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SUSTAVIANFEED

ALTERNATIVE ANIMAL FEEDS IN MEDITERRANEAN POULTRY BREEDS TO OBTAIN SUSTAINABLE PRODUCTS

SUSTAINABLE FEEDING PROGRAM DEFINITION

DELIVERABLE 2.1

This project (grant Number 2015), is part of the PRIMA programme, supported by the European Union

Partners

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SUMMARY

The development of a methodology for the development of sustainable feeding programs for the aviculture sector and the proposal of preliminary diets to be used in pilot project activities are the two main objectives of this document.

First of all, a methodology has been defined with the different steps followed during task development and which can be exploited to other context and farming sectors, beyond the aviculture one.

Then, all the different steps followed have been explained so a consistent document is developed.

The first step followed has been the development of a usual ingredients list to be contemplated for the elaboration of the diets. In this step, the first set of LL activities has taken places, in order to gain knowledge with from external actors. These activities have offered relevant and quality results which were contemplated in the diets proposals.

Then, the study of nutritional composition of the different ingredients proposed have been studied by using relevant bibliography as well as conducting some analysis when information was missing. This, together with the specification of the nutritional requirements of each pilot project, has led to the definition of the initial preliminary diets, proposed by each partner and which have been the basis for the whole task development.

These initial preliminary diets have been refined by several activities done:

- Internal evaluation of the initial preliminary diets made by ALIA and UMU.
- Feed safety and health evaluation of the diet (task 2.3).
- Environmental evaluation of the diet (task 2.4).
- Nutritional evaluation of the diet (task 2.5).
- Diet validation by external actors in the framework of LL activities (task 4.1).

After studying all these inputs, the sustainable feeding program has been proposed. Nevertheless, it should be considered as a proposal, as due to availability of ingredients for the project start and the nutritional characterization of insects and other ingredients, the sustainable program may have some deviations, although the methodology will be followed as indicated in this document.

All the sustainable feeding programs proposed perfectly satisfy the nutritional requirements of the animals, while achieving project objectives in terms of reduction of the environmental inputs, included of by-products and alternatives ingredients and reduction of imported ingredients. The different proposed diets reduce the environmental impact from the 17% (the most conservative one to the 50% (the most ambitious one).

This research has established a specific and tangible path for the development of alternative diets and, consequently, a sustainable farm sector. It can be adapted to different locations and context, which makes it an example of exploitation possibilities.

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Acronyms and abbreviations

Abbreviation	Description
DDGS	Distiller's Dried Grains with Solubles
FAO	The Food and Agriculture Organization of the United Nations
FEDNA	Fundación Española para el Desarrollo de la Nutrición Animal
GDA	Directorate-General for Agriculture and Rural Development
GDPR	General Data Protection Regulation
GHGs	Greenhouse Gases
INRA	Institut National de la Recherche Aagronomique (National Institute of Agronomic Research)
LCA	Life Cycle Assessment
LL	Living Lab
NRC	National Research Council
WP	Work Package

1 Introduction

Climate change and the ecological crisis are the most important challenge our society has ever faced. The global temperatures are higher each year due to GHGs emissions and the earth resources are more limited due to the vast paths of consumption that our society has.

The agri-food sector has a great impact on both aspects and it is responsible of about 31% of GHGs emissions as shown in recent studies. Among them, the livestock sector stands out, accounting about half of the emissions. In addition, the most important trend since 1990, is the increasingly important role of food-related emissions generated outside of agricultural land, in pre- and post-production processes along food supply chains, at all scales (Tubiello et al, 2022).

Inside the livestock, the feed production has a high impact in the whole supply chain. It is associated to deforestation issues (especially in the case of soybean), to a high transportation impact, land-use or water consumption, among others. In addition, the growth in world population, higher demand for animal protein, and its consequence pressure on natural resources, makes this problem bigger and it is expected to growth. Therefore, the development of alternative diets for animals must be the mainstream of the livestock sector in order to reduce its environmental impact.

Furthermore, due to the increasing environmental awareness, industries are working on the sustainability field so new market possibilities are opened.

Considering all this, SUSTAvianFEED project will develop sustainable diets for the aviculture sector by including alternative feed sources and insects in the nutritional formula, as well as through the fostering of local ingredients and resources use.

In the chicken meat supply chain, 78% of the emissions are associated to feed production and 69% is associated when talking about the egg supply chain. Consequently, to reduce the environmental impact of the feed production is remarkably the most important aspect for a sustainable aviculture approach.

Furthermore, the global context we are facing nowadays, after the pandemic and because of the current geopolitical situation, has created a period of uncertainty in the global economy and the food sector.

The energy crisis which has increased the electricity and gas prices, cost of transport (freight rates have more than doubled), lack of resources in several parts of the world as raw materials for feed production, makes it especially important to promote sustainable, resilient and local approaches for the sector.

As reported by the data of the Chicago Board of Trade, the international reference point for the future market of agricultural commodities, but trivially also the latest international updates, the quotations of the main elements of the animal diet have skyrocketed to historic highs, with corn recording the largest increase of the decade, while soybeans have reached the peak for almost seven years.

So, it's urgent that new food chains must be environmentally friendly, foster local economies and consider social aspects, and feed production will be the mainstream of this change.

2 Methodology

2.1 Scope

The core of SUSTAvianFEED project is to promote sustainable farming sectors by developing alternative feeding programs. Therefore, WP2 and task 2.1 have a vital importance in the whole project development.

The main objective of the sustainable feeding program definition is to develop a methodology and framework which can be applied to different scenarios along different countries and regions with environmental and socioeconomic conditions. These sustainable feeding program will be the mainstream of a sustainable aviculture sector.

Therefore, the project and WP objective is not only to define a single solution for a specific momentum and conditions, but also to develop a suitable way of working which can offer a solution for SUSTAvianFEED project pilots, and also for other projects, regions, and, in summary, for sustainable farming practices across the Mediterranean area.

The development of alternative diets has been marked by the idea of developing a resilient sector in which geopolitical scenarios, prices increase and other factors which may affect the sector do not have a crucial impact and the sector can continue growing in a sustainable way from the environmental, economic and social way.

The inclusion of local ingredients, the use of by-products which can establish synergies among feed producers and agri-food industries and the promotion of alternative and innovative protein sources coming from insects have been some of the aspects considered for the diet elaboration.

These approaches were evaluated from the environmental, safety, health and nutritional perspectives in order to define the most optimal feed programs.

It is vital to consider that the sustainability of poultry meat and eggs production is becoming critically important due to the increasing environmental awareness, the growth in world population, higher demand for animal protein, and its consequence pressure on natural resources. Broiler production is an important source in meeting this demand. On the other hand, compared to other meat-producing animals, broilers have one of the best feed conversion rates and the smallest environmental footprint (Ritchie, H. & Roser, M., 2021).

2.2 Methodology

In order to define the sustainable feeding programs for each pilot, several steps have been followed (Figure 1):

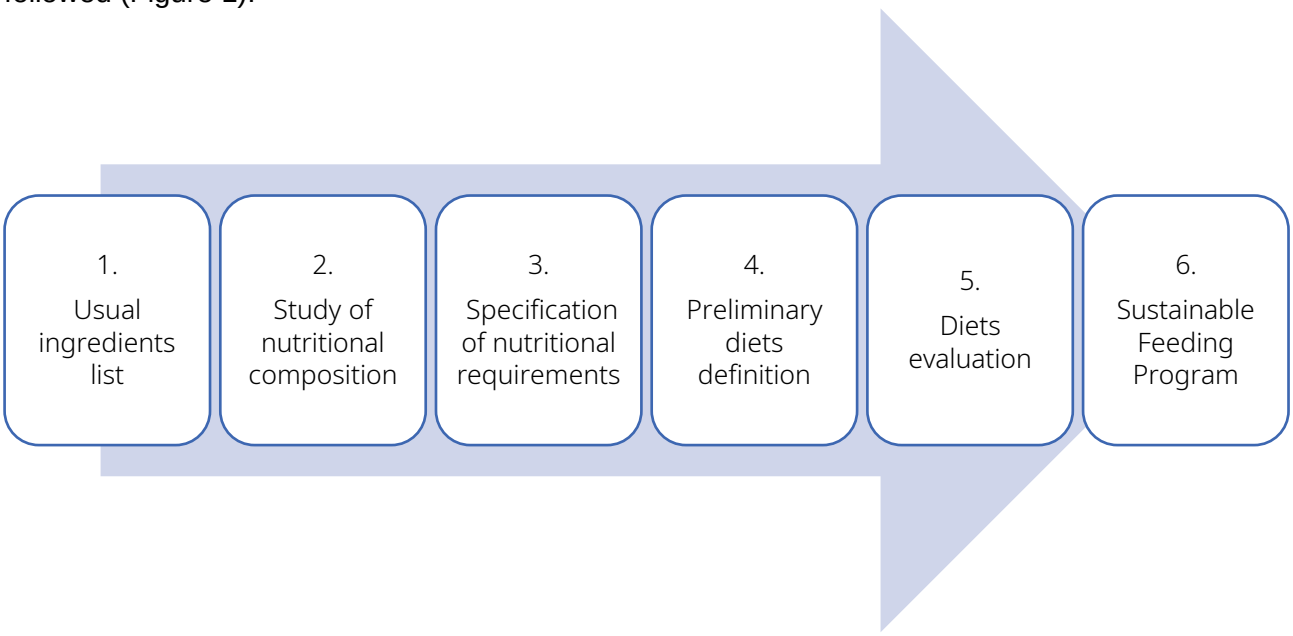


Figure 1. Methodology followed for the sustainable feeding program definition

1. **Development of a usual, alternatives, local ingredients, and by-products list.**

First of all, an extensive list of the usual ingredients, and by-products was elaborated in order to be analysed and to start the process of diets elaboration. Each pilot partner developed it for its own region.

For Tunisian case, a close cooperation was made from this step until the end of the process between ISA-CM and RAYHANA, because of the similarities of their regional circumstances. By checking this initial list, the first possibilities for further diets elaboration were thought internally.

One key aspect of this first step, was the development of the second living lab activity of the project “LL A2. Sustainable feeding program design: Interviews with local experts”. It had the objective of involving external actors in order to complement the list and to include more possibilities for the project. This LL activity is further developed in section 3 of the document.

2. **Study of nutritional composition of known ingredients, and by-products together with insects.**

After the first list of ingredients was developed, it was necessary to determine the nutritional value of the usual ingredients used in each area (Spain, Italy, Turkey and Tunisia), as well as to nutritionally characterize the alternative ingredients or by-products available.

In this step, each participant created a database with the nutritional value of the ingredients, which was used for the optimization of the diets in order to meet the requirements of the birds according to the different pilots, optimizing balanced diets.

A design of isoenergetic and isonitrogenous diets was studied according to the production phase and pilot breed. This was developed in order to compare feeding programs with usual diets (less sustainable) with alternative programs with the inclusion of more sustainable diets (with alternative ingredients and *Hermetia illucens* insect's larvae).

The nutritional characterization of usual ingredients of the feed for laying hens or/and meat-type chickens, as well as the possible local ingredients to be included in the sustainable feeding program (by-products or other alternatives) was done by using the most relevant existing bibliography.

On the other hand, for those ingredients that lack sufficient information about their nutritional value, chemical analysis (especially the by-products of some area) were performed.

In the case of insects, again, relevant bibliography was studied. In addition to this, some analyses were carried out by ALIA, UMU and EGE in order to complement this information. In this step, it is necessary to say that, due to the variability on insects' composition, before pilots starting point, the analysis will be conducted again so the diets composition is refined.

This information is further detailed in Deliverable 2.5 "Nutritional evaluation of the diet".

3. **Specification of nutritional requirements.**

The third step consisted in the selection of the genetic type of animal adapted to its environment to be used in the further pilots' activities within SUSTAvianFEED project so the nutritional requirements of the animals were defined. These requirements will depend on the genotype, the type and production system, as well as the environmental conditions.

The different pilot partners selected the animals to be used (Figure 2). This can be appreciated as follow:

- **UMU (Spain):** laying hens, crossing with breeds adapted to western-Mediterranean, for first phase of lay production, meeting the nutritional requirements of these animals according to FEDNA (2018).
- **UNITO (Italy):** meat chickens, Bianca di Saluzzo male (an Italian autochthonous breed), for Grower (d0 – d60) and Finisher (d61 – d150) periods, meeting the nutritional requirements of these birds according to low input diets for slow-growing chickens (Cerolini et al, 2019).
- **EGE (Turkey):** meat chickens, in this case the Anadolu-T (ecotype) and a commercial fast-growing strain (Ross 308), for Starter (d0 - d14), Grower (d15 - d28) and Finisher phases (d29 - slaughter age), meeting the nutritional requirements according Sarıca et al. (2019; 2021).
- **ISA-CM and RAYHANA (Tunisia):** meat chickens and laying hens, in this case the autochthonous Tunisian (ecotype), Geánt and Génosie (local) will be used, meeting

nutritional requirements according to TECHNA (Tunisian company expert in feed formulation).

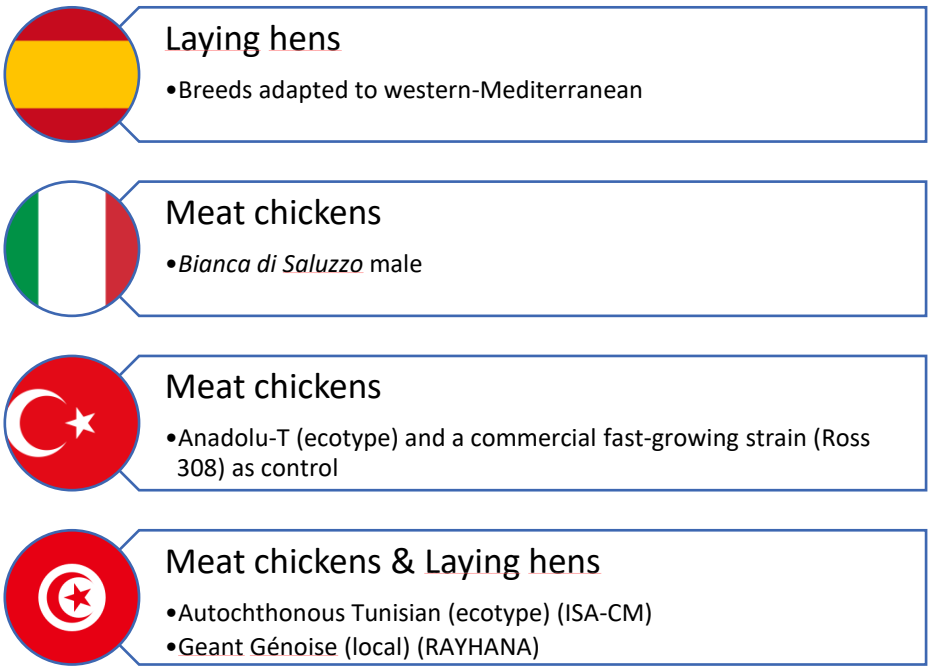


Figure 2. Selection of breeds for the pilot implementation

This information was complemented by the phase and production level of each of them in order to define the nutritional requirements of the animals. Detailed information is shown in (Table 1).

Table 1. Characterization of the birds, production and references of nutritional requirements

Pilot	Type of production	Avian breed/hybrid Performances	Phase evaluated for the pilot
Spain (UMU)	Laying hens	• Crossing with breeds adapted to western-Mediterranean • Body weight (1.5 kg at 18 weeks, and 2.3 kg at 78 weeks) • 290 eggs/year • Egg weight: 61.5 - 62 g	• Lay phase • First phase of lay up to 40 wk approximately
Italy (UNITO)	Meat chickens	• *Bianca di Saluzzo* male (Italian autochthonous breed) • Body weight (2,8 at 25 weeks) • Average daily intake (adult): 140 g/day	• Grower (d0 – d60) • Finisher (d61 – d150) • Slaughter age: 150d
Turkey (EGE)	Meat chickens	• Anadolu-T (ecotype) • Average 1,69 kg in 35 days and 2,3 g at 42 days • Feed consumption 4258-4566 (para UNITO, typing error g/bird at 42 d	• Starter (d0 - d14) • Grower (d15 - d28) • Finisher (d29 - slaughter age)

Tunisia	Meat chickens	• ISA-CM: Autochthonous Tunisian (ecotype)	• Starter (d1-d28) • Grower (d29-d66) • Finisher (d67 - slaughter age)
	Laying hens	• RAYHANA: Geánt and Génosie (local)	• Laying phase

This information is further detailed in Deliverable 2.5 “Nutritional evaluation of the diet”.

4. Proposal of preliminary diets

After the characterization of the ingredients and the animals’ nutritional requirements were defined, the first proposal of diets was developed per each pilot area.

For these proposals, some criteria were defined:

- Formulation of optimized diets to meet the requirements of birds according to type of poultry production and phase of each pilot.
- Implement a diet design, by phase and type of production, to compare a usual control diet (with non-sustainable ingredients), and other diets that include more sustainable ingredients (according to the criteria of deliverable 2.4 about feed impact).
- Sustainable diets should contain less imported soybean meal (or other imported ingredients), and incorporate alternative ingredients (unusual or by-product).
- At least one of sustainable diets must include larvae of the insect *Hermetia illucens*.
- At least the control and one alternative diet will be iso-energetic and iso-nitrogenous (for crude protein and/or amino acid), by phase and type of production, in each pilot.

These criteria resulted in the development of three different types of diets in which a standard diet could be compared with two sustainable diets in which insects would be included in at least one of them (Figure 3).

Figure 3. General criteria for the development of preliminary diets

In this step, the environmental impact of the ingredients was considered as well in order to make the preliminary diets with an environmental perspective. For this, in a first phase, a database has been built consisting of the most usual ingredients to manufacture poultry feed, and, in addition, possible alternative ingredients have been included.

This database has been made for both egg and meat production according to the information provided by each pilot. This data was used in order to have in mind that the alternative diets have to be at least 10-15% more sustainable than the controls one.

This is further developed in section 4 of the present document.

5. Diets evaluation

The definition of the initial preliminary diets was complemented by several actions in order to define the final sustainable feeding program. These actions are summarized below:

1. Review of the different pilot diets between task and WP leader (ALIA and UMU). The two partners have used their experienced in diets' formulation in order to offer feedback to the different groups and make changes accordingly. To this aim, the Cargill's Feed Management Systems Brill Formulation software was used, among others tool and relevant bibliography.
2. The feed safety and health evaluation of the diet, which was studied in detail in task 2.3, also checked that no conflicts were present among the diets' formulation proposed and health and safety issues, as well as the important aspects to be considered from the different ingredients.
3. The environmental evaluation of the diet, which was study in detail in task 2.4 and which offered key information for the development of the sustainable diets.
4. The nutritional evaluation of the diet, which was study in detail in task 2.5, was fundamental in order to define the final diet.

5. Finally, in the framework of LL activities “LL A4: Sustainable Feeding program Design: Workshop/Focus Group for farmers validation on the feed program” and “LL A5: Sustainable Feeding program Design: Interviews with farmers for validation on the feed program” were used for diets validation and to include relevant inputs in the diet definition.

This is further developed in section 5.

6. Sustainable Feeding Program definition

Finally, after all these steps, the sustainable feeding programme has been defined and proposed for the future pilot implementation (Figure 4). However, as already explained, the ingredients, by-products and raw materials availability may modify the original concept. The approach and philosophy would be similar, but some adjustments in the formulas are expected for the actual pilot activity. Therefore, this should be considered as a preliminary sustainable feeding program.

This step is further developed in section 6 of the present document.

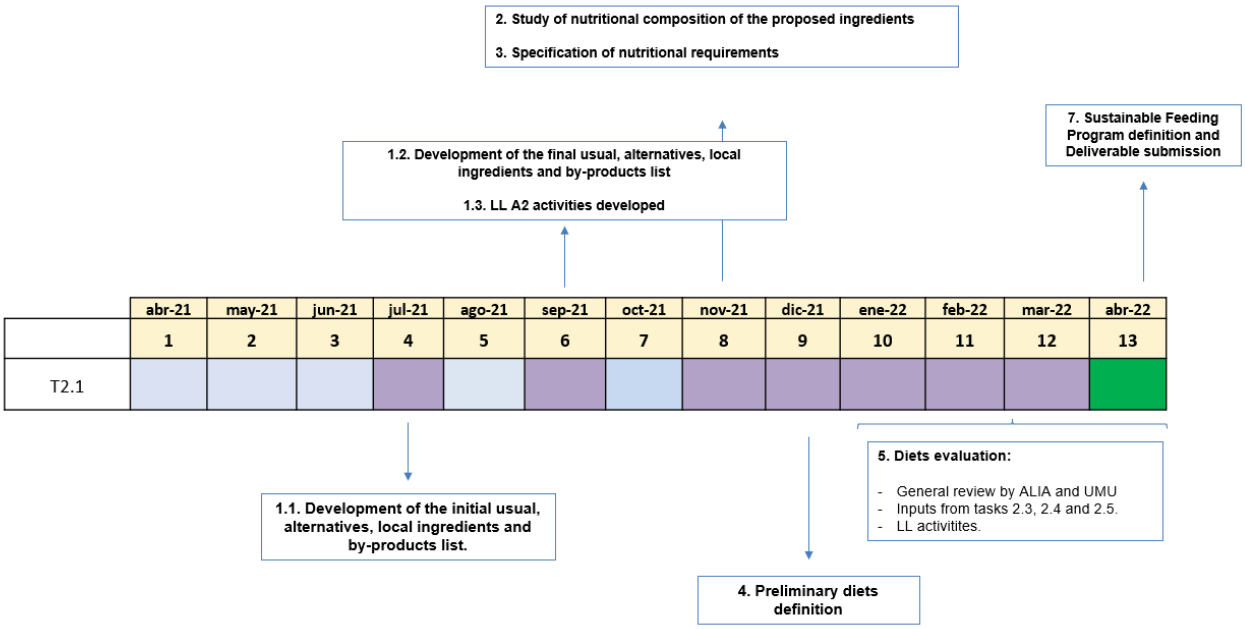


Figure 4. Timeline of task development

3 Interviews with local experts for the sustainable feeding program design

The second living lab activity developed in the framework of SUSTAvianFEED project (after the first one, about each partner's stakeholder mapping) was focused in the improvement of the ingredients list of possible by-products, local ingredients, etc., to be included in the alternative diet to be developed in the project. The main characteristics of this activity are:

- **Objective:** To elaborate an optimal, realistic and extensive list of possible by-products, local ingredients, etc., to be included in the alternative nutritional diet to be developed in the project.
- **Tasks Related:** Task 2.1
- **Partners involved:** ALIA, UMU, UNITO, ISA-CM, RAYHANA, ENTOMO, EGE
- **When:** From July to October 2021
- **Target group:** Professionals and experts in animal feed from academia and private sector
- **Location:** Online
- **Key themes:** List of possible by-products, local ingredients, etc., to be included in the alternative nutritional diet to be developed in the project.
- **Developed activities:** Semi-structured Interviews, survey, focus groups.
- **Expected Output:** to extract new insights to be included in the formula and to confirm or change the initial ideas.

Each pilot partner engaged relevant stakeholders to this aim. The summary of activities developed by country and the type of stakeholders engaged are shown in Figure 5 and Figure 6.

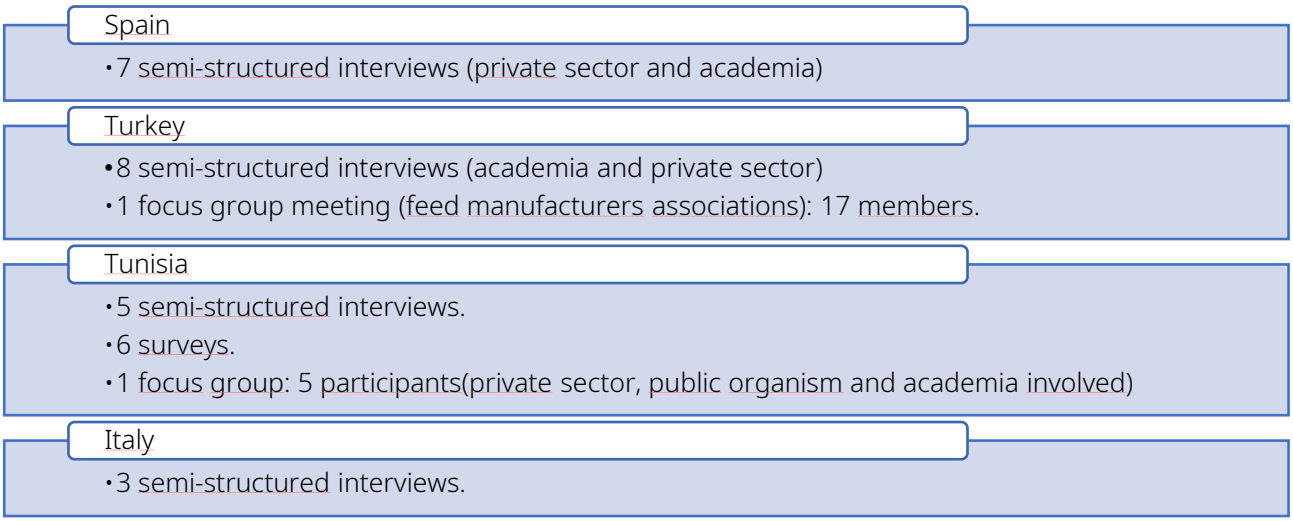


Figure 5. Activities developed per country

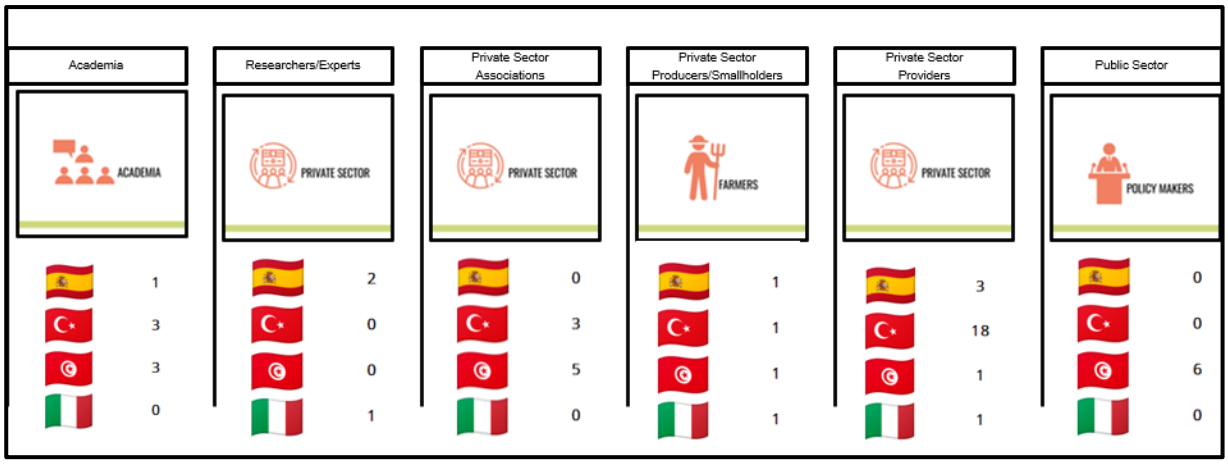


Figure 6. Type of stakeholders engaged per pilot country

For the development of the activity, common guidelines were developed for all the partners in order to have relevant results from the different countries and to study the suggestions in a similar format. In addition, a preliminary list of by-products and ingredients was developed by each partner which can be shown in Table 2:

Table 2. Preliminary list of ingredients and by-products

SPAIN			
Ingredients		By-products	
Maize	Rapeseed	Cookie flour	
Soybean meal	Animal fat	Sunflower cake	
Barley	Wheat grain	Sunflower flour	
Wheat middlings	Maize distiller grains	Carob flour	
Sodium bicarbonate	Monocalcium phosphate	Malt root	
Salt	Calcium carbonate	Citrus pulp	
Soybean oil	L- Lysine	Rapeseed cake	
Sunflower meal	DL-Metionine		
Soybean hulls	Peas		
ITALY			
Ingredients		By-products	
Maize	Soybean oil	Maize gluten meal	Wheat bran
Barley	Sunflower oil	Broken rice	
Soybean meal	Palm oil	FFP (former food products)	
Sunflower meal	Animal fat	BBP (bakery by products)	
Sodium bicarbonate	DL-methionine	Hazelnut skins (Nutella by-products - source of antioxidants)	

Salt	vitamin-mineral premix	Grape skins (wine by-products - source of antioxidants)	
Phosphates (mocalcium- and dicalcium-)	Pigments	Colza meal	
Calcium carbonate	Enzymes	Peas	
L-lysine		Fava beans	
TURKEY			
Ingredients		By-products	
Corn	Mineral premix	Tomato pomace (dried) (available from end of July to mid of September)	
Soybean meal	Aminoacids (DL-methionine,L-lysine)	Grape pomace (dried) (available from end of July to mid of September)	
Fish meal (*Engraulis encrasicolus*)	Marble dust	Whey powder	
Sunflower seed meal	Calcium sources (Limestone, DCP)	Whey powder (demineralized with high protein)	
Wheat	NaCl		
TUNISIA			
Ingredients		By-products	
Maize	Enzymes	Wheat bran	Olive pomace
Wheat	Antioxidants, acidifiers,	Soybean meal	Grape marc
Barley	Yeasts	Soybeans hulks	Beet pulp
Fava beans	Pigments	Rapeseed meal	Straw
Rapeseed	Anticoccidials	Tomato pulp	S/P of olive tree pruning
Alfalfa plugs	Amino-Acids	Brewer's grain	Carob pulp
Vitamins	Fat	Beet pulp	By-products of the date palm
Minerals	Licking stones	Molasses	
Premixed additives			

The different activities resulted in relevant and interesting key findings which were classified in the following topics (Figure 7):

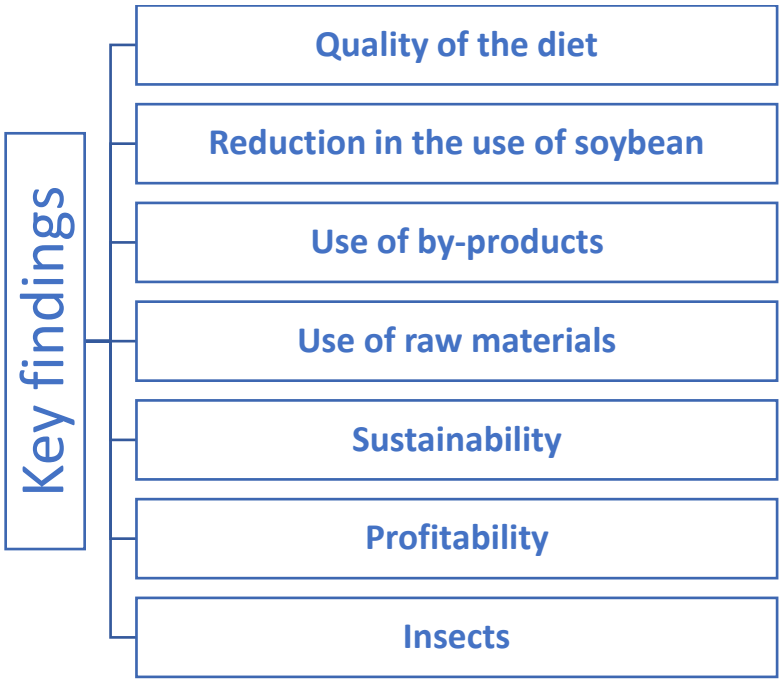


Figure 7. Key findings topics resulted from the LL A2

The most relevant inputs regarding this topic are listed below:

Quality of the diet:

- The fibre is one of the main obstacles for the inclusion of alternative source of proteins.
- Enzymes and other additives can be useful in order to improve the nutrient digestion. In addition, to they are important increase nutrient digestibility and improve feed conversion. It is emphasized that enzyme supplementation can reduce the soybean usage up to 30 %.
- Synthetic amino acids could be considered to adjust the amino acids profile and content particularly that of lysine and methionine,
- Phytobiotics (e.g., onion, garlic, fenugreek, moringa, marjoram, basil, tomatoes, nettle and essential oils...) could be used to promote the health of chicken to be raised under an alternative farming system.
- The search for alternative feed ingredients is more suitable for slow-growing broilers rather than for fast-growing broilers. Slow-growing birds are characterized by lower nutritional requirements than broiler chickens; furthermore, these genotypes would be adapted to free-range farming systems and would provide high quality carcasses.
- It is stated that R&D studies are required at the regional level to make alternatives sustainable, stable and easy to use with considering antinutritional factors.
- It is important of updating data relating to the nutritional value of local ingredients and by-products, in particular their levels of metabolizable energy and digestible amino acids, to make the appropriate diet formulation adjustments.

Reduction in the use of soybean:

- Nowadays, there is a big dependence of soybean and basic cereals, so we need to find adequate alternative ingredients.

- Economic constraints and the competitive market seem to be the limiting factors in terms of solutions.
- Even if there is no complete alternative to soybean, it is important if it is even slightly substituted.
- It is stated that every option that will minimize the raw material cost and make it sustainable is precious.
- Participants suggested to use, if the soybean is needed, certified or national soybean as a way to reduce its environmental impact, although the availability is very limited.

Use of by-products:

- The price of by-products and their heterogeneity could be a restraining for their use.
- Participants agreed on the possibility of the regional by-products and ingredients inclusion for the improvement of the sustainability.
- The possibility of the use of local feed crops and by-products to develop sustainable nutritional formulas is conditioned on they are available in sufficient quantities (at least for half a year) and at a reasonable cost.
- The use of by-products/industry waste products is limited due to their high moisture content. This high moisture level raises energy costs for drying.
- Bread, chips, and crackers whose expiration date is approaching and are collected in markets can be used in feed factories by adding them to chicken diet. They should be analysed first and processed immediately to avoid any food contamination problems.
- Some participants mentioned the use of animal by-products in the nutritional formula (if the legislation would allow it) would be an excellent alternative ingredient.

Other alternative ingredients:

- To consider the local raw materials is fundamental to reduce the cost and increase the sustainability, however, is difficult to compete with the nutritional value of the soybean.
- Alternative raw materials should always be readily available, cheap and sustainable.
- Non-standard sizes of rice and bulgur can be used.
- There are interesting raw materials (fava beans, barley, and triticale) and by-products (rapeseed meal) that can just be used in a limited way because of their low availability.

Sustainability:

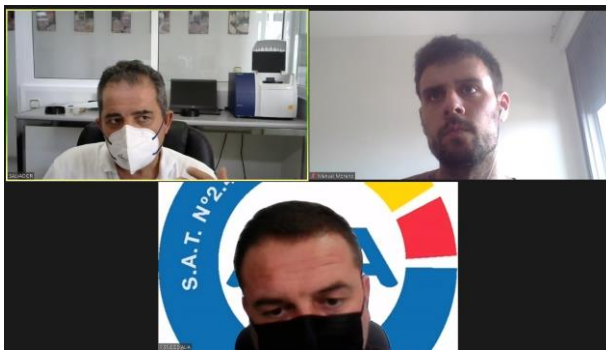
- Better conditions for animals and more sustainability should be a priority for the sector.
- It is important to think about the change of the system as a whole: short cycles, short transports, regional and local synergies, etc.
- National development priorities and strategies must be revised in the light of several environmental constraints, in particular global warming and water scarcity.
- Alternative poultry farming should take conscious steps towards its long-term sustainability action plan by focusing on the circular economy concept and embedding it firmly within the farming systems processes. That includes the use of sustainably and locally produced feed resources and the application of farming techniques easily reproducible by medium and small farmers, mainly rural women.

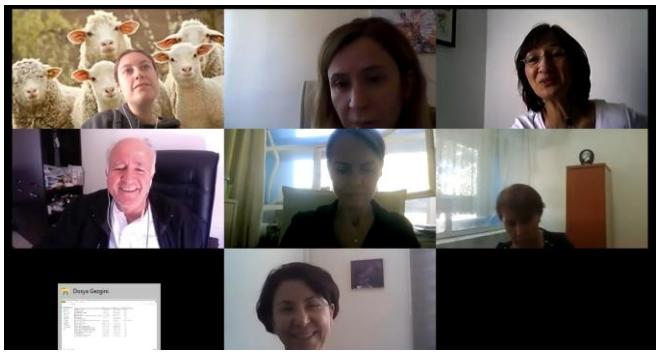
Profitability:

- In order to make a competitive nutritional formula which could provide consumers with products affordable for everyone (and not the eco products which sometimes double the price of the normal ones), the cost of the sustainable diet should be reasonable.

Insects:

- Most of the participants mentioned the importance of including insects as an alternative source of protein, even though that the insects were not part of the scope of the activity,
- There are legislative issues regarding the use of insects in poultry feed which have to be addressed in the different territories.
- The SUSTAvianFEED approach of using insects for animal feeding was shown as great innovation and results will be very inspiring for the sector.





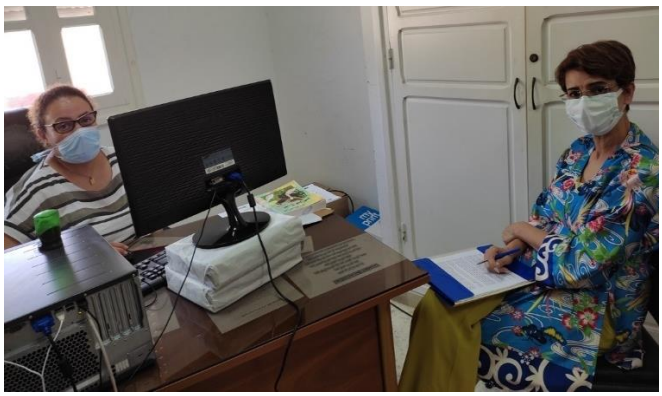


Figure 8. Stakeholders involved in LL A2

In summary, the participatory activities showed that there is room for the sustainability improvement through the elaboration of alternative diets with some limiting factors due to market competitiveness, products availability and legislation issues. There was a big consensus in the fact that the sector needs to increase the whole sustainability and that the diet should be its mainstream.

After all these topics, lots of possible alternative ingredients resulted in order to complement the first one developed by partners (Table 3):

Table 3. By-products and ingredients suggested by the involved actors

SPAIN			
Ingredients		By-products	
Pig mucosa	Omega3 ingredients	Olive by-product	
Corn gluten meal	Brewer's yeast		
Rice	Synthetic additives beyond lysine and methionine in order to balance and reduce soybean content		
Oatmeal	Single cell protein		
Carob flour	Meat meal		
Purslane	Aquatic protein		
Camelina oil			
ITALY			
Ingredients		By-products	
Meat meal	Corn gluten	Wheat bran	
Sunflower meal	Wheat gluten	Crushed rapeseed seeds	
Broken rice	Fish meal	Bakery by-products	
Alfalfa protein concentrate	Processed Animal Proteins (PAP) from pigs		
Pea			
TURKEY			
Ingredients		By-products	
Safflower meal	Lupin	Dry brewer residue	Bread left unsold in the bakery
Camelina meal	Egg-processing by-products	Olive mill waste	Egg-processing by-products
Rapeseed (canola) meal	Rendering products	Dairy waste	
Sunflower meal	Rice, bulgur	Tomato processing waste	

Algae	Crackers, biscuits, chips just before expiration date	Black cumin (*Nigella sativa*) seed meal	
TUNISIA			
Ingredients		By-products	
Dried brewing grains	Millet	Chopped vegetable crops waste	
Dried tomato pulp	Triticale	Chopped wholesale and local markets (vegetables, fruits and fish) waste	
Dried citrus pulp	Oat	Organic household waste.	
Peas	Full fat extruded soy	Synthetic amino acids beyond lysine and methionine to balance and reduce soybean content	
Lentils	Expeller soy	Milling by-products	
Vetch (*Vicia narbonensis*)	Sunflower meal	Poultry slaughterhouse by-product	
Prickly pears	Insects' meal		
Medicago arborerea and creeping Medicago	Fish meal		
Lupine	Algae (e.g., Spirulina)		
flax	Azolla		
Rye			

Finally, a summary of the participants involved per country are listed. Some of them are missing in this public deliverable due to GDPR (Table 4).

Table 4. Stakeholders involved in LL A2

SPAIN
Pablo Catalá Gregori. CECAV´s director (Centro de Calidad Avícola de Alimentación Animal de la Comunidad Valenciana- Poultry Quality Center for Animal Feed of the Valencian Community. Private sector.
Miguel José López Asensio. Member of the technical department of INTEGA (Industrial Técnica Ganadera S.L). Private sector.
Sandra García Carmona. Manager of Granja AGAS. Private sector.
Salvador Escobar. Avícola Levantina SA. Animal Feeding production company. Department of nutritional formula definition. Private sector.
Eva Armero. Technical University of Cartagena and technical director of the Association of Friends for the Murcian Chicken (AGAMUR). Academia and private sector.
Javier Prieto. MIAVIT. Aviculture department. Nutritional formulas development. Private sector.
José Ángel Ayala. Lorca nutrición animal. Guadalen. Technical director and nutrition department. (Private sector).
ITALY
Dr. Franco CALINI, DVM Poultry nutritionist, TECNAS.

Dr. Piero GAIDANO, DVM Poultry nutritionist – Feed mill supervisor, Mangimificio F.lli Borello.
Dr. Oreste MASSIMINO, DVM Poultry health expertise and commercial consultant, ORA Società Agricola.
TURKEY
Engin Yenice, Ankara University, Department of Animal Science.
Mehmet Bozkurt, Adnan Menderes University, Department of Animal Science.
Necmettin Ceylan, Ankara University, Department of Animal Science.
Bedri Girit, President of "Poultry Promotion Group" and "Ege Fisheries and Animal Products Exporters's Association", Member of "Egg Producers Association".
TÜRKİYEMBİR, Turkish Feed Manufacturers Association. TÜRKİYEMBİR participated with 17 executive committee members.
TUNISIA
Mr. Atef SAY. Delmon Poultry Company.Department of Poultry production and nutrition. Private sector.
Mr. Taha SASSI.Adisseo Company: Regional technical manager for the North Africa and French-speaking West Africa region. Private sector.
Dr. Mahmoud GANNOUN. DVM and Head of the Interprofessional Group of Poultry and Rabbit Products (Groupement Interprofessionnel des produitsavicoles et cunicoles, GIPAC). Public sector.
Mr. Lotfi Jouirou. Head of the Regional (Sousse) Direction of Livestock and Pasture office (Office de l'Elevage et du Pâturage, OEP). Public sector.
Mrs Sonia Halwani. General engineer Technical Center for Organic Agriculture (Centre Technique de l'AgricultureBiologique, CTAB) –Chott Mariem –Sousse-Tunisia. Public sector.
Pr. Ridha BARGAOUI, Academician.
Pr. Hédi ABDOULI,Academician.
Pr. Taha NAJAR,Academician.
Mr. Kaïs JEMMALI. Zootechnician engineer. Livestock and Pasture office (Office de l'Elevage et du Pâturage, OEP). Public sector.
Dr. Sonia RJIBA. Ph.D. Ministry of Agriculture, Hydraulic Resources and Fisheries. Public sector.
Mr. Aymen OTHMAN. Zootechnician engineer. Regional Commissariat of Agricultural Development of Sousse (Commissariat Régional au Développement Agricole de Sousse, CRDA). Public sector.
Focus Group: members from the Agricultural Development Group "Drahem": Livestock association and the facilitator.

4 Preliminary diets definition

SUSTAvianFEED project is formed by four countries in which five pilot activities will be conducted by following a similarly methodology. Therefore, a normalized approach for the sustainable feeding program definition has been defined and followed by each pilot partner. In the methodology section, it has been already explained that three diets per pilot were proposed from its draft version:

1. Standard diet as control one.
2. Alternative diet with alternative ingredients in which insects can be included or not.
3. Alternative diet with alternative ingredients in which insects are included.

Each pilot has designed a feeding program according to the type of genotype, poultry production, expected performance and evaluated period. The three diets per pilot are described below:

- For Spanish pilot (UMU), three experimental treatments were developed: one diet control with inclusion of usual ingredients) (Control), and two more sustainable ones: with alternative ingredients, and 3% or 6% of *Hermetia illucens* dried larvae (3-HERM and 6-HERM, respectively).

- For Italian pilot, three experimental treatments have been developed for each productive phase (Grower and Finisher): a control (with inclusion of usual ingredients) (Control), and two more sustainable: diets with alternative ingredients (ALTER); and other with ALTER diets supplemented with *Hermetia illucens*, so a reduction in intake of the alternative diets is expected between 3 and 6% (approximately a mean of 4.5%, 4.5-HERM).

- To design of diets for the Turkish pilot (EGE), three experimental treatments were established for each productive phase: one control (with inclusion of usual ingredients) (Control), and two more sustainable: one with alternative ingredients, and other with and 5% of *Hermetia illucens* dried larvae (ALTER and 5-HERM, respectively).

- In the design of diets of Tunisian pilot (ISA-CM and RAYHANA) three experimental treatments were developed for each productive phase: a control (with the inclusion of usual ingredients) (Control), and two more sustainable: one with alternative ingredients without insects (ALTER), and other with alternative ingredients and a 5 % of dry larvae of *Hermetia illucens* (5-HERM). Also, this general design was used for poultry production of meat or eggs.

The initial preliminary diets of each pilot are included in the following sections.

On the other hand, it has to be remarked the relevance of the inclusion of insects in the diet as a way of reducing the environmental impact in an innovative way, even though the percentages of inclusion do not represent the biggest amount in the diet. The use of insects for feeds is widely recognized as one of the potential solutions for the environmental problem of livestock sector and face the expected growth of consumer demand (Sogari et al, 2019). It is also important to remark that chickens with access to outdoor areas pick up insects at all life stages and eat them voluntarily, so insects can be considered as part of their natural diet (Bellezza et al, 2019) (Star et al, 2019) ((Veldkamp & Niekerk, 2019).

The analysis of the different diets alternatives has been directly included in section 6 Sustainable Feeding Program (from Table 5 to 13 and Figure 9 to 18).

4.1 Spain

Table 5. Initial preliminary laying hen diets of Spanish pilot: control, alternative diet with 3% and 6% *Hermetia illucens* (3-HERM and 6-HERM diets, respectively)

Diet Composition (%)	Control	3-HERM	6-HERM
Maize	55.00	36.01	21.25
Wheat	1.00	10.0	24.54
Soybean meal	22.00	15.21	11.02
Sunflower meal	6.29	7.0	7.0
Bakery by-product		6.0	6.0
Wheat middling	1.00	1.00	1.00
Pea		7.81	10.00
Hermetia illucens		**3.00**	**6.00**
Soybean oil	3.20	2.87	2.47
Calcium carbonate	9.49	9.08	8.70
Monocalcium phosphate	0.69	0.69	0.69
Sodium chloride	0.24	0.23	0.23
Sodium bicarbonate	0.15	0.15	0.15
Vitamin-mineral premix plus enzymes	0.80	0.80	0.80
DL-methionine	0.15	0.14	0.13
L-threonine		0.02	0.03
Calculated value[1]	Control	3-HERM	6-HERM
Metabolizable Energy (kcal/kg)	2750	2750	2750
Crude protein (%)	16.50	16.57	16.80
Ether extract (%)	5.68	5.89	5.77
Methionine (%)	0.42	0.42	0.42
Methionine + cystine (%)	0.70	0.71	0.72
Lysine (%)	0.82	0.82	0.82
Threonine (%)	0.62	0.62	0.62
Crude fiber (%)	4.44	4.87	5.04
Calcium (%)	3.83	3.83	3.83
Phosphorus (%)	0.51	0.54	0.55
Na (%)	0.15	0.17	0.17
Linoleic acid (%)	2.81	2.52	2.19

[1]Calculated value according to FEDNA (2019).

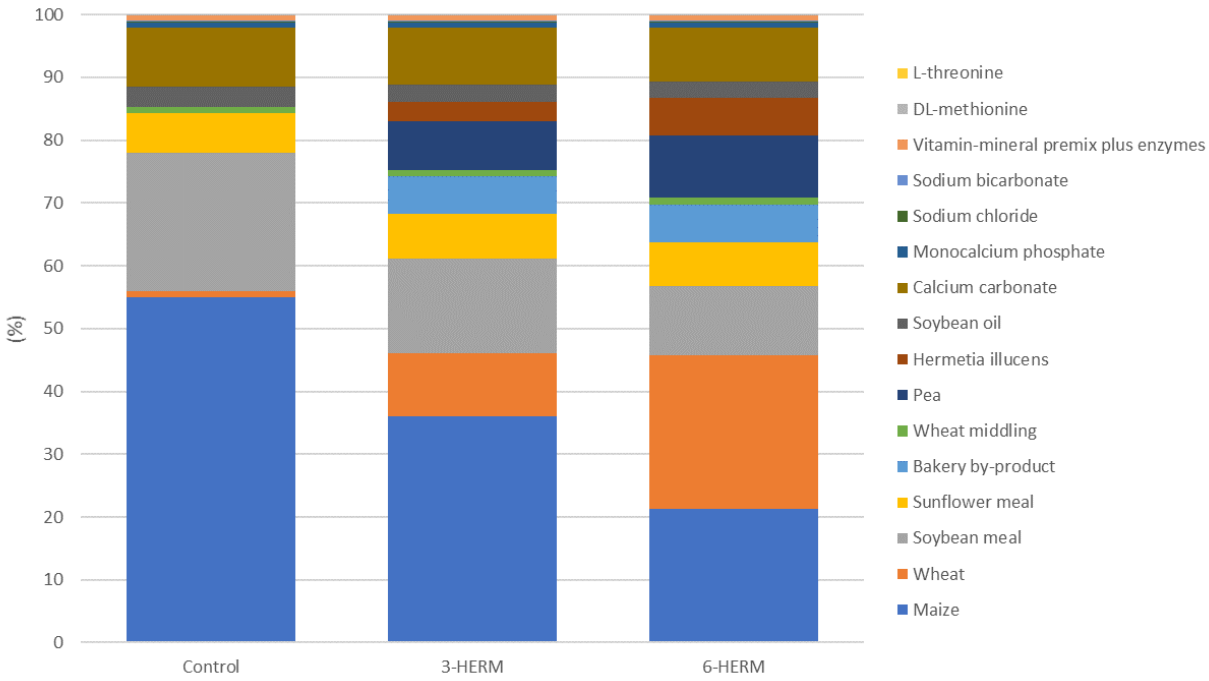


Figure 9. Initial preliminary laying hen diets of Spanish pilot

4.2 Italy

Table 6. Initial preliminary meat chicken diets of Italian pilot: two period (grower and finisher) per three programs: control, alternative diet without insect (ALTER), and alternative diet with expected *Hermetia illucens* substitution between 3 and 6% (4.5-HERM)

	Grower period			Finisher period		
Diet Composition (%)	**Control**	**ALTER**	**4.5-HERM**	**Control**	**ALTER**	**4.5-HERM**
Maize	60	54.7	52.24	61.77	54.5	52.05
Soybean meal	34.57	6.57	6.27	32	4.04	3.86
Fava beans		8.6	8.21		9.5	9.07
Pea		8.6	8.21		9.5	9.07
Alfalfa meal					0.2	0.19
Sunflower meal		5	4.78		5	4.78
Maize gluten		12	11.46		12	11.46
Soybean oil	1.2			2	0.6	0.57
Dicalcium phosphate	1.35	1.35	1.29	1.35	1.35	1.29
Calcium carbonate	1.9	1.9	1.81	1.9	2	1.91
Sodium chloride	0.15	0.15	0.14	0.15	0.15	0.14
Sodium bicarbonate	0.14	0.14	0.13	0.14	0.14	0.13
DL-methionine	0.1	0.1	0.10	0.1	0.04	0.04
L-lysine		0.3	0.29		0.4	0.38
Vitamin-mineral premix	0.59	0.59	0.56	0.59	0.59	0.56
Hermetia illucens			**4.5**			**4.5**
Calculated values[1]	**Control**	**ALTER**	**4.5-HERM**	**Control**	**ALTER**	**4.5-HERM**
Metabolizable Energy (kcal/kg)	2761.51	2796.40	> ALTER[2]	2831.60	2834.84	> ALTER
Crude protein (%)	20.52	20.31	> ALTER	19.50	19.61	> ALTER
Ether extract (%)	4.08	2.73	> ALTER	4.89	3.30	> ALTER
Methionine (%)	0.40	0.47	> ALTER	0.38	0.39	> ALTER
Lysine (%)	1.00	0.84	> ALTER	0.94	0.87	> ALTER
Threonine (%)	0.71	0.64	> ALTER	0.67	0.61	> ALTER
Crude fiber (%)	3.39	3.92	> ALTER	3.28	3.92	> ALTER
Calcium (%)	1.20	1.15	> ALTER	1.19	1.19	> ALTER
Phosphorus (%)	0.60	0.60	> ALTER	0.59	0.59	> ALTER

[1]Calculated value according to INRA (2004).
[2]Expected nutritional value higher than alternative diet.

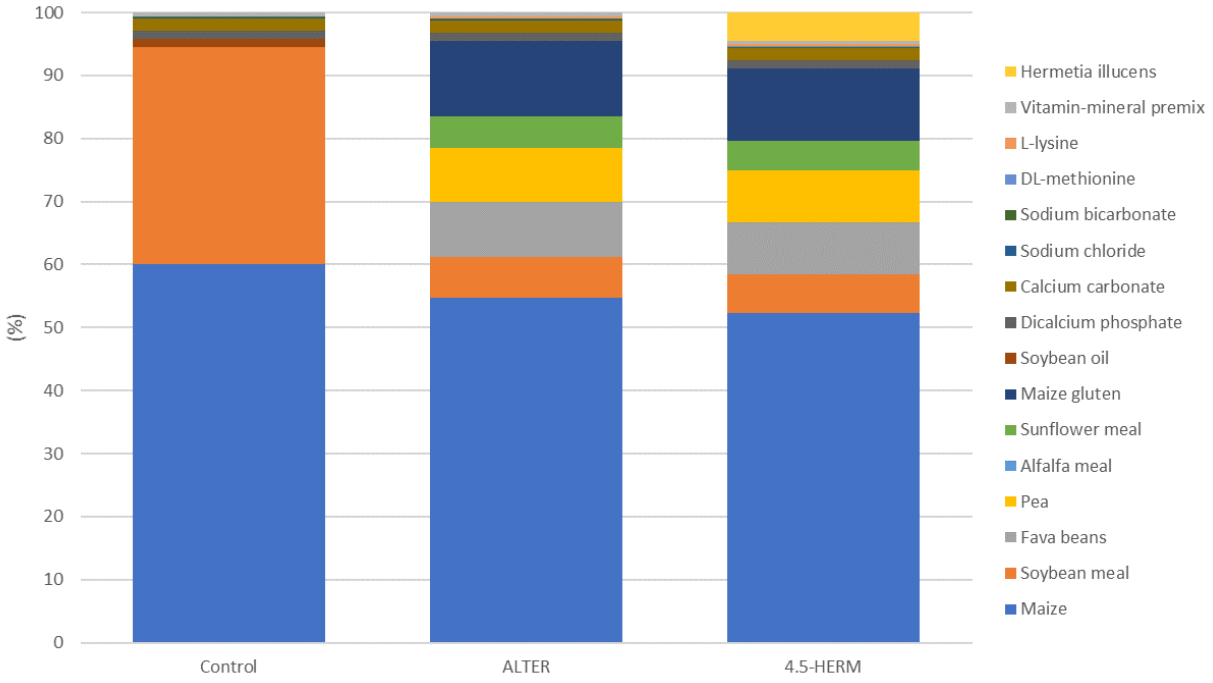


Figure 10. Initial preliminary meat chicken diets of Italian pilot: Grower

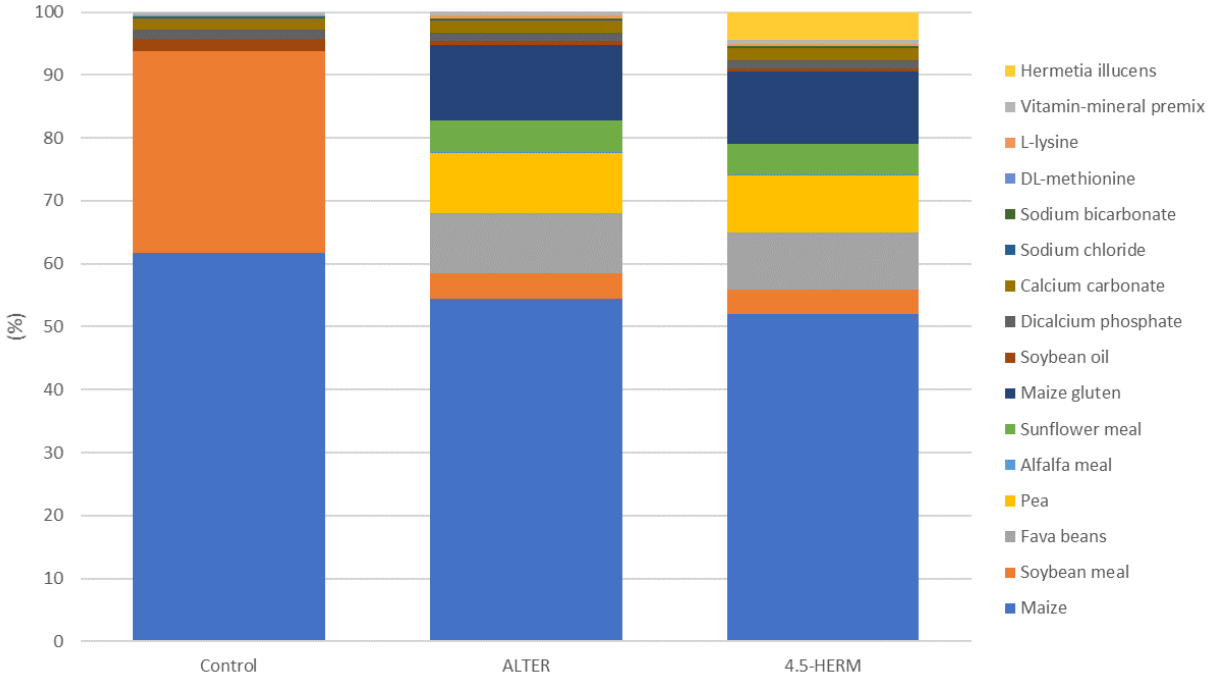


Figure 11. Initial preliminary meat chicken diets of Italian pilot: Finisher

4.3 Turkey

Table 7. Initial preliminary meat chicken diets of Turkish pilot for the starter period

Diet Composition (%)	Control	5-HERM	10-HERM
Maize	44.401	40.501	35.301
Wheat	10	5	8.4
Sunflower meal		12	8.8
Soybean meal	33	24	20
Fish meal	5		
Brewers' dried grain		5	10
Hermetia illucens		**5**	**10**
Sunflower oil	5	6	5.1
Dicalcium phosphate	1.468	1.468	1.468
Vitamin-mineral premix	0.35	0.35	0.35
Sodium chloride	0.481	0.481	0.481
L-lysine	0.1	0.1	0.05
DL-methionine	0.2	0.1	0.05
Calculated values[1]	**Control**	**5-HERM**	**10-HERM**
Metabolizable Energy (kcal/kg)	3031.76	3017.17	3005.11
Crude protein (%)	22.41	22.08	22.02
Ether extract (%)	7.20	9.42	9.96
Crude fiber (%)	4.05	5.98	6.53
Calcium (%)	1.01	1.10	1.12
Phosphorus (%)	0.65	0.63	0.65

[1]Calculated value according to analysis, NRC (1994) and Sari et al. (2008).

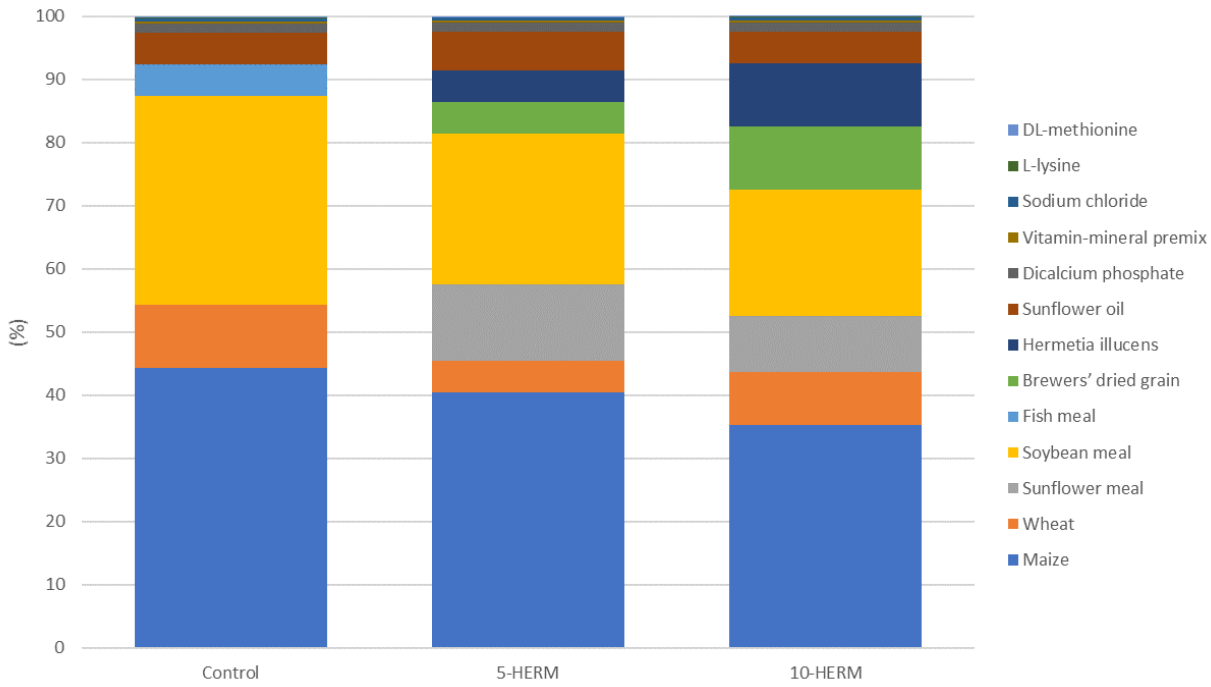


Figure 12. Initial preliminary meat chicken diets of Turkish pilot for the starter period

Table 8. Initial preliminary meat chicken diets of Turkish pilot for the grower period

Diet Composition (%)	Control	5-HERM	10-HERM
Maize	47.401	41.801	41.601
Wheat	14	10	9
Sunflower meal		12.2	10.9
Soybean meal	26	18	14
Fish meal	5		
Brewers' dried grain		4	7
Hermetia illucens		**5**	**10**
Sunflower oil	5	6.5	5.1
Dicalcium phosphate	1.468	1.468	1.468
Vitamin-mineral premix	0.35	0.35	0.35
Sodium chloride	0.481	0.481	0.481
L-lysine	0.1	0.1	0.05
DL-methionine	0.2	0.1	0.05
Calculated values[1]	**Control**	**5-HERM**	**10-HERM**
Metabolizable Energy (kcal/kg)	3098.91	3116.03	3100.28
Crude protein (%)	20.07	20.03	20.07
Ether extract (%)	7.32	9.98	10.09

Crude fiber (%)	3.71	5.57	6.09
Calcium (%)	1.05	1.10	1.13
Phosphorus (%)	0.63	0.61	0.62

[1]Calculated value according to analysis, NRC (1994) and Sari et al. (2008).

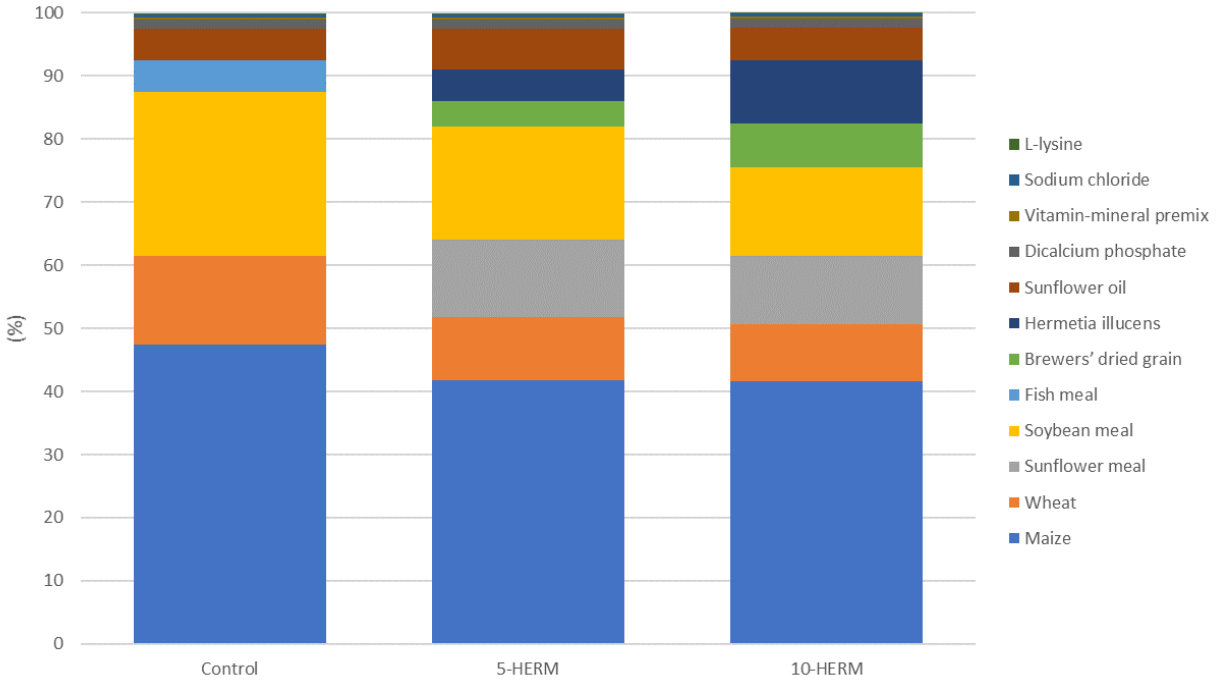


Figure 13. Initial preliminary meat chicken diets of Turkish pilot for the grower period

Table 9. Initial preliminary meat chicken diets of Turkish pilot for the finisher period

Diet Composition (%)	**Control**	**5-HERM**	**10-HERM**
Maize	46.901	42.301	42.701
Wheat	14	11	9
Sunflower meal		13.7	10.8
Soybean meal	30.6	13	10
Brewers' dried grain		6	10
Hermetia illucens		**5**	**10**
Sunflower oil	5.9	6.5	5.1
Dicalcium phosphate	1.468	1.468	1.468
Vitamin-mineral premix	0.35	0.35	0.35
Sodium chloride	0.481	0.481	0.481
L-lysine	0.1	0.1	0.05
DL-methionine	0.2	0.1	0.05
Calculated values[1]	**Control**	**5-HERM**	**10-HERM**

Metabolizable Energy (kcal/kg)	3101.67	3112.1	3089
Crude protein (%)	19.04	19.04	19.06
Ether extract (%)	7.73	10.06	10.18
Crude fiber (%)	2.73	5.95	6.36
Calcium (%)	0.98	1.16	1.2
Phosphorus (%)	0.55	0.61	0.61

[1]Calculated value according to analysis, NRC (1994) and Sari et al. (2008).

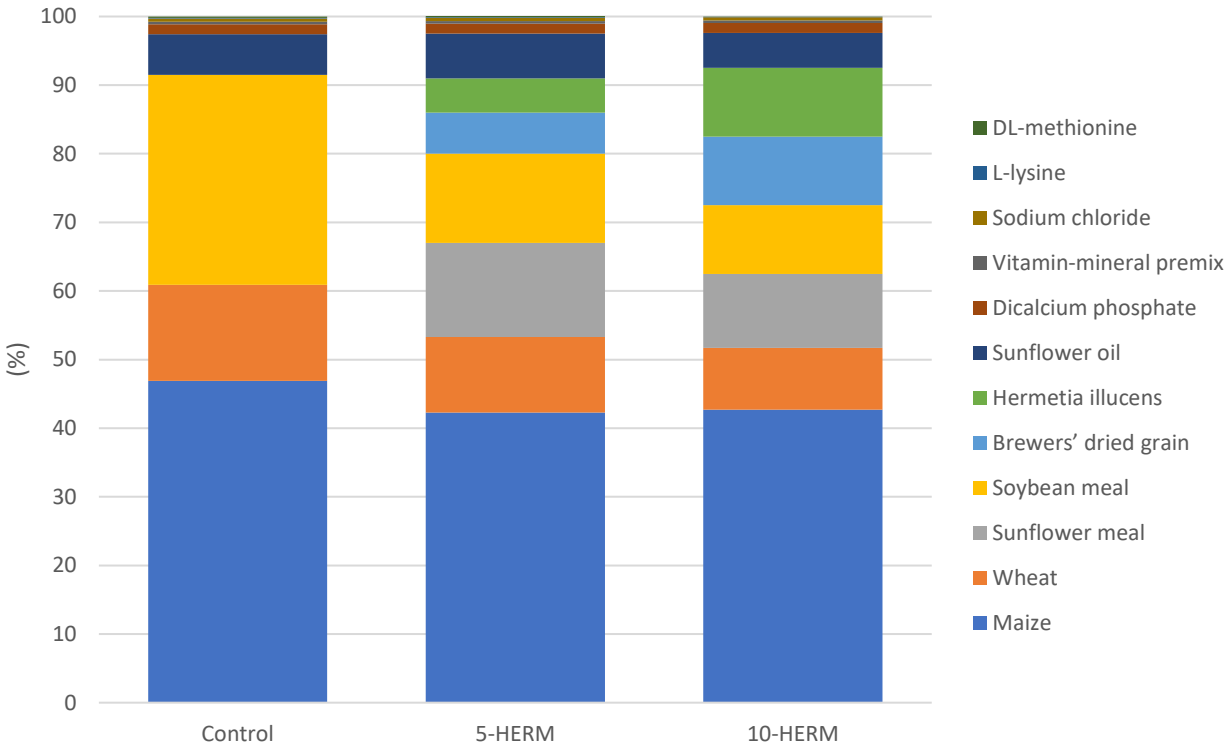


Figure 14. Initial preliminary meat chicken diets of Turkish pilot for the finisher period

4.4 Tunisia

Table 10. Initial preliminary meat chicken diets of Tunisian pilot for starter period: control, alternative diet without insect (ALTER), and alternative diet with 5% of *Hermetia illucens* (5-HERM)

Diet Composition (%)	Control	ALTER	5-HERM
Soybean meal	36.3	26.45	23
Maize	59.7	33.24	33.24
Soybean oil		1.5	
Pasta wastes		5	5
Rapeseed meal		10	5
Fava beans		5	10
Triticale		15	15
Hermetia illucens			**5**
DL-methionine	0.15	0.15	0.16
L-lysine	0.03	0.03	0.03
Sodium chloride	0.39	0.38	0.37
Vitamin-mineral premix	0.5	0.5	0.5
Calcium carbonate	1.08	1	1
Dicalcium phosphate	1.85	1.75	1.7
Calculated values[1]	**Control**	**ALTER**	**5-HERM**
Metabolizable Energy (kcal/kg)	2853.18	2857.96	2856.79
Crude protein (%)	21.56	21.55	21.54
Ether extract (%)	2.86	5.09	4.63
Methionine (%)	0.49	0.49	0.49
Methionine + cystine (%)	0.85	0.88	0.85
Lysine (%)	1.22	1.19	1.19
Threonine (%)	0.83	0.82	0.82
Crude fiber (%)	2.83	3.81	3.85
Calcium (%)	1.13	1.14	1.11
Phosphorus (%)	0.69	0.72	0.7
Linoleic acid (%)	1.34	2	1.32
Na (%)	0.16	0.16	0.16
Cl (%)	0.28	0.28	0.29
K (%)	0.96	0.94	0.91

[1]Calculated value according to INRA (2004).

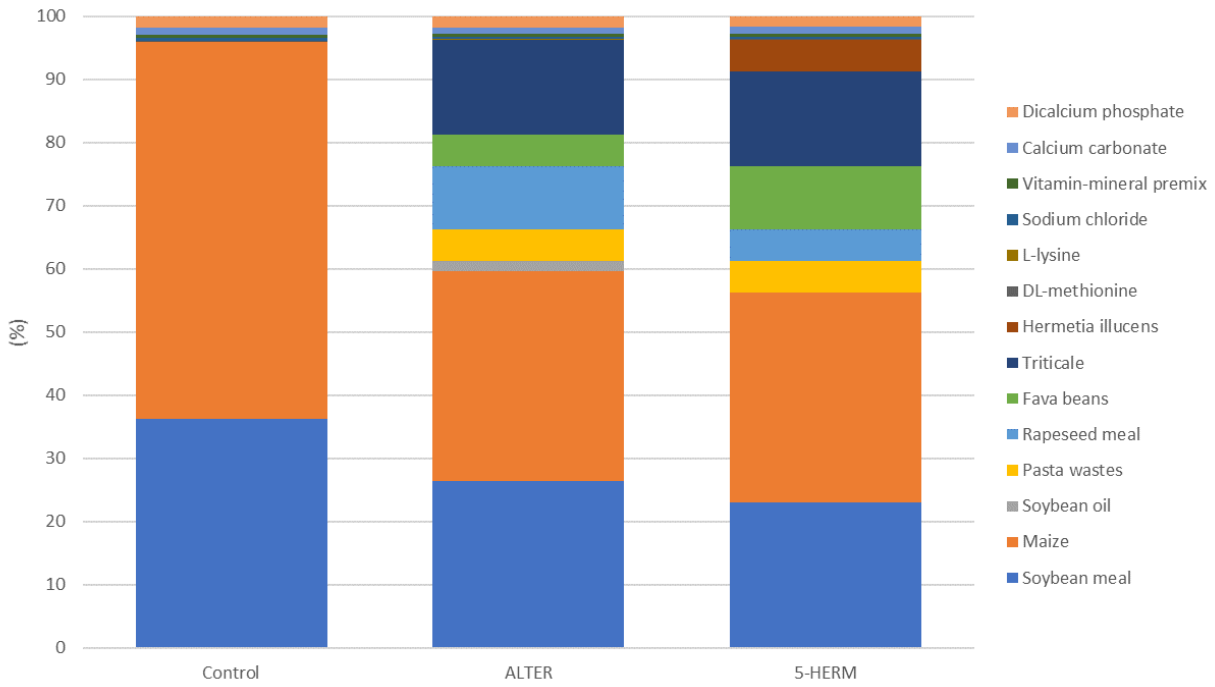


Figure 15. Initial preliminary meat chicken diets of Tunisian pilot for starter period

Table 11. Initial preliminary meat chicken diets of Tunisian pilot for grower period: control, alternative diet without insect (ALTER), and alternative diet with 5% of *Hermetia illucens* (5-HERM)

Diet Composition (%)	Control	ALTER	5-HERM
Soybean meal	28	18	13.7
Maize	68.3	36	34.8
Soybean oil		1.5	
Pasta wastes		13	12
Rapeseed meal		7	7
Fava beans		7	7
Triticale		14	17
Hermetia illucens			5
DL-methionine	0.1	0.1	0.1
L-lysine	0.01	0.01	0.01
Sodium chloride	0.39	0.39	0.39
Vitamin-mineral premix	0.5	0.5	0.5
Calcium carbonate	1	0.9	1
Dicalcium phosphate	1.7	1.6	1.5
Calculated values[1]	**Control**	**ALTER**	**5-HERM**
Metabolizable Energy (kcal/kg)	2933.88	2937.11	2933.53

Crude protein (%)	18.28	18.2	18.19
Ether extract (%)	3.03	4.62	4.86
Methionine (%)	0.39	0.38	0.39
Methionine + cystine (%)	0.72	0.73	0.72
Lysine (%)	0.98	0.94	0.93
Threonine (%)	0.71	0.68	0.68
Crude fiber (%)	2.63	3.37	3.57
Calcium (%)	1.04	1.02	1.05
Phosphorus (%)	0.64	0.64	0.64
Linoleic acid (%)	1.44	1.93	1.35
Na %	0.16	0.16	0.17
Cl %	0.28	0.29	0.31
K %	0.82	0.78	0.75

[1]Calculated value according to INRA (2004).

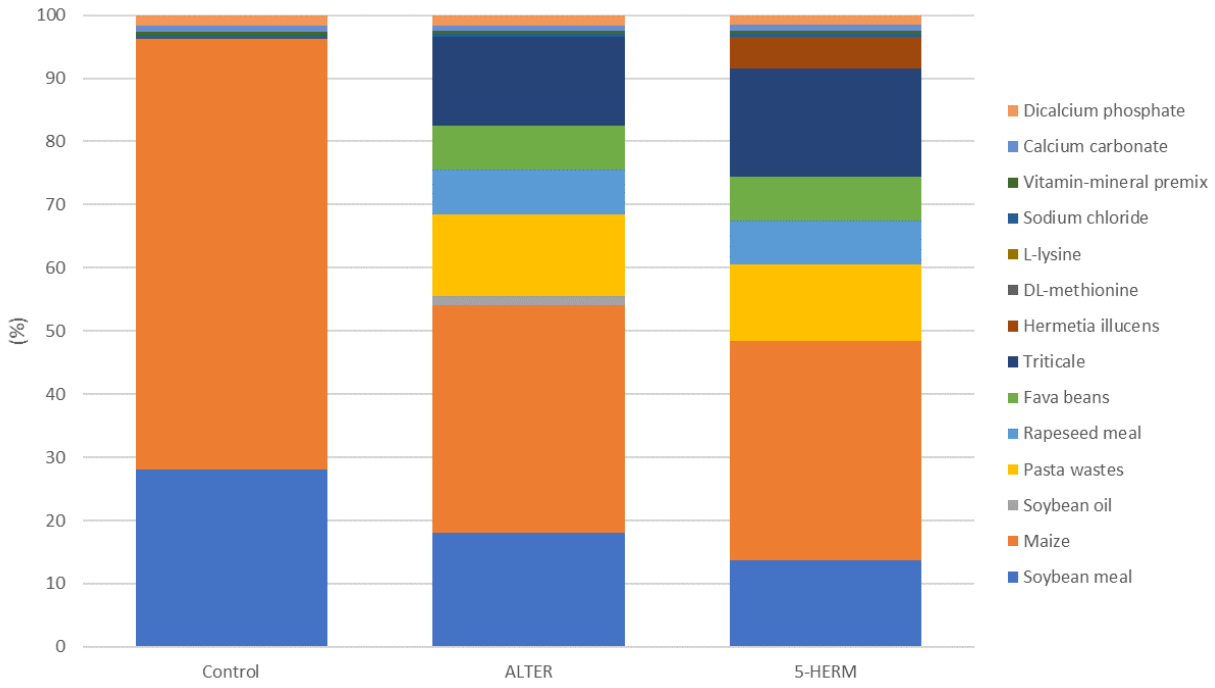


Figure 16. Initial preliminary meat chicken diets of Tunisian pilot for grower period

Table 12. Initial preliminary meat chicken diets of Tunisian pilot for finisher period: control, alternative diet without insect (ALTER), and alternative diet with 5% of *Hermetia illucens* (5-HERM)

Diet Composition (%)	Control	ALTER	5-HERM
Soybean meal	24	13.1	10.1
Maize	72.7	27	40.75

Soybean oil		2.2	
Pasta wastes		10	11
Rapeseed meal		7	7
Fava beans		7	7
Triticale		30.55	16
Hermetia illucens			**5**
DL-methionine	0.05	0.05	0.05
L-lysine	0.01	0.01	0.01
Sodium chloride	0.39	0.39	0.39
Vitamin-mineral premix	0.5	0.5	0.5
Calcium carbonate	0.9	0.9	0.9
Dicalcium phosphate	1.45	1.3	1.3
Calculated values[1]	**Control**	**ALTER**	**5-HERM**
Metabolizable Energy (kcal/kg)	2980.32	2978.79	2979.91
Crude protein (%)	16.71	16.7	16.69
Ether extract (%)	3.12	5.15	4.99
Methionine (%)	0.33	0.31	0.32
Methionine + cystine (%)	0.63	0.64	0.63
Lysine (%)	0.88	0.83	0.83
Threonine (%)	0.64	0.61	0.62
Crude fiber (%)	2.54	3.39	3.49
Calcium (%)	0.93	0.94	0.96
Phosphorus (%)	0.58	0.59	0.59
Linoleic acid (%)	1.49	2.21	1.42
Na %	0.16	0.16	0.17
Cl %	0.28	0.29	0.31
K %	0.75	0.71	0.68

[1]Calculated value according to INRA (2004).

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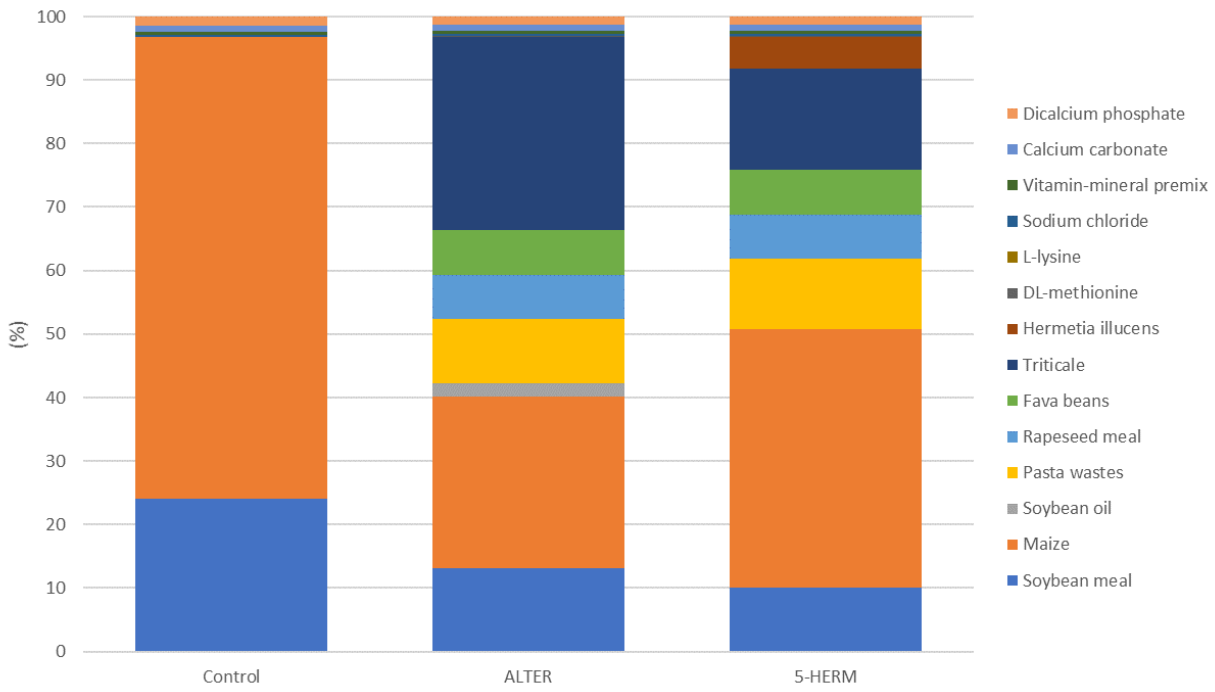

Figure 17. Initial preliminary meat chicken diets of Tunisian pilot for finisher period

Table 13. Initial preliminary laying hen diets of Tunisian pilot: control, alternative diet (ALTER) and alternative diet with 5% of Hermetia illucens (5-HERM)

Diet Composition (%)	Control	ALTER	5-HERM
Soybean meal	24	22	16.5
Maize	57	40	31.3
Pasta wastes		12	12.3
Triticale		10	10
Hermetia illucens			**5**
Wheat bran	8	5	14
DL-methionine	0.04	0.04	0.05
L-lysine		0.05	0.08
Calcium carbonate	9	9	8.9
Sodium chloride	0.36	0.36	0.36
Vitamin-mineral premix	0.2	0.2	0.2
Dicalcium phosphate	1.4	1.35	1.31
Calculated values	**Control**	**ALTER**	**5-HERM**
Metabolizable Energy (kcal/kg)	2636.53	2638.08	2639.08
Crude protein (%)	16.72	16.68	16.73
Ether extract (%)	2.86	2.3	4.04
Methionine (%)	0.34	0.34	0.35

Methionine + cystine (%)	0.64	0.64	0.64
Lysine (%)	0.88	0.88	0.88
Threonine (%)	0.64	0.62	0.61
Crude fiber (%)	2.86	2.58	3.23
Calcium (%)	3.92	3.9	3.88
Phosphorus (%)	0.61	0.57	0.62
Na %	0.15	0.15	0.16
Cl %	0.26	0.28	0.3
K %	0.78	0.74	0.74

[1]Calculated value according to INRA (2004).

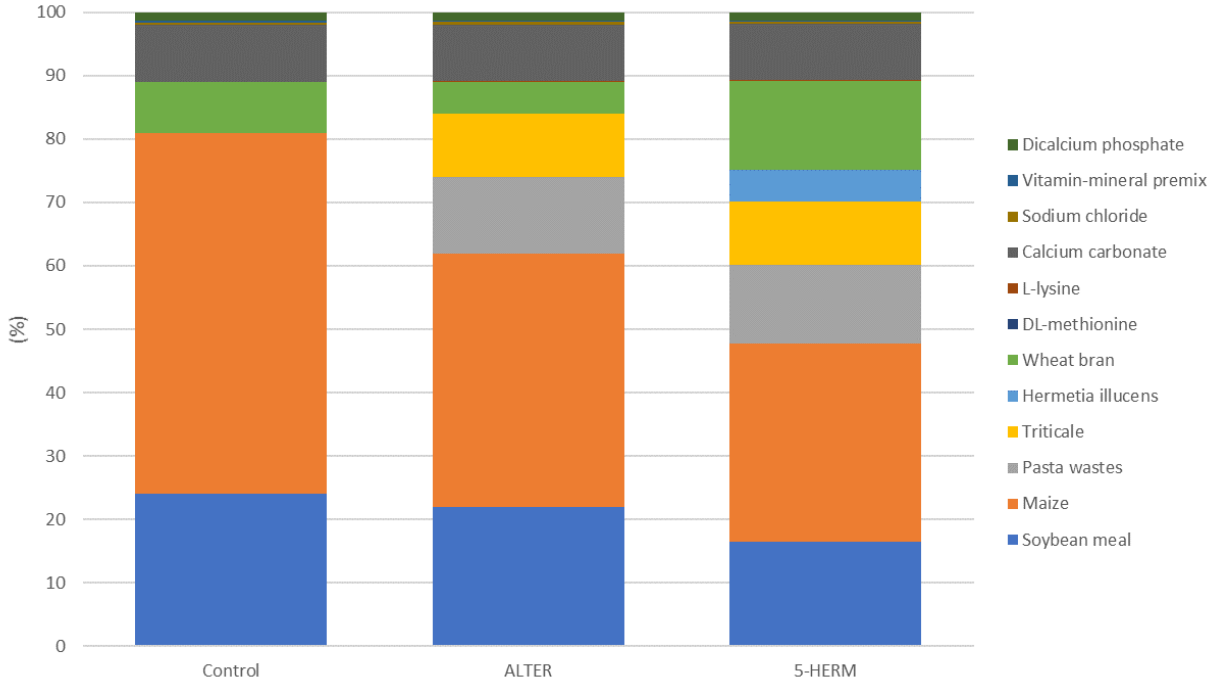

Figure 18. Initial preliminary laying hen diets of Tunisian pilot

5 Diets evaluation

5.1 Internal evaluation of the initial preliminary diets

The first step for the diets evaluation and review for improvement has been the internal evaluation of the preliminary diets made by ALIA and UMU. The pilots' feeding programs have been reviewed in order to evaluate the diets, and make practical recommendations. This review has focused on the following items:

- **Availability of raw materials and other ingredients**. The offer and the possibility of acquiring ingredients in the different pilots were evaluated. Therefore, a list of raw materials and alternative ingredients and by-products from each location was used as an input in the review. ALIA's knowledge of the market led to the proposal of places to purchase products to reduce availability problems, as well as reducing the environmental impact of transportation.

- The **nutritional value of the ingredients** was considered, incorporating the knowledge of ALIA's nutritionists. This was done in order to assign the nutritional value to the different ingredients proposed by the pilots, so that the programs designed included ingredients that could totally or partially replace the less sustainable ingredients, always considering some limitations to avoid problems in the performance or quality of the productions.

- In addition, the needs of **equipment and technology** necessary for the handling of the ingredients were taken into account. Some ingredients may block silos, granulating machines and other type of equipment. Thus, this was also considered for avoiding some ingredients which in a real-life environment may not be optimal.

- Regarding the evaluation of the **global feeding program** of each pilot, ALIA's nutritionists considered for the evaluation the adequacy of the diets for each phase and type of production developed in each pilot, as well as the adequacy of the sustainability criteria. By taking this approach, it was analysed if the diets effectively covered the needs for energy, protein and other nutritional requirements, evaluating the result of the feed formulation. Finally, final adjustments were made in order to achieve a design of feeding programs in accordance with the project objectives.

A summary of the initial preliminary review per pilot region is listed below:

- **Spain and Italy**

UMU, ALIA and UNITO had several conversations in order to define the sustainable feeding program of both pilots.

In the first review carried out by ALIA, the incorporation of the vitamin and micromineral corrector was adapted, substituting part of the ingredient called sepiolite in the formulas for laying hens. In addition, ALIA warned of the unavailability of peas at the present time, although their acquisition was considered feasible at the time the pilots began. Furthermore, ALIA recommended not using bakery by-products, if the feed was offered in the form of mash, in such a way that it suggested the incorporation of maize DDGS and rapeseed meal. Finally, maize DDGS and peas were included in the final preliminary

formulations for laying hens, and rapeseed meal was not included due to its possible negative effect on egg flavour.

- **Turkey**

The first formulas made by Turkey needed some clarification about the ingredients and nutrients that were incorporated. The previous evaluation carried out by ALIA showed that the diets covered the needs of the chicken meat, although a high level of linoleic acid was detected in the finishing phase. These appreciations were studied by the Turkish pilot, incorporating some modifications in the final preliminary formulas.

Some of the modifications for the final diet were about the reduction of the insect's incorporation to just one diet, in order to define a control diet which could be used for comparison and which may be used with commercial broilers in the pilot activities.

- **Tunisia**

For the Tunisian pilot, the ALIA nutritionists assessed the preliminary diets, and they were considered to be in accordance with the established criteria, so no further modifications were needed.

5.2 Feed safety and health evaluation of the diet

The feed safety evaluation of the diet is a crucial aspect in order to develop a sustainable feeding program with all the necessary guarantee for animal and human health.

The feed safety has been confirmed by assessing the microbiological status and the potential contaminants (mycotoxins and heavy metals). In the same way, it should be confirmed during pilot project activities. For each ingredient, the regulations, and laws of each country partner of the project have been collected and summarized.

This is especially important in a context in which during the last few decades, controls, regulations and quality and safety standards have increased substantially. In addition, the need of including alternative ingredients in feed, may arise new potential risks.

All the feed ingredients proposed by the different partners in order to be included in the diets have been analysed. They are subjected to EU regulation for Italy and Spain or local regulation for Tunisia and Turkey. For those ingredients which are not under local regulations, pesticides, heavy metals and mycotoxins should be analysed before including them in the diet.

A relevant example of the potential risks and changes in regulation for alternative feed is the use of insects. The "COMMISSION REGULATION (EU) 2021/1372 of 17 August 2021", have been definitively authorized in the feeding of poultry and pigs. This regulation has taken several years to be enacted and has followed the numerous positive opinions of EFSA (European Food Safety Authority).

However, despite the European Union's authorization about the use of insects as a protein source, the supervision of their safety is active and will probably be modified and improved over the years. In a four years' project as SUSTAvianFEED, legislation may change, so

partners will study these modifications in order to determine if new potential risks are identified.

This information is further developed in Deliverable 2.3 “Feed safety and health evaluation of the diet”.

5.3 Environmental evaluation of the diet

SUSTAvianFEED project has as one of its key pillars the reduction of the environmental impact of the sector by the inclusion of sustainable diets. Therefore, the environmental impact of the different ingredients has been evaluated from the very beginning in order to consider it for diets formulation and to achieve project goals.

The environmental evaluation of the diet has been important for the definition of the sustainable feeding program. During the process, even though it was clear that soybean and other imported ingredients should be reduced in favour of by-products and local ingredients, lots of possible ingredients were studied in order to have the whole picture.

For the environmental evaluation, LCA methodology has been followed and SimaPro 9.2.0.1 has been used. Two different methods have been used:

1. ReCiPe method, which includes a global punctuation in points (pt.) and has also been employed with the aim of classifying the damage in three category indicators:

    - Human health, points
    - Biodiversity, points
    - Resources, points

2. ILCD method studies the impact on 16 category indicators, but we studied the following categories according to bibliography consulted (Castanheira et al., 2019; Loyola et al., 2021; Ogino et al., 2021):

    - Climate change, kg CO_2eq/t.
    - Acidification, molc H+eq/t.
    - Land use, kg C deficit/t.
    - Marine eutrophication, kg N eq/t.
    - Human toxicity, non-cancer effects, CTUh/t.
    - Human toxicity, cancer effects, CTUh/t.

In order to check the GHGs emissions reduction, the Climate change category was used.

Some of the most important recommendations and conclusion of the LCA, which can be studied in detail in Deliverable 2.4 “Environmental evaluation of the diet”, are described below:

- Environmental results of ingredients vary quite widely due to differences in data and scenarios.
- It is interesting to note how little impact the alternative ingredients have in general compared to the usual ones.

- Alternative ingredients and agricultural by-products allow reducing the climate change impact associated with soybean meal and imported cereals inclusion.
- Importing protein sources from remote areas can be reduced when insect larvae is used in poultry feeding.
- Local crops should be favoured to reduce imports of feed ingredients.

In summary, it has to be remarked that all the preliminary proposals of the project have a tangible reduction in the environmental impact, satisfying project objectives. The results of the different sustainable feeding program of each pilot are included in section 6.

5.4 Nutritional evaluation of the diet

The nutritional evaluation of the diet was other crucial aspect for the sustainable feeding program definition. For nutritional characterization of usual ingredients of the feed for laying hens or/and meat-type chickens, as well as the possible local ingredients (by-products or other alternatives) that could be used in the manufacture of the experimental feeds, the most relevant bibliography, national and international databases was used to assign the nutritional value of these ingredients.

Internationally recognized procedures of analyses were developed, when the ingredients had lack sufficient information about their nutritional (especially the by-products of some area, and the insects usable in the development of the project). Thus, each pilot has selected the usual and alternative ingredients that could be used in the formulation of the diets by assigning nutritional values from available scientific and technical information, and/or through chemical analysis.

To estimate the nutritional requirements of the birds, each pilot characterized the type of poultry production, laying hens or/and meat chickens, the breed or hybrid, and the phase and level of production. Thus, nutritional recommended for poultry, adapted from scientific and technical references was used to assign the requirements of the animals.

For the design and formulation of the diets, a set of general criteria established in the different meetings held during the development of the project was followed as described in the methodology section.

Optimized diets to meet the requirements of birds of each pilot have been established to compare a control diet (with usual ingredients, no sustainability criteria), with other diets more sustainable (according to the criteria of deliverable 2.4 about feed impact).

All pilots have achieved a design of three feeding programs that met the established criteria. Sustainable diets have lower levels of soybean meal and including alternative ingredients (unusual or by-products), and at least one of them incorporates *Hermetia illucens* insect larvae. In addition, at least the control and one alternative diet have been iso-energetic and iso-nitrogenous (for crude protein and/or amino acid).
These diets are considered final preliminary diets, but they must be adapted, in each pilot, to the nutritional characterization of the *Hermetia illucens* larva used, and to the availability of ingredients at the formulation moment for the in vivo trials. This fact is especially important due to the highly volatile situation, created by the international circumstances, and as the countries of Eastern Europe are an important source of materials for animal feed.

5.5 Diet validation by external actors

The last aspect of the diet's evaluation process has been the development of a new Living Lab activity focused on the diet validation by farmers and other experts. The aim of this activity was to obtain the opinion of this farmer on the current situation in the poultry feed sector and on the introduction of factors such as sustainability, concern for the environmental impact derived from the production of diets, the introduction of more sustainable alternative ingredients, the introduction of insects, etc., in order to obtain an approach directly from the most affected sector. The main characteristics of this activity are:

- **Objectives:**
  1) To collect information about attitudes, values, and preferences of the farmers and consumers towards poultry feed sources.
  2) To discuss and rank possible feeding options.
  3) To consult the acceptance of the farmers on sustainable feeding.
- **Tasks Related**: Task 2.1
- **Partners involved**: ALIA, UMU, UNITO, ISA-CM, RAYHANA, EGE
- **When**: From December 2021 to March 2022
- **Target group**: Farmers (with participation of feed producers and experts)
- **Location**: Online/Visit to farmers
- **Developed activities**:
  4) Semi-structured interviews using questionnaire
  5) Focus groups/workshops
- **Expected Output:** This second set of Living Lab Activities had the main objective of co-creating, co-implementing, and co-evaluating the experimental diets from the first stage of LL activities.

Each pilot partner engaged relevant stakeholders for the development of the activity. Individual semi-structured Interviews (all) and a focus group (Turkey) were done. The semi-structured interview meetings were planned to evaluate the standard and sustainable diets, and to reveal the interviewees' attitudes, preferences towards ingredients, and their acceptance of alternative sustainable diet formulations, drawing on their expertise and experience.

The first preliminary diets were shared with the participants and explained. The farmers, experts and feed producers who decided to participate in this interview were asked two open questions in which they could express their thoughts on this topic, and questions in which they had to give a value on a scale from 1 to 5 based on their opinion.

The main topics addressed were:

- Producers' opinion on reduction in the soybean meal in the diet and awareness about imported soybean.
- Producers' opinion on 1) specific local ingredients and by-products and on 2) introduction of insects into the diets of chickens.
- Producers' opinions on the consumers' acceptance regarding the introduction of insects in chickens' diets.

- Producers' willingness to use the designed Sustainable Diets (No 1 and 2) and concerns that might prevent producers from using each sustainable diet.
- Producers' expectations regarding the environmental impact of each sustainable diet and their interest in the reduction of LCA values by these sustainable diets.
- Opinions of producers regarding the reflection of environmentally friendly diets on broiler meat, eggs and diet prices.
- Intention to pay more for these hypothetic more sustainable diets.
- Opinion about proximate environmental impact reduction related to the use of more sustainable diets.

The activities developed by country as well as the type of stakeholders engaged are summarized in Figure 19 and Figure 20.

Figure 19. Activities developed per country

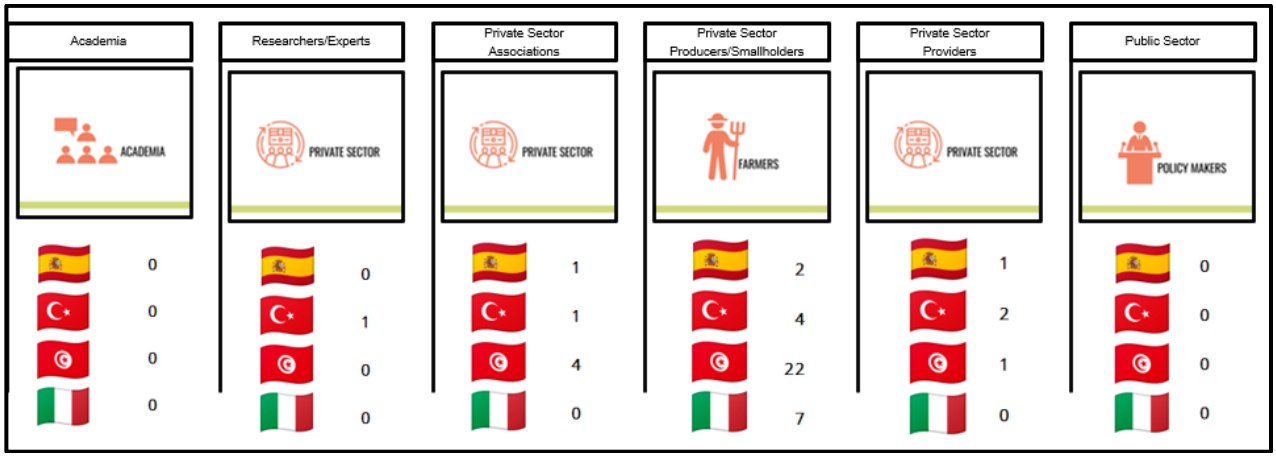

Figure 20. Type of stakeholders engaged per pilot country

The different activities resulted in relevant and interesting key findings which were classified in the following topics (Figure 21):

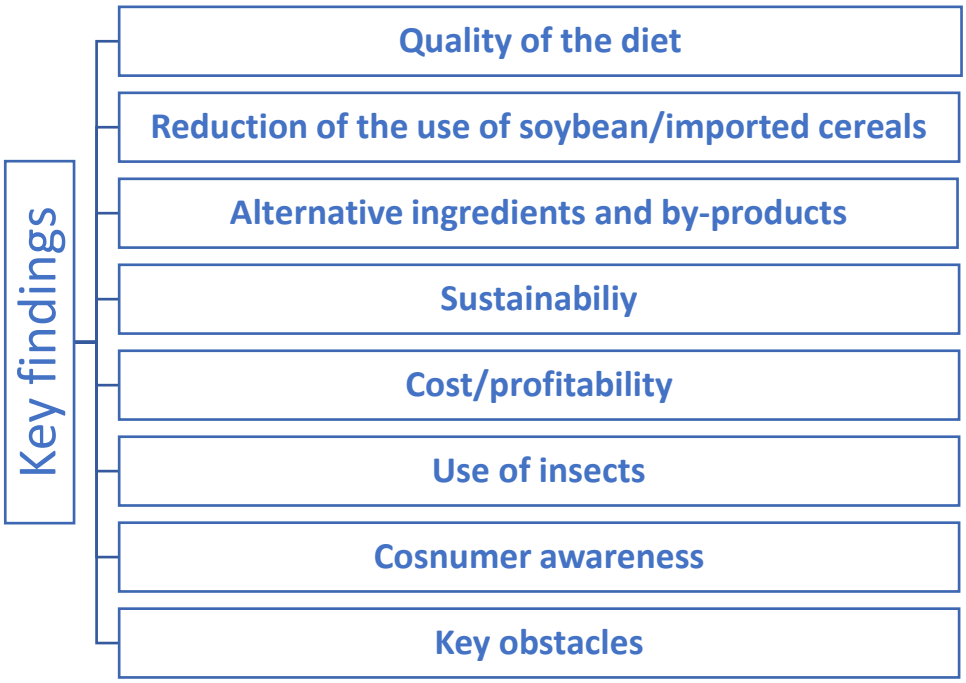

Figure 21. Key findings topics resulted from the LL A2

Quality of the diet

The main conclusions classified by partners regarding the quality of the alternative diets are explained in Figure 22.

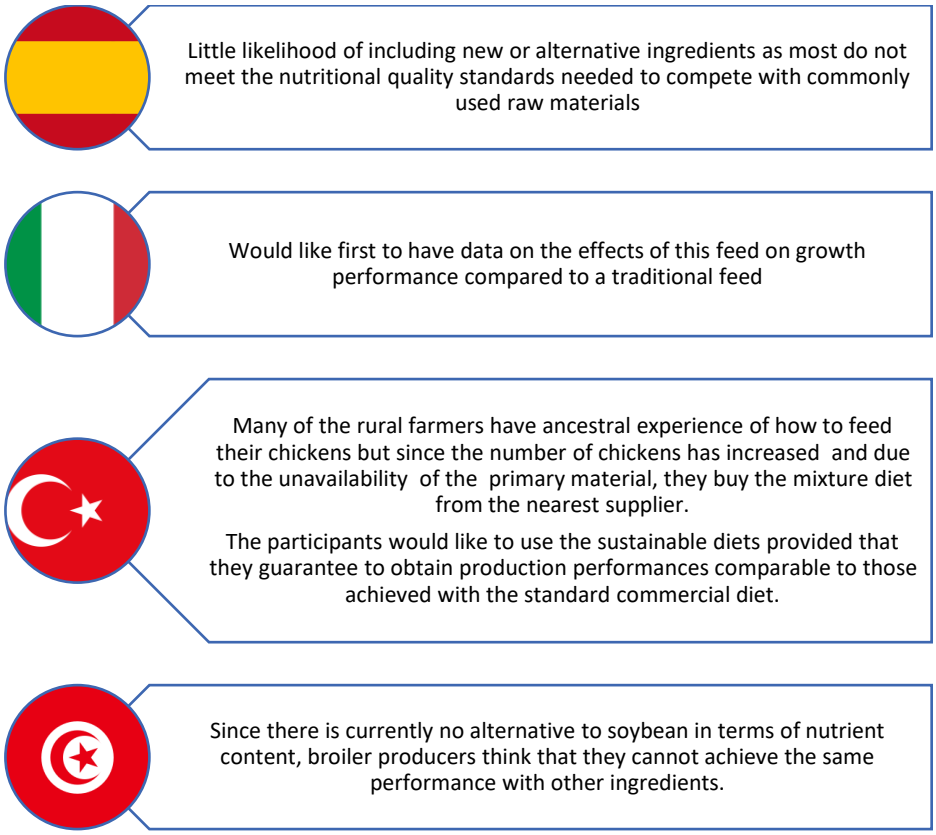

Figure 22. Quality of the diet key findings

Reduction of the use of soybean/imported cereals

The reduction of imported ingredients, especially soybean, has been another important topic addressed. Figure 23 lists the main conclusions obtained from this topic.

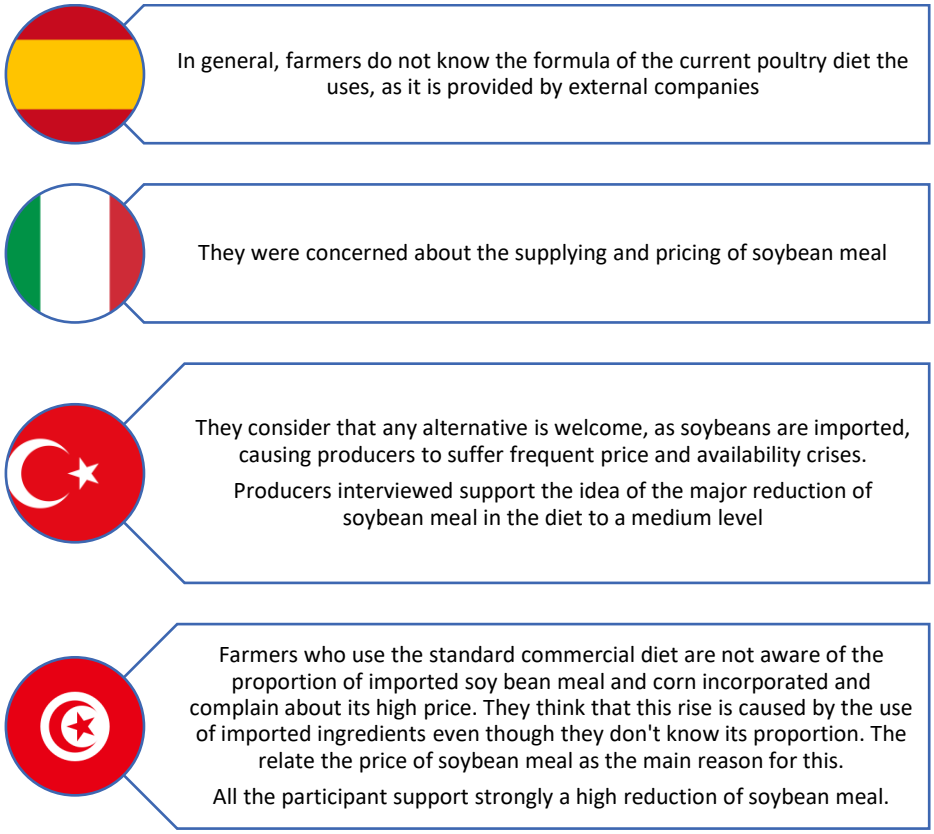

Figure 23. Reduction of the use of soybean/imported cereals key findings

Alternative ingredients and by-products

When discussing about the alternative ingredients and by-products that the alternative diets included, there were new suggestions for adding new ingredients un many occasions. In addition, some other comments were added as it is shown in Figure 24.

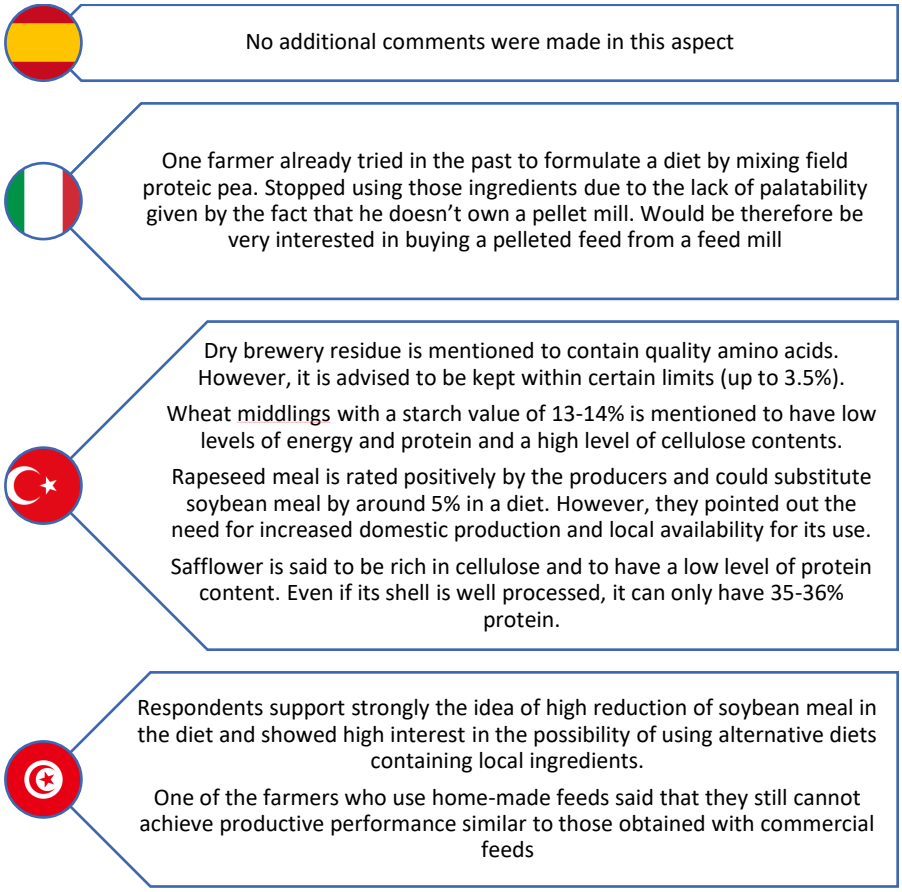

Figure 24. Alternative ingredients and by-products key findings

Sustainability

The sustainability is without any doubt the main pillar of the project. In that sense, it is also important to know the environmental concern of farmers and other stakeholders in this aspect. The main feedback obtained is explained in Figure 25.

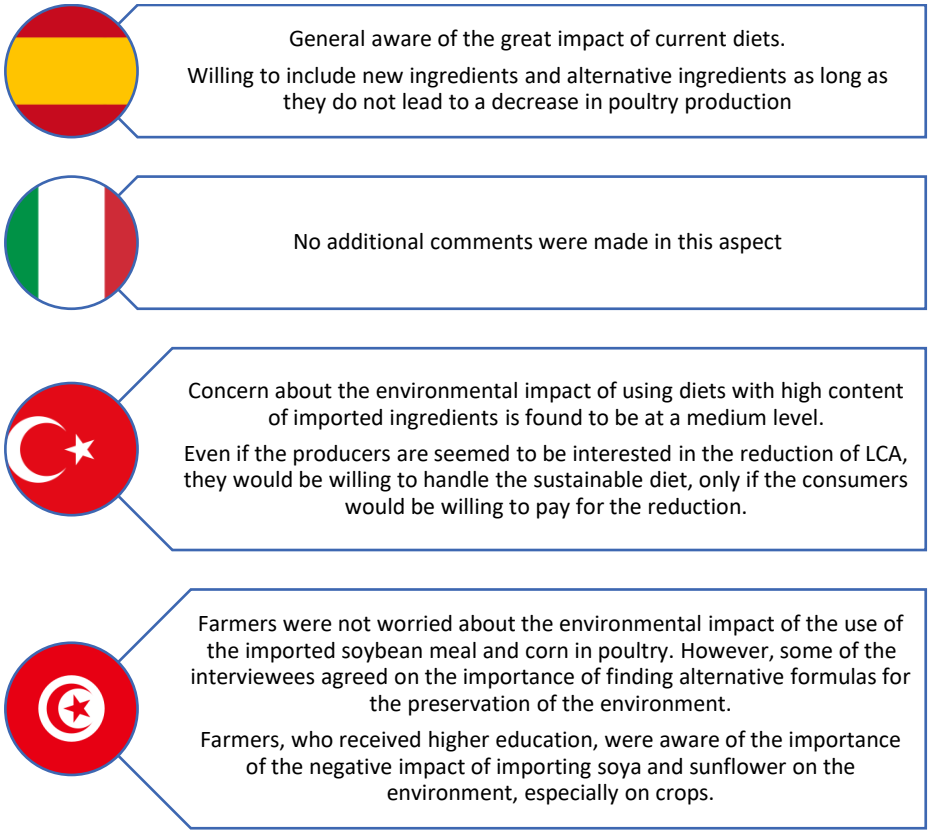

Figure 25. Sustainability key findings

Cost/Profitability

The profitability is, in many occasions, a critical point for the farmers and other actors of the supply chain. The loss of competitiveness or the no willingness of the consumers of paying more for sustainable products are some of the most important aspects mentioned. The summary of all of them is included in Figure 26.

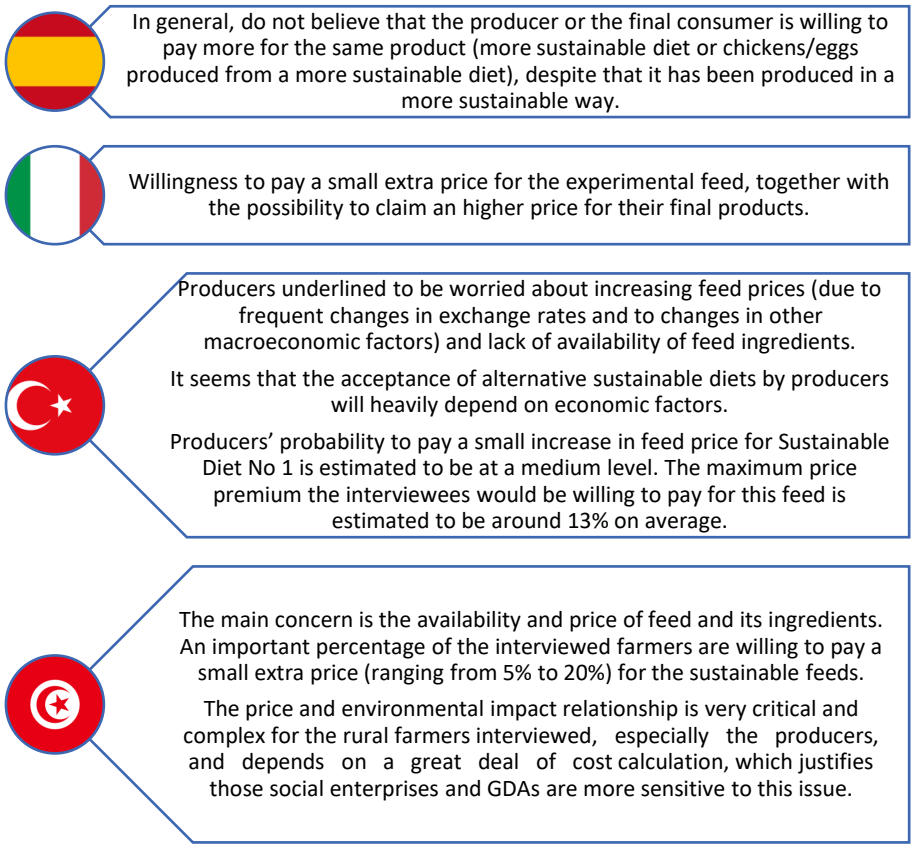

Figure 26. Cost/Profitability key findings

Use of insects

The use of insects, one of the most important innovations of SUSTAvianFEED approach, has attracted a lot of interest from the participants because of the possibilities its use arises. Some of the key aspects mentioned about them are listed in Figure 27.

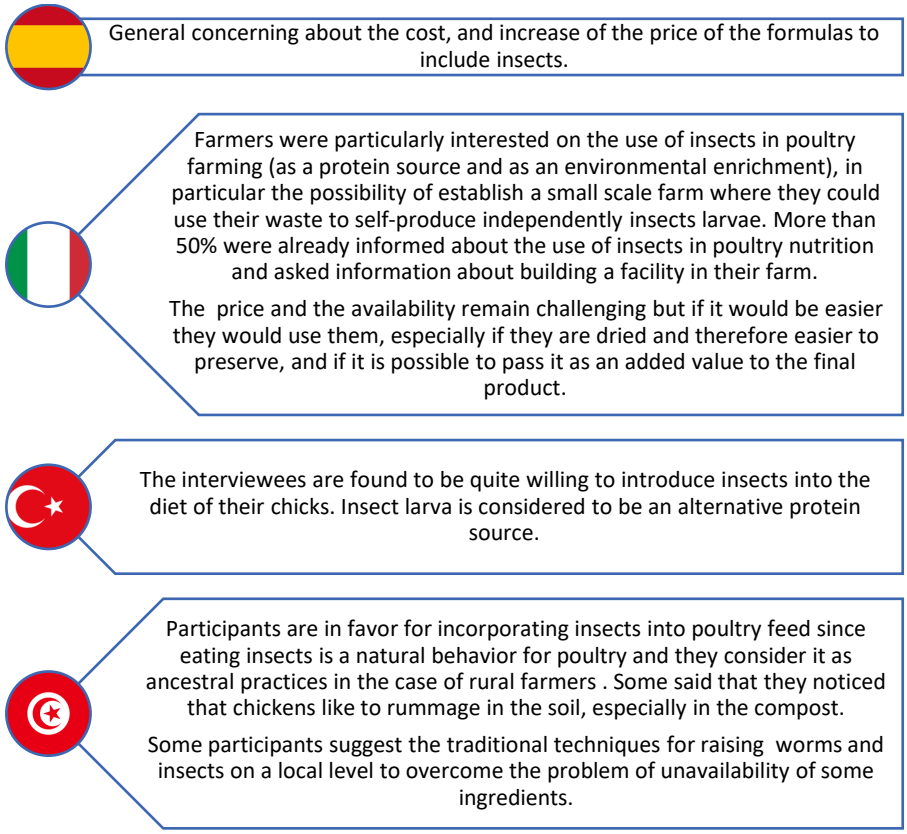

Figure 27. Use of insect's key findings

In addition, other key findings had more general comments and are listed below:

Consumers on price and sustainability

- It has been stated that if the reduction in environmental impact increases the cost of feed, this increase should be reflected in the price of meat. It is thought that - even if limited to a small group – there would be consumers who are willing to pay this price premium, especially if the environment-friendly characteristic of the product is accurately communicated to them (Turkey).
- Most of the interviewees mentioned that a price premium would be paid by a tiny group of consumers (3 to 10% of the whole consumer population) and that the demand of the consumers would depend on the way the product is presented (Turkey and Spain).
- Not believe that consumers are prepared and informed enough to understand an increase in the price of chicken resulting from a possible inclusion of alternative ingredients in their diet (Spain).
- Farmers are convinced that consumers do not look at the label for quality seals, that there are very few who actually do that and as consequence, willing to pay and increase for this reason (Spain).

Consumers on the use of insects

- Consumer acceptance regarding the inclusion of insect larva in broiler feed does not appear to be a major problem for the producer. The producers are rather optimistic regarding the consumers' probability of having concerns about the introduction of insects in chickens' diets. However, they placed some level of uncertainty on this topic. Most of them agreed that the perception of the consumers would depend upon promotional efforts (Turkey).
- Farmers somewhat don't worry about the consumer acceptance regarding the inclusion of insects in poultry feed. According to the majority of participants, the consumer does not seek to know the composition of the feed. He is rather concerned about the price and quality of the product (Tunisia).
- Farmers suggest awareness campaigns on the benefits of the product to convince certain consumers which will not accept buying products from animal fed with insects (Tunisia).

Key obstacles

- **Alternative ingredients and by-products**
  - It is noted that sunflower meals, dry brewery residue, and wheat middling are already being used at low rates in diet formulations. But there are questions on the efficiency; i.e., growth is expected to be slow. Besides, digestibility issues are pointed out relating to the high cellulose content.
  - Organizations need to improve the infrastructure to generate decorticated sunflower, as wholemeal cannot be administered in large quantities.
  - According to the producers, sunflower meals can be found with 34-36% protein content only, and it would not be feasible to find it with 38% protein content in the domestic market.

- **Use of Insects**
  - Insect protein was not accepted in Helal labeling, which could prevent them from being able to export poultry products to the Muslim countries which constitute Turkey's major market (Turkey).
  - Price, cost, and supply. Concern regarding the use of insects as well was rather on the side of economic factors, such as costs and availability in high quantities. (Turkey, Spain and Italy)

In the last stage of the interviews, several questions were made to the participants in order to quantify some of the discussed aspects:

When asking about the environmental awareness of using diets with a high content of imported ingredients, Spain and Italy shows higher awareness than Tunisia and Turkey (Figure 28). The higher socioeconomic level seems to be relevant to this issue.

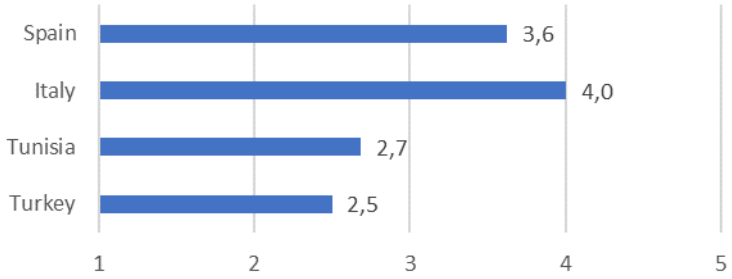

Figure 28. Environmental awareness of the participants per country (being 1 the less and 5 the top)

When asking about the acceptance of changes in the farming sector (Figure 29), there is a consensus in the need of reduction of the soybean, in the introduction of insects in diets, as well as in the need of increasing the final products price in case alternative diets have a higher cost.

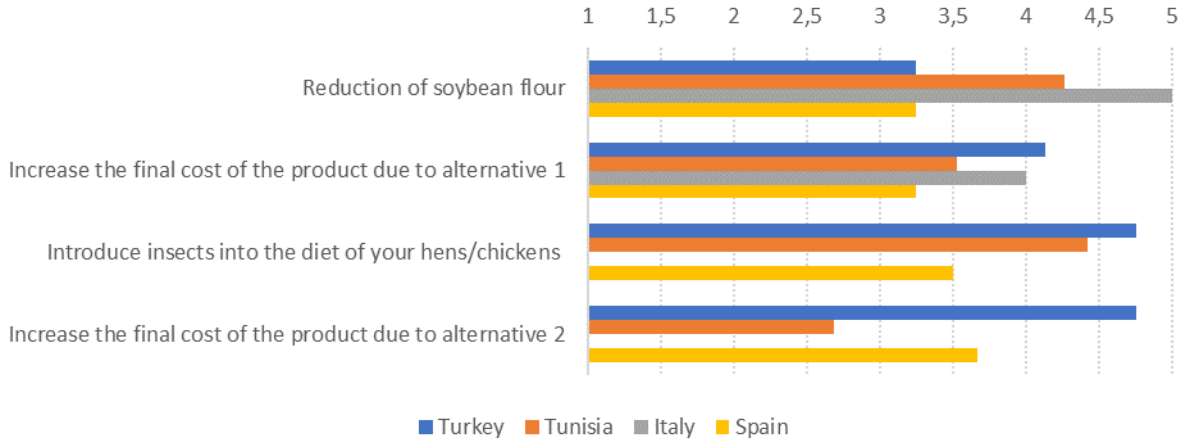

Figure 29. Acceptance of changes in the farming sector (being 1 the less and 5 the top)

However, even though this is accepted, when asking about the likelihood of consumers to pay more for the products, the participants did not give to it a high possibility (Figure 30). This is something which has been reduntant to in the whole LL activity: nowadays, most of the consumers will pay more attention to the price than sustainability aspects, especially those from lower socioeconomic levels. However, this trend may change in the future.

According to participants it is more possible that farmers pay an increase in the feed price and there is little possibility that consumers could have problems with insects' inclusion in diets.

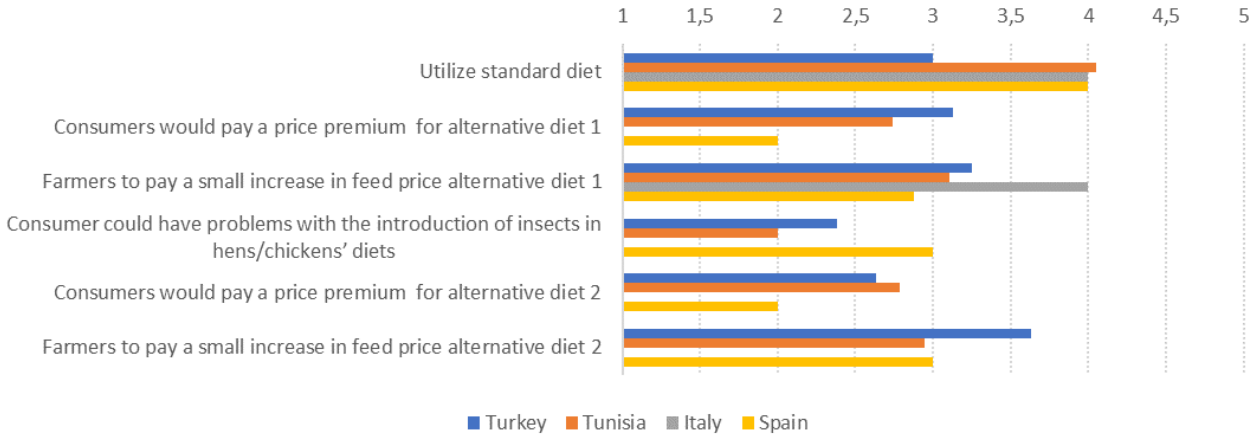

Figure 30. Likelihood of actions to be implemented (being 1 the less and 5 the top)

Finally, regarding the interest in the different diets (Figure 31) Italy and Spain, followed by Turkey, seems to be the most interested in the incorporation of the diets to its projects. Tunisia is a bit behind, however, still with a punctuation beyond 3.

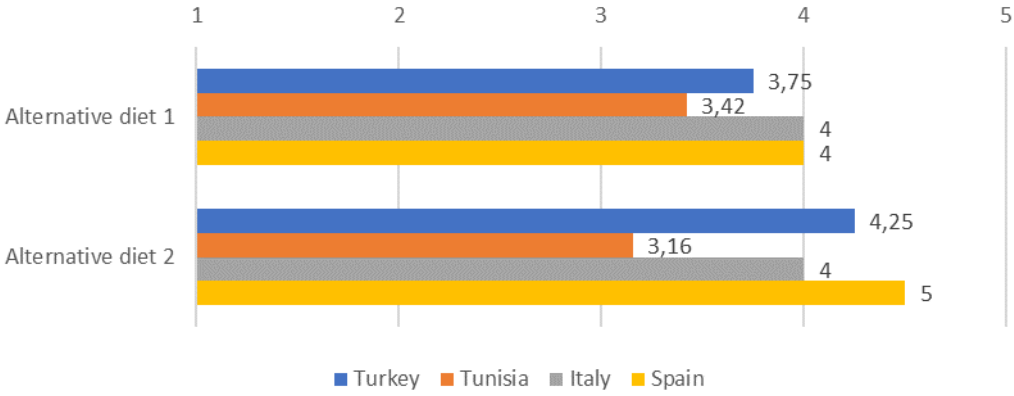

Figure 31. Interest in the proposed alternatives (being 1 the less and 5 the top)

It is important to mention that LL activities and the surveys developed have a limited impact as in a formal survey. This kind of massive surveys will be developed in Task 3.6 of the project "Economic evaluation of pilot activities". However, by the development of LL activities, the process can be cocreated in a continuous way, without waiting for final results, so all the considerations are included in the project development.

In summary, the reduction of import dependency, the animal welfare, the valorisation of traditional techniques, the insects' potential for a sustainable diet development and the key obstacle about the price are the most important aspects for its consideration. Most important remarks of the activities are included in Figure 32.

Reduction of import dependency

Price

Animal welfare

Valorization of traditional techniques

Insects' potential

Figure 32. Most important remarks of the activities

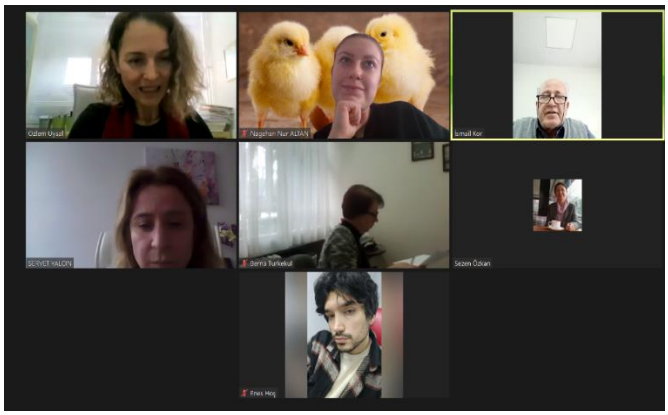

Figure 33. Stakeholders involved in LL A4-A5

Finally, a summary of the participants involved per country are listed. Some of them are missing in this public deliverable due to GDPR (Table 14).

Table 14. Stakeholders involved in LL A4-A5

SPAIN
Director of the company's health and production department. Big Spanish egg company.
Small chicken producer 1
Veterinarian, animal feed company, and private smallholder.
Ramón Quiñonero Bravo. Smallholder. Animal farmer for its own consumption.
ITALY
Azienda Agricola Mellano Emanuele
Società Agricola La Gallinella
Azienda Agricola Bertero
Societa' Agricola Bertea Francesco E Paola S.S.
Cascina Losetta, Stradale Baudenasca
Perotti Pinuccia, Backyard holding of Gallina Bianca Di Saluzzo
Azienda Agricola Monge
TURKEY
Damla Konca, Damii Organik, Private sector, producer.
Abdullah Koç, Dilek Tavukçuluk, Private sector, producer, and President of Poultry Producers Central Association.
İsmail Kor, Agro Organik Gıda, Private sector, producer.
Mehmet Erdemir, Başarı Yem, Private sector, provider.
Yavuz Erten, Abalıoğlu Yem A.Ş., Private sector, Feed factory manager of broiler integrated enterprise.
Adnan Zaimoğulları, A-Z Yem Danışmanlık Hizmetleri, Consultant.
Onur Sarıbaş, Yeşilküre Organik, Private sector, producer.
Bircan Uyar, Özlem Tarım, Private sector, Feed factory manager of broiler integrated enterprise.

TUNISIA
Wahida GONDI; smallholder
Salem BRICHNI; smallholder
Yassin ZAYANI; smallholder
Salem CHNITI; smallholder
Samir CHNITI; smallholder
KhlifaCHNITI; smallholder
Ali NEFZI; smallholder
Mahbouba LAHMAR; smallholder
Hanen ABDENNOUR; smallholder
MarouanBHIRI; smallholder
Mahrez TRABELSI; provider/producer
Entreprise sociale Maamlakat Errich
Groupement Agricole Ghraghiz
Groupement Agricole T'Zyout
Kawther Ghanney; smallholder
Saida Mezni; smallholder
Samia Rebhi; smallholder
Omayma Ouechtati; smallholder
Nejib Taboui; smallholder
Kawther Ghanney; smallholder

6 Sustainable Feeding Program

Once all the different activities for evaluation of the diets were conducted, a sustainable feeding program was defined. This feeding program should be considered as a draft version of the diet to be used during pilot activities. As it has been already mentioned, the availability of the ingredients and the nutritional characterization of the products before pilot activities start, may modify these proposals.

The following sections include this preliminary diet proposed for the pilot activities in each territory.

6.1 Spain

The main characteristics of the three proposed diets of the Spanish pilot are:

- **Control diets (usual ingredient)**: high amounts of imported maize (55 percentage units), soybean meal (22 percentage units) and little use of local ingredients.

- **3-HERM diet (with sustainable ingredients and insect)**:
  - Reduction imported maize (-12.51 percentage units), soybean meal (-5.95 percentage units) and other imported cereals.
  - Introduction of alternative ingredients: more national wheat (+7.03 percentage units); DDGS (+2 percentage units); peas (+6.68 percentage units) and dried larvae at 3%.

- **6-HERM diets (with sustainable ingredients and with insect)**:
  - Reduction imported maize (-29.60 percentage units), soybean meal (-10.84 percentage units).
  - Introduction of alternative ingredients: more national wheat (+24.03 percentage units); DDGS (+1.5 percentage units); peas (+10 percentage units) and dried larvae at 6%.

The formulated diets are close to be iso-energetic and iso-nitrogenous. The design of dietary treatments has applied the contrast between usual diet (with non-sustainable ingredients), and other two diets that contain more sustainable. Therefore, these diets include less imported cereal and soybean meal, and incorporate more alternative ingredients (unusual or by-product). In addition, these sustainable diets include larvae of insect, at 3 or 6%, respectively (Table 15 and Figure 34).

Table 15. Final preliminary laying hen diets of Spanish pilot: control, alternative diet with 3% and 6% *Hermetia illucens* (3-HERM and 6-HERM diets, respectively)

Diet Composition (%)	Control	3-HERM	6-HERM
Maize	55.00	42.49	25.40
Wheat	1.00	8.03	25.03
Soybean meal	22.00	16.05	11.16
Sunflower meal	6.29	6.50	6.50
Maize DDGS		2.00	1.50
Wheat middling	1.00	1.00	1.00

Pea		6.68	10.00
Hermetia illucens		**3.00**	**6.00**
Soybean oil	3.20	3.13	2.67
Calcium carbonate	9.49	9.10	8.73
Monocalcium phosphate	0.69	0.69	0.69
Sodium chloride	0.24	0.23	0.23
Sodium bicarbonate	0.15	0.15	0.15
Vitamin-mineral premix plus enzymes	0.80	0.80	0.80
DL-methionine	0.15	0.14	0.13
L-threonine		0.01	0.02
Calculated value[1]	**Control**	**3-HERM**	**6-HERM**
Metabolizable Energy (kcal/kg)	2750	2750	2750
Crude protein (%)	16.50	16.70	16.80
Ether extract (%)	5.68	6.04	5.78
Methionine (%)	0.42	0.42	0.42
Methionine + cystine (%)	0.70	0.71	0.72
Lysine (%)	0.82	0.82	0.82
Threonine (%)	0.62	0.62	0.62
Crude fiber (%)	4.44	4.84	5.00
Calcium (%)	3.83	3.83	3.83
Phosphorus (%)	0.51	0.54	0.55
Na (%)	0.15	0.16	0.16
Linoleic acid (%)	2.81	2.73	2.32

[1]Calculated value according to FEDNA (2019)

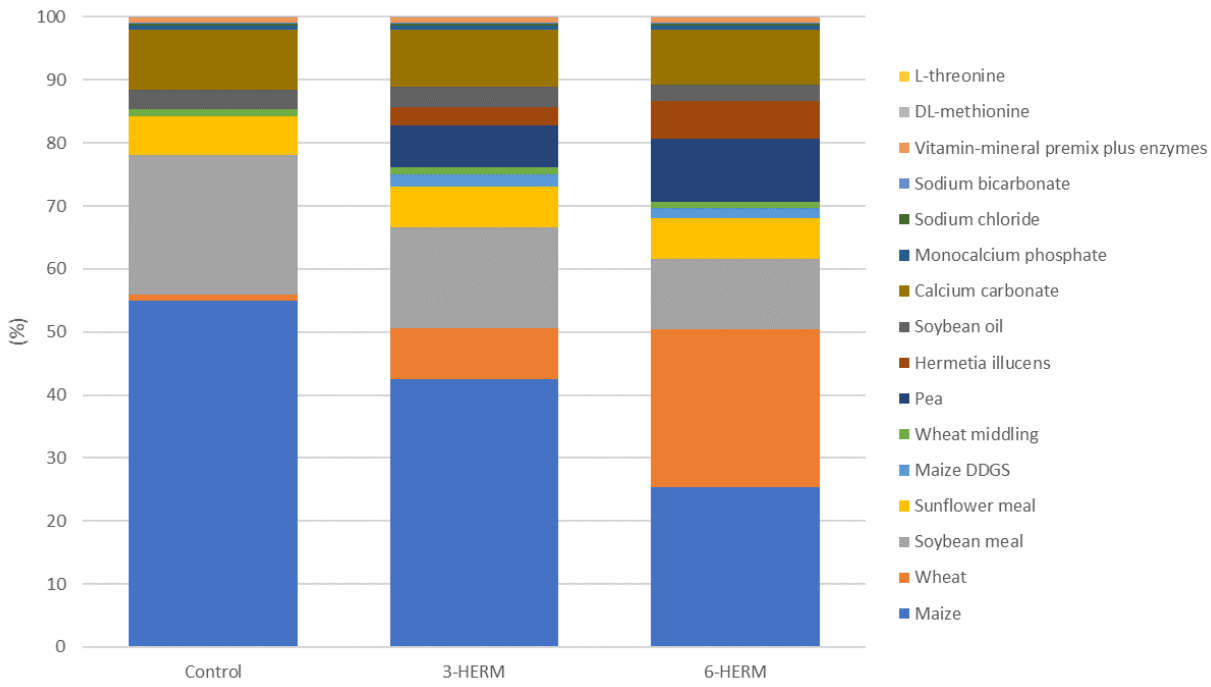

Figure 34. Final preliminary laying hen diets of Spanish pilot

Regarding the environmental evaluation of the diets, it can be appreciated that a great reduction has been achieved in comparison with the control diet. The reduction varies from the 17% to the 33%, dependant of the diet and the method followed (Table 16 and Figure 35).

For the Spanish case, as well as for the rest of them, the Impact on climate change estimated with ILCD 2011 Midpoint+ V1.10 / EC-JRC Global method (Impact (kg CO_2 eq/t)) and the Estimated impact obtained with ReCiPe 2016 Endpoint (H) method have been studied.

Table 16. Environmental impact of Spanish diets

	Impact (kg CO_2 eq/t)	Total Impact (pt)
Control	2600	124
3-HERM	2160 (17% less)	92.6 (25% less)
6-HERME	1850 (29% less)	83.1 (33% less)

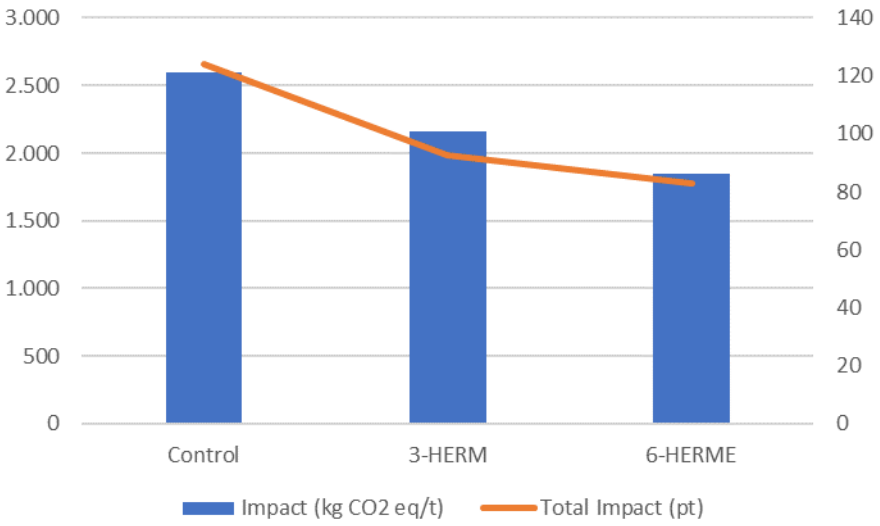

Figure 35. Environmental impact of Spanish diets

6.2 Italy

The main characteristics of the three proposed diets of the Italian pilot are:

- **Control diets (usual ingredient):** high amount of maize (60.0 and 61.77 for grower and finisher, respectively), high amount of soybean meal (34.57 and 32.0% for grower and finisher, respectively) and soybean oil (1.2 and 2.0% for grower and finisher, respectively).
- **ALTER diets**:
  - Reduction maize (-5.3, and -7.27 percentage units for grower and finisher, respectively), soybean meal (-28 and -27.97 percentage units for grower and finisher, respectively) and oil soybean (-1.2, and -1.4 percentage units for grower and finisher, respectively).
  - Introduction of alternative ingredients: fava beans (+8.6, and +9.6 percentage units for grower and finisher, respectively), pea (+8.6, and +9.6 percentage units for grower and finisher, respectively), sunflower meal (+5 percentage units for grower and finisher) and maize gluten (+12 percentage units for grower and finisher).
- **4.5-HERM diets (ALTER diets plus insect larvae):**
  - Reduction maize, soybean meal and oil soybean, according ALTER diets and dependent on the percentage of substitution of ALTER feeds by *Hermetia illucens*.
  - Introduction of alternative ingredients: fava beans, sunflower meal and maize gluten, according ALTER diets and dependent of the percentage of substitution of ALTER feeds by *Hermetia illucens*.

The diets of Italy pilot meet the requirements according to the type of poultry production. The formulated preliminary diets by phase are close to be iso-energetic, and iso-nitrogenous for crude protein, at least the controls and alternatives without insect. In the design of the diets, three experimental programs have been developed for each productive phase: a control (with inclusion of usual ingredients), and two more, that include a program with alternative ingredients; and other with alternative diet plus *Hermetia illucens* dried larvae, expecting a reduction of intake of the alternative diets between 3 and 6% (Table 17, Figure 36 and Figure 37).

Table 17. Final preliminary meat chicken diets of Italian pilot: two period (grower and finisher) per three programs: control, alternative diet without insect (ALTER), and alternative diet with expected *Hermetia illucens* substitution between 3 and 6% (4.5-HERM)

	Grower period			Finisher period		
Diet Composition (%)	**Control**	**ALTER**	**4.5-HERM**	**Control**	**ALTER**	**4.5-HERM**
Maize	60	54.7	52.24	61.77	54.5	52.05
Soybean meal	34.57	6.57	6.27	32	4.04	3.85
Fava beans		8.6	8.21		9.6	9.17
Pea		8.6	8.21		9.6	9.17
Sunflower meal		5	4.78		5	4.78
Maize gluten		12	11.46		12	11.46
Soybean oil	1.2			2	0.6	0.57

Dicalcium phosphate	1.35	1.35	1.29	1.35	1.35	1.29
Calcium carbonate	1.9	1.9	1.81	1.9	2	1.91
Sodium chloride	0.15	0.15	0.14	0.15	0.15	0.14
Sodium bicarbonate	0.14	0.14	0.13	0.14	0.14	0.13
DL-methionine	0.1	0.1	0.10	0.1	0.04	0.03
L-lysine		0.3	0.29		0.4	0.38
Vitamin-mineral premix	0.59	0.59	0.56	0.59	0.59	0.56
Hermetia illucens			**4.5**			**4.5**
Calculated values[1]	**Control**	**ALTER**	**4.5-HERM**	**Control**	**ALTER**	**4.5-HERM**
Metabolizable Energy (kcal/kg)	2761.51	2796.40	> ALTER[2]	2831.60	2837.72	> ALTER
Crude protein (%)	20.52	20.31	> ALTER	19.50	19.61	> ALTER
Ether extract (%)	4.08	2.73	> ALTER	4.89	3.30	> ALTER
Methionine (%)	0.40	0.47	> ALTER	0.38	0.39	> ALTER
Lysine (%)	1.00	0.84	> ALTER	0.94	0.87	> ALTER
Threonine (%)	0.71	0.64	> ALTER	0.67	0.61	> ALTER
Crude fiber (%)	3.39	3.92	> ALTER	3.28	3.89	> ALTER
Calcium (%)	1.20	1.15	> ALTER	1.19	1.19	> ALTER
Phosphorus (%)	0.60	0.60	> ALTER	0.59	0.59	> ALTER

[1]Calculated value according to INRA (2004).
[2]Expected nutritional value higher than alternative diet.

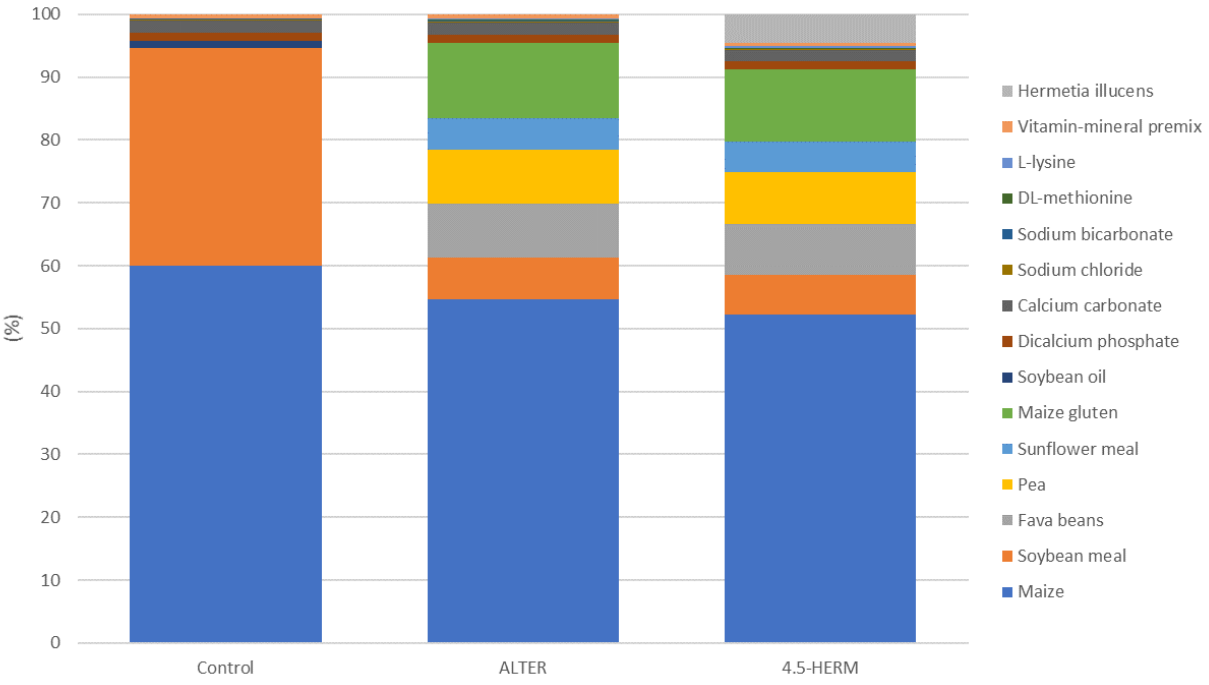

Figure 36. Final preliminary meat chicken diets of Italian pilot: Grower

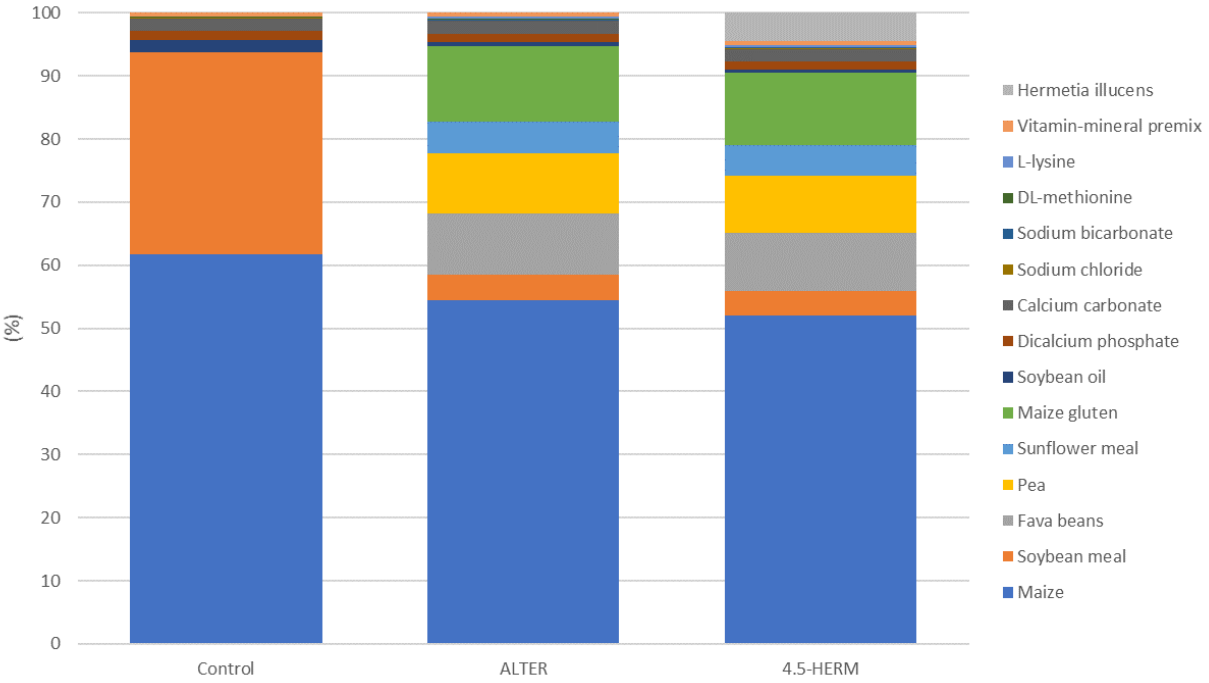

Figure 37. Final preliminary meat chicken diets of Italian pilot: Finisher

For the Italian pilot, it can be appreciated that a great reduction has been achieved in comparison with the control diet as well. The reduction varies from the 36% to the 50%, dependant of the diet, the phase and the method followed (Table 18 and Figure 38).

Table 18. Environmental impact of Italian diets

Phase		Impact (kg CO_2 eq/t)	Total Impact (pt)
Grower (0-60d)	Control	1940	95.3
	ALTER	972 (50% less)	57.6 (40% less)
	4.5-HERM	1063 (45% less)	59.8 (37% less)
Finisher (61-150d)	Control	1910	94.9
	ALTER	980 (44% less)	58.4 (38% less)
	4.5-HERM	1070 (49% less)	60.5 (36% less)

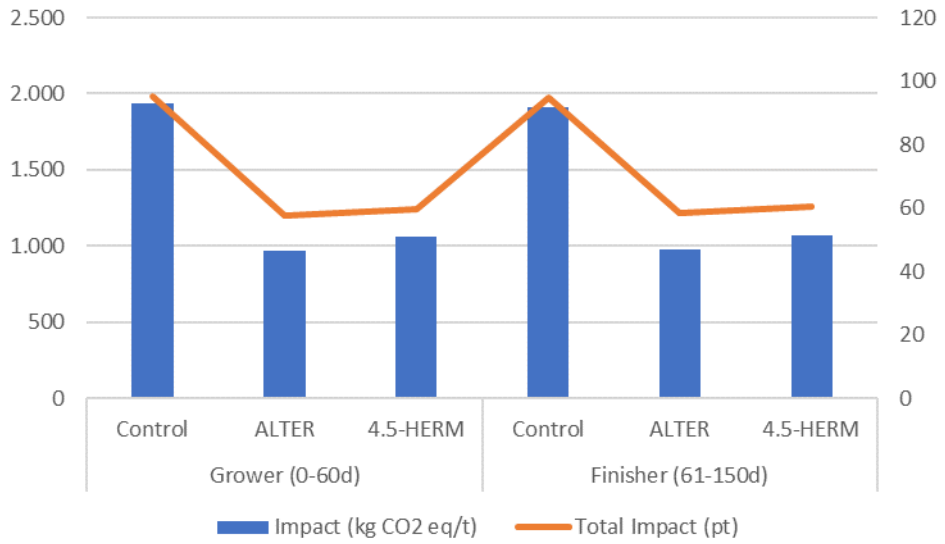

Figure 38. Environmental impact of Italian diets

6.3 Turkey

The main characteristics of the three proposed diets of the Turkish pilot are:

- **Control diets (usual ingredient):** high amount of imported maize and high amount of soybean meal for starter, grower and finisher period.
- **ALTER diets:**
  - Reduction maize (-15.65, -5.15, and -9.5 percentage units for starter, grower and finisher, respectively) and soybean meal (-11, -8.2, and -13 percentage units for starter, grower and finisher, respectively).
  - Introduction of alternative ingredients: Brewers´ dried grain (+5, +3, and +5 percentage units for starter), wheat middling (+7, +4, and +5.7 percentage units for starter, grower and finisher, respectively) and sunflower meal (+8, +7.4, and +11.9 percentage units for starter, grower and finisher, respectively).

- **5-HERM diets:**
  - Reduction maize (-12, -3.65, and -4.9 percentage units for starter, grower and finisher, respectively) and soybean meal (-18.4, -15.5, and -16.5 percentage units for starter, grower and finisher, respectively).
  - Introduction of alternative ingredients: Brewers´ dried grain (+5, +3, and +3.9 percentage units for starter), wheat middling (+4, +3, and +5 percentage units for starter, grower and finisher phases, respectively) and sunflower meal (+12, +11, and +11.1 percentage units for starter, grower, and finisher phases, respectively), and dried larvae at 5%.

The preliminary formulas of the Turkish pilot meet the requirements of birds according to the type of poultry production and phase, and the diets are close to being iso-energetic and iso-nitrogenous. Also, the diet design has implemented the comparation between usual diets (with non-sustainable ingredients), and other diets that include more sustainable. Sustainable diets contain less imported soybean meal and incorporate alternative ingredients (unusual or by-products). In addition, at least one sustainable program includes larvae of the insect. Proposed diets are shown from (Table 19 and Figure 39 to Table 21 and Figure 41).

Table 19. Final preliminary meat chicken diets of Turkish pilot for the starter period

Diet Composition (%)	Control	ALTER	5-HERM
Maize	48.473	32.823	36.473
Wheat	7	10.5	10.5
Sunflower meal		8	12
Soybean meal	36	25	17.6
Brewers' dried grain		5	5
Wheat Middlings		7	4
Hermetia illucens			**5**
Sunflower oil	5.3	8.35	6.1
Lime Stone		1	1
Dicalcium phosphate	1	1	1
Vitamin-mineral premix	0.1	0.1	0.1
Sodium chloride	0.177	0.177	0.177
Enzymes	0.05	0.05	0.05
L-lysine (HCl)	0.75	0.75	0.75
DL-methionine	0.1	0.2	0.2
L-threonine	0.05	0.05	0.05
Calculated values[1]	**Control**	**ALTER**	**5-HERM**
Metabolizable Energy (kcal/kg)	3004.95	3003.58	3000.28
Crude protein (%)	21.15	21.05	21.00
Ether extract (%)	6.95	9.94	9.44
Methionine (%)	0.69	0.69	0.64
Lysine (%)	1.2	1.21	1.21
Crude fiber (%)	2.93	5.24	5.94
Calcium (%)	1.28	1.24	1.32
Phosphorus (%)	0.48	0.57	0.57
Linoleic acid (%)	3.44	4.94	4.01

[1]Calculated value according to analysis, NRC (1994) and Sari et al. (2008).

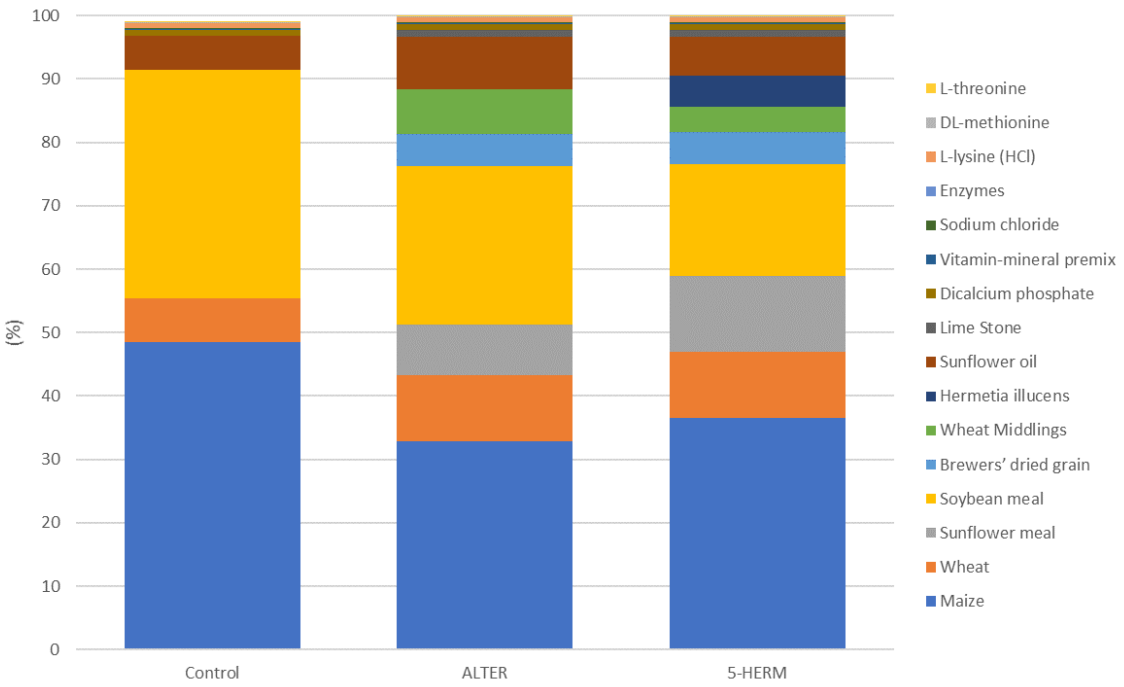

Figure 39. Final preliminary meat chicken diets of Turkish pilot for the starter period

Table 20. Final preliminary meat chicken diets of Turkish pilot for the grower period

Diet Composition (%)	Control	ALTER	5-HERM
Maize	48.273	43.123	44.623
Wheat	9	6	6
Sunflower meal		7.4	11
Soybean meal	33	24.8	17.5
Brewers' dried grain		3	3
Wheat Middlings		4	3
Hermetia illucens			**5**
Sunflower oil	6.5	8.35	6.55
Lime Stone	1	1	1
Dicalcium phosphate	1	1	1
Vitamin-mineral premix	0.1	0.1	0.1
Sodium chloride	0.177	0.177	0.177
Enzymes	0.05	0.05	0.05
L-lysine (HCl)	0.75	0.75	0.75
DL-methionine	0.1	0.2	0.2
L-threonine	0.05	0.05	0.05
Calculated values[1]	**Control**	**ALTER**	**5-HERM**
Metabolizable Energy (kcal/kg)	3102.39	3102.15	3102.42

Crude protein (%)	20.07	20.01	20.00
Ether extract (%)	8.10	10.00	9.95
Methionine (%)	0.67	0.67	0.63
Lysine (%)	1.17	1.17	1.17
Crude fiber (%)	2.78	4.50	5.24
Calcium (%)	1.28	1.29	1.34
Phosphorus (%)	0.47	0.52	0.53
Linoleic acid (%)	4.02	4.96	4.26

[1]Calculated value according to analysis, NRC (1994) and Sari et al. (2008).

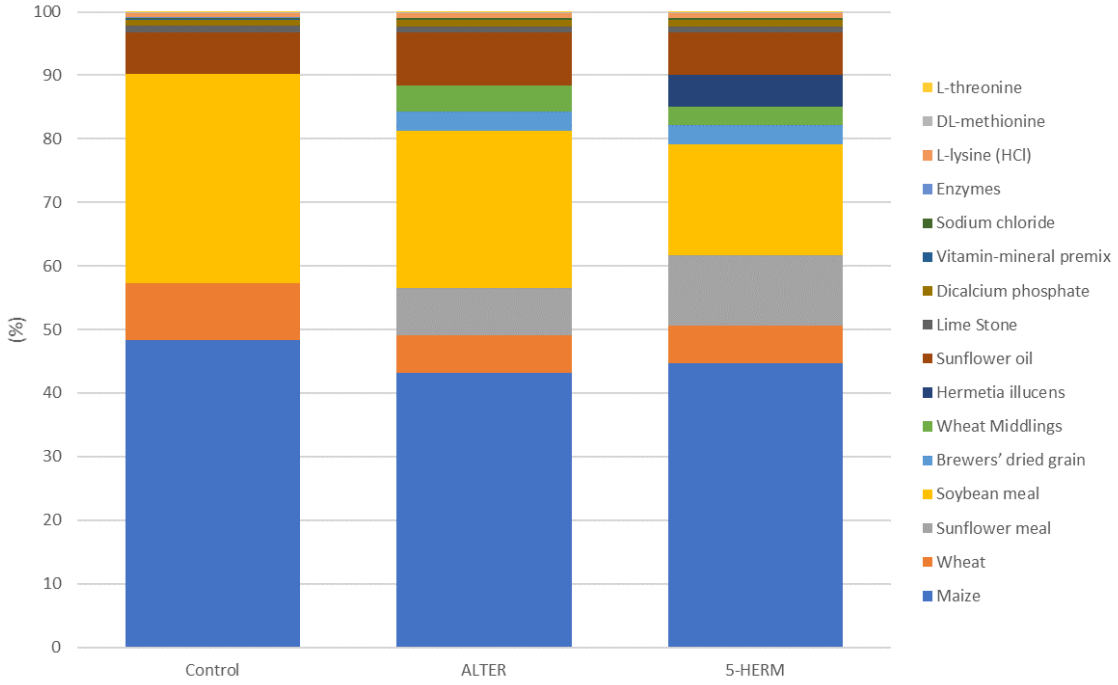

Figure 40. Final preliminary meat chicken diets of Turkish pilot for the grower period

Table 21. Final preliminary meat chicken diets of Turkish pilot for the finisher period

Diet Composition (%)	Control	ALTER	5-HERM
Maize	49.073	39.573	44.173
Wheat	15.5	13.9	10
Sunflower meal		11.9	11.1
Soybean meal	26.5	13.5	10
Brewers' dried grain		3.9	5
Wheat Middlings		5.7	5
Hermetia illucens			**5**
Sunflower oil	5.7	8.2	6.4
Lime Stone	1	1	1
Dicalcium phosphate	1	1	1
Vitamin-mineral premix	0.1	0.1	0.1
Sodium chloride	0.177	0.177	0.177
Enzymes	0.05	0.05	0.05
L-lysine (HCl)	0.75	0.75	0.75
DL-methionine	0.1	0.2	0.2
L-threonine	0.05	0.05	0.05
Calculated values[1]	**Control**	**ALTER**	**5-HERM**
Metabolizable Energy (kcal/kg)	3111.38	3102.34	3100.99
Crude protein (%)	18.11	18	18.01
Ether extract (%)	7.42	9.98	9.95
Methionine (%)	0.63	0.59	0.57
Lysine (%)	1.1	1.1	1.11
Crude fiber (%)	2.51	5.14	5.37
Calcium (%)	1.31	1.31	1.39
Phosphorus (%)	0.45	0.53	0.53
Linoleic acid (%)	3.68	4.94	4.26

[1]Calculated value according to analysis, NRC (1994) and Sari et al. (2008).

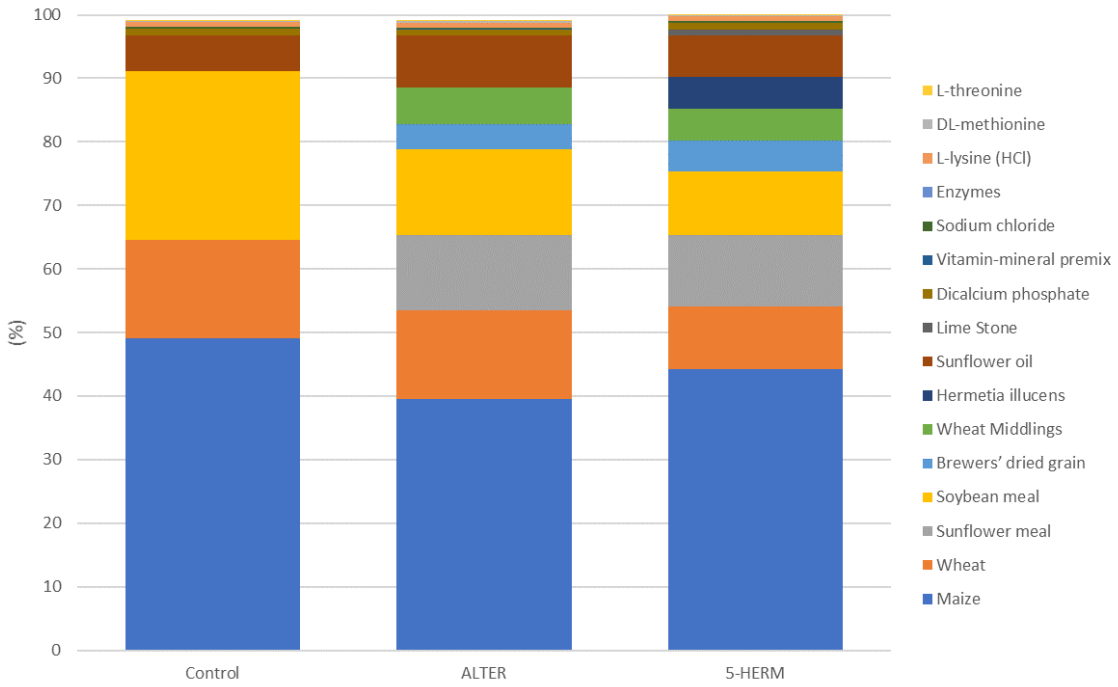

Figure 41. Final preliminary meat chicken diets of Turkish pilot for the finisher period

For the Turkish pilot, it can be appreciated that a great reduction has been achieved in comparison with the control diet as well. The reduction varies from the 29% to the 46%, dependant of the diet, the phase and the method followed (Table 22 and Figure 42).

Table 22. Environmental impact of Turkish diets

Phase		Impact (kg CO_2 eq/t)	Total Impact (pt)
Starter (0-14d)	Control	3460	138
	ALTER	1870 (46% less)	87.6 (37% less)
	5-HERM	1950 (44% less)	89.8 (35% less)
Grower (15-28d)	Control	3360	135
	ALTER	1980 (41% less)	91.8 (32% less)
	5-HERM	2030 (40% less)	92.5 (31% less)
Finisher (29d-slaughter)	Control	3080	126
	ALTER	1870 (39% less)	86.8 (31% less)
	5-HERM	1970 (36% less)	89.8 (29% less)

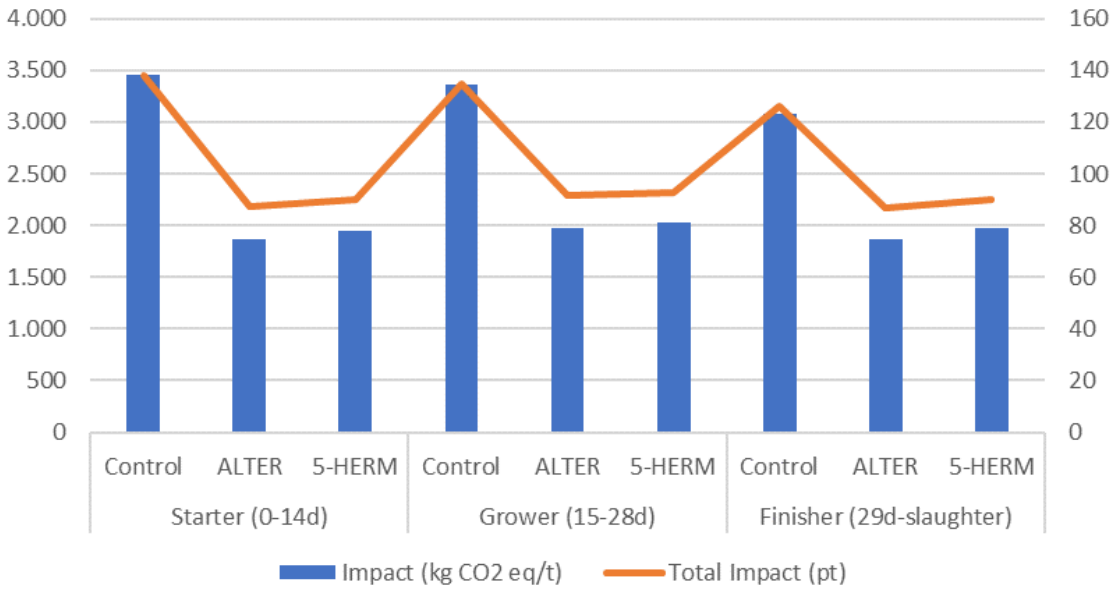

Figure 42. Environmental impact of Turkish diets

6.4 Tunisia

The main characteristics of the three proposed diets of the Tunisian pilot are:

For meat production,

- **Control diets (usual ingredient):** high amounts of imported maize (59.7, 68.3 and 72.7 % for starter, grower and finisher, respectively); and soybean meal (36.3, 28, and 24 % for starter, grower and finisher, respectively).

- **ALTER diets (with sustainable ingredients without insect):**
  - Reduction imported maize (-26.46, -32.3 and -45.7 percentage units for starter, grower and finisher, respectively) and soybean meal (-9.85, -10 and -10.9 percentage units for starter, grower and finisher, respectively).
  - Introduction of alternative ingredients: national triticale +15, +14 and +30.55 percentage units for starter, grower and finisher, respectively); rapeseed meal (+10, +7 and +7 percentage units for starter, grower and finisher, respectively); Fava beans (+5, +7 and +7 percentage units for starter, grower and finisher, respectively); and pasta waste (+5, +13 and +10 percentage units for starter, grower and finisher, respectively).
- **5-HERM diets (with sustainable ingredients and with insect):**
  - Reduction imported maize (-26.46, -33.5 and -31.95 percentage units for starter, grower and finisher, respectively) and soybean meal (-13.3, -14.3 and -13.9 percentage units for starter, grower and finisher, respectively).
  - Introduction of alternative ingredients: national triticale +15, +17 and +16 percentage units for starter, grower and finisher, respectively); rapeseed meal (+5, +7 and +7 percentage units for starter, grower and finisher, respectively); Fava beans (+10, +7 and +7 percentage units for starter, grower and finisher, respectively); pasta waste (+5, +12 and +11 percentage units for starter, grower and finisher, respectively) and dried larvae at 5% for all periods.

For egg production,

- **Control diets (usual ingredient):** high amounts of imported maize (57 percentage units); and soybean meal (24 percentage units).

- **ALTER diets (with sustainable ingredients without insect):**
  - Reduction imported maize (-17 percentage units) and soybean meal (-2 percentage units).
  - Introduction of alternative ingredients: national triticale +10 percentage units); and pasta waste (+12 percentage units).

- **5-HERM diets (with sustainable ingredients and with insect):**
  - Reduction imported maize (-25.7 percentage units) and soybean meal (-7.5 percentage units).
  - Introduction of alternative ingredients: national triticale +10 percentage units); pasta waste (+12.3 percentage units) and dried larvae at 5% for all periods.

The preliminary formulas of Tunisian pilot meet the requirements of birds according type of poultry production (meat or eggs), and the diets are close to be iso-energetic and iso-

nitrogenous. Also, the diet design has implementing the comparation between usual diet (with non-sustainable ingredients), and other diets that include more sustainable ingredients (according to the criteria of deliverable 2.4 about feed impact). Sustainable diets contain less imported soybean meal and incorporate alternative ingredients (unusual or by-product). In addition, the one sustainable diet includes larvae of the insect at 5%.

There were no modifications from the initial preliminary diets.

For the Tunisian pilot, in the case of meat production, the reduction varies from the 27% to the 44%, depending on the diet, the phase and the method followed (Table 23 and Figure 43).

Table 23. Environmental impact of Tunisian pilot for meat production

Phase		Impact (kg CO2 eq/t)	Total Impact (pt)
Starter (0-14d)	Control	2600	140
	ALTER	1900 (27% less)	96.4 (31% less)
	5-HERM	1550 (40% less)	87.1 (38% less)
Grower (15-28d)	Control	2450	138
	ALTER	1450 (41% less)	79.8 (42% less)
	5-HERM	1390 (43% less)	77.2 (44% less)
Finisher (29d-slaughter)	Control	2390	137
	ALTER	1440 (40% less)	79.9 (42% less)
	5-HERM	1390 (42% less)	76.7 (44% less)

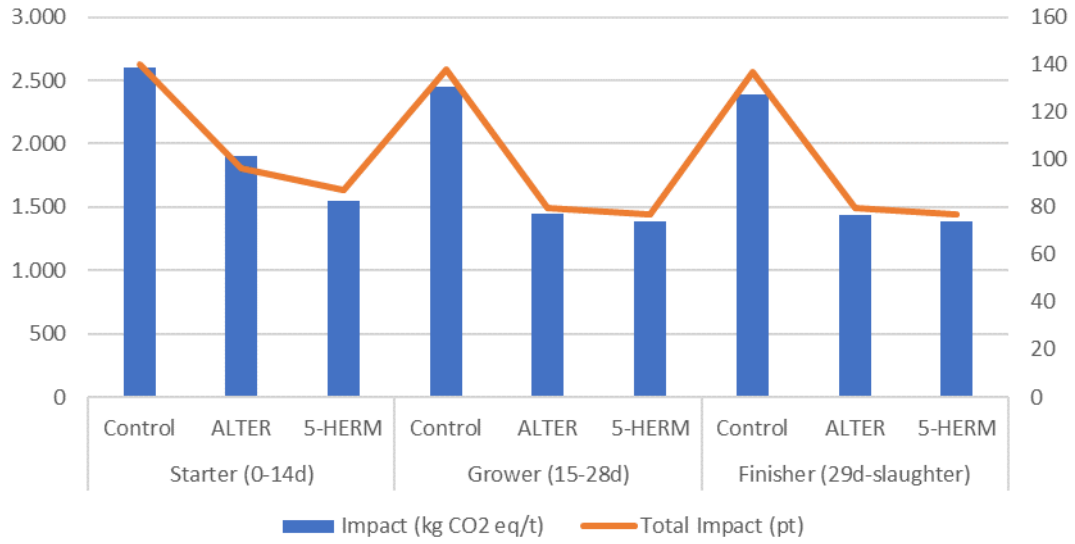

Figure 43. Environmental impact of Tunisian pilot for meat production

Regarding eggs production, the reduction is still significant, although a bit lower, from 17% to 33% (Table 24 and Figure 44).

Table 24. Environmental impact of Tunisian pilot for eggs production

	Impact (kg CO2 eq/t)	Total Impact (pt)
Control	2600	124
ALTER	2160 (17% less)	92.6 (25% less)
5-HERME	1850 (29% less)	83,1 (33% less)

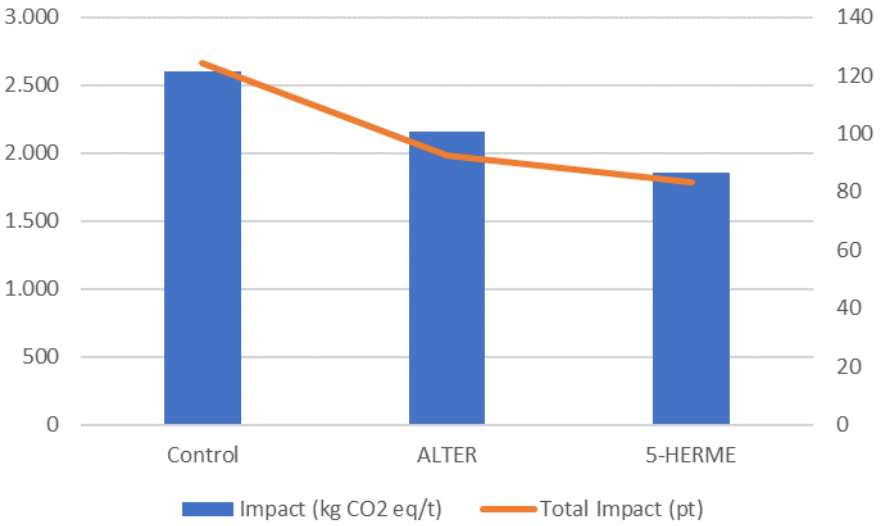

Figure 44. Environmental impact of Tunisian pilot for eggs production

Finally, Table 25 offers the environmental reduction of all the different diets. It can be shown that the environmental reduction is high, going from the 17% in the most conservative case, to the 50% in the most ambitious one.

Table 25. Summary of the environmental impact reduction of the alternative diets in relation to the control one

	Impact (kg CO_2 eq/t) reduction (%)		Total Impact (pt) reduction (%)	
	Alternative diet 1	Alternative diet 2	Alternative diet 1	Alternative diet 2
Spain laying hen diets	17%	29%	25%	33%
Italy grower diet for meat-type chickens	50%	45%	40%	37%
Italy finisher diet for meat-type chickens	49%	44%	38%	36%
Turkey starter diet for meat-type chickens	46%	44%	37%	35%
Turkey grower diet for meat-type chickens	41%	40%	32%	31%
Turkey finisher diet for meat-type chickens	39%	36%	31%	29%
Tunisia starter diet for meat-type chickens	27%	40%	31%	38%

Tunisia grower diet for meat-type chickens	**41%**	**43%**	**42%**	**44%**
Tunisia finisher diet for meat-type chickens	**40%**	**42%**	**42%**	**44%**
Tunisia laying hen diets	**17%**	**29%**	**25%**	**33%**

On the other hand, the price issue is other aspect that should be considered for the diet formulation in order to make sustainable formula which can be competitive in a real market. At the moment, we are facing a scenario in which prices are increasing and there is a huge volatility.

The pandemic, the geopolitical crisis, the energy crisis derived from this one, has reduce the availability of raw materials (Ukraine is one of the most important providers of maize, barley and wheat among others) and has drastically increased the prices of the daily life, in general, and the feed production, in particular. Therefore, the economic evaluation of pilot activities will consider all these aspect during project implementation.

As reported by the data of the Chicago Board of Trade, the international reference point for the future market of agricultural commodities, but trivially also the latest international updates, the quotations of the main elements of the animal diet have skyrocketed to historic highs, with corn recording the largest increase of the decade, while soybeans have reached the peak for almost seven years.

In this context, it's urgent that new food chains foster local economies, short production cycles and the resilience of the agri-food systems. It is crucial to reduce the huge dependency from other countries we have by this time, so these external events are not so damaging for regional and local agri-food systems. In addition, it is important as several actors involved during living lab activities, to think in the holistic systems which may make benefit for the region, create added value, new jobs position, etc.

The insect's approach is other positive aspect regarding price. Despite that currently the prices are not so cheap due to low technological maturity (still at initial phases), it is expected that they will be a sustainable and cheap alternative in the future and also a way of managing the organic waste. The positive legislation changes in the last few years makes this aspect consistent.

During the Task 3.6 "Economic evaluation of pilot activities", all these aspects will be studied in detailed.

7 Conclusions

As it has been studied in this document, there are a great number of possibilities which can increase the sustainability of the feed production and, consequently, the aviculture sector.

During this deliverable a methodology for a sustainable feeding program definition and a preliminary set of diets to be used in project pilots' activities have been developed.

It has been remarked the importance of the nutritional characterization of the diets, as well as the crucial step of the environmental evaluation by using the appropriate LCA methods. In addition, the sustainable feeding program includes a feed safety and health evaluation so potential risks are avoided.

On the other hand, the importance of the external actor's engagement has been highlighted. With the two sets of activities developed in the framework of living labs, relevant insights have been obtained for the project itself and for future activities in an exploitation phase.

The task 2.1 has resulted in a preliminary diets proposal for pilot implementation which reduce consistently the environmental impact of diets, the presence of imported products and which perfectly satisfies the nutritional characteristics of the animals for a satisfactory growth.

All these aspects revealed that the transition towards a more sustainable aviculture sector is possible and, considering the moments we are living, should be a must for our world. The climate change is already a reality, Mediterranean countries are suffering its effects dramatically and this is expected to be increased in the future. In addition, the social perspective also makes it important to mention that those who contribute the less to climate change are the ones which suffer the most its effects.

The farming sector is one of the main contributors to climate change, so it is crucial that new food chains are environmentally friendly, foster local economies and consider social aspects and feed production will be the mainstream of this change.

It is time to act, and SUSTAvianFEED propose a tangible approach for this which will be complemented by results in the following project activities.

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