PROMOTING CIRCULAR ECONOMY BY THE ASSESSMENT AND VALIDATION OF MUSHROOM INDUSTRY BY-PRODUCTS AS SUSTAINABLE INGREDIENTS FOR RAINBOW TROUT *Oncorhynchus mykiss* DIETS

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INTRODUCTION

The European Union produces more than one million metric tons of mushroom per year, with Spain ranking as the third producer country in Europe, contributing approximately 10% of the total production. According to the European Mushroom Growers Group, from **each metric ton of mushrooms** cultivated and sold, **150 kg of organic residues are produced.** Under this scenario, the potential use of these agriculture wastes (mushroom stems or not marketable mushrooms) as alternative protein and functional ingredient sources in aquafeeds may be a promising strategy for promoting circularity in this industry and valorize a by-product that is generally used for producing manure. Therefore, the **present study aimed to evaluate the suitability of this agricultural by-products in aquafeeds** by assessing the ***in vitro* (A) and *in vivo* digestibility (B) of mushroom by-products** from three selected species like *Agaricus bisporus (Ab), Lentinula edodes (Le) and Pleurotus ostreatus (Po)* in rainbow trout (*Oncorhynchus mykiss*) juveniles, as well as correlating their dietary inclusion to selected **key performance indicators, KPI (B.1).**

MATERIALS AND METHODS

Tested raw materials

- **FM LT70** — CP: 70%, CL: 9%
- ***Agaricus bisporus* (Ab)** — CP: 20%, CL: 2.3%
- ***Lentinula edodes (Le)*** — CP: 13%; CL: 1.7%
- ***Pleurotus ostreatus (Po)*** — CP: 17%, CL: 1.9%

(A) *IN VITRO* DIGESTIBILITY ASSAY

Two-step hydrolysis (closed reactor for acid and alkaline digestion)

Morales & Moyano (2010) Aquaculture 306, 244-251.

(B) *IN VIVO* DIGESTIBILITY TRIAL

Ingredients (%)	Experimental diets			
	Control	Ab	Le	Po
Fish meal LT70 (NORVIK)	35.00	24.50	24.50	24.50
Soy protein concentrate (Soycomil P)	10.00	7.00	7.00	7.00
Corn gluten meal	10.00	7.00	7.00	7.00
Soybean meal Hipro (Alphasoy 530)	10.00	7.00	7.00	7.00
Wheat meal	19.28	13.50	13.50	13.50
WISIUM MIX AQUA 1.5%	1.00	0.70	0.70	0.70
Choline chloride 50% SiO2	0.20	0.14	0.14	0.14
Yttrium oxide (Amperit)	0.02	0.01	0.01	0.01
Fish oil-MIXTURE	5.00	3.50	3.50	3.50
Rapeseed oil-MIXTURE	9.50	6.65	6.65	6.65
Mushroom meal	-	30.00	30.00	30.00

Glencross et al. (2023) Aquaculture 565, 739137.

Experimental design

Diet composition:
40-48% CP
16-21% CL

Feeding rate:
1.6-1.8 % of fish biomass

BWi = 120 ± 0.4 g

T = 15.7 ± 0.2 °C
O_2 = 8.8 ± 0.3 ppm

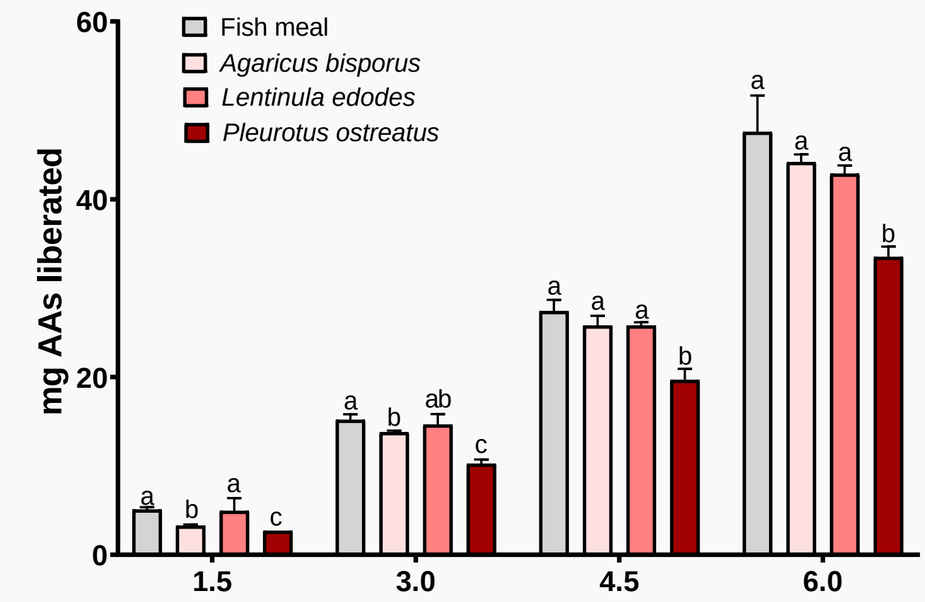
RAS unit IRTAmar®
3 replicates per diet
45 fish per 2 m^3 tank

(B.1) SAMPLING & KPI

- Fish performance
- Digestive enzymes
- Blood biochemistry
- Liver morphology

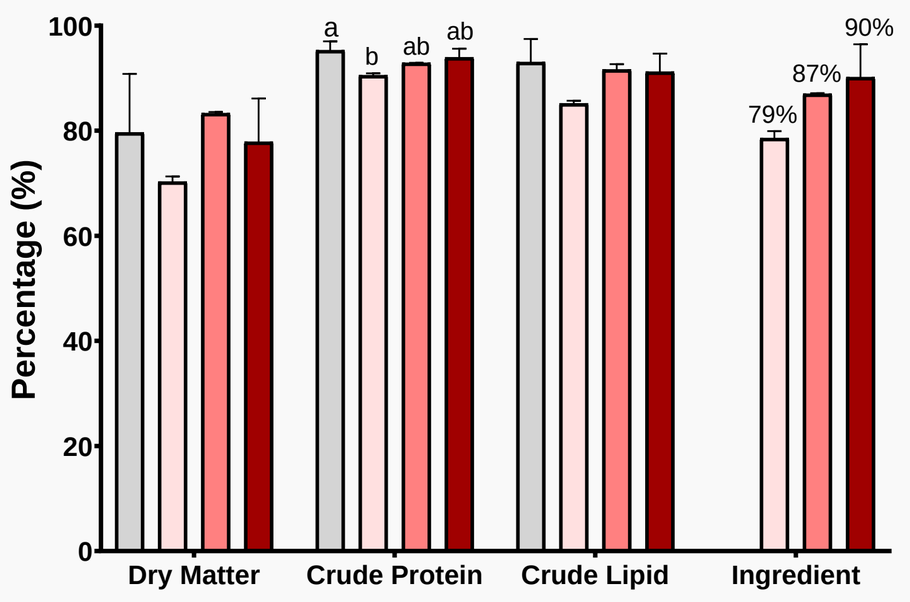
RESULTS

(A) *IN VITRO* DIGESTIBILITY



Total amount of amino acids (AAs) released after hydrolysis of the different tested raw ingredients within the double chamber reactor. Data are expressed as mean ± SD (n = 3), different letters indicate significant differences among dietary treatments ($p < 0.05$).

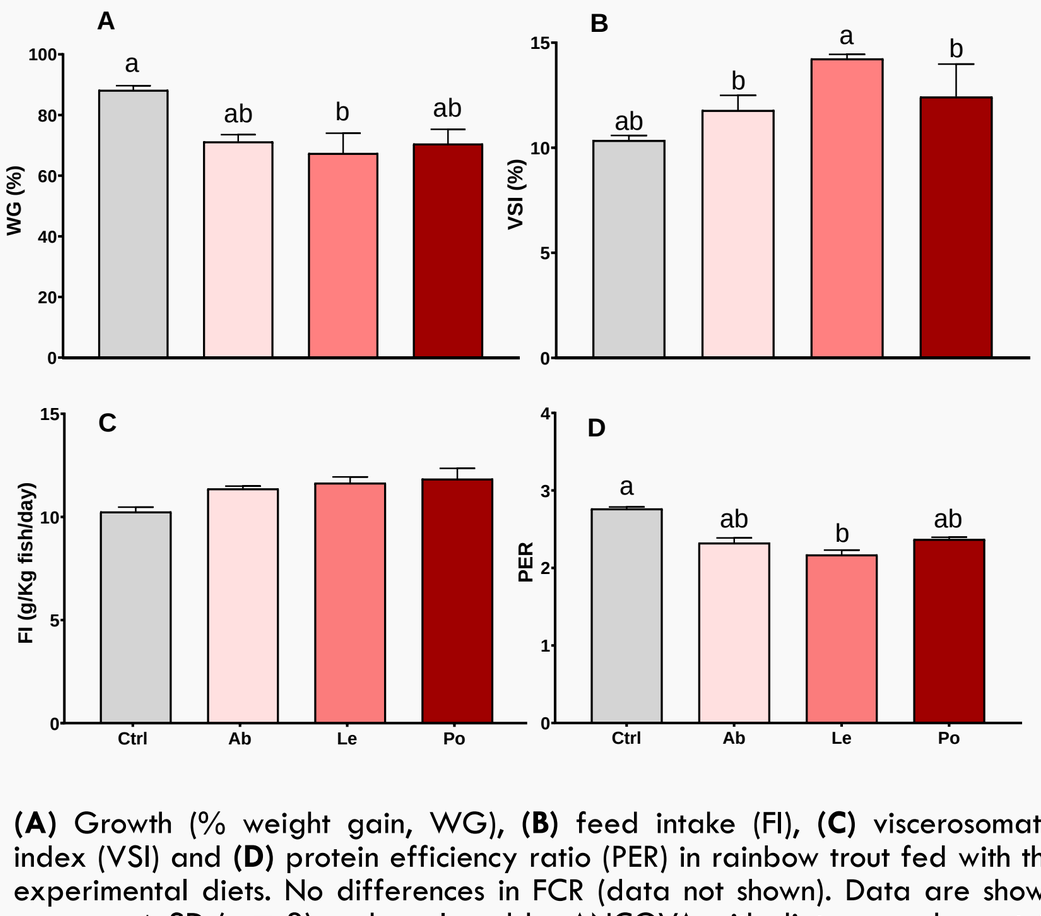
(B) *IN VIVO* DIGESTIBILITY



Apparent Digestibility Coefficient

In vivo apparent digestibility coefficients (ADC) of dry matter, protein and lipid in tested diets fed to rainbow trout for 42 days. Data are expressed as mean ± SD (n = 3), different letters indicate significant differences ($p < 0.05$).

(B.1) KEY PERFORMANCE INDICATORS



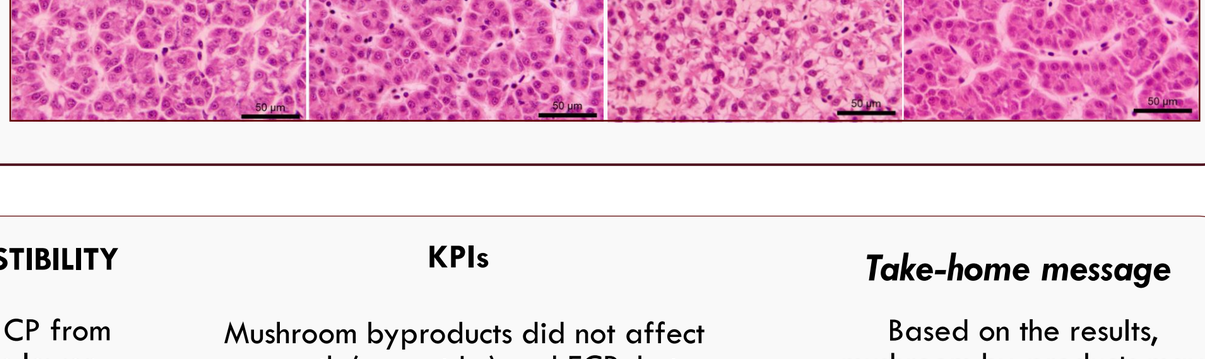
(A) Growth (% weight gain, WG), **(B)** feed intake (FI), **(C)** viscerosomatic index (VSI) and **(D)** protein efficiency ratio (PER) in rainbow trout fed with the experimental diets. No differences in FCR (data not shown). Data are shown as mean ± SD (n = 3) and analyzed by ANCOVA with dietary crude protein as covariant. Different letters indicate significant differences ($p < 0.05$).

Digestive enzymes activity of rainbow trout showed no significant differences when fed with the experimental diets for 42 days. Data are shown as mean ± SD (n = 3) and analyzed by ANCOVA with dietary crude protein as covariant.

Activity (U/mg protein)	Control	Ab	Le	Po
Pepsin	0.59 ± 0.08	0.51 ± 0.04	0.50± 0.19	0.71± 0.14
Trypsin	0.17 ± 0.03	0.18 ± 0.00	0.17 ± 0.04	0.12 ± 0.02
Alkaline protease	1.22 ± 0.11	1.01 ± 0.10	1.07 ± 0.23	0.82 ± 0.09
Alpha-amylase	44.84 ± 5.46	38.30 ± 13.72	35.22 ± 12.38	29.57 ± 16.32
Lipase	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.02

Blood and biochemical parameters in serum of rainbow trout showed no significant differences when fed with the experimental diets for 42 days.

Liver histology of rainbow trout fed with the experimental diets for 42 days. The Le fed group presented higher level of hepatocytes vacuolization compared to the group fed with the control diet.



CONCLUSIONS

- ***Agaricus bisporus* (Ab)**
- ***Lentinula edodes* (Le)**
- ***Pleurotus ostreatus* (Po)**

***IN VITRO* DIGESTIBILITY**

A. bisporus and *L. edodes meals* showed similar *in vitro* digestibility values compared to fish meal

***IN VIVO* DIGESTIBILITY**

ADC values for CP from *A. bisporus* was lower than FM, whereas *L. edodes* and *P. ostreatus* showed similar values

CP > 90%; CL > 80%; Ingredient > 78%

KPIs

Mushroom byproducts did not affect growth (except Le) and FCR, but tend to increased FI values to compensate lower dietary CP levels. No major changes in enzymes activity and blood biochemestry. *L. edodes* increased hepatic vacuolization.

Take-home message

Based on the results, mushroom by-products may be a potential ingredient for diversifying the basket list in aquafeed formulation. Current studies are on-going to determine their optimal level of inclusion.