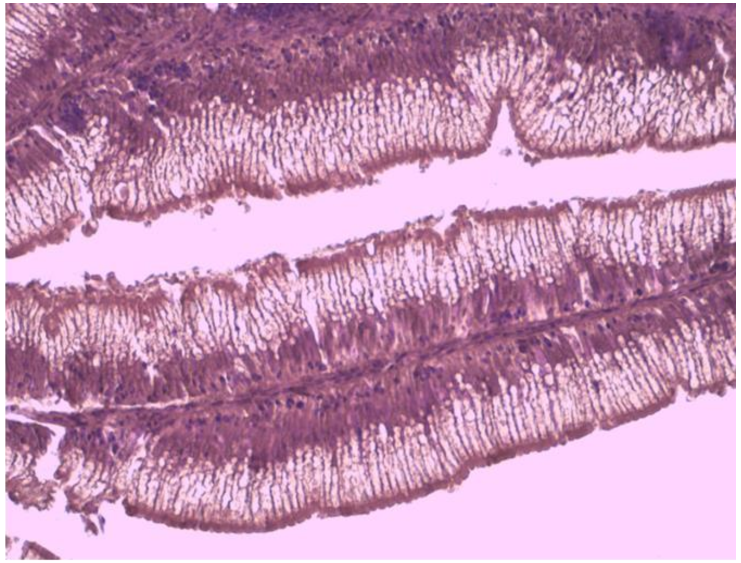
CTRL + CHITIN

PROXIMAL INTESTINE

DISTAL INTESTINE



10 X



10 X

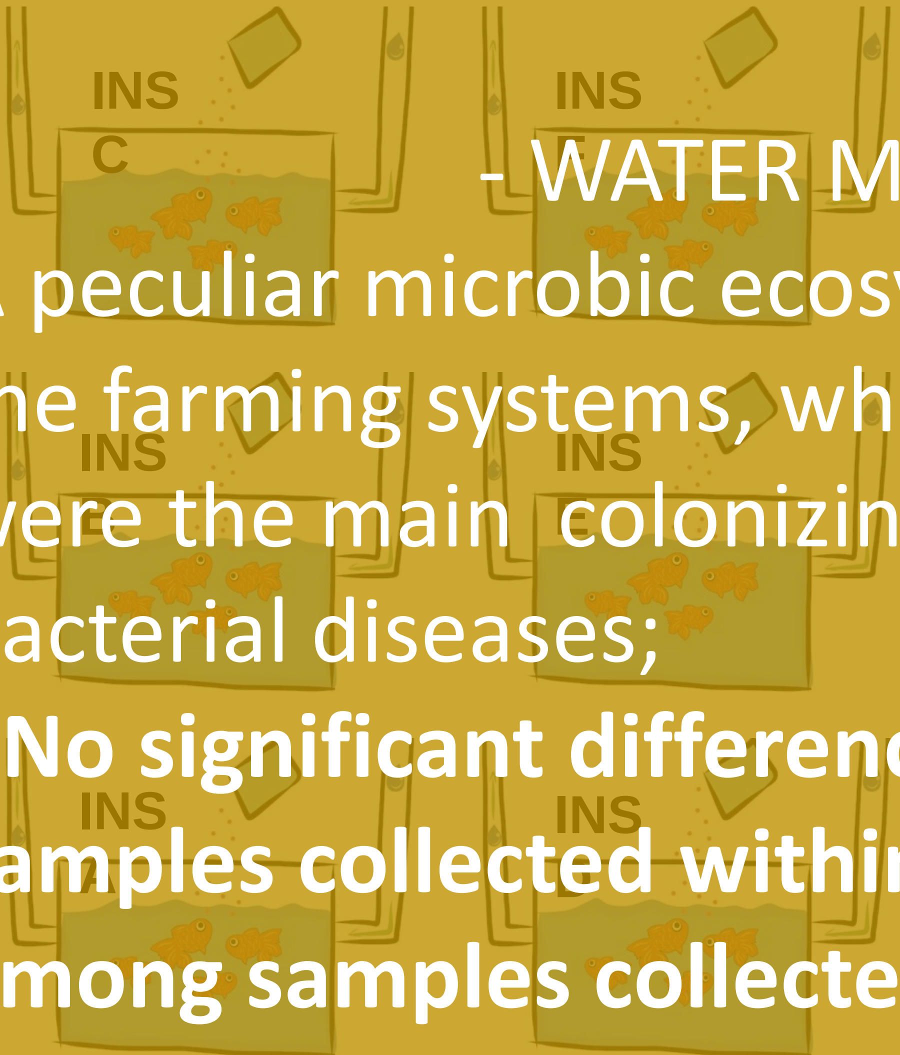
WATER AND GUT MICROBIOTA AS MARKER OF ENVIRONMENTAL CONDITIONS AND NUTRITIONAL WELFARE

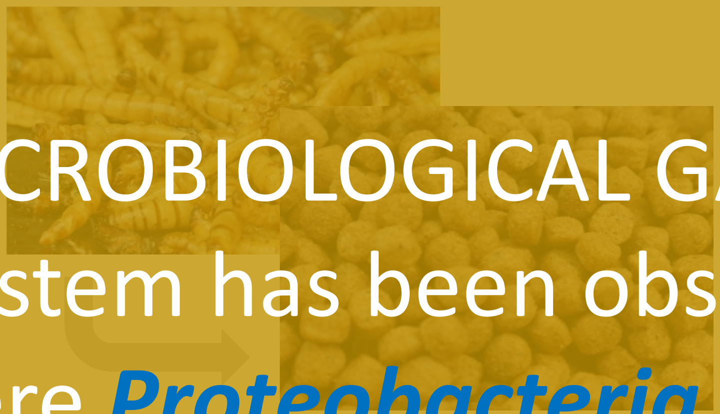
- WATER MICROBIOLOGICAL GARDEN

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.

SAMPLING DESIGN





Tank Name	Feed Formulation
INS A	Substitution of fish meal with insect 0%
INS B	Substitution of fish meal with insect 50%
INS C	Substitution of fish meal with insect 75%
INS D	Substitution of fish meal with insect 100%
INS E	Substitution plant oil with insect oil 33%
INS F	Substitution plant oil with insect oil 66%

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Growth performances

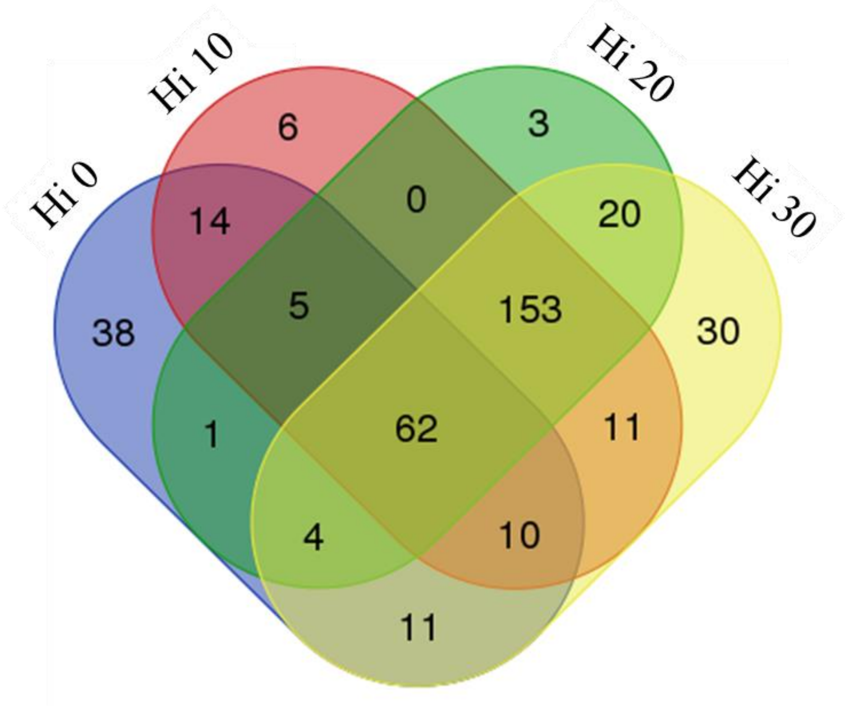
	Hi 0	Hi 10	Hi 20	Hi 30
BW_i (g)	67.01 ± 1.71	66.38 ± 2.51	65.63 ± 0.42	66.95 ± 2.31
BW_f (g)	223.20 ± 23.67	220.34 ± 29.60	216.97 ± 26.16	221.74 ± 22.25
WG (g)	156.86 ± 4.33	154.20 ± 6.04	146.89 ± 8.03	152.30 ± 10.18
SGR	1.42 ± 0.01	1.40 ± 0.06	1.38 ± 0.04	1.38 ± 0.04
FCR	0.90 ± 0.02	0.93 ± 0.04	0.95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

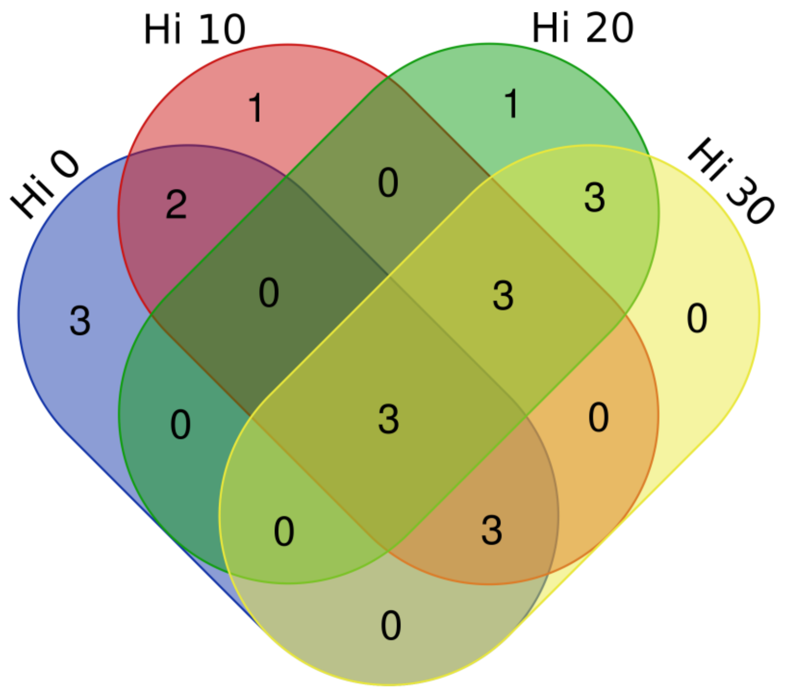
Analysis of gut microbiota structure

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.

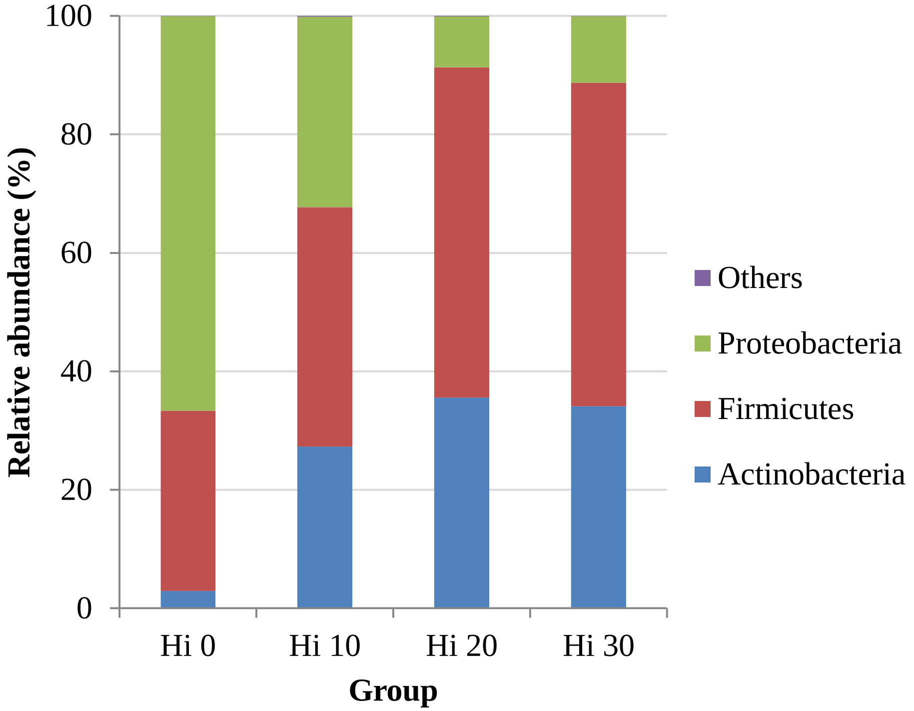
MUCOSA



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

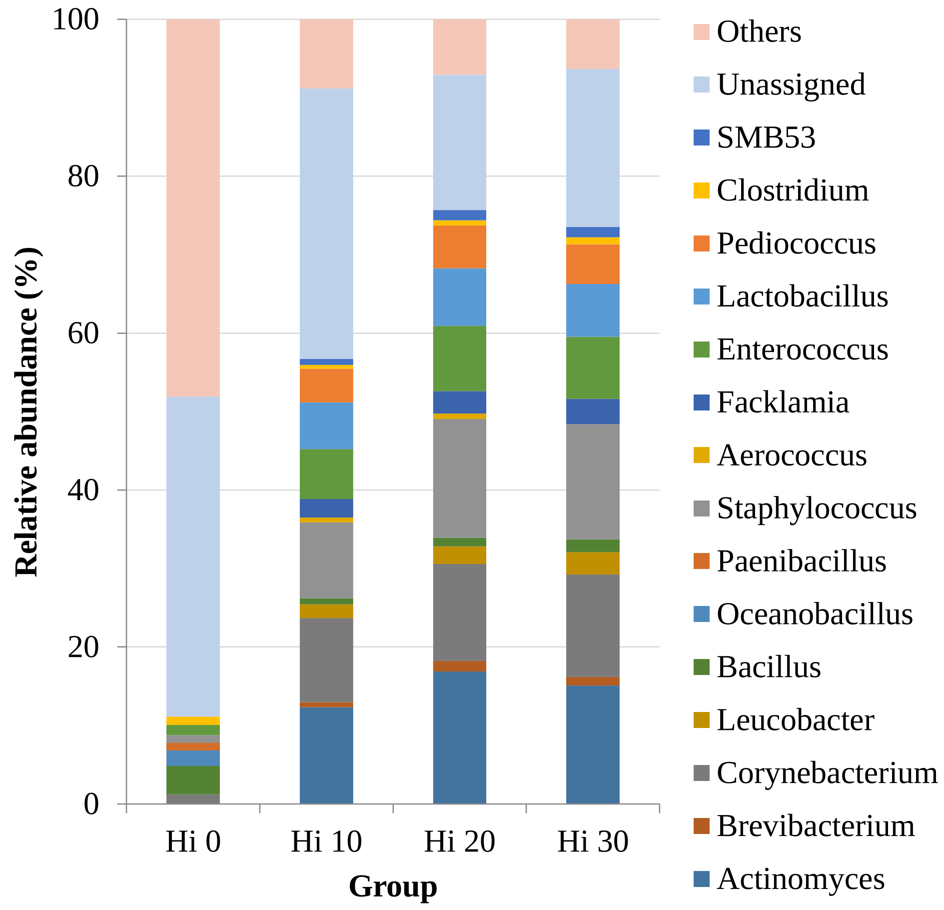
The whole microbial community profile of fecal samples was mainly comprised of 7 phyla, 12 classes, 26 orders, 68 families, 98 genera, and 55 species



At phylum level

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

	Hi 0	Hi 10	Hi 20	Hi 30
Phylum				
Actinobacteria	2.94 ± 1.16 [b]	27.30 ± 3.31[a]	35.56 ± 1.37 [a]	34.08 ± 3.58 [a]
Firmicutes	30.39 ± 9.76	40.38 ± 4.79	55.77 ± 1.55	54.66 ± 4.32
Proteobacteria	66.62 ± 9.43 [a]	32.16 ± 7.59[a]	8.59 ± 2.13 [b]	11.21 ± 7.77 [b]



At genus level

Genus	Hi 0	Hi 10	Hi 20	Hi 30
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 ± 0.32 a
Corynebacterium	1.23 ± 0.42 b	10.74 ± 1.78 a	12.33 ± 0.94 a	13.04 ± 1.40 a
Leucobacter	0.14 ± 0.08 b	1.76 ± 0.16 a	2.27 ± 0.25 a	2.83 ± 0.48 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 ± 0.04 ab	0.19 ± 0.03 ab	0.10 ± 0.01 b
Paenibacillus	0.99 ± 0.52	0.12 ± 0.03	0.23 ± 0.05	0.27 ± 0.06
Staphylococcus	0 .97 ± 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 ± 0.02 b	2.34 ± 0.46 a	2.84 ± 0.20 a	3.22 ± 0.57 a
Enterococcus	1.32 ± 0.48 b	6.36 ± 0.77 a	8.35 ± 0.27 a	7.93 ± 0.87 a
Lactobacillus	0.32 ± 0.08 b	5.98 ± 1.00 a	7.33 ± 0.59 a	6.75 ± 0.43 a
Pediococcus	0.23 ± 0.14 b	4.28 ± 0.68 a	5.45 ± 0.29 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 ± 0.02 b	0.77 ± 0.12 ab	1.30 ± 0.23 a	1.32 ± 0.11 a

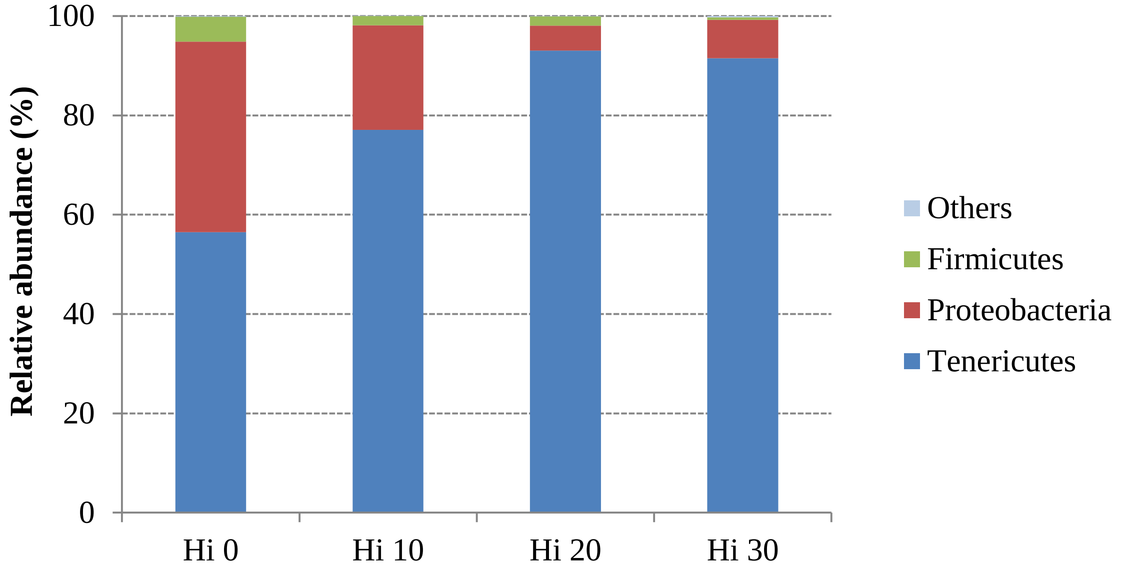
Actinomyces, *Brevibacterium*, *Corynebacterium*, *Leucobacter*, and *Staphylococcus*

LAB: *Aerococcus*, *Facklamia*, *Enterococcus*, *Lactobacillus*, and *Pediococcus*

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



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 ± 0.96

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

- ✓ 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"

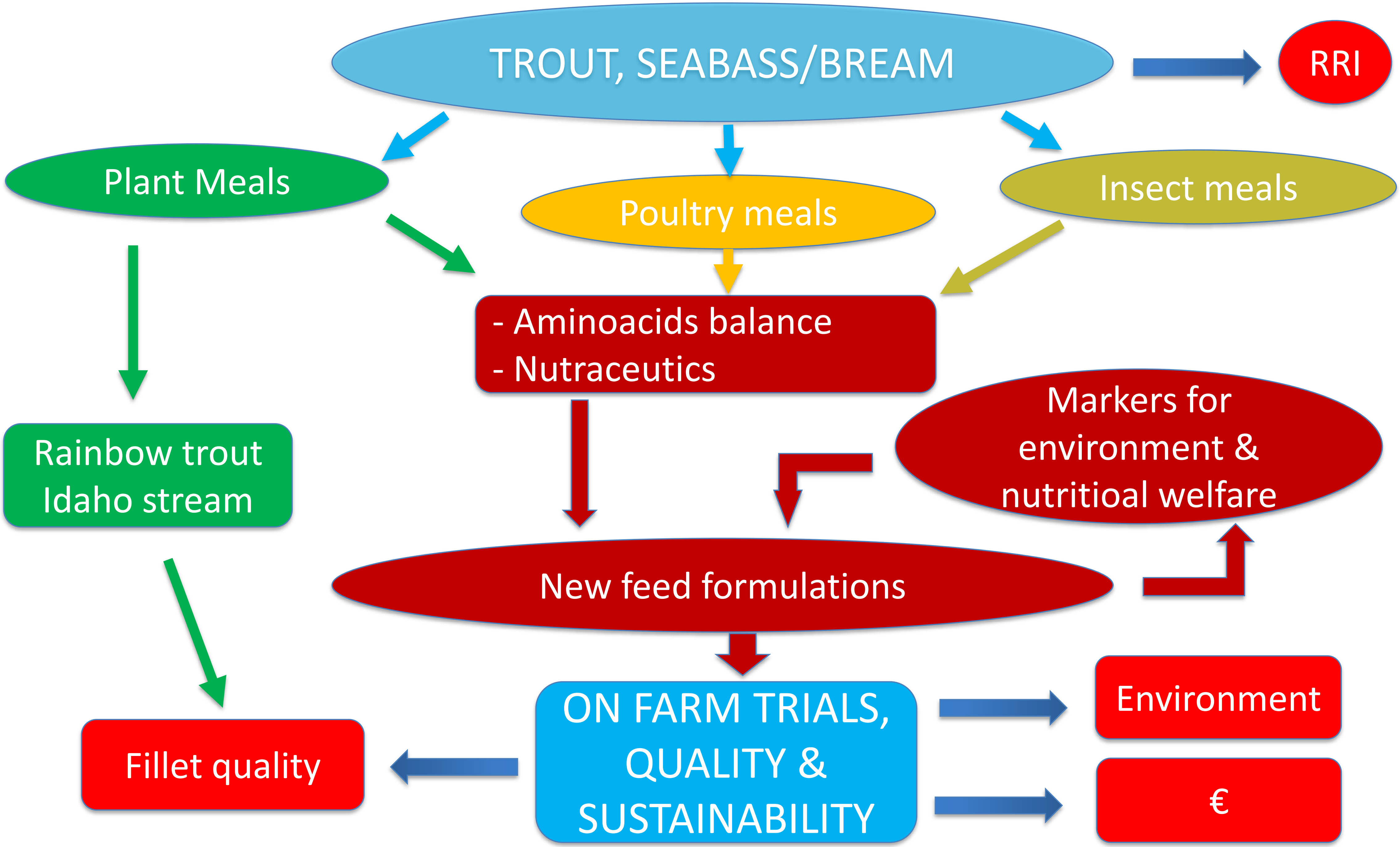

3 diets (4 replicates)

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


	CTRL	PBP	HI
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


TROUT, SEABASS/BREAM
RRI
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Fillet quality

DISSEMINATION AND RESPONSIBLE RESEARCH & INNOVATION (RRI)

Acquacoltura & Sostenibilità

Comunicazione e disseminazione dei risultati a cura di Consorzio Italbiotec

Consorzio Italbiotec

AGER 2 4F

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**
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- **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

PROGETTO AGER AGROALIMENTARE E RICERCA | FONDAZIONI IN RETE PER LA RICERCA AGROALIMENTARE

Economic f
meal in aqu
Italian s

L. Mulazzani[1], G. Malorgio[1], F. Ma
[1]University of Bologna, [2]University of S

EAFE CONFERENCE 20

ALMA MATER STUD

PeerJ

Effect of a specific composition of short- and medium-chain fatty acid 1-

Rev Fish Biol Fisheries
https://doi.org/10.1007/s11160-019-09558-y

Check for updates

ORIGINAL RESEARCH

Rainbow
modulate
in the die

Genciana Tero
Chiara Ceccott

Received: 5 Octob

animals

MDPI

Article

The Effects
illucens Pre
of Rainbow

Simona Rimoldi [1]
and Genciana Terov

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Received: 8 March 20

animals

MDPI

Article

A First Attempt to Produce Proteins from Insects by Means of a Circular Economy

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Anna Rizzolo [4], Giova
Andrea Badaile [6], Giuse
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Received: 26 March 2019;

animals

MDPI

Article

Protective Effect of Dietary Taurine from ROS Production in European Seabass under Conditions of Forced Swimming

Chiara Ceccotti [1], Basim S.A. Al-Sulaivany [2], Omar A.M. Al-Habbib [3], Marco Saroglia [1],
Simona Rimoldi [1] and Genciana Terova [1,*]

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Received: 10 July 2019; Accepted: 20 August 2019; Published: 26 August 2019

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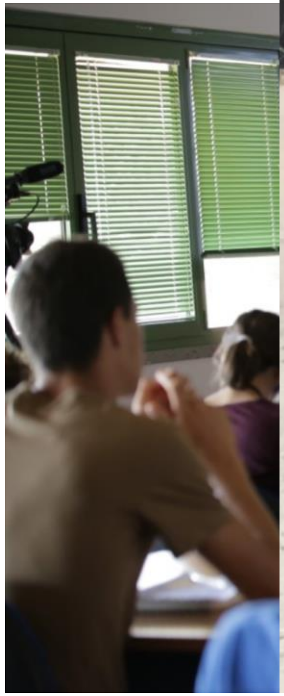


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(and coll.)

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