



# MilkQua

## **Milk quality along the dairy chain for a safe and sustainable milk**

Deliverable Nb: D1.2

First Mid-Term technical report

Part B

**Period covered by the report:** from 01/04/2019 to 31/10/2020



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# Document Abstract

This report aims at synthesising the main achievements within MilkQua during the first reporting period (April 2019-October 2020).

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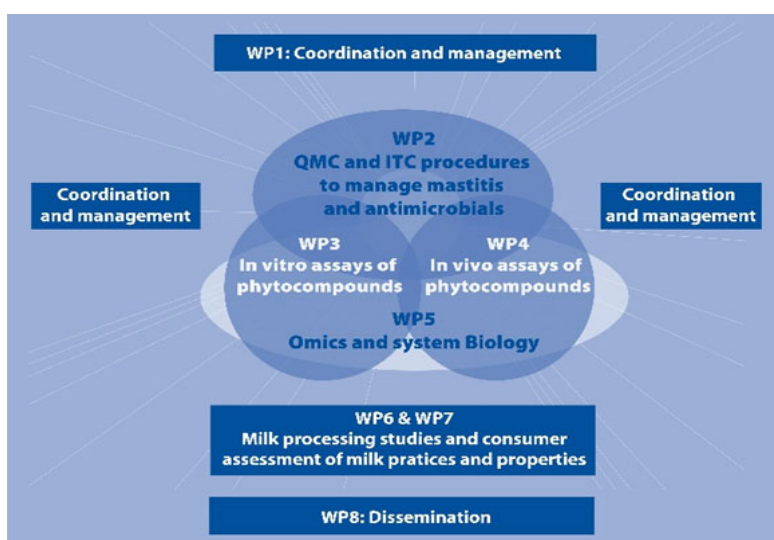




# 1. Explanation of the work carried out by the beneficiaries and Overview of the progress

*Explain the work carried out during the reporting period in line with the proposal submitted.*

MilkQua's strategic aim is to enhance global food security and dairy food quality by reducing antimicrobial use on Tunisian farms. Our main objective is to improve the milk quality and sustainability of Tunisian agriculture sector by addressing a high priority species-specific disease, mastitis, which is of great importance to animal and agriculture sector economy in Tunisia. To achieve this objective, MilkQua is organized into 8 WPs represented in the figure listed below:



During this first reporting period, with the support of different partners (Idele, OEP, Delice Danone) and Tunisian national organizations and agriculture ministry, and the participation of stakeholders involved at strategic levels in the Tunisian dairy chain among them the General Directorate of the Veterinarian Services (DGSV) in Tunisia, a first evaluation of the state of use of antimicrobial in Tunisia in dairy farms was initiated in the frame of WP2. Surveys have been created in order to better know the infection prevalence, the use of antibiotics in herds and to go on the etiological work. Idele trained to make the survey to identify the preventive and curative practices that are used by breeders. Moreover, in order to determine the most important determinants of changes in practices by the breeders to improve milk quality and the rationalization of the use of antibiotics, a socio-technical survey has been built to describe the brakes, the motivations and assess the difficulties of the breeders. The implementation of this program was delayed due to the situation of the COVID crisis.

As a second objective, the MilkQua tested, in the frame of WP3 and WP4, the use of autochthonous Tunisian medicinal and aromatic plants as alternate to reduce the use of antibiotics and antibiotic-resistance for cattle and consumers. *In vitro* studies were conducted and have permitted to select a set of 10 plants as the potential most promising ones to use in animal diets and health based on their *in vitro* biological properties and ability as antimicrobial and anti-inflammatory bioactive molecules. Some of them (for which toxicity was also proved) are under *in vivo* testing and the expected results will be delivered at the beginning of 2021.

With the support of Absiskey (subcontracting), WP8 has delivered on a communication and dissemination plan. It has been developing communication tools (social media accounts, logo & visual identity, website, poster, pilot video, etc...) to inform about the project right after the start during events. Some of those tools have already been adjusted into a second version. Time is now to report on first results, using a Newsletter, in addition to a website and social media. A Scientific, Intellectual Property, Valorisation and Exploitation Committee has been established to finetune agreements between partners and boost valorisation and exploitation, together with data management.



*Include an overview of the project results towards the objective of the action in line with the structure of the proposal submitted, including **summary of deliverables and milestones**, and a **summary of exploitable results and an explanation about how they can/will be exploited**.*

See Appendixes:

- 💧 Appendix 1 - Overview of project results towards the objectives of the action
- 💧 Appendix 2 - Table 1: Summary of deliverables
- 💧 Appendix 3 - Table 2: Summary of Milestones
- 💧 Appendix 4 - Table 3: Summary of exploitable results

## 1.1 Objectives

*List the specific objectives for the project as described in section 1.1 of the proposal submitted and describe the work carried out during the reporting period towards the achievement of each listed objective. Provide clear and measurable details.*

Specific objectives and achievements of the project towards objectives are outlined below:

<b>Objective 1</b>	<b>Helping producers to prevent monitor and reduce food safety risks in their farms and companies by designing and implementing an on-farm food safety program, the Quality Milk Program (QMP).</b>
Work carried out towards the objective	<p><i>Describe the work carried out during the reporting period towards the achievement of each listed objective. Provide clear and measurable details.</i></p> <p>To achieve this objective 1, a QMP program was defined in the frame of WP1 and conducted by IDELE, OEP, ENMV and Delice Danone with the involvement of several Tunisian dairy stakeholders. Different analytical approaches and surveys were elaborated to determinate the etiology of mastitis in the Tunisian dairy herds and identify the socio and economic determinants of milk quality.</p>
<b>Objective 2</b>	<b>Reducing the use of antibiotics and antibiotic-resistance for cattle and consumers, and improving animal yield by implementing an innovative phytochemicals-based anti-microbial approach and prevention in dairy farms.</b>
Work carried out towards the objective	<p><i>Describe the work carried out during the reporting period towards the achievement of each listed objective. Provide clear and measurable details.</i></p> <p>To achieve this objective, an innovative research based program involving LPAM, UPFF CISC, LPAF and Idele took place. The studies enabled to select and characterize the 10 most promising Tunisian aromatic and medicinal plants to be used as alternate to antibiotics. <i>In vitro</i> approaches were developed to study their potential beneficial <i>in vitro</i> properties to reduce inflammation and as antimicrobials against specific bacteria involved in the development of mastitis in Tunisian dairy herds. <i>In vivo</i> assays were initiated in June 2019 to evaluate the effect of specific essential oils after addition in the diet of young animals on long term animal performance and health status. A recent experimental <i>in vivo</i> assay was established (Ralph Nehme's Thesis) to evaluate their properties as preventive molecule to treat mastitis (for more details see WP3 and WP4). The samples (Milk and other biological samples) were sent to UMIL for evaluating microbiome (WP5) and to INRAE for milk characterization (WP6).</p>



<b>Objective 3</b>	<b>Improving daily handling practices by implementing an educational program and the use of Information and Communication Technology (ICT), providing farmers with customable tools as a sustainable resource of reducing mastitis and improving antimicrobial stewardship for dairy stakeholders that will enhance food security and viability of rural communities.</b>
Work carried out towards the objective	<i>Describe the work carried out during the reporting period towards the achievement of each listed objective. Provide clear and measurable details.</i> This part of the work has not started yet.
<b>Objective 4</b>	<b>Applying new technologies (OMICS) to milk analysis and conservation throughout all the dairy chain.</b>
Work carried out towards the objective	<i>Describe the work carried out during the reporting period towards the achievement of each listed objective. Provide clear and measurable details.</i> This part of the work has not started yet.

Table 1 - Achievements of the project towards objectives over the reporting period

## 1.2 Explanation of the work carried out per WP

*Explain the work carried out in WP1 during the reporting period giving details of the work carried out by each beneficiary/linked third party involved. Please detail Task by Task as described in the project proposal. (About one page and a half per work package: the report shall be concise and readable. Any duplication should be avoided).*

### 1.2.1 Work Package 1 - Coordination and management

**Responsible:** Latifa Najar (Idele - France)

**Partners involved:** Idele: Florence Macherez (project manager), Djamila Meliani, (assistant)

**Status of tasks:**

**Task 1.1 Scientific management monitoring the status of the WP's.** Leader: Idele, Latifa Najar (M1-M36)

**Management Committee:** The management of MILKQUA is being carried out through the Management Committee composed of **Latifa Najar (coordinator), Florence Macherez (project manager) and Djamila Meliani (assistant), Idele.**

**Monthly Project Executive Meetings:** the Management Committee has organised monthly Project Executive Meetings. Those meetings have regularly addressed issues around the scientific daily work, achievement of specific results and their delivery on time and within the budget allocation. **All Work Package Leaders** took part in Project Executive Committees. The project Management Committee has provided support to partners in reporting on scientific, financial and administrative procedures and quality control of the performances, milestones and deliverables.

**Project Steering Boards:** **All partners** participated in Project Steering Boards on April 2019 (Launch meeting), in Paris and on June 2020 (first Project Steering Board), as a remote meeting.

**Project Scientific, Intellectual Property, Valorisation and Exploitation Committee:** To reinforce links between partners and facilitate decision making around intellectual property, valorisation and exploitation of results, the Management Committee has set up a Project Scientific, Intellectual Property, Valorisation and Exploitation Committee that will meet twice a year starting in November, 2020. It will mobilise **Work Package Leaders and some partners** on an ad hoc basis.





Scientific management monitoring: Complementarily to monthly Project Executive meetings and yearly Project Steering Board meetings, the scientific management monitoring has also been operated on a daily basis by **Latifa Najar (Idele)** bilaterally with **all partners**, in particular Work Package leaders. Specific scientific meetings will soon be organised to go deeper on certain topics in smaller sub-groups.

Deliverable D1.1. Management Committee constitution: The project management bodies and internal rules are summarised into Deliverable D1.1. Management Committee constitution that has been validated among partners and submitted at M18.

💧 **Task 1.2: Project Reporting.** Leader: Idele, Latifa Najar (M1-M36)

Internal and external reporting: **Latifa Najar and Florence Macherez (Idele)** have been carrying out the internal and external reporting, with the contribution of **all partners**, especially **Work Package Leaders**. So far, this report is the only one that has been prepared in writing. Two informal reports have been delivered to PRIMA-IS on April 14<sup>th</sup> and October 16<sup>th</sup>, 2020, on a remote basis, using a powerpoint presentation. Annual Project Steering Boards also serve as a basis to share project advancements among partners.

Reporting to funding agencies: **Each partner** has the responsibility to prepare reports to deliver to their national funding agencies.

💧 **Task 1.3: Administrative and financial management.** Leader: Idele, Latifa Najar (M1-M36)

The Management Committee composed of **Latifa Najar (coordinator)**, **Florence Macherez (project manager)** and **Djamila Meliani (assistant)**, **Idele**, supported by **Sylvie Leconte and Corinne Thibault (Idele)** for financial issues, in close relation with **all partners**, ensures the appropriate use of grant between the participants. Regular reporting of man-power and other expenses status has been made by **partners** on the [Smartsheet](#), allowing for regular overview of expenses and work progress.

Status of expected deliverables:

- 💧 D1.1. Management committee constitution (MC) (M1): **submitted**
- 💧 D1.2. Financial and administrative report (M18, M36): **on-going**

## 1.2.2 Work package 2 - Innovative QMP practices in Tunisia

Engineers in charge: Bénédicte Fusai and Philippe Roussel (Idele – France)

Partners involved: ENMV, OEP, Delice Danone, LPAF (INRAT)

Status of tasks:

💧 **Task 2.1: Assessment of mastitis occurrence in Tunisian dairy herds.** Leader: OEP (Henda Hanafi), ENMV (Monia Daaloul). Partners involved: Idele (Philippe Roussel, Marlène Guyadeur), Delice Danone (M1-M24)

This task started at the beginning of the project in April 2019. 4 areas of work have been launched:

- **2.1.1/ analysis of available performance control data in Tunisia (OEP) linked with mammary infections since 10 years (individual Somatic Cellular Concentration results and tank)**  
The recovery of the existing data was done by **OEP** between June and October 2019, then was forwarded to **Idele** in order to carry out a detailed analysis of the data during the last quarter of 2019.  
On the basis of 1/the herd cell concentration averages by month for herds enrolled on milk recording, 2/the indicator of new infection during milking, 3/the indicator of infection for primiparous cows, 3/the indicators of new infection and cure during the dry period, we have highlighted some specific evolutions depending on regions, years and herd sizes.  
The validation of this work conclusions by the task force members was initially scheduled for June 2020, and then delayed to the last quarter of 2020.
- **2.1.2/ retrospective analysis of bacteriological data on mastitis milk available at ENMV**



This work carried out by the **ENMV** of Tunis in 2019 provided the opportunity to study 187 bacteriological results on mastitis milk samples collected between 2009 and 2016. In these population of mastitis milk samples, we can see a high prevalence of major pathogenic bacteria (enterobacteria/staphylococcus aureus and streptococcus).

This work should have continued in 2020 by collecting further samples, but due to the Covid-19 crisis, visiting farms was forbidden in Tunisia during this year. A new sampling campaign is planned during the epidemiological/etiologiical surveys.

#### - **2.1.3/ Retrieval and analysis of data on the presence of antibiotic residues in the collected milk (OEP)**

The objective of this work is to better understand the exact situation of collection with regards to antibiotic residues and thus improve technical information on this subject for livestock farmers and collectors. Engineers from **Idele** went to Tunisia several times (April, July and October 2019), and with the help of **OEP's** representatives, met the different partners of the dairy sector in charge of the milk quality in Tunisia.

We faced difficulties for data recovery: 1/ data mainly exist in collect centers and in dairies but very hardly at the scale of each herd, 2/ these data are accessible only in books or sheets but very seldom in digital version. In 2021, if possible, we intend to retrieve some collect centers results in IT version with the aim to assess the situation.

#### - **2.1.4/ Achievement of epidemiological/etiologiical surveys**

In order to better know the infection prevalence (in particular mastitis), the use of antibiotics in herd and to go on the etiologiical work (bacteria responsible for mastitis) in the Tunisian herds, a survey has been created with the participation of the **ENMW** and veterinarians from the General Directorate of the Veterinarian services (DGSV) in Tunisia.

**Idele** prepared a draft survey, then tested and adapted it during the missions in October 2019 and February 2020. During the mission in February, the DGSV veterinarians were trained to make the survey. This survey will allow identifying the preventive and curative practices that are used by breeders to prevent mastitis and the treatments used on farms.

#### 💧 **Task 2.2: Social determinants of milk quality.** Leader: Idele (Bénédicte Fusai, Florence Kling, Amandine Menet, Philippe Roussel) (M13-M26). Partners involved: ENMV, Delice Danone, LPAF (INRAT)

The aim of the task 2.2 is to identify the determinants of changes in practices by the breeders in order to improve milk quality and the rationalization of the use of antibiotics.

A socio-technical survey has been built by **Idele** and **partners involved** to describe the brakes, the motivations and assess the difficulties of the breeders. The aims of the survey are to know 1/ How milk quality is understood by the dairy farmers? 2/ What are their current practices? Are they willing to change them? What are their motivations and obstacles to change? 3/ Which information media do they use?

A draft survey was made by Idele, then tested and adapted during the missions in October 2019 and February 2020. Then the content was adapted. In February 2020, **OEP's** technicians were trained to conduct the survey and its specificities.

The task 2.1.4 and 2.2 should have been done on 88 herds in various dairy Tunisian regions since June 2020 but due to the sanitary situation, it is delayed and will begin as soon as possible.

#### 💧 **Task 2.3: Economic determinants of milk quality.** Leader: Idele (Bénédicte Fusai) (M13-M26). Partners involved: ENMV, Delice Danone, LPAF (INRAT)

During the mission from 21st to 25th October, 2019, many breeding visits were organised by partners involved in herds of different sizes and areas. The results were used in a first economic analysis of the dairy sector.

This first approach has highlighted the following main points:

- The price of the milk is decided by the government.





- In particular, in the small herds, the financial products are exclusively provided by the sale of the milk. We can consider that the price is too low to ensure the quality of milk. Quality can only be achieved thanks to financial incentives, training and awareness. The capacity of investment or maintenance of the production tool reflected in the cost price is very low for these herd. They don't seem to be financially able to fight against the multiplication of mastitis and consequently to the presence of antibiotics in the milk.
- An external economic support (subsidies) or a milk price (incentive grid) are necessary. In this context, the livestock stakeholders, advice organisms and collect centres, have a preponderant role to play in the control and awareness program.

We can observe that during august 2020, the milk price decided by the government has been revalued up to 130 millimes: 95 millimes for the farmer, the rest for milk collect centres and industrialists.

During 2021, based on this new context and the elements collected in the different surveys, we will study the economic impact of the implementation of means to control mastitis.

💧 **Task 2.4: Communication practices and tools.** Leader: Idele (Bénédicte Fusai). Contributing partners: OEP (Henda Hanafi), ENMV (Monia daloul), idele (Philippe Roussel) (M17-M34)

Not started yet.

#### Status of expected deliverables:

- 💧 D2.1 - Evaluation report and epidemiology and etiology of mastitis to identify main pathogens are present on Tunisian farms (M24): **on-going**
- 💧 D2.2 - Milk quality improvement report (M24) and D2.3 -Elaboration of a milk quality payment grid, included in a report on recommendations for the support of dairy farmers and the mobilisation of the stakeholders to improve the milk quality and the rationalisation of the use of antibiotics in the Tunisian dairy herds. (M30): **not started**
- 💧 D2.4 - Guidelines for educational module – important for dissemination WP8 (M34): **not started**
- 💧 D2.5 - Development of a communication and training plan for the Tunisian dairy farmers through the use of an IT platform (SMS, mail, website, network...). (M34): **not started**

## 1.2.3 Work package 3 - In vitro evaluation of bioactive molecules and extracts

**Responsible:** David Pereira (UPFF - Portugal)

**Partners involved:** CSIC, INRA, LPAM

#### Status of tasks:

💧 **Task 3.1: Standardization of plant sampling procedures.** Leader: LPAM, Riadh Ksouri (M1-M3). Contributing partners: UPFF

As one of the earlier tasks in the project, **LPAM** and **UPFF** have developed a harmonized procedure to ensure that all samples were collected and stored in equivalent conditions, thus ruling out possible variations in results that could arise from sampling. The information resulting from this task has constituted Deliverable 3.1 and was used in all steps of the project in which vegetable material had to be collected.

💧 **Task 3.2: Optimization of extraction protocols; Obtention of bioactive extracts; Phytochemical characterization of bioactive extracts and sampling.** Leader: LPAM, Riadh Ksouri (M1-M8)



This task has benefited from the experience of **LPAM** in obtaining and characterizing EOs (Essential Oils). After the protocol has been established, the phytochemical profile of the 10 samples was determined by GC-FID, as included in Deliverable 3.3. The information related to the phytochemical profile is important for subsequent tasks as the biological activity of EO may be mimicked by using the major compounds in the samples.

💧 **Task 3.3: To evaluate the *in vitro* effects of plants and essential oils on ruminal fermentation parameters and methane production when included in the diet of ruminants.** Leader: CSIC Sonia Andres (M5-M12)

The protocol proposed initially (RUSITEC) has been changed by **CSIC** into an *in vitro* screening in order to test multiple doses of EOs and combinations of pure compounds. The purpose of this methodology will be finding the best option to improve ruminal fermentation parameters, thus increasing animal performance/feed efficiency when feeding essential oils to dairy cows. A second challenge of this trial will be finding the best dose or combination of pure compounds to reduce methane production and therefore the environmental impact of livestock. This trial will be carried out in two different phases. This first one, dealing with the best dose of EO has been finished in the first week of November and now samples are being analysed (e.g, volatile fatty acids, methane). Samples will be delivered to **UMIL** to study microbiome after ruminal fermentation. In December the second phase will take place to study the best combination of pure compounds, using the original EO and dose selected in the first phase as a control. If any of these combinations provides encouraging results, a patent can be attempted. After consideration in SIPVEC, the results will be published in peer review journals and presented in national and international conferences.

💧 **Task 3.4: To elucidate the anti-inflammatory, antioxidant and antimicrobial activity of EO and bioactive extracts.** Leader: UPFF, David Pereira (M5-M15). Contributing partner: LPAM

Initial studies on the bioactivity of EO towards microorganisms was conducted by **LPAM**. All samples obtained in Task 3.2 were studied for their bacterial growth inhibition potency by using the disc diffusion method using two bacterial strains isolated from infected cow breasts. This has allowed the identification of one sample that was clearly superior than the others (*Thymus capitatus*). This result was further confirmed via MBC values, obtained by the dilution method.

The EO samples obtained from Task 3.2 were also evaluated for their potential anti-inflammatory activity. As a model, human THP-1 cells differentiated into macrophages by the means of the molecule phorbol 12-myristate 13-acetate was used. As a screening method, we have evaluated the ability of the samples to inhibit or lower the activation of NF- $\kappa$ B caused by the pro-inflammatory molecule lipopolysaccharide. To achieve this, cells bearing a NF- $\kappa$ B-inducible Luc reporter construct were used. This assessment has allowed the reduction of the initial samples (n=10) into just 4. Subsequent steps, which are currently taking place, will evaluate the mechanism of action of the EO samples exhibiting anti-inflammatory activity and will be part of Deliverable 3.5.

💧 **Task 3.5: To assess the potential toxicity of plant materials.** Leader: UPFF David Pereira (M9-M18). Contributing partner: LPAM

In order to assure that the EO did not cause significant toxic or genotoxic effects, their toxicity was evaluated *in vitro*. Initially, the non-toxic concentrations of the samples were evaluated towards the THP-1 cell line. This cell line was relevant as it was the same used for the assessment of anti-inflammatory activity (Task 3.4), thus guarantying that only non-toxic concentrations were being used in the assays regarding bioactivity.

Additionally, the impact of the samples towards the viability of human keratinocyte cell line HaCaT was determined and the micronucleous assay conducted, in order to identify potential genotoxicity. Among all samples tested, none exhibited genotoxic effects at the highest non-toxic concentration. These results were included in Deliverable 3.6.



#### **Status of expected Deliverables:**

- 💧 D3.1 - Sampling/extraction protocol and list of plants (M3): **submitted**
- 💧 D3.2 - Bioactive extracts for subsequent biological assessment (M9): **submitted**
- 💧 D3.3 - Phytochemical profile of selected samples (M9): **submitted**
- 💧 D3.4 - Report on the effects of plants/essential oils on ruminal fermentation parameters and environmental impact (M12): **delayed to March, 2021**
- 💧 D3.5 - Report on the anti-inflammatory, antimicrobial and immunomodulatory properties of selected plant species (M18): **on-going, expected in January/February 2021**
- 💧 D3.6 - Safety report on selected samples (M15): **submitted**

### **1.2.4 Work package 4 - In vivo evaluation of bioactive molecules and extracts**

**Responsible:** Sonia Andres (CSIC - Spain)

**Partners involved:** Idele, LPAF (INRAT), ENMV

#### **Status of tasks:**

- 💧 **Task 4.1: Standardization of feeding trials and methods for data and sample collection.** Leader: CSIC Sonia Andres (M5-M10). Contributing partners: LPAF (INRAT) Hichem Ben Salem

This task has involved **CSIC** and **LPAF (INRAT)**. It is almost finished, pending minor details from experiments carried out in Tunisia. Then, it will be submitted to PRIMA, probably before the end of 2020.

- 💧 **Task 4.2: Potential use of EOs and medicinal plants to improve the feed efficiency, milk yield and quality and health status of dairy cows from early to the adult phase.** Leader: CSIC Sonia Andres and LPAF (INRAT) Hichem Ben Salem (M13-M24)

- Sub-task 4.2.1. Feed efficiency and health status during the replacement period of dairy calves supplied with EOs along the first two months of life in Spain (CSIC)

Sixteen newborn dairy calves were born at **CSIC** between June-July 2020. These animals were divided in two groups, a control (no EO) and a treatment group (EO supplied in the milk replacer during the first 45 days of life). The dry matter intake was controlled during this period (only milk replacer was fed during this phase), and animals were weighed at the beginning and at the end of the EO administration period, so feed efficiency in terms of weight gain was measured. Blood samples to study different types of lymphocytes (flow cytometry), biochemical profiles and microbiome in faeces were collected at birth and at the end of EO administration. Dry matter intake and feed efficiency will be measured again in May-July 2021 to test the long terms effects of feeding EO during the early life. Also, blood samples and faeces will be collected again during this phase to measure all the parameters related to biochemical profile, immune status and microbiome, so this information will be useful to explain the differences (if any) in feed efficiency of calves. All this information will be shared in the context of SIPVEC before attempting any patent (in case we found encouraging results), before being published in peer review journals, or presented in national and international conferences.

- Sub-task 4.2.2. Feed efficiency, milk yield and quality and health status of adult dairy cattle supplied with EOs during the mid-lactation in Tunisia- LPAF (INRAT)

The experiment on dairy cattle finished in September at **CSIC**. Two groups of 17 dairy cattle each were used. The experimental group received 6 ml EO-Thym/head/day. Dry matter intake and milk production was measured individually for each animal. Analysis (feeds, milk, blood, etc) are in progress. The results and information obtained from this trial will be managed according to the same rules explained in previous tasks.





- 💧 **Task 4.3: Testing EOs as curative solution to manage mastitis in in vivo trials under controlled conditions.** Leader: Latifa Najar (M13-M32) (Ralph Thesis: supervisors Latifa Najar Idele/Said Bouhallab, INRAE).

Twelve dairy calves with subclinical mastitis have been used by **Idele** in the present study. Six cows were treated with a mixture of thymus, whereas another 6 cows were treated with a milking grease (without adding the EO, control group). Treatments were applied daily on the udder of dairy cows by using a massage. Immunological studies of the animals were evaluated to determine the effect of the treatment on PBMC. Several biochemical parameters were assessed before and after the treatment to evaluate the physiopathological status of the animal and to determine whether the thymus improved the health status of the animal. Samples of milk, skin and blood were sent to partners **UMIL** and **INRAE** to evaluate respectively the impact of the treatment on microbiota (WP5) and milk quality (WP6). All this information will be shared in the context of SIPVEC before attempting any patent (in case we found encouraging results).

- 💧 **Task 4.4: Testing EOs as curative solution to manage mastitis in in vivo trials: large scale study in Tunisian farms.** Leader: ENMV Monia Daaloul (M21-M32): involvement OEP (Flocks identification, Idele (results of WP4) and LPAM (Essential oils preparation)

The experimental design and details of the study are still under consideration. This trial will be started in 2021.

#### **Status of expected Deliverables:**

- 💧 D4.1 Protocols and data collection (M10): ***first version submitted at M11, March 2020. Second version under preparation, lacking some minor details of some experiments [IDELE, ENMV and LPAF (INRAT)]***
- 💧 D4.2 Report on EOs newborn calves and feed efficiency (M24): ***delayed between 6-12 months***
- 💧 D4.3 Report on EOs newborn fed calves and health status (M24): ***delayed between 6-12 months***
- 💧 D4.4 Report on the effect of EOs fed on milk yield, quality and health in adult cows (M24): ***on time***
- 💧 D4.5 Report on the curative effect of EOs against mastitis in small controlled conditions (M32) ?
- 💧 D4.6 Report on the effect of EOs against mastitis (Large scale study, Tunisian Farms) (M32) ?

## **1.2.5 Work package 5 - System biology assessment of the effects of essential oils after in vitro and in vivo studies**

**Responsible:** Fabrizio Ceciliani (UMIL - Italy)

**Partners involved:** Idele, INRA, CSIC, LPAF (INRAT)

**Status of tasks:** This WP has not started yet.

- 💧 **Task 5.1: Microbiome determination (on faeces, rumen, milk).** Leader: UMIL Fabrizio Ceciliani (M19-M32). Partners involved: CSIC, LPAF (INRAT), Idele. Not started yet.
- 💧 **Task 5.2 MicroRNAome determination on milk.** Leader: UMIL Fabrizio Ceciliani (M19-M32). Partners involved: LPAF (INRAT) and Idele. Not started yet.
- 💧 **Task 5.3: Metabolome determination.** Leader: UMIL Fabrizio Ceciliani (M19-M32). Partners involved: CSIC, LPAF (INRAT), Idele. Not started yet.
- 💧 **Task 5.4: Proteome determination.** Leader: UMIL Fabrizio Ceciliani (M19-M32). Partner involved: LPAF (INRAT). Not started yet.

#### **Status of expected Deliverables:**

- 💧 D5.1 A list of bacterial at genus level that are present in faeces and milk of cows fed with different extracts from WP4 and WP5 (M32)
- 💧 D5.2 The miRNome of milk as related to feeding with different extracts (WP5) (M32)



- 💧 D5.3 A list of milk metabolites involved in the immune response which are modified after different extracts regimen (WP5) (M32)
- 💧 D5.4 A list of differentially abundant proteins after feeding with different extracts (WP5) (M32)

## 1.2.6 Work package 6 - Milk and dairy products processing and consumer studies

**Responsible:** Saïd Bouhallab (INRAE - France)

**Partners involved:** Idele, LPAM (INRAT), UPFF

**Status of tasks:** This WP has not started yet.

- 💧 **Task 6.1 Analysis of milks from the in vivo trials (WP4) and from processed dairy products.** Leader: Idele Faustine Noël (M12-M24). Common PhD between Idele and INRAE STLO after M18, Ralph Nehme. This task has not started yet.
- 💧 **Task 6.2. Comparative mechanisms of two encapsulation strategies of EOs in milk and dairy products.** Leader: INRA STLO Saïd Bouhallab (M12-M24). Common PhD between Idele and INRAE STLO after M18, Ralph Nehme. This task has not started yet.
- 💧 **Task 6.3 Supplemented milk properties assessment.** Leader: LPAM Riadh Ksouri (M12-M30). This task has not started yet.

**Status of expected Deliverables:**

- 💧 D6.1 Comparative efficiency of two strategies of EOs encapsulation (M26). Not started yet
- 💧 D6.2 Mechanisms of EOs encapsulation and selection of the most stable and efficient one (M26). Not started yet
- 💧 D6.3 Antimicrobial activity of encapsulated Eos (M24). Not started yet
- 💧 D6.4 Nutritional and sensorial properties and characteristics of EOs UHT milk and cheese (M30). Not started yet

## 1.2.7 Work package 7 - Consumer assessment of milk

**Responsible:** Ibtissem Hamrouni (LPAM - Tunisia)

**Partners involved:** LPAM

**Status of tasks:** This WP has not started yet.

- 💧 **Task 7.1: Sensorial analysis of milk enriched with encapsulated EOs.** Leader: LPAM Ibtissem Hamrouni (M11-M16). This task is delayed for better consistency with the rest of the project activities.
- 💧 **Task 7.2: KAP survey of Tunisian consumers toward milk and dairy products.** Leader: LPAM Ibtissem Hamrouni (M13-M18)
- 💧 **Task 7.3: Preliminary pilot testing.** Leader: LPAM Ibtissem Hamrouni (M17-M20)
- 💧 **Task 7.4: Data collection/report.** Leader: LPAM Ibtissem Hamrouni (M17-M24)
- 💧 **Task 7.5: Data analysis of the survey's results.** Leader: LPAM Ibtissem Hamrouni (M21-M30)

**Status of expected Deliverables:**

- 💧 D7.1 Sensorial properties of milk enriched with encapsulated Eos (M18). This deliverable is delayed to M28 – August 2021.



- 💧 D7.2 Questionnaire establishment on milk consumption (M20)
- 💧 D7.3 Validation of the questionnaire (M23)
- 💧 D7.4 Data Collection and verification (M28)
- 💧 D7.5 Report on consumer survey (M30)

## 1.2.8 Work package 8 - Communication and dissemination

**Responsible:** Florence Macherez (Idele), with the subcontracted support of Absiskey (Rémy M’Gadmi)

**Partners involved:** all partners

**Status of tasks:**

- 💧 **Task 8.1: Dissemination and Communication activities.** Leader: Idele, Florence Macherez, with the support of Absiskey, Rémy M’Gadmi (M1-M36). Partners involved: all partners

Communication issues have been discussed monthly during Communication and Dissemination Committee meetings gathering **all WPLs**, and **Rémy M’Gadmi, Absiskey**, established at M2 – May 2019. Task 8.1. has started developing and implementing an appropriate dissemination strategy and supportive materials for the communication plan.

- The Twitter account was set up at M1 – April 2019 by **Idele (Florence Macherez and Fanny Mazza)**, then it has been regularly updated by **Rémy M’Gadmi (Absiskey)** with inputs from **all partners**. To date, the Twitter account has 907 followers and 96 following.
- the LinkedIn account was created at M4 - July 2019 by **Rémy M’Gadmi (Absiskey)**
- The website was launched at M10 – January 2020, by **Rémy M’Gadmi (Absiskey)**, after consultation of **all partners** during Project Executive Committee meetings, making use of the project proposal and of inputs from **all partners**.
- A first draft of the poster was delivered in December 2019, prepared by **Latifa Najar, Djamila Meliani, and Idele**, for a scientific congress in Dakar. It has been updated by **Rémy M’Gadmi (Absiskey)** and validated by **all partners** in September 2020.
- Powerpoint and word templates were delivered by **Rémy M’Gadmi (Absiskey)** in March 2020.
- The flyer has been designed by **Rémy M’Gadmi (Absiskey)** with the contribution of **Florence Macherez (Idele)** and validated by **all WPLs** in September 2020. It needs to be translated into French and Arabic.
- Participation in events have started in June 2019 (3rd International Symposium “Milk, vector of development”, Dakar, Senegal - <https://colloque.inra.fr/lait2019/>, then IDELE Innovations Comice 2019 [http://idele.fr/no\\_cache/recherche/publication/idelesolr/recommends/linstitut-de-lelevage-organise-un-comice-pour-mettre-en-valeur-ses-innovations-et-partenariats.html](http://idele.fr/no_cache/recherche/publication/idelesolr/recommends/linstitut-de-lelevage-organise-un-comice-pour-mettre-en-valeur-ses-innovations-et-partenariats.html)
- A first pilot video has been prepared by **Latifa Najar and who???, Idele** with the support of **project partners** for the Comice of innovations, in December 2019. A first official video will be prepared later in 2021 when travels will be possible.

- 💧 **Task 8.2: Knowledge and Data Management.** Leader: Idele, Florence Macherez. Partners involved: all partners (M1-M36).

The Data Management Plan has been under discussion among partners since the first version provided by **Fabrizio Ceciliani, UMIL**, in April 2019. **Sonia Andres (CSIC)**, **Latifa Najar and Florence Macherez (IDELE)**, and **Chiara Mariani (UMIL)** provided contributions. A Data Management plan survey has been prepared by **Chiara Mariani (UMIL)** that should be filled out by partners in order to finetune the Data Management Plan. We expect to finish it by M20.

- 💧 **Task 8.3: Sustainability of MILKQUA project activities.** Leader: Idele, Florence Macherez. Partners involved: all partners (M24-M36).

This activity has not started yet.





**Status of deliverables:**

- 💧 D8.1 Communication and dissemination materials (M12, M24, M36): **submitted at M18, October 2020**
- 💧 D8.2 Plans for dissemination and exploitation of the results (M12, M24, M36): **submitted at M18, October 2020**
- 💧 D8.3 Completed and planned communication activities (M12, M24, M36): **submitted at M18, October 2020**
- 💧 D8.4 Report on sustainability of MILKQUA project activities and results (M36)
- 💧 ADDITIONAL D8.5 Data Management Plan (M18, M24, M36): **on-going**

## 1.3 Impact

*Include in this section whether the information on section 2.1 of the proposal submitted (how your project will contribute to the expected impacts) is still relevant or needs to be updated. Include further details in the latter case.*

Nothing to mention.

## 2. Update of the plan for exploitation and dissemination of result (if applicable)

*Include in this section whether the plan for exploitation and dissemination of results as described in the proposal submitted needs to be updated and give details.*

Not applicable, see Appendix 3 – Summary of exploitable results

## 3. Update of the data management plan (if applicable)

*Include in this section whether the data management plan as described in the proposal submitted needs to be updated and give details.*

Not applicable, data management plan under construction (see section on WP8 activities).



## 4. Follow-up of recommendations and comments from previous review(s) (if applicable)

*Include in this section the list of recommendations and comments from previous reviews and give information on how they have been followed up.*

Not applicable.

## 5. Deviations from the proposal submitted (if applicable)

*Explain the reasons for deviations (scientific aspects, planned work, estimated budget, etc.) from the proposal submitted, the consequences and the proposed corrective actions.*

Some deviations in the planning of activities due to COVID-19 were reported on due time to PRIMA-IS. They result in a request of an extension of 6 months of the project duration.

### 5.1 Tasks

*Include explanations for tasks not fully implemented, critical objectives not fully achieved and/or not being on schedule. Explain also the impact on other tasks on the available resources and the planning.*

Not applicable.

### 5.2 Use of resources

*Include explanations on deviations of the use of resources between actual and planned use of resources in proposal submitted, especially related to person-months per work package.*

*Include explanations on transfer of costs categories (if applicable).*

*Include explanations on adjustments to previous financial statements (if applicable).*

Not applicable.

#### 5.2.1 Unforeseen subcontracting (if applicable)

*Specify in this section:*

*a) the work (the tasks) performed by a subcontractor which may cover only a limited part of the project;*

*b) explanation of the circumstances which caused the need for a subcontract, taking into account the specific characteristics of the project;*

*c) the confirmation that the subcontractor has been selected ensuring the best value for money or, if appropriate, the lowest price and avoiding any conflict of interests*

Not applicable.



### **5.2.2 Unforeseen use of in-kind contribution from third party against payment or free of charges (if applicable)**

*Specify in this section:*

*d) the identity of the third party;*

*e) the resources made available by the third party respectively against payment or free of charges;*

*f) explanation of the circumstances which caused the need for using these resources for carrying out the work.*

Not applicable.

## **6. Appendix**





## 6.1 Appendix 1 – Summary of deliverables

WP	Task	Title	Partner	Dissemination level	1 <sup>st</sup> reporting period																2 <sup>nd</sup> reporting period																	
					2019								2020								2021												2022					
					Apr	May	June	Jul	Aug	Sept	Oct	Dec	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb
WP1	D1.1	Management committee constitution (MC)	IDELE	PU	I																																	
	D1.2	Financial and administrative report	IDELE	CO																																		I
WP2	D2.1	Evaluation and epidemiology reports	OEP	PU																																		
	D2.2	Milk quality improvement report	OEP	PU																																		
	D2.3	Elaboration of a milk quality payment grid	IDELE	PU																																		
	D2.4	Guidelines for educational module	IDELE	PU																																		
	D2.5	ITC Communication and training plan	OEP	PU																																		
WP3	D3.1	Sampling/extraction protocol and list of plants	LPAM	PU			✓																															
	D3.2	Bioactive extracts for subsequent biological assessment	LPAM	PU									I		✓																							
	D3.3	Phytochemical profile of selected samples	LPAM	PU									I		✓																							
	D3.4	Report on the effects of plants/essential oils on ruminal fermentation parameters and environmental impact	CSIC	PU												I																						
	D3.5	Report on the anti-inflammatory, antimicrobial and immunomodulatory properties of selected plant species	UPFF	PU																																		
	D3.6	Safety report on selected samples	UPFF	PU																																		
WP4	D4.1	Protocols and data collection	CSIC	PU																																		
	D4.2	Report on EOs newborn calves and feed efficiency	CSIC	CO																																		
	D4.3	Report on EOs newborn fed calves and health status	CSIC	CO																																		
	D4.4	Report on the effect of EOs fed on milk yield, quality and health in adult cows	LPAF	CO																																		
	D4.5	Report on the curative effect of EOs against mastitis in small controlled conditions	IDELE	PU																																		
	D4.6	Report on the effect of EOs against mastitis (Large scale study,Tunisian Farms)	ENMV	CO																																		
WP5	D5.1	A list of bacterial at genus level that are present in faeces and milk of cows fed with different extracts from WP4 and WP6	UMIL	CO																																		
	D5.2	The miRNome of milk as related to feeding with different extracts (WP5)	UMIL	CO																																		
	D5.3	A list of milk metabolites involved in the immune response which are modified after different extracts regimen (WP5)	UMIL	CO																																		
	D5.4	A list of differentially abundant proteins after feeding with different extracts (WP5)	UMIL	CO																																		
WP6	D6.1	Comparative efficiency of two strategies of EOs encapsulation	STLO	PU																																		
	D6.2	Mechanisms of EOs encapsulation and selection of the most stable and efficient one	LPAM	PU																																		
	D6.3	Antimicrobial activity of encapsulated Eos	LPAM	PU																																		
	D6.4	Nutritional and sensorial properties and characteristics of EOs UHT milk and cheese	IDELE	PU																																		
WP7	D7.1	Sensorial properties of milk enriched with encapsulated Eos	LPAM	CO																																		
	D7.2	Questionnaire establishment on milk consumption	LPAM	PU																																		
	D7.3	Validation of the questionnaire	LPAM	PU																																		
	D7.4	Data Collection and verification	LPAM	PU																																		
	D7.5	Report on consumer survey	LPAM	PU																																		
WP8	D8.1	Communication and dissemination materials	IDELE	PU																																		
	D8.2	Plans for dissemination and exploitation of the results	IDELE	PU																																		
	D8.3	Completed and planned communication activities	IDELE	PU																																		
	D8.4	Report on sustainability of MILKQUA project activities and results	IDELE	PU																																		
	D8.5	Data management plan	IDELE	CO																																		

Table 1 - Table of deliverables of the first and second years

	Deliverable in progress
	In progress : delay validated by the Coordinator
	Late
	Delivered, validation process ongoing
	Finished

## 6.2 Appendix 2 – Summary of milestones

### MilkQua Milestones status

All the Milestones (validated) are in the "Milestones" Room on the Collaborative Platform

Last update:

17/11/2020

Legend			
COLOUR		PICTO	
	Deliverable in progress	I	= Project Month in which the deliverable is initially due
	In progress : delay validated by the Coordinator	P	= New date of delivery if Postponed
	Late	L	= New date of delivery expected if Late
	Delivered, validation process ongoing	O	= Date when the deliverable was delivered to the Coordinator
	Finished	✓	= Date when the deliverable was delivered to the EC

WP	Task	Title	Partner	1 <sup>st</sup> reporting period																		2 <sup>nd</sup> reporting period																	
				2019									2020									2021									2022								
				Apr 1	May 2	June 3	Jul 4	Aug 5	Sept 6	Oct 7	Dec 8	Jan 9	Feb 10	Mar 11	Apr 12	May 13	June 14	Jul 15	Aug 16	Sept 17	Oct 18	Nov 19	Dec 20	Jan 21	Feb 22	Mar 23	Apr 24	May 25	June 26	Jul 27	Aug 28	Sept 29	Oct 30	Nov 31	Dec 32	Jan 33	Feb 34	Mar 35	Apr 36
WP1	M1.1	Management committee constitution (MC)	IDELE			✓																																	
WP2	M2.1	Recommendations for the support of dairy producers and the mobilization of the stakeholders to improve the milk quality and the rationalization of the use of antibiotics in the Tunisian dairy herds	IDELE																							I													
	M2.2	Guidelines of every educational module	IDELE																																	I			
	M2.3	Communication and training plan for the Tunisian dairy farmers through the use of ICT	OEP																																I				
WP3	M3.1	Obtention of bioactive extracts	LPAM														✓																						
	M3.2	Safety report	UPFF														I			✓																			
	M3.3	Selection of EOs and doses	UPFF															✓																					
WP4	M4.1	WP4 delivers samples for analysis to STLO and OMICS	CSIC																							I													
WP5	M5.1	System biology data collected and reported	UMIL																																I				
WP6	M6.1	Ability of heteroprotein coacervates to encapsulate EOs molecules	LPAM																	I																			
	M6.2	Stability of encapsulated EOs without adverse effect	LPAM																							I													
	M6.3	Demonstration of antimicrobial activity of EOs ones encapsulated	LPAM																																			I	
	M6.4	Dairy products quality determination	IDELE																														I						
WP7	M7.1	Acceptance of encapsulated EOs milk by consumer	LPAM																	I																			
	M7.2	Tunisian milk aptitude and practices	LPAM																															I					

Table 2: Summary of Milestones



## 6.3 Appendix 3 – Summary of exploitable results

This table will be filled out within the SIPVEC (Scientific, Intellectual property, Valorisation and Exploitation Committee meetings).

*Table 3: Summary of exploitable results*

Main project results/deliverables	Dissemination status	Potential users	Dissemination strategy	Potential use/exploitation
D2.1 Evaluation report and epidemiology of mastitis in the Tunisian dairy herds. (WP2)	PU	Scientists Pre- and post- farm gate industries Farmer organizations, advisory services General public, NGO, policy makers	Peer-reviewed publications, communications at conferences, newsletters, website, social media MilkQua final seminar, training events, stakeholder workshops	
D2.2 Report on recommendations for the support of dairy producers and the mobilization of the stakeholders to improve the milk quality and the rationalization of the use of antibiotics in the Tunisian dairy herds.	PU	Pre- and post- farm gate industries, Farmer organizations	Newsletters, communications at conferences, MilkQua final seminar, training events, stakeholder workshops, social media, public website	
D2.3 Co-construction of the payment grid to the quality of milk with the all actors of the Tunisian dairy sector.	PU	Pre- and post- farm gate industries, Farmer organizations Advisory services	Newsletters, communications at conferences, MilkQua final seminar, training events, stakeholder workshops, social media, public website	
D2.4 Realization of guidelines of every educational module	PU	Pre- and post- farm gate industries, Farmer organizations Advisory services	Newsletters, communications at conferences, MilkQua final seminar, training events, stakeholder workshops, social media, public website	
D2.5 Development of a communication and training plan for the Tunisian dairy farmers through the use of ICT (SMS, mail, website, networking).	PU	Pre- and post- farm gate industries, Farmer organizations Advisory services	Newsletters, communications at conferences, MilkQua final seminar, training events, stakeholder workshops, social media, public website	
D3.1 Sampling/extraction protocol and list of plants	PU	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D3.2 Bioactive extracts for subsequent biological assessment	PU	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D3.3 Phytochemical profile of selected samples	PU	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D3.4 Report on the effects of plants/essential oils on ruminal fermentation parameters and environmental impact	PU	Scientists Pre- and post- farm gate industries, Farmer organizations Advisory services	Peer-reviewed publications, communications at conferences, newsletters, website, social media MilkQua final seminar, training events, stakeholder workshops,	
D3.5 Report and scientific papers on the anti-inflammatory, antimicrobial and immunomodulatory properties of selected plant species	PU	Scientists Pre- and post- farm gate industries, Farmer organizations Advisory services	Peer-reviewed publications, communications at conferences, newsletters, website, social media MilkQua final seminar, training events, stakeholder workshops,	

D3.6 Safety report on selected samples	PU	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D4.1 Protocols and data collection	PU	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D4.2 Report on EOs newborn calves and feed efficiency	CO	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D4.3 Report on EOs newborn fed calves and health status	CO	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D4.4 Report on the effect of EOs fed on milk yield, quality and health in adult cows	CO	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D4.5 Report on the curative effect of EOs against mastitis in small controlled conditions	PU	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D4.6 Report on the effect of EOs against mastitis (Large scale study, Tunisian Farms)	CO	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D5.1 A list of bacterial at genus level that are present in faeces and milk of cows fed with different EOs (WP5), and from in vitro samples from WP4	CO	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D5.2 The miRNome of milk as related to feeding with different EOs	CO	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D5.3 A list of milk metabolites involved in the immune response which are modified after different EOs regimen (WP5)	CO	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D5.4 A list of differentially abundant proteins after feeding with different EOs	CO	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D6.1 Comparative efficiency of two strategies of EOs encapsulation	PU	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D6.2 Mechanisms of EOs encapsulation and selection of the most stable and efficient one	PU	Scientists	Peer-reviewed publications, communications at conferences, newsletters, website	
D6.3 Antimicrobial activity of encapsulated EOs	PU	Scientists Pre- and post- farm gate industries, Farmer organizations Advisory services General public, NGO, policy makers	Peer-reviewed publications, communications at conferences, newsletters, website, social media MilkQua final seminar, training events, stakeholder workshops	
D6.4 Nutritional and sensorial properties and characteristics of EOs UHT Milk and cheese	PU	Scientists Pre- and post- farm gate industries, Farmer organizations Advisory services General public, NGO, policy makers	Peer-reviewed publications, communications at conferences, newsletters, website, social media MilkQua final seminar, training events, stakeholder workshops	
D7.1 Sensorial properties of milk enriched with encapsulated Eos	PU	Scientists Pre- and post- farm gate industries, Farmer organizations Advisory services	Peer-reviewed publications, communications at conferences, newsletters, website, social media MilkQua final seminar, training events, stakeholder workshops,	

		General public, NGO, policy makers		
D7.4 Data report on consumer survey	PU	Scientists Pre- and post- farm gate industries, Farmer organizations Advisory services General public, NGO, policy makers	Peer-reviewed publications, communications at conferences, newsletters, website, social media MilkQua final seminar, training events, stakeholder workshops	