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Insects derived products in fish feeding: factors to consider

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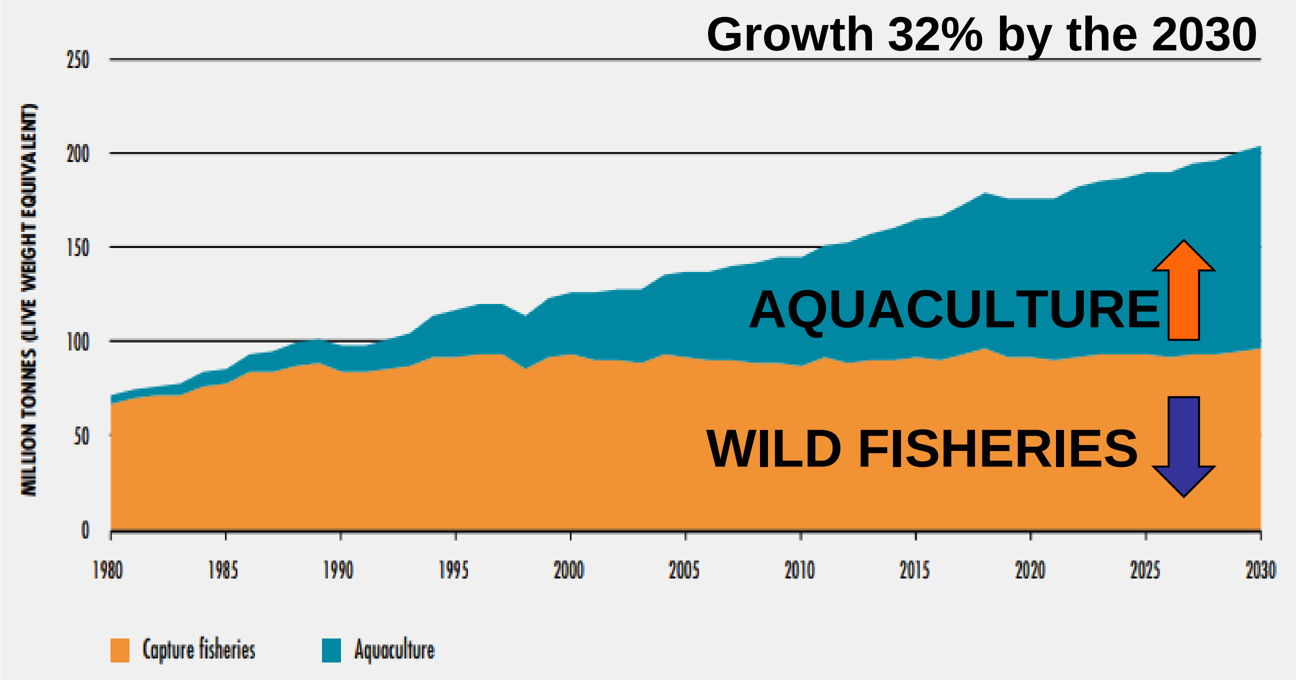
Aquaculture is expanding to meet the World Food Demand

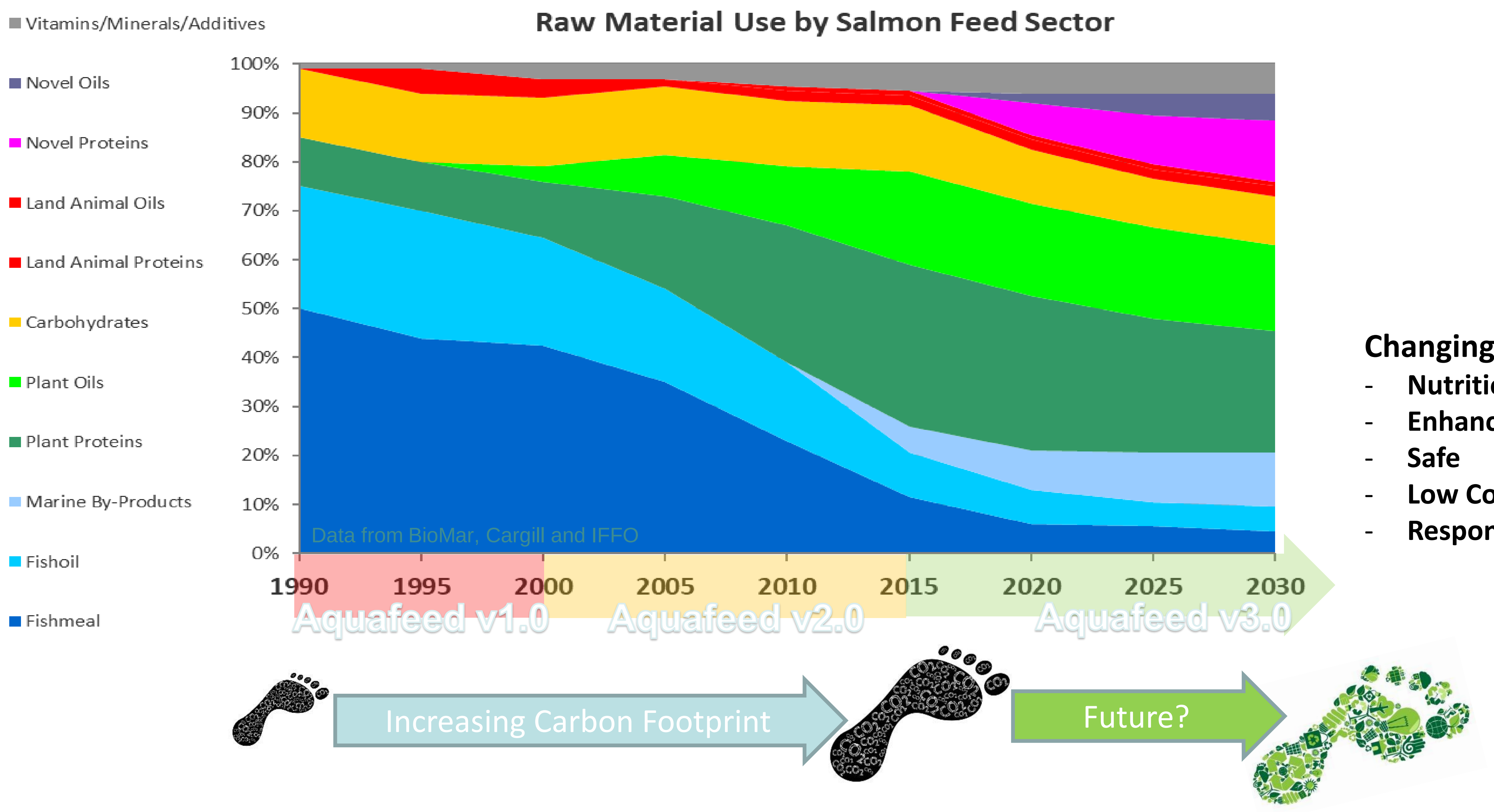


But we need to ensure that it grows
SUSTAINABLY

Changing Expectations

- **Nutritious**
- **Enhanced Qualities**
- **Safe**
- **Low Cost**
- **Responsible**

Changing Ingredient Base

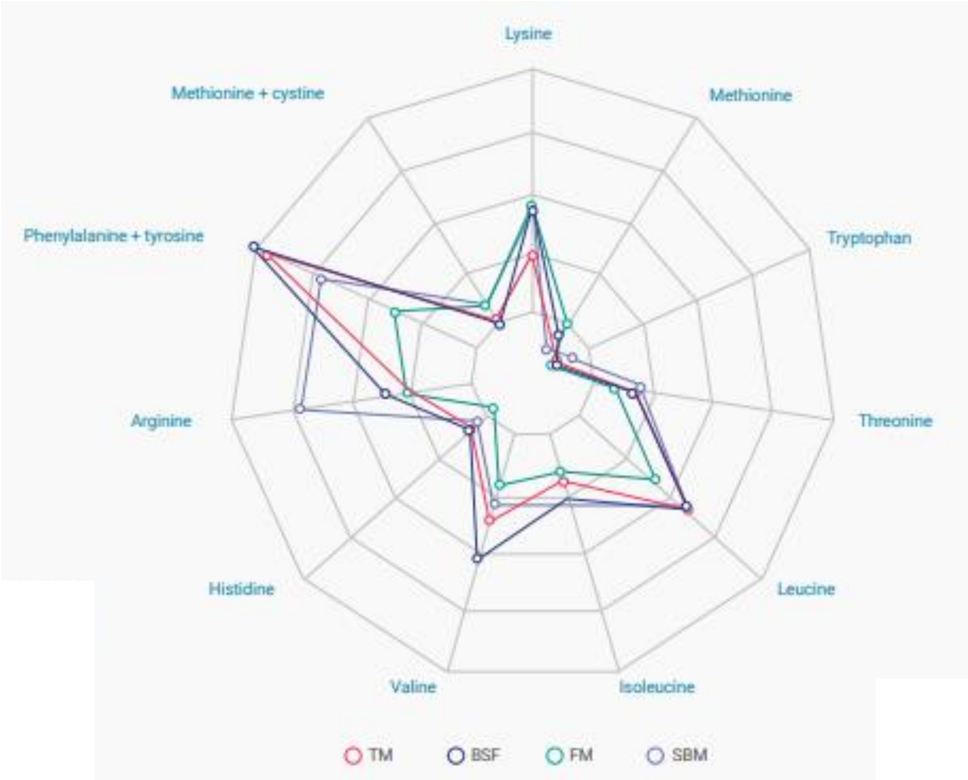


Changing Expectations

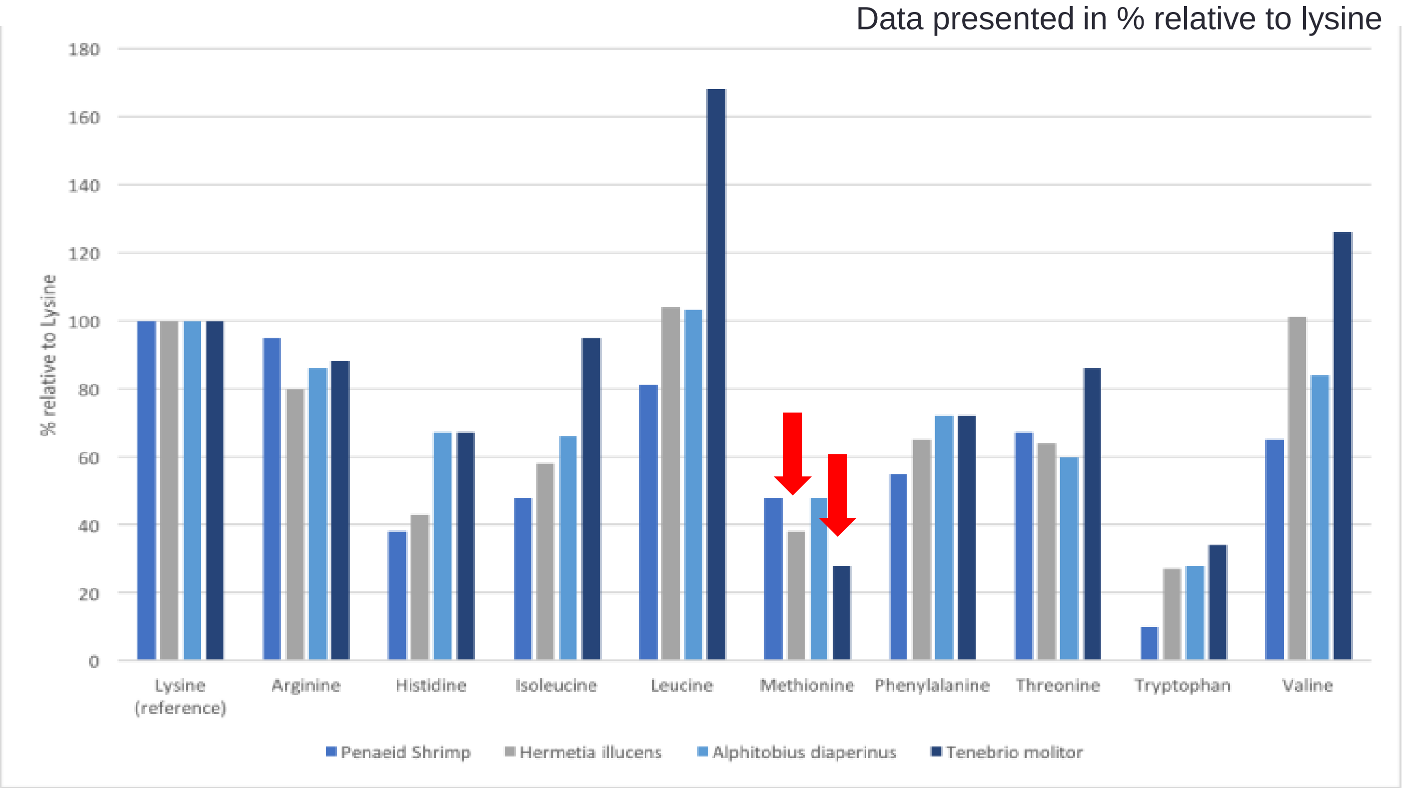
- **Nutritious**
- **Enhanced Qualities**
- **Safe**
- **Low Cost**
- **Responsible**

HOW VALUABLE ARE INSECTS?

Comparison of EAA profile of Insects Meals vs conventional raw material



Comparison of ideal amino acid profile of Penaeid shrimp vs insect meals



positive results in terms of growth rates, development as well as animal health and welfare

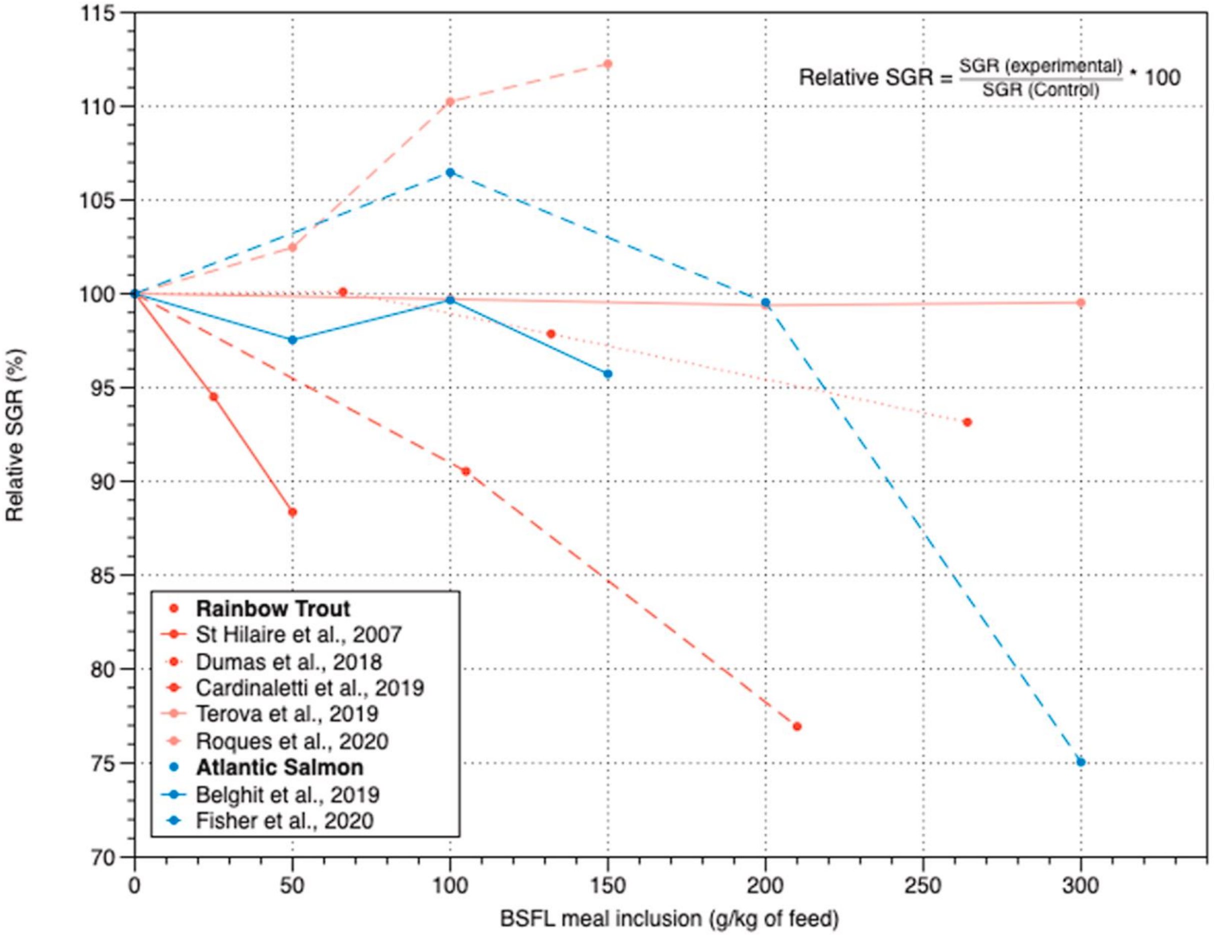
Data from NRC, 2011; Veldkamp et al., 2012

- **Lipids** (10-60% DM depending by substrate, stage and species)
- **Vitamins** (B group, unless B1 and B3)
- **Minerals** (Ca, Mg, Mn, P, Se, Fe, Zn)
- **Specific compounds**

Nutritional components	**Silkworm pupae meal**	**Black soldier fly larvae**	**Housefly maggot meal**	**Yellow Mealworm**	**Lesser mealworm**	**House cricket**[b]	**Fishmeal**	**Soymeal**
Crude protein	60.7 (81.7)	42.1 (56.9)	50.4 (62.1)	52.8(82.6)	57.3 (62.6)	63.3 (76.5)	70.6	51.8
Lipids	25.7	26.0	18.9	36.1	8.5	17.3	9.9	2.0
Calcium	0.38	7.56	0.47	0.27	0.13	1.01	4.34	0.39
Phosphorus	0.60	0.90	1.6	0.78	0.11	0.79	2.79	0.69
Ca/P ratio	0.63	8.4	0.29	0.35	1.18	1.28	1.56	0.57
EPA, 20:5n-3	0.21–0.79	0.06–0.59	–	–	–	–	0.3–11.1	–
DHA, 22:6n-3	1.96–5.52	0.03–1.66	–	–	–	–	3.7–29.1	–

(Alfiko et al., 2022)

Relative growth performance of HI-fed salmonids

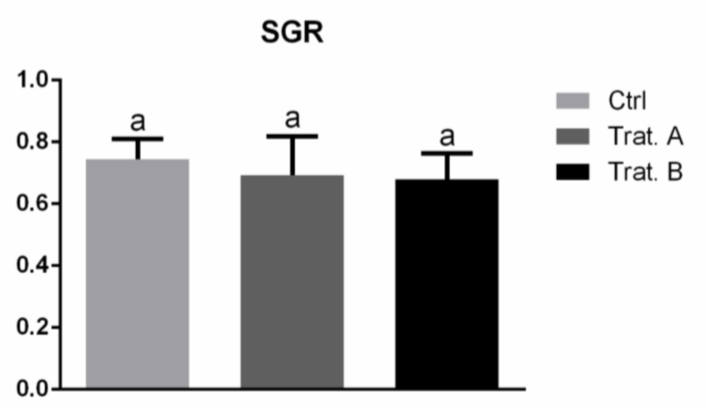


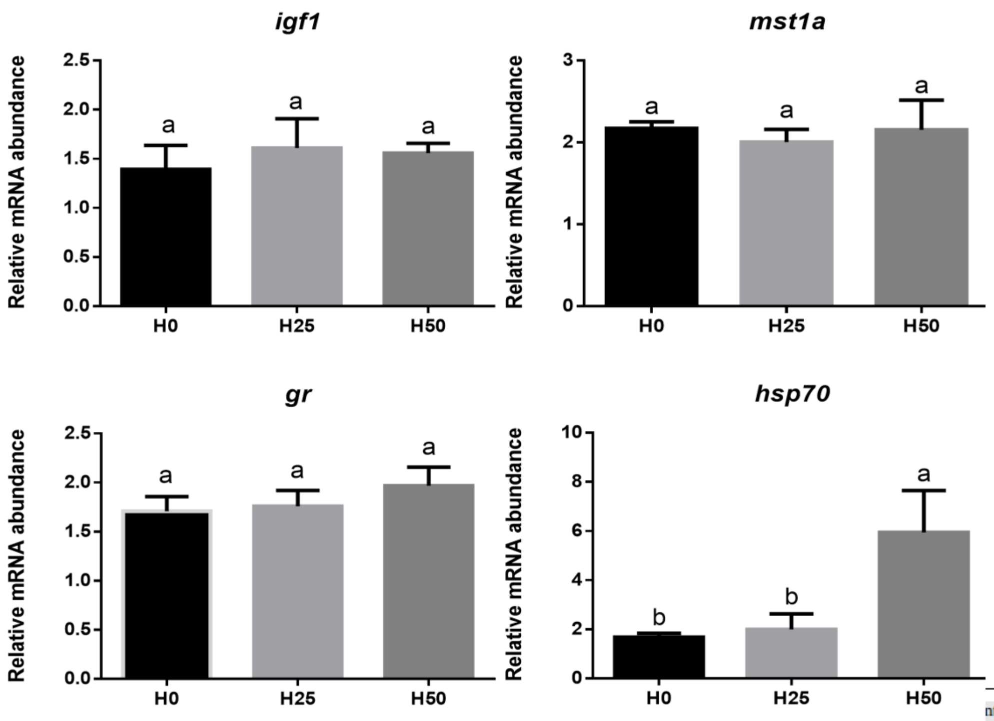
Insect meal digestibility in Rainbow trout

Insect meal	DM	Proteins	Energy	Lipids
Tenebrio	**83.4 b**	**89.9 b**	**87.2 c**	**104.4 c**
Hermetia	76.6 a	83.0 a	53.9 a	93.7 a
Hermetia + Chitinase	77.3 a	82.9 a	**81.2 b**	**100.4 b**
	P<0.001	*P<0.001*	*P<0.001*	*P<0.001*

- Higher digestibility observed for *Tenebrio* meal
- Chitinase can be added to improve digestibility of BSF meal

Effect of 25 and 50% replacement of FM with HI in diets for rainbow trout





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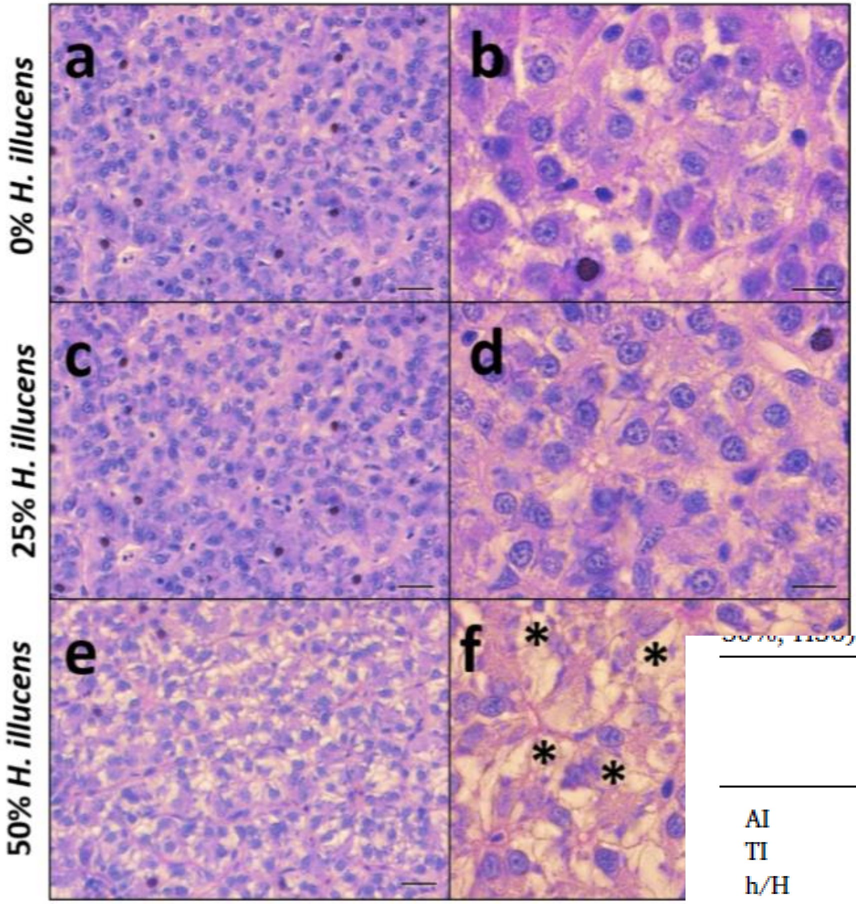
Dietary inclusion of full-fat *Hermetia illucens* prepupae meal in practical diets for rainbow trout (*Oncorhynchus mykiss*): Lipid metabolism and fillet quality investigations

Leonardo Bruni[a,*], Basilio Randazzo[b], Gloriana Cardinaletti[c], Matteo Zarantoniello[b], Fabio Mina[c], Giulia Secci[a], Francesca Tulli[c], Ike Olivotto[b], Giuliana Parisi[a]

Incresing liver lipid deposition (PAS coloration)



animals

Article

Effects of Graded Dietary Inclusion Level of Full-*Hermetia illucens* Prepupae Meal in Practical Diet for Rainbow Trout (*Oncorhynchus mykiss*)

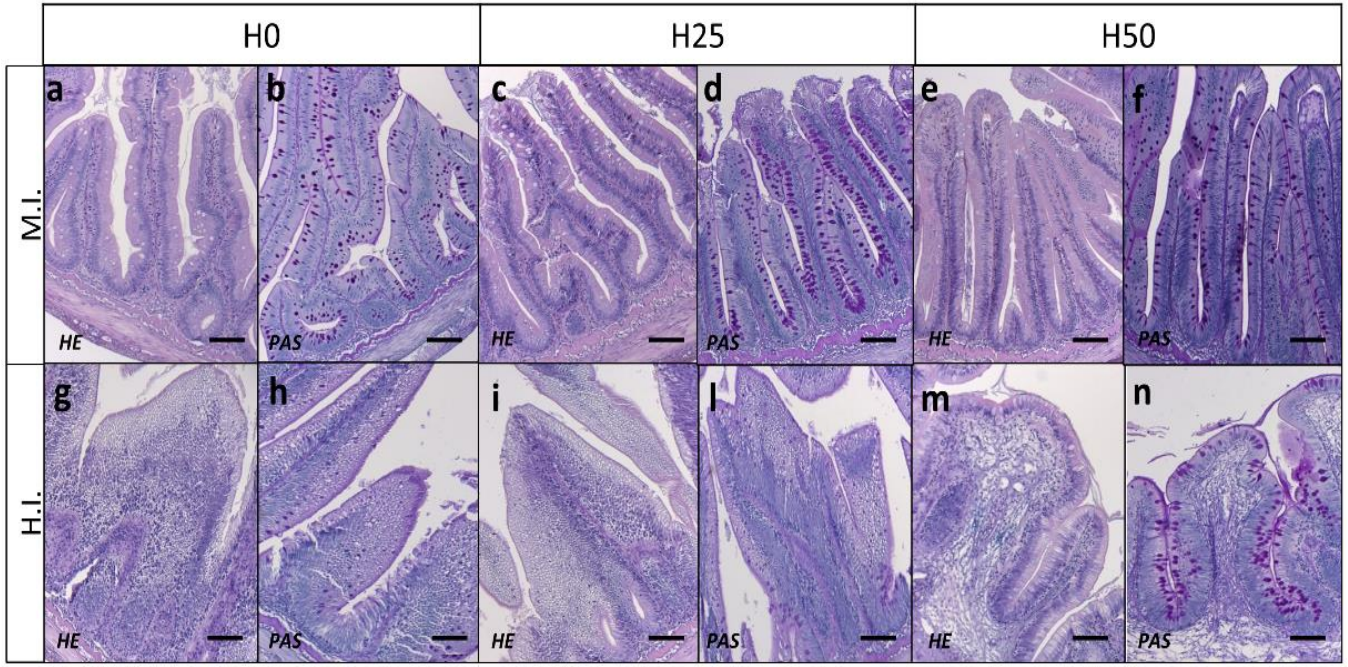
Gloriana Cardinaletti [1,*,†], Basilio Randazzo [2,*,†], Maria Messina [1], Matteo Zarantoniello [2], Elisabetta Giorgini [2], Andrea Zimbelli [2], Leonardo Bruni [3], Giuliana Parisi [3], Ike Olivotto [2] and Francesca Tulli [1]

Limited lipid fillet quality effect

	Diet			SEM[1]	*p-value*[2]
	H0	H25	H50		
AI	0.34[b]	0.36[b]	0.48[a]	0.019	***
TI	0.25	0.23	0.26	0.008	*ns*
h/H	3.30[ab]	3.63[a]	3.18[b]	0.080	*
n-3/n-6	1.41	1.42	1.35	0.089	*ns*

AI: atherogenicity index; TI: thrombogenicity index; h/H: hypocholesterolaemic/Hypercholesterolaemic fatty acid ratio.

Alteration in gut morphology in trout fed full fat BSF prepupae meal



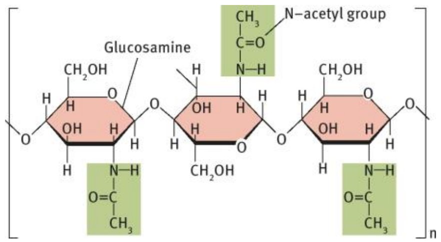
- No inflammatory events were shown in both M.I. and H.I.
- No significant differences in fold length between H0 and H25 groups
- Increase in mucus cell of H.I. at the highest BSF inclusion level.
- Significant shortening of the folds (reduced absorptive surface) in fish fed insect diets

(Data Cardinaletti et al., 2019)

Fish Performance are Based on Nutrients NOT Ingredients

Which are the Specific compounds present in Insects meal?

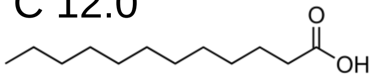
CHITIN



- Cellulose-like amino polysaccharide.
- Copolymer of **N-acetyl-D-glucosamine** and **D-glucosamine units** linked with **β-(1,4) glycosidic bonds**
- Contained in the **exoskeleton** of **insects**

LAURIC ACID

C 12:0



Dominant FA in **Black Soldier Fly (BSF)**: 13-52% FA
Beneficial effect of **MCFAs** and **Lauric acid** for animal health has being recognized

Effects of **chitin** from insect meals in fish diets appear to be complex…

Not digestible for monogastric species…

…but **teleost fish have chitinase** (Ikeda et al. 2017)

NEGATIVE EFFECTS ON GROWTH PERFORMANCE AND FEED UTILIZATION (Karlsen et al. 2015; Belforti et al. 2016; Gasco et al. 2016; Piccolo et al. 2017)

NO NEGATIVE EFFECTS ON GROWTH PERFOMANCE (Belghit et al. 2019; Bruni et al., 2020; Fawole et al., 2020; Li et al. 2017; Magalhães et al. 2017; Wang et al. 2019)

Is there a dose-response effect of chitin?

Is it due to chitin or other components of insect meal?

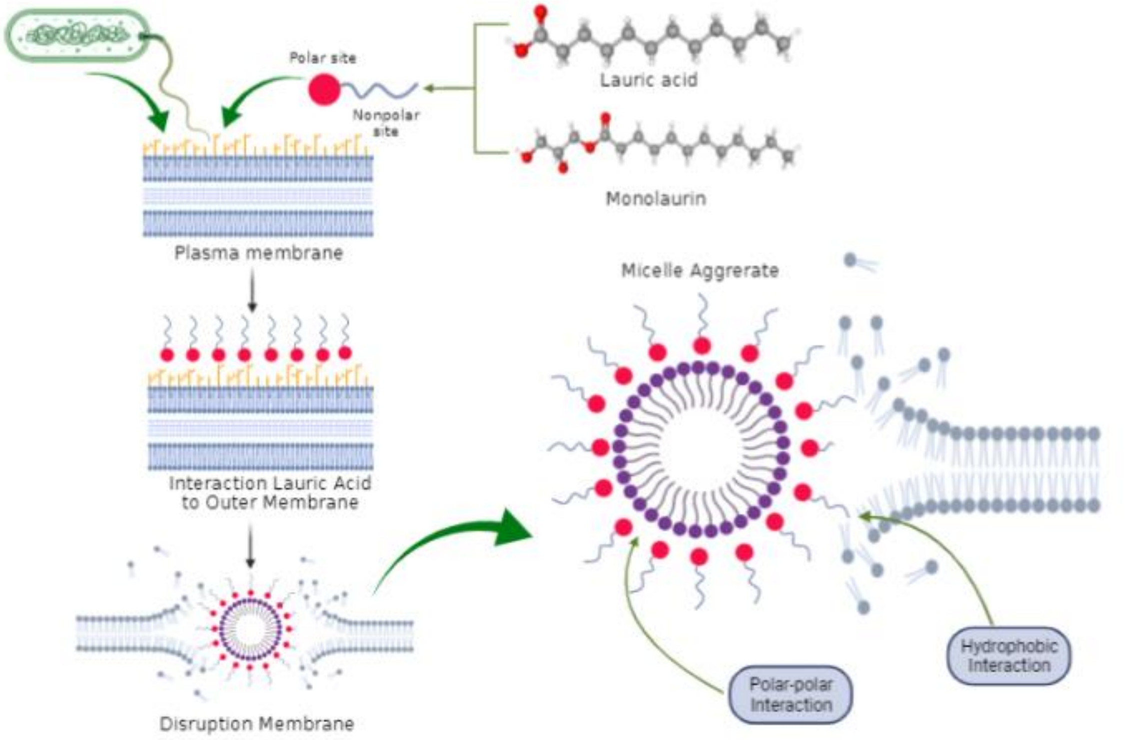
LAURIC ACID

Dodecanoic acid (C12:0) is the major fatty acid in Black Soldier Fly (29–50% of the total FA).

"Lauric acid and monolaurin have a strong ability to destroy gram-positive bacteria, especially *S. aureus*, fungi such as *C. albicans*, and viruses including vesicular stomatitis virus (VSV), herpes simplex virus (HSV), and visna virus (VV)"
(Nitbani et al. 2022)

***In vitro* study**

- Pathogen cells membrane rupture (activity against *Aeromonas hydrophila* and *Ichthyophthirius multifiliis* (Do Couto et al. 2021).
- Reduce the virulence of *Saprolegnia parasitica* (Lone and Manohar, 2018)



Cell membrane disruption by lauric acid and monolaurin

AIM

General aim

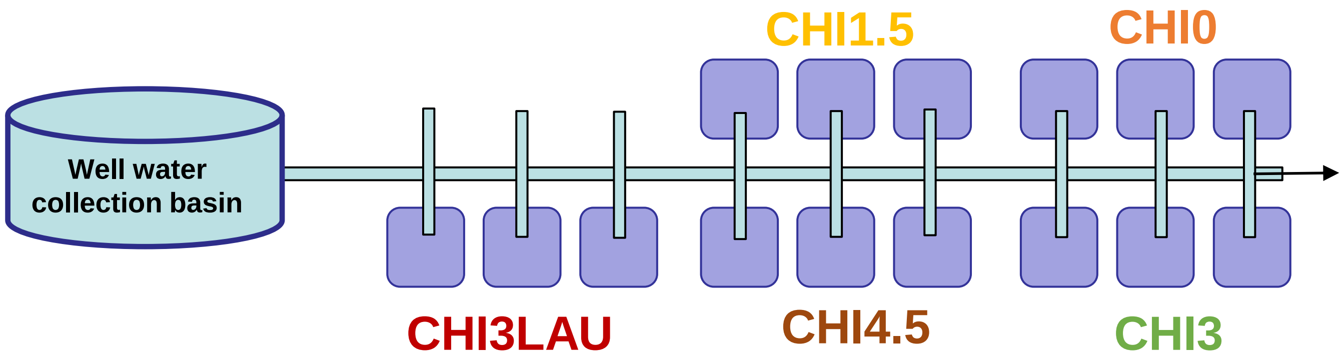
Investigate the potential of insect meal inclusion in animal feeds for environmental sustainability and animal one health

Research aim

Effect of dietary **chitin** and **lauric acid** on the metabolism and gut functionality of **rainbow trout** (*Oncorhynchus mykiss*)

EXPERIMENTAL DESIGN

EXPERIMENTAL DESIGN: MONOFACTORIAL WITH TRIPLICATES PER TREATMENT



EXPERIMENTAL TREATMENTS

Basal semipurified diet +

↑

Highly digestible diet formulation

CHI0	CHI1.5	CHI3	CHI4.5	CHI3LAU
0% chitin	1.5% chitin	3% chitin	4.5% chitin	3% chitin and 1.1% lauric acid
	↑ 150 g/kg BSF	↑ 300 g/kg BSF	↑ 450g/kg BSF	↑ 300g/kg BSF

DIET FORMULATION

Formulation of the experimental diets

g/100 g	CHI0	CHI1,5	CHI3	CHI4,5	CHI3LAU
Fish Meal (67%)	34	34	34	34	34
Casein	28	28	28	28	28
Fish oil	12	12	12	12	11
Dextrine	3.4	3.3	3.2	3	3.2
Wheat flour	14	14	14	14	14
Cellulose	**4.2**	**2.8**	**1.4**	**0.1**	**1.3**
Soy lecithin	1	1	1	1	1
Chitin	**0**	**1.5**	**3**	**4.5**	**3**
Lauric acid	0	0	0	0	**1.1**
Min + vit premix	1.4	1.4	1.4	1.4	1.4
Agar Agar	1	1	1	1	1
Celite®	**1**	**1**	**1**	**1**	**1**

Chemical composition of the experimental diets

% DM	CHI0	CHI1,5	CHI3	CHI4,5	CHI3LAU
Crude Protein	50.15	50.14	50.67	50.01	51.42
Crude Lipid	15.8	15.51	15.81	16.3	15.22
Crude Fiber	1.12	2.36	3.37	4.36	3.68
Ash	7.24	7.42	7.43	7.95	7.64
Chitin	0	1.5	2.88	4.08	2.82
NFE	25.69	23.07	19.84	17.3	20.22

1. Diets Manifacturing at the University of Udine

2. Diets mixed with a grinder

3. Cold extruded Pellet 3 mm

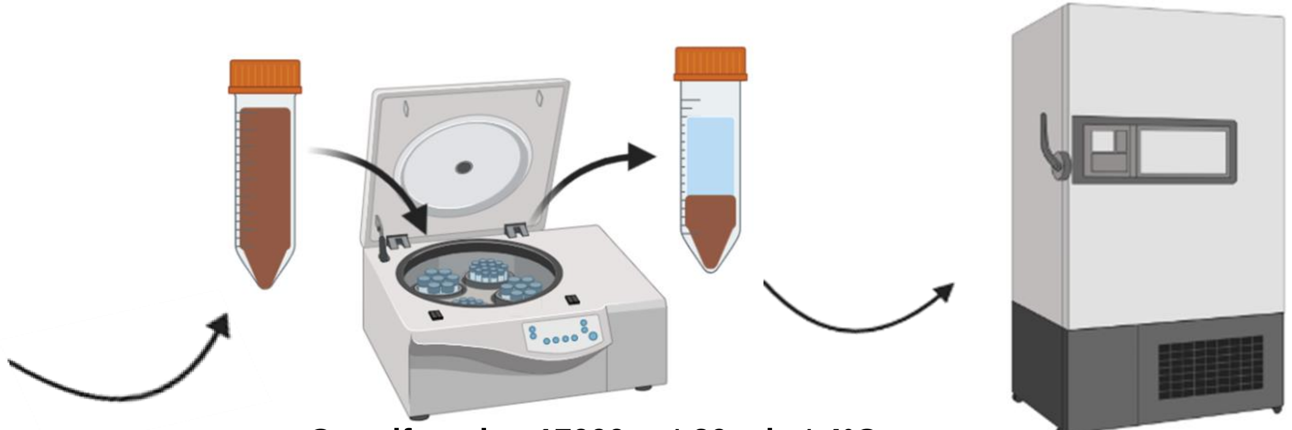
In vivo TRIAL

Settling column Guelph system

(Cho & Slinger, 1982)

Feces collection for *in vivo* nutrient digestibility evaluation

- **225 Rainbow trout juveniles** (27.4±2.4g IBW) randomly distributed in the 5 units of 3 tanks (15 fish/tank)
- Experimental trial lasted 10 weeks
- All diets were offered once a day at 9:00am, at 1% of live weight (uneaten feed was weighted and registered)
- Monitored rearing conditions (Temperature 13.6±0.6°C, Dissolved oxygen 9.4±0.3 mg/L, pH 8.2±0.1)



Centrifugation 17000xg * 20 min * 4°C

Storage at -20°C until analysed

DIET AND FECES CHEMICAL DETERMINATIONS

Dry matter: drying in an oven at 105 °C, up to constant weight (16-18 h) (AOAC 934.01)

Ash: by gravimetric method after combustion at 550 °C until constant weight (AOAC 942.05)

Crude lipid: as an ether extract using the Soxhlet method (AOAC 2003.05)

Kjeldahl nitrogen by distillation and the crude protein was calculated as N × 6.25 (AOAC 2001.11)

Acid Insoluble Ash: ash fraction insoluble in an HCl solution (AOAC 941.12-1941)

Acid detergent Fiber: by gravimetric method after digestion with an acid solution (cellulose, lignin, cutine and insoluble minerals)

Acid detergent Lignin: determined gravimetrically as the residue remaining upon ignition after 72% H_2SO_4 treatment.

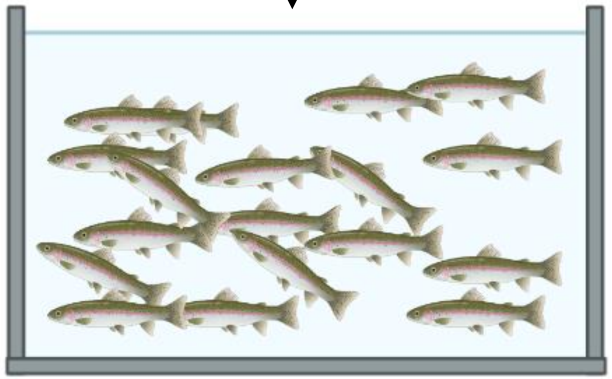
Chitin: was estimated as ADF – ADL (Hahn et al ,2018)

NUTRIENT AVAILABILITY ESTIMATION:

Protein and **lipid** apparent digestibility coefficients (ADC) of the diets were computed as follows:

$$ADC_{Diet} = 1 - \frac{\%\ \text{indicator in the diet}}{\%\ \text{indicator in feces}}\ x\ \frac{\%\ \text{nutrient in feces}}{\%\ \text{nutrient in the diet}}$$

After **6 hrs fasting**

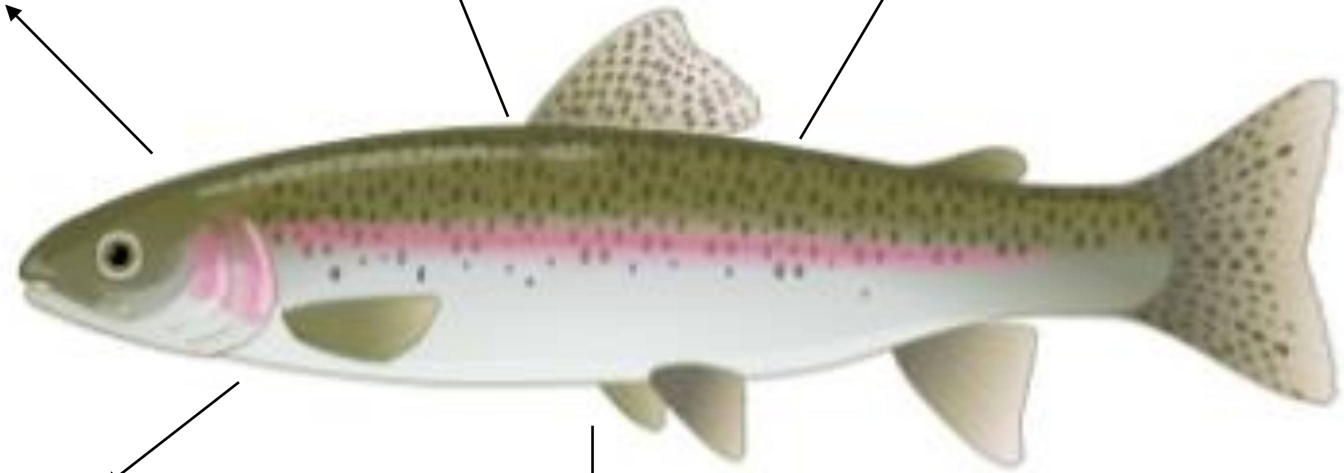


Suppression with a lethal dose of anesthetic
(OPBA authorization prot. 8/2021)

Zootechnical performance
(n=3)

In vivo
Nutrient availability
(n=6)

Gut physiology
(BBM enzymes: sucrase & maltase, ALP and L-ANP)
(n=6)



Intestinal microbiota
(n=6)

Gene Expression
(Gchiom and PEPS)
(n=6)

Metabolic status
(Plasma metabolites and enzymes)
(n=8)

Zootechnical performance

	CHI0	CHI1.5	CHI3	CHI4.5	CHI3LAU
[1]RFI (g/kg/d)	9,67 ± 0,5^{ab}	10,30 ± 0,9^{ab}	10,16 ± 0,3^{a}	11,4 ± 0,8^{b}	10 ± 0,3^{a}
FBW (g)	56,65 ± 4,7^{a}	54,94 ± 4,9^{a}	59,03 ± 2,9^{a}	42,48 ± 3,4^{b}	59,34 ± 2,8^{a}
[2]SGR (%)	0,97 ± 0,2^{b}	1,08 ± 0,1^{ab}	1,18 ± 0,1^{a}	0,73 ± 0,1^{c}	1,04 ± 0,0 ^{ab}
[3]FCR (%)	1,07 ± 0,2^{a}	1,02 ± 0,0^{a}	0,93 ± 0,1^{a}	1,65 ± 0,2^{b}	1,03 ± 0,1^{a}

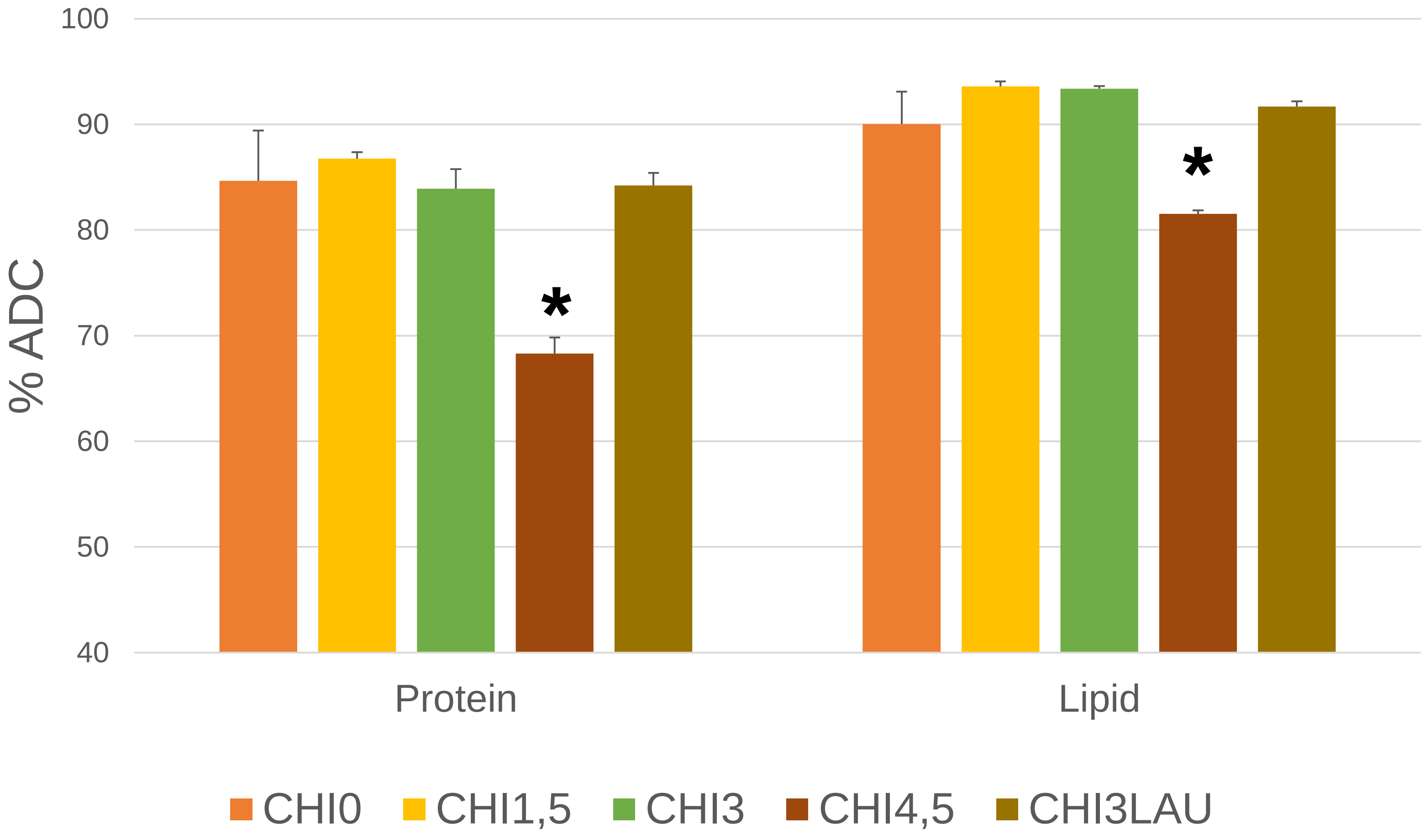
Row means with different superscript letters are significantly different (P<0.05).

RFI: Feed intake /(Final Body Weight + Initial Body Weight) / 2 / days
SGR: 100 × [(ln final body weight − ln initial body weight) / days]
FCR: feed intake / weight gain

Dietary chitin at highest inclusion level (CHI4.5) negatively affects overall growth performance parameters over 10 weeks feeding.

Nutrient Digestibility

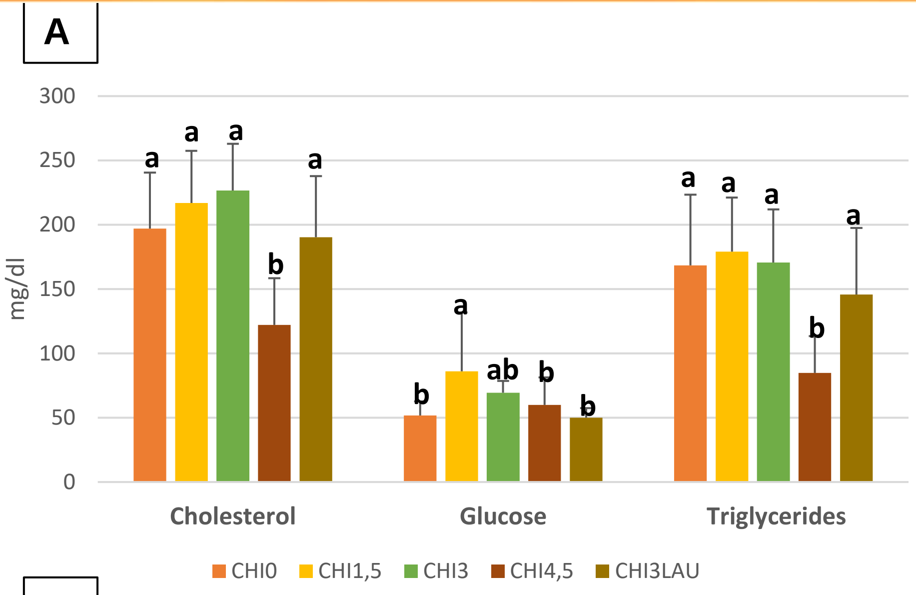
Protein and lipid digestibility was negatively affected by the highest dietary chitin inclusion level (CHI4.5) (P<0.05)

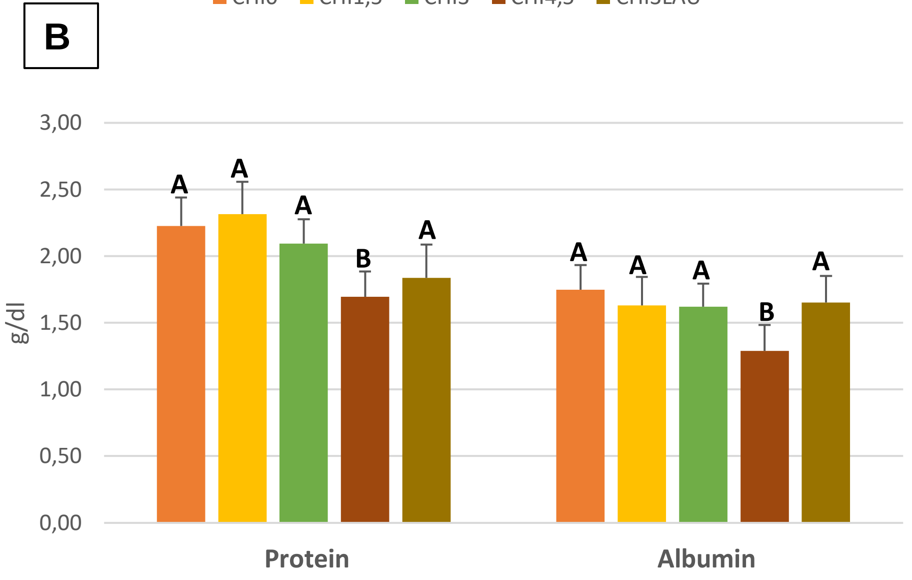


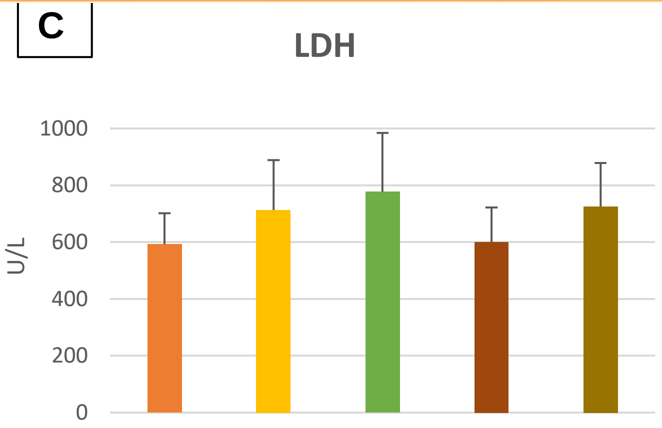
Metabolic status

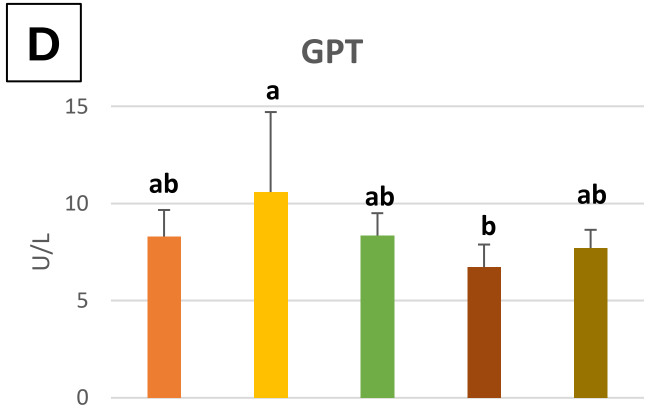
Plasma metabolites related to nutritional conditions (cholesterol, protein and albumin) were reduced in fish fed the CHI4.5 diet (**A** and **B**). Intermediate values were observed for the Glucose (**A**)

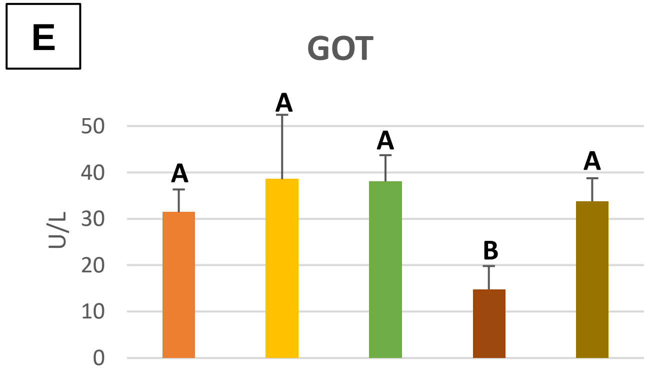
Glutamic oxalacetic transaminase, (GOT) has been downregulated by the highest % of Chitin (**E**). Intermediate values were observed for the glutamic pyruvic transaminase (GPT) (**D**)





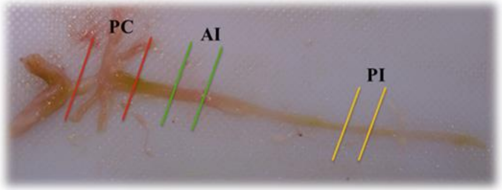


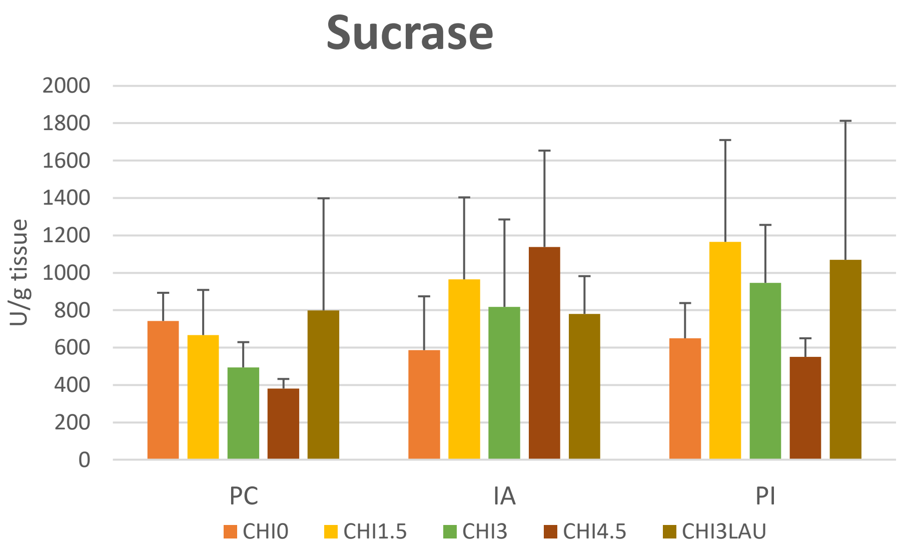


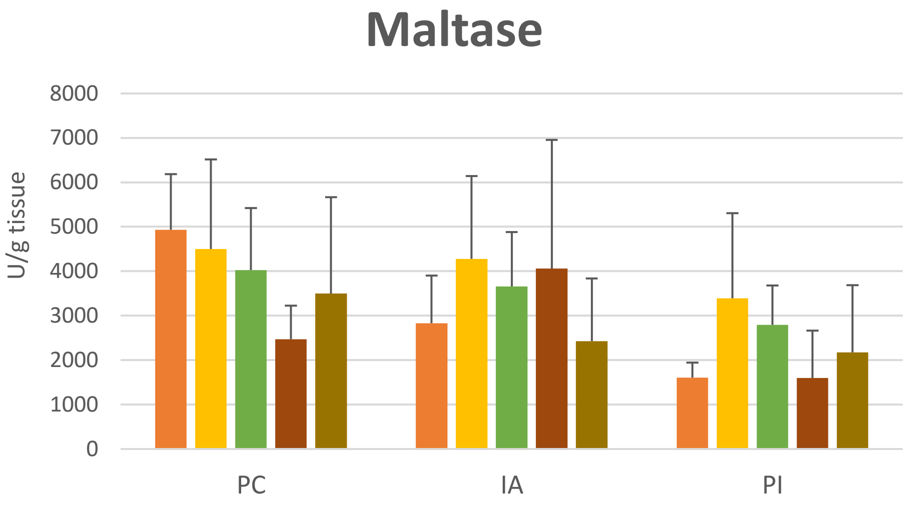


(n=6; lower case letter P<0.05; capital letter P<0.001)

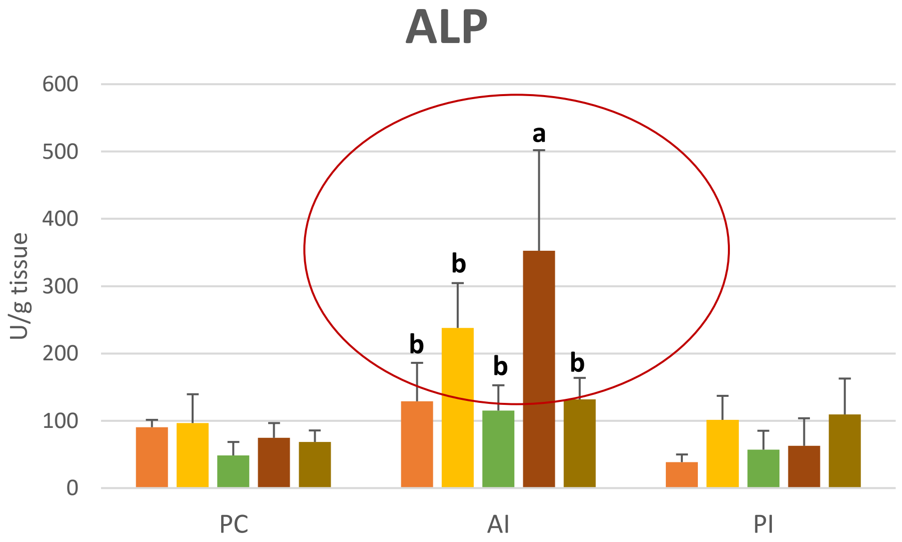
Gut functionality

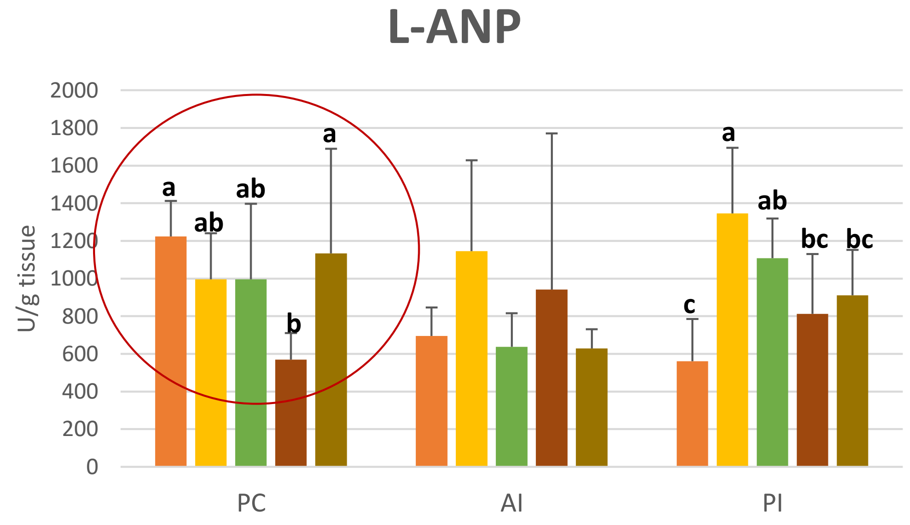






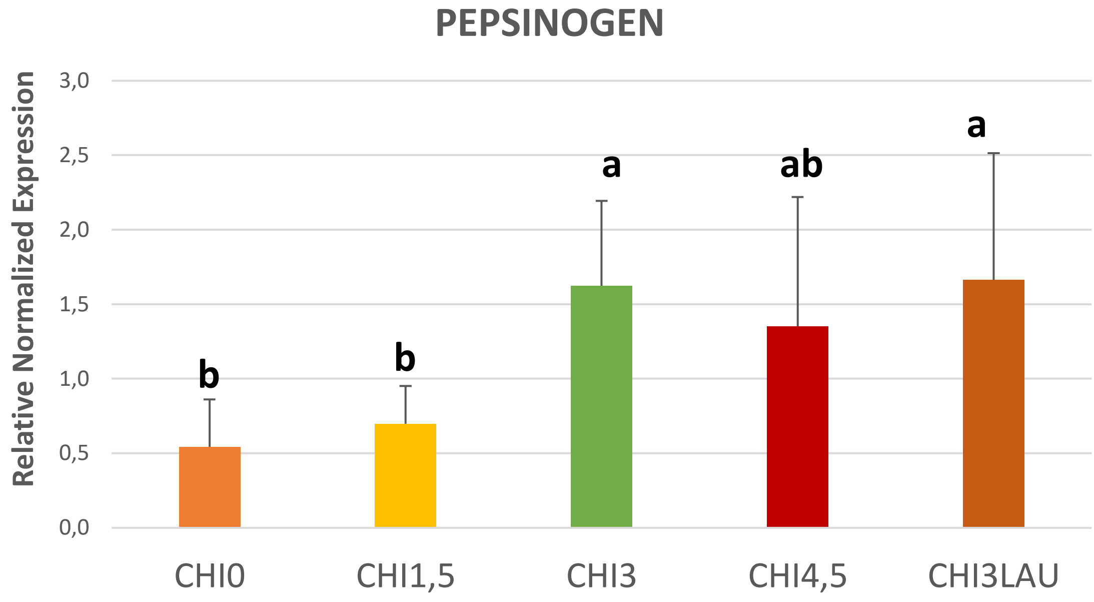
Dietary chitin level affected the activity of Alkaline Phosphatase (ALP) and Leucine-amino peptidase (L-ANP) of the brush border membrane enzymes



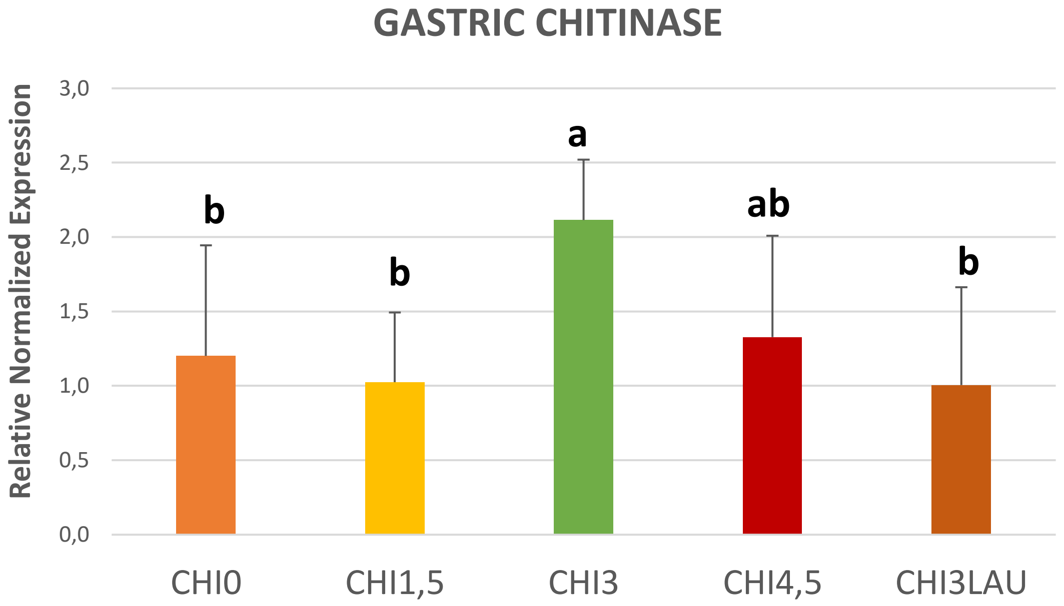


(n=6)

Gene expression



Pepsinogen gene expression in the stomach was upregulated for chitin inclusion level higher than 3% ($P<0.05$). Probably, this is due to a lower digestive transit.



Gastric chitinase gene expression is slightly modulated by the dietary chitin levels in rainbow trout.

Chitin Effect Evaluation

GROWTH PERFORMANCE

- Final body weight (g)
- Lenght (cm)
- Total of feed intake (g)

DIET DIGESTIBILITY

- Feces collection for the apparent digestibility coefficient (**ADCs**) calculations

DIGESTIVE TRACT INTEGRITY & FUNCTIONALITY

- Stomach, intestine, and liver **histomorphology**
- **BBM** Enzyme activity
- **Digestive** enzyme activity
- **Expression of gene** involved in the digestive process

ANIMAL WELFARE

- Plasma & serum collection
- Intestinal microbiota

as **CHITIN** **could**

- act as **immunostimulant** (Henry et al. 2015, 2018a, 2018b)
- have prebiotic effects in the **modulation** of fish **gut microbiota**

(Bruni et al. 2022; Terova et al. 2019; Gaudioso et al. 2021)

Alpha-diversity indices of the analysed samples (n = 5).

Index	p-value	
	Diet effect	Sample origin effect
Observed ASVs	0.7147	0.0015
Pielou's evenness	0.1835	0.1097
Faith's phylogenetic diversity	0.0233	0.4959

ASVs, amplicon sequence variants.

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Effect of diets containing full-fat *Hermetia illucens* on rainbow trout microbiota: A dual cultivation-independent approach with DGGE and NGS

Leonardo Bruni[a,*], Vesna Milanović[b], Francesca Tulli[c], Lucia Aquilanti[b], Giuliana Parisi[a]

Conclusion

- Dietary level of chitin up to **3%** is tolerated by juvenile rainbow trout as demonstrated by **growth performance** parameters and protein and lipid **ADCs**

- Higher dietary levels (**4.5%)** negatively affect both fish **growth performance** both **nutrient absorption** supported by the lowest activity of the Leucine-amino peptidase (L-ANP) registered in pyloric caeca and interference with the **lipid metabolism** as suggested by triglycerides and cholesterol circulating levels

- **Alkaline Phosphatase** activity suggest a possible inflammatory action of chitin at high level.

- **Gastric pepsinogen gene** expression was activated for chitin inclusion level higher than 3% probably, for the lower digestive transit.

Present data indicate that a dietary chitin level up to 3% is well tolerated by rainbow trout without hampering growth and physiological response.

up to 3 % of chitin — **CHI3** → **up to 300 g/kg of BSF meal**

animals

MDPI

Article

Replacing Fish Meal with Defatted Insect Meal (Yellow Mealworm *Tenebrio molitor*) Improves the Growth and Immunity of Pacific White Shrimp (*Litopenaeus vannamei*)

Constant Motte [1,*], Alfredo Rios [1,*], Thomas Lefebvre [1], Hong Do [1], Morgane Henry [2] and Orapint Jintasataporn [3]

THANK YOU FOR YOUR ATTENTION

Fr

ANY QUESTIONS?

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hic sunt futura

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