## *BIOVITAE BUFFALO PROJECT*

How food is produced has strong environmental and health implications that cannot be neglected.

Almost all of the products of animal origin that can be found in large-scale distribution circuits come from intensive farms, therefore from farms that are characterized by the high concentration of animals in confined and controlled environments.

In these environments, the ideal conditions are created for the development and propagation of known and emerging diseases that can have important repercussions on society, from a health and economic point of view.

After the Second World War, the so-called "Green Revolution" changed the scenario of agricultural environments, open pastures gave way to large sheds where animals are confined, and farms that diversify crops and livestock opt for for the cultivation of a few, if not a single crop.

The goal of the Green Revolution is to guarantee food for everyone after the difficult years of the war, and to this end, governments encourage the cultivation of plants such as corn, the production of which becomes profitable thanks to state aid. Farmers can buy cereals at low prices, feed animals and sell their meat on acceptable terms. Inevitably, the low production costs of meat affect the living conditions of the animals.

Disease is a rare occurrence in nature, usually affecting individuals with weaker immune systems: puppies, older or weakened animals. Furthermore, the genetic predisposition exposes some individuals more than others to infectious risks caused by exposure to pathogens.

Genetic diversity, personal and environmental hygiene are the factors that determine the good state of health of a population or a group of individuals: in intensive farming these factors are irremediably compromised.

The living conditions of animals in massive farms are responsible for their poor state of health, to remedy which it is often necessary to

resort to drugs, in particular antibiotics. Intensive farming could not function without pharmacological support.

The first antibiotic used in the livestock sector, in the 1940s, was penicillin which proved to be much more effective than the treatments previously used in cases of bovine mastitis in dairy cows. Later, streptomycin, added to the chicken diet, was found to increase their weight. Over the years and with the progressive decrease in the cost of antibiotics, their use as drugs and as supplements for their growth promoting effect has been increasing.

In addition to the constant administration of doses of drugs, feeding stress is also very important in intensive farming. The animals are given excessively nutrient-rich foods that accelerate the metabolism such as to force the early slaughter of animals that would not stand up to this stress.

If in nature wild animals are the main reservoirs of pathogens, intensive farming can be considered biological reactors in which microorganisms are able to mutate rapidly and unpredictably, causing new uncontrollable pathologies. If in an industrial farm an animal contracts an infectious disease, unlike what would happen in nature, the agent that caused it is quickly transmitted to all the others.

It therefore becomes clear how high the risk is in these environments with a high rate of promiscuity and how important it is to implement preventive measures useful to contain the micro-pandemics that occur very often in intensive farming.

In the past we have seen several examples of the risk deriving from intensive farming and how these, when managed lightly, give rise to real problems that are very difficult to manage, as well as cruel attitudes towards animals deprived of any form of comfort and freedom.

The use of minced meat from sheep that died of scrapie (HIV) was used in the form of flours to feed lactating cows, which in turn were transformed into meat for human food. The use of these foods is believed to be closely related to CDJ disease in humans (Creutzfeldt-Jakob disease) which was believed to be in close connection with the PRION derived from mad cow disease [1,2].

We have produced white meat calves fed until the desired weight is reached with milk only, preventing the normal functioning of the esophageal shower. By completely depriving them of the share of trace elements they were forced to a chronic anemic state, they were kept tied

in a fixed position (fixed post), tied to the chain, for the sole purpose of selling this type of meat on pediatric advice. DES (diethylstilbesterol) was used, this behavior was blocked, and specific regulatory references were issued [3,4].

In rabbit farms, we have shortened the reproductive intervals to 38 days in order to obtain 8/9 parts per year. The concentrations of chickens, turkeys, guinea fowl, in rotating industrial sheds that reached up to half a million head [5,6].

Breeding of mustelids, lagomorphs, and others, in very small cages to produce furs. The preservation of the aesthetic quality of the fur turned into a series of cruel actions [7]: creation of "artificial" genetic lines with the sole purpose of deforming the animals in the edible parts involved in the greatest conversion and development (pigs, chickens, cows, beef cattle, etc.).[8] Egg laying rhythms by laying hens in order to reach values of 98% / year, i.e. one egg per day, except for sending animals below the 90% threshold to the slaughterhouse [8].

It is well understood that this model of breeding is unsustainable from all points of view. Due to the presence of numerous highly complex elements, it is necessary to approach the problem from different points of view with the aim of achieving sustainable living conditions for animals, as well as ensuring safe nutrition throughout the food chain and taking great care of health safety.

## GENERALITY

The term zoonosis refers to a group of infectious diseases that have the characteristic of being transmissible from animals to humans and vice versa.

These are diseases that can be transmitted through all the most common routes of contagion: directly from animal to animal or indirectly through animated vehicles (parasites, insects, other animals) or inanimate vehicles.

Despite the epidemics, the attention on the consequences for the health of workers has often been overlooked or taken into little consideration, the risk of contracting a direct zoonosis is very high for operators in the livestock sector, given the frequent contact with the animal. with its secretions or excretions and with the environment potentially contaminated by them.

Today, this risk appears to be considerably increased due to the preminent typology of workers in charge of animal management, mainly of foreign origin, with linguistic difficulties, different habits, poor understanding of the usefulness of adopting protective devices and/or often incorrectly and properly trained.

## EXECUTION

The project was divided in two phases:

**Phase 1**: involved the De Vita livestock farm, based in Capaccio (SA), and the laboratory at the University of Naples Federico II that verified the effectiveness of the Biovitae technology on bacterial strains collected inside the milking parlor. The different microorganisms isolated during the different sampling were brought to the laboratory to be subjected to tests that had the aim of verifying the efficacy of a lighting source with standard Biovitae technology against these microorganisms. The list of microorganisms detected during sampling both on surfaces and in cow's milk is the following: Gram−: *Escherichia coli, Klebsiella spp., Enterobacter spp., Pseudomonas spp.* Gram+: *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Streptococcus dysgalactiae, S.microti*.

**Phase 2**: involved the design of lighting devices with Biovitae technology suitable for providing the correct lighting required by law in the milking parlor as well as guaranteeing continuous sanitation of the environment. Microbiological tests were carried out in order to verify the correct application of the technology in milking environments in terms of containment of the microbial load dangerous for living beings and the preservation of the natural microbial flora that plays an important role

thanks to its continuous interaction and collaboration with living beings. The samples collected included freshly milked buffalo milk, swabs from the parlor surfaces and swabs made on the hands and nostrils of the farm workers during milking.
The farm raises around 200 buffalo cows and uses a 2 x 3 tandem milking parlor for lactating buffaloes.

The results of the sampling showed that the pathogenic microorganisms present were predominantly Gram−, there were few potentially pathogenic Gram+. Most interesting was the detection of a specific family of *Staphylococcus microti* a microorganism actively involved in bovine mastitis.

## *Laboratory testing*

*Ethical Statement: the study's protocol was approved by the Ethical Animal Care and Use Committee of the University of Naples Federico II (Certificate Number: PG/2020/009228 del 06/11/2020), in compliance with the Italian Legislative Decree 26/2014, Article 2.*

### MATERIALS AND METHODS

The bacterial strains, i.e. *Escherichia coli, Klebsiella spp., Enterobacter spp., Pseudomonas spp., Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Streptococcus dysgalactiae, Staphylococcus microti*, were collected in the milking parlor, properly stored and transported to Microbiology Laboratory of the Department of Veterinary Medicine and Animal Production of the University of Naples Federico II (Naples, Italy), stored at −80°C in Microbank™ vials (Pro-lab Diagnostics, Richmond Hill, ON, Canada). The strains were identified by proteomic analysis using matrix assisted laser desorption/ionization - time of flight mass spectrometry (MALDI-TOF-MS) (Bruker Daltonik, Germany). The strains were transferred from the bacterial stock into appropriate growth media, McConkey agar for E. coli and Columbia CNA agar for S. aureus respectively. The plates were placed in an incubator for 24 hours at 37°C.

To perform the tests, a loop of fresh bacterial colonies was transferred into 3 mL of Heart and Brain Infusion Broth (BHI Broth), which is a non-selective enrichment medium for aerobic bacteria, and then incubated aerobically at 37° C. Until the desired bacterial population is obtained for experimental use, equal to 0.5 McFarland turbidity. The plates were enumerated and 10 µl of suspension were

distributed, for each plate. Soil MacConkey agar were used for Gram− microorganisms, and Columbia CNA agar, for Gram+ microorganism. The inoculated drops on the plate were allowed to dry and then exposed uncovered under the Biovitae LEDs. Exposure times were 2 and 4 hours for the Master Light strip, at controlled temperature and humidity in BSC.

A LED strip supplied by Nextsense Srl (Biovitae Masterlight) and powered with Biovitae technology was used in this study (Fig. 1). Master light strip Biovitae ® uses a special combination of frequencies covering the visible spectrum with energy humps at 400–420 nm, 400–450 nm, 400–700 nm at an intensity of 3.51 mW $cm^2$, 5.85 mW $cm^2$, 12.53 mW $cm^2$, respectively (Fig.2).

The Biovitae device was installed in biosafe cabinet (BSC) at 30 cm distance from the work surface, operating in continuous wave (CW) throughout the experimental period. To prevent any sample heating during exposure, the lamp is set up with a heat sink for thermal management.

**Fig. 1** - Biovitae Masterlight

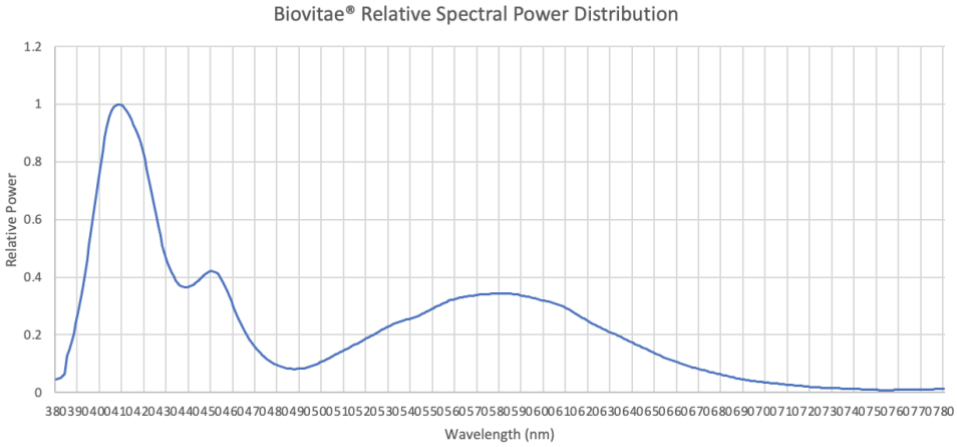



**Fig. 2** - In the main experiment, a master Biovitae LED strip was used. The strip is made of 13 Biovitae LEDs delivering 2.24 W/nm in the 400-420 nm range at 55° angle, and of 37 White LEDs (Osram Oslon (R) Square GW CSSRM2.EM-MFN2-XXX5-1). The LED strip is powered at 500mA constant current, supplied with a TCI MP 80/500 SLIM LED driver

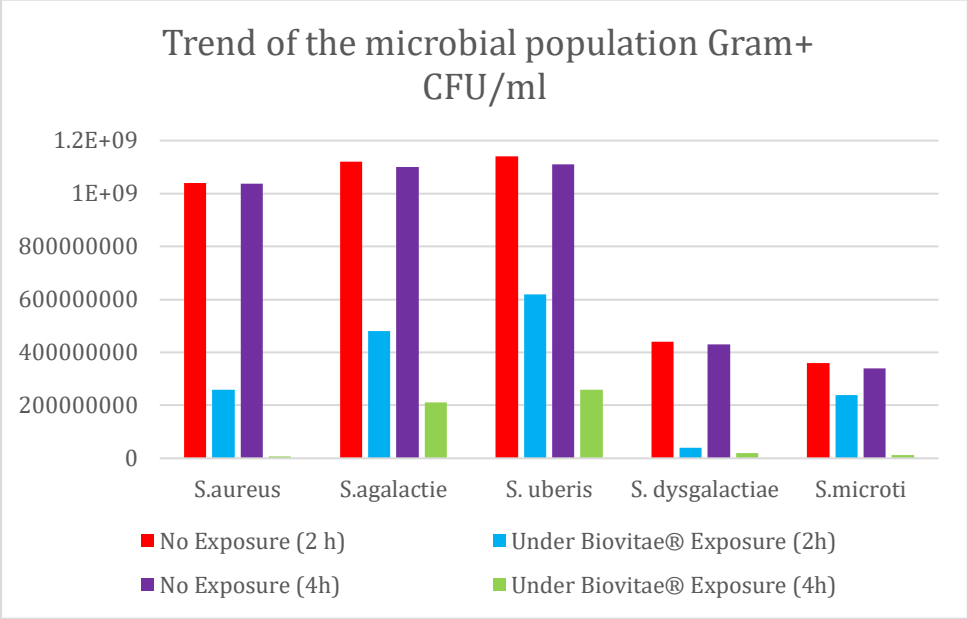



**Fig. 3** The microbial strains subjected to the action of Biovitae Masterlight showed a reduction of more than 95% after 2 hours, reaching 99% reduction in 4 hours. All the tests were performed in quadruplicate the result is given by the average of the four repetitions.

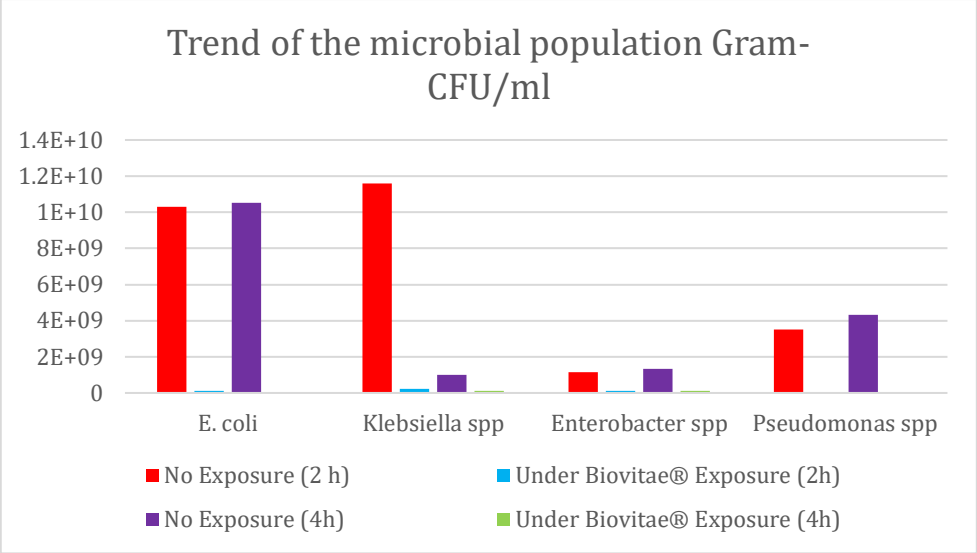



**Fig. 4** The microbial strains subjected to the action of Biovitae Masterlight showed a reduction of more than 97% after 2 hours, reaching 99.9% reduction in 4 hours. All the tests were performed in quadruplicate the result is given by the average of the four repetitions.

## *Tests in Real Environment*

The tests in real environment were designed in order to verify that the lighting products made with Biovitae technology were used correctly by the operators and where necessary to allow the fine adjustment of the lamps so that they operate in the spirit for which they were designed, that is to contain the microbial load without ever reaching the complete sterility of the environments in which it is installed.

### SAMPLING MODEL

Each time two milk samples were taken per lactating animal, precisely two aliquots of milk were taken, one of 50 ml and 15 ml, each of which was taken from the pool of quarters. The first aliquot was used for the somatic cell count and product examination, while the second for the microbiological study.

The samplings carried out with sterile swabs involved different surfaces within the milking parlor and immersed in transport soil. Samples were taken both before the start of the milking phase, therefore inside the room without animals, and during the milking phase, when about 20% of the animals had been milked. In addition, room workers

were sampled by taking two swabs from their hands (one swab for each hand) and a single swab for both nostrils.

The samples, once taken from the farm, were transported within 24 hours in an isothermal box to the Veterinary Microbiology Laboratory of the Department of Veterinary Medicine and Animal Production (NA), for the microbiological study, and to the Institute of Zooprophylactic of Southern Italy (NA) for the count of somatic cells and for the commodity examination.

## TEST SETUP

The sampling setup provided that the normal cleaning routine was respected in both phases of the research. Before the sampling began, the milking operators were informed about the best practices to use on themselves and in the environment before and during milking operations. The hours of use of the lamps with Biovitae technology were calculated by Nextsense technicians to ensure control of the microbial load present inside the milking parlor.

In the first phase of the tests (without the use of lamps with Biovitae technology) in the real environment, normal lighting was used by applying the same protocol in terms of hours of use. The lighting devices with Biovitae technology were switched on 1 hour before the start of milking and left on for the 4 hours following the end of milking. Milking was done twice a day in the morning from 05:00 AM to 06:30 AM and in the afternoon from 04:00 PM to 05:30 PM.

## MILK samples: Bacterial growth of Gram– and Gram+ in the samples before the installation of the lamps

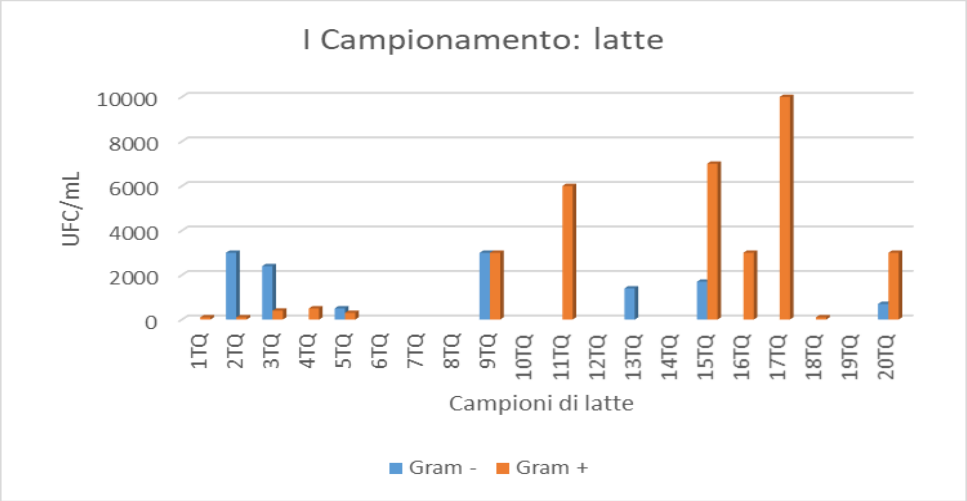



**Fig.5.** First milk samples before Biovitae lamps installation.

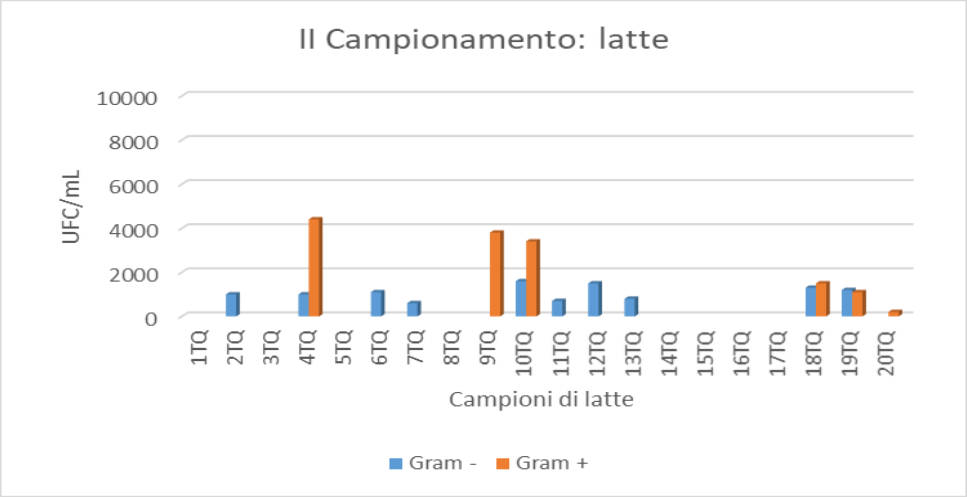
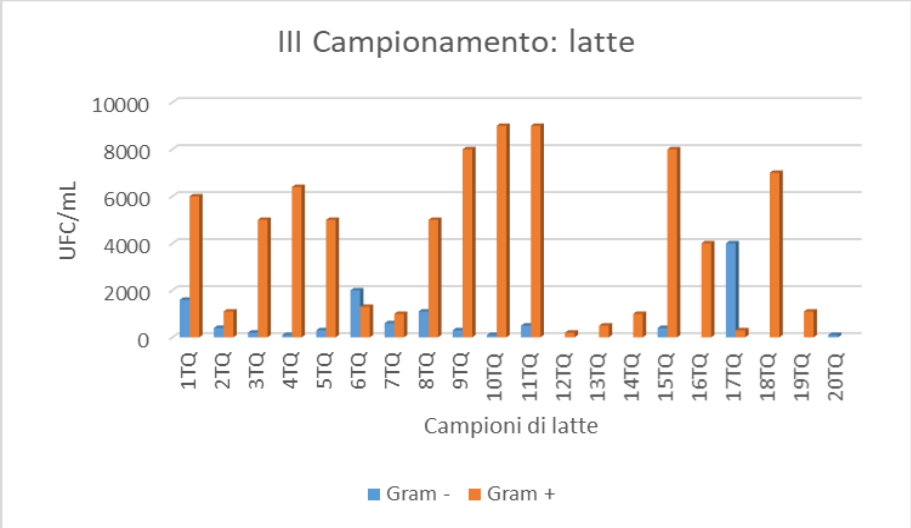
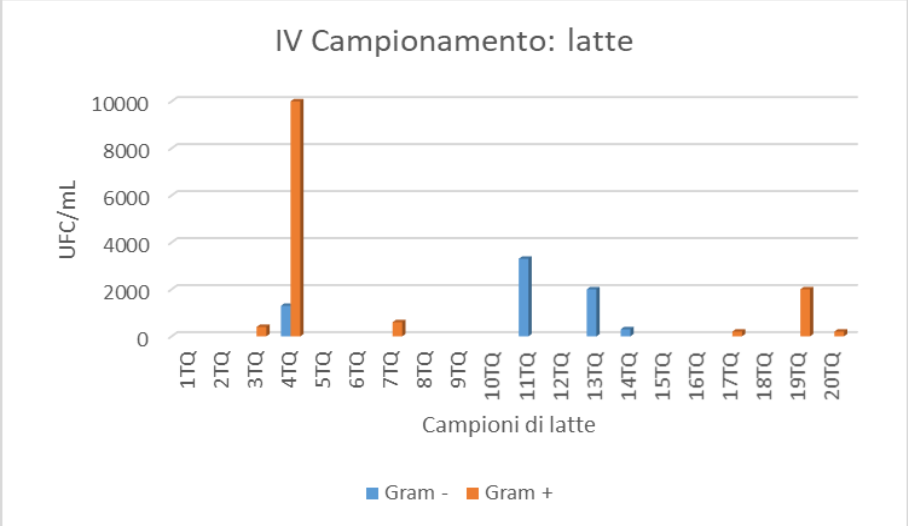



**Fig.6.** Second milk samples before Biovitae lamps installation.

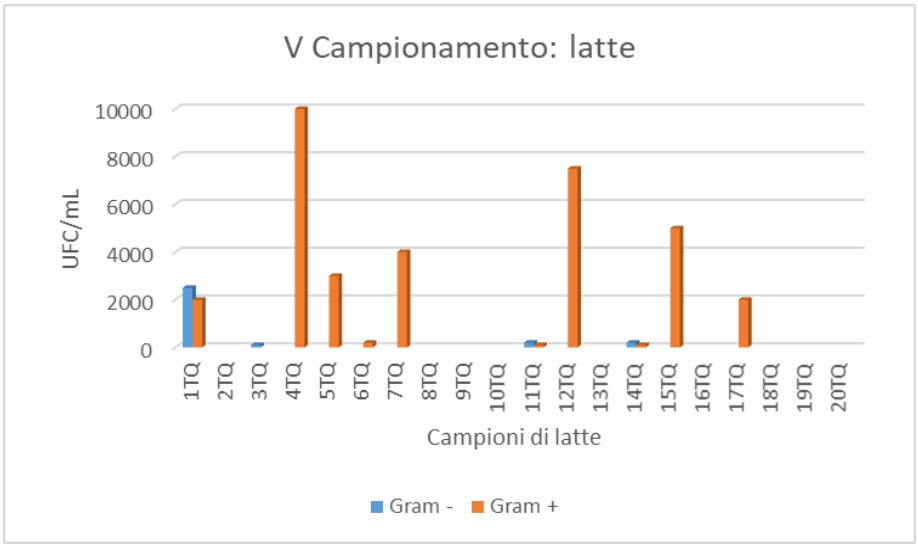
***TQ**: milk sample as collected (Tal Quale);* **–:** Es. Escherichia coli, Klebsiella spp., Enterobacter spp., Pseudomonas spp.; **Gram+:** Es. *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Streptococcus dysgalactiae.*

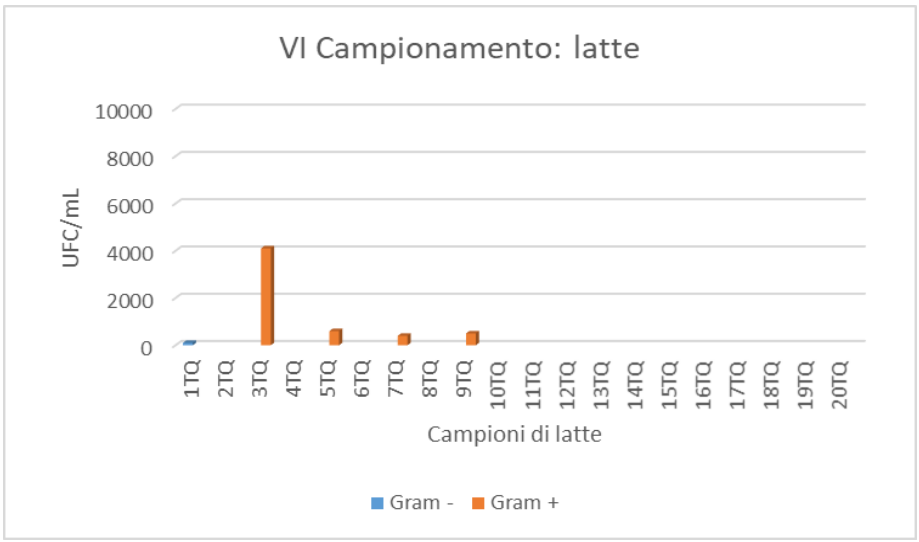
## MILK samples: Bacterial growth of Gram– and + in the samples afer the installation of the lamps




***TQ**: milk sample as collected (Tal Quale);* **Gram−:** Es. Escherichia coli, Klebsiella spp., Enterobacter spp., Pseudomonas spp.; **Gram+:** Es. *Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Streptococcus dysgalactiae.*

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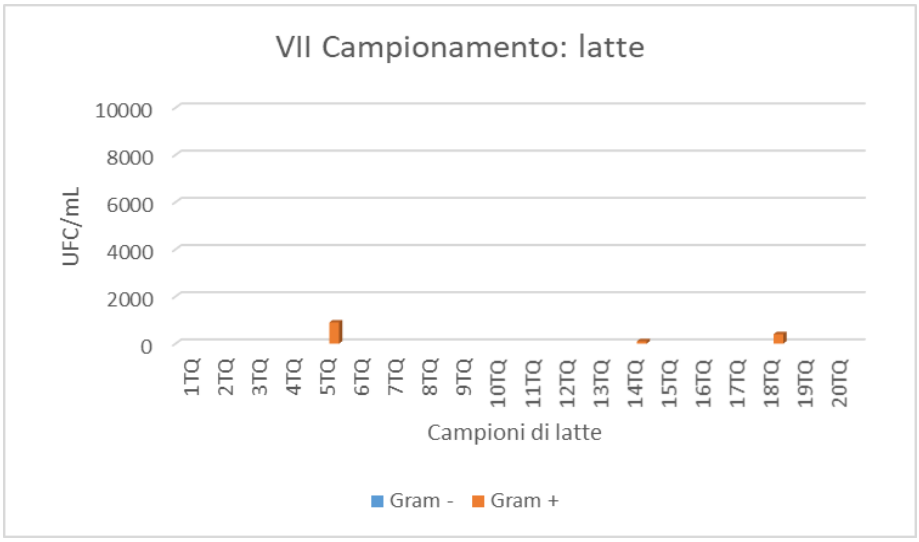


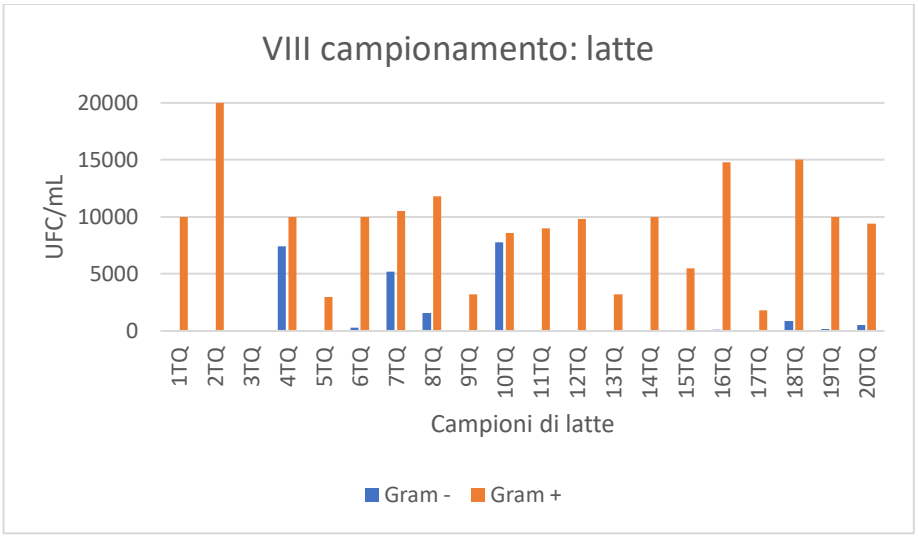


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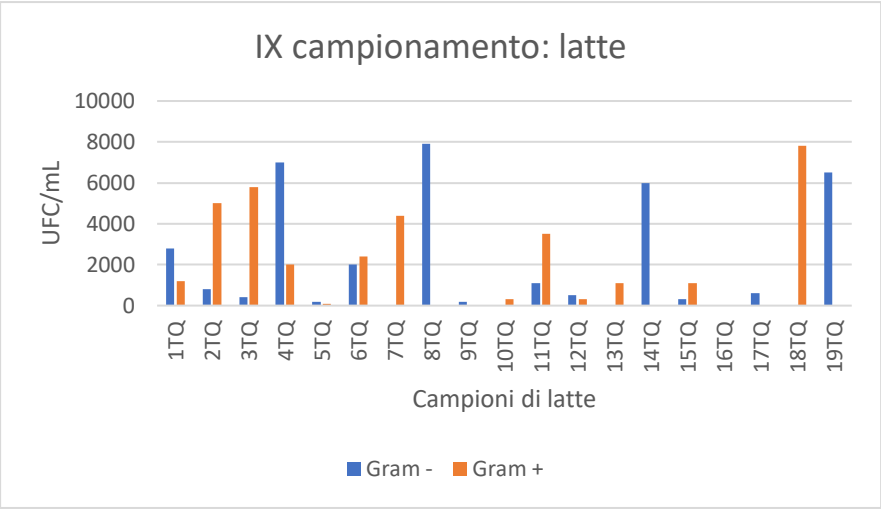


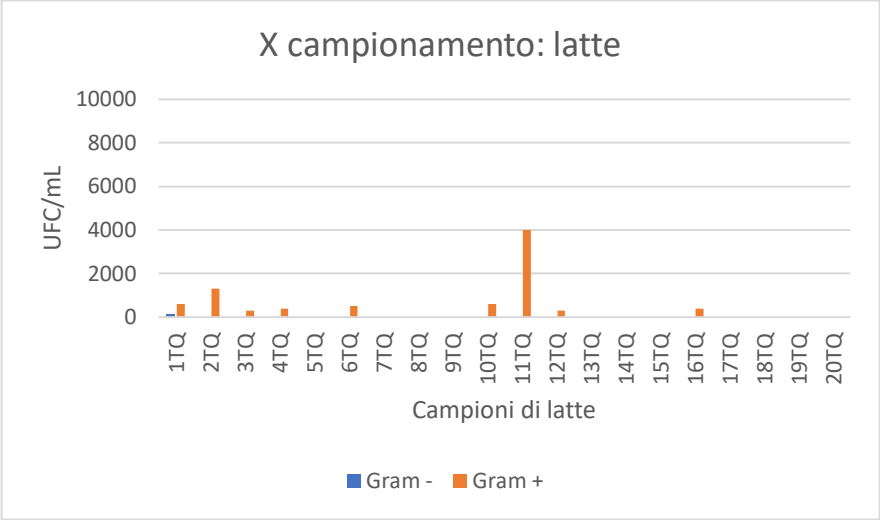


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The histograms show how, from the sampling following the installation and continuous use of the Biovitae lamps, there was a strong decrease in the total bacterial load in the milk samples.

It is very interesting to note that in the sampling relative to samples VIII and IX there was a reappearance of the Gram− microorganisms and an increase in the Gram+ populations. In this case, a new milking operator did not follow the agreed usage protocol, turning off the lights immediately after milking operations were over.

It can be noted that already from the IX sampling immediately following the restoration of the protocol of use of the lamps with

Biovitae technology the microbial load begins to decrease rapidly until it reaches a very low microbial load.
In fact, in the last sampling, the trend decreased again, presenting a reduced presence of Gram+ and an absence of Gram−.

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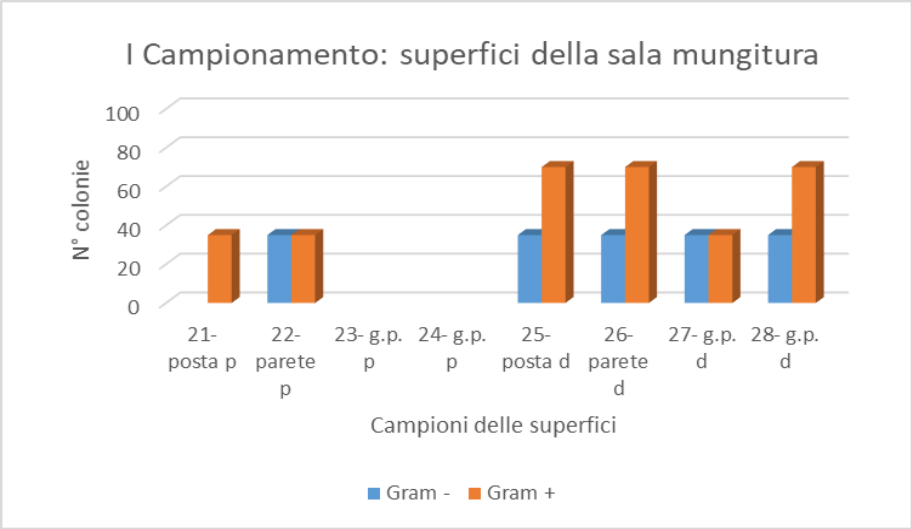
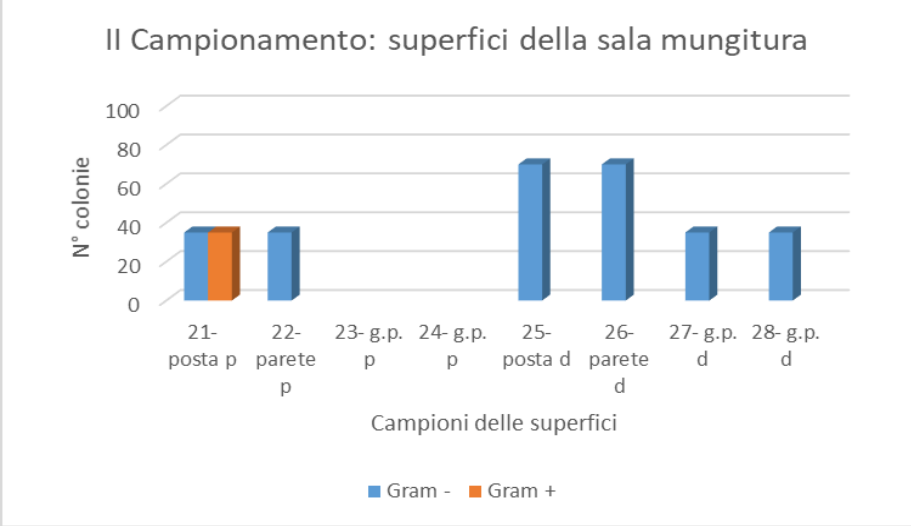
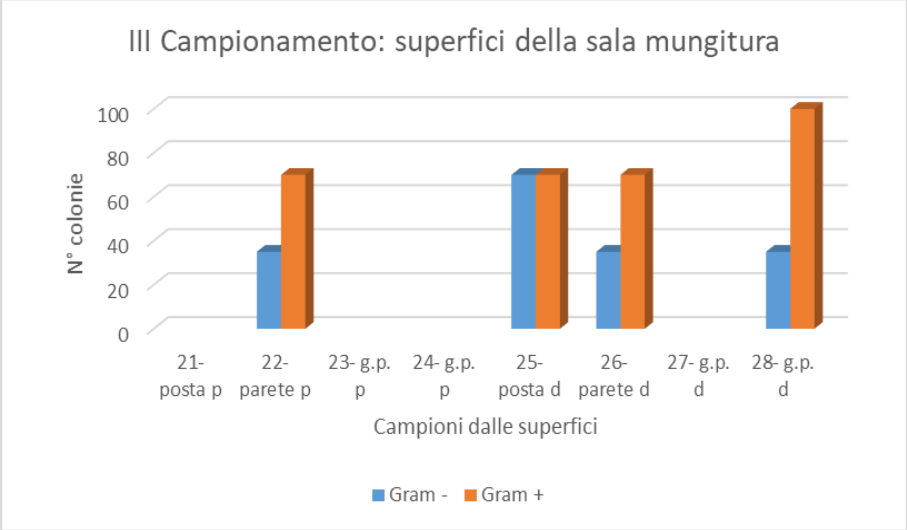
*SURFACES OF THE MILKING ROOM: Bacterial growth of Gram– and Gram+ in samples from surfaces before the installation of the lamps*

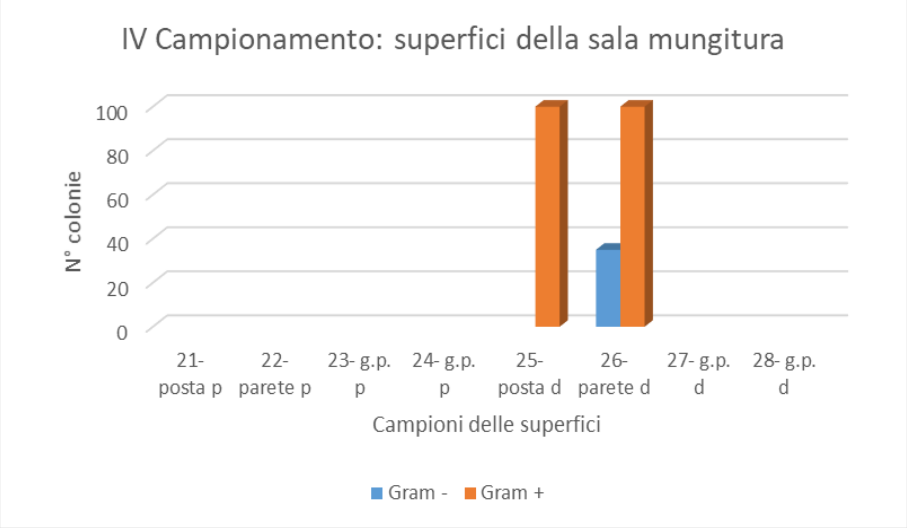



***Posta p**: pre-milking animal station. **Parete p**: pre-milking parlor wall. **g.p.p** : pre milking nipple-taking group; **post d**: post milking animal station; **wall d**: post milking parlor wall; **g.p.d** : post milking nipple-taking group*; **Gram–**: *Eg Escherichia coli, Klebsiella spp., Enterobacter spp., Pseudomonas spp.* **Gram+**: *Eg Staphylococcus aureus, Streptococcus agalactiae, Streptococcus uberis, Streptococcus dysgalactiae.*

*Bacterial growth of Gram– and Gram+ in the samples after the installation of the lamps*

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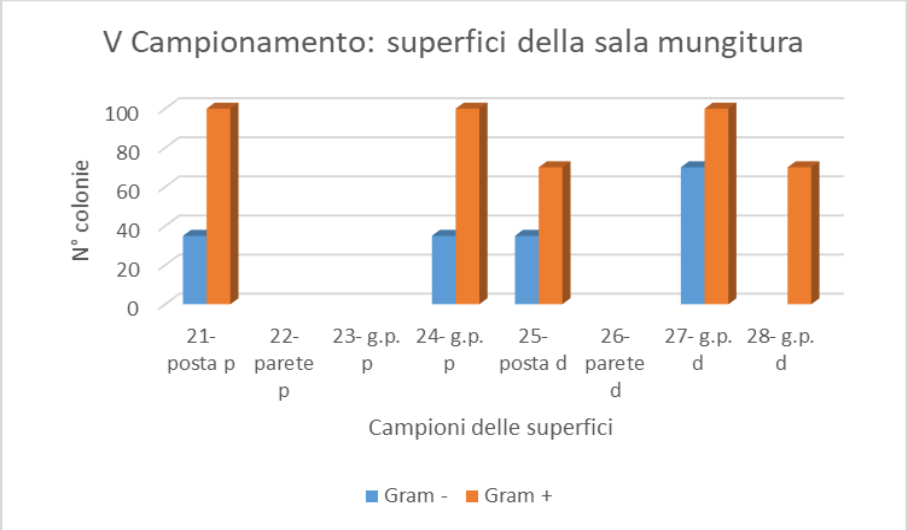


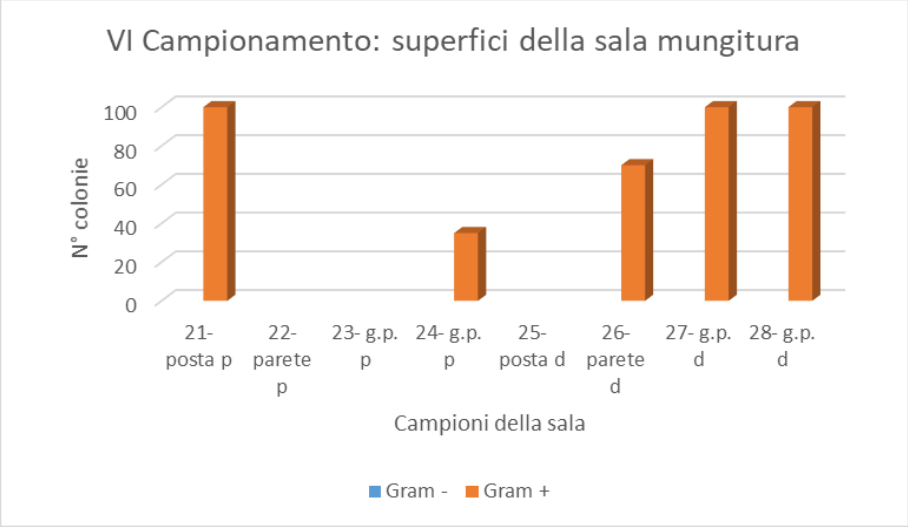


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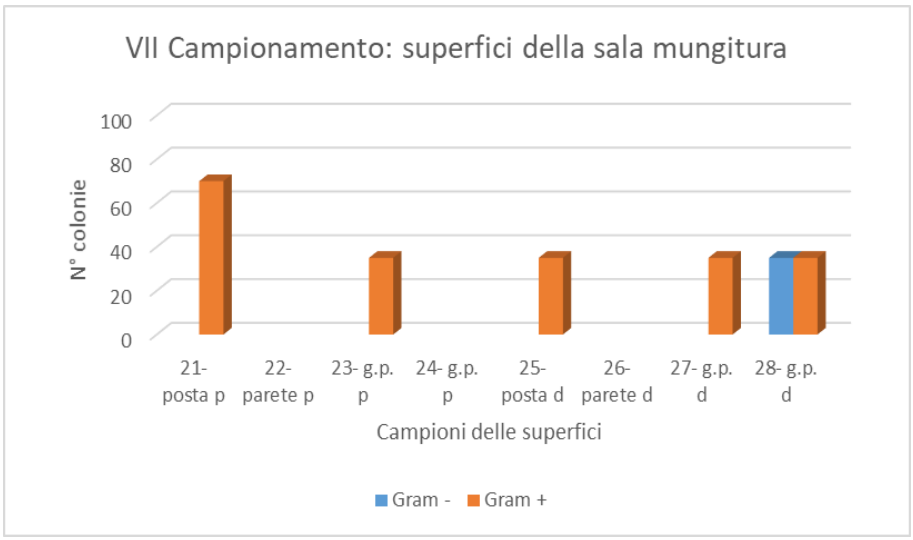


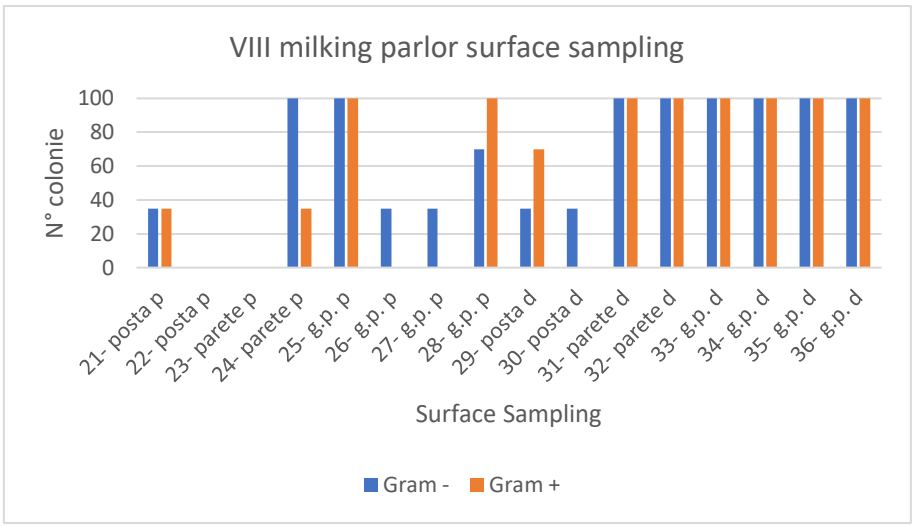


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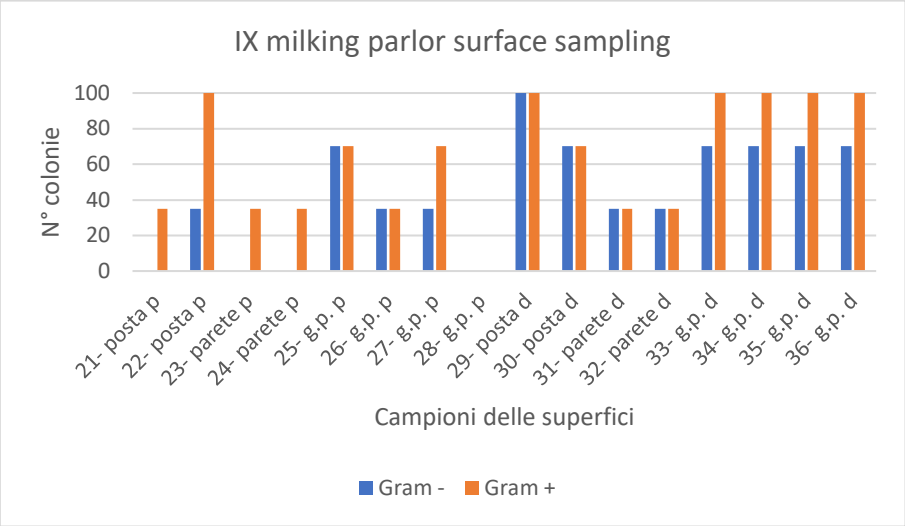


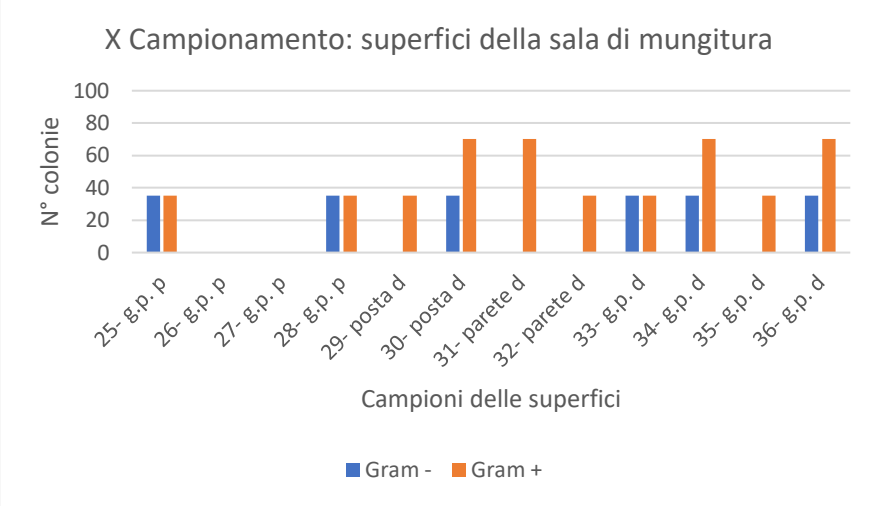


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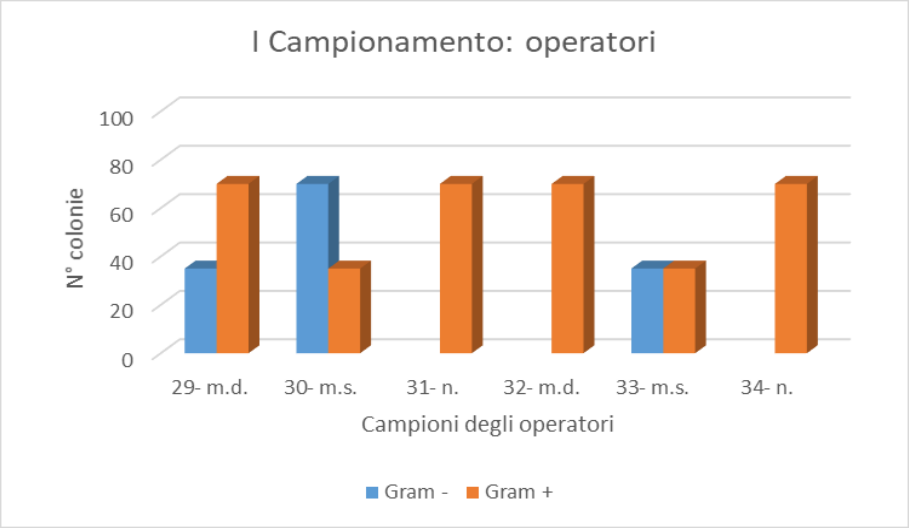
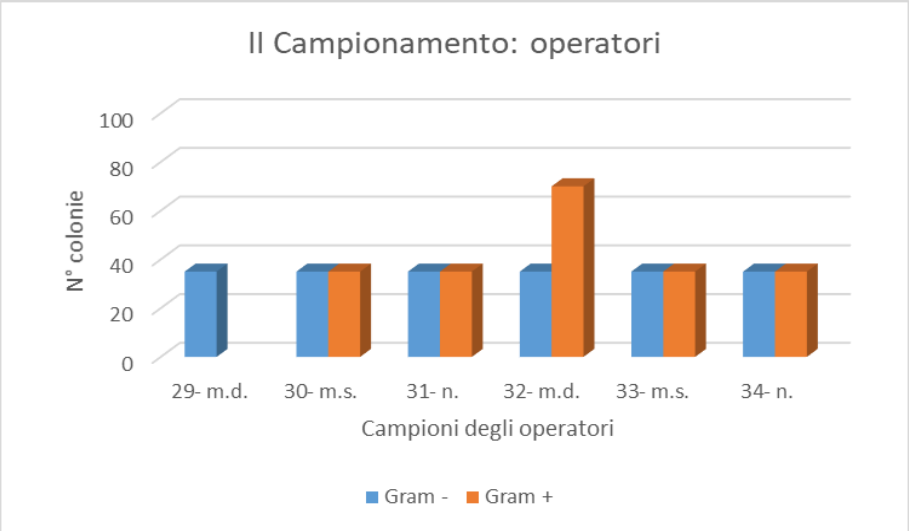
The swabs taken from the surfaces of the milking parlor show a reduction in the total bacterial load for each sampling subsequent to the installation of the Biovitae lamps. Specifically, there appears a notable decrease in the growth of Gram− bacteria and a consequent decrease in Gram+.

All swabs were also tested for the growth of fungi, reporting the presence of Candida on the surfaces of the room only in the two pre-installation samples and in the first post-installation. In the last samplings after the installation of the lamps there was also an absence of fungi. Even the surfaces are affected by the incorrect application of the protocol as detected in the milk samples at the VIII sampling. The immediate restoration of the correct conditions of use also promptly checked the microbial load present on the surfaces.

*Samples taken from the hands and nostrils of the MILKING ROOM OPERATORS*

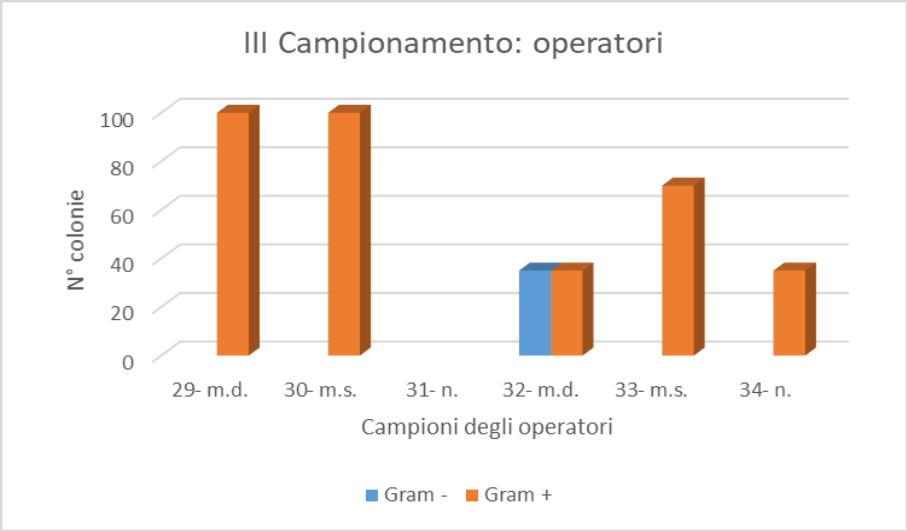


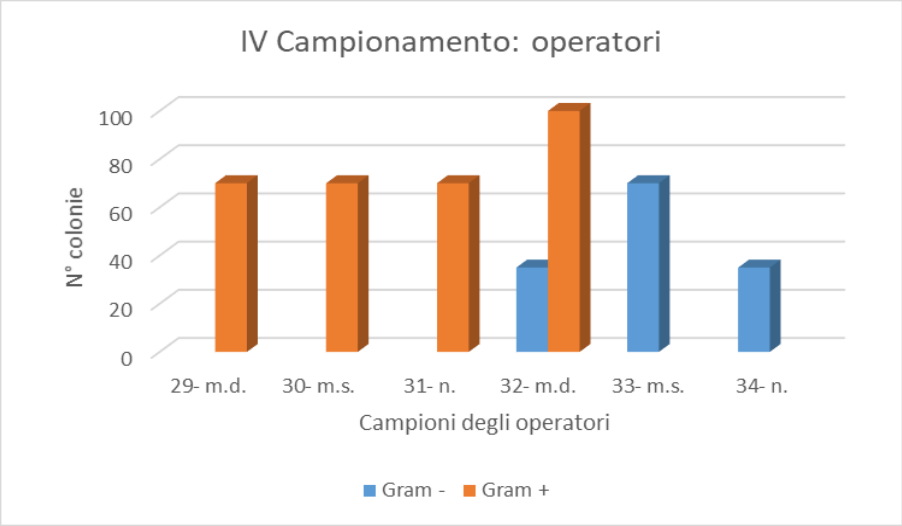




Gram– and Gram+ microbial load in the samples of the room operators without Biovitae technology.

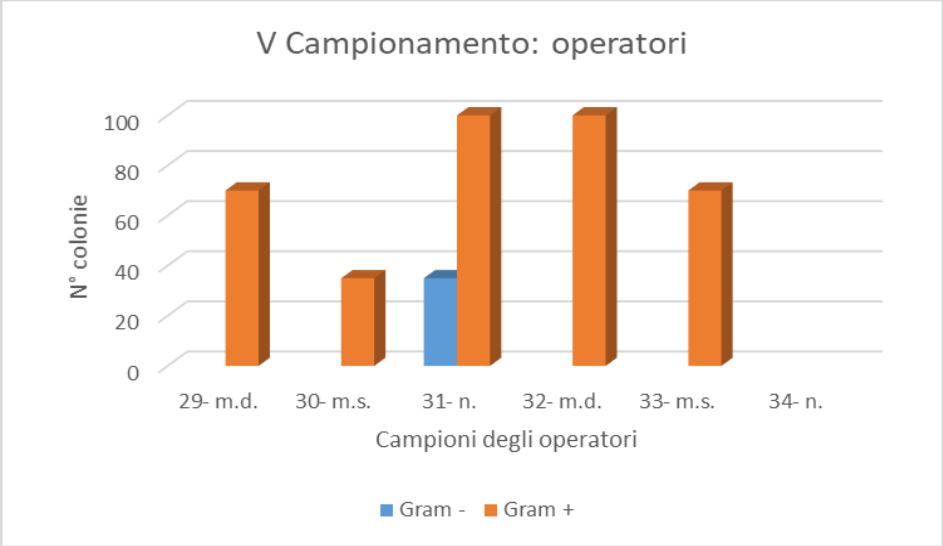
***m.d** .: right hand of an operator; **m.s** .: left hand of an operator; **n** .: both nostrils of an operator*

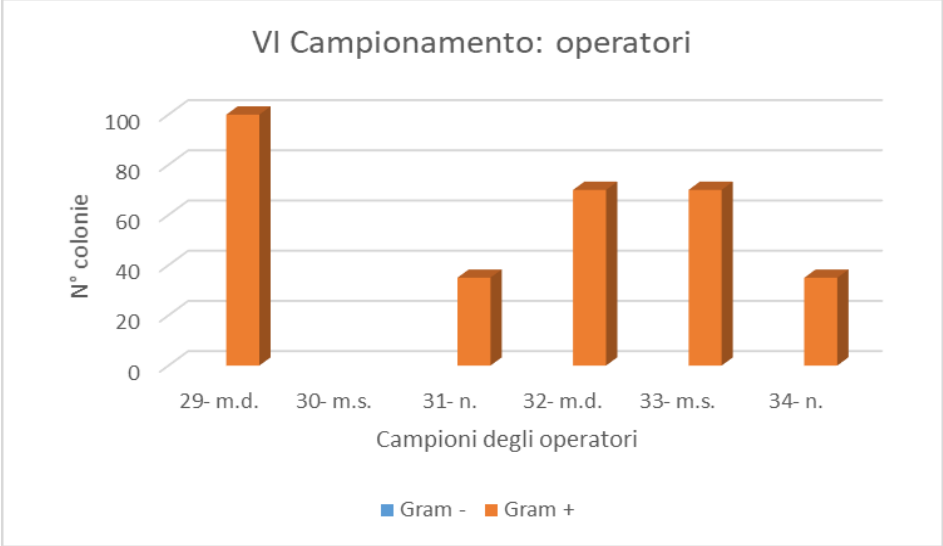
*Bacterial growth of Gram– and Gram+ in samples after the installation of lamps with Biovitae technology*



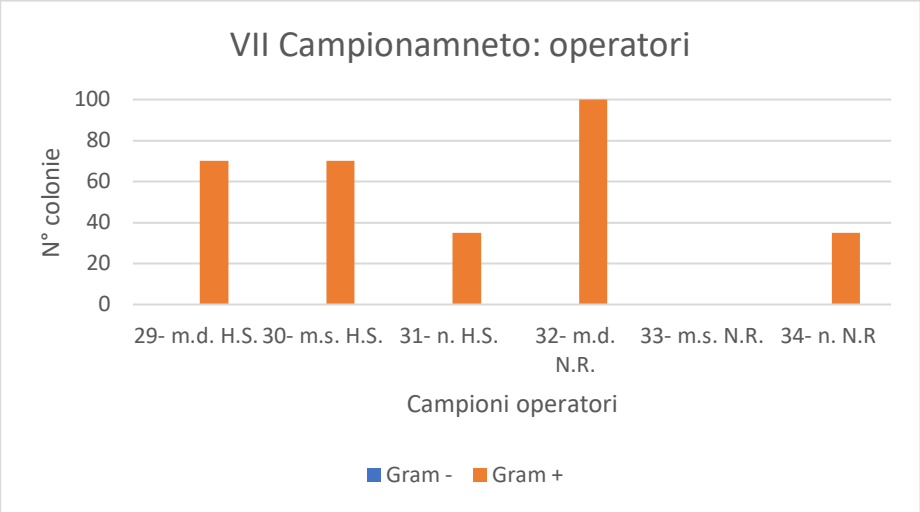



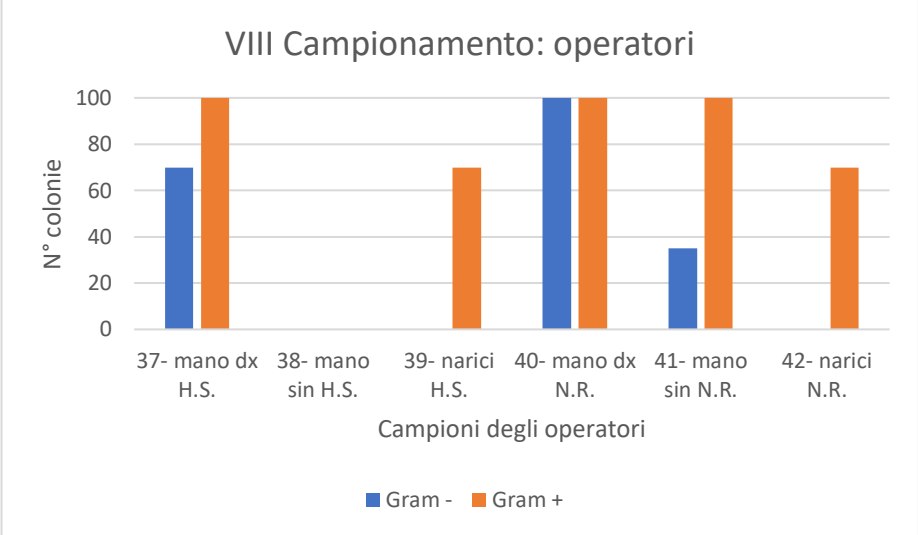

***m.d** .: right hand of an operator; **m.s** .: left hand of an operator; **n** .: both nostrils of an operator*



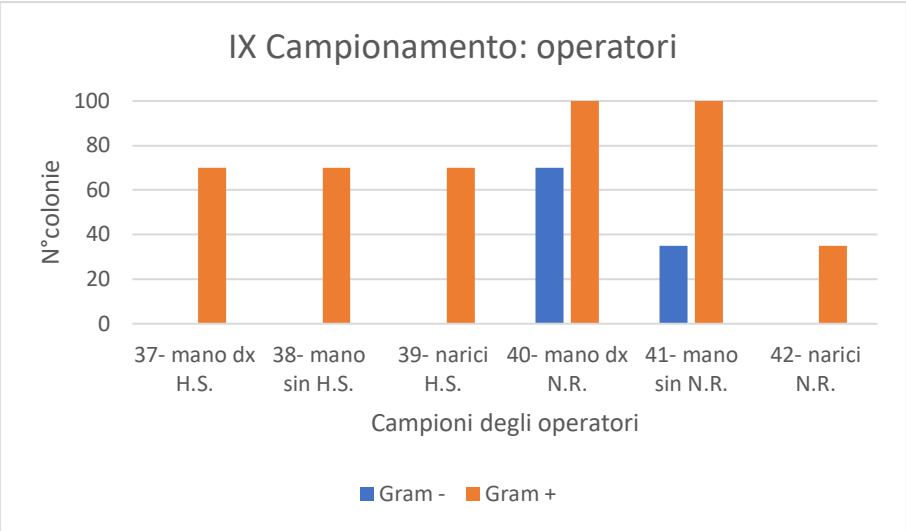



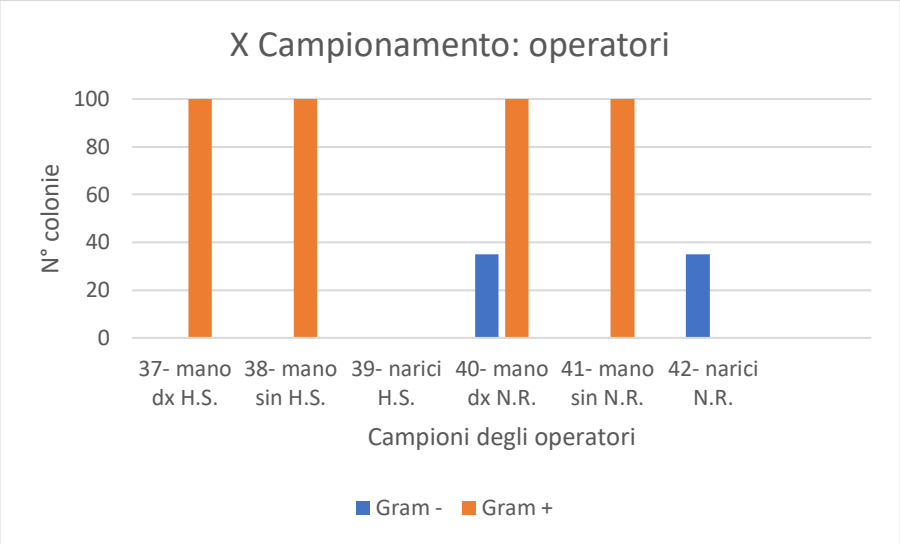

***m.d** .: right hand of an operator; **m.s** .: left hand of an operator; **n** .: both nostrils of an operator*




***m.d .****: right hand of an operator;* ***m.s .****: left hand of an operator;* ***n .****: both nostrils of an operator*




***m.d*** *.: right hand of an operator;* ***m.s*** *.: left hand of an operator;* ***n*** *.: both nostrils of an operator*

## *Conclusions*

Buffalo breeding is characterized by free housing and the concentration of more than 60/70 animals in boxes of about 800/900 square meters. The litter and the high concentration of buffaloes in large boxes is the first considerable difference with cattle breeding. Buffaloes are wild animals that love to live in open spaces. This determines a broad conformation of the stables, built with galvanized iron fences and covered, only in the upper part, by an iron or even wooden roof. Only in some cases, often due to the transformation from cattle to buffalo breeding, do we find stables partially closed on the sides.

This morphology is also favored by the high concentration of buffalo herds along the Tyrrhenian coast, characterized by a mild and dry climate; it should not be overlooked that a greater bacterial load is not recommended for close environments. For this reason, the environment of greatest microbiological risk, in the case of buffalo farms, is represented by the milking parlors, closed environments to which the animals have access only for short periods. Very often the structures are of old conception and do not allow easy sanitation of either the animals or the milking stalls.

The use of lighting systems with a continuous sanitization function has been shown to significantly lower the microbial load present in the milking parlor.

It has been possible to verify that even the operators directly benefit from this protection as the microbial load detected on the hands and in the nostrils was found to be considerably decreased, contributing to concretely decrease the infectious risk for the operators.

Milk, a primary source of income for buffalo breeders, also recorded significant improvements in the microbial load. The whole study showed that the use of Biovitae continuous sanitization technology is of fundamental importance in the entire milk production cycle.

**NEXTSENSE S.r.l.**

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COO, Director of Scientific Research

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