

## Article

# Alfalfa for a Sustainable Ovine Farming System: Proposed Research for a New Feeding Strategy Based on Alfalfa and Ecological Leftovers in Drought Conditions

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**Citation:** Viti, C.; Bellabarba, A.; Daghighio, M.; Mengoni, A.; Mele, M.; Buccioni, A.; Pacini, G.C.; Bekki, A.; Azim, K.; Hafidi, M.; et al. Alfalfa for a Sustainable Ovine Farming System: Proposed Research for a New Feeding Strategy Based on Alfalfa and Ecological Leftovers in Drought Conditions. *Sustainability* **2021**, *13*, 3880. <https://doi.org/10.3390/su13073880>

Academic Editors: Fabian Capitanio, Pier Paolo Miglietta, Donatella Porrini, Giulio Fusco and Anastasios Michailidis

Received: 3 March 2021

Accepted: 18 March 2021

Published: 1 April 2021

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**Abstract:** In the past 10 years, the average demand for meat and milk across the world has significantly increased, especially in developing countries. Therefore, to support the production of animal-derived food products, a huge quantity of feed resources is needed. This paper does not present original research, but rather provides a conceptual strategy to improve primary production in a sustainable way, in relation to forthcoming issues linked to climate change. Increases in meat and milk production could be achieved by formulating balanced diets for ovines based on alfalfa integrated with local agricultural by-products. As the central component of the diet is alfalfa, one goal of the project is increasing the yield of alfalfa in a sustainable way via inoculating seeds with symbiotic rhizobia (i.e., *Sinorhizobium meliloti*). Seed inoculants are already present on the market but have not been optimized for arid soils. Furthermore, a part of the project is focused on the selection of elite symbiotic strains that show increased resistance to salt stress and competitiveness. The second component of the experimental diets is bio-waste, especially that obtained from olive oil manufacturing (i.e., pomace). The addition of agro-by-products allows us to use such waste as a resource for animal feeding, and possibly, to modulate rumen metabolism, thereby increasing the nutritional quality of milk and meat.

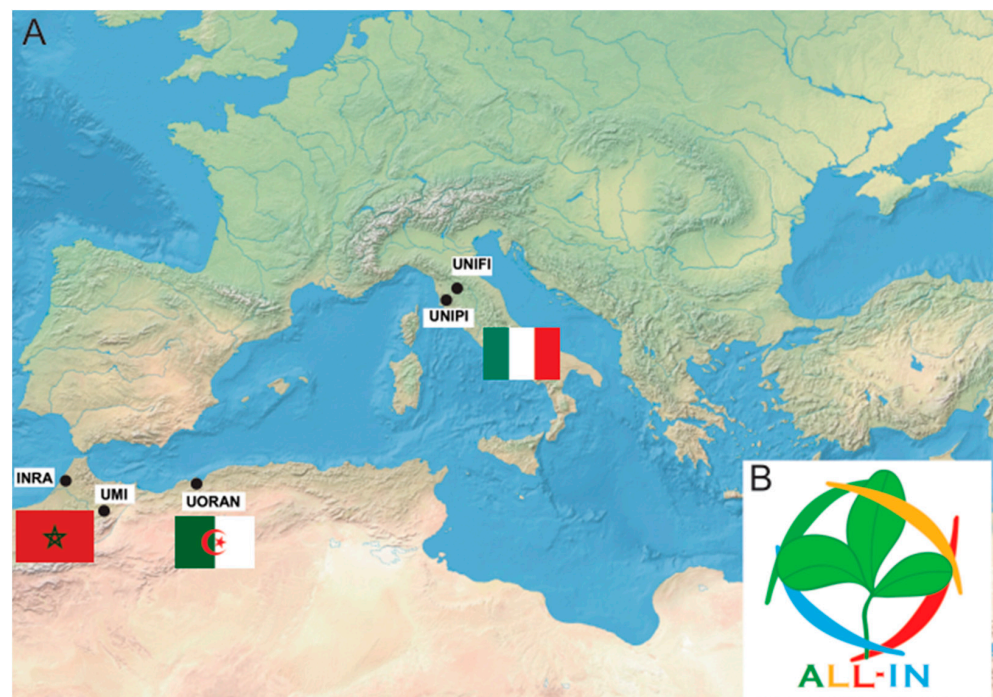
**Keywords:** legume; alfalfa; crop yield; rhizobia; nitrogen fixation; livestock; milk and meat; climate change; circular economy; food loss and waste

## 1. Introduction

Considering the constantly growing human population and the concomitant increases in lifespan, a huge increase in demand for milk and meat derived products is expected [1]. Hence, we are faced with the challenge of how to meet consumers' needs while also minimizing the impact of animal breeding on the environment. This scenario is worsened when considering the issues related to pollution and climate change, which directly affect

primary production [2]. Here, we propose a research project (ALL-IN—ALfalfa for sustainable Livestock farming systems: Improve alfalfa–rhizobia symbioses and New feeding strategies based on ecological leftovers) to promote the development of local, sustainable food societies by adopting a new feeding strategy for ovines based on the principle of “ecological leftovers”.

Ovines are widely bred in the Mediterranean basin [3], and so, the countries to be involved in the project (Morocco, Algeria, and Italy) have been selected because they are situated within this area (Figure 1). Diets will be designed to optimize the reuse of agricultural bio-wastes, using alfalfa as the basal component. The supplementation of diets with agro-food by-products that originate from food production systems matches with the principles of a circular economy (i.e., the three Rs—reduce, reuse, and recycle), which are strongly supported by FAO (Food and Agriculture Organization) and EU (European Union) programs to achieve sustainable development. Alfalfa, which is classed as a high-quality feed due to its high protein content, can be combined with several by-products [4] that originate from food production and processing. Alfalfa is a crop that is able to face water shortages in the form of irrigation or rainfall deficiencies. However, water deficiency affects the yield of alfalfa directly by influencing plant parameters and indirectly by decreasing rhizobia nodulation and influencing the first steps of the symbiotic process [5]. Indeed, leguminous plants can establish mutualistic symbioses with nitrogen-fixing rhizobia, contributing substantially to BNF (biological nitrogen fixation). Rhizobial inoculants are widely used in agriculture, as they represent one of the most cost-effective ways to boost legume performance through the provision of the nitrogen requirements of the plant, thus minimizing the use of fertilizers. The primary goals of the project are (i) to increase the yield of alfalfa (*Medicago sativa* L.) crops via the development of new rhizobial inoculants and (ii) to formulate a balanced diet for livestock based on alfalfa and integrated with local agricultural by-products.



**Figure 1.** (A) Countries involved in the project (UNIFI = University of Florence, Italy; UNIFI = University of Pisa, Italy; UORAN University Oran1, Algeria; INRA = National Institute of Agricultural Research (INRA), Morocco; UMI = Moulay Ismail University, Morocco). (B) ALL-IN (ALfalfa for sustainable Livestock farming systems: Improve alfalfa–rhizobia symbioses and New feeding strategies based on ecological leftovers) logo.

Alfalfa has been defined as the “Queen of the Forages” [6]. *Medicago* species have high agronomic value worldwide and are essential for bioenergy and forage for animals [7,8]. ALL-IN aims to advance the knowledge on bacterial competitiveness and adaptation to water deficiency, combining classic microbiology approaches with state-of-the-art genomics. To date, the screening of rhizobia for inoculant production has been mostly based on their nitrogen fixation efficiency; however, this should not be the sole criterion for their selection [9]. In *Medicago sativa*–*Sinorhizobium* association, the relationship is not exclusive, and different strains with different nitrogen fixation efficiency may colonize the same plant individual, creating a mixed nodule [10]. Consequently, as the plant could not select a priori which is the best strain to be nodulated, the presence of highly competitive cheating strains in a soil could severely affect its yield [11]. Therefore, two main aspects should be taken into consideration for the development of inoculants to be used in arid regions: (i) a general one is the development of a methodology for screening highly competitive strains, and (ii) the second one is the selection of new rhizobial strains able to survive in areas characterized by high temperatures, salinity, and drought. The development of elite rhizobial inoculants is important to enhance alfalfa yield in sustainable agriculture and to improve its growth and also the recovering process of marginal areas.

The single-gene approach has been mainly used to uncover the genetic and molecular bases in the nodulation process, but there is still a gap about the main features that a priori make rhizobial strains able to outcompete other indigenous rhizobia [12]. The first objective of the project is the development of a method to infer the competitive capabilities of a strain solely based on its genome content. A phenotypic measure of competitiveness for nodulation was evaluated in different symbiotic associations [13,14], but the genetic basis involved in this phenomenon is still not completely understood. Comparative genomic analyses and genome-wide association studies (GWAS) are powerful approaches for identifying genetic determinants of important traits in symbiotic bacteria [15], especially for more complex phenotypes that would remain undetected as the competition phenotypes [9,16]. The employment of user-friendly software tools able to predict phenotypes of interest from the genomic sequence, such as PhenotypeSeeker [17], could be a useful approach for the identification of genomic biomarkers controlling competitiveness. As mentioned above, the other aspect to take into consideration in the screening process is the selection of strain inoculants able to boost alfalfa in areas characterized by high temperatures and salinity. In these sub-optimal growing conditions, the increase in the rhizobial fitness will help improve crop productivity [18,19]. This could be more easily achieved using classic approaches. Obtained results will be valuable not only from a scientific point of view, but also for their potential application in the pre-screening of indigenous bacteria, where the same program could be exploited for the formulation of elite inoculants. Since chemical fertilizers are expensive inputs that small farmers or farmers at economic risk cannot afford, nitrogen-fixing legumes offer an economic advantage for plant-protein production under limited nitrogen inputs. Therefore, ALL-IN may contribute to improving farmers’ profits by reducing the cost of production and preserving good environmental quality.

The second part of the project is instead focused on the use of alfalfa, specifically in the formulation of a balanced diet for livestock based on alfalfa and integrated with local agricultural by-products. Alternative feed resources may give an essential contribution to sustainable production systems. In this context, thanks to its higher protein content, alfalfa is a high-quality feed that may be combined with several by-products originating from food production and processing. For livestock, alternative feed resources to the conventional energy and protein sources, and not in competition with human nutrition, may give an important contribution to sustainable production systems [20]. Bio-waste selection is based among those available in the countries involved in the project. The inclusion of bio-waste in an animal’s diet has multiple advantages: (i) reducing the environmental impact of livestock production and the competition for arable land between food and feed products, (ii) recycling material that must be disposed or otherwise processed, and (iii) exploiting the specific content of the agro-by-products used for rumen modulation.

In the last few decades, animal nutritionists have increasingly focused on the physiology of the rumen and on its microorganisms [21]. It is widely accepted that feed utilization in the rumen is dependent on the whole consortium of ruminal microorganisms rather than on the action of specific groups or strains of microorganisms [21]. Several studies reported that shifts in ruminal microorganism composition and abundance are induced not only by the type of feed (i.e., grain-rich diet vs. fresh-forage-based diets), but also by dietary plant secondary compounds (PSC), such as phenolic compounds, saponins, and essential oils present in feed either naturally or supplemented [22]. PSC can have both positive and adverse effects on livestock physiology. The effects of PSC may vary in relation to their structure, the amount of PSC ingested, and their availability within the gastrointestinal tract of the animal [23,24]. Among the positive effects that can be obtained by adding PSC to an animal's diet are: anti-bloat properties, the improvement of nitrogen utilization, a reduction in methane production, anti-helminthic effect, interference with the biohydrogenation of fatty acids, and consequently, the modulation of the fatty acid profile of ruminant products. However, these effects may be achieved in relation to the kind and amount of PSC added to the diet [23,24]. Therefore, the correct use of agro-industry by-products and food waste containing PSC (i.e., olive pomace, grape marc, citrus pulp) is an important part of the global strategy to ameliorate an animal's welfare and the quality of the final products.

One of the by-products used as a complement in experimental diets is olive oil pomace. Olive oil is produced in many countries of the Mediterranean area, and every year, a huge amount of pomace needs to be discarded [25]. Olive oil pomace disposal is difficult because of its chemical nature (high polyphenolic content) [25]. However, olive oil pomace could be a powerful resource in animal nutrition because of its antimicrobial activity, allowing us to modulate rumen metabolism [21,26]. Olive oil pomace can be useful to modulate the rumen metabolism because of the action of polyphenols, which can interact with rumen microbiota [26]. The use of olive oil pomace in dairy sheep feeding could then lead to an increase in the amount of functional fatty acids in rumen, which can be transferred to milk and meat, increasing their quality. Therefore, livestock diets formulated in this project will reduce the farm bio-waste by using discarded organic material (coupled with alfalfa) while also optimizing animal diet.

## 2. Proposed Approach

The project is positioned in the spectrum "from idea to application". This section describes how the project will be developed. No results are reported in the following paragraphs; rather, the rationale of each Work Package (WP) and task, the type of data that will be obtained, and the methodology used for data collection will be discussed. The project is running on three Work Packages (WPs) to be carried out in three years: (i) The main goal of WP1 is the selection of elite bacterial inoculants to boost legume production (24 months), (ii) WP2 is focused on the development of the ovine's experimental diet center on alfalfa in combination with farm organic by-products (25 months partially overlapping with WP1), and (iii) WP3 is devoted to knowledge diffusion and will be carried out for the entire length of the project (Figure 2). The project will be carried out by five different institutions (University of Florence UNIFI, University of Pisa UNIPI, University Oran1 UORAN, National Institute of Agricultural Research INRA, and Moulay Ismail University UMI) located in three countries: Italy, Algeria, and Morocco (Figure 1).



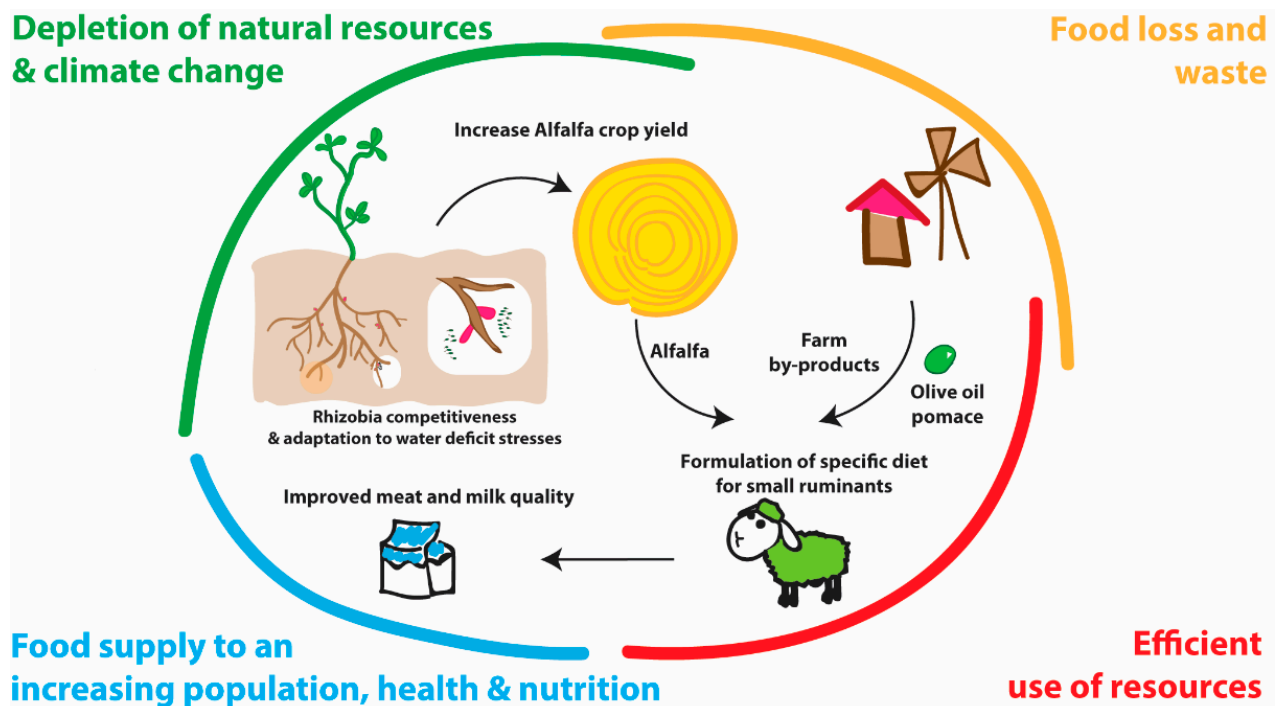


Figure 2. Objectives and deliverables of the project.

### 2.1. First Objective: Elite Rhizobial Inoculants to Improve Alfalfa Yield (WP1; Months 1–24)

The main objective of work package 1 (WP1) is the development of an elite inoculant to boost alfalfa production in harsh conditions. WP1 is subdivided into five tasks, and its accomplishment requires the selection of rhizobial strains based on two main phenotypes: high competition capabilities and resistance to dry environments. Therefore, two goals will be assessed in WP1: (i) build a model able to simulate the competition capabilities of a given strain on the basis of its genome content (Task 1.1), and (ii) select and characterize rhizobial strains able to enhance alfalfa production under water deficiency (Task 1.2). The experimental strategies to achieve these two goals are unlinked; therefore, they could progress at the same time for an estimated length of 14 months. Rhizobial strains isolated in Algeria will be characterized for their resistance to water stress and nitrogen fixation efficiency in dry conditions. In contrast, competition capabilities will be determined using a panel of *Sinorhizobium meliloti* strains whose genome is fully sequenced and available on a public repository (not linked to saline stress). Combining results obtained from competition tests with genomic sequences, a list of candidate genes will be compiled, and a model able to predict the competitiveness phenotype will be constructed. The model will allow the most competitive strains among those with a high tolerance to water deficiency to be selected. Strains with both features will then be used to develop elite inoculants (Task 1.3). Finally, the evaluation of inoculants' effects on alfalfa yield and soil health will be performed (Task 1.4, 1.5; Figure 3).

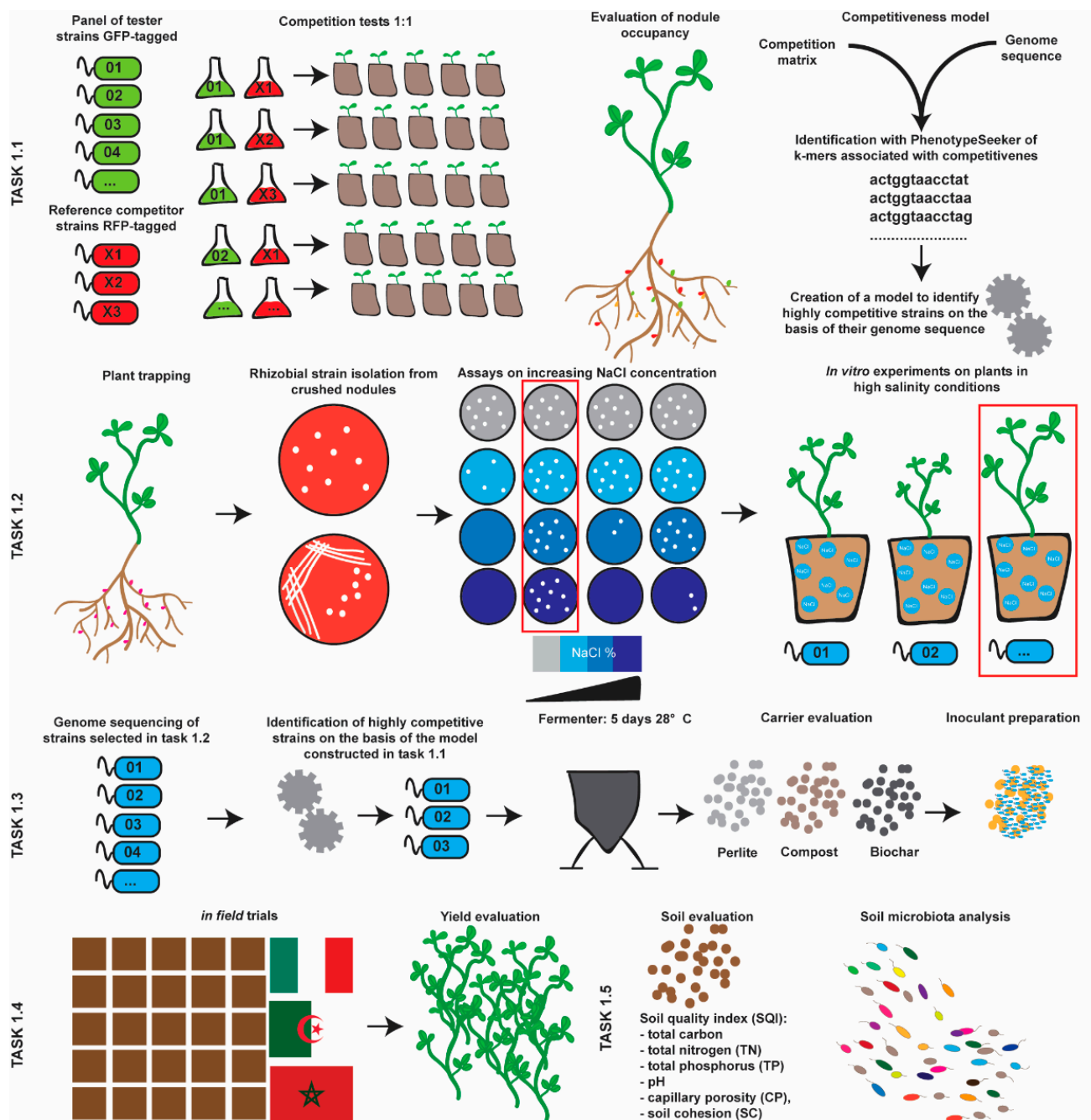


Figure 3. Experimental workflow of work package 1.

### 2.1.1. Competitiveness Model Construction (Task 1.1; Months 1–14)

Decoding the competitive pattern that occurs in the rhizosphere is challenging in the study of social interaction strategies of bacteria. It is fundamental to understand which traits make different rhizobial strains able to win the competition for plant infection over the other indigenous rhizobia. In this first task, a model to evaluate strain competitiveness will be built. The model will be constructed using *meliloti* strains whose genome assembly is complete; currently, out of 237 sequenced genomes, only 26 are completely assembled. A panel of *S. meliloti* strains (at least 13) will be tested against three different *S. meliloti* reference competitor strains. Competition tests will be performed using sterile vermiculite and nitrogen-free solution in controlled growth conditions. Alfalfa seedlings will be inoculated with a rhizobial mix of two different fluorescent-labelled strains at a time. In particular, the

13 *S. meliloti* tested strains will be green fluorescent protein GFP-tagged, while the three reference strains will be red fluorescent protein RFP-tagged. After 28 days post-inoculation, the nodule occupancy will be assessed using an epifluorescence stereomicroscope. PhenotypeSeeker will be employed to identify the genetic features responsible for the competition phenotype assessed in these tests [17]. PhenotypeSeeker can identify competition-specific k-mers using bacterial genomic sequences and a matrix with the phenotypic data, i.e., the results of the competition tests (in other words, the single nodule occupancy expressed as the percentage of green nodules found for each strain in each competition). The most significantly associated k-mers ( $p < 0.05$ ) with the competition phenotype will be mapped on the genomic sequences of the 13 *S. meliloti* strains to recognize the genetic biomarkers determining competitiveness, and a model to fish highly competitive rhizobial strains will be constructed.

#### 2.1.2. Selection of Rhizobial Strains Tolerant to Water Deficiency Stress (Task 1.2; Months 1–14)

This task will be devoted to the isolation, characterization, and selection of rhizobial strains with enhanced tolerance to water deficiency. Rhizobia will be isolated in Algeria by plant trapping. Briefly, alfalfa plants (variety California) grown in different fields (at least three) of the Oran region in the North-West of Algeria will be used to trap rhizobia from soils. Alfalfa nodules will be detached from the roots, disinfected with sodium hypochlorite solution, and then washed with distilled water and crushed. The homogenate will be spread on Petri dishes containing Yeast Extract Mannitol Agar (YEMA) supplemented with Congo red [27]. The colonies obtained, representing the characteristics of rhizobia, will be purified by repeated streaking and further characterized to confirm their identity and resistance to water deficiency. At least 100 different isolates will be analyzed. Isolates will be identified by sequencing the gene coding for 16S rRNA (Ribosomal RNA). The tolerance of each rhizobial strain to NaCl will be determined by evaluating the development of distinct colonies after three to seven days of growth on YEMA medium containing increasing doses of NaCl. On more promising isolates, a deep characterization using Phenotype Microarray technology will be conducted [28], and each strain will be evaluated for its nitrogen fixation efficiency in normal and stressed (high salt concentration) conditions.

#### 2.1.3. Elite Inoculants Formulation (Task 1.3; Months 14–18)

The genome of rhizobial strains selected in Task 1.2 will be fully sequenced. The model obtained in Task 1.1 will be used to infer which strains have highly competitive capabilities. Strains characterized by high nitrogen fixation efficiency in water-stress conditions and high competitiveness will be selected and used as inoculants for in field experiments. Inoculants will be produced in a pilot fermenter at 28 °C for about five days. The growth of the inoculant will be followed by centrifugation at  $8000 \times g$  to increase the inoculum concentration by one order of magnitude. Three different carriers will be evaluated: perlite, compost, and biochar from pyrolysis. For inoculum preparation, all the carrier materials will be ground, passed through an 80 mm sieve, and autoclaved. The inoculum obtained, as indicated above, will be combined with recognized cell protector consisting of 1% locust bean plus 1% trehalose (weight:volume). The cellular suspension will be uniformly and septically mixed with the carrier. After the preparation, the inoculum will be immediately transferred to 4–6 °C.

#### 2.1.4. Inoculants Evaluation (Task 1.4 and 1.5; Months 16–24)

In Task 1.4, the agronomic performance of the rhizobial inoculants will be evaluated. Inoculants will be tested in field trials in Italy, Morocco, and Algeria to appraise the superiority of the inoculant containing the selected strains compared to the autochthonous rhizobia. In Italy, field trials will be located at MoLTE (Montepaldi long term experiment, [www.dagri.unifi.it/p473.html](http://www.dagri.unifi.it/p473.html) (accessed on 1 March 2021)), the longest experiment on organic farming of the whole Mediterranean area that features a number of studies on agronomic and biodiversity performances of organic agriculture practices, including inves-

tigations on nitrogen dynamics in the crop-soil system [29,30]. The experimental design will follow a statistical pattern of randomized complete blocks with three replications. The experimental unit will be about 25 m<sup>2</sup> (5 × 5) plot, with rows 0.5 m apart and spaces between plants of 0.15 m. Experimental groups will be spaced 2 m apart to prevent the spread of inoculants in the soil solution. After the first cut, alfalfa plants will be harvested at the late bud stage. Harvested material will be weighed fresh and after oven-drying at 50 °C for 48 h to evaluate the yield.

Inoculants will be evaluated not only in terms of increase in crop yield but also for their effect on soil health (fertility and microbial diversity). In Task 1.5, the impact of selected inocula on soil health will be measured through the soil quality index (SQI) system, which consists of measuring total carbon, total nitrogen (TN), total phosphorus (TP), pH, capillary porosity (CP), and soil cohesion (SC). Moreover, the soil microbial community structure and diversity will be characterized. MiSeq metagenomics Illumina pipeline, targeting the V3-V4 region of the 16S rRNA gene, will be employed [31]. Data will be analyzed to derive bacterial taxa presence and abundance using well-established software (QIIME/DADA2 [32,33]) and ad hoc software developed by the proponents.

## 2.2. Second Objective: Balanced Diets for Ovines (WP2; Months 12–36)

The development of animal feeding strategies using alternative feed resources plays a key role in reducing the feed/food competition and moving towards sustainable production systems. Furthermore, the use of diets able to reduce the environmental impact of livestock farming is desirable. WP2 has four objectives: (i) the formulation of livestock diets coupling agro-food by-products and alfalfa, thus decreasing the production of farm bio-wastes, (ii) the optimization of animal diet by satisfying the animal requirements and possibly reducing methane emissions and nitrogen excretions [21], (iii) the description of the effects of the tested diet on the complex rumen microbial communities and on rumen metabolism exploiting powerful -omic technologies, and (iv) increasing the quality of animal products (i.e., ovine meat and milk) by providing new balanced diets formulated using alfalfa and by-products. WP2 will be divided into two tasks. In Task 2.1, preliminary characterization of the diets and *in vitro* fermentation will be performed to select the diets for small ruminants for the following *in vivo* feeding trials. In Task 2.2, the activities that will be performed are (i) production trials, (ii) the metagenomic and metabolomic characterization of rumen microbial communities and rumen content, and (iii) the characterization of animal meat and milk (Figure 4).

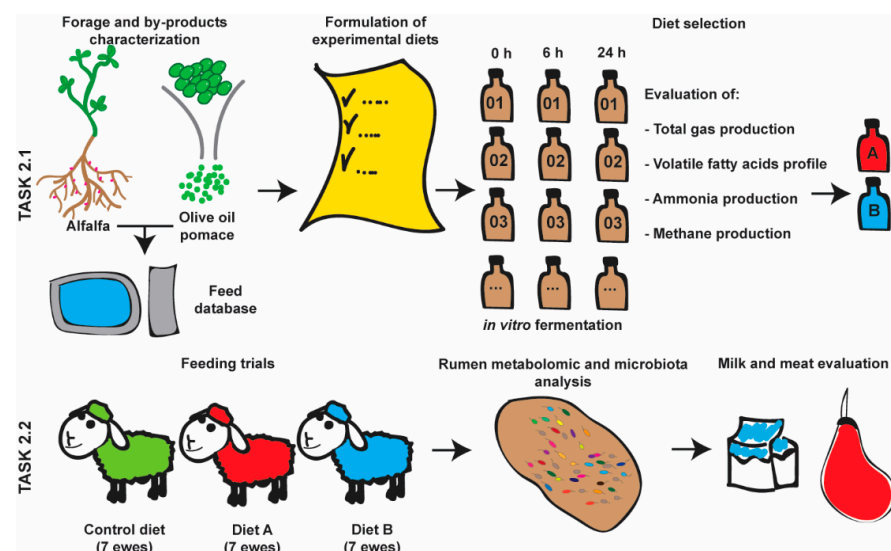


Figure 4. Experimental workflow of work package 2.



A detailed nutritional evaluation of legume forage alfalfa and by-products obtained from food production and processing will be conducted to identify nutritional complementarities. Olive oil pomace will be used in the project since it is widely produced in the Mediterranean area and can modulate rumen metabolism due to its high polyphenolic content [34].

#### 2.2.1. In Vitro Rumen Fermentation Trials (Task 2.1; Months 12–20)

In Task 2.1, the experimental diets, which will be tested in preliminary in vitro rumen fermentation trials, will be formulated. Before diet formulation, a characterization of forages (alfalfa) and by-products will be performed to identify nutritional complementarities. The characterization will be performed by analyzing their chemical composition (e.g., carbohydrates, proteins, liposoluble vitamins, fatty acids, macro- and micro-minerals, and secondary plant metabolites). In vitro digestibility and buffer solubility tests will be performed to evaluate the nutritive value of feedstuffs. Data obtained by this preliminary characterization will be included in a feed database and used to design the optimal theoretical diets.

The information obtained will be used to formulate different diets in order to meet animal requirements according to the Cornell net carbohydrate and protein system [35]. Diets will then be fermented in vitro using ovine rumen liquor for a preliminary diet screening [36,37]. The whole rumen content will be collected at the slaughterhouse from different animals and used to inoculate the fermenters. The rumen content of each animal will be mixed to obtain a representative sample and filtered through cheese cloth into a flask under a flux of CO<sub>2</sub> [38]. Samples will be collected from the fermenters at different sampling times (e.g., 0 h, 6 h, and 24 h), and total gas production, volatile fatty acids profile, and ammonia and methane production will be determined [38,39]. The obtained data will be used to evaluate the best combination of the ingredients in the diets for the in vivo feeding trials.

#### 2.2.2. In Vivo Feeding Trials (Task 2.2; Months 18–36)

In Task 2.2, the feeding trials on dairy sheep and light lambs will be performed to evaluate the effects of the selected diets (according to the results obtained in Task 2.1) on animal performance and milk and meat quality. Twenty-one ewes will be allotted into three groups (seven ewes per group). The ewes will be previously synchronized for the estrus and inseminated in order to have lambing in the same week. Four weeks before lambing, the ewes will be assigned to the diets: a control diet (cereal grains and soybean) and two experimental diets (cereal by-products, alfalfa, and stoned olive cake at two levels of inclusion). Diets will be administered from 4 weeks before lambing to 12 weeks after lambing. Each ewe will be allotted an individual box in order to measure individual feed intake. After lambing, lambs will be maintained with the mother for one month and fed mainly with maternal milk. Lambs' individual weight will be recorded weekly from birth until slaughtering (40 days), then ewes' milk yield and quality will be recorded weekly until 12 weeks after lambing. Milk composition and quality will be analyzed by an infrared analyzer. Rumen content will be analyzed by DNA targeted metagenomics to characterize the microbiota and by mass spectrometry-based metabolomics to describe the effects of the diets on rumen metabolic pathways and functional or productive responses. Nutritional characterizations of milk and meat, oxidative stability, proximate chemical composition, fatty acid composition, cholesterol, liposoluble vitamins, and polyphenols content will be determined. Meat color and tenderness will be evaluated.

#### 2.3. Third Objective: Exchange of Knowledge and Scientific Cooperation among Countries (WP3; Months 1–36)

Cooperation among countries and knowledge improvement are among the main pillars in ALL-IN. The dissemination plan is inextricably linked to scientific and extensive audience communication, stakeholder engagement activities, and the exploitation of the results. Therefore, ALL-IN will approach these aspects in a coherent and integrated way

by drawing them together in a comprehensive Dissemination and Exploitation Plan (DEP). A DEP will be developed using social media communication strategies, public digital information on climate change and the sustainable use of water resources in the Mediterranean agricultural production and the importance of circular economy reusing agriculture residuals. The exchange of knowledge of the innovative solutions will also be achieved by creating a project web portal platform (ALL-IN platform) involving technical staff and stakeholders. The platform will include a repository, ICT (Information and Communications Technology) tools for data gathering, and use and communication areas. Educational activities will be developed for training professionals (including young researchers) and local farmers. Training programs (e.g., a summer school) on massive DNA sequencing technologies and bioinformatics analyses for PhD and postdoc students will be performed. Educational activities organized by UORAN and UMI will involve local farmers together with Algerian and Moroccan stakeholders.

The Stakeholder Engagement Strategy, which will be included in the DEP, will (i) clearly identify the different stakeholders, in particular, ALL-IN end users, and (ii) develop targeted exchanges tailored to best fit specific and relevant stakeholder groups and general communication tools and channels for broader uptake. Existing local linkages with growers and local farmers already established in other initiatives will help to conduct field and animal experiments. In Italy, the ALL-IN project will be supported by “Gruppo Operativo”, which includes a large number of actors in the production of milk, cheese, and other dairy products. In Algeria, the ALL-IN project obtained the endorsement of the “Chamber of Agriculture of Wilaya of Oran”, which is a public company in charge of the agricultural development of the area of Wilaya of Oran. The mission of this institution is the development of green fodder production used to intensify milk and red meat production. In Morocco, the ALL-IN project is supported by the “Fédération Interprofessionnelle Marocaine de la Filière Biologique—FIMABIO”. FIMABIO was created, as stipulated in the statutes, by including three exclusive members (L’Association Nationale des Producteurs de la Filière Biologique, l’Association Nationale pour la Valorisation des Produits Biologiques, and l’Association Nationale des Distributeurs et Exportateurs des Produits Biologiques), who represent the activities (or colleges) of production, enhancement, and marketing/export, respectively, which make up the entire organic sector in Morocco [40].

Stakeholder dialogue will be performed by means of a blog section created inside the ALL-IN platform, where activities, needs, and problems will be posted. The meetings established in the dissemination plan will also ensure dialogue. Stakeholders will perform exploitation activities in terms of added value to grain seeds using the newly developed bacterial inoculant (thereby increasing the market for seed coating companies) and possibly apply for patents on some of the developed technologies (e.g., seed coating, inoculant formulation).

### 3. Expected Impacts

The results of this project will allow chemical fertilizers for crop production to be reduced. The environmental impact is expected to be positive due to the beneficial effects of bacteria for enhanced nitrogen fixation and plant growth and yield. As a result, dependency on fossil-based energy (needed for manufacturing, distribution, and use of fertilizers) can be reduced, creating an indirect economic benefit. The results of this research are then directly applicable to local crop production; the increasing yield will then benefit both producers and consumers. Increased production of legumes will reduce actual needs for importation in some cases, whereas in others, it will increment the exportation to demanding markets. Discarded products from food processing will be reused in animal feeding in combination with alfalfa, concurring in reducing feed-food competition. Results obtained by testing a balanced diet will be available for farmers, allowing the sustainable recycling of local farm by-products. Relevant results will be available on a dedicated website, where both scientific data and a lay summary of results and their impact will

be reported. In this way, though the primary recipient of the results will be the scientific community, professionals and citizens can access the obtained results.

#### 4. Conclusions

In the past decade, the awareness of promoting responsible food consumption and production is progressively increasing, leading to a gradual change in our agricultural and livestock systems. From a circular economy perspective, the research project presented in this paper describes a new strategy to increase alfalfa yields, which could act as a driver to improve the quality and quantity of animal products. In particular, the reported approach will allow three important milestones in environmental, social, and economic sustainability to be achieved: (i) the formulation of effective rhizobial inoculants for drought soil conditions will allow the increment of alfalfa yields and the recovery of marginal soils, thus lowering the antagonism between food and feed production for the acquisition of arable land, (ii) the valorization of food by-products as sources of bioactive molecules capable of ameliorating the nutritional quality of animal products will be a key component to reduce the environmental impact of livestock production, and lastly, (iii) the formulation of specific ovine diets by using the optimal combination of alfalfa with farm organic by-products will be fundamental to replace the massive use of feedstock with an economic model based on the reuse of resources.

In conclusion, the transition towards a circular economy may contribute to the transformation of the rural economy in the Mediterranean area and generate new holistic and integrated approaches that will create local job opportunities and chances for social integration and cohesion.

**Author Contributions:** Conceptualization, F.P., A.M., M.M., A.B. (Agnese Bellabarba), M.D., A.B. (Arianna Buccioni) and C.V.; methodology, F.P., A.M., M.M., A.B. (Agnese Bellabarba), M.D., A.B. (Arianna Buccioni) and C.V.; writing—original draft preparation, F.P., A.M., M.M., A.B. (Agnese Bellabarba), M.D., A.B. (Arianna Buccioni) and C.V.; writing—review and editing, F.P., A.M., M.M., A.B. (Agnese Bellabarba), M.D., A.B. (Arianna Buccioni), G.C.P., A.B. (Abdelkader Bekki), K.A., M.H., C.V. and visualization, F.P. All authors have read and agreed to the published version of the manuscript.

**Funding:** The authors acknowledge the financial support for this project provided by transnational funding bodies, partners of the H2020 ERA-NETs SUSFOOD2 and CORE Organic Cofund, under the Joint SUSFOOD2/CORE Organic Call 2019.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

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