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SUSTAVIANFEED

ALTERNATIVE ANIMAL FEEDS IN MEDITERRANEAN POULTRY BREEDS TO OBTAIN SUSTAINABLE PRODUCTS

ENVIRONMENTAL EVALUATION OF PILOT ACTIVITIES

DELIVERABLE 3.5

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SUMMARY

The SUSTAvianFEED project, funded by the European Union's PRIMA program, aims to reduce the environmental impact of the poultry sector in the Mediterranean region through the implementation of more sustainable feeding programs. This approach includes the partial or total replacement of conventional protein ingredients, such as soybeans, and other imported ingredients, such as corn, with alternative ingredients of local or nearby origin. To assess the effectiveness of these programs, five pilot studies were conducted in the Mediterranean countries participating in the project (Italy, Spain, Tunisia, and Turkey), where the environmental impact of the new feeding programs on meat and egg production was evaluated. These studies were carried out in five different settings, providing a broad view of the environmental benefits of the new diets for both laying hens and broilers. To evaluate the environmental impact of the various feeding programs, a Life Cycle Analysis was conducted using the ILCD Midpoint and ReCiPe Endpoint methods with the SimaPro v.9 software. These methodologies allowed for measurement of the effects of the experimental diets across multiple impact categories, such as greenhouse gas emissions (CO_2 equivalent), human health, ecosystems, and resource use.

In each pilot, two alternative experimental diets were compared to a standard control diet. One experimental diet included at least two local plant-based ingredients, varying by country, and was named the ALTER diet. In addition, the other experimental diet incorporated dried larvae of the Black Soldier Fly as a more sustainable and innovative protein source than soy and was labeled ALT+INSECT. The larvae were fed on agro-industrial by-products, promoting circular economy principles by reducing dependence on imported inputs and increasing the use of locally available resources. Furthermore, the pilot studies used locally adapted breeds suited to each country's environmental conditions, supporting biodiversity preservation.

The Italian pilot, conducted by the University of Turin, involved 144 slow-growing male broilers of the Bianca di Saluzzo breed. The study was conducted from 39 to 174 days of age, with an intermediate slaughter at 147 days to assess the environmental impact of meat production at both ages. Three experimental diets were evaluated: a Control diet based on corn and soybean meal, the ALTER diet in which total soybean meal and part of the corn meal were replaced by alternative ingredients such as fava beans (11%), peas (10.8%), sunflower meal (9.50%), barley (4.7%), and maize gluten meal (11.6%); and the ALT+INSECT diet, in which the ALTER diet was supplemented with 5% whole dehydrated Black Soldier Fly larvae. For the environmental analysis of the latter diet, two scenarios were considered: one where the insect was raised in UNITO and another where it was sourced from Entomo, an insect production company in Cehegín, Spain. The results showed that the alternative diets reduced kg CO_2 eq/kg diet emissions in all scenarios studied. Specifically, the ALTER diet achieved a 32% lower kg CO_2 eq/kg diet emission than the Control diet, while ALT+INSECT reduced the impact by 29-24%, depending on the insect's origin. In terms of impact on Human Health, Ecosystems, and Resources, the ALTER diet reduced impact compared to Control by 28%, 11%, and 23% kg CO_2 eq/kg diet, respectively, while ALT+INSECT achieved slightly less reduction than ALTER. However, when the study was conducted in relation to producing 1 kg of live weight (LW) or 1 kg of ready-to-cook meat (RTCC), with productive parameters achieved by animals fed different diets considered, the ALTER diet had a significantly lower impact than the Control, and the ALT+INSECT diets achieved the largest reductions across all categories studied. Thus, in the scenario where the insect was raised in Turin, it also had a lower impact than the ALTER diet, achieving a 40% reduction in kg CO_2 eq/kg LW and kg CO_2 eq/kg RTCC compared to the Control diet. Notably, slaughter at 147 days reduced emission of kg CO_2 eq, impact on Human health, Ecosystem and Resources about 10% compared to slaughter at 175 days.

The Spanish pilot, conducted at the University of Murcia, involved 120 23-week-old Isazul laying hens. The hens were distributed in 15 floor pens and randomly assigned to one of the three experimental treatments. The study lasted until the hens were 39 weeks old. The feeding program consisted of: a Control diet with conventional ingredients such as corn and soybean meal, an ALTER diet in which these ingredients were reduced by approximately by 13% globally, with other alternative plant ingredients such as Distillers Dried Grains with Solubles (DDGs) (7.46%), peas (5.56%), and sunflower meal (6%) included, being isoenergetic and isoproteic compared to the Control.; and a third ALT+INSECT diet consisting of the same ALTER diet supplemented with 5% whole dehydrated Black Soldier Fly larvae. In terms of CO_2 emissions, the alternative experimental diets achieved a 10-12% reduction compared to the Control diet, with the ALTER diet producing a slightly lower impact than the ALT+INSECT diet. Overall, experimental diets also achieved reductions in impact on Human health, Ecosystems, and Resources. When studying the impact associated with the production of 1 kg of egg mass considering the hens' productive data, it was observed that the alternative diets showed a significantly lower impact than the Control over the entire study period in relation to kg CO_2 eq/kg egg mass, with 10.4% and 15% less for the ALTER and ALT+INSECT, respectively. Regarding Human Health, there was also a significant reduction with the ALTER and ALT+INSECT diets compared to the Control by 6% and 13%, respectively. However, for Ecosystems and Resources, the ALT+INSECT diet significantly reduced impacts by 15% and 9%, respectively, while the ALTER diet had less evident effects in these last two categories.

Two pilot studies were conducted in Turkey at EGE University: one with a local slow-growing broiler breed (Anadolu-T) from day 1 to day 55, and another with a commercial breed (Cobb-500) from day 1 to day 40. In both cases, a three-phase feeding program was used: starter (from 0 to 14 days), grower (from 15-28 days), and finisher (from 29 days to slaughter age). The Control diet was mainly based on corn and soybean meal, while in the ALTER diet, these ingredients were reduced and alternative ingredients such as brewer's dried grain (up to 4%), wheat middling (up to 4%), and sunflower meal (up to 7%) were incorporated. In the ALT+INSECT diet, 5% Black Soldier Fly larvae meal were added, with all three experimental diets being isoenergetic and isoproteic. The results showed significant reductions in greenhouse gas emissions with the alternative experimental diets, with both alternative diets achieving similar reductions of around 38-43% kg CO_2 eq/kg diet. In the categories of Human Health, Ecosystems, and Resources, reductions were between 23% and 38%, depending on the phase and diet, compared to the Control diet. In studying the impact of producing 1 kg of LW, it was observed that local chicken breed had a 31% greater impact than the commercial breed in the four categories studied. However, the alternative diets achieved very significant impact reductions in CO_2 eq/kg LW emissions compared to the Control diet, for both the local and commercial breeds, with around 38-39% less CO_2 eq/kg LW. In the other categories studied, impact reductions compared to the Control diet per kg LW were also significant: 34% for Human Health, 23-27% for Ecosystems, and around 36-38% for Resources. Therefore, the reductions achieved by the alternative diets were very similar in both breeds.

In Tunisia, two pilots were conducted at the Institut Supérieur Agronomique de Chott Mariem (ISA CM) and another at the Rayhana Association. The ISA CM pilots focused on raising slow-growing chickens (Sasso T44) from 37 to 84 days of age and commercial laying hens from 30 to 40 weeks of age. The feeding program used in the slow-growing chicken study in Tunisia involved two phases: a growth phase (from 37 to 64 days of age) and a finishing phase, from 65 to 84 days of age. As in the other pilot studies, the Control diets were based on corn and soybean meal. In the experimental ALTER diet, imported soybean meal and corn were reduced by 7.5% and 20%, respectively, in both phases, and alternative ingredients such as rapeseed meal (6% in both phases), beans (6% in both phases), canola (6% only in the grower phase), and triticale (14% and 15% in grower and finisher phases, respectively) were added. The ALT+INSECT diet included the same ingredients as

ALTER but in different proportions as 5% Black Soldier Fly larvae were added. In this pilot, two scenarios were studied for the insect diet: one assuming the insect was raised near the pilot site and one assuming it was provided by Entomo, a company located in Cehegín, Spain. The results showed a 12% reduction in kg CO_2 eq/kg diet emissions for the ALTER diet compared to the Control diet, and a 19–21% reduction for the ALT+INSECT diets, with slightly more favorable environmental outcomes when insect production occurred closer to the pilot site. For Human Health and Resources, the reductions achieved by these alternative diets were even higher (up to 24%), with the Ecosystem category showing the least impact reductions. When examining the impact of producing 1 kg of LW, the ALTER diet achieved a significant 16% reduction in kg CO_2 eq/kg LW emissions compared to the Control diet, while diets incorporating insects in both scenarios analyzed reached a significant reduction of around 22% compared to the Control. For other categories studied, the reductions relative to the Control diet varied but were notably lower in the experimental diets, ranging between 18–26% in Human Health and Resources, and approximately 4–9% in Ecosystem.

In the laying hen study, a single-phase feeding program was used with a Control diet based on corn and soybean meal. The ALTER diet reduced the inclusion of imported ingredients such as corn (over 27%) and soybean meal (by about 9%), while introducing alternative local ingredients such as triticale (20%), fava beans (10%), and rapeseed meal (5%). The ALT+INSECT diet consisted of 95% of the ALTER diet plus 5% dried whole Black Soldier Fly larvae. The environmental impact study showed a 16.4% reduction in kg CO_2 eq/kg diet with the ALTER diet, and 14.7% with the ALT+INSECT diet when the insects were reared near the pilot site, or 13.8% if provided by Entomo. For Human Health and Resources, reductions ranged from 18.4% to 23%, with the ALTER diet, which incorporated local alternative plant ingredients, achieving the highest impact reduction. The Ecosystem category showed the least reductions with the studied diets. In terms of producing 1 kg of egg mass, the alternative diets showed similar and significantly lower impacts compared to the Control diet, with 17–18% reductions in kg CO_2 eq/kg egg mass and 22–23% reductions in Human Health and Resources impacts, but only diets containing insects were able to reduce the ecosystem category by over 4%.

Regarding the Rayhana pilot, a study was conducted with 14 women farmers using commercial laying hens. The feeding program was composed of two diets: a Control diet with local plant-based products (corn, bran, barley, dry bread, and wheat), and another diet based on the Control diet plus 5% Black Soldier Fly larvae (ALT+INSECT). Half of the farmers used the Control diet, and the other half used the ALT+INSECT diet. The impact of the diet per kilogram on CO_2 emissions, Human health, Ecosystem, and Resource use was greater with the ALT+INSECT diet than with the Control diet. However, when considering the productive parameters of the hens, the ALT+INSECT diet reduced the environmental impact in all the categories studied. Specifically, the emission per egg produced was reduced by 17.6% in kg CO_2 eq, 21.2% in Human health, 27.5% in Ecosystem impact, and 10% in Resource use. In addition, in a scenario where the insects were reared with less energy expenditure, the impact reductions were even greater. Therefore, under such rural egg production conditions, the insect-enriched feed is more sustainable due to the extra nutrients it provides for the hens.

In conclusion, the SUSTAvianFEED project has demonstrated that using local and alternative ingredients, as well as Black Soldier Fly larvae, while decreasing the dependency of soybean meal and imported cereals is an effective strategy to reduce the environmental impact of the poultry sector in the Mediterranean region. The experimental diets not only significantly lowered CO_2 emissions but also reduced resource usage and minimized negative impacts on Human health and Ecosystems. Furthermore, the inclusion of Black Soldier Fly larvae as a protein source in the ALTER diet was the best strategy in terms of impact per production unit (kg of live weight or kg of egg mass) in all the pilots. On the other hand, it has also been observed that slaughtering chickens of the same breed at an earlier age reduces the environmental impact per kilogram of

live weight or of ready to cook carcass. In addition, it has been observed that the reductions achieved by the alternative experimental diets are similar in both local breeds and commercial breeds, which demonstrates the usefulness of the alternative diets studied in commercial breeds, although the environmental impact produced per kilogram of live weight was lower in commercial breeds. These innovations in poultry feeding also promote the circular economy by utilizing local by-products and closing production loops. Beyond environmental benefits, these sustainable diets have the potential to drive socioeconomic growth in rural areas by decreasing reliance on imported ingredients and strengthening local resilience. In addition, the use of alternative ingredients may contribute to more sustainable and responsible production, aligning with current consumer demands for more environmentally friendly products.

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Acronyms and abbreviations

Abbreviation	Description
ALTER	Alternative diet with local feedstuffs
ALT+INSECT	ALT diet with Black Soldier Fly
BSF	Black Soldier Fly
DDGs	Distiller's Dried Grains with Solubles
EGE	Ege University
EU	European Union
ISA-CM	Institut Supérieur Agronomique de Chott Mariem
LCA	Life cycle assessment
LW	Live weight
mpt	milli points
RAYHANA	Rayhana Association for Women of Jendouba
RTCC	Ready to cook carcass
UMU	University of Murcia
UNITO	University of Turin

1 Introduction

The poultry sector continues to grow and develop globally, driven primarily by population growth, increased purchasing power, and urbanization. Genetic advancements, along with the development and transfer of technologies in feeding, slaughtering, and processing, have improved the safety and efficiency of poultry production. However, these changes have favored industry concentration in large-scale operations, with significant environmental repercussions, including greenhouse gas emissions and soil and water pollution (Cappelaere et al., 2021). As chicken meat is the most widely consumed meat globally, poultry and egg production are expected to continue increasing to meet growing demand (Mottet et al., 2017). Given the relevance of the poultry sector, a transition toward more sustainable practices is essential. There is considerable interest in reducing the environmental impact associated with feeding, as feed production is the most significant factor in the environmental footprint of poultry sector (Andretta et al., 2021; Cappelaere et al., 2021). Currently, the EU imports a large quantity of raw materials for animal feed, particularly soybeans and cereals (CESFAC, 2022). Therefore, the livestock sector as a whole requires a shift towards more sustainable production, where local raw materials are prioritized, and dependency on imported ingredients is reduced.

In recent years, alternatives such as the use of agro-industrial by-products in animal feed have been studied, as their use helps to mitigate environmental issues and reduces the carbon footprint when locally produced ingredients are employed. These by-products are largely not suitable for human consumption, avoiding direct competition with human food sources (Fiorilla et al., 2024). In addition, insects offer an interesting option with environmental and nutritional benefits (Gasco et al., 2019). Since insects are part of the natural diet of poultry and contain a high percentage of protein, fats, minerals, and vitamins. Some species, like Black Soldier Fly, have been included in European regulations as possible feed ingredients for monogastric animals, including chickens and laying hens (Regulation (EU) 2021/1372 of 17 August).

In this context, it is essential to test new, more sustainable and environmentally friendly poultry feeding programs through the development of pilot studies tailored to the diverse conditions of the countries participating in the SUSTAvianFEED project. The following sections describe the methodology used, as well as the results and conclusions obtained regarding Deliverable 3.5: Environmental evaluation of pilot activities.

2 Methodology

2.1 Scope

The objective of the SUSTAvianFEED project is to reduce the environmental impact of the poultry sector. To achieve this, pilot studies have been carried out in different Mediterranean countries, developing sustainable nutritional formulas aimed at lowering the overall carbon footprint of poultry production. The project was conducted across five different environments. In most of these studies, local or native chicken breeds, which are well-adapted to their environments, were used. This approach not only supports sustainable feeding practices but also plays an important role in preserving biodiversity and promoting the resilience of local ecosystems.

Each of the five pilot studies formulated experimental diets aimed at reducing dependence on imported ingredients, which are often sourced from distant countries. These imported ingredients were replaced with local or regionally sourced alternatives, favoring the use of ingredients and by-products of closer origin. This strategy aligns with the principles of the circular economy, as it seeks to make better use of local resources and reduce the environmental costs associated with long-distance transportation.

An innovative aspect of the project was the consideration of black soldier fly (BSF) larvae as a potential ingredient for poultry feed. The larvae proved to be an excellent and nutrient-rich source of protein and energy, enabling the replacement of more environmentally harmful protein sources, such as soybean meal. The larvae used in the pilot studies were either directly integrated into the poultry feed or offered as a supplement. In the latter case, the larvae not only provided an efficient and sustainable source of protein and energy but also acted as an enrichment factor, being able to improve their natural behavior.

Furthermore, to adhere even more closely to the principles of the circular economy, the BSF larvae themselves were fed on agricultural by-products, creating a closed-loop system that minimizes waste and promotes resource efficiency.

This report will present the detailed results obtained from each of the five pilot studies, which employed different feeding programs and worked with various poultry breeds, including both meat chickens and laying hens. In addition, all the pilots included similar criteria in the formulation of feed, using available alternative ingredients that were capable of reducing the environmental impact.

2.2 Methodology

UMU as Task leader of WP 3.5 requested from all pilot's information on the final diets used, pilot study design, as well as the production parameters obtained in each of them in order to be able to carry out the environmental impact study of the pilots.

Impact assessment was performed with the software package SimaPro 9.3.0.3 software developed by PRé Consultants, which classifies and combines the flows of materials, energy, and emissions into and out of each product system by the type of impact their use or release has on the environment. In addition, SimaPro provides advanced tools for environmental impact assessment throughout the product life cycle, allowing for a detailed analysis that covers everything from resource extraction to final disposal, thus contributing to a deeper understanding of the environmental effects of industrial activities. To calculate the environmental impact the ILCD 2011 Midpoint+ V1.11 / EC-JRC Global (kg CO_2 eq), and the ReCiPe 2016 Endpoint (v.1.06) method were used. Midpoint orientation means that the results are expressed in terms of their potential damage rather than their actual damage levels. Inputs related to on-farm production of raw materials were obtained from inventory data from the economic allocation database "Agri-foodprint 5". In the case of imported ingredients, an American and a European average was estimated according to the origin of provenance with data available from Agri-foodprint 5. In addition, to build our model for the experimental diets of each pilot, the impact was considered proportional to the number of origins of each ingredient, as well as transportation information provided by feed manufacturer, including the transportation method (truck, train or ship) and the distance between the different feed ingredient production sites and the feed mill in each pilot.

The ReCiPe Endpoint (H) method includes global punctuation in millipoints (mpt) and has also been employed with the aim of classifying the damage in three category indicators:

- Human health, mpt
- Ecosystem, mpt
- Resources, mpt

The ILCD method studies the impact on 16 category indicators:

- Climate change, kg CO_2 eq
- Ozone depletion, kg CFC-11 eq
- Human toxicity, non-cancer, CTUh
- Human toxicity, cancer, CTUh
- Particulate matter, kg PM2.5 eq
- Ionizing radiation HH, kBq U-235 eq
- Photochemical ozone formation, kg NMVOC eq
- Acidification, molc H+eq
- Eutrophication terrestrial, molc N eq
- Eutrophication freshwater, kg P eq
- Eutrophication marine, kg N eq
- Freshwater ecotoxicity, CTUe
- Land use, kg C deficit,
- Water resource depletion, m^3 water eq
- Mineral, fossil, &ren resource depletion, kg Sb eq

For the crop cultivation model in Agri-Footprint, the following aspects are considered:

- Crop yield (kg crop product / ha cultivated)
- Energy inputs (type and quantity / ha cultivated)
- Land use change (m^2/ ha cultivated)
- Land use change related emissions:
    - Carbon dioxide emissions
- Water use (m^3/ha cultivated)
- Artificial fertilizer and lime inputs (type and application rate/ha cultivated)
- Animal manure inputs (type and application rate/ha cultivated)
- Fertilizer / manure related emissions:
    - Nitrous oxide emissions
    - Carbon dioxide emissions (from lime and Calcium Ammonium Nitrate)
    - Ammonia and nitrate emissions
    - Heavy metal emissions
- Emissions from pesticides application (type and kg active ingredient/ha cultivated)

Effects on the acidification and marine eutrophication categories are usually due to fertilizers during crop production, and energy expenses. Land use is related to the land used change. Human toxicity is mainly related to the use of pesticides and fuel combustion.

In addition, we analyzed the impact of a new ingredient: Black soldier fly (BSF) larvae considering the production system, feeding, and transport to the Entomo facilities according to data provided by ENTOMO company. To incorporate them into the experimental diets of the pilots in Italy, Murcia, and Tunisia, whole dehydrated larvae were used—a novel form of inclusion that, when provided as a supplement, can serve as an environmental enrichment element since the ingestion of insects is part of the natural behavior of birds. This could improve animal welfare, as it represents a natural component of their diet (Schiavone and Castillo, 2024). Insects were reared by ENTOMO company for Italian, Spanish pilots, under highly technical and intensive conditions, with controlled environmental characteristics (29°C and 70% HR). Adult colony was maintained for larvae production. Larvae at 5 days of age began to fatten for 12 days, at which time they were slaughtered by bleaching. Subsequently, the larvae were dried to be supplied to UMU and UNITO pilots. In the case of the pilots in Turkey and Tunisia, the larvae were purchased from local companies, although a scenario assuming they had been purchased in Spain was also studied on Tunisian pilot since initially the larvae would be provided by Entomo company (Spain). In the previous Deliverable (2.4) the impact of the larva production system was calculated, and this determination was incorporated into the present Deliverable as an item for the impact assessments.

All the pilot studies conducted at the Universities in Spain, Italy, Turkey, and Tunisia followed a unified experimental design for the feeding program, with slight variations based on the specific conditions of each location. The design consisted of three experimental diets:

1. Control Commercial Diet: This was the standard diet commonly used by poultry farmers in each respective country, adapted to the type of chicken farming involved—whether it was egg production or meat production. The control diet served as the baseline for comparison against the experimental diets in each country.
2. Experimental diet with Local Ingredients: This diet incorporated more locally sourced ingredients, such as regional by-products, while reducing reliance on imported cereals and protein sources like

corn and soybean meal. The goal was to make the diet more sustainable by utilizing ingredients that were readily available in each country, thus lowering the environmental impact associated with long-distance feed imports as well as avoiding dependence on other countries.

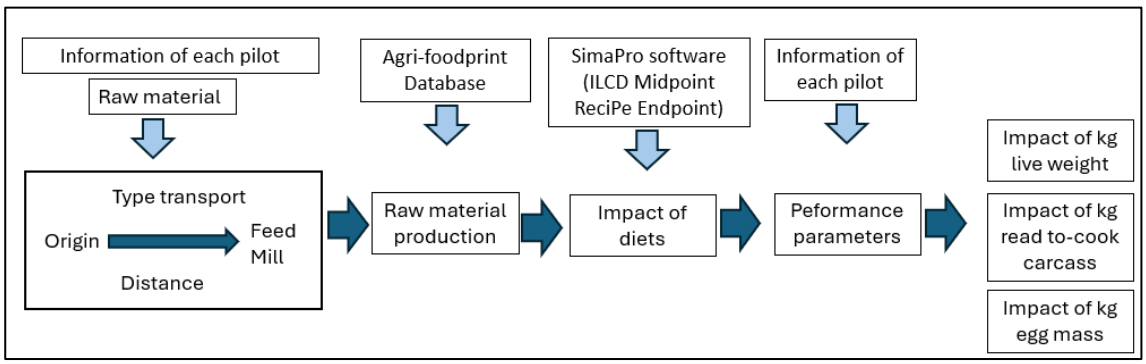
3. Experimental Diet with BSF Larvae: This diet included 5% inclusion of BSF larvae as a protein source. The inclusion of insect larvae aimed to assess the potential of insects as a sustainable and alternative protein source in poultry diets and as an element of environmental enrichment, according to the pilot. The addition of insect protein was explored as a strategy to reduce the environmental impact of conventional poultry feeds while maintaining or improving production efficiency and animal health.

Lastly, the environmental impact of diets used in pilot studies was estimated considering at least 96% of the composition for each formula. This deliverable will present the total environmental impact scores of each diet, providing detailed breakdowns across several critical categories. Specifically, it will include the impact on Human Health, Ecosystems, and Resources, as calculated using the Recipe 2016 H Endpoint method, which assesses the long-term consequences of dietary choices on these vital areas. Additionally, the report will highlight the impact of diets on Climate change, using the ILCD Midpoint+ method, which focuses on greenhouse gas emissions and other climate-related factors. By utilizing these two complementary methodologies, a comprehensive view of the environmental impact will be provided, allowing for a nuanced understanding of how each diet affects sustainability from multiple perspectives.

Once the environmental impacts of the sustainable feeding programs implemented in each pilot were established, we proceeded to analyze the specific impact of producing 1 kilogram of live weight in the pilots that focused on meat chickens, namely those conducted in Italy, Turkey and Tunisia. For these pilots, the aim was to determine how the different feeding strategies influenced the environmental footprint of producing meat from chickens. In contrast, for the pilot conducted in Spain and other in Tunisia, which worked with laying hens, they assessed the impact of producing 1 kilogram of egg mass. Thus, this double proposal allowed us to capture the environmental effect of the feeding programs across both meat and egg production systems.

The calculations were based on detailed production data provided by each pilot, considering factors such as feed conversion rates, performance parameters, or egg-laying performance. By using this data, we were able to estimate the environmental impact not just at the feed level, but at the level of final animal product output, providing a more holistic understanding of how different feeding strategies affect sustainability. **Figure 1** shows the methodology used.

Figure 1. Flow diagram of the methodology followed



This analysis was crucial for drawing meaningful comparisons between the pilots that focused on meat chickens and those that dealt with laying hens, allowing us to determine which feeding strategies and local ingredients had the most significant positive impact on reducing the environmental footprint of both meat and egg production. Furthermore, it provided insights into the broader implications of transitioning to more sustainable feeding programs and how these programs might contribute to the overall reduction of greenhouse gas emissions, resource depletion, and other environmental concerns in poultry production systems.

By looking at the full production cycle—from feed intake to final animal product—this study offers a comprehensive view of how sustainable feed practices can improve environmental outcomes in both meat and egg industries across different geographical and operational contexts.

In those parameters in which we had several replicates, such as performance parameters, a statistical analysis was carried out with the help of the SPSS statistical program, using a general linear model considering diet as a fixed factor. Statistical differences were set at $P<0.05$. When differences between dietary treatments were found, Tukey's test was used to analyze the differences between diets. However, each pilot briefly describes the statistical model used.

3 Results and Discussion

3.1 Italian pilot

The Italian pilot study, conducted by the University of UNITO, focused on meat-type chickens using the Bianca di Saluzzo males. This is an indigenous, slow-growing breed, which is well-suited to traditional farming systems in the region. The feeding program was designed around a single-phase approach, where three different diets (one control and two alternative diets) were evaluated. A total of 144 chickens, starting at 39 days of age, were randomly assigned to one of the three experimental diets, with 6 pen per diet and 8 chickens per pen (each pen acting as a replicate), ensuring robust data collection. The chickens were fattened from day 39 until 174 days of age. However, at 147 days, an intermediate culling was performed, where 2 chickens per replicate were slaughtered. This slaughter was important for assessing the environmental impact of producing both live weight and meat at two distinct time points. By analysing the data at 147 days and again at 174 days, the study aimed to provide comprehensive insights into how different feeding strategies affect both environmental sustainability and production efficiency over the entire fattening period.

The feeding program of the Italian pilot consisted of the following diets:

- **Control diet wa**s based on wheat and soybean meal, which are common ingredients in poultry feed in Italy. Both of these ingredients have a significant environmental impact. Additionally, this diet includes soybean meal as the main source of protein**.** This diet would serve as a baseline for comparing the environmental impact of the other two alternative diets.
- **Alternative Diet 1 (ALTER**), in this diet, soybean meal was completely removed and more sustainable ingredients such as fava beans (11%), peas (10.8%), barley (4.70%), sunflower meal (9.50%), and maize gluten meal (11.6%) were incorporated. Unlike other experimental diets, this one did not include insect larvae as a protein source.
- **Alternative diet 2 (ALT+INSECT)** includes the same ingredients as the ALTER diet but also incorporates whole dried larvae of BSF (Black Soldier Fly). Due to the high digestibility and nutrient density of the insect protein, a reduction in the overall intake of the alternative diet by 3 to 6% is expected, so the average incorporation was 5% (calculation based on dry matter intake). This diet is named ALT+INSECT. In this pilot the insect larva was provided by Entomo Agroindustrial company (Spain).
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The composition of experimental diets is shown in **Table 1**.

Table 1. Ingredients of the experimental diets used in the Italian pilot (g/100g as fed basis)

Ingredients	CONTROL	ALTER	ALT + INSECT
Maize meal	61.7	46.1	43.8
Soybean meal 44	32.0	-	-
Field bean	-	11.0	10.5
Pea protein	-	10.8	10.3
Barley	-	4.70	4.46
Sunflower meal	-	9.50	9.00
Maize gluten	-	11.6	11.0
Soybean oil	2.00	1.60	1.52
Dicalcium phosphate	1.35	1.35	1.28
Calcium carbonate	1.90	2.00	1.90
Sodium chloride	0.15	0.15	0.14
Sodium bicarbonate	0.14	0.14	0.13
DL-methionine	0.17	0.07	0.07
L-lysine	-	0.40	0.38
Vitamin- mineral Premix[2]	0.59	0.59	0.56
Hermetia Illucens larvae	-	-	5.0

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet; ALT+INSECT: diet ALTER plus 5% whole dehydrated Black Soldier Fly. [2]Vitamin A, Vitamin D_3, Betaine anhydrous 600.48mg, Biotin 0.04mg, Choline chloride 333.07mg, Folic acid 0.81mg, Niacinamide 25.01mg, Calcium pantothenate 7.28mg, Vitamin B1 0.75mg, Vitamin B12 0.02mg, Vitamin E 18.50mg, Vitamin K3 2.50mg, Copper 10.00mg, Iodine 1.50mg, Iron 44.01mg, Manganese 62.01mg, Selenium 0.25mg, Zinc 50.01mg

3.1.1. Impact of the experimental diets in the Italian pilot study

Table 2 shows the impact on Climate change, expressed in kg CO_2 equivalent per kg of diet (kg CO_2 eq/kg diet), calculated using the ILCD Midpoint method, considering more than 99% of the composition of the diets. When the analysis was performed using the ReCiPe Endpoint method, the potential impacts of 1 kg of diet, expressed in Total millipoints (mPt), impact on Human health (mPt), Ecosystems (mPt), and Resources (mPt) are presented in **Table 3.** The ILCD Midpoint method assesses the impact across 16 categories. However, for the sake of brevity in this report, only the impact on climate change is highlighted, as it is considered the

category with the most significant social relevance. The full study, which estimates the impact across all 16 categories, can be found in **Figure 1.1** in Annex.

For the study of the impact of the diet containing BSF larvae, two distinct scenarios were analyzed to better understand the environmental consequences of sourcing and transporting insect-based ingredients. In the first scenario, it was assumed that the larvae were produced locally in UNITO (Turin), the same location where the pilot study was conducted. This scenario represents a situation where production and consumption are geographically close, minimizing the need for long-distance transportation.

In the second scenario, which was the real situation in this pilot, the larvae were assumed to be produced in Cehegín, Spain, at the Entomo Agroindustrial facility, which specializes in insect production. This scenario involved evaluating the additional environmental impact caused by the transportation of the insect larvae from Cehegín to Turin. This second case reflects a more complex supply chain, accounting not only for the production of the larvae but also for the logistical factors, such as the emissions associated with transporting the larvae between these two locations.

Table 2. Impact of 1 kg of Italian pilot experimental diets on climate change (ILCD Midpoint method) considering > 99% of the diet composition in the two scenarios studied: if larva was produced in UNITO and if larva was produced in Spain

	Experimental diets[1]			
	CONTROL	ALTER	ALT+INSECT (UNITO)	ALT+INSECT (Spain)
Climate change (Kg CO_2 eq/kg diet)	2.05	1.39	1.46	1.55

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet; ALT+INSECT (UNITO): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Turin (UNITO). ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain).

The Climate change impact of the Control diet was 2.05 kg CO_2 equivalent per kg of diet, while for the ALTER diet, it was 1.39 kg CO_2 equivalent per kg of diet, representing a 32% reduction. The impact of the diet that included 5% dehydrated whole insect BSF in the scenario where the insect was raised in Turin was 1.46 kg CO_2 eq/kg diet. In the scenario where the insect was raised at the Entomo Agroindustrial company located in Cehegín (Murcia, Spain), the impact was 1.55 kg CO_2 eq/kg diet. This means that feeding chickens with a diet where soybean meal was replaced and supplemented with dehydrated whole insects raised in Turin reduced the climate change impact by nearly 29% compared to the Control diet, and by 24% when the insect was raised in Spain.

When considering the impact expressed in total millipoints (mpt), using Recipe Endpoint method (**Table 3** and **Figure 2**), the Control diet had a total impact of 88.1 mpt. The ALTER diet reduced the total impact by 26%, while the insect-based diet reduced the total impact by 25% when the insects were raised in Turin and by 21% when raised in Cehegín (Spain) and transported to Turin (Italy).

Of the three categories studied using the ReCiPe Endpoint method, Human Health was the most affected by the animal diets. A similar trend was observed as with the Total impact: the ALTER diet reduced the impact by 28%, and the diets incorporating insects reduced the impact by 26% and 23%, depending on whether the insects were raised in Turin or Spain, respectively. For the Ecosystem category, the reductions compared to the Control diet were 11% for the ALTER diet and 13% and 12% for the ALT+INSECT diet in the Turin and Spain scenarios, respectively. In the case of Resources, the reductions were 23% for the ALTER diet and 19% and 11% for the ALT+INSECT diets in the two scenarios studied.

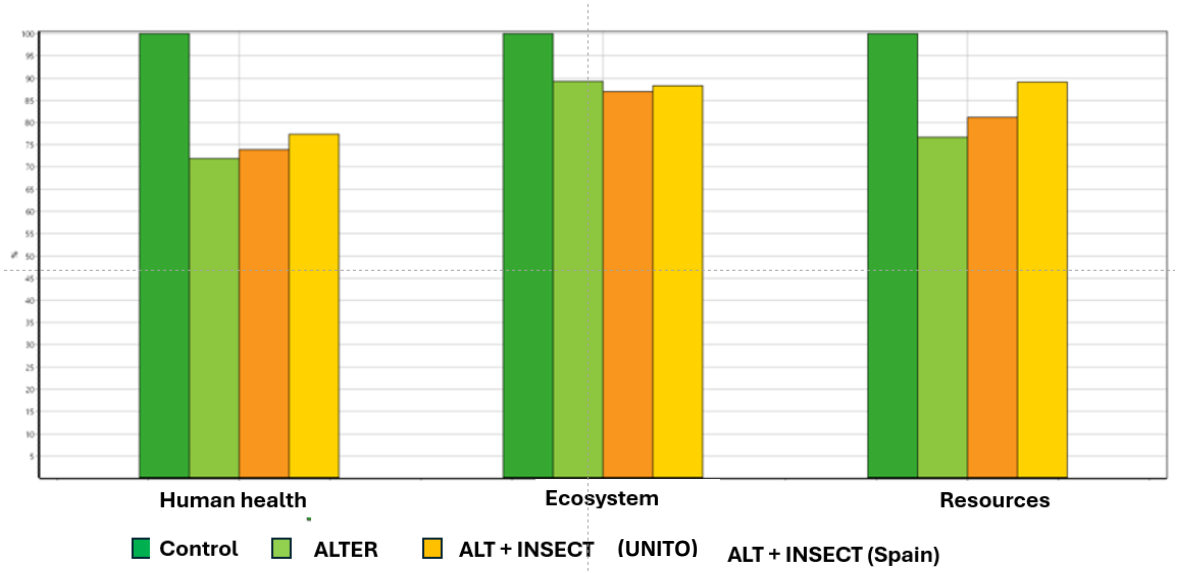
In conclusion, we observed that all the experimental diets managed to reduce the impact across all categories by more than 10%, which was the minimum reduction target set for more sustainable diets in the project's objectives.

Table 3. Impact of 1 kg of experimental diets of the Italian pilot on different categories studied, considering >99% of the diet composition in the two scenarios studied: if larva was produced in UNITO and if larva was produced in Spain

	Experimental diets[1]			
	CONTROL	ALTER	ALT+INSECT (UNITO)	ALT+INSECT (Spain)
Total impact, mpt	88.1	65.1	66.4	69.3
Human health, mpt	77.4	55.7	57.2	59.9
Ecosystem, mpt	9.44	8.43	8.20	8.32
Resources, mpt	1.19	0.91	0.97	1.06

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet; ALT+INSECT (UNITO): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Turin. ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain).

Figure 2. Impact of 1 kg of experimental diets of the Italian pilot weighted against the diet with the highest impact on different categories studied, considering >99% of the diet composition.



CONTROL: conventional diet; ALTER: Alternative experimental diet; ALT+INSECT (UNITO): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in UNITO (Italy). ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain).

3.1.2. Impact of Italian diets on the production of 1 kg of live weight at slaughter

Since the Italian pilot was working with a slow-growing breed of chickens, the environmental impact of fattening these animals at two points in their growth was studied. So, a part of the chickens was slaughtered at 147 days of age, and another group of chickens at 174 days, in order to study the impact as a function of slaughter age. For the estimation of the impact of 1 kg live weight (LW), the amount of feed consumed per animal and the slaughter weight were each time studied. These data were analyzed with a bifactorial model, with diet and age of slaughter, and their interaction as fixed factors. The data obtained are shown in **Table 4.** A general linear model analysis was then performed considering the effect of diet on each of the times studied. The results are shown in **Tables 5** and **6**.

Table 4. Impact of 1 kg live weight production of chickens fed the experimental diets on Global warming, Human health, Ecosystems and Resources of the Italian pilot experimental diets at 147 and 174 days of age in the two scenarios studied: if larvae are produced in UNITO and if larva are produced in Spain

	Experimental Diets[1]				Age		SEM[2]	P-value		
	CONTROL	ALTER	ALT+INSECT (UNITO)	ALT+INSECT (Spain)	147d	174d		Diet (D)	Age (A)	DxA
Global warming, kg CO_2 eq/kg LW	8.24^{a}	5.29^{b}	4.93^{c}	5.23^{b}	5.59	6.25	0.035	<0.001	<0.001	0.237
Human health/kg LW, mpt	311.0^{a}	211.9^{b}	193.0^{c}	202.1^{bc}	216.7	242.2	1.334	<0.001	<0.001	0.302
Ecosystem/kg LW, mpt	37.93^{a}	32.07^{b}	27.67^{c}	28.07^{c}	29.69	33.19	0.180	<0.001	<0.001	0.597
Resources/kg LW, mpt	4.78^{a}	3.47^{b}	3.26^{c}	3.58^{b}	3.56	3.98	0.022	<0.001	<0.001	0.535

LW. Live weight; [1]CONTROL: conventional diet; ALTER: Alternative experimental diet; ALT+INSECT (UNITO): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Turin. ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain). [2]SEM: standard error of mean; DxA: interaction diet x age. a,b,c: different letters in the same row show significant differences at P<0.05

Table 5. Impact of 1 kg live weight production of chickens fed the experimental diets on Global warming, Human health, Ecosystems and Resources of the Italian pilot experimental diets at 147 days of age in the two scenarios studied: if larvae are produced in UNITO and if larvae are produced in Spain

	Experimental diets[1]				SEM[2]	P-value
	CONTROL	ALT	ALT+INSECT (UNITO)	ALT+INSECT (Spain)		
Climate change, kg CO_2/kg LW	7.79^{a}	4.97^{b}	4.66^{b}	4.95^{b}	0.052	<0.001
Human health, mpt/kg LW	293.95^{a}	199.33^{b}	182.55^{c}	191.16^{bc}	1.986	<0.001
Ecosystem, mpt/kg LW	35.82^{a}	30.17^{b}	26.17^{c}	26.55^{c}	0.264	<0.001
Resources, mpt/kg LW	4.52^{a}	3.26^{bc}	3.08^{c}	3.38^{b}	0.032	<0.001

LW. Live weight; [1]CONTROL: conventional diet; ALTER: Alternative experimental diet; ALT+INSECT (UNITO): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Turin. ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain). [2]SEM: standard error of mean; a,b,c: different letters in the same row show significant differences at P<0.05

Table 6. Impact of 1 kg live weight production of chickens fed the experimental diets on Global warming, Human health, Ecosystems and Resources of the Italian pilot experimental diets at 174 days of age in the two scenarios studied: if larvae are produced in UNITO and if larvae are produced in Spain

	Experimental diets[1]				SEM[2]	P-value
	CONTROL	ALTER	ALT+INSECT (UNITO)	ALT+INSECT (Spain)		
Climate change, kg CO_2/kg LW	8.69[a]	5.60[b]	5.19[c]	5.51[bc]	0.046	<0.001
Human health, mpt/kg LW	328.00[a]	224.5[b]	203.4[c]	213.0[bc]	1.782	<0.001
Ecosystem, mpt/kg LW	40.00[a]	33.98[b]	29.16[c]	29.59[c]	0.246	<0.001
Resources, mpt/kg LW	5.04[a]	3.67[b]	3.43[c]	3.77[b]	0.029	<0.001

LW. Live weight; [1]CONTROL: conventional diet; ALTER: Alternative experimental diet; ALT+INSECT (UNITO): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Turin. ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain). [2]SEM: standard error of mean; a,b,c: different letters in the same row show significant differences at P<0.05

As shown in **Table 4**, both diet and slaughter age had a very significant impact (P<0.001) on the production of 1 kg of live weight, with the Control diet having the greatest impact across all categories studied. No significant interaction effect (diet x age) was observed. For CO_2 eq emissions, the production of 1 kg LW with Control diet potentially emitted 8.24 kg CO_2 eq. The ALT+INSECT (UNITO) diet, which included 5% BSF larvae (in the scenario where the larvae were raised in Turin), had the lowest impact, at 4.93 kg CO_2 eq/kg LW, representing a 40% reduction compared to the Control diet. The diet where soybean meal was completely replaced by alternative local plant-based ingredients reduced CO_2 eq emissions per kg LW by 36%, similar to the scenario in which the diet incorporated insect protein raised in Spain, with a 37% reduction. The ALTER and ALT+INSECT (Spain) diets did not show statistically significant differences (P>0.05) between them.

In the study of the impact on Human health/kg LW, the behavior of the diets was similar to that observed for CO_2 emissions, with the ALT+INSECT diet (insect raised in UNITO) showing the lowest impact, reducing it by 38% compared to the Control diet. The diet with insects in the scenario where insect was reared in Spain had a 35% lower impact than the Control, while the ALTER diet reduced the impact on this category by 32% compared to the Control.

For the Ecosystem category, experimental diets behaved similarly to the Human Health category. The diet incorporating 5% BSF (in the scenario where the larvae were raised in Turin, where the pilot study was conducted) achieved the greatest reduction, lowering the impact by 27% compared to the Control diet. This was followed by the ALT+INSECT diet with insects raised in Spain, which reduced the impact by 26%, and finally the ALTER diet, which reduced the impact by 15% compared to the Control.

Furthermore, the reductions in the Resources category compared to the Control diet were around 32%, 27%, and 25% for the ALT+INSECT (UNITO) diet, ALTER diet, and ALT+INSECT (Spain) diet, respectively.

Note that in this pilot, the insects were supplemented to the ALTER diet, and the ALT+INSEC diet had a greater environmental impact than the ALTER diet as seen above; however, considering the productive parameters obtained in the chickens fed with these diets, the impact per kg of live weight was reduced in the ALT+INSECT diet with respect to the ALTER diet.

Therefore, we observed that all the experimental diets managed to reduce the impact in all categories studied by more than 10%, both in terms of emissions per 1 kg of diet and emissions to produce 1 kg of live weight. The 10% reduction was the minimum goal set in the project's objectives.

When considering the slaughter age, we observed a lower environmental impact for all diets when chickens were slaughtered at 147 days instead of 174 days. This result was expected, as slaughtering at an older age requires more feed consumption and the feed conversion ratio worsen with age. The impact reductions when slaughter occurred at 147 days were approximately 10% lower across all categories analyzed.

The results obtained on the impact on the production of 1 kg of LW when the animals were slaughtered at 147 days (**Table 5**), show a very significant effect of the experimental diets ($P<0.001$), with the greatest impact of the Control diet with respect to all the alternative diets studied in all the categories analyzed. ($P<0.001$). Regarding Climate change, alternative diets did not show significant differences among them ($P>0.05$). However, in the impact on Human health and Ecosystem, the diets that incorporated insects stood out for their lower impact, and on Resources, the ALT+INSECT (UNITO) diet was the one that caused the lowest impact.

Under the assumption of slaughtering animals at 174 days (**Table 6**), the impact on Climate change, Human health and Ecosystem was significantly lower when diets incorporating insects were used in the two scenarios studied, although it was also observed that the ALTER diet had a significantly lower impact than the Control diet. In the Resource use category, the ALT+INSECT (UNITO) diet stood out as having the lowest potential impact.

3.1.3. Impact of Italian diets on the production of 1 kg of ready-to-cook carcass

After slaughter, the animals' heads, necks, and legs were removed, and the resulting carcass was weighed, considering it as a ready to cook carcass (RTCC). This allowed us to estimate the impact of each diet on the production of 1 kg of RTCC. The data analyzed with a bifactorial model and one way model similar to the previous one are presented in **Table 7-9**, providing a clear comparison of how each dietary approach influences environmental categories per kilogram of carcass.

Regarding the impact on CO_2 emissions, we observed a significant effect of both diets and the slaughter age ($P<0.001$). However, no significant effect of the interaction between these two factors was detected. Among the experimental diets analyzed, the Control diet showed the highest environmental impact, with 12.56 kg CO_2 eq required to produce 1 kilogram of RTCC. In contrast, the ALT+INSECT diet, which incorporated BSF reared in Turin, significantly reduced this impact by nearly 40%. Similarly, the alternative plant-based diet (ALTER) achieved a 35% reduction in CO_2 emissions, which was comparable to the 36% reduction observed with the ALT+INSECT diet where the larvae were reared in Spain. The insect reared in Spain scenario also considered the additional environmental cost of transporting the larvae from Cehegín (Spain) to Turin (Italy), yet it still managed to significantly lower emissions. These results highlight the potential of alternative and insect-based feeds to substantially reduce the carbon footprint of poultry production. By replacing traditional ingredients like soybean meal with more sustainable options—whether locally sourced plant-based proteins or insect larvae—the environmental impact, particularly in terms of greenhouse gas emissions, can be significantly reduced. This is especially relevant when considering both the environmental cost of feed production and the overall impact on the final product, such as the RTCC. The findings reinforce the importance of incorporating innovative feed strategies to improve the sustainability of meat production, while also factoring in logistical considerations, such as the geographic origin of ingredients.

Table 7. Potential environmental impact of 1 kg of ready to cook carcass (RTCC) on Global warming, Human health, Ecosystems, and Resources of the experimental diets of Italian pilot at 147 and 174 days of age in the two scenarios studied: if larvae are produced in UNITO and if larvae are produced in Spain

	Experimental Diets[1]				Age		SEM[2]	p-value		
	CONTROL	ALTER	ALT+INSECT (UNITO)	ALT+INSECT (Spain)	147d	174d		Diet	Age	DxA
Global warming, kg CO_2 eq/kg RTCC	12.56^{a}	8.14^{b}	7.57^{c}	8.04^{ab}	8.59	9.55	0.069	<0.001	<0.001	0.481
Human health/kg RTCC, mpt	473.3^{a}	326.2^{b}	296.5^{c}	310.5^{bc}	333.2	370.1	2.69	<0.001	<0.001	0.512
Ecosystem/kg RTCC, mpt	57.72^{a}	49.37^{b}	42.51^{c}	43.13^{c}	45.64	50.73	0.365	<0.001	<0.001	0.588
Resources/kg RTCC, mpt	7.28^{a}	5.34^{b}	5.00^{c}	5.50^{b}	5.48	6.08	0.043	<0.001	<0.001	0.674

[1]CONTROL: conventional diet; ALTER: Alternative experimental with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (UNITO): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Turin. ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain). [2]SEM: standard error of mean; DxA: interaction diet x age. RTCC: ready to cook carcass. a,b,c: different letters in the same row show significant differences at P<0.05

Table 8. Potential environmental impact of 1 kg of ready to-cook-carcass (RTCC) on Global warming, Human health, Ecosystems, and Resources of the experimental diets of Italian pilot at 147 days of age in the two scenarios studied: if larvae are produced in UNITO and if larvae are produced in Spain

	Experimental diets[1]				SEM[2]	P-value
	CONTROL	ALT	ALT+INSECT (UNITO)	ALT+INSECT (Spain)		
Global warming, kg CO_2/kg RTCC	11.90^{a}	7.62^{b}	7.21^{b}	7.66^{b}	0.082	<0.001
Human health, mpt/kg RTCC	449.37^{a}	305.23^{b}	282.36^{c}	295.69^{bc}	3.191	<0.001
Ecosystem, mpt/kg RTCC	54.81^{a}	46.20^{b}	40.48^{c}	41.07^{c}	0.435	<0.001
Resources, mpt/kg RTCC	6.91^{a}	5.00^{bc}	4.76^{c}	5.23^{b}	0.051	<0.001

[1]CONTROL: conventional diet; ALTER: Alternative experimental with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (UNITO): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Turin. ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain). [2]SEM: standard error of mean; DxA: interaction diet x age. RTCC: ready to cook carcass. a,b,c: different letters in the same row show significant differences at P<0.05

Table 9. Potential environmental impact of 1 kg of ready to-cook-carcass (RTCC) on Global warming, Human health, Ecosystems, and Resources of the experimental diets of Italian pilot at 174 days of age in the two scenarios studied: if larvae are produced in UNITO and if larvae are produced in Spain

	Experimental diets[1]				SEM[2]	P-value
	CONTROL	ALT	ALT+INSECT (UNITO)	ALT+INSECT (Spain)		
Global warming, kg CO_2/kg RTCC	13.17[a]	8.66[b]	7.93[b]	8.42[b]	0.112	<0.001
Human health, mpt/kg RTCC	497.17[a]	347.20[b]	310.69[c]	325.35[bc]	4.319	<0.001
Ecosystem, mpt/kg RTCC	60.64[a]	52.55[b]	44.54[c]	45.19[c]	0.585	<0.001
Resources, mpt/kg RTCC	7.64[a]	5.69[b]	5.24[b]	5.76[b]	0.069	<0.001

[1]CONTROL: conventional diet; ALTER: Alternative experimental with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (UNITO): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Turin. ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain). [2]SEM: standard error of mean; DxA: interaction diet x age. RTCC: ready to cook carcass. a,b,c: different letters in the same row show significant differences at P<0.05

Regarding Human Health, the impact reductions observed were quite significant. The diet containing 5% BSF larvae reared in Turin achieved a reduction of 37% compared to the Control diet. This was the highest reduction observed in this category. The ALTER diet, which utilized alternative plant-based ingredients, resulted in a 31% reduction in this category of impact. The diet incorporating 5% BSF larvae reared in Spain achieved an intermediate reduction of approximately 34%, highlighting the benefits of both local and insect-based feeding strategies.

When examining the effects on Ecosystems, the diet with 5% BSF larvae reared in Turin again led to the most substantial reduction, achieving a 26% decrease in impact compared to the Control diet. In the scenario where the larvae were reared in Spain, the reduction was slightly lower at 25%. The ALTER diet provided a 15% reduction in Ecosystem impact relative to the Control diet, also reflecting a considerable improvement over conventional feeding practice.

In terms of Resources, the reductions varied among the diets. The diet containing 5% BSF larvae reared in Turin resulted in a notable 31% reduction in resource use. The ALTER diet achieved a 27% reduction, while the diet with 5% BSF larvae reared in Spain resulted in a 25% reduction. These results indicate that incorporating insect larvae and alternative ingredients can significantly decrease resource consumption, with varying degrees of effectiveness depending on the specific diet and its sourcing.

Overall, these findings demonstrate that integrating sustainable feeding practices, such as using locally sourced plant-based ingredients and insect larvae, can substantially reduce the environmental impacts related to Human Health, Ecosystems, and Resources. The variations observed across different diets and sourcing scenarios underscore the importance of considering both ingredient types and production locations to optimize sustainability outcomes in poultry production.

When the analysis was performed at each slaughter age separately, the impacts on the production of 1 kg RTCC showed a similar behavior to the production of 1 kg LW, with a very significant effect of the diet (P<0.001), highlighting the Control diets as those that caused the greatest impact on Global warming (P<0.001), showing no significant differences between the experimental diets (P>0.05), both at 147 (**Table 8**)

and 174 days of age (**Table 9**). On Human health and Ecosystem, the ALT+INSECT diets had the least impact, especially in the scenario of being reared in UNITO. In the Resources category, at 147 days, the ALT+INSECT(UNITO) diet stood out as having the least impact, together with the ALTER diet, while in the 174-day study, the three alternative diets had a similar impact (P>0.005).

In summary, **Table 10** displays the impact reductions achieved across all studied scenarios in Italian pilot. Overall, the complete elimination of soybean meal from the diet of slow-growing chickens, replaced by local plant-based alternatives, significantly reduced the impact per kilogram of diet. However, considering the effects on production parameters of diets that included 5% insect larvae, especially in scenarios where the insects were reared close to the pilot location, these diets achieved even greater reductions in impact compared to the ALTER diet. Effect related to improvements in production rates observed when insects are incorporated into diets at 5%.

Table 10. Summary of the reductions achieved by the experimental diets of the Italian pilot on the impact on the different categories analysed with respect to the Control diet

		Experimental Diets[1]		
		ALTER	ALT+INSECT (UNITO)	ALT+INSECT (Spain)
kg diet	Global warming, kg CO_2 eq/kg diet	-32	-29	-24
	Human health, mpt/kg diet	-28	-26	-23
	Ecosystem, mpt/kg diet	-11	-13	-12
	Resources, mpt/kg diet	-23	-19	-11
kg LW	Global warming, kg $CO_{2\ eq}$/kg LW	-36	-40	-37
	Human health, mpt /kg LW	-32	-38	-35
	Ecosystem/, mpt/kg LW	-15	-27	-26
	Resources, mpt /kg LW	-27	-32	-25
kg RTCC	Global warming, kg $CO_{2\ eq}$/kg RTCC	-35	-40	-36
	Human health, mpt /kg RTCC	-31	-37	-34
	Ecosystem, mpt /kg RTCC	-15	-26	-25
	Resources, mpt /kg RTCC	-27	-31	-25

[1]ALTER: Alternative experimental with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (UNITO): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Turin. ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain). LW: live weight; RTCC: ready to cook carcass

Additionally, in general per LW a reduction of 10.56% was observed (**Table 11**) when the chickens were slaughtered at 147 days of age instead of at 174 days. This decrease is significant, as it suggests that shortening the growth period by nearly a month can lead to a notable reduction in environmental impact, possibly due to lower feed consumption and the effects of age on conversion rates. By reducing the time, the chickens are kept before slaughter, not only are operational costs potentially minimized, but the overall carbon footprint associated with poultry farming is also lessened. This adjustment in the growth period could also contribute to a more efficient use of farm

resources, such as water, energy, and feed, which tend to increase the longer the birds are raised. By optimizing the growth timeline while still maintaining the health and quality of the chickens, this approach could offer a practical and impactful method to enhance the sustainability of poultry farming. This reduction aligns with the growing demand for more sustainable agricultural practices and demonstrates how small adjustments in production processes can result in meaningful improvements in environmental outcomes. On the other hand, by reducing growth period we will reduce chickens' weight consequently, if chickens are slaughtered at 147 days instead of 174 more production cycles of meat chickens the farmer can have, more birds/year he can slaughter with less average weight of carcass.

Table 11. Summary of the reductions achieved by slaughtering the chickens at 147 days of age instead of at 174 days in the different categories studied

	147 d vs 174 d	
	LW	**RTCC**
Global warming, kg CO_2 eq/kg LW	-10.6	-11.2
Human health/kg LW, mpt	-10.5	-11.1
Ecosystem/kg LW, mpt	-10.5	-11.2
Resources/kg LW, mpt	-10.6	-10.9

LW: Live weight; RTCC: ready-to-cook carcass

Therefore, the alternative experimental diets studied in the Italian pilot reduced CO_2 eq emissions substantially with respect to the Control diet. The impact of the alternative diet where soybean meal was totally replaced by other vegetable ingredients achieved the greatest reduction in kg CO_2 eq, as well as in the other categories studied. However, when the study was carried out in relation to the impact to produce 1 kilogram of live weight, or 1 kilogram of ready-to-cook carcass, for which the productive parameters achieved by the animals fed with the different diets were considered, the diets supplemented with insects, in the two scenarios studied, were those that achieved the greatest reductions in all the categories studied, with diet ALT+INSECT (UNITO) achieving a reduction of 40% in kg CO_2 eq, per kg LW and per kg RTCC, with respect to the Control diet.

3.2 Environmental impact of Spanish pilot study

The pilot study conducted in Spain at the University of Murcia (UMU) involved a laying hen breed known as ISAZUL, specifically chosen for its adaptation to the Mediterranean climate. 120 laying hens at 17 weeks of age were randomly distributed across 15 floor pens. Each pen was assigned to one of three experimental diets, with 5 pens designated per diet. After a 6-week adaptation period, during which the hens adjusted to their new environment and feeding regimen, the study continued for an additional 15 weeks, starting from the point when the hens began laying eggs. All the hens were maintained under consistent husbandry

management, including feed and water ad libitum, a 16:8 h light–dark photoperiod, and ambient temperature conditions (13.2–18.2 ◦C) and relative humidity (63.1–69.0% RH).

The feeding program for this study was designed as a single-phase diet, covering the period from the initiation of egg laying up until approximately the midpoint of the laying cycle, which was around 40 weeks of age. This approach allowed for a consistent evaluation of the impact of diets over a significant portion of the laying period. Throughout the study, three assessments were conducted at 28, 63, and 105 days after the start of trial. These assessments provided critical performance data, which were then used to estimate the potential emissions associated with each diet. This data collection was essential for understanding how different feeding strategies influenced environmental impacts, such as greenhouse gas emissions, Human health, Ecosystem and Resource use.

The experimental diets used in the UMU pilot were specifically formulated to include various sustainable ingredients, and their impacts were compared to determine the most effective strategies for reducing the environmental footprint of egg production. The experimental diets used in the UMU pilot were as follows:

- **Control diet** was a diet based on corn and soybean meal. These are common ingredients in Spain with a high environmental impact because they are imported from distant countries. It would serve as a baseline for comparing the environmental impact of the other two alternative diets.
- **Alternative diet 1** (ALTER): In this diet, corn and soybean meal were reduced, approximately by 13% globally, and alternative ingredients such as peas (5.56%) and Distillers Dried Grains with Solubles (DDGs) (7.46%) were included. In addition, the incorporation of sunflower meal increased up to 6%. Both Control and Alternative 1 diets were isoenergetic and isoaminoacidics.
- **Alternative diet 2** (ALT+INSECT): In this diet, the same Alternative diet 1 was used but supplemented with 5% of dehydrated whole larvae of BSF. This addition was adjusted weekly according to the total dry matter intake from the previous week. The BSF larvae was provided by Entomo company.

The whole dehydrated larvae were administered in a metal feeder every day at 10:00 a.m. In those replicates that did not consume larvae, a similar but empty feeder was provided to avoid any handling effect for alternative diet 2.

The composition of the experimental diets is shown in **Table 12**.

Table 12. Ingredients of the experimental diets used in the Spanish pilot (g/100g as fed basis)

Ingredients	Experimental diets[1]		
	CONTROL	ALTER	ALT+INSECT
Corn	41.58	35.29	33.53
Soybean meal	21.97	15.00	14.25
Wheat	14.00	14.68	13.95
Calcium carbonate	8.69	8.90	8.45

Soybean hulls	3.05	-	-
Soybean oil	2.50	2.50	2.38
Barley	2.00	2.50	2.38
Wheat middlings	1.67	0.09	0.09
Monocalcium Phosphate	0.67	0.52	0.49
Diatomaceous earth	0.50	0.50	0.47
Premix vitam-mineral[2]	0.33	0.33	0.31
Salt	0.25	0.20	0.19
Methionine DL	0.19	0.18	0.17
Sodium bicarbonate	0.07	0.12	0.11
L- Lysine 50	0.03	0.16	0,15
Sunflower meal	2.50	6.00	5.70
DDGs	-	7.46	7.09
Peas	-	5.56	5.28
Black Soldier Fly Larvae (dried)	-	-	5.00

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: ALTER diet plus 5% whole dehydrated Black Soldier Fly.[2] Provided per kilogram of feed: vitamin A, 7,500 IU; vitamin D3, 1500 mg; vitamin K3, 1.5 mg; vitamin B2, 3 mg; vitamin B12, 10 µg; nicotinamide, 15 mg; D-calcium pantothenate, 7 mg; Pantothenic acid, 6.44 mg; Betaine, 54.15 mg; choline chloride, 127.5 mg; Fe, 18 mg as Ferrous sulfate monohydrate; Cu, 4 mg as copper sulfate pentahydrate; Zn, 37 mg as zinc oxide; Mn, 65 mg as manganese (II) oxide; I, 1.9 mg as potassium iodate; selenium, 0.1 mg as sodium selenite; 600 FTU as 6-Phytase EC 3.1.3.26 (1 FTU is the amount of enzyme which liberates 1 micromole of inorganic phosphate per minute from sodium phytate at pH 5,5 and 37 °C); and 1500 EPU endo-1,4-β-xylanase EC 3.2.1.8 (1 EPU is the amount of enzyme which liberates 0,0083 micromoles of reducing sugars (xylose equivalents) from oat spelt Xylan per minute at pH 4,7 and 30 °C).

3.2.1 Impact of the experimental diets in Spanish Pilot

Table 13 shows the impact on Climate change expressed in kg CO_2 eq/kg of diet obtained with the ILCD Midpoint method considering more than 98% of the composition of the experimental Spanish diets. The impacts on the rest of the categories analyzed with this method are reflected in **Figure 1.2** of Annex.

Table 13. Impact of 1 kg de experimental diets from Spanish pilot on Climate change considering more than 98% of their composition

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	Experimental diets[1]		
	CONTROL	ALTER	ALT + INSECT
Climate change (Kg CO_2 eq/kg diet)	2.50	2.21	2.25

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: ALTER diet plus 5% whole dehydrated Black Soldier Fly

The potential impact obtained with 1 kg of experimental diet in the case of the pilot study in Spain was 2.5, 2.21, and 2.25 kg CO_2 eq for the Control, ALTER, and ALT+INSECT diets, respectively. This represented a reduction of 11.6% and 10% with the two alternative experimental diets respected to Control diet, which had reduced soybean meal and imported maize, and other alternative plant-based of closer-sourced ingredients incorporated. It is worth noting that the diet supplemented with dehydrated whole larvae of the BSF insect had a slightly higher impact than the ALTER diet. This is because the larvae used in the pilot study in Spain were produced under highly technical and intensive conditions, achieving a kg CO_2 eq production per kg of dry larvae slightly higher than the combination of ingredients resulting from the formulation of the ALTER diet. However, the inclusion of insects in the diet with alternative ingredients was also considerably advantageous compared to the control diet.

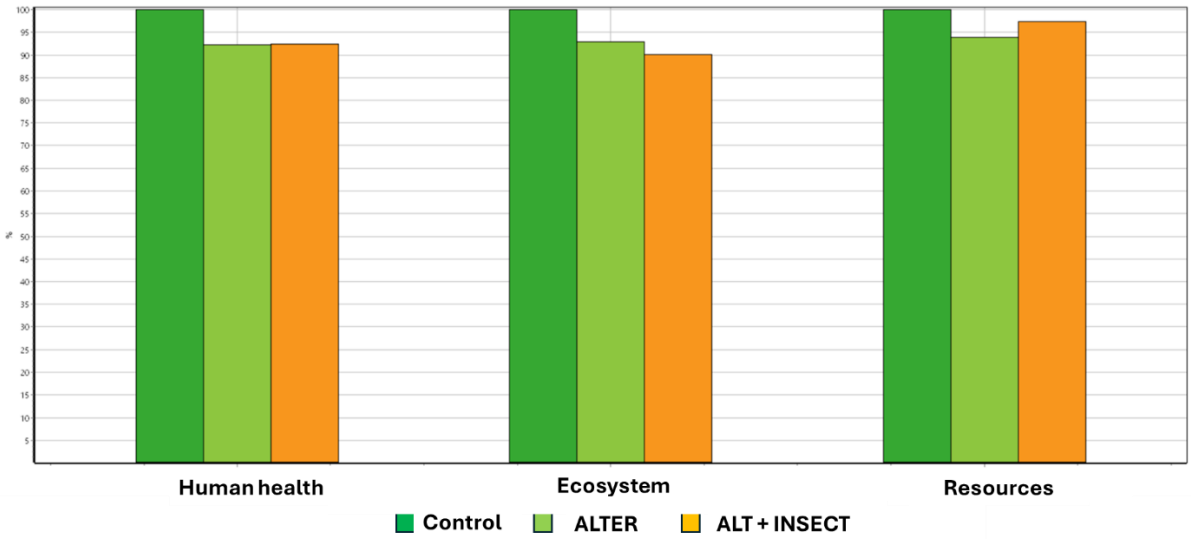
When the analysis was carried out with the Recipe endpoint method, the potential impacts of 1 kg of diet, expressed as milli points (mpt) of Total impact, and on Human health, Ecosystem and Resources are shown in **Table 14**. In **Figure 3**, the impact results obtained from experimental diets, weighed against the diet with the highest impact, are shown.

Table 5. Impact of 1 kg of experimental diets of Spanish pilot on Total impact, Human Health, Ecosystem and Resources considering >99% of the composition of the diets

	Experimental diets[1]		
	CONTROL	ALTER	ALT +INSECT
Total impact, mpt	106.0	97.7	97.6
Human health, mpt	94.3	87	87.2
Ecosystems, mpt	10.2	9.49	9.2
Resources, mpt	1.29	1.21	1.25

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: ALTER diet plus 5% whole dehydrated Black Soldier Fly

Figure 3. Impact of 1 kg of experimental diets of the Spanish pilot weighed against the diet with the highest impact on Human health, Ecosystem and Resources, considering >99% of the diet composition



CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: ALTER diet plus 5% whole dehydrated Black Soldier Fly

It can be observed that the Control diet had the highest impact in all categories, while the potential impacts of the ALTER and ALT+INSECT diets were quite similar, achieving reductions of 8% compared to Control when considering Total impact and Human health categories. Regarding the Ecosystem category, reductions were 7% and 9.8% for ALTER and ALT+INSECT, respectively. The Resources category showed the lowest impact reduction when using the experimental diets, as the impact was reduced by 6.2% and 3.1% with the ALTER and ALT+INSECT diets, respectively.

After evaluating the potential impacts of the experimental diets used in the Spain pilot study, we estimated the potential impacts per hen by considering several factors. These included diets, feed intake and duration of the experiment. Additionally, we assessed the impact per kilogram of egg mass, considering the performance parameters. Finally, we analyzed the impacts per hen per day.

This comprehensive approach allowed us to evaluate the effects of diets on various levels, from individual hens to overall production metrics. By examining these different perspectives, we aimed to gain a detailed understanding of how experimental diets influenced outcomes throughout the study. The analysis provided insight into the efficiency and effectiveness of diets in reducing impact across multiple dimensions, helping to identify the most beneficial dietary interventions for sustainable poultry production.

3.2.2 Impacts of the hens from Spanish pilot

Additionally, the potential impacts of the hens used in the UMU pilot have been calculated based on the feed intake, diet ingested and duration of trial. These data are shown for the subperiods analyzed (0-28 d; 28-63 d; 63-105 d) and for the total period (0-105 d).

Table 15 shows the impact of each hen when consuming the different experimental diets on Climate change expressed in kg CO_2 eq obtained with the ILCD Midpoint method considering more than 99% of the composition of the diets, and with the Recipe endpoint method on Total impact, Human health, Ecosystem, and Resources (expressed in mpt).

Table 65. Impact of the hens with Spanish diets on Climate Change, Human health, Ecosystem and Resources, considering more than 98% of the composition of the diets, in each period analysed and throughout the entire study

	Experimental diets[1]			SEM[2]	P-value
	CONTROL	ALT	ALT+INSECT		
Climate change, kg CO_2 eq/hen/period					
0-28 d	8.24	7.58	7.76	0.142	0.191
28-63 d	11.16^{a}	9.95^{b}	9.80^{b}	0.167	0.012
63-105 d	13.98^{a}	12.45^{ab}	12.32^{b}	0.218	0.016
0-105 d	33.38^{a}	29.98^{b}	29.89^{b}	0.467	0.015
Human health, mpt /hen/period					
0-28 d	310.76	298.45	300.87	5.506	0.637
28-63 d	420.95^{a}	391.83^{ab}	379.93^{b}	6.509	0.063
63-105 d	527.35^{a}	490.14^{ab}	477.46^{b}	8.540	0.084
0-105 d	1259.06	1180.42	1158.25	18.253	0.100
Ecosystem, mpt /hen/period					
0-28 d	33.61	32.56	31.74	0.592	0.457
28-63 d	45.53^{a}	42.74^{ab}	40.08^{b}	0.703	0.026
63-105 d	57.04^{a}	53.46^{ab}	50.37^{b}	0.926	0.038
0-105 d	136.19^{a}	128.76^{ab}	122.20^{b}	1.973	0.042
Resources, mpt /hen/period					
0-28 d	4.25	4.15	4.31	0.077	0.696
28-63 d	5.76	5.45	5.45	0.091	0.310
63-105 d	7.214	6.82	6.84	0.119	0.349
0-105 d	17.22	16.42	16.60	0.256	0.429

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT: ALTER diet plus 5% whole dehydrated Black Soldier Fly.[2]SEM: standard error of the mean. a,b: different letters in the same row show significant differences at P<0.05

The results demonstrate a significant effect (P<0.05) of the experimental diets on greenhouse gas (CO_2) emissions, beginning from the first month of consumption. Initially, in the first control period (28 days of

consuming the experimental diets), no significant effect on CO_2 emissions was observed (P>0.05). However, in the subsequent control periods, differences became significant (P<0.05). During the second control period (28-63 days), the impact reductions for CO_2 emissions were 10.8% and 12.2% for the ALTER and ALT+INSECT diets, respectively. This trend continued into the third control period (63-105 days), where reductions were 10.9% and 11.9%, respectively. Over the entire experimental period, the ALTER diet achieved a 10.2% reduction in CO_2 eq emissions, while the Alt + INSECT diet achieved a 10.5% reduction compared to the baseline CO_2 eq emissions.

Regarding the impact on Human health, no significant reductions were observed in any of the study periods. Nonetheless, a statistical trend was noted, indicating a potential lower impact in the second (p=0.063) and third (p=0.084) periods for the ALT+INSECT diet compared to the Control. Despite this trend, there were no significant differences observed throughout the study overall (P>0.05).

The diets also had a significant impact (P<0.05) on Ecosystems starting from 28 days into the study. Among the diets tested, the ALT+INSECT diet was most effective in reducing Ecosystem impact. This diet reduced the impact by 12.0%, 11.7%, and 10.3% for the second, third, and overall periods, respectively. In contrast, the ALTER diet did not achieve a significant reduction in Ecosystem impact compared to the Control diet, though it did show some numerical reduction.

Regarding the impact on the Resources category, no significant effects of the diet were observed (P>0.05) across the various analyzed periods, nor in the overall period for the Spain pilot study. This indicates that dietary changes did not significantly influence the consumption or utilization of resources in the context of this study.

Overall, these findings highlight the potential of experimental diets, particularly ALT+INSECT, in mitigating CO_2 emissions and impacting ecosystems, while the effects on Human health or Resources do not seem to be affected by alternative diets.

3.2.3 Impact of Spanish diets on the production of 1 kg of egg mass

Additionally, we analyzed the impact associated with the production of 1 kg of egg mass for these same categories, considering the laying index and egg weight. The results are detailed in **Table 16**, providing a comparative overview of resource use and impact in relation to egg mass production under the experimental conditions.

Table 7. Impact of the production of 1 kg of egg mass from Spanish pilot on Climate Change, Human health, Ecosystem and Resources, considering more than 99% of the diet composition, in each period analysed and throughout the entire study

	Experimental diets[1]			SEM[2]	P-value
	CONTROL	ALTER	ALT+INSECT		
Climate change, kg CO_2 eq/kg egg mass					
0-28 d	7.36^{a}	6.82^{ab}	6.36^{b}	0.154	0.063
28-63 d	6.67^{a}	5.91^{b}	5.56^{b}	0.089	0.001
63-105 d	6.79^{a}	6.03^{ab}	5.74^{b}	0.159	0.049
0-105 d	6.86^{a}	6.15^{b}	5.82^{b}	0.084	0.001
Human health, mpt /kg egg mass					
0-28 d	277.43	268.38	246.32	6.003	0.136
28-63 d	251.55^{a}	232.67^{ab}	215.61^{b}	3.419	0.040
63-105 d	256.21	237.45	222.35	6.064	0.115
0-105 d	258.59^{a}	241.97^{b}	225.57^{b}	3.207	0.004
Ecosystem, mpt /kg egg mass					
0-28 d	30.01^{a}	29.28^{ab}	25.99^{b}	0.652	0.060
28-63 d	27.21^{a}	25.38^{b}	22.75^{b}	0.370	0.010
63-105 d	27.71^{a}	25.90^{ab}	23.46^{b}	0.655	0.062
0-105 d	27.97^{a}	26.39^{a}	23.80^{b}	0.347	0.001
Resources, mpt /kg egg mass					
0-28 d	3.80	3.73	3.53	0.084	0.428
28-63 d	3.44^{a}	3.24^{ab}	3.09^{b}	0.047	0.033
63-105 d	3.51	3.30	3.19	0.084	0.327
0-105 d	3.56^{a}	3.37^{ab}	3.23^{b}	0.044	0.048

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT: ALTER diet plus 5% whole dehydrated *BSF*.[2]SEM: standard error of the mean. a,b,c: different letters in the same row show significant differences at P<0.05

Tables 17-20 displays the overall reductions achieved with the different experimental diets analyzed in Spanish pilot study.

The studied diets had a significant effect (P<0.05) on CO_2 emissions per kilogram of egg mass during the second and third periods, as well as over the entire experimental period. In the first period (0-28 days), although the reduction in kg CO_2 equivalent emissions per kilogram of eggs with the alternative diets (ALTER and ALT+INSECT) was not statistically significant (p=0.063), there was a discernible trend towards lower emissions compared to the Control diet. In the second period (28-63 days), the ALTER diet achieved an 11.4% reduction in CO_2 emissions per kilogram of egg mass compared to the Control diet, while the ALT+INSECT diet resulted in a more substantial reduction of 16.6%. During the third period (63-105 days), the reductions were 11.2% for the ALTER diet and 15.5% for the ALT+INSECT diet. Although the reduction for the ALTER diet

in the third period was not statistically significant compared to the Control, it still represented a quantitative decrease. When analyzing the entire experimental period, the ALTER diet resulted in an overall reduction of 10.4% in CO_2 eq emissions per kilogram of egg mass, whereas the ALT+INSECT diet achieved a 15% reduction. Both alternative diets showed significantly lower emissions compared to the Control diet ($P<0.05$), with no significant difference between the two alternative diets themselves ($P>0.05$). This indicates that both alternative diets were effective in reducing CO_2 emissions, with the ALT+INSECT diet providing a slightly greater reduction. Thus, it is noteworthy that the inclusion of insects could have a favorable effect on the productive indices, which seems to be reflected in a quantitatively lower impact of this diet in terms of kg CO2 eq/kg egg mass.

Table 8. Summary of the reductions achieved by the experimental diets of the Spanish pilot on the impact on Climate change with respect to the Control diet

Climate change, kg CO2 eq	ALT	ALT + INSECT
Climate change, kg CO_2 eq/kg diet	-11.60	-10.0
Climate change, kg CO_2 eq/ hen/period		
0-28 d	-8.0	-5.8
28-63 d	-10.8	-12.2
63-105 d	-10.9	-11.9
0-105 d	-10.2	-10.5
Climate change, kg CO_2 eq/ kg egg mass		
0-28 d	-7.3	-13.5
28-63 d	-11.4	-16,6
63-105 d	-11.2	-15.5
0-105 d	-10.4	-15.0

ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: ALTER diet plus 5% whole dehydrated Black Soldier Fly.

Table 9. Summary of the reductions achieved by the experimental diets of the Spanish pilot on the impact on Human health with respect to the Control diet

Human health, mpt	ALT	ALT + INSECT
Human health, mpt/ kg diet	-7.7	-7.5
Human health, mpt/hen/period		
0-28 d	-4.0	-3.2
28-63 d	-6.9	-9.7
63-105 d	-7.1	-9.5
0-105 d	-6.2	-8.0
Human health, mpt/kg egg mass		

0-28 d	-3.2	-11.2
28-63 d	-7.5	-14.3
63-105 d	-7.3	-13.2
0-105 d	-6.4	-12.8

ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: ALTER diet plus 5% whole dehydrated Black Soldier Fly.

Table 10. Summary of the reductions achieved by the experimental diets of the Spanish pilot on the impact on Ecosystem with respect to the Control diet

Ecosystem, mpt	ALT	ALT + INSECT
Ecosystem, mpt/ kg diet	-7.0	-9.8
Ecosystem, mpt /hen/period		
0-28 d	-3.2	-5.6
28-63 d	-6.2	-11.7
63-105 d	-6.1	-12.0
0-105 d	-5.5	-10.3
Ecosystem, mpt /kg egg mass		
0-28 d	-2.4	-13.4
28-63 d	-6.7	-16.4
63-105 d	-6.5	-15.4
0-105 d	-5.6	-14.9

ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: ALTER diet plus 5% whole dehydrated Black Soldier Fly.

Table 11. Summary of the reductions achieved by the experimental diets of the Spanish pilot on the impact on Resources with respect to the Control diet

Resources, mpt	ALT	ALT + INSECT
Resources, mpt/ kg diet	-6.2	-3.1
Resources, mpt /hen/period		
0-28 d	-2.4	-1.5
28-63 d	-2.3	-2.4
63-105 d	-5.5	-5.5
0-105 d	-4.7	-3.6
Resources, mpt /kg egg mass		
0-28 d	-1.6	-7.0
28-63 d	-6.0	-10.2
63-105 d	-5.8	-9.1
0-105 d	-5.4	-9.1

ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: ALTER diet plus 5% whole dehydrated Black Soldier Fly.

In conclusion, regarding CO_2 emissions, the alternative experimental diets achieved a reduction of 10-12% compared to the Control diet. Also, experimental diets also achieved reductions in the impact on Human Health, Ecosystems and Resources. When studying the impact associated with the production of 1 kg egg mass considering the hens' production data, it was observed that the alternative diets showed a significantly lower impact than the Control during the whole study period in relation to kg CO_2 eq/kg egg mass, with 10.4% and 15% less for ALTER and ALT+INSECT, respectively. For Human Health, there was also a significant reduction with the ALTER and ALT+INSECT diets compared to the Control by 6% and 13%, respectively. However, for Ecosystems and Resources, the ALT+INSECT diet significantly reduced impacts by 15% and 9%, respectively, while the ALTER diet had less evident effects in the latter two categories.

3.3 Environmental impact of Turkish pilot study

The pilot study conducted by EGE University in Turkey focused on poultry meat production using two different types of chickens: a local ecotype: Anadolu-T and a commercially popular broiler strain (Cobb-500). The objective of this comparison was to study how the new feeding programs affected both breeds. The local ecotype was a breed that has evolved to thrive in the specific environment of Turkey. The use of local breeds would favor the preservation of biodiversity, and the possibility of rearing in conditions that the commercial line probably does not tolerate as well. In this pilot, these animals were slaughtered at 55 days of age. The commercial broiler line is widely used in industrial poultry production due to its fast growth rate, efficient feed conversion and high meat yield. It serves as a benchmark for productivity in the poultry industry. In the pilot study in Turkey, Cobb chickens were slaughtered at 40 days of age.

The feeding program in both breeds was the same and was divided into three phases based on the developmental stages of the birds, with the objective of adjusting to the nutritional needs of the chicks as they grew. The phases were: starter (from 0 to 14 days), grower (from 15-28 days) and finisher (from 29 days to slaughter age). In each phase, three diets (1 control and 2 alternative diets) were studied:

- **Control diet** was a corn, wheat, and soybean meal-based diet. They are usual ingredients in Turkey for chickens feeding with high environmental impact. This diet would serve as a baseline for comparing the environmental impact of the other two alternative diets.
- **Alternative diet 1 (ALTER)** where corn and soybean meal were reduced, and it included alternative ingredients such as Brewers´ dried grain, wheat middling, and sunflower meal.
- **Alternative diet 2 (ALT+INSECT)** was a diet where corn and soybean meal were reduced and alternative ingredients such us Brewers´ dried grain, wheat middling, and sunflower meal were included. In addition, in this diet, BSF larvae meal were incorporated at 5%. The insect meal used in this diet was purchased from a Turkish company (Germina Tarım Teknolojileri Tic. Ltd., ¸Sti., Ankara, Türkiye).

Soybean meal reductions in this pilot ranged from 13 to 58% depending on the phase and diet. Thus, in the starter phase, the reductions were 13 and 27% in the ALTER and ALT+ INSECT diets, respectively. In the grower phase, soybean meal reductions were 24 and 42% in the ALTER and ALT+ INSECT diets, respectively; and in the finisher phase, they were 37 and 58% in the ALTER and ALT+ insect diets, respectively. Alternative ingredients were incorporated in different proportions according to the phase studied and diet. Therefore, 9 diets were used for each chicken breed. The composition of each experimental diet is shown in **Table 21**.

Table 21. Ingredients of the experimental diets used in the Turkish pilot (g/100g as fed)

Ingredients	Control			ALTER			ALT+INSECT		
	Starter	Grower	finisher	Starter	Grower	finisher	Starter	Grower	finisher
Corn	45.28	51.24	57.34	39.18	44.44	47.44	41.30	46.54	49.64
Wheat	11.86	14.86	15.00	12.50	14.50	15.50	12.18	14.50	15.50
Soybean meal	34.33	27.90	23.20	29.80	21.10	14.60	25.1	16.30	9.70
Sunflower meal	5.88	4.00	3.00	3.58	6.3	8.00	3.63	6.30	8.00
Brewer's Dried Grain	-	-	-	2.58	3.08	4.00	2.63	3.08	4.00
Wheat Middlings	-	-	-	2.58	3.08	4.00	2.63	3.08	4.00
Sunflower oil	-	-	-	7.13	5.50	5.00	4.88	3.20	2.70
Limestone	0.50	0.30	0.20	0.50	0.30	0.20	0.50	0.30	0.20
Dicalcium Phosphate	1.00	0.80	0.60	1.00	0.80	0.60	1.00	0.80	0.60
Vitamin-mineral Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine HCL (78%)	0.30	0.30	0.15	0.50	0.30	0.15	0.50	0.30	0.15
Methionine DL (99%)	0.10	0.05	0.01	0.10	0.05	0.01	0.10	0.05	0.01
Threonine	0.05	0.05	-	0.05	0.05	-	0.05	0.05	-
Rovabio+Natuphos E BASF	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Black Soldier Fly Larvae (dried)	-	-	-	-	-	-	5.00	5.00	5.00

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal

3.3.1 Impact of the experimental diets in Turkish Pilot

Table 22 shows the potential impact on Climate change expressed in kg CO_2 eq/kg of diet obtained with the ILCD Midpoint method considering more than 98% of the composition of diets. In addition, when the analysis was carried out using the Recipe Endpoint method, the potential environmental impacts of 1 kilogram of diet was expressed in total milli points (mpt), and the impact was presented in terms of their effects on three key categories: Human Health, Ecosystems, and Resources, as illustrated in **Table 23** and **Figure 4**.

The ILCD Midpoint method evaluates the impact on 16 categories. However, to summarize this report, the impact on Climate change is shown as the category with the highest social impact and the complete impact estimation study on the 16 categories is shown in **Figure 1.3-1.5** in Annex 1.

Table 12. Impact of 1 kg of experimental Turkish pilot diets on Climate change (Kg CO_2 eq/kg diet) considering more than 98% of the diet compositions

Kg CO_2 eq/kg diet	Experimental diets[1]		
Phase	Control	ALTER	ALT+INSECT
Starter	3.34	1.90	1.93
Grower	3.13	1.88	1.90
Finisher	3.03	1.87	1.89

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal

Among the phases analyzed, the diet from the first stage, the starter phase, had the most significant impact on Climate Change compared to the subsequent grower and finisher phases. During the starter phase, the Climate change impact of the Control diet was estimated at 3.34 kg CO_2 eq/kg diet. In contrast, the ALTER diet reduced this impact to 1.90 kg CO_2 eq/kg of diet, representing a substantial reduction of 43.1%. Similarly, the diet that included 5% insect meal had an impact of 1.93 kg CO_2 eq/kg of diet. This data demonstrates that when chickens were fed a diet in which soybean meal content was reduced and alternative ingredients, including 5% of insect meal were incorporated, the Climate change impact was reduced by 42.2%.

When shifting focus to the grower phase, we observe that the ALTER diet resulted in a reduction of 39.9% in the Climate change impact compared to the Control diet, while the ALT+INSECT diet also achieved a notable reduction of 39.3%. In the final stage, known as the finisher phase, reductions in environmental impact continued, with the ALTER diet showing a decrease of 38.3%, and the ALT+INSECT diet following closely behind with a reduction of 37.6%, both compared to the conventional Control diet.

It should be noted that the experimental diets used in the Turkish pilot significantly reduced the inclusion of soybean meal, but the reduction varied depending on the production phase. In the starter phase, the soybean meal content was reduced by 13% and 27% in the ALTER and ALT+INSECT diets, respectively. During the grower phase, the reduction was more substantial, with soybean meal being cut by nearly 24% and 42% in the ALTER and ALT+INSECT diets, respectively. In the finisher phase, the reductions were even more pronounced, with soybean meal decreasing by 37% and 58% for the ALTER and ALT+INSECT diets, respectively. This progressive reduction of soybean meal, especially in the grower and finisher phases, played a key role in the significant reductions in emissions. Soybean meal is known for its high environmental impact, particularly due to the land use and deforestation associated with its production, as well as the associated greenhouse gas emissions. By replacing such a large proportion of soybean meal with alternative protein sources balancing nutrients, the diets not only maintained adequate nutritional profiles but also achieved substantial reductions in the environmental footprint.

In conclusion, the experimental alternative diets, both ALTER or ALT+INSECT, were effective across all phases of the feeding program. These diets consistently produced significant reductions in the environmental footprint, particularly in terms of greenhouse gas emissions. In all cases, reductions were consistently above 37%, underscoring the potential of these alternative feed formulations to significantly lower the climate change impact of poultry production. This highlights the importance of integrating more sustainable feed options, such as alternative ingredients and insect-based protein sources, into animal diets to achieve meaningful environmental benefits.

The results obtained with the ReCipe Endpoint methodology, on the total impact of the experimental diets (expressed in mpt/kg diet) are shown in **Table 19**.

Table 23. Results obtained with the ReCipe Endpoint methodology, on the Total impact of the experimental diets (expressed in mpt/kg diet) by Turkish experimental diets

Total impact, mpt	Experimental diets[1]		
Phase	Control	ALTER	ALT+INSECT
Starter	124.0	80.3	80.7
Grower	118.0	79.5	79.7
Finisher	116.0	78.9	79.1

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal

Respect to Total impact, of the three phases of the feeding program used, the first phase (starter) had the greatest impact with all experimental diets (124.0, 80.3, and 80.7 mpt/kg diet for Control, ALTER, and ALT+INSECT, respectively). The grower diets had a total impact of 118.0, 79.5, and 79.7 mpt for Control, ALTER, and ALT+INSECT, respectively, while the finisher diets had slightly less impact than the previous phase: 116, 78.9, and 79.1 mpt/kg diet for Control, ALTER, and ALT+INSECT, respectively. These results highlight the significant reduction in the total impact of the alternative diets designed by the Turkey pilot, which reduced the total impact by approximately 31-35%, far exceeding the 10% reduction requirement set in the project.

When observing the impacts of the three categories studied with the ReCiPe 2016 Endpoint method, the most affected category was Human health. Furthermore, of the three phases of the feeding program, the feed in the first phase (starter) had a greater impact than the grower phase, which in turn was slightly higher than the finisher phase, whose impact was the lowest of all.

In the starter phase, the impact of 1 kg of diet on Human health, expressed in millipoints (mpt), was 110 mpt for the Control diet (**Table 24**), while the ALTER, and ALT+INSECT diets reduced the impact by about 35%. In the grower and finisher phases, the impact reductions in this category were around 32-34%. It is worth noting that the impact of the diet with reduced soy and incorporation of alternative ingredients was slightly lower than the one incorporating insects in this category across all phases of the feeding program.

Table 13. Impact of 1 kg of experimental diets of Turkish pilot on Human Health (mpt) considering more than 98% of the composition of the diets

Human Health, mpt	Experimental diets [1]		
Phase	Control	ALTER	ALT+INSECT
Starter	110.0	70.6	71.3
Grower	106.0	70.1	70.6
Finisher	104.0	69.8	70.3

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + ALT+INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal

Regarding the impact on the Ecosystem category (**Table 25**), the starter phase diets had a greater impact than the grower and finisher diets. In addition, as expected, the Control diet had a greater impact than the experimental alternatives. In the starter phase the alternative diets managed to reduce the impact by 23-24% for the ALTER diet and 26-27% for the ALT+INSECT diet.

Table 14. Impact of 1 kg of experimental diets of Turkish pilot on Ecosystem (mpt) considering more than 98% of the composition of the diets

Ecosystem, mpt	Experimental diets[1]		
Phase	Control	ALTER	ALT + INSECT
Starter	10.9	8.29	7.96
Grower	10.4	7.99	7.66
Finisher	10.0	7.69	7.35

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + ALT+INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal

With respect to the Resources category, the impacts of the Control diet were reduced by 37% with both experimental diets, as can be seen in **Table 26**.

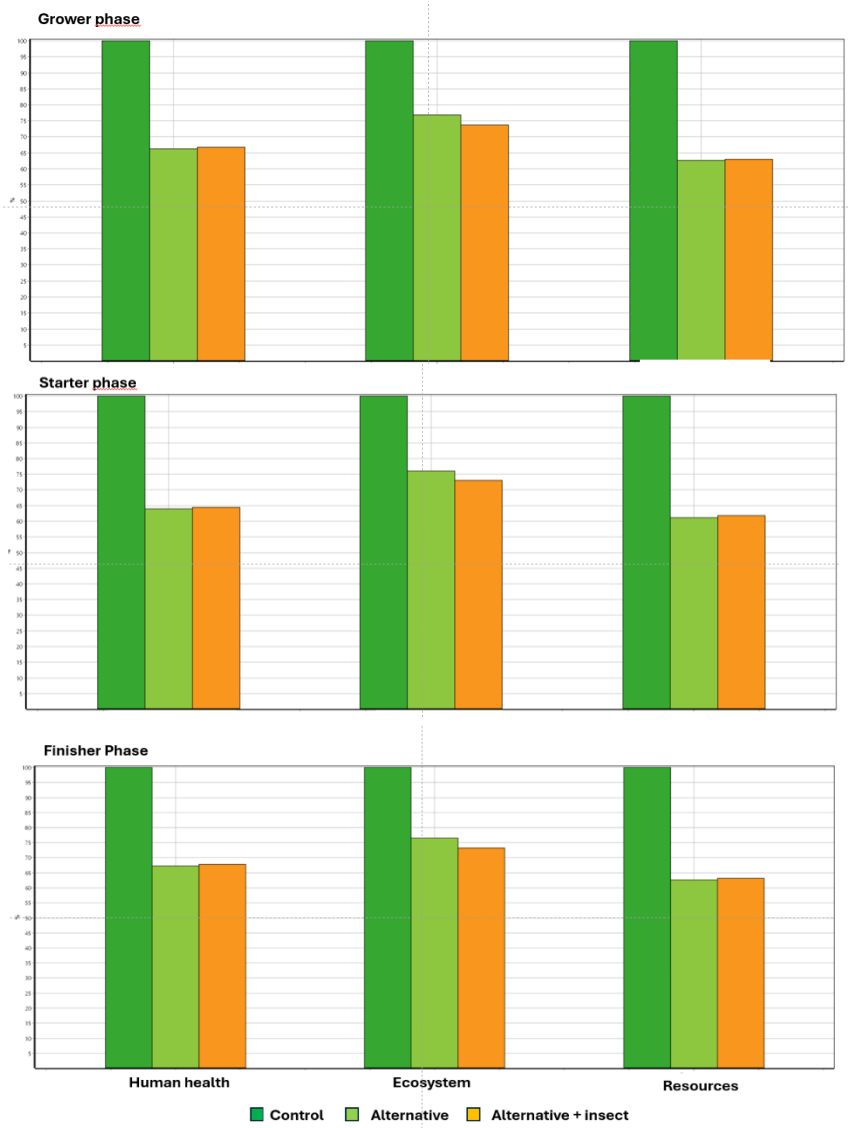
Table 26. Impact of 1 kg de experimental diets of Turkish pilot on Resources (mpt) considering more than 98% of the composition of the diets

Resources, mpt	Experimental diets[1]		
Phase	Control	ALTER	ALT + INSECT
Starter	2.34	1.43	1.44
Grower	2.24	1.40	1.41
Finisher	2.22	1.39	1.40

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + ALT+INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal

In **Figure 4** plots the impacts of the diets for each phase of the Turkey pilot weighted by the impact of the diet with the highest impact.

Figure 4. Impact of 1 kg of experimental diets of the Turkish pilot weighed against the diet with the highest impact on Human health, Ecosystem and Resources, in each feeding phase, considering >98% of the diet composition



CONTROL: conventional diet; Alternative: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; Alternative + insect: diet with alternative ingredients and 5% Black Soldier Fly larvae meal

After analyzing the impacts of the experimental diets, we will now discuss the results related to the production of 1 kg of meat (live weight) obtained from the different diets across the two breeds studied. In this evaluation, both the environmental impact of diets and the production performance parameters of the animals used in the pilot studies have been carefully considered. This comprehensive approach allows for a more accurate assessment of the overall effectiveness and sustainability of each diet in the context of meat production.

3.3.2 Impact of Turkish diets on the production of 1 kg of live weight at slaughter

The feed intake per kilogram of live weight (LW) and the final slaughter weight were analyzed to estimate the environmental impact of producing 1 kg of live weight. For the statistical analysis of the data, we first conducted a two-factor analysis, with diet and breed as fixed effects, along with their interaction. Following this, we performed a one-way linear model within each breed, using diet as a fixed factor. Differences between means were examined using the Tukey test. A p-value of less than 0.05 was considered statistically significant.

Table 27 presents the results of the bifactorial analysis (experimental diet and breed, together with their interaction as fixed effects) to produce 1 kg live weight of meat chicken obtained in the pilot study in Turkey.

Table 15. Impact of the production of 1 kg live weight of chickens fed the experimental diets Turkish pilot on Climate change, on Global warming, Human health, Ecosystems and Resources in local and commercial breed (Cobb)

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	Experimental Diets[1]			Breed		SEM[2]	P value		
	CONTROL	ALTER	ALT + INSECT	Local	Cobb		Diet (D)	Breed (B)	DxB
Global warming, kg CO_2 eq/kg LW	6.23[a]	3.79[b]	3.85[b]	5.25	4.00	0.043	<0.001	<0.001	<0.001
Human health/kg LW, mpt	212.4[a]	141.4[b]	143.2[b]	188.3	143.0	1.538	<0.001	<0.001	0.005
Ecosystem/kg LW, mpt	20.57[a]	15.77[b]	15.21[b]	19.48	14.88	0.156	<0.001	<0.001	0.029
Resources/kg LW, mpt	4.52[a]	2.81[b]	2.86[b]	3.87	2.94	0.032	<0.001	<0.001	0.001

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + ALT+INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal. Cobb: commercial breed. [2]SEM: standard error of the mean; DxB: interaction Diet x breed. a.b: different letters in the same row indicate statistical differences P<0.05.

For all impact categories. A highly significant effect of diet (P<0.001) and breed (P<0.001) was observed, as well as a significant interaction between diet and breed (P<0.05).

Regarding diet, chickens fed the Control diet showed a significantly higher potential impact per kilogram of live weight (P<0.001) compared to those on the experimental alternative diets, in which soybean meal was reduced and other alternative ingredients, alone or with BSF larvae were incorporated. This difference was consistent across all studied categories: Global Warming, Human Health, Ecosystems, and Resources. No significant differences (P>0.05) were observed between the two experimental diets, ALTER and ALT+INSECT, for any of the categories studied. Therefore, all the alternative ingredients incorporated in the case of the Turkey pilot feeding program were valid to reduce the environmental impact of feeding these birds.

In relation to breed, local breed chickens had a greater impact (P<0.001) per kilogram of live weight, about 31% more than the commercial breed (Cobb) in the four categories studied. This can partly be explained by the slaughter age, as local breed chickens were slaughtered at 55 days of age, unlike commercial breed chickens (Cobb500). Furthermore, the dietary efficiency in these animals is worse than commercial breed which due to the intense genetic selection they have undergone, have significantly reduced their fattening

days and improved feed conversion ratio. Thus, Cobb chickens were slaughtered at 40 days of age, achieving a similar weight. These results are consistent with those obtained by Italian pilot and the published in the literature (Fiorilla et al., 2024).

In all the categories studied, a diet per breed interaction was observed, as the experimental diets managed to reduce the impact in both breeds, but the magnitude of the impact was different, thus, the impact produced in the finishing phase is more pronounced in the local breed than in the commercial breed.

The following are the results obtained from the analysis of each meat chicken breed used in the Turkish pilot separately.

3.3.3 Impact to produce 1 kg live weight using local breed in Turkish pilot

The local breed chickens used in the Turkey pilot were fed on a three-phase program with the following distribution: starter phase (1–10 days), grower phase (11–25 days), and finisher phase (26–55 days), at which point the chickens were slaughtered, with an average weight of 2.26 kg. The feed conversion ratio (FCR) for the local breed chicken was 2.30 kg/kg. The mortality rate throughout the study was 1.26%. These values were higher than those observed in commercial breed chickens.

In terms of environmental impacts, a significant effect of diet (P<0.001) was observed across the four categories studied. Chickens fed the Control diet had an average impact throughout their entire growth period (55 days) of 7.118 kg CO2 eq/kg live weight (LW) (**Table 28**). In contrast, chickens fed the ALTER and ALT+INSECT diets reduced the impact per kilogram of LW by approximately 39%.

Regarding the impact on Human health, the Control diet had an impact of 242.94 mpt/kg LW, while the ALTER and ALT+INSECT diets reduced this impact by 34% and 33%, respectively. On Ecosystems, the reductions were around 25–27%, and on Resources, the reduction was approximately 38% for both experimental alternative diets. No differences were observed between the alternative diets (P>0.05). These findings suggest that the use of alternative diets, where partial substitution of soybean meal with locally sourced alternative ingredients can drastically reduce the impact on the environment.

Table 16. Average impact of the production of 1 kg live weight of local breed chickens fed the experimental diets of Turkish pilot on Global warming, Human health, Ecosystems and Resources

	Experimental diets[1]			SEM[2]	P value
	Control	ALTER	ALT + INSECT		
Global warming, kg CO_2 eq/kg LW	7.118[a]	4.282[b]	4.353[b]	0.059	<0.001
Human health/kg LW, mpt	242.94[a]	160.07[b]	161.82[b]	183.92	<0.001
Ecosystem/kg LW, mpt	23.50[a]	17.78[b]	17.14[b]	19.056	<0.001
Resources/kg LW, mpt	5.178[a]	3.19[b]	3.23[b]	3.773	<0.001

[1]Control: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal. [2]SEM: standard error of the mean. a.b: different letters in the same row indicate statistical differences P<0.05.

In addition, when we performed the analysis model on the Local breed, considering the diet in each growth phase, the impacts obtained per chicken produced are shown in the **Table 29.** As expected, the finisher phase has the greatest potential impact on all the categories studied, since feed consumption at this age is much higher than in the previous phases and the duration of the phases is also different, being finisher phase the longest. Diet continues to have a very significant effect (P<0.001) on Climate change, Human health, Ecosystem and Resources, with the Control diet having the greatest impact, well above the ALTER and ALT+INSECT diets in each phase and globally, the latter referring to the accumulated impact during the entire test. It should be noted that the ALTER and ALT+INSECT diets had very similar impacts in this pilot.

It's important to mention that in this pilot, the experimental diet incorporating insects had a slightly lower quantitative impact than the one incorporating vegetables ingredients on global period.

Table 17. Impact of the production of one local breed chicken during each growth phase according to Turkish experimental diets

	Experimental Diets[1]			SEM[2]	P value
	CONTROL	ALTER	ALT + INSECT		
Climate Change, kg CO_2 eq/chicken					
Starter	1.21[a]	0.74[b]	0.86[b]	0.022	<0.001
Grower	3.09[a]	1.82[b]	1.87[b]	0.027	<0.001
Finisher	11.74[a]	7.21[b]	7.00[b]	0.144	<0.001
Global[1]	16.03[a]	9.77[b]	9.73[b]	0.145	<0.001
Human health, mpt/chicken					
Starter	39.80[a]	27.49[b]	31.66[b]	0.770	<0.001
Grower	104.66[a]	67.88[b]	69.51[b]	0.967	<0.001
Finisher	402.79[a]	273.58[b]	260.34[b]	5.321	<0.001
Global	547.25[a]	368.94[b]	361.51[b]	5.258	<0.001
Ecosystem, mpt/chicken					
Starter	3.942[a]	3.227[b]	3.54[b]	0.084	0.015
Grower	10.27[a]	7.73[b]	7.54[b]	0.101	<0.001
Finisher	38.73[a]	29.65[b]	27.22[b]	0.506	<0.001
Global	52.94[a]	40.61[b]	38.29[b]	0.517	<0.001
Resources, mpt/chicken					
Starter	0.84[a]	0.56[b]	0.66[b]	0.016	<0.001
Grower	2.21[a]	1.36[b]	1.39[b]	0.020	<0.001
Finisher	8.60[a]	5.44[b]	5.19[b]	0.111	<0.001
Global	11.65[a]	7.36[b]	7.21[b]	0.110	<0.001

[1]Global: corresponds to the cumulative impact over the course of the study. Control: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT: diet with alternative

ingredients and 5% Black Soldier Fly larvae meal.[2]SEM: Standard error of the mean. a,b: different letters in the same row indicate statistical differences P<0.05.

3.3.4 Impact to produce 1 kg live weight using commercial breed (Cobb 500) in Turkish pilot

Regarding the Cobb-500 breed, this is a commercial hybrid selected for fast growth with low feed consumption, and they are typically slaughtered around 6 weeks of age. It is one of the most widely used commercial lines globally. In this project, in the pilot conducted at EGE, the same diets formulated for the local breed were used but with different trial duration. The feeding program for chickens of this breed was as follows: the starter phase lasted from birth until day 10, the grower phase until day 25, and the finisher phase until day 40, at which point the animals were slaughtered, with an average weight of 2.23 kg. The average FCR was 1.79 kg. The average mortality was 1.6% throughout the fattening period. It can be observed that the fattening period was shorter than for the local breed, and the FCR is lower than that obtained for local breed chickens.

Table 30 shows the estimated impacts on the 4 categories studied, to produce 1 kg LW obtained with the 3 experimental diets provided to Cobb chickens. We observe a highly significant effect of diet (P<0.001) on the categories analyzed (Global warming, Human health, Ecosystems, and Resources).

Chickens fed with the Control diet had an impact of 5.34 kg CO_2 eq/kg LW, while those fed with the ALTER diet had an impact of 3.29 kg CO_2 eq/kg LW, and those fed with the ALT+INSECT diet had an impact of 3.36 kg CO_2 eq/kg LW. These data represent reductions of about 37% in CO_2 emissions, 32% in Human Health, 25-22% in Ecosystems, and 36% in Resources. The reductions achieved with the ALTER and ALT+INSECT diets are very similar, although slightly lower in all categories, compared to those obtained for the local breed.

Table 18. Average impact of the production of 1 kg live weight of commercial chickens fed the experimental diets of Turkish pilot on Global warming, Human health, Ecosystems, and Resources

	Experimental diets[1]			SEM[2]	P value
	CONTROL	ALTER	ALT + INSECT		
Global warming. kg CO_2 eq/kg LW	5.34[a]	3.29[b]	3.36[b]	0.063	<0.001
Human health/kg LW, mpt /kg LW	181.79[a]	122.68[b]	124.68[b]	2.289	<0.001
Ecosystem/kg LW, mpt/kg LW	17.64[a]	13.74[b]	13.28[b]	0.239	<0.001
Resources/kg LW, mpt/kg LW	3.87[a]	2.45[b]	2.49[b]	0.047	<0.001

[1]Control: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal. [2]SEM: standard error of the mean. LW: live weight. a.b: different letters in the same row indicate statistical differences P<0.05.

When we conducted the study considering diet per phase to produce one commercial chicken, the data obtained show results similar to those observed in the Local breed (**Table 31**). A highly significant effect of diet (P<0.001) was observed across the four categories studied. The Control diet had the greatest potential impact, and a much larger impact was estimated for the finisher phase compared to the starter and grower phases. This behavior was observed in all the phases for analyzed categories: Global warming, Human health, Ecosystems, and Resources.

Table31. Impact of the production of one commercial breed chicken during each growth phase according to Turkish experimental diets

	Experimental Diets[1]			SEM[2]	P value
	CONTROL	ALTER	ALT + INSECT		
Climate Change, kg CO_2 eq/chicken					
Starter	1.19[a]	0.71[b]	0.80[b]	0.022	<0.001
Grower	3.51[a]	2.16[b]	2.21[b]	0.023	<0.001
Finisher	6.64[a]	4.56[b]	4.48[b]	0.139	<0.001
Global[1]	11.64[a]	7.43[b]	7.49[b]	0.146	<0.001
Human health, mpt/chicken					
Starter	39.22[a]	26.29[b]	29.60[b]	0.787	<0.001
Grower	118.82[a]	80.60[b]	81.99[b]	0.834	<0.001
Finisher	238.34[a]	170.18[b]	166.78[b]	4.990	<0.001
Global	396.38[a]	277.06[b]	278.27[b]	5.275	<0.001
Ecosystem, mpt/chicken					
Starter	3.89 [a]	3.09[b]	3.30[b]	0.088	0.006
Grower	11.66[a]	9.19[b]	8.90[b]	0.091	<0.001
Finisher	22.92[a]	18.5[b]	17.44[b]	0.514	0.002
Global	38.46[a]	31.02[b]	29.64[b]	0.550	<0.001
Resources, mpt/chicken					
Starter	0.83[a]	0.53[b]	0.60[b]	0.016	<0.001
Grower	2.51[a]	1.61[b]	1.64[b]	0.017	<0.001
Finisher	5.09[a]	3.39[b]	3.32[b]	0.102	<0.001
Global	8.43[a]	5.53[b]	5.56[b]	0.108	<0.001

[1]Global: corresponds to the cumulative impact over the course of the study. Control: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal. [2]SEM: standard error of the mean. a.b: different letters in the same row indicate statistical differences P<0.05

In conclusion, the experimental diets studied in the pilot in Turkey where soybean meal considerably reduced and BSF insect larvae were incorporated managed to reduce the environmental impact very significantly with respect to the Control diet. **Table 32** and **Table 33** shows a summary of the reductions achieved by the experimental diets of this pilot.

Table 19. Summary of the reductions achieved by the experimental diets of the Turkish pilot study on the impact on Climate change, Human health, Ecosystem and Resource, with respect to the Control diet

	ALT	ALT + INSECT
Climate change, kg CO_2 eq/kg diet		
Starter	-43.1	-42.2
Grower	-39.9	-39.3
Finisher	-38.3	-37.6
Human health, mpt/kg diet		
Starter	-35.8	-35.2
Grower	-33.9	-33.4
Finisher	-32.9	-32.4
Ecosystem, mpt/kg diet		
Starter	-23.9	-27.0
Grower	-23.2	-26.3
Finisher	-23.1	-26.5
Resources, mpt/kg diet		
Starter	-38.9	-38.5
Grower	-37.5	-37.1
Finisher	-37.4	-36.9

ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal. [2]SEM: standard error of the mean.

Table 20. Summary of the reductions achieved to produce 1 kg live weight of local breed and commercial breed chickens (Cobb) of the Turkish pilot study on the impact on Climate change, Human health, Ecosystem, and Resources with respect to Control diet

	LOCAL BREED		COMMERCIAL BREED	
	Experimental diets[1]			
	ALT	ALT + INSECT	ALT	ALT + INSECT
Climate change, kg CO_2 eq/kg LW	-39.8	-38.8	-38.9	-37.1
Human health, mpt/kg LW	-34.1	-33.4	-32.5	-31.4
Ecosystem, mpt /kg LW	-25.2	-27.1	-22.1	-24.7
Resources, mpt/kg LW	-38.4	-37.6	-36.7	-35.6

[1]ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: diet with alternative ingredients and 5% Black Soldier Fly larvae meal.

Although commercial breed animals had less impact than local breed animals, it is interesting to note that the impact reductions achieved by the experimental diets were quite similar in both breeds for all categories analyzed, although slightly larger for the local breed.

3.4 Environmental impact of Tunisian pilot study

The pilot study in Tunisia was developed by the Institut Supérieur Agronomique de Chott Mariem (ISA-CM) and by the Rayhana Association.

The ISA CM pilot study was conducted using both slow-growing meat chickens and laying hens. First, we will discuss the study with the meat chickens, followed by the study with the laying hens.

3.4.1. ISA-CM: Trial with slow-growing meat chickens

The meat chickens used were from an autochthonous Tunisian ecotype (SASSO T44), which is well adapted to the climatic conditions of Tunisia. A total of 180 birds, aged 37 days, were used, and the trial was extended until they reached 84 days of age. The chickens were distributed into 15 pens and randomly assigned to one of the three experimental diets, with five pens per diet. Each pen housed 12 birds, meaning there were 60 animals per treatment group.
This study aimed to evaluate the effects of the different dietary treatments on various categories of environmental impact.

The **feeding program** used in the slow-growing meat chicken study in Tunisia involved two phases: a growth phase (from 37 to 64 days of age) and a finishing phase, from 65 to 84 days of age, at which point the chickens were slaughtered with an average weight of 3 kg.

In each phase, three diets (1 control and 2 alternative diets) were studied. The experimental diets were as follows:

- **Control Diet**: This consisted mainly of a diet based on corn and soybean meal, which is commonly used in Tunisia for meat chickens. However, this diet has a high environmental impact due to the fact that these ingredients must be imported from distant locations. This diet would serve as a baseline for comparing the environmental impact of the other two alternative diets.
- **Alternative Diet 1 (ALTER)**: In this diet, the amount of corn (over 20% in both growth phases) and soybean meal (over 7.5% in both phases) was reduced, and alternative ingredients were introduced, such as rapeseed meal (6% in both phases), beans (6% in both phases), and triticale (14 and 15% in grower and finisher phase, respectively).
- **Alternative Diet 2 (ALT+INSECT)**: This diet used the same ingredients as Alternative 1 but in different proportions as shown **Table 34**. Additionally, dried Black Soldier Fly larvae was added on top and adjusted to account for 5% of the estimated daily feed intake. The larva was purchased from a local company (NextProtein, Grombalia, Tunisia).

  All experimental diets were isoenergetic and isoproteic in this trial.

Table 34. Ingredients of the experimental diets used in the slow-growing chickens oh the Tunisia pilot (g/100g as fed basis)

Ingredients	Experimental diets[1]					
	CONTROL		ALTER		ALT+INSECT	
	GROWER	FINISHER	GROWER	FINISHER	GROWER	FINISHER
Corn	68.7	70.3	48.1	50.9	39.9	37.05
Soybean meal	27.5	25.5	20.0	18.0	14.25	12.35
Soybean oil	0.15	0.80	1.40	1.90	0.475	1.330
Phosphate bicalcium	1.79	1.67	1.6	1.5	1.52	1.425
Calcium carbonate	0.88	0.75	0.90	0.71	0.855	0.76
Premix-poulet	0.50	0.50	0.50	0.50	0.475	0.475
Sodium cloride	0.39	0.39	0.39	0.39	0.3705	0.3705
Methionine	0.09	0.09	0.10	0.10	0.095	0.095
Rapeseed meal	-	-	6.00	6.00	6.65	4.75
Fava beans	-	-	6.00	6.00	6.65	7.885
Triticale	-	-	15.0	14.0	23.75	28.5
Lysine HCl	-	-	0.01	-	0.0095	0.0095
Black Soldier Fly Larvae (dried)	-	-	-	-	5.00	5.00

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: diet ALTER with 5% whole dehydrated Black Soldier Fly

3.4.1.1. Impact of the experimental diets in the Tunisian pilot study: Slow-growing chickens

The potential impacts of the experimental diets used in the Tunisian pilot (Control, ALTER, and ALT+INSECT) were calculated. However, in the case of the ALT+INSECT diet, two scenarios were considered: the first scenario assumed that the insect was produced by the company Entomo Agroindustrial from Spain (ALT+INSECT (Spain)) as initially scheduled, however the larvae were finally purchased from a local insect production company, so a second scenario where larvae production was close to the development of the pilot was studied (ALT+INSECT (Tunisia)). Therefore, **Table 35** shows the impact on Climate change expressed in kg CO_2 eq/kg of diet obtained using the ILCD Midpoint method considering more than 96% of their compositions for the 4 possible diets. The ILCD Midpoint method evaluates the impact on 16 categories. However, for the sake of summarizing this report, the impact on Climate change is shown as it is considered

the category with the most social impact, and the complete study estimating the impact on all 16 categories is shown in **Figure 1.6-1.7** in Annex. Additionally, an analysis was conducted using the Recipe Endpoint method, and the potential impacts of 1 kg of diet expressed as millipoints (mpt) on Total impact, Human health, Ecosystem, and Resources are shown in **Table 36**. In addition, **Figure 5** shows the weighted impacts relative to the highest-impact diet on Human Health, Ecosystems, and Resources.

Table 21. Impact of 1 kg of the experimental diets of Tunisia pilot on Climate change (Kg CO_2 eq/kg diet) considering more than 96% of the composition of the diets in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

Climate change (Kg CO_2 eq/kg diet)	Experimental diets[1]			
	CONTROL	ALTER	ALT+INSECT (Spain)	ALT+INSECT (Tunisia)
Grower	2.50	2.20	2.02	2.00
Finisher	2.50	2.20	1.99	1.98

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT + INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia.

As can be observed in **Table 31**, during the grower phase, the impact of ALT+INSECT diets was slightly higher than that of the finisher phase in terms of kg CO_2 eq. Furthermore, experimental diets had a lower impact on Climate change in both phases of the feeding program than CONTROL diet. In the grower phase, the ALTER diet reduced the impact on this category by 12%, the ALT+INSECT (Spain) diet, in the scenario in which the insect was reared in Spain, reduced it by 19.2%, and in the case of the ALT+INSECT (Tunisia) diet, in the scenario in which the insect was reared in ISA CM, near to the pilot, it reduced by 20%. In the case of finishing diets, the reductions were even greater, being 12%, 20.4%, and 20.8% for the ALTER, ALT+INSECT (Spain), and ALT+INSECT (Tunisia) diets, respectively. Given that transportation is reduced if the insects are produced near the pilot, the impacts on Climate change were lower in the ALT+INSECT (Tunisia) diet than in the ALT+INSECT (Spain) diet.

In **Table 36**, the results of impacts on Total impact (mpt) obtained with the experimental diets from the Tunisian pilot are shown.

Table 226. Impact of 1 kg of the experimental chicken diets of Tunisian pilot on Total Impact considering more than 96% of the composition of the diets in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

Total impact mpt/kg diet	Experimental diets[1]			
	CONTROL	ALTER	ALT+INSECT (Spain)	ALT+INSECT (Tunisia)
Grower	130	112	103	103
Finisher	130	113	102	101

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT + INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia.

The Total impact of the Control diet was the same in both phases; and with respect to the alternative diets, the impacts were very similar in both phases, and less than those of the Control diet. For the grower phase, the reductions were 13.8% for the ALTER diet and 20.8% for the ALT+INSECT diet in both scenarios. In the finisher phase, the reductions achieved by the experimental diets were 13.1%, 21.5%, and 22.3% for the ALTER, ALT+INSECT (Spain), and ALT+INSECT (Tunisian) diets, respectively. It can be observed that insect-based diets (regardless of where they are produced) had a lower impact than the ALTER diet.

The behavior of experimental diets regarding the impact on Human health is shown in **Table 37** and **Figure 5**. The Control diet had the same impact on Human health in both the grower and finisher phases. However, the experimental diets varied slightly and were always lower than the impacts of the Control diet. In the grower phase, the impact reductions were 15.3%, 21.9%, and 22.5% for the ALTER, ALT+INSECT (Spain), and ALT+INSECT (Tunisia) diets, respectively. In the finisher phase, the reductions achieved by the experimental diets were 14.4%, 23.5%, and 24.1% for the ALTER, ALT+INSECT (Spain), and ALT+INSECT (Tunisia) diets, respectively. Once again, it is observed that the diet with insects has the lowest impact of all the diets studied.

Table 23. Impact of 1 kg of the experimental chicken diets of Tunisian pilot on Human health (mpt) considering more than 96% of the diet compositions in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

Human health mpt/kg diet	Experimental diets[1]			
	CONTROL	ALTER	ALT+INSECT (Spain)	ALT+INSECT (Tunisia)
Grower	118	100	92.2	91.5
Finisher	118	101	90.3	89.6

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT + INSECT (Tunisia): diet ALTER with 5% whole dehydrated BSF in the scenario in which the insect was reared in ISA-CM, Tunisia.

Regarding the impact on the ecosystem, the results obtained from the experimental diets are detailed in **Table 38** and **Figure 5**.

In terms of Ecosystem impact, the Control diet showed no difference between the grower and finisher phases. However, the alternative diet that included insects (specifically dried BSF) shows a reduction in Ecosystem impact, particularly during the grower phase. So, reduction was by approximately 7% during the grower phase and 4% during the finisher phase, indicating that the incorporation of insect larvae can contribute to a more sustainable feeding strategy. On the other hand, the ALTER diet, which incorporated alternative plant-based ingredients, did not result in a noticeable reduction in environmental impact during either the grower or finisher phases, suggesting that not all alternative ingredients are equally effective in mitigating ecosystem impact. These results highlight the potential of insects as a source of protein in poultry diets in Tunisia to reduce the environmental burden associated with poultry production, especially during the early stages of growth.

Table 24. Impact of 1 kg of the experimental chicken diets of Tunisian pilot on Ecosystem (mpt) considering more than 96% of the diet composition in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

Ecosystem mpt/kg diet	Experimental diets[1]			
	Control	ALTER	ALT+INSECT (Spain)	ALT+INSECT (Tunisia)
Grower	10.7	10.8	9.94	9.91
Finisher	10.7	10.7	10.2	10.1

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT+INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia.

When we analyzed the impact on Resources/kg diet from the Tunisian pilot, the results are shown in **Table 39**. Similar to the previous category, the Control diet had the same impact in both the grower and finisher phases (1.5 mpt/kg diet), while the ALTER diet and ALT+INSECT diets showed a lower impact. Compared to the Control diet, the ALTER diet reduced Resource impact by 16% in both phases while the ALT+INSECT diets performed even better, achieving a 20-21.3% reduction in Resource impact during the growing phase and a 22-24% reduction during the finishing phase. The extent of the reduction in resource use depended largely on the proximity of the insect larval production site to the pilot location. When larvae were produced closer to the pilot study, the diets were even more efficient, further reducing the environmental load and making

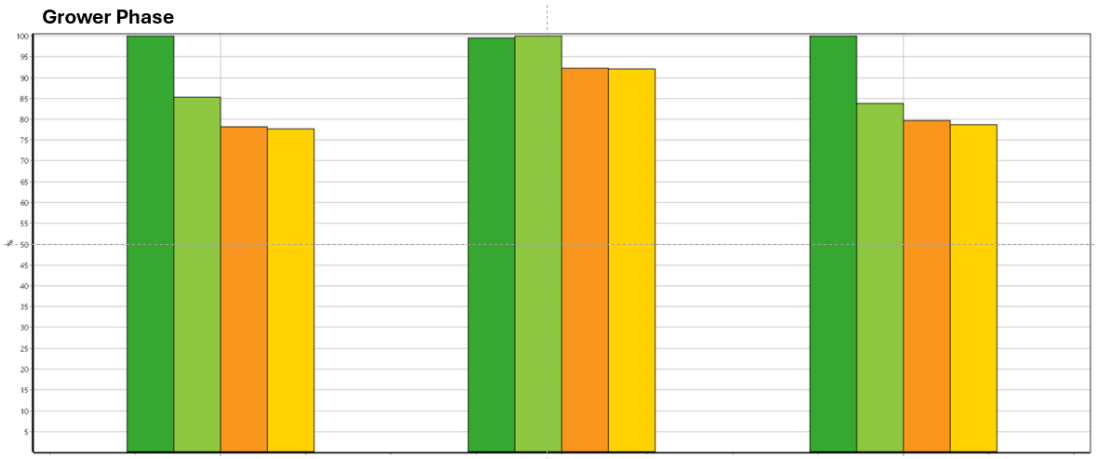
these diets incorporating insects as a protein and energy source a more sustainable alternative to the Control diet. This highlights the importance of sourcing ingredients locally, as reducing the transportation of raw materials can significantly improve the overall environmental sustainability of livestock production systems.

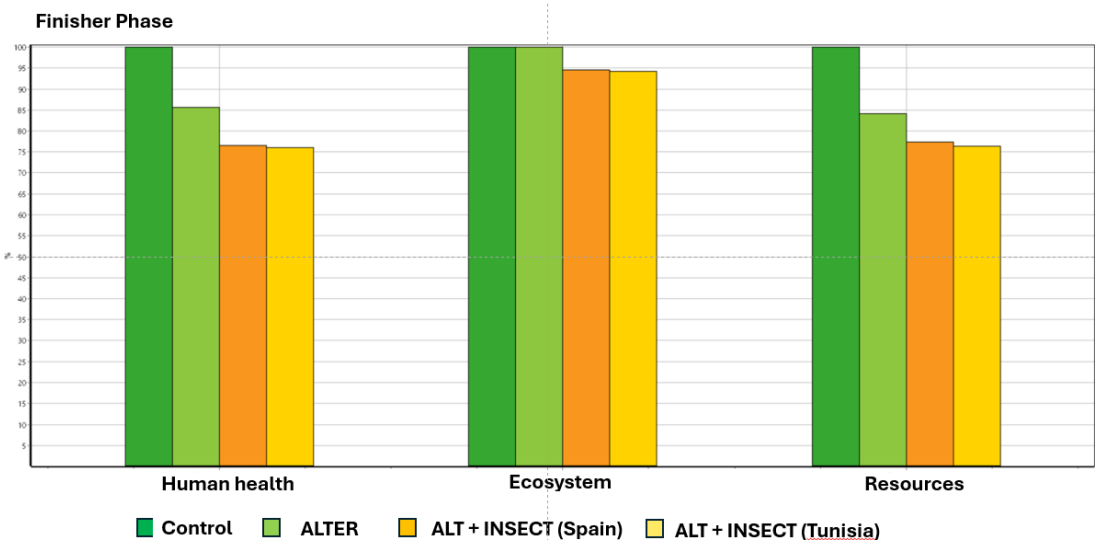
Table 25. Impact of 1 kg of the experimental chicken diets of Tunisian pilot on Resources (mpt) considering more than 96% of the composition of the diets in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

Resources mpt/kg diet	Experimental diets[1]			
	CONTROL	ALTER	ALT+INSECT (Spain)	ALT+INSECT (Tunisia)
Grower	1.5	1.26	1.20	1.18
Finisher	1.5	1.26	1.16	1.14

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT+INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia.

Figure 5. Impact of 1 kg of experimental diets of the Tunisian slow-growing chickens' pilot weighed against the diet with the highest impact on Human health, Ecosystem and Resources, in each feeding phase, considering >98% of the diet composition and in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia





[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT+INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia.

It is worth noting that in this pilot, unlike the previous ones, the diets that included insects were more favorable in mitigating impacts than those that only included plant-based alternatives. This effect was due to the fact that the reduction of high-impact ingredients, such as soybean meal, was more severe in the diet that included larvae compared to the alternative without insects.

The impact of the production of slow-growing Tunisian chickens at different growth phases was then studied.

3.4.1.2. Impact of slow-growing chicken production in Tunisia by phases

Considering the impacts of each diet and the feed consumption of the chickens fed with the different experimental diets, we studied the impact produced during each of the phases of the feeding program: grower and finisher, as well as globally, during the whole experimental period. **Table 40** shows the impacts obtained on the categories studied.

The type of diet had a highly significant effect ($P<0.001$) on the impacts related to Climate Change, Human health, Ecosystem, and Resource use across all phases of the study. These effects were not only evident in each individual phase but also contributed substantially to the cumulative impact over the entire study period. The marked influence of diet on these key environmental aspects suggests that the choice of feed formulation plays a crucial role in shaping the overall sustainability of poultry production, emphasizing the need for careful consideration of dietary strategies to mitigate long-term ecological impacts.

Table 40. Impact of the chickens with Tunisian diets on Climate Change, Human health, Ecosystem and Resources, considering more than 96% of the composition of the diets, in each period analysed and throughout the entire study, in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

	Experimental diets[1]				SEM[2]	P-value
	Control	ALTER	ALT+INSECT (Spain)	ALT+INSECT (Tunisia)		
Climate change, kg CO_2 eq/period						
Grower	5.88^{a}	5.12^{b}	4.70^{c}	4.65^{c}	0.025	<0.001
Finisher	6.94^{a}	5.95^{b}	5.21^{c}	5.19^{c}	0.011	<0.001
Total period	12.82^{a}	11.07^{b}	9.91^{c}	9.84^{c}	0.030	<0.001
Human health, mpt /period						
Grower	277.6^{a}	232.7^{b}	214.6^{c}	213.0^{c}	1.180	<0.001
Finisher	327.7^{a}	273.0^{b}	236.5^{c}	234.7^{c}	0.513	<0.001
Total period	445.7^{a}	373.0^{b}	328.7^{c}	326.2^{c}	0.513	<0.001
Ecosystem, mpt /period						
Grower	25.17^{a}	25.13^{a}	23.14^{b}	23.07^{b}	0.117	<0.001
Finisher	27.95^{ab}	28.92^{a}	26.72^{c}	26.45^{c}	0.051	<0.001
Total period	40.42^{a}	39.72^{b}	36.66^{c}	36.66^{c}	0.051	<0.001
Resources, mpt /period						
Grower	3.53^{a}	2.93^{b}	2.79^{bc}	2.75^{c}	0.015	<0.001
Finisher	4.17^{a}	3.41^{b}	3.04^{c}	2.99^{c}	0.007	<0.001
Total period	5.67^{a}	4.67^{b}	4.24^{c}	4.17^{d}	0.030	<0.001

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT+INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia. [2]SEM: standard error of the mean. a,b,c,d: different letters in the same row show significant differences P<0.05.

The reductions achieved with the alternative diet based on locally sourced plant-based ingredients for Climate Change were around 13% and 14% during the grower and finisher phases, respectively. This led to a reduction on Climate Change impact of 13.7% across the entire pilot study when comparing the ALTER diet to the Control diet. When diets incorporating insects were used in the two scenarios studied—whether the insect was reared in Spain or near the location of the pilot study—the reductions were significantly greater than with the ALTER diet, showing a statistically significant difference compared to both the Control and ALTER diets. Specifically, reductions of around 20% and 25% were observed during the grower and finisher phases, respectively, when the insect was raised in Spain, and approximately 21% and 25% when the insect was raised in Tunisia. Overall, throughout the entire study, the reduction achieved with the ALT+INSECT (Spain) diet was 22.7%, and with the ALT+INSECT (ISA-CM) diet, it was 23.2 %, with no significant differences found between them (P>0.05).

Regarding Human Health, it was observed that experimental diets significantly reduced the impact in this category compared to the Control diet (P<0.001). The ALTER diet achieved a reduction of 16.2% in impact during the grower phase and 16.7% during the finisher phase. This resulted in an overall reduction of 16.3% in the Human Health category over the entire study period compared to the Control diet. On the other hand, the diets incorporating 5% insect further reduced the impact in both scenarios studied, with reductions of approximately 23% and 28% for the grower and finisher phases, respectively. Considering that the insect reared in Spain required a longer transport distance, the overall reduction in impact throughout the entire study was 26.3%. When the insect was reared in Tunisia (closer to the pilot study location), the total impact reduction was 26.8%. No significant differences were found between the two scenarios in terms of the impacts achieved, but both were significantly different from the Control diet and the ALTER diet.

The Ecosystem category was the least affected by the experimental diets, as the reductions were smaller compared to the other categories. Moreover, the ALTER diet did not show significant differences compared to the Control diet (P>0.05). However, the diets incorporating insects (in both scenarios studied) achieved a significantly lower impact than the Control diet and the ALTER diet. The reductions were approximately 8% in the grower phase and 4-5% in the finisher phase. Over the entire study period, the reductions achieved with these diets were around 9% in both scenarios.

The impact on the Resources category was significantly reduced by the experimental diets (P<0.001). The ALTER diet reduced the impact on Resources by around 17-18%, depending on the phase, compared to the Control diet. The diets incorporating insects farmed in Tunisia achieved even greater reductions, approximately 21%, 28%, and 26% in the grower, finisher, and total period, respectively. The reductions in the scenario with insects farmed in Spain were slightly lower with respect to reared in ISA CM, in each phase studied.

In conclusion, in the pilot study conducted in Tunisia with a slow-growing chicken line, where three experimental diets and two insect farming scenarios were evaluated, the diets incorporating insects—regardless of the farming scenario—proved to be the most environmentally friendly. These diets had a lower environmental impact than the diet where imported ingredients were reduced and replaced with more locally sourced alternatives because a higher level of restriction of the highest-impact ingredients could be achieved by incorporating dried larvae.

The impact of producing 1 kg live weight at slaughter age in the Tunisian pilot chickens, i.e. at 84 days, is shown below.

3.4.1.3. Impact of Tunisian diets on the production of 1 kg of live weight at slaughter

The feed intake per kilogram of live weight (LW) and the final slaughter weight at 84 days were evaluated to estimate the potential environmental impact of producing 1 kg of LW **(Table 41).** For the statistical analysis, a one-way linear model was applied, with diet as the fixed factor. Mean differences were assessed using the Tukey test, with statistical significance set at a p-value of less than 0.05.

Table 41. Impact of producing 1 kg live weight production of chickens fed the experimental diets on Climate change, Human health, Ecosystems and Resources of the Tunisian pilot experimental diets at 84 days of age in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

	Experimental diets[1]				SEM[2]	P value
	Control	ALTER	ALT+INSECT (Spain)	ALT+INSECT (Tunisia)		
Global warming, kg CO_2 eq/kg LW	4.35^{a}	3.65^{b}	3.39^{c}	3.37^{c}	0.024	<0.001
Human health/kg LW, mpt	151.5^{a}	123.1^{b}	112.5^{c}	111.6^{c}	0.743	<0.001
Ecosystem/kg LW, mpt	13.73^{a}	13.11^{ab}	12.55^{bc}	12.45^{c}	0.078	<0.001
Resources/kg LW, mpt	1.93^{a}	1.54^{b}	1.45^{c}	1.43^{c}	0.009	<0.001

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT+INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia.[2]SEM: standard error of mean; [3]LW. Live weight

As shown in **Table 42**, the diet had a very significant effect (P<0.001) in producing 1 kg of LW across the four environmental impact categories studied.

Regarding Climate Change, expressed as kg CO_2 eq/kg LW, we observed that all experimental diets significantly reduced the impact (P<0.001) compared to the Control diet. The ALTER diet reduced it by 16%, and the diets with 5% insects, in the two scenarios analyzed, reduced the impact by approximately 22%, with a slightly greater reduction when the insect was raised in Tunisia, near the location where the pilot was conducted (22.5%). Additionally, the diets containing insects significantly reduced (P<0.05) CO_2 emissions compared to the ALTER diet.

Regarding the impact on Human Health when producing 1 kg of LW, it can be seen that the alternative diets used reduced the impact by 18.8% with the ALTER diet and by around 26% when using the diets with insects in any of the studied scenarios. Furthermore, the latter had a lower impact than the ALTER diet (P<0.001). The impact on the Ecosystem category remained the one with the smallest reduction when using the alternative diets in the Tunisia pilot, achieving reductions of 8-9% with the insect-based diet in both analyzed scenarios. Although the ALTER diet achieved a 4.5% reduction, it was not significantly different from the Control diet or the insect-diet when the insect was raised in Spain (P>0.05).

Finally, in the Resources category, all the experimental alternative diets managed to reduce the impact of producing 1 kg of LW, with the diets with insects (whether the insect was raised in Spain or Tunisia) achieving the greatest impact reduction, around 25%.

The following **Table 42-44** shows the summary of the reductions achieved by the experimental diets in the Tunisia pilot study in all of the categories studied.

Table 42. Summary of the impact reductions achieved by experimental diets in Tunisia's pilot project on slow-growing chickens **in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia**

	ALTER	ALT + INSECT (Spain)	ALT + INSECT (Tunisia)
Climate change, kg CO_2 eq/kg diet			
Grower	-12.00	-19.2	-20.8
Finisher	-12.00	-20.4	-20.8
Human health, mpt/kg diet			
Grower	-15.3	-21.9	-22.5
Finisher	-14.4	-23.5	-24.1
Ecosystem, mpt/kg diet			
Grower	0.90	-7.10	-7.38
Finisher	0.00	-4.67	-5.61
Resources, mpt/kg diet			
Grower	-16.0	-20.0	-31.3
Finisher	-16.0	-22.67	-24.0

[1]ALT + INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT + INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia

Table 43. Summary of impact reduction by production phase achieved by alternative experimental diets in Tunisia's slow-growing chicken pilot in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

	ALTER	ALT + INSECT (Spain)	ALT + INSECT (Tunisia)
Climate change, kg CO_2 eq/period			
Grower	-12.9	-20.1	-20.9
Finisher	-14.3	-24.9	-25.2
Total period	-13.7	-22.7	-23.2
Human health, mpt/period			
Grower	-16.2	-22.9	-23.3
Finisher	-16.7	-27.8	-28.4
Total period	-16.3	-26.3	-26.8
Ecosystem, mpt/period			
Grower	-0.16	-8.10	-8.34
Finisher	3.47	-4.40	-5.37
Total period	-1.73	-9.30	-9.30
Resources, mpt/period			

Grower	-17.0	-21.0	-22.1
Finisher	-18.2	-27.1	-28.3
Total period	-17.6	-25.2	-26.5

[1]ALT + INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT + INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia

Table 26. Summary of impact reduction achieved by kg live weight by alternative experimental diets in Tunisia's slow-growing chicken pilot in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

	ALTER	ALT + INSECT (Spain)	ALT + INSECT (Tunisia)
Climate change, kg CO_2 eq/kg LW	-16.09	-22.07	-22.53
Human health, mpt/kg LW	-18.75	-25.74	-26.34
Ecosystem, mpt/kg LW	-4.52	-8.59	-9.32
Resources, mpt/kg LW	-20.21	-24.87	-25.91

[1]ALT + INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT + INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia. LW: live weight.

In conclusion, in the pilot study conducted in Tunisia using a slow-growing chicken ecotype, where insects were included in the diet as a new ingredient, these diets proved to be the most environmentally advantageous. They were more beneficial than diets where imported ingredients, primarily soy and corn, were reduced and other alternative plant-based ingredients were incorporated. However, alternative diets without insects had a lower environmental impact than the conventional diet in almost all categories studied.

3.4.2. ISA-CM: Trial with laying hens

ISA-CM also conducted a pilot study to assess the environmental impact of experimental diets using commercial-line laying hens. A total of 150 Lohman White line, 30 weeks old, were used in the study and divided into 15 pens floor, with 10 hens per pen. The pens were randomly assigned to one of the three experimental treatments, ensuring that there were five replicates per treatment. The study lasted for 70 days, allowing for an extended period to observe the effects of diets on the hens under consistent conditions. The pens were maintained under natural temperature conditions with a controlled photoperiod regime of 16 hours of light and 8 hours of darkness to simulate typical commercial production environments. Since the hens started the study at peak laying, around 96-98% of lay, **the feeding program** used for the pilot consisted of a single feeding phase with 3 experimental diets:

The experimental diets were as follows:

- **Control Diet**: This diet consisted of a diet based on corn and soybean meal, which is commonly used in Tunisia for laying hens. However, this diet has a high environmental impact due to the fact

that these ingredients must be imported from distant locations. This diet would serve as a baseline for comparing the environmental impact of the other two alternative diets.

- **Alternative Diet 1 (ALTER)**: In this diet, the amount of imported ingredients such as corn (over 27%) and soybean meal (about 9%) was reduced, and alternative local ingredients were introduced, such as triticale (20%), fava beans (10%), and rapeseed meal (5%).
- **Alternative Diet 2 (ALT+INSECT)**: This diet consisted of 95% Alternative 1 diet plus 5% dried whole Black Soldier Fly) larvae. Therefore, this diet was not iso energetic and isoproteic with respect to the other diets.

Table 45 shows the composition of the experimental diets used in laying hens' Tunisian pilot.

Table 27. Ingredients of the experimental diets used in the laying hens of the Tunisia pilot (g/100g as fed basis)

	Experimental diets[1]		
	CONTROL	ALTER	ALT+INSECT
Corn	62.6	35.2	33.44
Soybean meal	26.2	17.3	16.435
Soybean oil	-	1.35	1.2825
Phosphate bicalcium	1.50	1.39	1.3205
Calcium carbonate	8.97	9.00	8.55
Sodium cloride	0.35	0.37	0.37
Methionine	0.08	0.09	0.0855
Premix	0.30	0.30	0.285
Triticale	-	20.0	19.0
Fava beans	-	10.0	9.50
Rapeseed meal	-	5.00	4.75
Black Soldier Fly Larvae (dried)	-	-	5.00

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: diet ALTER with 5% whole dehydrated Black Soldier Fly

3.4.2.1. Impact of the experimental diets in the Tunisian pilot: Laying hens

The potential impacts of the diets used in the laying hen pilot project in Tunisia on Climate change are presented in **Table 46** (ILCD Midpoint method). The rest of the categories studied with this method are shown in **Figure 1.8** in Annex. The Control diet resulted in an impact of 2.25 kg CO_2 eq/kg diet, whereas the ALTER

diet, which incorporated only local plant-based ingredients, reduced this impact to 1.88 kg CO_2 eq/kg diet. For the diet supplemented with insects (ALT + INSECT), CO_2 emissions were 1.94 and 1.92 kg CO2 eq/kg diet, depending on whether the insects were reared in Spain or Tunisia, respectively. This represents a reduction in CO_2 emissions of 16.4% for the ALTER diet and 13.8% or 14.7% for the insect-supplemented diets, depending on the rearing location of the insects.

It is important to note that when insects are added as an additional supplement to the diet, there is a slight increase in impact compared to the diet without the insect component. However, this increase is less pronounced if the insects are reared locally, near the site where the pilot project took place, in this case near ISA-CM. By sourcing insects closer to the point of use, emissions associated with transportation are minimized, further contributing to the environmental benefits of local production. In any case, the reductions achieved by the experimental diets in Tunisia are greater than the 10% set by the project proposal, making the diets used in this pilot a substantial environmental improvement.

Table 28. Impact of 1 kg de experimental diets from laying hen Tunisian pilot on Climate change considering more than 99% of the diet compositions in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

	Experimental diets[1]			
	CONTROL	ALTER	ALT + INSECT (Spain)	ALT + INSECT (Tunisia)
Climate change (Kg CO_2 eq/kg diet)	2.25	1.88	1.94	1.92

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT + INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia.

When using the Recipe Endpoint method (**Table 47** and **Figure 6**) to assess the Total impact in milli points (mpt), the Control diet scored 117 mpt, while the experimental diets showed significant improvements. The ALTER diet scored 94.9 mpt, the ALT + INSECT (Spain) diet 95.5 mpt, and the ALT + INSECT (Tunisia) diet 94.8 mpt. These results indicate that the alternative experimental diets achieved an 18-19% reduction in Total impact compared to the Control diet.

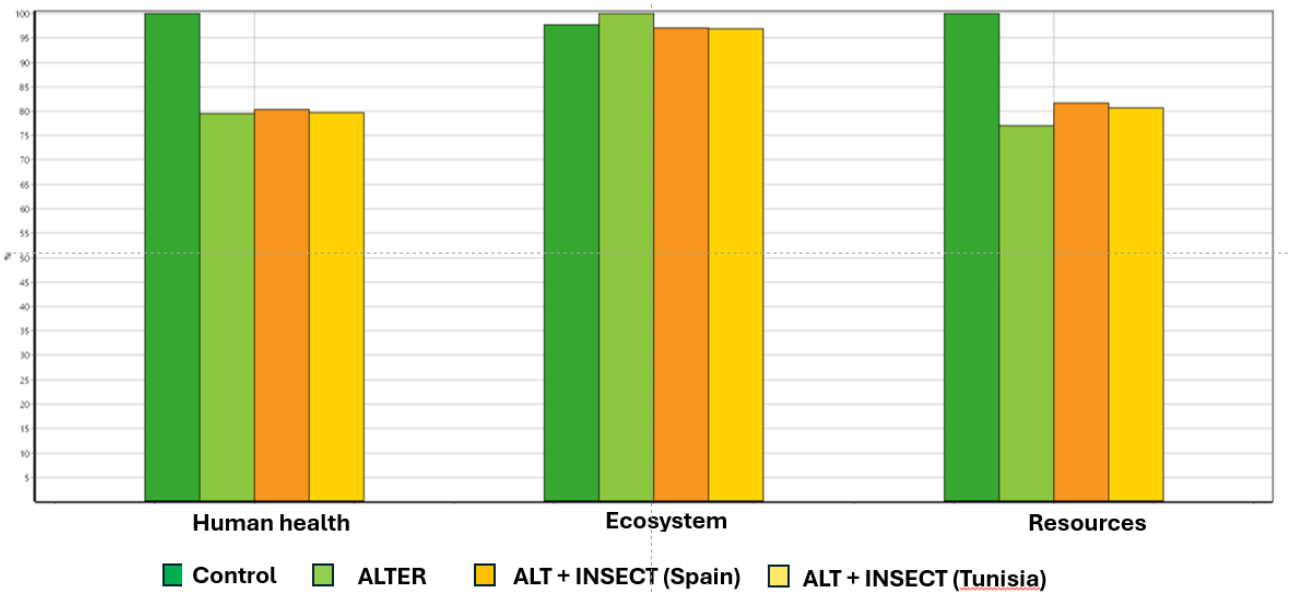
In the Human Health category, the Control diet had an impact of 106 mpt/kg diet, while the experimental diets reduced this value by 20-21%, depending on the specific diet. This suggests that experimental diets offered notable improvements in terms of reducing health-related impacts. Regarding the Ecosystem impact, the experimental diets did not manage to achieve any reductions when compared to the Control diet. However, in the Resources category, the ALTER diet led to a substantial 22.8% reduction in impact compared to the Control diet. For the insect-supplemented experimental diets, where 5% of BSF larvae was supplemented, the reduction in Resource impact ranged from 18-19% in both scenarios analyzed (insects reared in Spain and Tunisia, respectively).

Table 29. Impact of 1 kg of experimental diets of Tunisian pilot on Total impact, Human Health, Ecosystem and Resources (Recipe Endpoint method) considering more than 99% of the composition of the diets in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

	Experimental diets[1]			
	CONTROL	ALTER	ALT +INSECT (Spain)	ALT +INSECT (Tunisia)
Total impact, mpt	117	94.9	95.5	94.8
Human health, mpt	106	84.0	84.8	84.2
Ecosystems, mpt	9.60	9.84	9.55	9.52
Resources, mpt	1.36	1.05	1.11	1.1

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT+INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia.

Figure 6. Impact of 1 kg of experimental diets of the Tunisian laying hens' pilot weighed against the diet with the highest impact on Human health, Ecosystem and Resources, in each feeding phase, considering >98% of the diet composition.



[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was

reared in Cehegín, Spain. ALT+INSECT (Tunisia): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in ISA-CM, Tunisia.

3.4.2.2. Impact of Tunisian diets on the production of 1 kg of egg mass

To study the impact on the product obtained, in this case on egg mass production, we considered the data on laying percentage of laying hens during the study period and egg weight, thus calculating egg mass to estimate the impact of the experimental diets per kilogram of egg mass. The impact data obtained on Climate change, Human health, Ecosystem, and Resources are shown in **Table 48**.

Table 30. Impact of the production of 1 kg egg mass with Tunisian diets on Climate Change, Human health, Ecosystem and Resources, considering more than 98% of the composition of the diets in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

	Experimental diets[1]				SEM[2]	P-value
	CONTROL	ALTER	ALT+INSECT (Spain)	ALT+INSECT (Tunisian)		
Climate change, kg CO_2 eq/kg egg mass	5.46^{a}	4.50^{b}	4.54^{b}	4.50^{b}	0.026	<0.001
Human health, mpt /kg egg mass	257.5^{a}	200.9^{b}	198.6^{b}	197.2^{b}	1.145	<0.001
Ecosystem, mpt /kg egg mass	23.32^{ab}	23.53^{a}	22.37^{b}	22.29^{b}	0.123	0.004
Resources, mpt /kg egg mass	3.30^{a}	2.51^{b}	2.60^{b}	2.58^{b}	0.015	<0.001

[1]CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT+INSECT (Tunisia): diet ALTER with 5% whole dehydrated BSF in the scenario in which the insect was reared in ISA-CM, Tunisia.[1]SEM: standard error of the mean. a,b: different letters in the same row show significant differences at P<0.05

A highly significant effect of the diet on CO_2 emissions was observed (P<0.001) in relation to egg mass production, with the Control diet showing the highest environmental impact, generating 5.46 kg CO_2 eq/kg egg mass. In comparison, the alternative diets, ALTER and ALT+INSECT (Tunisia), had lower emissions, at 4.50 kg CO2 eq/kg egg mass both diets, while the ALT+INSECT (Spain) diet emitted 4.54 kg CO_2 eq/kg egg mass. This represents a reduction of approximately 17% in CO_2 emissions when alternative diets were used compared to the Control diet. Importantly, no significant differences were found between the CO_2 emissions of the three alternative diets (P>0.05).

Regarding the impact on Human Health, the Control diet had a score of 257.5 mpt/kg egg mass, while the alternative diets significantly reduced this impact by about 22-23% (p<0.001). Both the ALTER diet and the

insect-supplemented diets (ALT+INSECT) achieved similar reductions in Human health impact, and there were no statistically significant differences between them (P>0.05).

In the Ecosystem category, the reduction in environmental impact was smaller, with no significant differences observed between the alternative diets and the Control diet (23.32, 23.53, 22.37, and 22.29 mPt /kg egg mass for Control, ALTER, ALT+INSECT (Spain), and ALT+INSECT (Tunisia), respectively). However, it is worth noting that the insect-supplemented diets, both in the scenario of being reared in Spain and in the scenario of being reared in Tunisia, showed a lower impact on the ecosystem compared to the ALTER diet (P<0.001).

Finally, in the Resources category, the Control diet had an impact of 3.30 mPt. The alternative diets had significantly lower impacts (P<0.001), achieving reductions of 21-23%. Specifically, the impacts were 2.51 mpt for the ALTER diet, 2.60 mt for the ALT+INSECT (Spain) diet, and 2.58 mpt for the ALT+INSECT (Tunisia) diet, with no significant differences found between the three alternative diets in this category (P>0.05).

The **Table 49** and **Table 50** summarize the impact reductions in each category achieved by the experimental diets used in the pilot for laying hens in Tunisia.

In conclusion, the achievement of reductions obtained with the experimental diets tested in the Tunis pilot with laying hens, well above the expected threshold, highlights the effectiveness of these experimental diets in reducing environmental impacts, such as carbon emissions, resource use and health effects.

The reduction levels achieved with the Tunisian experimental diets represent a major step forward in terms of sustainability in poultry farming, offering a tangible solution to reduce the environmental footprint of egg production in this country. By exceeding the 10% reduction target, the pilot project demonstrates that these diets could play an important role in shaping more environmentally friendly farming practices, providing a model for other sectors to follow. The results underscore the potential of these alternative feeding strategies to contribute significantly to global efforts to mitigate climate change and conserve natural resources, ultimately making feed systems more sustainable.

Table 31. Summary of impact reduction achieved by alternative experimental diets in Tunisia's laying hen pilot per kilogram of diet in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

	Experimental diets[1]		
	ALTER	ALT + INSECT (Spain)	ALT + INSECT (Tunisia)
Climate change, kg CO_2 eq/kg diet	-16.4	-13.8	-14.7
Human health, mpt/kg diet	-20.5	-20.6	-18.4
Ecosystem, mpt/kg diet	2.50	-0.83	-0.52
Resources, mpt/kg diet	-22.8	-19.1	-18.4

[1]ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT+INSECT (Tunisia): diet ALTER with 5% whole dehydrated BSF in the scenario in which the insect was reared in ISA-CM, Tunisia

Table 50. Summary of impact reduction achieved by alternative experimental diets in Tunisia's laying hen pilot per kilogram of egg mass in the two scenarios studied: if the larvae are produced in Spain or if they are produced in Tunisia

	Experimental diets[1]		
	ALTER	ALT + INSECT (Spain)	ALT + INSECT (Tunisia)
Climate change, kg CO_2 eq/kg egg mass	-17.6	-17.6	-16.8
Human health, mpt/kg egg mass	-22.2	-23.2	-22.7
Ecosystem, mpt/kg egg mass	0.9	-4.4	-4.1
Resources, mpt/kg egg mass	-23.9	-21.8	-21.2

[1]ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER with 5% whole dehydrated Black Soldier Fly in the scenario in which the insect was reared in Cehegín, Spain. ALT+INSECT (Tunisia): diet ALTER with 5% whole dehydrated BSF in the scenario in which the insect was reared in ISA-CM, Tunisia

3.4.3. Rayhana pilot

As mentioned above, pilot studies were carried out in Tunisia both at ISA-CM, under experimental conditions, and at Rayhana. Rayhana is an association that supports rural women and promotes their work. In the context of SUSTAvianFEED, Rayhana conducted a pilot study with 14 women farmers laying hens in real conditions on its farm using a total of 300 laying hens.

The profile of the participants was as follows:

- All belong to the governorate of Jendouba, to agricultural delegations.
- Most of them belong to a middle social class.
- Their age ranged between 41-55 years, thus being of adult category. **Figure 7** shows the age of the participants.
- They are still in good ancestral practices inherited from parents and grandparents in the production system. They preserve traditional knowledge and maintain rural communities.

Figure 7. Age of participants (rural women) in the pilot developed by Ryahana

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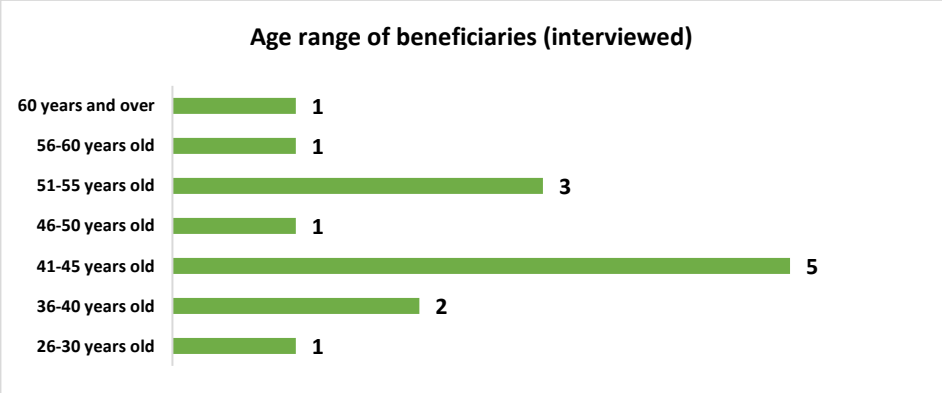

The participating women followed a feeding program consisting of two diets. One was a Control diet based on 30% feed mix made from locally sourced maize, wheat bran, and barley, 25% wheat, 25% barley, and 20% dry bread (**Table 51**). The other diet was based on the Control diet supplemented with 5% BSF larvae (ALT+INSECT). Half of the farmers used the Control diet, and the other half used the AL+INSECT diet.

It should be noted that in this pilot the Control diet was based exclusively on local products, without incorporating soybean meal. The use of this diet is a common practice in these very unspecialized and non-technician rural rearing systems, so that the diet was not balanced and lacked a protein source per se. In this case, supplementation with insect larvae provided a significant protein and energy enrichment compared to the usual diet.

Table 51. Composition of experimental diets of the Rayhana pilot in Tunisia (g/kg as fed basis)

	Control[1]	ALT + INSECT
Barley	35.0	33.25
Wheat	25.0	23.75
Dry bread	20.0	19.0
Maize	10.0	9.5
Wheat bran	10.0	9.5
Black Soldier Fly larvae	-	5.0

[1]Control diet: diet with local vegetable products. ALT+INSECT 95% Control diet plus 5% insect

With respect to the environmental impact obtained, **Table 52** shows the impact of 1 kg of diet with respect to kg CO_2 eq emissions, Total impact, Human health, Ecosystem, and Resources. In this pilot, the larvae were purchased from a local company, assuming a high level of technology in their production with very controlled environmental conditions, and therefore a high energy expenditure (similar to Entomo company), which

caused that the impact of the alternative diet with insects, in terms of kg CO_2 eq/kg diet, to be 14% higher than that of the Control diet, as shown in Table 49. Therefore, an alternative scenario was studied where the larvae were reared under medium technology conditions, where only the minimum requirements of air conditioning and lighting for insect rearing were imputed (see apart 3.5). In this scenario, called medium technology, the ALT+INSECT diet reduced its kg CO_2 eq/kg diet impact by 9% with respect to the high technology ALT+INSECT diet, and with respect to the Control diet, the impact increased by only 4%.

Figure 16 in Annex 1 shows the impact on the rest of the categories analyzed with ILCD method.

Table 52. Impact of 1 kg of experimental diets of Rayhana pilot on Total impact, Human Health, Ecosystem and Resources considering 100% of the diet composition in the two scenarios studied: if the larvae are produced with high technology or if they are produced with medium technology

		Experimental diets[1]	
	CONTROL	ALT + INSECT (high technology)	ALT + INSECT (medium technology)
Climate change, kg CO_2 eq/kg diet	0.743	0.848	0.772
Total impact, mpt/kg diet	39.5	42.2	39.0
Human health, mpt/kg diet	33.6	36.3	34.0
Ecosystem, mpt/kg diet	5.38	5.29	5.19
Resources, mpt/kg diet	0.46	0.53	0.47

[1]Control diet: diet with local vegetable products. ALT+INSECT (high technology) 95% Control diet plus 5% insect reared under high-tech conditions and high energy expenditure. ALT+INSECT (medium technology): 95% Control diet plus 5% insect considering medium energy expenditure for its reared

The Total impact/kg diet of the Control diet was 39.5 mpt, while the ALT+INSECT diet when the insect was produced under high technology conditions was 6.8% higher, and in the scenario where the insect was reared under medium technology conditions it was very similar to the Control diet. On the Human health and Resources categories, while the Control diet had a score of 33.6 and 0.46 mpt/kg diet, respectively, the ALT+INSECT (high technology) diet was 8% and 15% higher, and in the medium technology scenario, the impact on these categories was only 1% and 2% higher than in the Control diet (**Figure 8**). The damage on the Ecosystem was reduced with the alternative diets, by 1.7 and 3.5% for ALT+INSECT (high technology) and ALT+INSECT (medium technology), respectively.

Figure 8. Impact of 1 kg of experimental diets of the Rayhana laying hens' pilot weighed against the diet with the highest impact on Human health, Ecosystem and Resources, considering 100% of the diet composition in the two scenarios studied: if the larvae are produced with high technology or if they are produced with medium technology.

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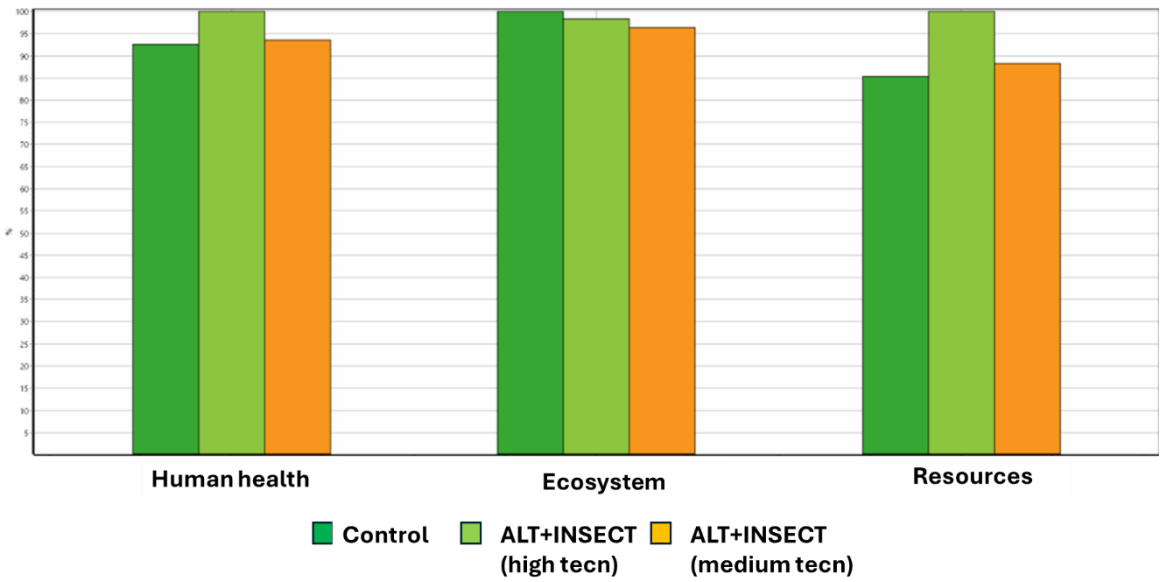

[1]Control diet: diet with local vegetable products. ALT+INSECT (high tecn) 95% Control diet plus 5% insect reared under high-technology conditions and high energy expenditure. ALT+INSECT (medium tecn): 95% Control diet plus 5% insect considering medium energy expenditure for its reared

It can be observed that the incorporation of the insect has increased the impact in almost all the categories studied, even in medium technology rearing conditions, except on Ecosystem. This is because the impact of the insect is greater than that of any of the ingredients used in their Control diet, since these were all locally sourced. In the hypothetical scenario studied where the insect was produced with medium energy expenditure, the potential impacts generated were slightly higher than those of the Control diet, but very similar to this diet.

During the 2 months of the study, the egg production of the hens was monitored. The average production of the hens that consumed the Alt+INSECT diet was between 18 and 24 eggs per month, (mean of 21 eggs/month), while those that consumed the same Control diet but without insect supplementation had a laying between 15 and 16 eggs per month (mean of 15.5 eggs/month). Considering these data and an estimation of the hens' feed intake (an average of 115 g hen/d), we calculated the impact per egg produced as shown in Table **53**. It is observed that diets supplemented with insects, even reared under high-tech conditions, had a lower impact than the Control diet, with 17.6 and 23.5% less kg CO_2 eq/egg, 21.2 and 27.1% on Human health, 27.5 and 29.1% on the Ecosystem and 10 and 20% on Resources in the high-tech and medium-tech insect rearing scenarios, respectively, due to the higher egg production achieved by supplementing the diet with insects.

Table 53. Impact obtained on the different categories with the experimental diets of Rayhana pilot for one egg production composition in the two scenarios studied: if the larvae are produced with high technology or if they are produced with medium technology

		Experimental diets[1]	
	CONTROL	ALT + INSECT High technology	ALT + INSECT Mediun technology
Kg CO_2 eq/egg	0.17	0.14	0.13
Human health, mpt/egg	8.79	6.93	6.41
Ecosystem, mpt/egg	1.20	0.87	0.85
Resources, mpt/egg	0.10	0.09	0.08

[1]Control diet: diet with local vegetable products. ALT+INSECT (high technology) 95% Control diet plus 5% insect reared under high-tech conditions and high energy expenditure. ALT+INSECT (medium technology): 95% Control diet plus 5% insect considering medium energy expenditure for its reared

Therefore, under such rural egg production conditions, the insect-enriched feed is more sustainable because of the extra nutrients it will provide to the hens, which will have a positive impact on egg laying. In this pilot, as the Control diet was more deficient in protein, the insect supplementation had a greater beneficial effect, not only from a productive point of view, but also environmentally.

In addition, according to the appreciation of the livestock breeders, there were also apparent changes in behavior and feather quality, which seemed to be better for the hens eating the insect-supplemented diet.

3.5 Black Soldier Fly Larvae rearing scenario in medium technology conditions

A way to make insect farming more sustainable could be by using less technologically advanced systems than those employed by Entomo Agroindustrial, which is a company with a high degree of technification and highly controlled environmental conditions. For this reason, a scenario was studied to produce BSF larvae under less technical conditions than those of Entomo. Thus, only the minimum requirements for climate control and lighting for larval rearing and development, as well as their subsequent dehydration, were considered. The automation systems for feeding and filtering were eliminated. Additionally, production was based on the rearing surface area, in this case using the surface of the container type designed in the project by Entomo. The impacts obtained in relation to high-tech and medium technology farming are shown in **Table 54**.

Table 32. Impact of Black Soldier Fly breeding under high and medium technology conditions

	Fresh Black Soldier Fly larvae		Dried Black Soldier Fly larvae	
	High Technology	Medium Technology	High Technology	Medium Technology
Climate change, kg CO_2 eq/kg insect	7.72	5.32	26.9	21.3
Human health, mpt/kg insect	25.2	17.5	87.7	69.7
Ecosystem, mpt/kg insect	1.07	0.77	3.59	2.90
Resources, mpt/kg insect	0.636	0.491	2.02	1.68

The impacts of the ALT+INSECT diets of the different pilots in the scenario where the insect is reared under medium technology conditions are shown in **Table 55**. A slight decrease in the impact of all the diets of the different pilots can be observed, since part of the energy expenditure has been omitted for its calculation.

Table 33. Impacts obtained by the diets of the different pilots in the scenario where the insect is produced under medium technology conditions

Climate change, kg CO_2 eq/kg diet	Impact/kg ALT+INSECT diet (Medium technology)	mpact/kg ALT+INSECT diet (High technology)	Reductions on kg CO_2 eq/kg diet
Italy	1.39	1.55	-10.32
Spain	2.17	2.25	-3.56
Turkish			
Starter	1.79	1.93	-7.25
Grower	1.82	1.90	-4.21
Finisher	1.81	1.89	-4.23
Tunisia- chicken meat			
Grower	1.92	2.00	-4.00
Finisher	1.85	1.98	-6.57
Tunisia- laying hens	1.85	1.92	-3.65
Rayhana	0.77	0.85	-9.41

As shown in **Table 55**, the alternative diets incorporating insects (ALT+INSECT), when considering a medium technology larval production scenario, reduce their impact on kg CO_2 eq emissions from 3.5, the smallest reduction for the Spanish diet, to 10.32 in the case of the Italian diet. It is important to highlight that, despite incorporating approximately 5% of insects in all the Alt+INSECT diets of the pilots of this project, the impacts are also influenced by the other ingredients of each diet.

Therefore, if it has been proven that the incorporation of dried insect larvae to alternative diets has been an environmentally beneficial strategy due to the improvements obtained from the productive point of view, if the insect rearing was under medium technology conditions, the environmental advantages obtained would be even greater.

In short and as a **final reflection** we can say that SUSTAVianFEED proposes researching new feeding programs in which commonly imported ingredients, such as soybean meal and imported cereals, are total or partially replaced with more sustainable local alternatives. This shift aims to support the poultry sector's transition toward more environmentally responsible farming practices. The alternative ingredients being evaluated include both plant-based sources (ALTER diet), such as DDGs (dried distillers grains), peas, brewers´ dried grain, fava beans, triticale, rapeseed meal, sunflower meal, as well as animal-based sources, specifically the larvae of the Black Soldier Fly, which are used either whole and dehydrated or processed into meal, depending on the pilot program (ALT+ÌNSECT diet). Additionally, the insect has been valued not only for its high nutritional content, especially in protein and energy, but also as an environmental enrichment resource and an extra nutrient in pilot programs in Italy, Spain, and Tunisia, where it is provided whole as a supplement to the experimental diet.

In all the pilot studies carried out in Italy, Spain, Turkey and Tunisia, a significant reduction in the environmental impact on poultry production has been achieved through the use of experimental diets, both with the ALTER diet and the ALT+INSECT diet. It should be noted that, although the conditions of each study varied from country to country, in all trials a reduction of more than 10% in kg CO_2 eq/ kg diet was achieved, which was one of the main objectives of the project. Spain, where the soy reduction in the alternative diets was only 7% achieved the lowest reductions, but pilots such as Italy or Turkey where soy reductions were higher, the reduction of kg CO2 eq/kg diet reached up to 40-43%. It is important to highlight that the reduction is not always directly proportional to the reduction of soy in the diet, since the impact of the diet is caused by the sum of the impacts of all the ingredients of the diets weighted by their percentage of incorporation.

The results of ALTER diets, designed specifically for each pilot according to the availability of alternative ingredients, confirm the robustness and flexibility of this strategy in countries of the Mediterranean region. Reducing the use of soybean meal in the current situation, an ingredient with a high environmental impact due to its cultivation and transport, is key to making poultry production more sustainable and environmentally friendly. In addition, this strategy not only reduces greenhouse gas emissions, but also reduces other impact factors, such as risks to human health, damage to ecosystems and consumption of natural resources.

Regarding the ALT+ISECT diet, in all pilots except the Tunisian meat chicken pilot, the environmental impact of this diet has been slightly higher than that of the ALTER diet. This is because, under current farming conditions, the production of dehydrated insect larvae has a greater environmental impact than most of the ingredients used. However, it is likely that, in the near future, large-scale insect farming will reduce both the environmental impact per kilogram of insect and the production costs. However, in all pilots, when considering the productive parameters of animals fed diets that include insects, the impacts improve per unit of production (kg of meat or kg of egg mass) compared to ALTER diets. This is due to the production improvements observed when incorporating insects into the animals' feed.

Another of the objectives of this project is the use of local or native animals as a strategy to promote the preservation of biodiversity. For this reason, animals of this type were used in all the pilots developed. When analyzing the results of impact per kg of LW and RTCC, obtained with the local breeds of the pilots in Italy, Turkey and Tunisia, these were higher compared with other values reported in literature obtained studying broiler chickens (Costantini et al., 2021; Kiss et al., 2022). In fact, the pilot study developed by Turkey used local and commercial breed chickens using the same diets and obtained lower impacts per kg LW in the commercial breed. This is an expected result, as these breeds are less selected and therefore less efficient,

requiring longer fattening periods than commercial breeds to achieve similar weights. However, these autochthonous chickens offer significant advantages because of their unique adaptation to local environments and their alignment with regional agricultural and culinary traditions. Their resilience and close ties to local cultures also help preserve local economies and food traditions, while maintaining biodiversity and genetic diversity, which are crucial to the poultry industry's resilience in the face of future challenges. Nevertheless, the percentages of reduction over kilo of chicken weight when using the alternative experimental diets have been similar in both breeds, which supports the validity of these diets in commercial breeds as a strategy to reduce the impact of the poultry sector. With respect to the results of the impact on the production of 1 kilogram of eggs, we observed a greater impact on the local Spanish breed than on the Tunisian commercial breed, for the same reason explained above. In the case of using commercial laying hens breed, the literature reports data quite similar to ours in terms of impact per kilogram of eggs (Estrada-González et al., 2020).

In addition, in this project, it has been shown that early slaughter of native breed animals is more environmentally favorable than later slaughter, as was observed in the pilot in Italy.

The success of these pilot studies demonstrates that the approach of the new experimental poultry feeding programs can have a significant positive impact on the entire poultry production chain in the Mediterranean region, contributing to the development of more environmentally responsible animal husbandry and the adoption of practices that favor the sustainability and resilience of the sector in the face of current and future global challenges. These results are crucial for both producers and consumers, as they provide evidence of the effectiveness of dietary changes in meeting sustainability goals and satisfying consumer demand for environmentally friendly animal products.

4 Conclusions

Having commented and discussed the results of the environmental impact obtained by the different pilot studies within the framework of the SUSTAVianFEED project, can be concluded in a remarkable way and of great interest:

- The partial replacement of soybean meal and other imported ingredients in poultry diets with local or regionally sourced alternatives can reduce the environmental impact by more than 10% in terms of kg CO_2 eq/kg of diet or kg CO_2 eq/kg live weight and kg CO_2 eq/kg egg mass across all trials, with reductions of up to 43%, 39% and almost 18%, respectively, in some cases. This result highlights the effectiveness of these diets as a strategy to reduce the environmental impact of the poultry sector in Mediterranean countries. Additionally, they offer significant benefits for human health, ecosystems, and the sustainable use of natural resources.

- The incorporation of dried Black Soldier Fly larvae in poultry diets, as a partial substitute for soybean meal, initially presents a slightly higher impact than insect-free diets, although it achieves a reduction of at least 10% kg CO_2 eq/kg diet, with respect to the standard diet. In addition, it should be noted that when considering the productive parameters of the animals, reductions in impact per kilogram of live weight or egg mass are achieved, surpassing those of exclusively plant-based diets, achieving reductions of up to 38% kg CO_2 eq/kg live weight and 15% kg CO_2 eq/kg egg mass compared to a Control diet.

- The alternative experimental diets used in SUSTAvianFEED are equally effective in reducing the environmental impact of the poultry sector, both for local breeds and for the commercial breeds commonly used in intensive production systems.

In conclusion, this project highlights the effectiveness of replacing imported soybeans and cereals with local alternative ingredients, contributing to a more sustainable and responsible production system, less dependent on imported ingredients, and aligned with the growing consumer demand for more environmentally friendly products. Although larval production has a slightly higher environmental impact than other ingredients, its nutritional benefits, especially in terms of protein and energy, together with its ability to improve production parameters, position it as a promising and environmentally beneficial ingredient. Furthermore, the results observed in this project reveal that these experimental diets are effective in both local and commercial breeds, offering us a new perspective to implement new and more sustainable feeding programs in commercial poultry production, not only covering local breeds, but also the usual commercial breeds used in intensive poultry production, supporting the long-term sustainability of the sector and contributing to a more sustainable and resilient poultry production.

5 References

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6 ANNEX 1

This Annex shows the impacts caused by the experimental diets of all the pilots when carrying out the environmental impact study with the ILCD 2011 Midpoint+ V1.11 method, where, in addition to the impact on Climate change, the impacts on the 16 categories analyzed are shown.

Figure 1.1. Impacts obtained per ton of experimental diet of the Italian pilot on all categories studied with the ILCD Midpoint method

Category	Unit	Control	ALTER	ALT+INSECT (UNITO)	ALT+INSECT (Spain)
Climate change	kg CO2 eq	2,05	1,39	1,46	1,55
Ozone depletion	kg CFC-11 eq	2,65E-8	2,97E-8	3,15E-8	3,17E-8
Human toxicity, non-cancer effects	CTUh	1,68E-6	2,67E-6	2,54E-6	2,55E-6
Human toxicity, cancer effects	CTUh	2,59E-8	5,06E-8	4,86E-8	4,87E-8
Particulate matter	kg PM2.5 eq	0,000783	0,000513	0,000527	0,000538
Ionizing radiation HH	kBq U235 eq	0,0324	0,0366	0,0482	0,0484
Ionizing radiation E (interim)	CTUe	7,46E-7	8,2E-7	8,89E-7	8,91E-7
Photochemical ozone formation	kg NMVOC eq	0,0148	0,00796	0,00832	0,00925
Acidification	molc H+ eq	0,0246	0,018	0,018	0,0188
Terrestrial eutrophication	molc N eq	0,0982	0,077	0,0762	0,0801
Freshwater eutrophication	kg P eq	0,000215	0,000317	0,000302	0,000302
Marine eutrophication	kg N eq	0,0145	0,0134	0,013	0,0134
Freshwater ecotoxicity	CTUe	24,1	26,7	25,5	25,6
Land use	kg C deficit	27	26,3	25,1	25,1
Water resource depletion	m3 water eq	0,0899	0,0749	0,0769	0,0769
Mineral, fossil & ren resource depletion	kg Sb eq	1,57E-6	2,62E-6	2,76E-5	2,76E-5

CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (UNITO): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Turin. ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain). E: exponential

Figure 1.2. Impacts obtained per ton of experimental diet of the Spanish pilot on all categories studied with the ILCD Midpoint method

Category	Unit	Control	ALTER	ALT+INSECT
Climate change	kg CO2 eq	2,5E3	2,21E3	2,25E3
Ozone depletion	kg CFC-11 eq	1,87E-5	1,96E-5	2,21E-5
Human toxicity, non-cancer effects	CTUh	0,00302	0,0029	0,00276
Human toxicity, cancer effects	CTUh	3,52E-5	3,24E-5	3,11E-5
Particulate matter	kg PM2.5 eq	0,906	0,868	0,866
Ionizing radiation HH	kBq U235 eq	23,9	33,4	45,8
Ionizing radiation E (interim)	CTUe	0,00206	0,0022	0,00221
Photochemical ozone formation	kg NMVOC eq	17	15,5	15,6
Acidification	molc H+ eq	30,6	28,6	28,2
Terrestrial eutrophication	molc N eq	125	115	113
Freshwater eutrophication	kg P eq	0,29	0,271	0,257
Marine eutrophication	kg N eq	17	15,8	15,3
Freshwater ecotoxicity	CTUe	1,69E4	1,73E4	1,65E4
Land use	kg C deficit	2,85E4	2,67E4	2,53E4
Water resource depletion	m3 water eq	39,5	104	105
Mineral, fossil & ren resource depletion	kg Sb eq	0,00134	0,00629	0,0326

CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT + INSECT: ALTER diet plus 5% whole dehydrated Black Soldier Fly. E: exponential

Figure 1. Impacts obtained per ton of experimental diet of the meat chickens' Turkish pilot on all categories studied with the ILCD Midpoint method on starter phase

Category	Unit	Control	ALTER	ALT+INSECT
Climate change	kg CO2 eq	3,34E3	1,9E3	1,93E3
Ozone depletion	kg CFC-11 eq	2,68E-5	2,59E-5	2,85E-5
Human toxicity, non-cance	CTUh	0,00167	0,00182	0,00171
Human toxicity, cancer effe	CTUh	2,63E-5	2,31E-5	2,22E-5
Particulate matter	kg PM2.5 eq	0,844	0,608	0,627
Ionizing radiation HH	kBq U235 eq	32,7	37,1	50,5
Ionizing radiation E (interir	CTUe	0,000471	0,000519	0,000623
Photochemical ozone form	kg NMVOC eq	28,3	15,8	15,5
Acidification	molc H+ eq	33,1	23,2	23,1
Terrestrial eutrophication	molc N eq	152	105	103
Freshwater eutrophication	kg P eq	0,248	0,227	0,208
Marine eutrophication	kg N eq	19,2	15,1	14,7
Freshwater ecotoxicity	CTUe	2,01E4	2,2E4	2,15E4
Land use	kg C deficit	2,66E4	2,21E4	2,07E4
Water resource depletion	m3 water eq	73,2	125	131
Mineral, fossil & ren resour	kg Sb eq	0,00152	0,00456	0,0314

Control: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT: diet Alternative with 5% whole dehydrated Black Soldier Fly larvae. E: exponential

Figure 2. Impacts obtained per ton of experimental diet of the meat chickens' Turkish pilot on all categories studied with the ILCD Midpoint method on grower phase

Category	Unit	Control	ALTER	ALT+INSECT
Climate change	kg CO2 eq	3,13E3	1,88E3	1,9E3
Ozone depletion	kg CFC-11 eq	2,71E-5	2,63E-5	2,89E-5
Human toxicity, non-cance	CTUh	0,0017	0,00176	0,00165
Human toxicity, cancer effe	CTUh	2,58E-5	2,24E-5	2,16E-5
Particulate matter	kg PM2.5 eq	0,828	0,617	0,635
Ionizing radiation HH	kBq U235 eq	33	38,6	51,8
Ionizing radiation E (interir	CTUe	0,00043	0,00049	0,000593
Photochemical ozone form	kg NMVOC eq	26,9	15,4	15,1
Acidification	molc H+ eq	32,4	23,2	23,1
Terrestrial eutrophication	molc N eq	149	104	102
Freshwater eutrophication	kg P eq	0,237	0,218	0,199
Marine eutrophication	kg N eq	19,3	15,1	14,7
Freshwater ecotoxicity	CTUe	2,09E4	2,33E4	2,27E4
Land use	kg C deficit	2,52E4	2,11E4	1,97E4
Water resource depletion	m3 water eq	78,6	135	140
Mineral, fossil & ren resour	kg Sb eq	0,00152	0,00519	0,0319

Control: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT: diet Alternative with 5% whole dehydrated Black Soldier Fly larvae. E: exponential

Figure 3. Impacts obtained per ton of experimental diet of the meat chickens' Turkish pilot on all categories studied with the ILCD Midpoint method on finisher phase

Category	Unit	Control	ALTER	ALT+INSECT
Climate change	kg CO2 eq	3,03E3	1,87E3	1,89E3
Ozone depletion	kg CFC-11 eq	2,77E-5	2,66E-5	2,91E-5
Human toxicity, non-cance	CTUh	0,00168	0,00168	0,00157
Human toxicity, cancer effe	CTUh	2,53E-5	2,16E-5	2,08E-5
Particulate matter	kg PM2.5 eq	0,825	0,622	0,641
Ionizing radiation HH	kBq U235 eq	33,7	40,8	54
Ionizing radiation E (interir	CTUe	0,000416	0,00049	0,000592
Photochemical ozone form	kg NMVOC eq	26,3	15,3	15
Acidification	molc H+ eq	32,3	23,1	23
Terrestrial eutrophication	molc N eq	149	103	101
Freshwater eutrophication	kg P eq	0,229	0,211	0,192
Marine eutrophication	kg N eq	19,4	14,9	14,6
Freshwater ecotoxicity	CTUe	2,18E4	2,38E4	2,32E4
Land use	kg C deficit	2,4E4	2E4	1,85E4
Water resource depletion	m3 water eq	84,4	150	155
Mineral, fossil & ren resour	kg Sb eq	0,00154	0,00634	0,0329

Control: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT: diet Alternative with 5% whole dehydrated Black Soldier Fly larvae. E: exponential

Figure 4. Impacts obtained per ton of experimental diet of the meat chickens' Tunisian pilot (ISA-CM) on all categories studied with the ILCD Midpoint method on grower phase

Category	Unit	Control	ALTER	ALT+INSECT (Spain)	ALT+INSECT (Tunisia)
Climate change	kg CO2 eq	2,5E3	2,2E3	2,02E3	2E3
Ozone depletion	kg CFC-11 eq	2E-5	1,84E-5	1,99E-5	1,98E-5
Human toxicity, non-cancer effects	CTUh	0,00404	0,00364	0,00329	0,00329
Human toxicity, cancer effects	CTUh	4,25E-5	4E-5	3,62E-5	3,62E-5
Particulate matter	kg PM2.5 eq	1,35	1,07	0,963	0,955
Ionizing radiation HH	kBq U235 eq	24,3	22,4	34,4	34,4
Ionizing radiation E (interim)	CTUe	0,000279	0,000267	0,000357	0,000357
Photochemical ozone formation	kg NMVOC eq	23,4	18,5	16,4	16,2
Acidification	molc H+ eq	43,3	35,2	31,7	31,5
Terrestrial eutrophication	molc N eq	171	142	128	127
Freshwater eutrophication	kg P eq	0,326	0,325	0,294	0,294
Marine eutrophication	kg N eq	22	19,2	17,6	17,5
Freshwater ecotoxicity	CTUe	2,24E4	2E4	1,75E4	1,75E4
Land use	kg C deficit	2,69E4	3,07E4	2,85E4	2,85E4
Water resource depletion	m3 water eq	59,6	129	177	177
Mineral, fossil & ren resource depletion	kg Sb eq	0,0013	0,00126	0,0284	0,0284

CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain); ALT+INSECT (Tunisian): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in ISA-CM, Tunisian. E: exponential

Figure 5. Impacts obtained per ton of experimental diet of the meat chickens' Tunisian pilot (ISA-CM) on all categories studied with the ILCD Midpoint method on finisher phase

Category	Unit	Control	ALTER	ALT+INSECT (Spain)	ALT+INSECT (Tunisia)
Climate change	kg CO2 eq	2,5E3	2,2E3	1,99E3	1,98E3
Ozone depletion	kg CFC-11 eq	2,03E-5	1,87E-5	2E-5	2E-5
Human toxicity, non-cancer effects	CTUh	0,00409	0,00372	0,00328	0,00327
Human toxicity, cancer effects	CTUh	4,3E-5	4,08E-5	3,64E-5	3,64E-5
Particulate matter	kg PM2.5 eq	1,35	1,08	0,92	0,912
Ionizing radiation HH	kBq U235 eq	24,6	22,8	34,6	34,5
Ionizing radiation E (interim)	CTUe	0,000283	0,000271	0,000361	0,000361
Photochemical ozone formation	kg NMVOC eq	23,2	18,5	15,6	15,3
Acidification	molc H+ eq	43,3	35,7	30,4	30,2
Terrestrial eutrophication	molc N eq	171	144	124	123
Freshwater eutrophication	kg P eq	0,331	0,331	0,298	0,298
Marine eutrophication	kg N eq	22,1	19,5	17,3	17,2
Freshwater ecotoxicity	CTUe	2,24E4	2,02E4	1,73E4	1,73E4
Land use	kg C deficit	2,7E4	3,04E4	2,99E4	2,99E4
Water resource depletion	m3 water eq	60,5	125	201	201
Mineral, fossil & ren resource depletion	kg Sb eq	0,00131	0,00128	0,0283	0,0283

CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain); ALT+INSECT (Tunisian): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in ISA-CM, Tunisian. E: exponential

Figure 6. Impacts obtained per ton of experimental diet of the laying hens Tunisian pilot (ISA-CM) on all categories studied with the ILCD Midpoint method

Category	Unit	Control	ALTER	ALT+INSECT (Tunisia)	ALT+INSECT (Spain)
Climate change	kg CO2 eq	2,25	1,88	1,92	1,94
Ozone depletion	kg CFC-11 eq	1,81E-8	1,61E-8	1,87E-8	1,87E-8
Human toxicity, non-cancer effects	CTUh	3,58E-6	3,14E-6	2,99E-6	2,99E-6
Human toxicity, cancer effects	CTUh	3,77E-8	3,53E-8	3,39E-8	3,4E-8
Particulate matter	kg PM2.5 eq	0,00121	0,00086	0,000857	0,000865
Ionizing radiation HH	kBq U235 eq	0,0231	0,0208	0,0338	0,0338
Ionizing radiation E (interim)	CTUe	2,09E-6	2,08E-6	2,09E-6	2,09E-6
Photochemical ozone formation	kg NMVOC eq	0,0211	0,0149	0,015	0,0152
Acidification	molc H+ eq	0,0387	0,0285	0,0281	0,0283
Terrestrial eutrophication	molc N eq	0,153	0,116	0,113	0,114
Freshwater eutrophication	kg P eq	0,000289	0,000295	0,00028	0,00028
Marine eutrophication	kg N eq	0,0196	0,0161	0,0156	0,0157
Freshwater ecotoxicity	CTUe	20	17,8	17	17
Land use	kg C deficit	24,1	29,4	28	28
Water resource depletion	m3 water eq	0,0527	0,142	0,141	0,141
Mineral, fossil & ren resource depletion	kg Sb eq	1,3E-6	1,22E-6	2,76E-5	2,76E-5

CONTROL: conventional diet; ALTER: Alternative experimental diet with fewer imported ingredients and new alternative ingredients incorporated; ALT+INSECT (Spain): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in Cehegín (Spain); ALT+INSECT (Tunisian): diet ALTER plus 5% whole dehydrated Black Soldier Fly reared in ISA-CM, Tunisian. E: exponential

Figure 7. Impacts obtained per ton of experimental diet of the laying hens Rayhana pilot on all categories studied with the ILCD Midpoint method

Category	Unit	Control	ALT+INSECT (High Technology)	ALT+INSECT (Medium Technology)
Climate change	kg CO2 eq	743	848	772
Ozone depletion	kg CFC-11 eq	1,52E-5	1,78E-5	1,67E-5
Human toxicity, non-cancer effects	CTUh	0,00206	0,00196	0,00196
Human toxicity, cancer effects	CTUh	1,71E-5	1,66E-5	1,64E-5
Particulate matter	kg PM2.5 eq	0,332	0,356	0,338
Ionizing radiation HH	kBq U235 eq	18,6	31,6	27,2
Ionizing radiation E (interim)	CTUe	0,000216	0,000319	0,000283
Photochemical ozone formation	kg NMVOC eq	4,37	4,93	4,3
Acidification	molc H+ eq	13	13,3	12,7
Terrestrial eutrophication	molc N eq	58,8	58,9	56,4
Freshwater eutrophication	kg P eq	0,164	0,156	0,155
Marine eutrophication	kg N eq	10,4	10,1	9,91
Freshwater ecotoxicity	CTUe	9,46E3	9,05E3	9E3
Land use	kg C deficit	1,69E4	1,61E4	1,61E4
Water resource depletion	m3 water eq	18,2	23,3	21,3
Mineral, fossil & ren resource depletion	kg Sb eq	0,000747	0,0271	0,0189

[1]Control diet: diet with local vegetable products. ALT+INSECT (High technology) 95% Control diet plus 5% insect reared under high-tech conditions and high energy expenditure. ALT+INSECT (Medium technology): 95% Control diet plus 5% insect considering medium energy expenditure for its reared. E: Exponential