



## Newsletter #1

Quarterly newsletter on project advancement and last results

March 2021



UNIVERSITÀ  
DEGLI STUDI  
DI MILANO



INSTITUT DE  
L'ÉLEVAGE  
Idele



DANONE



INRAE



INRAE



CSIC



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



### MilkQua project in a few words & advancement status

#### MilkQua in a few words:

##### Plant extracts to prevent mastitis in Tunisia.

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. MilkQua aims to reduce the use of antibiotics related to mastitis through the Tunisian plant extracts. The project brings together 10 partners in Tunisia, France, Italy, Spain and Portugal.

#### Advancement status at M22:

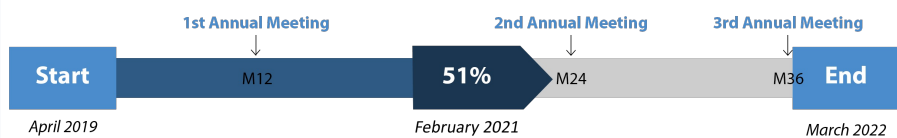
##### Advancement towards objective 1 - Helping producers to prevent monitor and reduce food safety risks in their farms and companies by designing and implementing an on-farm food safety program, the Quality Milk Program (QMP).

To achieve this objective 1, a QMP 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 determine the etiology of mastitis in Tunisian dairy herds and identify the socio and economic determinants of milk quality.

##### Advancement towards objective 2 - 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.

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 alternative to antibiotics. *In vitro* approaches were developed to study their potential beneficial *in vitro* properties to reduce inflammation and as antimicrobials against specific bacteria involved in the development of mastitis in Tunisian dairy herds. *In vivo* 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. *In vitro* fermentation trials have been initiated in November 2020 to evaluate the potential of these Essential Oils to modify ruminal fermentation and to reduce methane production, thus achieving to limit the environmental impact of livestock. Besides, a recent experimental *in vivo* assay was established (Ralph Nehme's Thesis) to evaluate their properties as preventive molecules to treat mastitis (for more details see WP3 and WP4 on the MilkQua website). The samples (milk and other biological samples) were sent to UMIL for evaluating microbiome (WP5) and to INRAE for milk characterization (WP6).

#### Project timeline:



MilkQua started in April 2019. The project just reached the mid term period last October. We are starting to get the first results mostly coming from the work implemented on WP2, WP3 & WP4.

[More information on MilkQua](#)

## Newsletter #1: Focus on WP2 and WP3

### WP2: Innovative Quality Milk Programme and communication technologies practices to manage mastitis and antimicrobial on dairy farm in Tunisia

#### WP2 objectives are:

1. To assess and understand the situation for determining a) the hygienic quality of raw milk and the prevalence and causes of mastitis in cows, b) for identifying bacterial species that cause mastitis and target the appropriate treatments with essential oils (EOs)
2. To describe the knowledge, brakes and motivations of the actors involved in order to identify and understand the factors of success, difficulties and efficiency of raw milk quality control and to reduce the use of antibiotics.
3. To identify the determinants of successful decision-making and a technical conduct as part of a change of direction to improve the raw milk quality and the rationalization of the use of antibiotics on a dairy farm scale.
4. To determine the economic stakes according to the types of processed products and the different markets of the hygienic quality of milks at the level of the Tunisian dairy industry (farmers, collectors and processors).
5. To identify the communication practices, the communication tools and consulting practices in order to improve the perception of the technical practices and the levers for the necessary change for a good mastery of milk quality and proper use of antibiotics.

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



*Training day for interviewers in the Beja region*

#### Activities performed so far:

##### **T2.1 Assessment of mastitis occurrence in Tunisian dairy herds (task leader: OEP)**

OEP, Idele and ENMV were strongly involved in this task in order to figure out the mastitis situation in Tunisian dairy herds. Idele analysed data linked to mammary infections since 10 years collected by OEP and found specific evolutions in mammary infections depending on herds size, region, years.

In the meantime, ENMV performed a retrospective analysis of bacteriological data on mastitis milk available at ENMV. 187 bacteriological results on mastitis milk samples collected between 2009 and 2016 were studied. In these populations of mastitis milk samples, we can see a high prevalence of major pathogenic bacteria (*enterobacteria/staphylococcus aureus* and *streptococcus*). Part of this work had to be postponed because of confinement measures applied in Tunisia.

A new sampling campaign is planned during the epidemiological/etiological surveys.

Idele and OEP also worked on information recovering regarding antibiotic residues in collected milk and consulted several actors of the Tunisian dairy sector in charge of milk quality.

Finally, Idele and ENMV created and tested an epidemiological/etiological survey aiming to better understand mammary infection prevalence and the use of antibiotics in herds. Last February 2020, DGSV veterinarians were trained to implement survey.

#### **T2.2 Social determinants of milk quality (task leader: Idele)**

Idele, ENMV, Danone Delice and INRRAT worked several months on this task to identify the determinants of change in practices by farmers towards the improvement of milk quality and a reduction of the use of antibiotics.

They built a socio-technical survey which aims to describe the brakes, the motivations and assess the difficulties of farmers as well as drivers for change. Last February 2020, OEP technicians were trained to implement the survey. This subtask has been on hold due to confinement measures.

The survey was supposed to be done on 88 herds in various Tunisian regions. However, it had to be postponed and will begin as soon as possible.

#### **T2.3 Economic determinants of milk quality (task leader: Idele)**

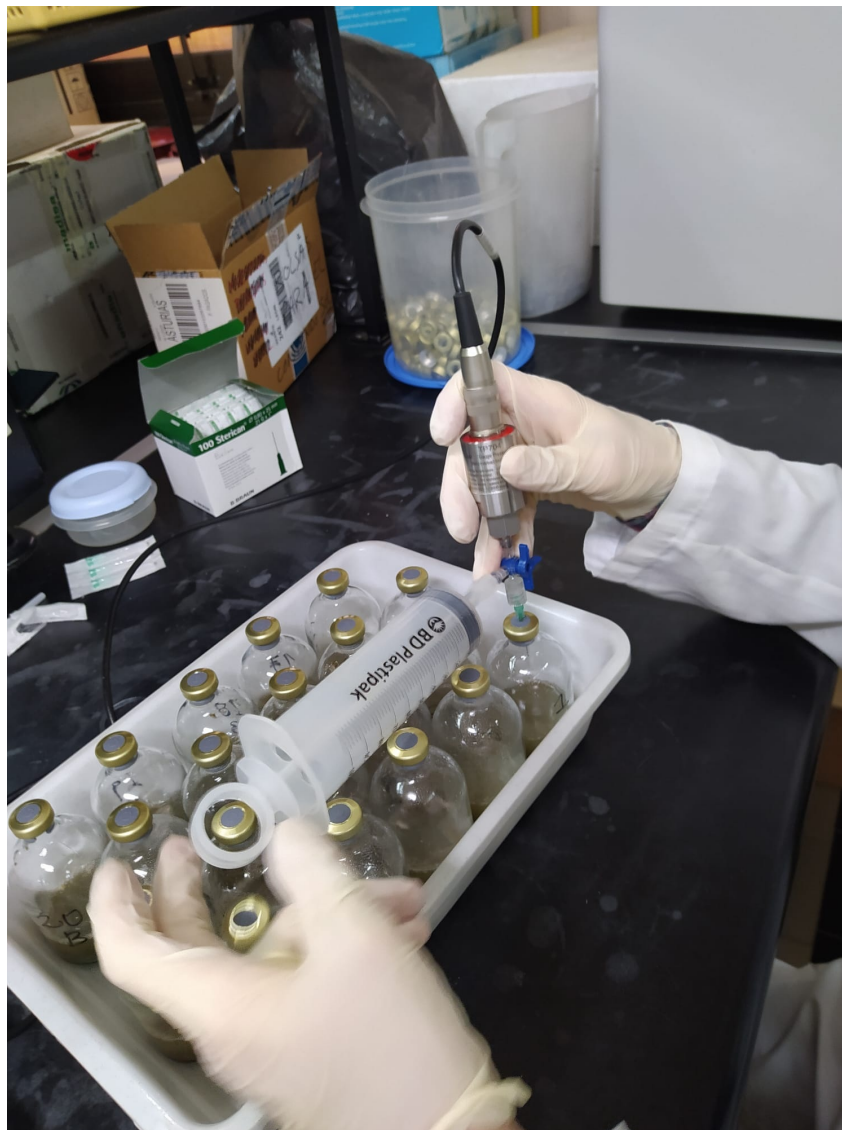
In order to identify the economic determinants of milk quality, Idele, OEP and ENMV led a survey on several herds and raised a major issues impacting the quality of milk linked to the fact that the government determines the price of milk at a level that is too low for farmers to reward milk quality.

Subsidies or milk price incentive grids are recommended to support farmers in achieving a better quality level, a better control of mastitis on herd and a more prudent use of antimicrobials.

**In recent months, the sanitary situation has made it impossible to continue the work undertaken (ban on going to farms, restriction of movement, curfew). Videoconferences are continuing between the Tunisian and French teams in order to sustain the success of this part of the program as soon as the situation permits.**

### **WP3: *In vitro* evaluation of bioactive molecules & extracts**

The overall objectives of this WP are to select the most promising plants regarding their biological potential and provide information regarding their phytochemical composition and mechanism of anti-inflammatory and antimicrobial activity.



*In vitro incubation*

### **T3.1 Standardization of plant sampling procedures (task leader: LPAM)**

Before starting collecting and studying plant samples, partners defined a protocol to ensure equivalent conditions for the collection and storage conditions of all samples in order to avoid variations in the results.

### **T3.2 Extraction protocols & obtention of bioactive extracts, phytochemical characterisation of bioactive extracts and sampling (task leader: LPAM)**

Based on the experience of LPAM with essential oils features and extraction process, GC FID helped determine the phytochemical profile of 10 samples of essential oils.

The results of the work carried out during this task are important for the rest of the project because the information contained on the phytochemical profile of these samples could be used to emulate the biological effects of the essential oils.

### **T3.3 *In vitro* effects of plants & Essential Oils on ruminal fermentation parameters/methane production when included in the diet of ruminants (task leader: CSIC)**

*In vitro* screening has been implemented by CSIC in order to test the best dose of Essential Oils or combination of pure compounds to be added to the diet of dairy cows with a double purpose: a) modify ruminal fermentation parameters to improve feed efficiency, b) reduce methane production, and therefore the environmental impact of livestock. This sub-task is still on-going.

### **T3.4 Anti-inflammatory, antioxidant & antimicrobial activity of Essential Oils & bioactive extracts (task leader: UPFF)**

Using the Essential Oils selected, extracted and the samples delivered in T3.2, LPAM studied their effects on two bacterial strains isolated from infected cow breasts. It appears that one sample was clearly more efficient than the others.

Samples were also used to assess their anti-inflammatory characteristics by testing their capacities to lower the activation of NF- $\kappa$ B caused by pro-inflammatory molecules. Considering the results, 4 of the samples have been selected for further characterization of their mechanism of action, which is now taking place.

### **T3.5 To assess the potential toxicity of plants materials (task leader: UPFF)**

Additional *in vitro* tests have been implemented in order to assess the toxicity of Essential Oils. The evaluation was made towards THP-1 cell line, also used for the anti-inflammatory activity assessment, thus they were the most relevant. None of the tested samples exhibited genotoxic effects at the highest non-toxic concentration and this toxicological assessment has allowed choosing the most adequate concentrations for other biological tests.

### Save the dates / events to come

- **19th March:** MilkQua Scientific meeting: presentation of first results from WP3, WP4, WP5
- **Coming date:** 4th International Symposium "Milk, vector of development" (CIRAD-INRA-IDELE-OEP), Tunisia
- **1st & 2nd June:** [19th Congress on Animal Production, on-line conference](#)
- **21st & 22nd June:** MilkQua project steering board
- **30th August - 3rd September :** [EAAP - 72nd Annual Meeting of the European Federation of Animal Science in Davos, Switzerland](#)
- **26th - 30th September :** [WBC World Buiatrics congress 2021](#)

### Deliverables already submitted

- D1.1 Management committee constitution
- D3.1 Sampling/extraction protocol and list of plants
- D3.2 Bioactive extracts for subsequent biological assessment
- D3.3 Phytochemical profile of selected samples
- D3.6 Safety report on selected samples
- D4.1 Protocols and data collection
- D8.1 Communication and dissemination materials
- D8.2 Plans for dissemination and exploitation of the results
- D8.3 Completed and planned communication activities

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