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AGRI-FISH: SUSTAINABLE AND INNOVATIVE FEEDS FROM AGRICULTURAL WASTES FOR A RESILIENT AND HIGH-QUALITY AQUACULTURE

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ABSTRACT

The AgrI-fiSh project has been funded under Horizon 2020 - PRIMA, with the research partnership of three Mediterranean countries: Algeria, Italy and Spain. The project will contribute to the transition to a more resilient agro-livelihood system through an innovative and circular farming approach, which promotes an efficient use of biological and natural resources with a valuable increase in productivity. New potential for farms, agri-food systems and local markets will arise from the valorization of agricultural wastes to be used for the creation of high-value plant-based feeds for a sustainable inland aquaculture. Nutraceutical potential of specific agricultural wastes (i.e grape seeds, legumes and cereals), in combination with other components, as Lactic Acid Bacteria (LABs) fermented whey and medicinal herbs) to create plant-based feeds for aquaculture, will foster animal and human health with consequent benefits for the environment, local economies and consumers.

1. INTRODUCTION

1.1 Project's aim

AgrI-fiSh project aims at increasing the environmental and socio-economic performances of small-scale farming systems thought the development of an innovative model, which will help in improving organizational aspects of the agri-systems, while creating a new value chain governance model, especially at the local levels. For the first time agriculture and aquaculture are linked together to achieve a common goal: the application of a circular economy model, defined as "from the field to the net", for the creation of sustainable and innovative feeds from agricultural wastes for a resilient and sustainable aquaculture. The overall objective of the project is to reinforce the resilience of local agri-food systems face to climate change, through capacity building and cooperation among the actors involved in the agriculture and aquaculture sectors, while adopting a circular model, which will effectively contribute to increase ecosystem services, biodiversity diversification and farmer's incomes. The scope is also to provide communities with evidence-based and promote a sustainable inland aquaculture in rural lands as an effective and cost-saving activity, thus promoting farming diversification in regions characterized by degraded soils, like Northern Africa and Southern Europe. The project is built on interconnected research activities, to support the sector's transition to sustainable agriculture and aquaculture and to the circular economy model proposed, which could be further on up-scaled for aquaculture at sea. The circular model is applied to conceive innovative feeds, with high-nutraceutical values and low environmental impact (from cradle-to-grave), for aquaculture productions starting from the reuse of agricultural wastes and with the combination of natural antimicrobial substances deriving from sustainable agriculture, such organic and climate-smart agriculture (CSA), LABs and some medicinal plant species, such as *Opuntia ficus-indica, Pistacia lentiscus, Eucalyptus globulus, Peganum harmala, Ziziphus lotus, Phoenix dactylifera, Thymus algeriensis and Allium spp.* among others.

1.2 Socio-economic impacts

The project itself contributes to achieve socio-economic impacts by scaling-up research activities from the laboratory dimension to the adoption of a circular model, adaptable and replicable according to inner territorial needs and characteristics, while generating new socio-economic opportunities. Agriculture and aquaculture are directly linked within this project, with the aim of enhancing their mutual potentials in a circular process, reinforcing the sustaina-

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bility of local communities, promoting knowledge transfer and creating an innovative sector of employment. Moreover, such combination constitutes an effective approach for the rehabilitation, development and the maintaining of local populations in abandoned and marginalized arid inlands, such as in Algeria. AgrI-fiSh intends to create plant-based feeds to limit the animal competition on biodiversity resources, and the principal sectors of research and investigations are grape, cereal and legume productions, as well as dairy products and medicinal herbs. Attention is also given to mitigation and adaptation to climate change, since the system is conceived to reduce greenhouse gas emissions from the sector, limit or eliminate the use of antibiotics, and promote the limitation of mycotoxins and fungal contamination in feeds used for aquaculture, through the use of natural ingredients from waste valorization.

2. BACKGROUND OF THE PROJECT

2.1 Acquaculture and its environmental impacts

The United Nations General Assembly has declared 2022 the International Year of Artisanal Fisheries and Aquaculture (IYAFA 2022) (FAO, 2022), and growing attention is given to aquaculture worldwide due to the increase in its demand and productions.

According to FAO, aquaculture is among fastest growing food production sectors worldwide and has become the main source of fish proteins for human consumption since 2016, as a consequence of the marine overfishing and depletion of wild fish stocks (FAO, 2022).

Unfortunately, intensive aquaculture represents a threat for climate change and biodiversity, especially due to the release of pollutants in the environment.

According to latest publications, The State of World Fisheries and Aquaculture from FAO and World Fish (FAO, 2022), the number of people employed in the primary fisheries and aquaculture sectors is 59.5 million, of which 14% are women. In the world, 20 million people are directly engaged in aquaculture and its production has increased by 527% since 1990. About 80% of world aquaculture production comes from developing countries. Globally, aquaculture produced 82.1 million tons of aquatic animals in 2018. Europe is the continent with the highest consumption of fish products (24.5 kg/capita), higher than the world average of 18 kg per year. It currently participates in 18% of world aquaculture production and the sector accounts for almost 20% of fish production, employing about 85,000 people. Aquaculture production is projected to increase by a third by 2030, reaching 109 million tons, and by 2050 it will provide most of the "aquatic" protein in people's diets. One of the most difficult challenges aquaculture faces is the climate crisis, since the heating of the water influences the metabolism of fish and therefore the production of the supply chain.

Thus, the combination of a higher demand and the increase of climatic threats that impoverish biological resources, result in the need of providing communities with resilient and sustainable aquaculture systems to guarantee their food security. The AgrI-fiSh project responds to such challenges.

2.2 Agriculture and environment

As known, the relationship between agriculture and climate change is two-sided, since agriculture is the economic sector most affected by the ongoing processes. It is a large emitter of greenhouse gases (GHGs), but it could also represent a solution in limiting GHG emissions through the development of sustainable agricultural approaches (FAO, 2013). Hence, to use agricultural discards as starting material for preparing fish feed is aimed at reducing environmental impact of both aquaculture and agriculture.

This sector emits substantial amounts of methane from enteric fermentation in ruminant animals and rice paddies, nitrous oxide from fertilized soils, and carbon dioxide from deforestation and fossil fuel use in farm operations. These emissions exacerbate the greenhouse effect, leading to global warming and associated climatic disruptions. Conversely, agriculture is also highly vulnerable to the impacts of climate change. Alterations in temperature, precipitation patterns, and the increased frequency of extreme weather events can severely affect crop yields, pest and disease dynamics, and water availability. These changes pose significant threats to food security and the livelihoods of farmers, making the agricultural sector one of the most affected by ongoing climate change processes. However, agriculture holds the potential to be part of the solution to climate change through the adoption of sustainable practices. Sustainable agriculture aims to reduce environmental impact while maintaining productivity. Organic farming, for instance, eschews synthetic fertilizers and pesticides, promotes soil health, and enhances biodiversity. CSA is another approach that focuses on increasing agricultural productivity sustainably, improving resilience to climate impacts, and reducing greenhouse gas emissions.

Thus, one innovative solution to reducing the environmental impact of both agriculture and aquaculture is the use of agricultural discards as a starting material for preparing fish feed. Traditionally, fish feed relies heavily on wild-caught fish or soy, both of which have significant environmental footprints. By repurposing agricultural by-products and discards, this approach not only minimizes waste that would otherwise contribute to methane emissions in landfills but also provides a sustainable alternative to conventional fish feed. This practice supports a more circular economy, where waste products are repurposed rather than discarded.

2.3 AgrI-fiSh model

The model proposed will improve the Mediterranean agroecosystems' resilience with focus on the concept of productivity and quality valorization, and the maintaining of the well-being of the environment and the population. In fact, not only wastes, especially issued from agroecological activities, will be used as quality materials due to their nutraceutical potential, but also the testing of the final product (plant-based feeds), will enhance the nutritional quality of the fish. Moreover, by integrating agricultural discards into fish feed, both agriculture and aquaculture will reduce their environmental footprints, enhance sustainability, and contribute to broader climate change mitigation efforts. This dual approach underscores the importance of innovative solutions and sustainable practices in addressing the complex interplay between agriculture and climate change.

Further on, consumers will find in the market a product of higher-quality level not only for their well-being but also for the well-being of the environment and the local economy. Consequently, producers (farmers and fishermen) will benefit from this virtuous system in terms of revenues and decrease in their vulnerability to climate change, since this model will guarantee yield prosperity and biodiversity abundance, while reducing the environmental impacts, by promoting sustainable agriculture and aquaculture and limiting the costs of adoption of the system.

Moreover, to respond to current environmental challenges in the Mediterranean area, the proposed model aims at identifying and undertaking innovative practices, suitable for aquaculture production and in line with Sustainable Development Goals (SDGs) and circular economy objectives, through adaptation and mitigation strategies.

3. AGRI-FISH ACTIVITIES

3.1 Partnership and support

The project's partnership is composed in Italy by the University of Camerino, being the coordinator, and the National Research Council – Institute of Marine Biological Resources and Biotechnologies (CNR-IRBIM), in Spain by the University of Valencia and in Algeria by the University Ibn Khaldoun of Tiaret.

The project is funded under Horizon 2020-PRIMA and started its implementations in June 2022, while the experimental phase of the pilot project in Italy in Visso (Marche Region) has started in October 2023 on 900 trouts (*Salmo trutta fario*) with trials on two diets and on 400 trouts (*Oncorhynchus mykiss*) with trials on one diet.

3.2 Activities

The main activities (Figure 1), relate to the definition of innovative feeds with a low environmental impact through the partial/total replacement of animal protein and lipid components with organic agricultural wastes (at least 70% of the composition of the feed), contributing to lessen CO_2 emissions from the sector; an in-depth knowledge of the bioactive compounds content of bioproducts, mainly of grape seeds from various local grapes (Italy, Spain and Algeria), legumes and medicinal plants, in order to better assign the biological properties of each compound to the desired objectives of the project in enhancing fish growth and resistance to microbial diseases, improving the taste, etc.; the valorization of organic agricultural wastes and reduction of the costs of disposal through the creation of sustainable and quality feeds, whit at least 20% lower costs; the improvement of animal growth and welfare by strengthening the immune system of fish with the bioactive compounds contained in the new feeds and consequent reduction in the use of antibiotics; the creation of a virtuous and circular local system that strengthens collaborations in the agri-food sector; the definition of a sustainable and economically favourable and competitive system that supports small-farmers in implementing organic productions, especially of legumes, which are a vegetable source of proteins and are soil nitrogen-fixing; the empowerment of local communities, with attention to gender and youth dimensions, strengthening the local dimension and the training system through direct collaboration at multiple levels (schools, universities and young entrepreneurs); the development of cooperation among Mediterranean countries through capacity building and knowledge sharing enhancement at different levels; the verification of the main organic and inorganic pollutants on the agricultural waste reused to develop the innovative feeds; and the development of a sampling protocol for sampling of water and fish in order to evaluate the state of the water quality of tanks and the well-being of the fish.

3.2.1 Testing of feed

Concerning the testing phase on fish on trout and tilapia, analysis will be conducted to evaluate the zootechnical performance and welfare of tested fish to assess the experimental diets and the setup of water quality and fish sampling protocol.

The feeding tests of the fish will be carried out to test the validity of the experimental diets containing the by-products of the sustainable supply chain. Each diet will be distributed to fish with an initial average weight of 30-40 gr for trout, and 100-150 gr for tilapia, will be reared at the same density and will be fed until a level close to satiety is reached. This will avoid subjecting the fish to forced feeding. After 90 days from the start of the test on Trout, the main growth performances will be measured (average weight, average length, average weight gain, specific growth rate, survival rate). The tests will continue by carrying out, at periodic intervals, the weight and length measurements on a representative sample of fish belonging to the different experimental and control groups. At the end of the tests, the zootechnical performance and the food conversion index for each test group will be determined. Such method will be replicated for targeted species in Spain and Algeria.

3.2.2 Water quality

Analysis will be carried out on the temperature and dissolved oxygen of the water and samples of 500 cc of water will be taken at the exit from the basins where the feeding tests of the trout with the experimental diets will be carried out, in comparison with the Control diet. To verify the functioning of the treatment path, the monitoring of the chemical-physical parameters (temperature and dissolved oxygen) and the analysis of the different nitrogenous forms (nitrites, nitrates and ammonium) present in the water from the tanks using a spectrophotometer, applying internationally recognized analytical methods. The concentration of dissolved oxygen directly affects both the self-purifying capacity of the water and the possibility of survival of the fish species. On a bi-weekly basis, as required by the protocol, the colorimetric analyzes (Strickland and Parson, 1972) are carried out after filtration (0.7 micron filter, Whatman GF/F) to quantify the presence of: NH3, NO3-, NO2-.

4. PROJECT IMPLEMENTATIONS

The Project intends to solve the following problems related to productions: i) waste volumes and disposal, by valorizing them; ii) unsustainable aquaculture, by creating innovative feeds which are plant-based; iii) use of pesticides and antibiotics in agri-food productions, by promoting sustainable agriculture and using LABs and medicinal herbs in the feeds; iv) post-harvest issues, by developing a new solution to prevent post-harvest issues based on non-toxic, eco-friendly biocide intended to substitute synthetic fungicides with non-toxic eco-friendly substances (GRAS, bio-stimulants, resistance inducers and natural substances) and biological control agents (antagonistic bacteria, yeasts and fungi) to prevent the storage fungal contaminants of aquaculture feeds; v) impoverishment of agricultural system, by developing a sustainable model, which is profitable for smallholders and creates competitiveness and new employment, especially in rural areas. During these first three months of project implementation, the main research activities focused on the characterization of agricultural wastes. In fact, the valorization of wastes has a strong adaptation potential, since an abundant agriculture production results in a high quantity of processing wastes. These wastes represent an important disposal cost to be borne by farmers and agriculture producing and processing companies. Hence, it is necessary to increase value of these wastes, both reducing their disposal and related costs, and valorizing their potential and intrinsic value. The production of vegetable by-products and discards leads to the formation of waste and effluents that are naturally rich in carbohydrates, proteins, fibers and lipids, as well as vitamins and other bioactive compounds, such as resveratrol, present in wine wastes. Studies have shown the health potential for human consumption of nutrients present in grape skin and seeds, such as resveratrol and other polyphenols, which can be transferred into meat (e.g., livestock or fish) and remain still available after consumption (Frombaum et al., 2012; Ragni et al, 2014). Polyphenols, among all, have the propriety to lower cholesterol levels in blood and limit the oxidative stress in the cells for human health (Frombaum et al., 2012). Analyzed wastes are: a) grape pomace, b) grape seeds, c) discard of pasta making of: -fava beans, -peas, -chickpeas, -lentils, -pasta made with Norberto wheat flour (wholemeal pasta), - pasta made with Senatore Cappelli wheat flour (both wholemeal and not), for a total of 9 discards/ingredients. The analysis on wastes have been directed, from one side, to the determination of nutrients in the wastes (proteins, lipids, carbohydrates, fibers), and from the other to bioactive and health promoting compounds, with special attention to macro- and micro-elements, fatty acid profile, including mono- and poly-unsaturated ones, aminoacidic profile, mineral profile with macro- and micro- elements, to end with Total Phenolic Content (TPC) and Total AntiOxydant Activity (AOA). Aminoacidic and fatty acid profiles were determined using High Performance Liquid Chromatography, coupled to a Mass Spectrometry and/or a Diode Array Detector. Mineral profile, related to 16 elements, was obtained using an ICP-MS apparatus. Finally, TPC and AOA were determined spectrophotometrically, with results given as Gallic Acid Equivalent and Trolox Equivalent, respectively.

5. RESULTS AND DISCUSSION

Table I shows some analytical results made prior to the definition of the main agricultural wastes. As expected, pomace and grape seeds showed a very high TPC (from 15 to 60 times higher than the other ingredients), while for Antioxidant activity the most effective ingredients showed to be Fava beans.

About elements, all discards showed to be from good to excellent source of them, especially for Ca, P, Mg, K, Zn, Cu, Mn.

More, pomace and, particularly, grape seed were excellent source of n-6 PUFA. First results on the testing of the two diets to trout in Itlay showed a fish growth rate of around 20% when compared to control diet but further assessments are needed since the tests will last until September 2024.

6. CONCLUSIONS

This study gives an overview of the main ongoing activities and the major expected impacts and outcomes, but it could not be exhaustive due to the recent inception of the project.

FIGURE 1: AgrI-fiSh model.

TABLE 1: Some analytical data of analyzed discards; all concentrations are expressed as mg/kg, except for fatty acid (n-6 PUFA), which is in g/100g.

	Peas	Fava beans	Lentils	Chick peas	Cappelli normal pasta	Cappelli wholemeal pasta	Norberto wholemeal pasta	Pomace	Grape seeds
Total phenolic content (mg GAE/kg)	844,55	1926,63	866,37	549,43	872,81	1293,95	1387,77	29828,9	30880,9
Antioxidant activity (mg TE/kg)	498,89	1277,82	691,31	533,35	619,2	628,26	697,77	1189,38	932,31
Ca	1101	865	303	714	243	569	804	6.299	7.543
P	3986	7998	4255	5523	1.933	4.702	5.149	3.209	3.582
Mg	1298	1788	941	1853	574	1.683	42,8	1.592	1.980
K	12994	19534	10693	15707	2.121	4.441	5.106	27.991	7.088
Zn	37,3	38,7	37,00	48,9	13,2	53,9	60,6	10,4	13,3
Cu	8,4	15,2	7,50	11,9	3,4	8,2	8,6	26,1	11,9
Mn	12,3	12,3	9,60	31,6	6,6	35,2	42,8	9,4	11,5
n-6 PUFA:									
18:2 n6 C	0,26	0,90	0,46	2,98	0,37	0,96	0,69	3,54	10,85

Beyond the state of the art, AgrI-fiSh will develop an innovative model, easily replicable, and will be crucial for pursuing sustainability and creating new business initiatives in the agri-food sectors.

Due to its high-degree of novelty it will represent an asset for national economies and for further investments in the sectors (1° innovation). The standardization in the use of medicinal plants and whey (fermented by LABs) as components of fish feeds for food quality and security issues will become a protocol (2° innovation). The creation of plant-based feeds from sustainable agricultural wastes will become a protocol of innovation to be released in the market (3° innovation). This sustainable model will provide fish food enriched with bioactive constituents useful for the consumers' health, with the scope to reinforce the Mediterranean lifestyle, develop food traceability and provide products with high added value (nutritionally and economically) (4° innovation).

Particular attention is given to the use of medicinal plants, which in aquaculture has exploded recently, and have been used since ages by rural fish farmers, since they produce various beneficial effects to aquaculture such as antistress, appetite stimulation, growth promotion, immunostimulation, and have antipathogens properties due to their active compounds (Reverter et al., 2017).

Antiviral and antifungal activities are also able to prevent high mortality rates in aquaculture and the addition of LABs to the innovative feed constitutes an asset (Dadras et al., 2023).

In fact, the widespread application of many probiotic LAB species and S. cerevisiae in foods and their status as Generally Recognized as Safe (GRAS) organisms, makes them suitable candidates for strategies devised to diminish oral exposure to chemical contaminants and reducing mycotoxins (Chiocchetti et al, 2019).

The combination of such technologies will result in the application of an effective model, which will contribute to reduce the vulnerability of the agri-food systems face to climate change.

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