The PRIMA programme is supported under Horizon 2020 the European Union's Framework Programme for Research and Innovation

SUSTAVIANFEED

ALTERNATIVE ANIMAL FEEDS IN MEDITERRANEAN POULTRY BREEDS TO OBTAIN SUSTAINABLE PRODUCTS

IMPLEMENTATION OF PILOT ACTIVITIES

DELIVERABLE 3.2

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

PROJECT INFORMATION	
Type of Project	PRIMA Foundation Project
Topic	Topic 1.2.1-2020 (RIA/IA) Genetic conservation and animal feeds: Sub-topic B) Alternative animal feeds (IA) of the Annual Work Plan 2020 for the Partnership for Research and Innovation in the Mediterranean Area (PRIMA)
Grant Agreement No.	2015
Project Duration	01/04/2021 – 31/03/2025 (48 months)
Project Coordinator	SOCIEDAD AGRARIA DE TRANSFORMACION 2439 (ALIA)
Project Partners	UNIVERSITY OF MURCIA (UMU), UNIVERSITY OF TURIN (UNITO), INSTITUT SUPERIEUR AGRONOMIQUE DE CHOTT MARIEM (ISA-CM), ASSOCIATION RAYHANA (RAYHANA), ENTOMO CONSULTING S.L. (ENTOMO), EGE UNIVERSITY (EGE), FONDAZIONE SLOW FOOD PER LA BIODIVERSITA' ONLUS (SLOWFOOD)

DOCUMENT INFORMATION	
Title	D3.2. Report on pilot evaluation activities
Version	3
Release Date (dd.mm.yy)	19.11.2024
Work Package	3
Dissemination Level	PU

DOCUMENT AUTHORS AND AUTHORISATION	
Document Responsible	EGE
Contributors	UMU, UNITO, ISA-CM, RAYHANA, EGE
Reviewed by	ENTOMO, UMU

DOCUMENT AUTHORS AND AUTHORISATION	
Approved by	Consortium

DOCUMENT HISTORY			
Version	Date (dd.mm.yy)	Description	Implemented by
1.00	22.10.2024	The first draft of the deliverable to be reviewed	UMU, UNITO, ISA-CM, RAYHANA, EGE
1.1	24.10.2024	Second draft	ISA-CM
2.0	5.11.2024	The second draft is to be reviewed	ENTOMO, UMU
3	19.11.2024	Third draft	UMU
3.1	27.11.2024	The third draft is to be reviewed	UMU

SUMMARY

Soybean is currently the main source of protein for chicken diets. Local plant-origin protein sources and agri-industrial by-products can be alternatives to soybean. The inclusion of insects such as Black soldier fly (BSFL, *Hermetia illucens*) larvae in chicken diets as an alternative protein source has also received significant attention in improving the circularity of the value chain and sustainability of chicken production. Within the scope of the SUSTAvianFEED project, pilot studies of Work Package (WP) 3 and Task 3.2, were conducted in Spain, Italy, Tunisia, and Turkey to develop sustainable chicken diets to increase productivity and sustainability, especially in small-scale chicken farms. These pilot studies, in a real farming context, aimed to demonstrate the usability and suitability of alternative diets to corn-soybean-based diets in local chicken breeds in the Mediterranean region and in commercial slow and fast-growing broilers and laying hens. For the chicken meat studies, a local meat-type chicken breed was used in pilot studies in UNITO, a commercial slow-growing broiler strain was used in ISA-CM, and a local meat-type chicken and a fast-growing broiler strain was used in EGE. For egg-type chicken studies, UMU used a local breed and ISA-CM used a commercial strain. To improve socioeconomic opportunities in the rural North Africa region, RAYHANA engaged rural women to participate in the project. Rural women were supported by supplying local meat- and egg-type chickens, chicken diets, BSFL, and technical information in their own production conditions. The sustainable diets used in the pilot studies included local feedstuffs available in the country with or without supplemented with BSFL to fully or partly replace soybean and corn in chicken diets.

Task 3.2 of the SUSTAvianFEED project demonstrated the usability and suitability of alternative diets to corn-soybean-based chicken diets to produce sustainable chicken eggs and meat while preserving genetic diversity.

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ACRONYMS AND ABBREVIATIONS

Abbreviation	Description
UMU	University of Murcia
UNITO	University of Turin
ISA-CM	Institut Supèrieur Agronomique de Chott Mariem
RAYHANA	Rayhana Association for Women of Jendouba
EGE	Ege University
CON	Control diet
ALT	Alternative diet with local feedstuffs
BSFL	Black soldier fly larvae
ALT+BSFL	ALT diet supplemented with BSFL
Work Package	WP

1. INTRODUCTION

As the global demand for poultry products increases, the sustainability of production also gains importance. Economical and environmentally friendly sustainable practices need to be adopted when meeting the demand for eggs and chicken meat. To meet consumer demand, poultry production systems are directed toward finding new high-quality and sustainable protein sources for chicken diets, since soybean as a main protein source is the costliest and most restrictive feedstuff in chicken diet formulations (El-Deek et al., 2020; Research and Markets, 2021; CBV, 2021; Marchal et al., 2024). SUSTAvianFEED project aimed to develop sustainable chicken diets through the partial or total replacement of soybean and corn with local plant-origin protein sources such as peas, field beans, canola, and fava beans, and agri-industrial by-products such as peas protein, corn DDGs, sunflower seed meal, maize gluten, and brewery by-products. Additionally, in recent years, black soldier fly larvae (BSFL) have gained great interest as a sustainable and environmentally friendly alternative protein source in chicken diets (Leiber et al., 2015; Velten et al., 2018; Schiavone et al., 2019). The use of alternative raw materials of plant origin or agro-industrial origin together with BSFL could help increase the efficiency and sustainability of small-scale chicken rearing systems.

The main objective of WP3.2 of the SUSTAvianFEED project was to develop the project's pilot poultry innovative farming activities by using local breeds and local plant- or agri-industrial by-product origin protein sources and BSFL in chicken diets in Spain, Italy, Tunisia, and Turkey. The chickens, which were locally adapted breeds or hybrids, were fed a standard diet, and two sustainable diets that were developed during the WP2 studies of the project. The sustainable diets included local feedstuffs, agri-industrial by-products, and BSFL.

The general approach of the pilot studies of WP3, task 3.2 was to develop sustainable chicken egg and meat production and develop appropriate policies to reduce the negative effects of intensive agricultural systems.

2. METHODOLOGY

EGE, who served as Task leader of WP 3.2., organized two internal online meetings with project partners at the beginning of the WP3.2 pilot studies.

1. January 2022: The meeting aimed to overview the general aim and task of the pilot studies.

2. April 2022: The meeting aimed to update the pilot activities of WP3.2. The facilities for the pilot studies, housing conditions, pens, number of animals, number of replications, the data collection procedure, and calendar of pilot studies were discussed during the meeting.

The sustainable diets developed in WP2 were used in farming activities in Italy (UNITO), Spain (UMU), Tunisia (ISA-CM and RAYHANA), and Turkey (EGE). The pilot studies were conducted with egg-type chickens in UMU, RAYHANA, and ISA-CM and meat-type chickens in UNITO, ISA-CM, RAYHANA, and EGE.

Each partner organized an online Co-creation workshop to share the objectives, methods, and expected outputs of the pilot activities with relevant stakeholders in the country. This activity also aimed to evaluate the relevance of the project's KPIs for the stakeholders. During the meeting, the aim of the SUSTAvianFEED project, expected impacts, concepts, work plan, and methodologies such as housing conditions, animal material, diets, and parameters to be measured were presented and discussed. At the end of the workshop, a survey was conducted to obtain the partners' opinions. Thus,

each stage of the pilot study was re-evaluated in line with the opinions of the partners before starting pilot studies in each country.

2.1. Ethical approval

2.1.1. UMU

Ethical approval for the experimental protocol (15.11.2022, registration number 202290000672437, identification number A13230109) was obtained from the Region de Murcia, Consejeria de Agua, Agricultura, Ganaderia, Pesca, Medio Ambiente y Emergencias, Direction General de Ganaderia, Pesca y Acuicultura, Murcia, Spain. In alignment with the European Union's strategies for the use and care of experimental animals, as outlined in Directive 2010/63/EU of the European Parliament and Council dated 22 September 2010.

1. 2.1.2. UNITO

All birds included in the study were reared under consistent management and environmental conditions, in accordance with the European Union's organic farming standards [Regulation (EC) No. 834/2007]. For the first 38 days of life, the chicks were kept in a brooder with controlled environmental settings.

2. 2.1.3. ISA-CM

Along both trials (Meat-type and egg-type), animals have been handled in accordance with the principles and specific guidelines of the Tunisian regulation N° 2005-95 (18 October 2005) concerning breeding and slaughtering. Ethical approval for the experimental protocol (CEEA-ENVM 71/23) was obtained from the National Animal Ethics Committee -National School of Veterinary Medicine, Sidi Thabet, Tunisia.

3. 2.1.4. EGE

The experimental procedures were approved by the Ege University Local Ethics Committee of the Agriculture Faculty (Approval no: 2022/02, 3-12-7316).

2. 2.2. Changes compared to the initial protocol

1. 2.2.1. UMU

The general protocol of the UMU pilot was not changed, only the diets that were presented as preliminary in WP2 were reformulated. Initially, three diets were proposed preliminarily: a control diet, an alternative diet with vegetable-based ingredients, and a 3% inclusion of *Hermetia illucens* larvae, and a second diet identical to the previous one but with a 6% inclusion of larvae. Ultimately, it was decided to formulate a control diet, as initially planned, an alternative diet using only vegetable-based alternatives (without insects), and a third diet identical to the alternative one but with a 5% inclusion of insect larvae. This change was made for two reasons. First, it was considered valuable to evaluate the effect of a diet with alternative vegetable ingredients separately, while another diet would incorporate a more substantial percentage of insects, rather than comparing two diets where the only difference would be a 3 percentage point increase in insect inclusion, as it was thought that the substantial differences could be diluted. The second reason was based on the observation of positive preliminary results from the UNITO pilot, which was already using a 5% inclusion of larvae.

2. 2.2.2. UNITO

There was no change in the protocol.

3. 2.2.3. ISA-CM

The experimental diets: Since the Tunisian regulations prohibit the use of pasta waste in animal feeds, the alternative ingredients used for the experimental diet formulation were restricted to triticale, canola meal, fava beans, and BSFL.

Animals and calendar: Initially it was planned that, for the trials with meat-type and egg-type chickens, local chicken eco-types, characterized by a very slow growth rate and thus a longer fattening or rearing period (before reaching the stage of laying), would be used, respectively. However, due to the significant delay (more than 2 and a half years delay) induced by the non-implementation of the insect farming unit in due time, it had been decided during a **General Assembly,** held on June 20, 2023, to apply a new working plan using a slow-growing broilers breed (SASSO T44) and a commercial laying strain (Lohmann White line), for the trials with meat-type and egg-type chickens, respectively. Indeed, it was almost impossible to obtain, during a short period (3 months from July to end of September 2023), a homogeneous flock of local chick ecotypes with the same sex, age, and weight to conduct the fattening trial in October 2023. The same applied to the laying trial. Since, it was difficult to have, at the beginning of the laying trial (April 2024), the required number of local pullets' ecotypes with a similar age and homogenous weight, a commercial strain (Lohmann White line) was used. This option also made it possible to reduce the duration of the laying trial so that it does not extend beyond June 2024 to avoid increased mortality and welfare concerns associated with high summer temperatures. A **clarification note justifying these changes has been developed and submitted.**

4. 2.2.4. RAYHANA

There is no change compared to the initial protocol, the farmers kept the same protocol.

5. 2.2.5. EGE

At the meeting held with Turkish feed industrialists and chicken meat industry representatives during the living lab activities, the sector's interest in the SUSTAvianFEED project and local alternative agro-industrial by-products and BSFL as protein sources attracted attention. It was brought up at the consortium meeting that the effect of diets should be tested not only on domestic lines but also on commercial breeds. EGE undertook this task and a commercial strain (Cobb-500) was also added as animal material to the experimental design of the pilot studies in Turkey.

3. 2.3. Definitive calendar of pilot implementation

1. 2.3.1. UMU

The pilot study with egg-type chickens was carried out between September 2022 and March 2023, covering everything from the reception of animals, adaptation, experimental tests, sacrifice, and sampling. The definitive calendar of the pilot studies is presented in Table 1.

Table 1. Definitive calendar of pilot studies at the UMU

Date	Days of age	Activity (measurements and sampling)
20/09/22	120	Reception and adaptation period Body weight measurement Leg mark
09/11/22	170	End of adaptation period (Initial weighting)
15/11/22	176	1st video recording

05/12/22- 15/12/22	196-206	1st Body weight measurement 1st welfare protocol 1st Tonic immobility test 1st Blood extraction 1st Egg quality analysis 1st Tasting event
14/12/22	205	2nd video recording
09/01/23- 24/01/23	231-245	2nd Body weight measurement 2nd Welfare protocol 2nd Tonic immobility test 2nd Blood extraction 2nd Egg quality analysis 2nd Tasting event
23/01/23	244	3th Video recording
16/02/23	268	4th Video recording
17/02/23- 08/03/23	269-290	3rd Body weight measurement 3rd Welfare protocol 3rd Tonic immobility test 3rd Blood extraction 3rdEgg quality analysis) 3rd Tasting event
08/03/23	290	5th video recording
14/03/23- 28/03/23	296-310	Digestibility trial Slaughtering Corticosterone feathers

2. 2.3.2. UNITO

The pilot study with meat-type chickens was started on May 27 and ended on October 9, 2022. A definitive calendar of studies is presented in Table 2.

Table 2. Definitive calendar of pilot studies at the UNITO

Date	Days of age	Activity (measurements and sampling
27/05/22	39	1st Body weight measurement Wing tags 1st Leg condition 1st Feather condition
30/05/22	42	1st Corticosterone feces 1st Microbiota feces 1st Tonic immobility
31/05/22	43	1st Avoidance Test
09/06/22	52	1st Video recording
17/06/22	60	2nd Body weight measurement
30/06/22	73	2nd Video
08/07/22	81	3rd Body weight measurement
18/07/22	91	2nd Corticosterone feces 2nd Microbiota feces 2nd Tonic immobility
19/07/22	92	2nd Avoidance Test
22/07/22	95	3rd Video recording
29/07/22	102	4th Body weight measurement 2nd Leg scoring 2nd Feather condition scoring
11/08/22	115	4th Video recording
19/08/22	123	5th Body weight
01/09/22	136	5th Video
05/09/22	140	3rd Corticosterone feces 3rd Microbiota feces 3rd Tonic immobility
06/09/22	141	3rd Avoidance Test
12/09/22	147	6th Body weight 3rd Leg scoring 3rd Feather condition scoring

13/09/22	148	1st Slaughtering
14/09/22	149	Meat quality
22/09/22	157	6th Video recording
01/10/22	166	4th Corticosterone feces 4th Microbiota feces 4th Tonic immobility
02/10/22	167	5th Avoidance Test
08/10/22	173	7th Body weight 4thth Leg scoring 4thth Feather conditions scoring
09/10/22	174	2nd Slaughtering

3. 2.3.3. ISA-CM

Pilot studies with meat-type chickens and egg-type chickens were carried out between September 19 and December 14, 2023, and March 7 and June 4, 2024, respectively. Tables 3 and 4 present the definitive calendar of pilot studies with meat-type and egg-type chickens at the ISA-CM.

Table 3. Definitive calendar of pilot studies with meat-type chickens at the ISA-CM

Date	Days of age	Activity (measurements and sampling)
19/09/23	1	Reception of chicks
18/10/23	30	Identification by wing mark Starting adaptation period
25/10/23	37	Start of fattening trial 1st Body weight
01/11/23	44	2nd Body weight
07/11/23	50	1st Video recording
08/11/23	51	3rd Body weight 1st Leg scoring

		1st Feather and skin condition scoring
09/11/23	52	1st Tonic Immobility 1st Feces corticosterone
10/11/23	53	1st Feces sampling 1st Envir Microbiota 1st Avoidance test
11/11/23	54	4th Body weight
20/11/23	63	2nd Tonic Immobility 2nd Feces corticosterone
21/11/23	64	2nd Video recording
22/11/23	65	5th Body weight 2nd Leg scoring 2nd Feather and skin condition scoring
23/11/23	66	1st Slaughter 1st Histological sampling of the intestine 1st Microbiota sampling
24/11/23	67	2nd Feces sampling 2nd Envir Microbiota 2nd Avoidance test
28/11/23	71	3rd Video recording
29/11/23	72	6th Body weight 3rd Leg scoring 3rd Feather and skin condition scoring
30/11/23	73	3rd Tonic immobility 3rd Feces corticosterone
01/12/23	74	3rd Feces sampling 3rd Envir Microbiota 3rd Avoidance test
06/12/23	79	7th Weighting 4th Leg scoring

		4th Feather and skin condition scoring
07/12/23	80	4th Video recording
08/12/23	81	4th Tonic immobility 4th Feces corticosterone
13/12/23	86	2nd Slaughter Final body weight and carcass traits
14/12/23	87	3rd Slaughter 2nd Histological sampling of the intestine 2nd Microbiota sampling

Table 4. Definitive calendar of pilot studies with egg-type chickens in the ISA-CM

Date	**Days of age**	**Activity[1] (measurements and sampling)**
07/03/24	188	Identification of chicken leg rings
25/03/24	206	Initial body weight measurement 1st Body scoring
01/04/24	213	1st Physical quality of eggs
03/04/24	215	1st Avoidance Distance test
08/04/24	220	2nd Physical quality of eggs
09/04/24	221	1st Novel Object test
11/04/24	223	1st Video recording
15/04/24	227	3rd Physical quality of eggs
23/04/24	235	3rd Avoidance Distance test
25/04/24	237	2nd Video recording
26/04/24	238	2nd Novel Object test
29/04/24	241	4th Physical quality of eggs
30/04/24	242	2nd Body scoring
06/05/24	248	4th Physical quality of eggs
09/05/24	251	3rd Video recording

10/05/24	252	4rd Avoidance Distance test
11/05/24	253	3rd Novel Object test
13/05/24	255	5th Physical quality of eggs
20/05/24	262	6th Physical quality of eggs
23/05/24	265	4th Video recording
25/05/24	267	4th Novel Object test
26/05/24	268	5th Avoidance Distance test
27/05/24	269	7th Physical quality of eggs
02/06/24	275	5th Video recording
03/06/24	276	Final body weight measurement 3rd Body scoring 8th Physical quality of eggs &yolk sampling
04/06/24	277	Slaughtering

4. 2.3.4. RAYHANA

The pilot study was conducted between 25 September 2023 and 3 March 2024. A definitive calendar of pilot studies in RAYHANA is given in Table 5.

Table 5. Definitive calendar of pilot studies with farmers in the RAYHANA

Date	Activity (measurements and sampling)
25-26/09/23	Distribution of feed to the farmers
30/09/23	Distribution of the rest of the feed
04/10/23	Chicks attend 56 days
05/10/23	The distribution of male chickens Body weight measurement
29/11/23	Distributions of females to the farmers (laying hens)
07/12/23	Measure the body weight of male chickens (roosters)
13/12/23	1st Control the behavior and quality of feathers

	2nd Activity level of hens and roosters
30/12/23	2nd Control the behavior and quality of feathers 2nd Activity level of hens and roosters
15/01/24	3rd Control the behavior and quality of feathers 3rd Activity level of hens and roosters
30/01/24	4th Control the behavior and quality of feathers 4th Activity level of hens and roosters
03/03/24	2nd Body weight measurement

RAYHANA is also in the state to assist farmers and support them with technical training. During the trial with meat-type chickens, body weight, feed consumption, and feed conservation were recorded in a table for each farmer, and a technician who collected the data also visited every week. Feather condition, foot pad, and hock burn were evaluated at the slaughter age.

During the trial with laying hens (duration was two months) egg production, cracked eggshell, mortality, distributed feed, and body weight were recorded at the beginning of the experiment (30 weeks of age), middle (34 weeks of age), and end of the trial (38 weeks of age). Welfare issues such as feather condition, foot pad, and huck burn were evaluated at the end of the trial.

5. 2.3.5. EGE

The pilot study was started on May 12, 20122 and ended on July 5, 2022. The calendar of pilot implementation is given in Table 6. During slaughtering, samples were collected for meat quality.

Table 6. Definitive calendar of pilot studies at the EGE

Date	Days of age	Activity (measurements and sampling)
12/05/22	0	Reception of chicks from Cobb500 (commercial) and Anadolu-T (local) 1st Body weight (both strains) Wing banding
23/05/22	10	2nd Body weight (both strains) 1st Feed consumption (both strains)
26/05/22	14	1st Behavior (both strains)
06/06/22	25	3rd Body weights (both strains) 2nd Feed consumption (both strains)
09/06/22	28	1st Tonic immobility duration (both strains)
16/06/22	35	2nd Behavior (both strains) 2nd Tonic immobility (Cobb500 chickens)
19/06/22	38	1st Feces collection (Cobb500)
20/06/22	39	4th Body weight (both strains) 3rd Feed consumption (both strains) 1st Scoring of footpad (both strains) 1st Scoring of feather cleanliness (both strains) 1st Blood and organ sampling (Cobb500 chickens)
21/06/22	40	1st Slaughtering (Cobb500 chickens)
29/06/22	48	3th Behavior (Anadolu-T chickens)

		3rd Tonic immobility (Anadolu-T chickens)
03/07/22	53	2nd Feces collection (Anadolu-T chickens)
04/07/22	54	5th Body weight (Anadolu-T chickens) 4th Feed consumption (Anadolu-T chickens) 2nd Scoring of footpad dermatitis (Anadolu-T chickens) 2nd Scoring of feather cleanliness (Anadolu-T chickens) 2nd Blood and organ sampling (Anadolu-T chickens)
05/07/22	55	2nd Slaughtering (Anadolu-T chickens)

4. 2.4. Experimental house, animals, and experimental design

1. 2.4.1. UMU

Experimental house: The pilot counted a total of 15-floor pens with a floor consisting of a mixture of straw and soil. Each treatment had 5 replicates. All pens measured 100 cm × 100 cm × 300 cm and were equipped with two water dispensers, a central feeder, a nest, and perches of sufficient length for resting.

Animals: A total of 120 Isazul-laying hens were used for the study. The Isazul breed, known for being a robust layer is well-suited to the climate of south-eastern Spain. The hens, aged 17 weeks, were sourced from a commercial farm (Granja Santa Isabel,

Córdoba, Spain). Each hen was weighed and then assigned to one of the 15-floor pens, with 8 hens per pen, ensuring an even distribution of body weight.

Experimental design: This study was carried out at the experimental farm of the Veterinary Faculty at the University of Murcia (Spain).

The experiment began once all replicates had entered the laying phase at 23 weeks of age. Following this, the pens were randomly allocated to one of three dietary treatments, with five replicates per treatment. Throughout the study, all hens were kept under uniform management conditions, including *ad libitum* access to feed and water, a 16-hour light and 8-hour dark cycle, and ambient environmental conditions (temperature ranging from 13.2°C to 18.2°C and relative humidity between 63.1% and 69.0%). The experiment lasted 15 weeks and was divided into three sub-periods (Period 1: 23 to 27 weeks of age; Period 2: 27 to 32 weeks of age; Period 3: 32 to 38 weeks of age). The animals were subjected to partial general sampling on three separate occasions, corresponding with the conclusion of each experimental sub-period. Alongside these general samplings, continuous data collection was carried out for feed intake and egg production throughout the entire experimental phase. Additionally, a comprehensive final sampling, which included the slaughter of the animals for tissue and other kinds of sample collection, was conducted at the end of the experiment to conclude the trial.

Figure 1. Pilot studies with egg-type layers at UMU

2. 2.4.2. UNITO

Experimental house: The experimental house had 18 pens, each measuring 2.0 x 3.2 meters, using rice husks as litter, and with an unrestricted outdoor area of the same dimensions as the pen. The environmental conditions in the poultry house, including temperature, lighting, ventilation, and humidity, were left natural and unmanaged throughout the study. On day one, they were exposed to 23 hours of light and 1 hour of darkness, with the dark period gradually extended by 1 hour daily from day two, reaching 18 hours of light and 6 hours of darkness by day six.

Animals: At 39 days old, 144 birds from the Bianca di Soluzza breed were moved to the experimental poultry facility, where they were evenly distributed into 18 pens, each holding 8 birds.

At this stage, each chick, hatched from eggs collected at the Avian Conservation Centre for Local Genetic Resources at the University of Turin (Italy), was individually tagged with wing markers and selected based on an average body weight of 316.8 ± 1.4 g. After hatching, the chicks were sexed by an expert, and only males were retained for the trial.

Experimental design: The birds were divided into three dietary treatment groups, each comprising 6 pens (with each pen acting as a replicate).

The study ran for 135 days, beginning when the chicks were 39 days old and concluding at 174 days with the final slaughter. An intermediate slaughter was also conducted at 147 days, where 2 birds per pen were selected for processing, amounting to a total of 36 birds.

The trial was conducted between May and October 2022. The average monthly temperatures during this period were: May, 20.5°C (min. 10°C, max. 31°C); June, 24°C (min. 15°C, max. 33°C); July, 26.5°C (min. 17°C, max. 36°C); August, 27.5°C (min. 18°C, max. 37°C); September, 20°C (min. 10°C, max. 30°C); and October, 17°C (min. 9°C, max. 25°C).



Figure 2. Pilot studies with meat-type chickens at UNITO

3. 2.4.3. ISA-CM

Experimental house: The trials with meat-type and egg-type chickens were conducted at the animal facility of ISA-Chott Mariem, Sousse. Birds were alloted over 15 pens (L = 6 m, W = 1.125 m, and H = 1.75 m) providing a total area of 6.75 m^2. The pen's internal area, representing 2/3 of the surface, was covered with sandwich panels and bedded with wood shavings litter. Each pen featured 2.25 meters of perches, a 10-liter- drinker, a suspended circular 10kg-feeder, and an 8-megapixel camera to monitor the behavior of the birds. For the laying trial, three nest boxes (840 cm^2 each) were placed in each pen. Lighting was provided by natural light supplemented with 5 white neon tubes light with a luminous efficacy of about 40 to 70 lm/W. The lighting program was 16 L:8 D.

Animals: The pilot studies were carried out on both meat-type and egg-type chickens in ISA-CM.

Meat-type chickens: A total of 180 SASSO T44 (slow-growing breed) male broiler chicks were used for meat-type chicken studies

Egg-type chickens: A total of 150 Lohmann White hens of 30 weeks of age were used for trials with egg-type chickens.

Experimental design: The meat-type chicks were randomly allocated to three diets, with 5 replicates, and 12 birds/pen/replication (3 diets x 5 pen replicates x 12 birds/pen replication).

Egg-type chickens were divided into three dietary treatment groups, each consisting of 5 pens and 10 hens/replicate (3 diets × 5 pen replicates × 10 hens/pen replicate).

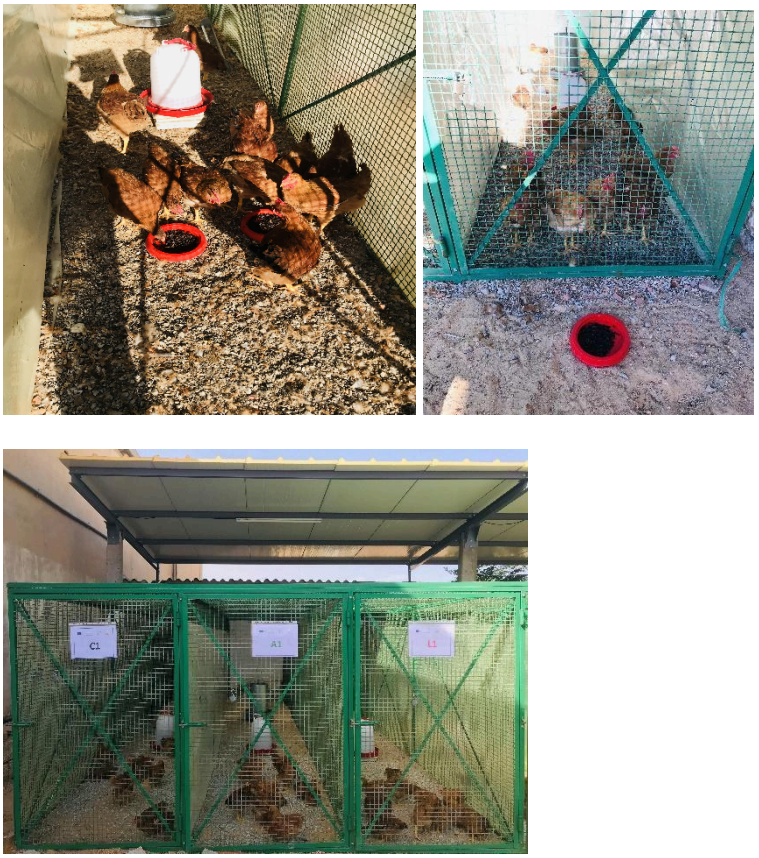

Figure 3. Pilot studies with egg-type (top) and meat-type (bottom) chickens at ISA-CM

4. 2.4.4. RAYHANA

Experimental house: There were 16 women farmers in total. Each farmer had two pens, each ranging between 8 and 12 m^2, 2/3 of each chicken house is closed, and 1/3 is open. Rayhana supported farmers with construction materials to ensure they had similar breeding conditions. The primary materials used in construction were clay, wood, cement, and wire mesh for open areas.

The farmers were located at Fernana, Bulla Regia, Souk Essebt, Ghardimaou, and Bouslem (Figure 4).

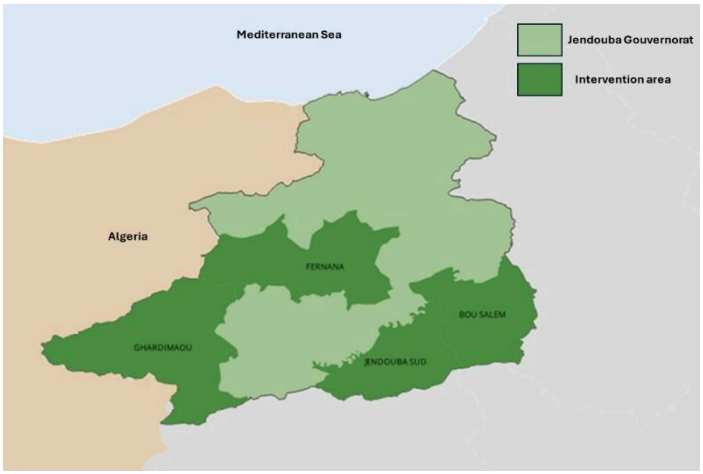

Figure 4. The area of the project implantation carried out by RAYHANA

Animals: Fertilized eggs of slow-growing Geant chickens were collected and incubated in a commercial hatchery (egg capacity is 1120) belonging to one of the farmers interviewed. After hatching the chicks were weighed and reared to 56 d by feeding a conventional corn-coy bean meals-based diet. At 56 d, after sexing, the males with average weight were distributed to 5-8 farmers between 50-60 chicks each for meat-type trials. The females were kept at the same farm till the laying age for laying-hen trial. The females were distributed to the same farmers when they reached to the laying age.

Experimental design: Each farmer used two diets as control and Control+ BSFL, for each experiment. Each farmer had two pens, one for the control diet and the other for the experimental diet. The experiment was ended at 112 d.

Figure 5. Pilot studies conducted under the women farmers' conditions at RAYANA

5. 2.4.5. EGE

Experimental house: The experimental pilot study was carried out at the Poultry Facilities of EGE University, Department of Animal Science, Faculty of Agriculture.

The experimental house was temperature and humidity-controlled. There were 4 rooms with 12 pens in each. The bedding consisted of wood shavings. A feeder and nipple drinker were provided in each pen. The dimensions of each pen were 1.4m X 1.2 m.

Animals: A total of 504 one-day-old chicks obtained from local (Anadolu-T dam line) and commercial (Cobb-500) breeders were used (252 chicks from each strain).

Anadolu-T is a registered genotype for broiler production in Turkey and within the scope of the selection and breeding program at the Transitional Zone Agricultural Research Institute, the Ministry of Agriculture and Forestry, in Eskişehir, Turkey. The pure lines of Anadolu-T and their crosses had lower body weights and impaired feed conversion ratio but higher livability compared to commercial strains (Erensoy and Sarıca, 2023). These chickens can be used as an alternative for niche markets and small local producers.

The birds were obtained from breeders of similar age. The chicks were weighed individually and wing-banded. All chicks were vaccinated against Newcastle disease, Gumboro disease, and infectious bronchitis at the hatchery. On d 10 and 18, Newcastle and infectious bronchitis vaccine recalls were performed.

Experimental design: The chicks were randomly placed in a total of 36 pens in three environmentally controlled rooms. The chicks from each strain were represented in 6 pens/room. There were 14 chicks per pen. The birds/strain in each pen received 3 diets, providing 6 replications/diet/strain.

The birds were reared at standard rearing conditions. The brooding temperature was maintained at approximately 32°C for the first 3 d, then decreased by 3°C weekly until 21 d. The temperature was kept between 21-24°C till the end of the experiment. The lighting regime was 23 L:1 D during the first 3 days, then gradually reduced to 18 L:6 D. The experiment ended at 40 d for Cobb50 and 55 d for Anadolu-T.

Figure 6. Pilot studies with meat-type chickens at EGE

5. 2.5. Experimental diets

1. 2.5.1. UMU

A standard diet containing soybean, corn, and wheat was developed as the control diet (CON). The second diet, referred to as the alternative (ALT) diet, included a partial replacement of soybean meal and corn with locally sourced plant-based alternatives, such as pea meal, corn-dried distillers' grains (DDGs), and a higher proportion of sunflower meal. For the third dietary treatment, the ALT diet was further supplemented with 5% whole dehydrated BSFL (ALT+BSFL). This addition was adjusted weekly according to the total dry matter intake from the previous week (ALT+DBSFL). The larvae were provided by ENTOMO Consulting S.L., based in Cehegín, Murcia, Spain. Both the CON and ALT diets were formulated to be isoenergetic and isonitrogenous, ensuring they met or exceeded the nutritional requirements for laying hens as established by the Spanish Foundation for the Development of Animal Nutrition (FEDNA, 2018). The feeds were administered ad libitum in identical feeders. The hole dry BSFL were provided separately in an additional metallic feeder, every day at the same hour (10:00 AM). To avoid influence on the behavior of the animals and interferences in the results, the rest of the replicates were provided with an identical metallic feeder (empty).

The ingredients of the CON and ALT diets are given in Table 7.

Table 7. Ingredients and analyzed proximate composition of the control and alternative diets used in pilot activities at the UMU

Ingredients, g/kg	Diets[1]	
	CON	ALT
Corn	415.8	352.9
Soybean meal (46% crude protein)	219.7	150.0
Wheat	140.0	146.8
Calcium carbonate	86.9	89.0
Corn DDGs[2]	-	74.6
Soybean hulls	30.5	-
Sunflower meal (28% crude protein)	25.0	60.0
Peas	-	55.6
Soybean oil	25.0	25.0
Barley	20.0	25.0
Wheat middling	16.7	0.9
Monocalcium phosphate	6.7	5.2
Diatomaceous earth	5.0	5.0
Premix [3]	3.3	3.3
Sodium chloride	2.5	2.0
DL–methionine (99%)	1.9	1.8
Sodium bicarbonate	0.7	1.2
L–lysine 50	0.3	1.6
Analyzed proximate composition		
Dry matter	909	912
Crude protein	161	162
Ether extract	78.0	71.0
Crude fiber	69.0	63.0
Ash	120	117
Starch	380	363
Calcium	38.4	37.3
Total phosphorus	5.95	5.70
Indispensable amino acid		
Arginine	7.91	8.27
Histidine	2.94	3.02
Isoleucine	4.88	4.60
Leucine	10.0	10.4

Lysine	8.27	8.48
Methionine	4.87	5.82
Methionine + cysteine [5]	8.86	9.14
Phenylalanine	5.64	5.69
Threonine	4.78	4.96
Valine	6.41	7.04

[1]CON: Control diet. ALT: Alternative diet with soybean meal partly replaced with alternative plant ingredients. [2] DDGs, dried distillers' grains with solubles. [3] Premix provided per kilogram of feed: Vitamin A, 7500 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 of 6-phytase EC 3.1.3.26 (1 FTU is the amount of enzyme that liberates 1 micromole of inorganic phosphate per minute from sodium phytate at pH 5.5 and 37 °C); and 1500 EPU of endo-1,4-β-xylanase EC 3.2.1.8 (1 EPU is the amount of enzyme that liberates 0.0083 micromoles of reducing sugars (xylose equivalents) from oat spelled xylan per minute at pH 4.7 and 30 °C).[4] Cysteine is a semi-essential amino acid.

Table 8 presents the analyzed proximate composition of BSFL.

Table 8. Analyzed the proximate composition of the BSFL (g/kg) used in pilot activities at the UMU

Composition	**BSFL [1]**
Dry matter	961
Crude protein	349
Ether extract	303
Crude fiber	77.5
Ash	118
Starch	-
Calcium	28.9
Total phosphorus	6.95
Indispensable amino acid	
Arginine	14.6
Histidine	7.95
Isoleucine	14.3
Leucine	22.2
Lysine	22.0
Methionine	16.9
Methionine + cysteine [2]	21.3
Phenylalanine	12.9
Threonine	12.8

Valine	20.1

[1] BSFL: whole dry black soldier fly larvae. [4] Cysteine is a semi-essential amino acid.

2. 2.5.2. UNITO

The CON group was given a standard commercial diet consisting of conventional ingredients like soybean meal, the ALT group was fed a diet where soybean meal was fully replaced with alternative ingredients, and the ALT+BSFL group received a diet that replaced soybean meal with alternative ingredients, supplemented with dehydrated BSFL, constituting 5% of the expected daily dry matter intake (Table 9).

Table 9. Ingredients and analyzed the proximate composition of control and alternative diets used in pilot studies at the UNITO

Ingredients, g/kg	**Diets[1]**	
	CON	**ALT**
Maize meal	617	461
Soybean meal 44	320	-
Field bean	-	110
Pea protein	-	108
Barley	-	47
Sunflower flour	-	95
Maize gluten	-	116
Soybean oil	20	16
Dicalcium phosphate	13.5	13.5
Calcium carbonate	19	20
Sodium chloride	1.5	1.5
Sodium bicarbonate	1.4	1.4
DL-methionine	1.7	0.7
L-lysine	-	4
Vitamin and mineral premix[2]	5.9	5.9
TOTAL	1000	1000
AME (Mj/kg)	11.8	11.9
Analyzed proximate composition, %		
Dry matter	90.81	90.27
Crude Protein	18.13	18.10
Ether Extract	3.59	3.63
Crude Fiber	3.28	4.80
Amino acid composition, g/100g		

Alanin	6.53	7.00
Arginin	6.53	5.92
Aspartic Acid	9.80	8.07
Glutamic Acid	17.42	17.76
Glycine	8.17	8.07
Histidin	2.45	2.64
Isoleucine	4.19	3.82
Leucine	8.17	9.69
Lysine	6.53	7.00
Methionine	2.12	2.05
Phenylalanine	5.12	5.17
Proline	6.53	7.00
Serine	4.79	4.52
Threonine	3.76	3.44
Tyrosine	3.10	3.28
Valine	4.79	4.57

[1]CON diet: Standard commercial diet consisting of conventional ingredients, ALT: A diet where soybean meal was fully replaced with alternative ingredients. [2]Vitamin A, Vitamin D3, 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.

Table 10 presents the analyzed proximate compositions of dehydrated and live BSFL.

Table 10. The analyzed proximate composition of dehydrated and live BSFL used in pilot activities at the UNITO

Composition	BSFL[1]	
	Dehydrated	Live
Dry matter g/100g	93.9	33.9
Crude protein g/100g (DM)[2]	33.9	43.3
Ether extract g/100g (DM)	31.4	16.1
Ash g/100g (DM)	10.8	11.7
Crude fiber g/100g (DM)	7.98	9.09
Gross energy MJ/kg	17.7	14.3
Amino acid composition (g/100 g CP)		
Alanine	3.10	3.73
Arginine	1.83	2.52

Aspartic Acid	3.54	4.41
Glutamic Acid	3.87	5.46
Glycine	2.41	2.98
Histidine	0.99	1.94
Isoleucine	1.70	2.12
Leucine	2.65	3.10
Lysine	2.63	3.36
Methionine	0.76	0.88
Phenylalanine	1.71	1.97
Proline	2.04	2.73
Serine	1.53	2.05
Threonine	1.50	1.92
Tyrosine	2.67	3.21
Valine	2.44	2.97

[1] BSFL: Black soldier fly larvae [2]DM: Dry matter

3. 2.5.3. ISA-CM

Alternative ingredients used to formulate the diets were: triticale, canola meal, fava beans, and full-fat dried BSFL. BSFL were purchased by ENTOMO for the ISA-CM benefit from a local commercial company (NextProtein, Grombalia, Tunisia).

Meat-type chickens: Three experimental diets were formulated to meet the nutrient recommendations of the SASSO T44 breed; a standard corn-soybean meal diet (CON), a diet containing local ingredients or by-products as partial substitutes for corn and soybean meal (ALT), and 5% full-fat dried BSFL was added on top of ALT each day to meet 5% of the estimated daily feed intake (ALT+BSF). All diets were isocaloric and isonitrogenous. For each diet, grower and finisher concentrates were distributed from 37 to 64 and 64 to 86 days of age, respectively.

The formula and proximate nutritive value of meat-type chicken diets are illustrated in Table 11. BSF larvae in ALT+BSFL were added separately on top of chick feeder trays.

Table 11. Ingredients and proximate composition of grower (d37-64) and finisher (d64-86) control and alternative diets used for meat-type chickens in pilot studies at the ISA-CM

	Diets[1]					
Ingredients,g/kg	**Grower**			**Finisher**		
	CON	**ALT**	**ALT+BSFL**	**CON**	**ALT**	**ALT+BSFL**
Corn	687.0	481.0	399.0	703.0	50.09	370.5
Soybean meal	275.0	200.0	142.5	255.0	180.0	123.5
Soybean oil	1.5	14.0	4.75	8.0	19.0	13.3
Phosphate bicalcium	17.9	16.0	15.2	16.7	15.0	14.25
Calcium carbonate	8.8	9.0	8.55	7.5	7.1	7.6
Premix	5.0	5.0	4.75	5.0	5.0	4.75
Sodium chloride	3.9	3.9	3.705	3.9	3.9	3.705
Methionine	0.9	1.0	0.95	0.9	1.0	0.95
Rapeseed meal	0	60	66.5	0	60.0	47.5
Fava beans	0	60	66.5	0	60.0	78.85
Triticale	0	150	237.5	0	140.0	285.0
Lysine HCl	0	0.1	0.095	0	0	0.095
Black Soldier Fly Larvae (dried)	0	0	50	0	0	50.0
Analyzed proximate composition, %						
ME, (Kcal/Kg)	2947.71	2948.32	2948.55	3010.81	3007.57	3009.24
Crude Protein	18.06	18.04	18.04	17.24	17.19	17.20

Crude Fiber	3.2	3.6	4.2	2.8	3	2.8
Crude Fat	3	3.8	3.2	2.4	2.6	2.3
Methionine	0.38	0.38	0.39	0.37	0.37	0.37
Methionine+Cysteine	0.70	0.72	0.71	0.68	0.70	0.67
Lysine	0.96	0.95	0.93	0.91	0.89	0.87
Threonine	0.70	0.69	0.68	0.66	0.65	0.64
Calcium	1.01	1.01	0.99	0.93	0.91	0.92
Na	0.16	0.16	0.16	0.16	0.16	0.16
K	0.81	0.80	0.76	0.77	0.76	0.72
VIT.A (KUI)	9.00	9.00	8.55	9.00	9.00	8.55
VIT.D3 (KUI)	4.00	4.00	3.80	4.00	4.00	3.80
VIT.E (KUI)	20.0	20.0	19.0	20.0	20.0	19.0

[1] CON: A standard corn-soybean meal diet, ALT: A diet containing local ingredients or by-products as partial substitutes for corn and soybean meal (ALT), ALT+BSFL: 5% full-fat dried BSFL was added on top of ALT each day to meet 5% of the estimated daily feed intake. [2]Premix: providing the following nutrients per 100 g; Mn 3.44mg, Cu 0.275 Se: 0.010, Fe 2.46 mg , Zn: 2.8 mg, I: 0.062 mg, Co: 0.013mg, Choline: 11.5 mg, vitamin A 360 UI, vitamin D3, 100 IU; vitamin E, 0.33 IU; vitamin K3, 0.036 mg; vitamin B1, 0.045 mg; vitamin B2, 0.135 mg, ; vitamin B5 0.27mg, vitamin B6, 0.067 mg; vitamin B12, 0.27 µg; niacin: 0.54 mg; folic acid, 9 µg

Egg-type chickens: Three experimental diets were formulated to meet the nutrient requirements of the Lohmann White line: A CON diet was a standard corn-soybean meal-based diet, the ALT diet contained local ingredients or by-products as partial substitutes for corn and soybean meal and 5% full-fat dried BSFL was added on top of ALT each day to meet 5% of the estimated daily feed intake (ALT+BSF).

The ingredients and proximate chemical composition of the experimental diets and dried BSFL are illustrated in Tables 12 and 13, respectively.

Table 12. Ingredients and proximate composition of control and experimental diet used for egg-type chickens in pilot studies at the ISA-CM

Ingredients, g/kg	**Diets[1]**	
	CON	ALT
Corn	626.0	352.0
Soybean meal	262.0	173.0
NaCl (salt)	3.5	3.70
Pure methionine	0. 8	0. 9
DCP	15.0	13.9
Lime carbonate	89.7	90.0
Premix[1]	3.0	3.0
Triticale	-	200.0
Canola[1]	-	50.0
Faba beans	-	100.0
Soybean oil	-	13.5
Analyzed proximate composition, %		
Me, (kcal/kg)	2732.86	2733.57
Crude protein	17.01	17.01
Crude fiber	2.43	3.37
Crude fat	2.79	4.19
Starch	42.6	41
Methionine	0.39	0.38
Methionine+cysteine	0.69	0.69
Lysine	0.91	0.90
Threonine	0.66	0.64
Tryptophane	0.19	0.19
Calcium	3.96	3.98
Na	0.15	0.16
K	0.76	0.76
VIT.A (K UI)	7.2	7.2
VIT.D3 (K UI)	1.98	1.98
VIT.E (K UI)	6.57	6.57

[1]CON: Diet was a standard corn-soybean meal-based diet, ALT: A diet contained local ingredients or by-products as partial substitutes for corn and soybean meal, and 5% full-fat dried BSFL was added on top of ALT each day to meet 5% of the estimated daily feed intake (ALT+BSF).Premix: providing the following nutrients per 100 g:: Mn 3.44mg, Cu 0.275 Se: 0.010, Fe 2.46 mg , Zn: 2.8 mg, I: 0.062 mg, Co: 0.013mg, Choline: 11.5 mg, vitamin A 360 UI, vitamin D3, 100 IU; vitamin E, 0.33 IU; vitamin K3, 0.036 mg; vitamin B1, 0.045 mg; vitamin B2, 0.135 mg, ; vitamin B5 0.27mg, vitamin B6, 0.067 mg; vitamin B12, 0.27 µg; niacin: 0.54 mg; folic acid, 9 µg

Table 13. Analyzed the proximal composition of the full-fat dried BSFL used in pilot studies at the ISA-CM

Composition	% Dry matter
Dry matter	93.90
Crude protein	41.00
Crude fat	25.30
Cellulose	7.60
Total ash	6.00
Chitin	2.78
Methionine	0.73
Methionine+cysteine	1.05
Lysine	2.24
Ca	0.61
Available Phosphorus	0.31
Na	0.10
Cl	0.32
K	1.09
Mg	0.20
S	0.34
Mn (mg)	75.00
Cu (mg)	9.10
Se (mg)	0.60
Fe (mg)	455.00
Zn (mg)	72.60

4. 2.5.4. RAYHANA

From October 2023 to February 2024, the farmers used two diets: half of the chickens were fed the CON diet and the other half were fed CON+BSFL with the addition of dried larvae to the CON diet. The CON diet consisted of maize, bran, and barley.

5. 2.5.5. EGE

Birds divided into three groups were fed control, ALT, or ALT+BSFL diets from day old to the slaughter age which was 40 and 55 days for commercial and local chickens, respectively. Table 14 shows the ingredients and analyzed composition of control and alternative diets for starter (d0-10), grower (d11-25), and finisher (d26-slaughter age) periods used in pilot studies at the EGE pilot.

Table 14. Ingredients and proximate composition of control and alternative diets for starter (d0-10), grower (d11-25), and finisher (d26-slaughter age) periods used in pilot studies at the EGE pilot

Ingredients	Diets[1]								
	CON			ALT			ALT+BSFL		
g/kg	0-10	11-25	26-S[2]	0-10	11-25	26-S	0-10	11-25	26-S
Corn	452.8	512.4	573.4	391.8	444.4	474.4	413.0	465.4	496.4
Wheat	118.6	148.6	150.0	125.0	145.0	155.0	121.8	145.0	155.0
Soybean meal	343.3	279.0	232.0	298.0	211.0	146.0	251.0	163.0	97.0
Sunflower meal	-	-	-	35.8	63.0	80.0	36.3	6.30	80.0
Brewers dried grain	-	-	-	25.8	30.8	40.0	26.3	30.8	4.00
Wheat middling	-	-	-	25.8	30.8	40.0	26.3	30.8	40.0
BSF larvae	-	-	-	-	-	-	50.0	50.0	50.0
Sunflower oil	58.8	40.0	30.0	71.3	55.0	50.0	48.8	32.0	27.0
Limestone	5.0	3.0	2.0	5.0	3.0	2.0	5.0	3.0	2.0
DCP	10.0	8.0	6.0	10.0	8.0	6.0	10.0	8.0	6.0
Vit+min premix[3]	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
NaCl	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

Lysine (HCL - % 78)	5.0	3.0	1.5	5.0	3.0	1.5	5.0	3.0	1.5
Methionine dl (% 99)	1.0	0.5	0.1	1.0	0.5	0.1	1.0	0.5	0.1
Threonine	0.5	0.5	-	0.5	0.5	-	0.5	0.5	-
Enzyme [4]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Analyzed proximate Composition[5]									
ME kcal/kg	2984	2923	2904	2992	2921	2904	2991	2919	2903
CP, %	20.78	18.68	17.00	20.74	18.65	17.05	20.78	18.64	17.04
EE, %	8.49	6.63	5.79	9.41	8.11	7.52	9.38	7.72	7.52
CF, %	2.91	2.61	2.35	3.70	3.69	3.77	3.94	3.92	3.99
Ca, %	1.08	1.04	1.02	1.08	1.03	0.99	1.13	1.08	1.05
Total P	0.50	0.44	0.38	0.55	0.52	0.47	0.55	0.52	0.48
Methionine,%	0.42	0.45	0.35	0.37	0.32	0.28	0.32	0.27	0.30
Lysine, %	1.00	0.86	0.78	0,78	0.73	0.65	0.54	0.52	0.43
Threonine, %	0.07	0.03	0.07	0.03	0.03	0.05	0.06	0.07	0.04

[1] CON: Control diet was a commercial soybean-corn-based diet. ALT: Alternative protein sources were used to partially replace soybean and corn. ALT+BSFL: A 5% BSFL was added to the ALT diet. [2]S: Slaughter age: It was 55 and 40 d for local and commercial strains, respectively. [3] Vitamin+mineral premix: Provided per 2.5 kg feed of diet. Vitamin A, 15,000,000 IU, Vitamin D3, 3,000,000 IU, vitamin E, 50,000 mg, Vitamin K3, 4,000 mg, vitamin B1, 3,000 mg, Vitamin B2, 6,000 mg, Niacinamid, 40,000 mg, Vitamin B6, 5,000 mg, Vitamin B12, 30 mg, Calcium-D-Pantothenate 15.000 mg, Biotin, 75 mg, Folic acid, 1,000 mg, Choline Chloride, 400,000 mg, Manganese 80.000 mg, Iron: 60.000 mg, Copper, 5000 mg, Zinc, 60.000 mg, Iodine, 2.000 mg, Selenium 150 mg. [4] Rovabio (50 gr) + Natuphos E (100 gr) BASF. [5] ME: Metabolizable energy, CP: crude protein, EE, ether extract, CF: crude fiber, Ca: calcium, Total P: total phosphorus.

The experimental diets were: A soybean and corn-based diet (CON), soybean and corn were partly substituted with sunflower meal, brewers dried grain, and wheat middlings (ALT), and 5 % dried BSFL meal was included in ALT (ALT+BSFL). Table 15 presents the analyzed proximal composition of dried BSFL.

Dried BSFL reared on vegetable and bakery by-products in Türkiye (Germina Tarım Teknolojileri Tic. Ltd., Sti., Ankara). Dried insect larvae were obtained from the company in vacuum-packed bags (approximately 1 kg). They were ground in a meat grinder to ensure homogeneity of the amount to be added (5%) to the diets.

Table 15. Analyzed the proximal composition of the full-fat dried BSFL meal used in pilot studies at the EGE pilot

Composition %	BSFL meal
Metabolizable energy, kcal/kg	5381
Dry matter	95.52
Crude protein	42.62
Ether extract	42.54
Crude fiber	11.04
Neutral detergent fiber	17.56
Acid detergent fiber	13.36
Acid detergent insoluble nitrogen	0.74
Crude ash	6.29
Total sugar	1.79
Methionine	0.65
Lysine	1.35
Threonine	2.73
Calcium	1.70
Total phosphorus	0.62
Amino acids,%	
∑Essential amino acids	10.62
∑Nonessential amino acids	8.25
∑Amino acids	18.87

3. CONCLUSION

The increase in global demand for chicken eggs and meat and sustainable products comes with significant challenges and new opportunities. In this context, pilot studies of the SUSTAvianFEED project were carried out to evaluate the acceptability of our innovative farming approach to be used in small-scale sustainable practices by investigating alternative protein sources to soybean and corn for sustainable poultry products.

The studies were conducted between March 2022 and June 2024 in UMU, UNITO, ISA-CM, and EGE. The pilot studies followed a similar protocol using the CON diet and two alternative diets including local protein sources, such as agri-industrial by-products, local feedstuffs, and BSFL meal as alternatives to soybean and corn. The ALT diet was formulated using available local protein (ALT) sources such as canola, fava beans, field beans, and peas which can be found in each country's conditions. Agro-industrial sector by-products such as DDGs, sunflower meal, brewers dried grain, and wheat middlings were also included in the ALT diets as substitutes for soybean. The BSFL meal was added either into the ALT diet or offered separately (ALT+BSFL). The experimental diet containing local feedstuffs (ALT) is expected to present a significantly lower environmental impact than the standard commercial diet when with BSFL (ALT+BSFL).

It has been hypothesized that local slow-growing chickens can effectively adapt to alternative protein sources. In the pilot studies, local egg-and-meat type chickens specific to the Mediterranean and slow- and fast-growing commercial broiler chickens were used to demonstrate the applicability of our approach to a wider range of producers (Table 16). Adding commercial strains to the pilot projects provided information on the performance, animal welfare, and environmental impact of local

protein sources as alternatives to soybean for large-scale egg and chicken meat producers.

The pilot studies also aimed to promote gender equality and empowerment of women by including rural women in pilot activities and thus develop a sustainable business model for women's socioeconomic growth. The pilot studies in RAYHANA were conducted in the women producers' own chicken houses with two diets; CON and ALT+BSFL

In conclusion, the current pilot studies have allowed the comparison of ALT and ALT+BSFL diets with soybean-corn-based diets for local meat-type and egg-type chicken birds as well as for commercial broilers and laying hens. The design of the current pilot studies conducted in Spain, Italy, Tunisia, and Turkey demonstrated the usability and suitability of alternative diets to corn-soybean-based diets while preserving genetic diversity. The farming activities enabled the comparison of chicken health and welfare issues (Task 3.3), the growth performance of egg- and -meat-type chickens, and egg and meat quality parameters (Task 3.4), in each pilot. Moreover, the results obtained from the pilot studies addressed the environmental (Task 3.5), economic (Task 3.6), and social (Task 3.7) evaluation of pilot activities.

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Appendix - 1

Summary of calendar of experimental pilot studies

	MAY 22	JUN 22	JUL 22	AUG 22	SEPT 22	OCT 22	NOV 22	DEC 22	JAN 23	FEB 23	MAR 23	APR 23	MAY… AUG 23	SEPT 23	OCT 23	NOV 23	DEC 223	JAN 24	FEB 24	MAR 24	APR 24	MAY 24
UMU EGG-TYPE																						
UNITO MEAT-TYPE																						
ISA-CM-MEAT-TYPE																						
ISA-CM EGG-TYPE																						
RAYHANA MEAT-TYPE																						
RAYHANA EGG-TYPE																						
EGE MEAT-TYPE																						

Appendix - 2

Summary of experimental design of pilot studies

Pilot study	Experimental period	Chickens	Breeds, number of animals, and replications	Alternative feedstuffs to soybean	
				ALT	ALT+BSFL
UMU	From Nov.1, 2022 to Mar 2, 2023	Egg-type chickens	Breed: Isazul-laying hens (23wks old) n=120 Replication=5 pens/diet	Sunflower meal, peas, wheat middlings, corn-dried distillers' grains (DDGs)	5% whole dehydrated BSFL was provided, separately
UNITO	From May 27, 2022 to Oct 10, 2022	Meat-type chickens	Breed: Bianca di Soluzza (39d old) n=144 Replication= 6 pens/diet	Field bean, pea protein, barley, sunflower flour, maize gluten	Dehydrated BSFL, constituting 5% of the expected daily dry matter intake was provided separately
ICA-CM	From Sept 19, 2023 to Dec 14, 2023	Meat-type chickens	Breed: Slow-growing male broilers SASSOT44 (37d old) n=180	Triticale, canola meal, fava beans	Full-fat dried BSFL, 5% of the estimated daily feed intake, was provided

Pilot study	Experimental period	Chickens	Breeds, number of animals, and replications	Alternative feedstuffs to soybean	
				ALT	ALT+BSFL
			Replication= 5 pens/diet		
	From Mar 25, 2024 to Jun 4, 2024	Egg-type chickens	Breed: Lohmann White (commercial) n=150 Replication=5 pens/diet	Triticale, canola meal, fava beans	Full-fat dried BSFL, 5% of the estimated daily feed intake, was provided
RAYHANA	From Oct 4, 2023 to Mar 3, 2024	Meat-type chickens	Breed: Geant (56d old) male n=50-60/farmer Replication=16 women farmers	-	Control (maize, bran, and barley)+Full-fat dried BSFL
	From Nov 29 2023 to Jan 30, 2024	Egg-type chickens	Breed: Geant (30 wks old, females) n=50-60/farmer Replication=16 women farmers	-	Control (maize, bran, and barley)+ Full-fat dried BSFL
EGE	From May 12, 2022 to July 5, 2024		Breed: ANADOLU-T (local) & Cobb500 (commercial)	Sunflower meal, wheat middling and brewers dried grain	5 % dried black soldier larvae included in the diet formulation

Pilot study	Experimental period	Chickens	Breeds, number of animals, and replications	Alternative feedstuffs to soybean	
				ALT	ALT+BSFL
			(day-old) n=252 /each breed Replication= 6/diet/breed		