Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXV

Development of circular economy in Marche Agrifood supply chain: from vegetable waste to the production of valuable fish species.

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BACKGROUND

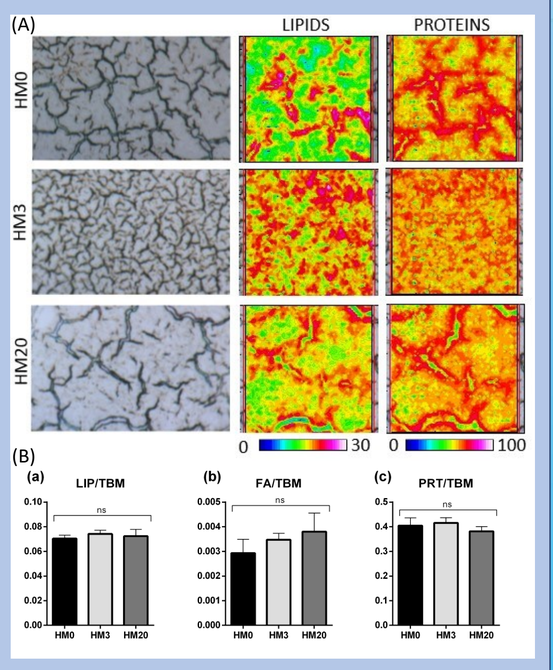
The 1st year of my PhD project provided results on the larval growth performance of *Hermetia illucens* and its nutritional value, as well as the best growth substrate, coffee silverskin with an inclusion of 15% of Spirulina (*Arthrospira platensis*). During the second year of PhD insect meal was used to formulate and produce aquafeeds for aquaculture trials. Specifically, a control diet based on fish meal, fish oil and vegetable protein was used and then 2 experimental diets including 3% and 20% of insect meal respect to fish meal have been formulated. A first trial on rainbow trout (*Oncorhynchus mykiss*) was performed at "Unità di Ricerca e Didattica di San Benedetto del Tronto (AP, Italy), URDIS-University of Camerino" and consisted in a feeding trial of 42 days. A second trial on giant freshwater prawn (*Macrobrachium rosenbergii*) was performed at the aquaponics facility "Cooperativa Agricola Tanto Sole" (Treia, Macerata, Italy) and lasting 60 days**.**

TROUT

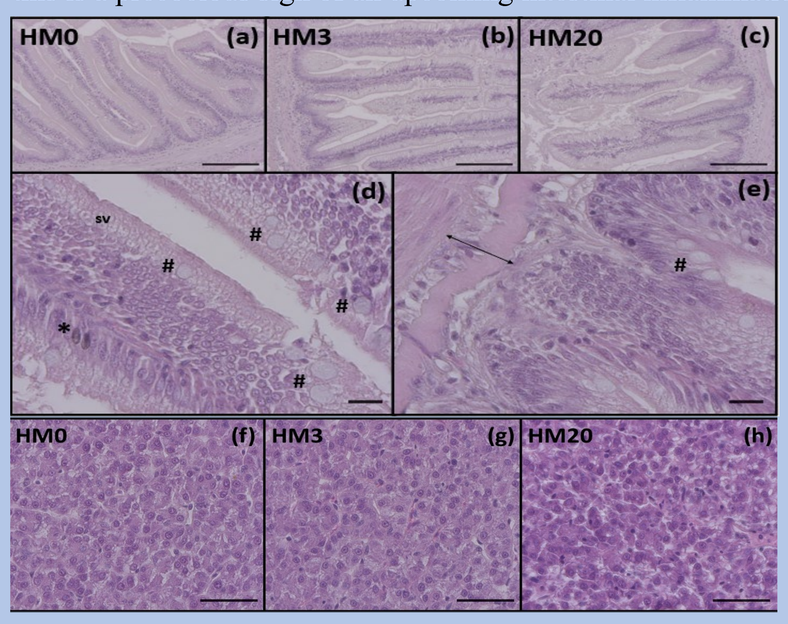
METHODS Five hundred forty rainbow trout juveniles were randomly divided into nine square fiberglass tanks (in triplicate). Fish were hand-fed on the three experimental diets at apparent satiety for 6 weeks. At the end of the trial, fish were individually measured and liver, pyloric caeca, distal intestine and whole fish were sampled. Dietary FA composition and the physiological response of fish to different diets were analyzed through a multidisciplinary approach including biometry, histology, spectroscopy, gas chromatography and molecular biology. **RESULTS S**urvival rate of fish was 100% in all the experimental groups and fish growth performance did not show significant differences among the experimental groups. This result was fully supported by the molecular analysis. Dietary FA analysis revealed an increasing percentage content of total PUFA, ω3 PUFA, DHA and EPA from HM0 to HM20 diets. As a consequence, the expression of genes related to lipid metabolism did not show significant differences among the experimental groups. Even carotenoids and total tocopherols showed the same increasing trend. Interestingly, the fatty acid profile of HM20 trout fillets was significantly enriched in ω3-PUFA, especially in C18:3-ω3, while HM3 contained higher level of DHA than the control group. Histological analysis showed that the mucosal fold height was significantly reduced in fish fed HM20 diet respect to HM0, but no sign of intestinal inflammation was detected in all experimental groups. However, most of the genes involved in the immune response showed an upregulation in HM20 group respect to HM3 and HM0 probably related to an upcoming intestinal inflammation. Microbiome analysis showed a slight but significant decrease in phylogenetic richness in fish fed HM diets that could generate a potential dysbiosis. Finally, no differences in the liver histology were observed and FT-IR analyses supported these results. In addition, stress gene expression did not show differences among experimental groups. **CONCLUSION** The study evidenced that lipids and bioactive molecules from *A. platensis* were successfully transferred to the fish diets. As regards fish fatty acids profile, a dose dependent ω3-PUFA increase was observed from H0 to H20 group. Furthermore, fish welfare was not negatively affected by the experimental diets, while an increased immuno-related gene expression was detected only in HM20; this result requires further studies in which longer feeding trials should be performed in order to understand if this is a precocious sign of an upcoming intestinal inflammation event.

Samples	Observations	Chao1	Shannon
HM0	582	589.03	8.60
HM3	494	499.90	8.03
HM20	496	527.68	8.21

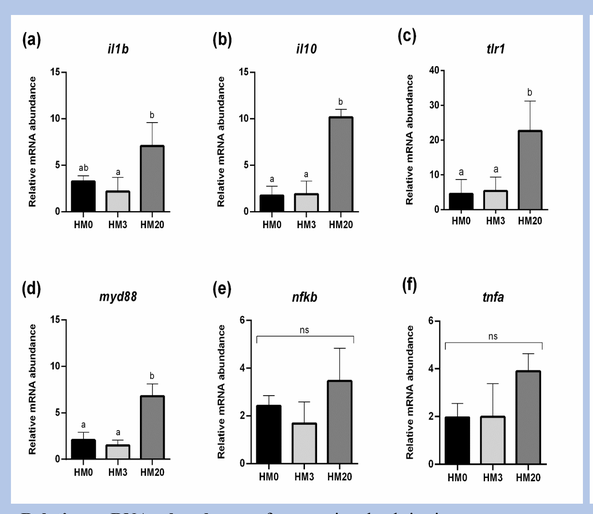
Microbiome analysis. Diversity richness (Chao 1) and diversity index (Shannon) in the gut content of rainbow trout fed experimental diets: HM0, HM3, and HM20.



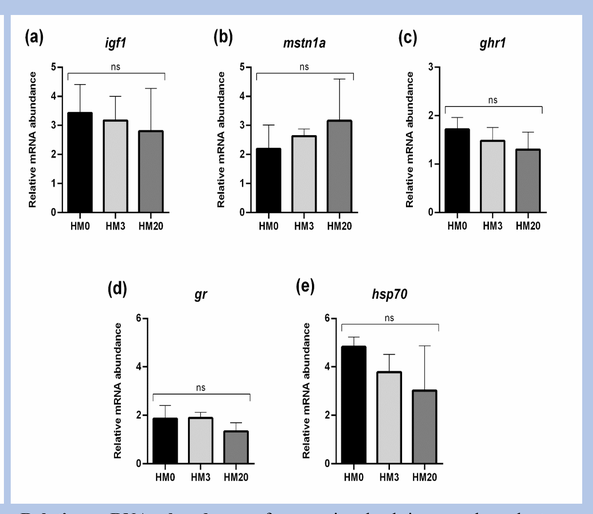
Hyperspectral imaging and biochemical composition of liver samples of rainbow trout fed the experimental diets. (A) False color images showing the topographical distribution of lipids and proteins. (B) Statistical analysis of: (a) LIP/TBM; (b) FA/TBM and (c) PRT/TBM band area ratios. (TBM = total body mass).



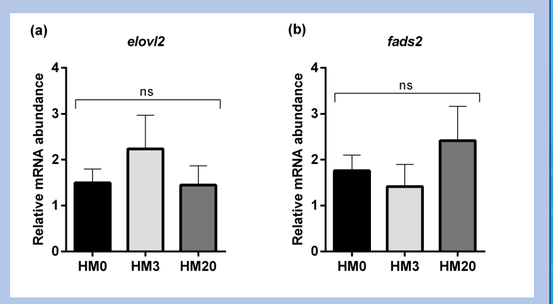
Example of histological images of distal intestine (a-e) and liver parenchyma (f-h) of rainbow trout fed experimental diets. (a,f) HM0, (b,g) HM3 and (c,h) HM20. (d,e) Details of mucosal folds (asterisk indicates melanomacrophages, hashtag indicate goblet cells, sv indicates supranuclear vacuoles and arrow indicate submucosa width).



Relative mRNA abundance of genes involved in immune response, analyzed in distal intestine samples of rainbow trout fed the experimental diets. (**a**) *il1b*, (**b**) *il10*, (**c**) *tlr1*, (**d**) *myd88*, (**e**) *nfkb*, (**f**) *tnfa*.



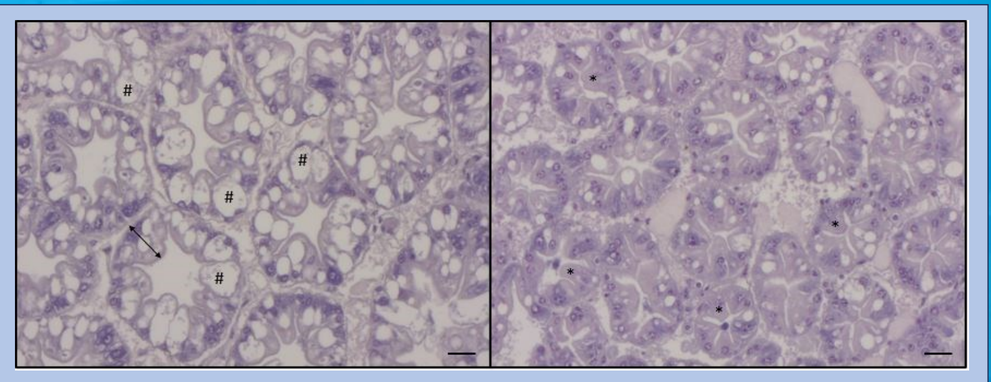
Relative mRNA abundance of genes involved in growth and stress response analyzed in liver samples of rainbow trout fed the experimental diets. (**a**) *igf1*, (**b**) *mstn1a*, (**c**) *ghr1*, (**d**) *gr*, (**e**) *hsp70.*



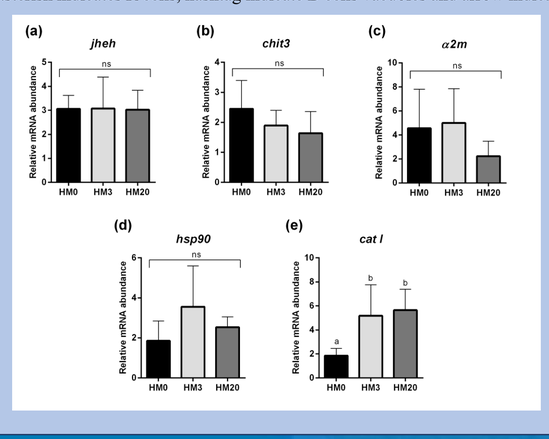
Relative mRNA abundance of genes involved in in lipid metabolism, analyzed in pyloric caeca samples of rainbow trout fed the experimental diets. (**a**) *elovl2*, (**b**) *fads2*.

PRAWNS

METHODS One thousand nine hundred seventy-one giant freshwater prawns juveniles were randomly divided into nine aquaponic systems according to the three dietary treatments (in triplicate). Each system consisted of a hydroponic unit for plants cultivation (15 *Lactuca sativa* seedlings) and a prawns tank. At the end of the trial, prawns were sacrificed for individual measurements and hepatopancreas were sampled. Biometrical, histological and molecular analyses were performed to evaluate the overall health status and the growth performance. **RESULTS** Survival rate was significantly higher in prawns fed HM3 diet, followed by HM0 and then HM20. However, prawns growth performances did not show significant differences among the experimental groups (results supported by molecular analysis of *jheh* gene expression). Gene expression related to enzymatic hydrolysis of chitin did not show significant differences among all the experimental groups. No signs of inflammation or structural alteration of hepatopancreas were evident in all the experimental groups and histological analysis did not reveal significant differences in mucosal fold height. Consequently, the expression of genes involved in stress and immune response did not highlight significant differences among the experimental groups. The degree of lipid accumulation in R cells did not show significant differences among the experimental groups; this result can relate to the life-stage of the prawns. The relative abundance of B cells-associated digestive vacuoles highlighted an increase in both groups fed on HM diets (HM3 and HM20) respect to HM0. This result was fully supported by the molecular analysis of *catl* gene expression that highlighted an upregulation in HM3 and HM20 groups respect to control diet (HM0). **CONCLUSION** The present study evidenced that a HM inclusion up to 20% in the diet does not negatively affect prawn welfare, proving that HM is a valid protein and lipid source for prawns. In addition, this trial confirms the feasibility of aquaponic systems for prawns culture.



Example of histological images of hepatopancreas of giant freshwater prawn fed experimental. Details of hepatopancreas duct (asterisk indicates R cells, hashtag indicate B cells vacuoles and arrow indicate submucosa width).



Relative mRNA abundance of genes involved in growth, enzymatic hydrolysis of chitin, immune and stress response and protein digestion analyzed in hepatopancreas samples of giant freshwater prawns fed the experimental diets. (**a**) jheh, (**b**) chit3, (**c**) a2m, (**d**) hsp90, (**e**) *catl.*