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XXIV CICLO

**INFLUENCE OF ATMOSPHERIC TEMPERATURE AND RELATIVE
HUMIDITY ON BULK MILK SOMATIC CELL COUNT IN DAIRY
HERDS**

VET 05

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ABSTRACT

The production loss and health issues due to the presence of high bulk milk tank somatic cell count in dairy herds makes it essential to implement a consistent effort to maintain this indicator at levels below those required by law. For veterinary practitioners, providing evidence-based advice to clients in order to reduce risk factors of increasing somatic cell count is a difficult task. Statistical Process Control tools allow to verify with statistical certainty when process performance is improving, staying the same, or getting worse and they can be used in dairy farms. The main purpose of the project was to improve understanding in bulk milk somatic cell count variation related to daily temperature and relative humidity, and to build a model which could be predictive of future performance of somatic cell count. Daily bulk milk samples of thirteen commercial dairy farms included in the study were collected and data on daily mean temperature and relative humidity were used. Statistical analysis was performed using Generalized Additive Mixed Models to assess the impact of climatic variables on somatic cell count. We could describe a regression model which shows that the effect of temperature on response appears approximately linear while the one of humidity varies in a more complex way. The model fits well for all herds except one, and explanations are provided. The model constitutes a solid basis for further study of the relationship between daily temperature and humidity, and daily bulk milk somatic cell count. It will allow to set up a quality control on dairy farm using atmospheric temperature and humidity data. Hence it will be possible to provide evidence-based advice to dairy farmers with the use of control charts created on the basis of our statistical model.

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INTRODUCTION

Milk somatic cell count (SCC) of dairy cows is considered an excellent indicator of udder health and milk quality at individual and at herd level. Somatic cells are a sign of inflammation of udder tissue, which is usually caused by bacterial infections (including yeasts and *Prototheca spp.*), even if it can be rarely caused by chemical, physical or mechanical trauma. Somatic cells are predominantly leukocytes (mostly polymorphonuclear neutrophilic leukocyte) and a few secretory epithelial cells¹. They reach the udder quarter to phagocytize and kill microorganisms that invaded it^{1, 2}. SCC from uninfected quarters is generally less than 200.000 cells/ml in pluriparous cows, and less than 100.000 cells/ml in primiparous cows^{3, 4}. Higher SCC without any evident sign of milk and/or quarter abnormality is considered as a subclinical mastitis⁴. Bulk milk tank somatic cell count (BMSCC) is limited by law in the European Union with a threshold of 400.000 cells/ml, considered as geometric mean of at least one sample per month for three consecutive months. The production loss due to the presence of high BMSCC⁵⁻¹⁰ makes it essential to implement a consistent effort to maintain this indicator at levels well below those required by law. The costs relating SCC with losses at the farm level were extensively reviewed by Fetrow¹¹ and those relating SCC with fluid milk and cheese quality by Schällibaum¹². Clinical^{13, 14} and subclinical mastitis reduce dairy farm profit because they reduce milk production, change milk composition^{15, 16}, increase therapeutic costs, increase labor, decrease income due to discarding milk containing drug residues, increase culling¹⁷ and decrease reproductive performance¹⁸⁻²³. Furthermore it must be considered the lack of gain in trade regime characterized by prizes and penalties related to SCC^{24, 25}. In addition to economic problems of dairy farms we highlight the impact on animal welfare due to a painful disease even in its subclinical stage²⁶⁻²⁸ (aspect, moreover, underestimated by veterinarians^{29, 30}) and the chance of affecting food safety e. g. by food contamination with toxins produced by *S. aureus*³¹⁻³³ or with zoonotic agents such as *Listeria spp.* or verotoxin producing *E. coli*³⁴⁻³⁶. An improved udder health

will lead to improved animal welfare, improved production efficiency and a reduction of the use of antibiotics. There are well-established relations between SCC and the herd prevalence of mastitis^{37, 38}, the milk composition^{15, 39} and the production losses.

RISK FACTORS

A great number of risk factors for new intramammary infections, determining an increase of SCC in dairy cows, have been described^{7, 37, 38, 40-52}.

Main risk areas are management and management practices of dairy herd (environment, milking parlour, milking routine, heat stress), cows and pathogens factors⁴⁴. Bedding hygiene and quality for lactating and dry cows^{37, 53-57}, temperature and humidity⁵⁸⁻⁶¹, nutrition, treatment protocols for clinical cases and dry-off, milking routine (including teat end cleaning, udder preparation, cluster attachment, teat disinfection), correct operation of milking machine (overmilking⁶², milking vacuum level, vacuum fluctuations, liner slips, pulsation rate and ratio, liners age⁶³), teat condition^{42, 64}, and cow's immune response to intramammary infections, are only a few of the risk factors described.

Recently Dufour *et al.*³⁷ conducted a standardized systematic review of the literature on associations between management practices used on dairy farms, and herd-level SCC. It was distinguished between management practices that have consistently shown association with SCC when applied at the herd level, and management practices for which evidence of an association with herd-level SCC is lacking. Surprisingly relatively few of the numerous management practices investigated demonstrated consistent associations with SCC. Furthermore, many of the practices frequently recommended in mastitis control programs had a limited amount of published information available on their effectiveness in a conventional dairy setting, with many showing inconsistent directions of association with SCC across studies. This could be because many practices are intended to help in preventing clinical

mastitis rather than high SCC and independence between these two udder health problems was described^{65, 66}. Practices associated with low SCC were: milkers wearing gloves during milking, the use of (well-adjusted) automatic milking unit take-offs, postmilking teat disinfection, milking high SCC cows and clinical mastitis cases last. Few housing-related interventions yielded very consistent associations with SCC: the use of freestall housing system with sand-bedded cubicles, cleanliness or frequency of cleaning of the calving pen, the administration of an approved intramammary antibiotic treatment to all cows at dry-off, frequent clipping of udder hairs and parenteral supplementation with selenium. The attitude of dairy producers toward culling needs to be modified to achieve lower SCC. They need to have proactive and well-defined culling strategies based on udder conformation, teat lesions, and clinical mastitis cases rather than simply reacting to udder health events. Although practices used from birth to first calving were investigated, most of the practices significantly associated with heifers' early lactation SCC were interventions used during the few weeks before and around calving time. This relatively short period is potentially of great importance for acquisition of new IMI.

It's really interesting to note, as described by Barkema³⁸, that there are aspects of management style which are associated to the increase of BMSCC and of the incidence of clinical mastitis and that "the most striking difference between farmers of herds with low and high bulk milk SCC was that the first group worked precisely rather than fast; the latter group of farmers worked quickly rather than precisely."

HEAT STRESS IN DAIRY COWS

Heat stress occurs when dairy cows suffer from hyperthermia when they fail to maintain thermoneutrality with increasing ambient temperature and humidity. Higher-producing cows are more at risk than lower producing cows, because high dry matter intake results in increased metabolic heat increment. Heat stress leads to

decreased milk production⁶⁷⁻⁷⁰ and changes in milk composition, to reduced reproductive performance^{71,72}, to an increased risk of mortality⁷³ and to an increase of clinical mastitis^{74, 75} and somatic cell count⁷⁶⁻⁷⁸. Dairy cows respond to high temperatures by seeking shade and wind, increasing water intake and respiration rate⁷⁹. The total body heat production of a cow is a combination of heat derived from normal metabolism, from the environment, and from physical and performance activities, such as milk production. Metabolic consequences of heat stress are increased heart rate, lower plasma glucose level⁸⁰, changes in the levels of stress hormones⁷¹ and an increase in rectal temperature⁸⁰. In order to lower body heat production, cows experiencing heat stress will voluntarily reduce dry matter intake, which results in depressed milk production. Other factors that may play a role in milk yield decline, associated with heat stress, are changes in hormone levels and an increase in maintenance requirements⁷¹. High temperatures may also affect susceptibility to infection, either by decreasing host resistance or by increasing the exposure to pathogens. Elevated temperature and high relative humidity enhance the survival and proliferation of pathogens in the environment. Under circumstances of heat stress, cows may lie in the alleyways of free stall barns or wallow in ponds, and streams in pastures or in mud holes in paddocks, in order to increase heat loss. This behaviour increases the risk of infection. Evidence for a direct effect of elevated environmental temperature on the immune system is limited and is related to a decrease in random migration and chemotaxis of leukocytes⁸¹ and in a decline in plasma immunoglobulins during the final two weeks of pregnancy, and for the first four milkings after calving⁸². An indirect effect on immunity may occur as a result of decreased feed intake and, consequently, insufficient uptake of essential nutrients, which are important to optimal immune function.

Evaluation of air temperature alone does not allow an accurate assessment of the effects of the thermal environment on physiology, welfare, health, and productivity in farm animals. For instance, high humidity in combination with high temperatures reduces the potential for evaporative heat loss, solar radiation adds heat to that

deriving from metabolic processes, and strong winds, especially in combination with precipitation, amplify the adverse effects of cold temperature. Different approaches have been used to quantify heat stress in farm animals including utilization of the temperature humidity index (THI). This is a practical tool and a standard for many studies and applications in animal biometeorology^{69, 73, 83-85}. Temperature and humidity are combined in a single value with a variety of formulas which differ from each other in the weight given to the effects of humidity.

UDDER HEALTH AND COMMUNICATION

Udder health control is based on control of risk factors, which are often concurrent in the same dairy herd. For dairy veterinary practitioners and extension agents, providing evidence-based advice to clients is a difficult task. Consultants must set the control plan bearing in mind the characteristics of veterinarians⁸⁶ and farmers⁸⁶⁻⁹⁰, trying to identify the risk factor which has the greatest impact on SCC on that specific dairy farm, thus reaching the best result with minimal effort. Importance of communicating to dairy farmers scientific evidences is becoming one of the most interesting topics in udder health research. A PhD thesis⁸⁶ in 2010 and the international conference⁹¹ in 2011 were entirely dedicated to udder health and communication.

Veterinarians' perceptions on their role as udder health advisor and their proactive advising skills in practice were studied in the Netherlands. Although veterinarians are considered the preferred udder health advisors by their clients, in daily practice there is room for improvement. Although most veterinarians have the intention of working on mastitis prevention and feel that proactive udder health advice comes within their professional remit, they seem to prefer a curative, demand-driven approach. They cope with this ambiguity between their intentions and actual behavior by citing many barriers. Most of these barriers are external, such as the perceived lack of willingness and motivation on the part of the farmer, the perceived inconsistency of information

on udder health, the perceived (economic) competition with other advisors, and the perceived expectations of farmers and colleagues about their role as veterinarian. Some barriers mentioned are internal, such as their perceived lack of knowledge and effective communication skills, their perceived self-identity as a professional curative-oriented veterinarian, and their tendency to shift the responsibility for udder health to the dairy farmer. Veterinarians could transform these perceived barriers into opportunities by adopting a customer-oriented, proactive approach and by applying elementary communication techniques in their advice. The following are considered essential communication skills for providing effective advice⁹²:

- 1) having a structured conversation,
- 2) active listening,
- 3) setting specific, measurable, achievable, relevant, time-based (SMART) goals,
- 4) specifically asking for farmers' goals and opinions by open questions,
- 5) having a balanced interaction in number of words, questions, and agenda setting between the persons involved.

Improvement of these skills could contribute to an optimization of veterinary consultancy and to the improvement of knowledge transfer to dairy farmers, with a consequent optimization of the effect of mastitis control programs⁸⁶.

Characteristics of farmers who seemed to be hard to reach with information on udder health were described. Such farmers were not always badly informed about udder health and did not always experience problems with mastitis. They could be divided into four categories based on their trust in external information sources regarding mastitis and their orientation toward the outside world: proactivists, do-it-yourselfers, wait-and-see-ers, and reclusive traditionalists (Fig. 1).

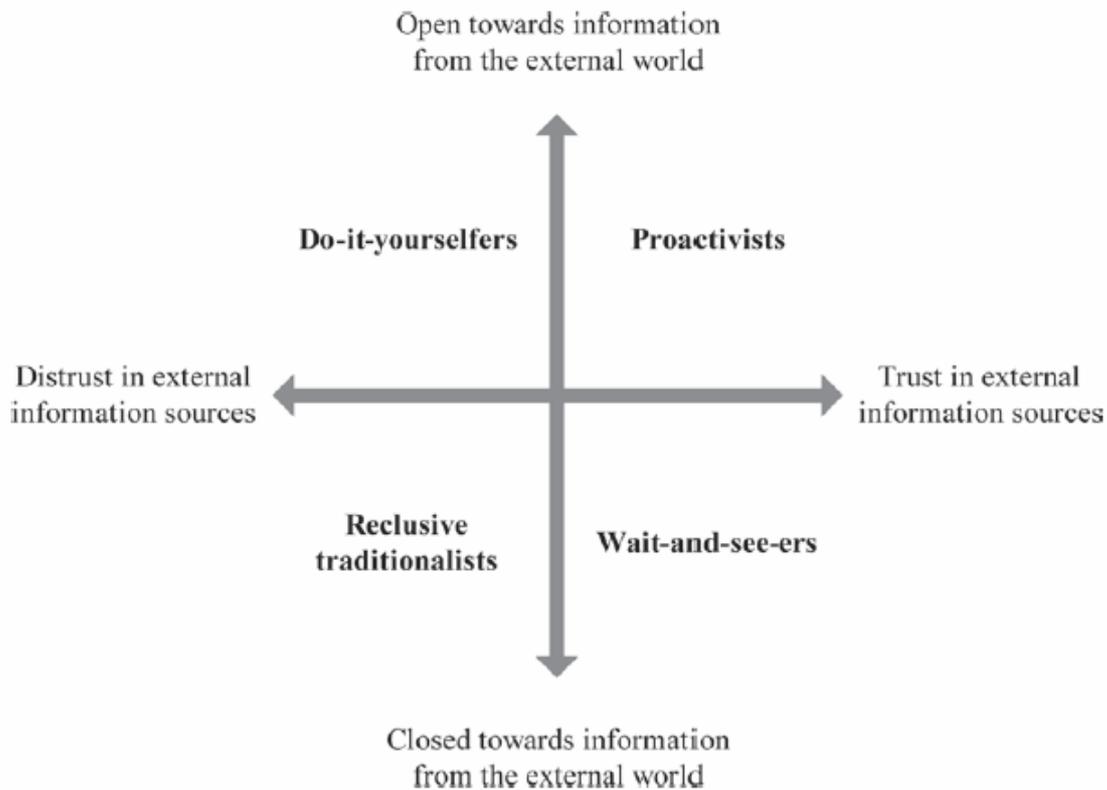


Figure 1 Different types of hard-to-reach farmers from Jansen *et al.* 2010

Proactivists are outward oriented, well informed, and interested in all kinds of new developments. They are almost all members of a study group, and some even participate in multiple study groups. Colleagues and peers are important information sources, and they discuss udder health openly. Most farmers in this group rate the Internet as an important information source, and they don't mind sharing milk inspection reports with their veterinarian online. They all state that they have a positive relationship with their veterinarian, but do not see their veterinarian as the only and most important information source because they use many different sources. *Do-it-yourselfers* are active and well informed but have a critical attitude toward external information. They often disagree with the available information and they perceive that they get a lot of contradictory information. They rely more on their own knowledge and experiences than on information from others. Although some of them are members of a study group, they don't talk much about their own mastitis situation with colleagues. Their most important information sources are farm magazines, and

some also use the Internet. Their relationship with their veterinarian is very pragmatic and businesslike. *Wait-and-see-ers* are in general open to advice from others, but rarely act on their own initiative to search for information and to change the management on the farm. Farmers in this group stated that they were easy to approach by others and had a good relationship with their veterinarian. *Reclusive traditionalists* are very inward oriented and they don't like the interference of others on their farm. They have few contacts with other farmers and don't feel the need to compare their farm with others'. They don't like exchanging information with others because they feel uncomfortable when others had access to their farm data. They perceive the relationship with their veterinarian as poor, costs being the main reason for having as little contact as possible. Their most important information source is farm magazines, which they appreciate and read thoroughly⁹³. Obviously, different types of farmers need to be approached in different ways and through different channels with information on udder health.

STATISTICAL PROCESS CONTROL TOOLS AND STUDY OF BULK MILK SOMATIC CELL COUNT VARIATION

In every dairy herds BMSCC is characterized by variation, which is an index of how inconsistent are the management and the performance during daily work activities. Statistical Process Control (SPC) tools are traditionally used by engineers to validate, monitor, and predict the expected behavior of processes or machines. This allows to verify with statistical certainty when process performance is improving, staying the same, or getting worse, i.e. not due to chance. We can think of the herd as a production process unit, designed to produce milk from its various inputs (feed, genetics, infrastructures, and management) that goes through the “machine” (cow) into the bulk milk tank. The outputs of this process are the batches of milk leaving the herd, each batch being the bulk milk tank's content. It is necessary to consider the

herd as a system of processes to facilitate a comparison between the herd production process and any type of industrial process and to apply total quality control methods at the herd level (Fig. 2). SPC methods can be used to signal emerging problems, evaluate the positive or negative impact of a change in a management practice or the implementation of a new product⁹⁴⁻⁹⁶.

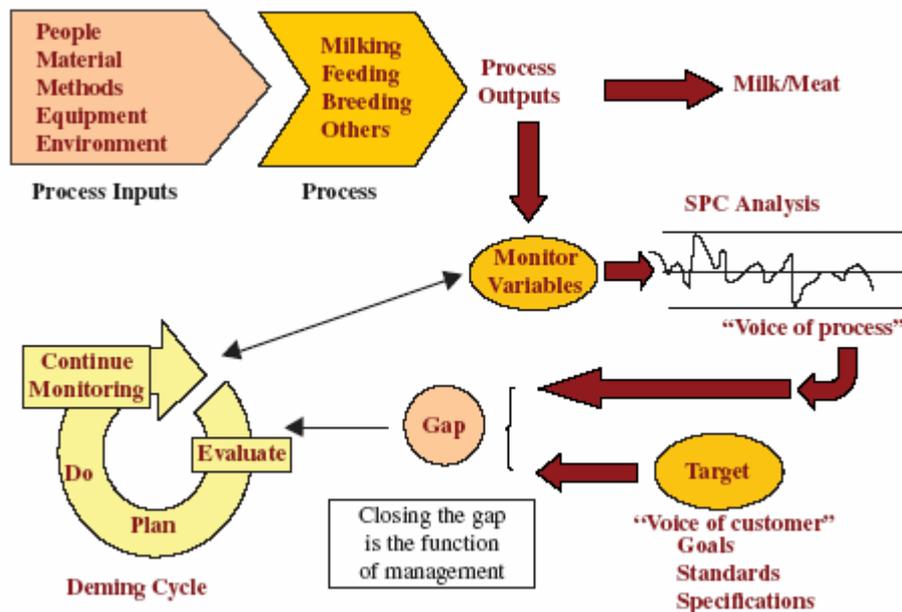


Figure 2 Process flow diagram from Reneau et al, 2006

The most common use of dairy herd management data is to compare this month's average with last month's average. Are we doing better or worse? The problem with such limited comparisons is that they are out of context. Context here means that the data should be interpreted in context of the time order in which they were generated. A simple way to present data is the use of graphs, but a simple time series chart doesn't have sufficient resolution for more meaningful interpretation. Shewhart control charts help to interpret data that is generated over time. Control charts provide further insight into data by displaying the level of normal, random variation in the data and by revealing the observations that indicate real change. This approach affords the practical application of statistical theory in a visual, easy to interpret context. The output of every process is characterized by a certain level of variation

(common cause variation) that is due to a cumulative effect of many factors that are out of our control. The level of common cause variation can be reduced by finding a way to control these contributing factors. Some level of variation is unavoidable: one may not know all of the possible factors that affect process output or it may not be economically justifiable to control some of the factors that are known. When only common cause variation is present in process output, the process is said to be operating under the state of statistical control. The process enters an out of control state when some aspect of the process that is usually under control changes and impacts process performance. The resulting variation is usually caused by machine/equipment problems, operator/personnel errors, or defective raw materials. This variation is called special cause variation because its source can usually be traced and eliminated, returning the process back to the state of statistical control⁹⁴.

Sigma (a measure of variation similar to standard deviation) is calculated for the data collected in a time frame called the “control period”. Control limits are set three sigma above and below the central line (mean). When initiating a control chart it is appropriate to use the first 20 data points collected as the control period. Once control charting is established the control period can be set depending the question being asked. If the intent is to monitor a process for the purpose of maintaining a stable process then using data from an apparently stable period makes sense. If the intent is use the control chart to evaluate the introduction of a new product or a change in process procedure etc. then the control period should be calculated from data collected just before the introduction of the product or change in procedure. It is generally safe to assume that the process is “out of control” when any one of the following rules (Western Electric rules) indicates a change:

1. A single point outside one of the control limits.
2. Two of three successive points fall on the same side and more than two sigma away from the central line.
3. Four out of five successive points on the same side of the line and are more than one sigma away from the central line.

4. Eight or more successive points on the same side of the central line.

Any time the conditions of any one of these four rules are met you can be certain the process is changed and is by definition “out of control”. When using all of the Western Electric rules, there will be a false alarm approximately 2% of the time, which means that for most management circumstances, there is a 98% probability of being right about whether a change is “real”⁹⁷. Since BMSCC is a reflection of many on-farm processes that contribute to milk quality (milking routine, milking system, bedding routine, dry/fresh cow management, and so forth), it is a good monitor of people, equipment, and animal performance. It can be monitored with I chart or EWMA (Exponentially-weighted moving average) chart. EWMA chart tracks the exponentially-weighted moving average of all prior sample means while I chart treat rational subgroups of samples individually. EWMA chart can be designed for optimal performance for a specific change in mean/variation and performs well when the magnitude of occurring change is reasonably close to the anticipated design value. However I chart signals a large shift sooner than EWMA chart. When large and small shifts are to be detected, it is recommended to plot both charts alongside each other. A different example of the use of SPC in dairy herds is the capability index (Cpk), which makes a direct relation between herd performance and the legal standard of European Union⁹⁸. To be properly described, any population needs at least the mean and the variance, thus geometric means used as legal standard

1. do not adequately describe the herd’s BMSCC distribution throughout the year,
2. is a poor indicator for compliance of the herd with the legal standard,
3. no predictive information about the expected future performance of the BMSCC can be derived from it.

The Cpk concept joins process performance statistics of the herd (a central tendency parameter and a dispersion one) with process performance specification criteria— in this case the European Union legal standard for SCC in the bulk milk tank. Therefore, the Cpk makes a direct relation between the herd performance and the legal standard. When compared with the traditional BMSCC average, the Cpk showed better

accuracy to describe the herd's ability to comply with the legal standard limits imposed on BMSCC. SPC tools were also used by Lukas et al. to estimate subclinical mastitis prevalence in the herd and observe for change in the subclinical mastitis status⁹⁹, analyze variation of BMSCC and evaluate the probability of exceeding a SCC standard¹⁰⁰, to develop consistency indices (CI) for 5 different SCC standards that would calculate the maximum variation allowed to meet a desired SCC level at a given mean BSCC, compare the percent correctly identified, detection probability, and certainty associated with a result of a test identifying future SCC standard violators based on herds' current CI or past violations, study the efficiency of SPC charts and CI as a warning system of future SCC standard violations¹⁰¹.

AIM AND DESIGN OF THE PROJECT

Until now, influence of single risk factors on variation of BMSCC was not described. Being able to verify the component of variation due to individual factors may allow a more effective indication to control risk factors that most influence the variation of BMSCC. In other words, if we could be able to break up BMSCC variation in the different contributions of different risk factors, we could rank risk factors on the basis of their greater or minor impact on BMSCC variation, thus allowing better suggestions to dairy farmers. The main purpose of the project was to improve understanding in BMSCC variation related to daily temperature and relative humidity, both as daily mean and its variation in a longer period and to build a model which could be predictive of future performance of BMSCC based on temperature and relative humidity variation.

PRELIMINARY STUDY

For the aim of the study we decided to collect and analyze milk samples once a week but we could not find bibliographic references on effect of time delay from sample collection and sample analysis on BMSCC in dairy herds. Thus we considered that weekly collection of milk samples could negatively influence the accuracy of SCC and we conducted a study to verify this assumption.

84 bulk milk samples from 12 commercial dairy herds, stored with bronopol at a temperature of +2° - +4° C were analyzed at the lab of Infectious Diseases Institute at the Faculty of Veterinary Medicine of Università degli Studi di Milano four times every three days till day 15 with Bentley Somacount 150™ (Bentley Instruments, Chaska, MN) for a total of 336 values of SCC. Statistical analysis was performed using R software¹⁰², and a mixed effects model with the single SCC used as random effect was performed.

The model obtained, using log(SCC) is :

$$\log(\text{SCC}) = 5.8366 - 0,002164 \times \text{number of days from sample}$$

β coefficient is statistically significant with $p < 0.0088$

An analogue model, using time I, II, III and IV instead of the number of days from sample to analysis, shows that only at time IV there is a significant, though low, decrease in SCC. The results of this study show that log(SCC) is influenced by time passed from sample collection to analysis, but the decrease is not biologically relevant, at least until 15 days from sample collection (Fig. 3). We concluded that bulk milk samples for somatic cell count, correctly preserved and analyzed till 15 days from sample, don't suffer any variation with biologically relevant significance¹⁰³.

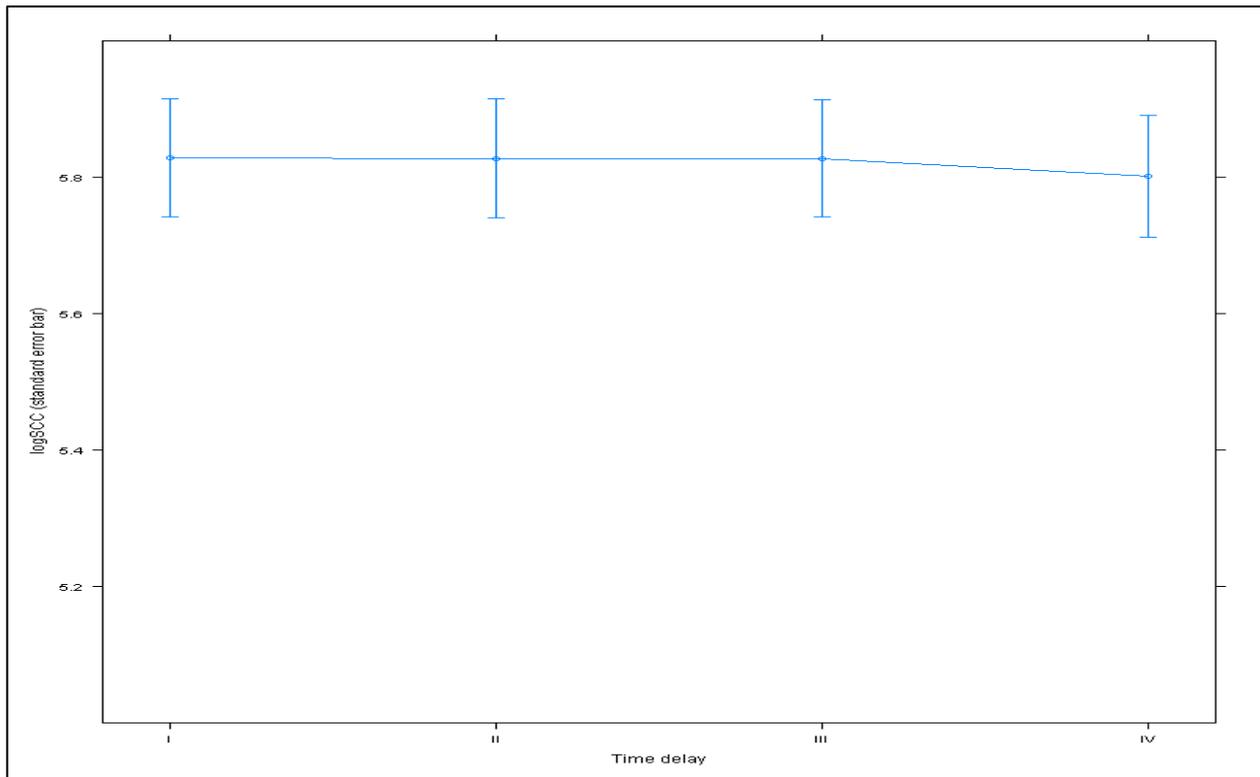


Figure 3 Trend of log(SCC) related to time delay from sample collection to analysis

MATERIALS AND METHODS

Herds

Thirteen commercial dairy farms were included in the study and identified with capital letters (A-L). Twelve herds are from the province of Bergamo and one from the province of Cremona (Tab. 1 – Fig. 4). Due to the particularly difficult economic period two dairy farms ceased the activity, one in August 2009 (J) and one in October 2010 (F), and therefore they were no more included in the research project. Another farm (M) started its activity on March 2010 and was then included in the study.

Inclusion criteria were:

1. minimum number of 50 cows per herd,
2. herds in a geographic area which allowed weekly samples collection in 3 hours,
3. farmers reliable and willing to perform the daily milk sample.

Herd	Latitude	Longitude	mean n° of cows
A	45.67386291505017	9.59557056427002	90
B	45.63363624890752	9.566302299499512	115
C	45.55284079318792	9.648635387420654	115
D	45.54824272251802	9.660935997962952	125
E	45.533938965880786	9.673832058906555	400
F	45.50784318067588	9.740951657295227	80
G	45.46910756273745	9.729600548744202	300
H	45.47481803911341	9.762430787086487	350
I	45.477323243369604	9.817062020301819	230
J	45.487546030331025	9.848207831382751	100
K	45.53676093018892	9.844554662704468	60
L	45.629637501949055	9.675151705741882	120
M	45.55779907527858	9.668773412704468	170

Table 1 Dairy herds coordinates and mean number of cows

All the dairy farms used freestall housing system for milking cows, except herd E, where freestalls were used only in the group of primiparous cows while all the other milking cows were on straw litter. Again, farm E differed from all the others in milking cows 3 times per day instead of 2 times per day.

Temperature and humidity data

Data on daily mean, minimum and maximum temperature and humidity were registered by Lombardy Agenzia Regionale per la Protezione dell’Ambiente (ARPA) at Capralba (Cr), Corzano (Bs) and Osio Sotto (Bg) meteorological control units (Tab. 2 – Fig. 4).

Meteorological control unit	Latitude	Longitude
Osio Sotto	45.6206454	9.61185138
Capralba	45.4441596	9.64584509
Corzano - Bargnano	45.4330569	10.0393246

Table 2 Meteorological control unit coordinates

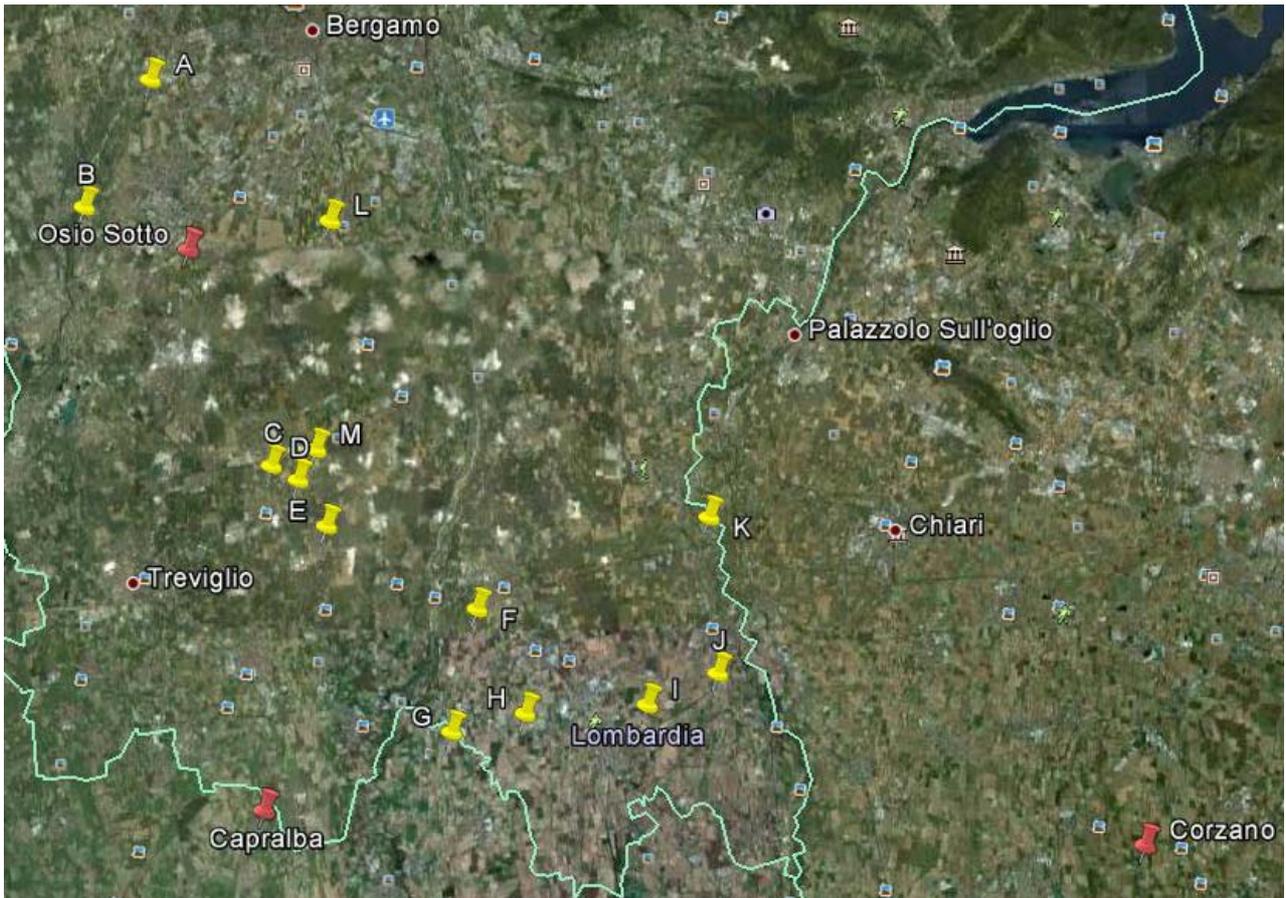


Figure 4 Map of dairy herds (yellow points) and meteorological control units (red points)

Data are labelled by ARPA as:

- complete and reliable
- complete but unreliable
- not present

Table 3 shows minimum and maximum values for “complete and reliable” data.

Temperature Capralba (°C)	from -6.90 to 26,6
Humidity Capralba (%)	from 35 to 100
Temperature Osio Sotto (°C)	from -6.20 to 29,3
Humidity Osio Sotto (%)	from 0 to 100
Temperature Corzano (°C)	from -5,4 to 27,8
Humidity Corzano (%)	from 40 to 100

Table 3 Minimum and maximum temperature and humidity registered

Minimum humidity in Osio Sotto (Fig. 5) seems too low even if data were validated by ARPA, compared with data from Capralba (Fig. 6).

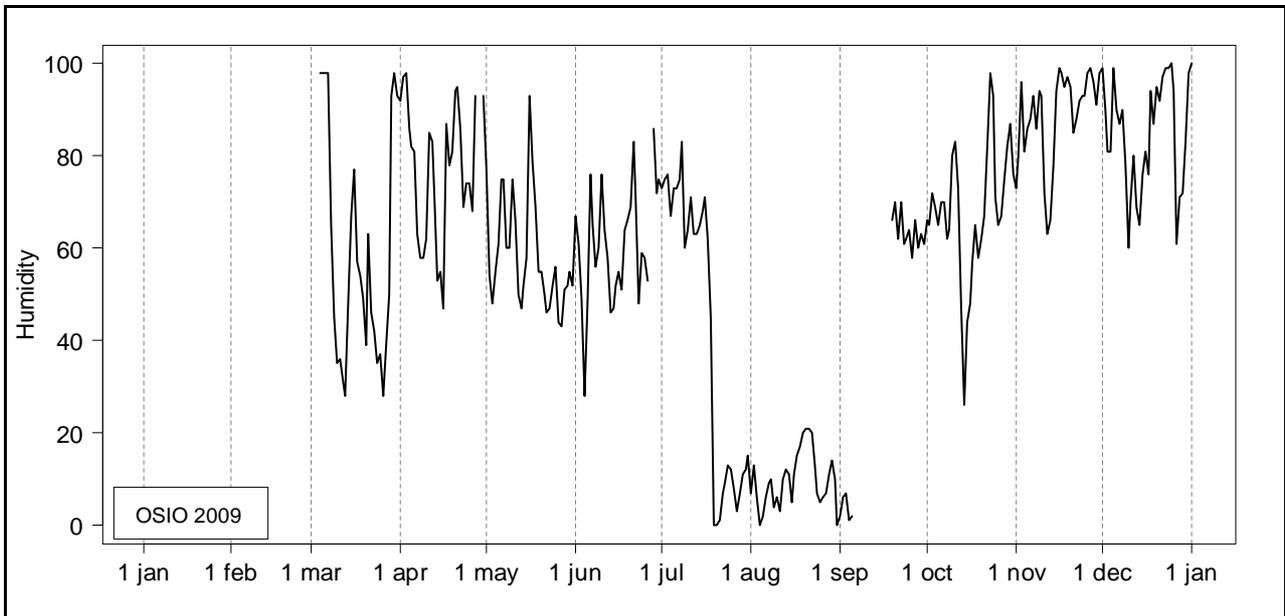


Figure 5 Daily humidity recorded in 2009 at the meteorological control unit of Osio Sotto

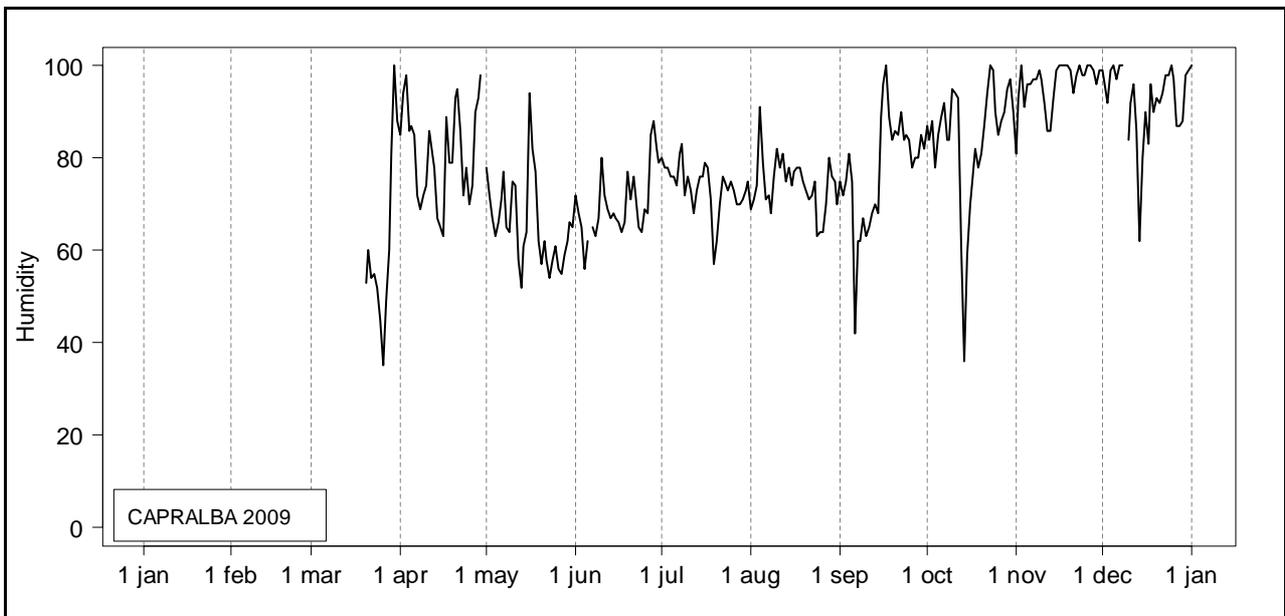


Figure 6 Daily humidity recorded in 2009 at the meteorological control unit of Capralba

Somatic cell count sampling time interval

The following Table (Tab. 4) shows start and end date of sampling, missing values and total number of records for each herd. In yellow are highlighted the greater differences due to farms F, J and M.

Herd	Start date	End date	missing	n° record
A	03/03/2009	02/10/2011	52	944
B	06/03/2009	03/10/2011	50	942
C	09/03/2009	02/10/2011	52	938
D	06/03/2009	03/10/2011	56	942
E	05/03/2009	02/10/2011	64	942
F	03/03/2009	25/10/2010	46	602
G	10/03/2009	03/10/2011	82	938
H	04/03/2009	02/10/2011	48	943
I	09/03/2009	02/10/2011	76	938
J	10/03/2009	19/08/2009	11	163
K	05/03/2009	02/10/2011	50	942
L	06/03/2009	03/10/2011	52	942
M	16/03/2010	02/10/2011	30	566
Total			669	10742

Table 4 Start and end date of sampling, missing values and total number of records for each herd

Sampling

Milk samples were taken daily at the end of the same milking session after milk was cooled and were stored with bronopol at a temperature of +2° - +4° C. Every week the samples collected were brought to the lab of Infectious Diseases Institute at the Faculty of Veterinary Medicine of Università degli Studi di Milano where they were analyzed with Bentley Somacount 150™ (Bentley Instruments, Chaska, MN) for somatic cell count.

Determination of somatic cells content in milk

The Somacount 150™ uses a proprietary fully automated process based on the principle of laser-based flow cytometry to determine the somatic cell counts in a milk sample.

- A milk sample is taken automatically and mixed with a fluorescent dye solution (ethidium bromide).
- This dye disperses the fat globules and stains the DNA in the somatic cells.
- An aliquot part of the stained suspension is injected into a laminar stream of carrier fluid.
- The somatic cells are separated by the stream and exposed to a laser beam.
- As the stained cells pass through the excitation source (laser beam), they begin to fluoresce creating a light pulse.
- Through a series of lenses, the fluorescent pulses are focused onto a photo multiplier tube, where they are converted into electrical pulses.
- The pulses are sorted and stored by size using a micro controller. By using a process known as pulse height analysis, the pulses are sorted, counted and translated into a somatic cell count.
- The Somacount 150™ meets the requirements of International Dairy Federation standards for somatic cell counting and is an AOAC (Association of Analytical Communities) approved methodology.

Statistical analysis

Statistical analysis was performed with R software¹⁰² to assess the impact of climatic variables: temperature (C) and relative humidity (H), on BMSCC, based on measurements that were taken in different dairy herds. We preferred to analyze temperature and humidity as separate variables, instead of analyzing THI index, to allow a flexible modelling of the two covariates and to study all the informations related to the variables without a priori constraints. For this purpose we used Generalized Additive Mixed Models (GAMMs), which allow a flexible exploration of the impact of variables without specifying a functional form of the effect, e.g. quadratic or logarithmic effect. GAMMs allow for flexible modelling of the covariate effects by replacing the linear predictor in GLMMs with an additive combination of nonparametric functions of covariates and random effects¹⁰⁴.

The choice of such a model does not require a function that may not fit satisfactorily to the complexity of the relationships between the variables studied, and it solves problems related to the potential complexity that would make the model unstable¹⁰⁵. As a matter of fact, mathematical description of complex functions may simply be done by using polynomials with variables raised up to the n^{th} power (n^{th} order polynomials). Considered the global nature of polynomial functions, the slightest variation of the variable, once elevated to the n^{th} power, e.g. 8^{th} or 10^{th} , causes large variations of the polynomial terms included in the regression model, thus rendering the model unstable (Runge's phenomenon). Statistical techniques were studied to find a compromise between the need to describe complex models with polynomials and the one to have models stable enough to be able to describe the phenomenon. To this purpose, spline functions were introduced as efficient and stable statistical techniques. They are sufficiently smooth piecewise-polynomial functions, and they consist of a set of polynomials connected with each other, whose purpose is to optimally approximate a set of points. GAMMs are therefore regression models:

- linear in the parameters,
- flexible in modeling a generic function: in our case BMSCC as a tensor product spline function of H and C,
- which adequately take into account the correlation of observations within a herd considering the typical random effect of the herd.

To fully exploit the potential information of the daily recorded data, a complex GAMM is to be specified taking into account the problem of missing information and the different sources of error. In order to ease these issues and to produce a first robust model the following choices were performed:

- We considered data from herds with larger periods of observation (10 herds): A, B, C, D, E, G, H, I, K, L.
- Values of BMSCC, temperature and humidity are considered on a monthly basis (mean).

- We decided to not use data from meteorological control unit of Corzano because they are complete only for limited periods over the time of the study. Temperature and humidity means were therefore calculated using mean daily values of meteorological control unit of Osio Sotto and Capralba.

In Table 5 the number of records for each month for all herds is reported. Only records with all daily data available (BMSCC, temperature and humidity) are included. Records where at least one of the values was missing were discarded. In yellow are highlighted the months not included in the study.

2009-03 131	2009-04 267	2009-05 305	2009-06 218	2009-07 308	2009-08 302	2009-09 170
2009-10 309	2009-11 295	2009-12 212	2010-01 272	2010-02 280	2010-03 305	2010-04 300
2010-05 305	2010-06 298	2010-07 149	2010-08 96	2010-09 279	2010-10 99	2010-11 0
2010-12 189	2011-01 0	2011-02 128	2011-03 286	2011-04 291	2011-05 276	2011-06 300
2011-07 308	2011-08 194	2011-09 299	2011-10 24			

Table 5 Monthly number of records for all herds, excluded records with at least one missing value
March 2009 and October 2011 were excluded because of the low number of data recorded in the start and end period of study observation.

In November 2010 and January 2011 we could not use any record because temperature or humidity are missing in at least one meteorological control unit.

Variables:

- *Outcome variable:*

Herd BMSCC as monthly mean.

To approximate the normal distribution, a natural logarithmic transformation of the BMSCC divided by 1,000 cells/ml was used, which is the usual transformation for SCC in milk¹⁰⁶.

- *Predictors:*

monthly mean temperature in Celsius degrees (C) and monthly mean relative humidity (H) of temperatures and humidities recorded in meteorological control unit of Osio Sotto and Capralba (variables included in the model are deviations from overall means: 14.56 °C for temperature , 70.93% for relative humidity).

- *Other variables of adjustment:*

year (A)

herd (AZ, random effect)

Herds are treated as a random effect as they are treated as a sample from a target population of dairy herds with similar characteristics to the herds in the study. This allows to estimate the effect of temperature and humidity "in the population", that means to estimate BMSCC expected on average over all dairy herds with characteristics similar to those measured, given temperature and humidity values. Factor "month" is not included in the model as predictor because temperature and humidity are strongly related to the month of measurement. The obtained model may be considered as an alternative to THI allowing the explanation of the relations between temperature, humidity and BMSCC.

The regression model has the following expression:

$$\log(\text{BMSCC}_{ijk}) = \beta_0 + A_j + S(C_{jk}, H_{jk}) + AZ_i + \varepsilon_{ijk}$$

where the following indexes are used:

- i, herd where BMSCC is measured

- j, year of measurement

- k, month in the year of measurement

and where β_0 = intercept, and ε_{ijk} = residual error.

Function $S(C,H)$ represents the flexible tensor product spline of variables temperature and humidity which was used to account for possible nonlinear and interaction effects.

RESULTS

Minimum and maximum daily BMSCC values per herd are tabulated in table 6.

BMSCC	A	B	C	D	E	F	G	H	I	J	K	L	M
Max	2850	552	840	816	1376	8970	695	1137	1306	870	946	616	298
Min	39	20	141	45	32	162	134	72	34	91	54	57	43

Table 6 Minimum and maximum BMSCC in dairy herds

The presence of outliers in farm F and in farm A is shown in Fig. 7.

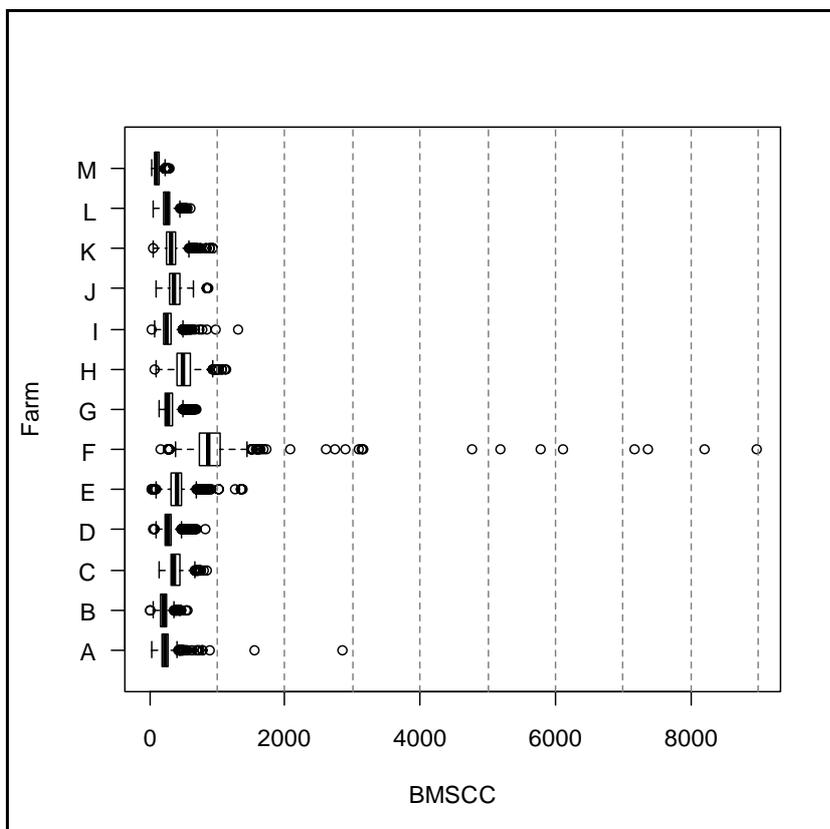


Figure 7 Values of BMSCC for all dairy herds

Fig. 8 shows the estimated values of the average monthly BMSCC as a function of monthly mean temperature values (y axis) and monthly average humidity (x axis) conditioning to year 2009. The choice of conditioning to year 2009 does not affect the interpretation of the effect of temperature and humidity because the effect of the year consists of a constant shift of predicted monthly BMSCC values for each C and H combination.

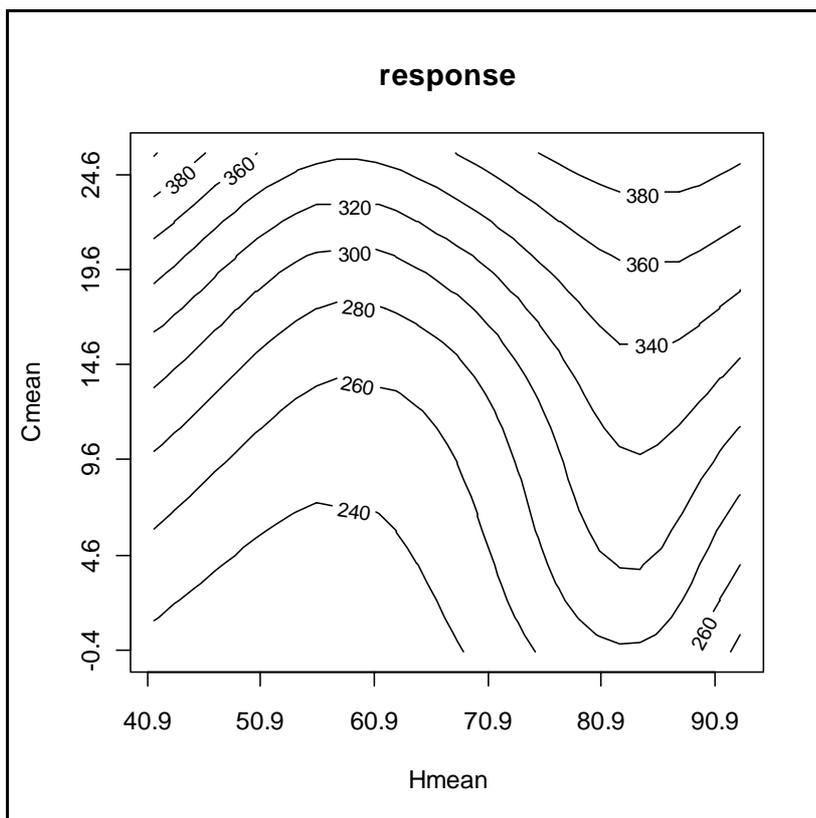


Figure 8 Estimated values of the average monthly BMSCC as a function of monthly mean temperature values and monthly average humidity

The graph shows the contour lines of the expected monthly average of BMSCC values. All the points of the contour lines identify the pairs of values of the variables associated to the Cartesian axes – mean temperature and mean humidity – which determine the same value of the response (BMSCC). Such values of BMSCC are shown by the numbers overlapping contour lines. If regions of the graph correspond to values of temperature and / or humidity which were not observed, values of the curves (average monthly BMSCC) are an extrapolation based on the model.

The evaluation of the combined effect of predictors can also be deduced from fig. 9, which can be considered as three-dimensional version of Fig. 8.

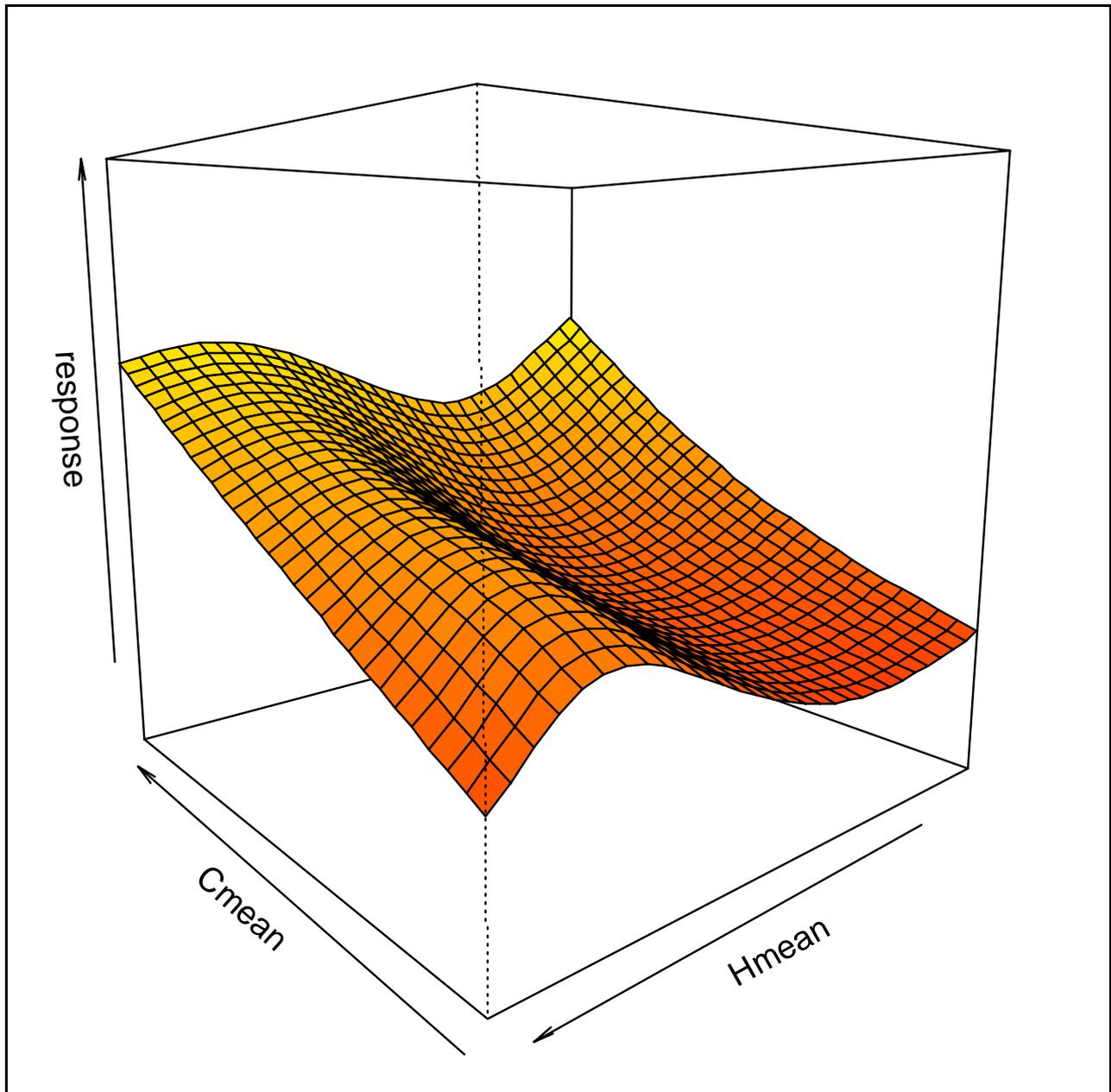


Figure 9 Spatial representation of the statistical model

Fig. 10 shows the average monthly BMSCC measured in each dairy herd, with expected values (red lines) estimated on the basis of the model.

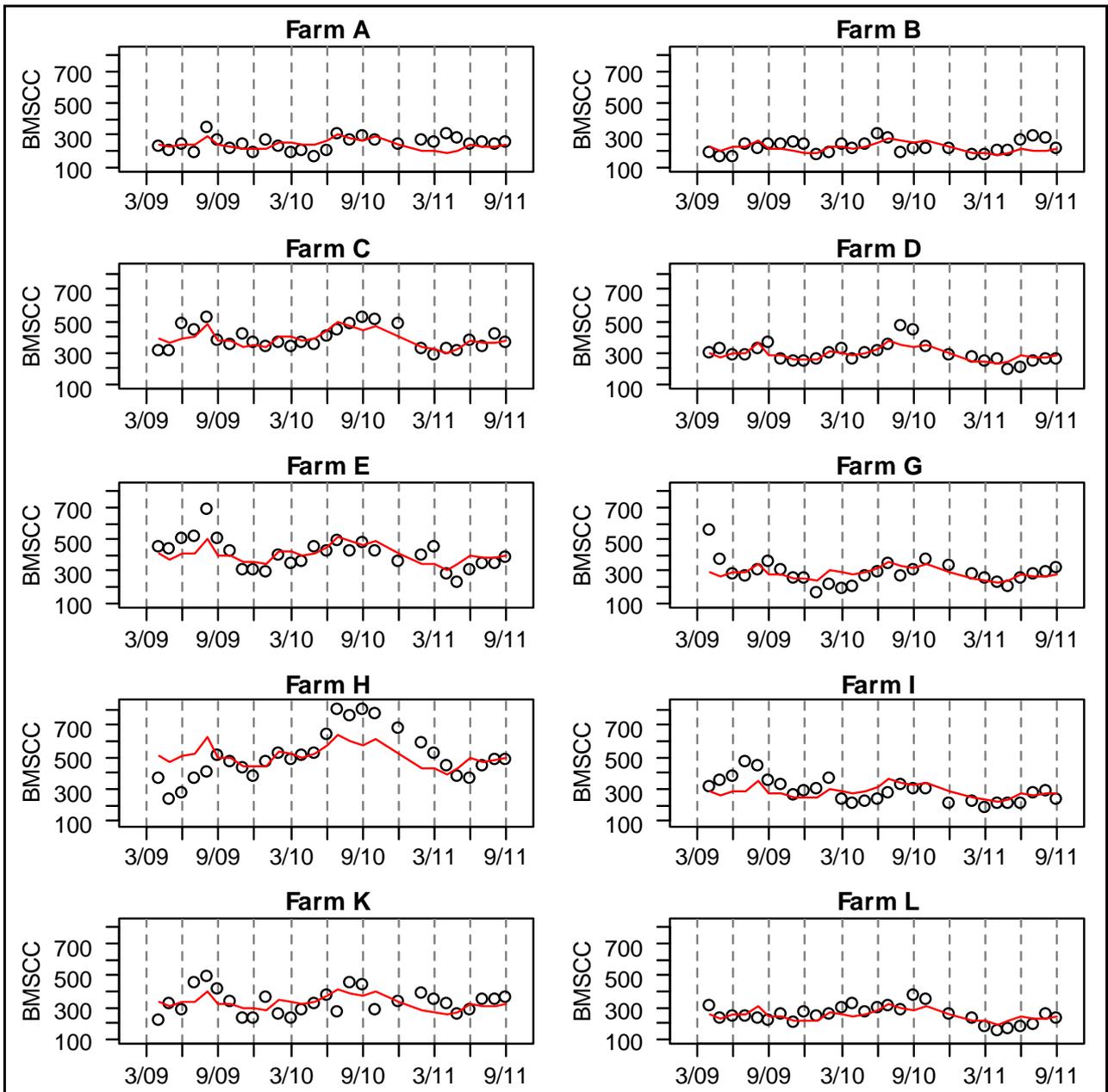


Figure 10 Expected values estimated on the basis of the model (red lines) and monthly measured means (circles) of BMSCC

The model has a good fit to the data, except for herd H.

DISCUSSION

Effect of temperature

First of all we can see that values of BMSCC increase with increasing temperature: therefore we expect monthly mean BMSCC in a herd increases with the rise of monthly mean temperature. Moreover vertical distances of contour lines are similar between each other. Actually, at least for C greater than 14° , an increase of monthly mean temperature of about 3° corresponds to an increase of monthly mean BMSCC of about 20. The effect of temperature on response appears approximately linear.

Effect of humidity

The effect of humidity on BMSCC can be evaluated by drawing horizontal lines in the graph, considering horizontal distances between contour lines at given values of temperature. The effect of humidity on the expected response of BMSCC varies in a more complex way, with a nonlinear effect: it decreases (range: 40.9-60.9), then it increases (range: 60.9-80.9), then it decreases again (range: 80.9 – 90.9). This is clear verifying BMSCC response at a fixed temperature value with different humidity values. This complex non-linear effect appears more evident for low values of temperatures with respect to high values.

Abnormal conditions

Both, bi-dimensional and three-dimensional graphical version of the model allow the detection of two abnormal conditions requiring control. In the first case the variable response increases at high temperature and low humidity (top-left corner in Fig. 8, top-right corner in Fig. 9), in the second case the variable response decreases in case of very low temperatures and high humidity (bottom-right corner of figure 8, bottom-left corner of figure 9). First of all we verified with Fig. 11 that areas presenting the anomalies were covered by actual observed points used for the estimation of the model, and not originated by extrapolation based on the model.

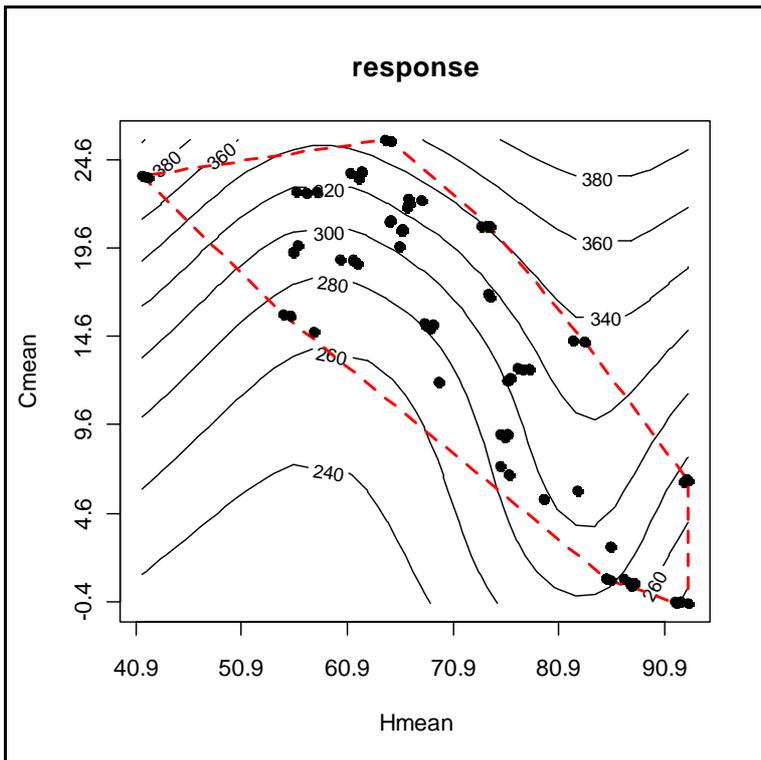


Figure 11 Same as Fig. 8 with measured points

In the graph the observed points are placed along the diagonal of the graph according to the correlation between C and H. The correlation has an impact on the estimation error of the estimated surface making the regression model less robust. It should be possible that the decrease of the estimated BMSCC could be determined by influential points potentially abnormal. In particular, the region in the upper left corresponds to a drop in humidity recorded in the meteorological control unit of Osio Sotto, which, although validated by ARPA, are hardly reliable (Fig. 12).

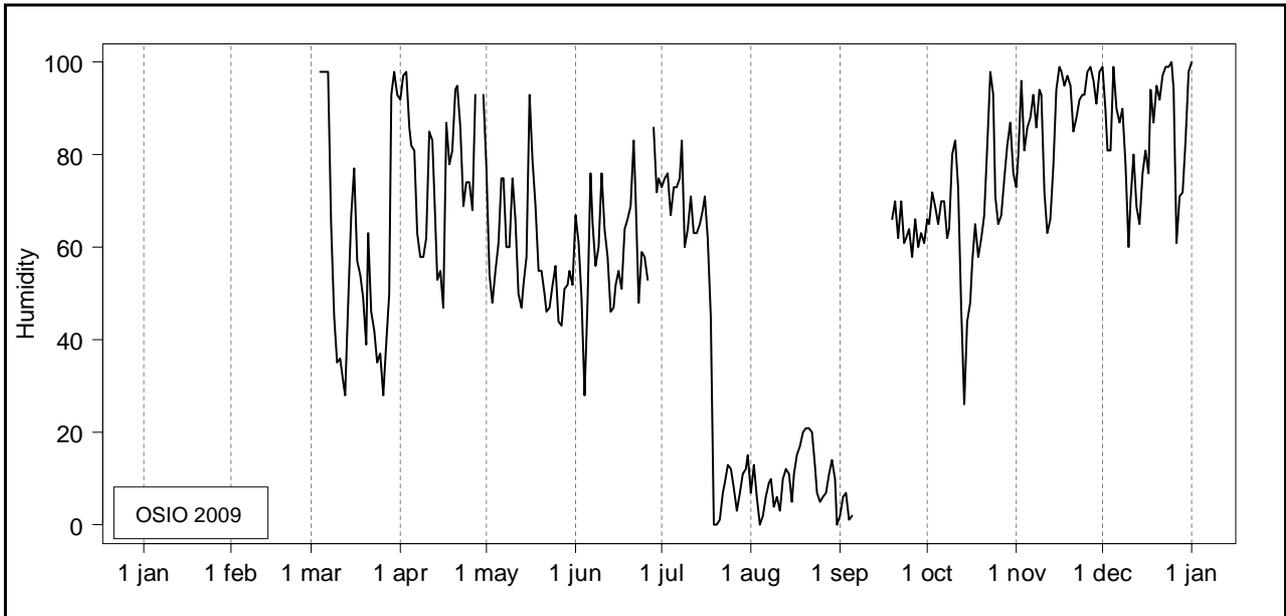


Figure 12 Daily humidity recorded in 2009 at the meteorological control unit of Osio Sotto

For this reason we prefer to consider very cautiously the phenomenon described in this region and we choose to not interpret this effect of climatic variables.

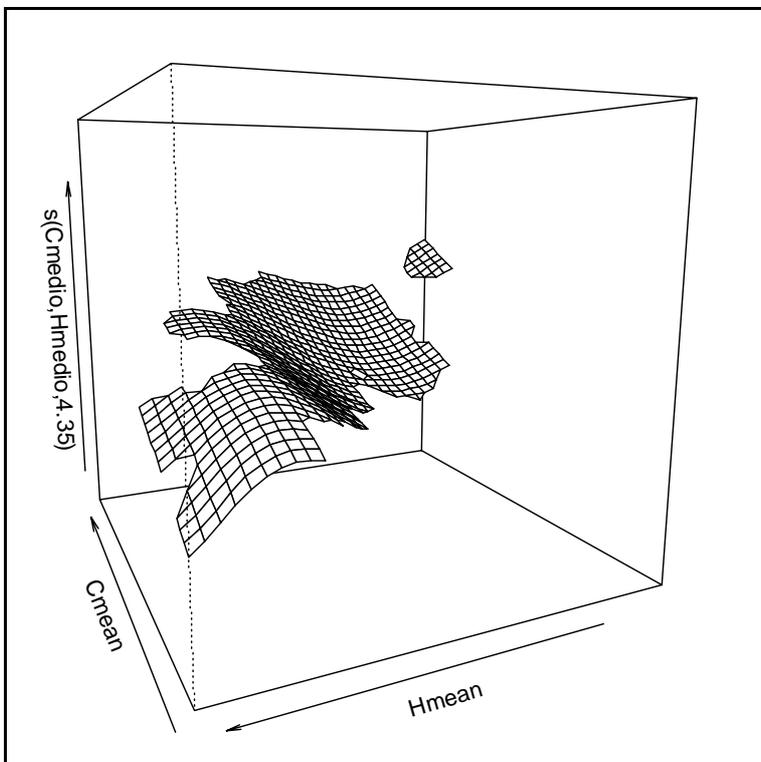


Figure 13 Spatial representation of the model with data actually measured

The region in the lower right (lower left in Fig.13), is characterized by a situation of high humidity and low temperature. It was not possible to find in the literature a study on BMSCC behavior in relation to atmospheric relative humidity, which could give an explanation to this phenomenon. Dakic *et al.* concluded their study on influence of year season on somatic cell count saying that cold stress during winter have considerable influence on the somatic cell count increase in cow milk¹⁰⁷ but humidity was not considered. We could think that an increase in humidity should lead to an increase of BMSCC, as it happens in the less extreme region of the graph, but this seems to be not true. All the predictions in this critical region are not based on an extrapolation from the model. To understand better this phenomenon we made a verification of the model estimation. First of all we decided to use trimmed means and medians of data but the graphs did not change. Afterwards we noted that the situations described above occurred in certain months of the observation period: November and December 2009, January, February and December 2010 (Fig. 14). There is an empirical evidence that in these months the observed BMSCC values seem to have a decreasing trend for increasing humidity. This means that the model does not fail in interpreting data. However, these values could be related to particular climatic conditions (which may be rain, snow, or something more complex linked to the winter season and immune response of dairy cows) that were not considered in the model.

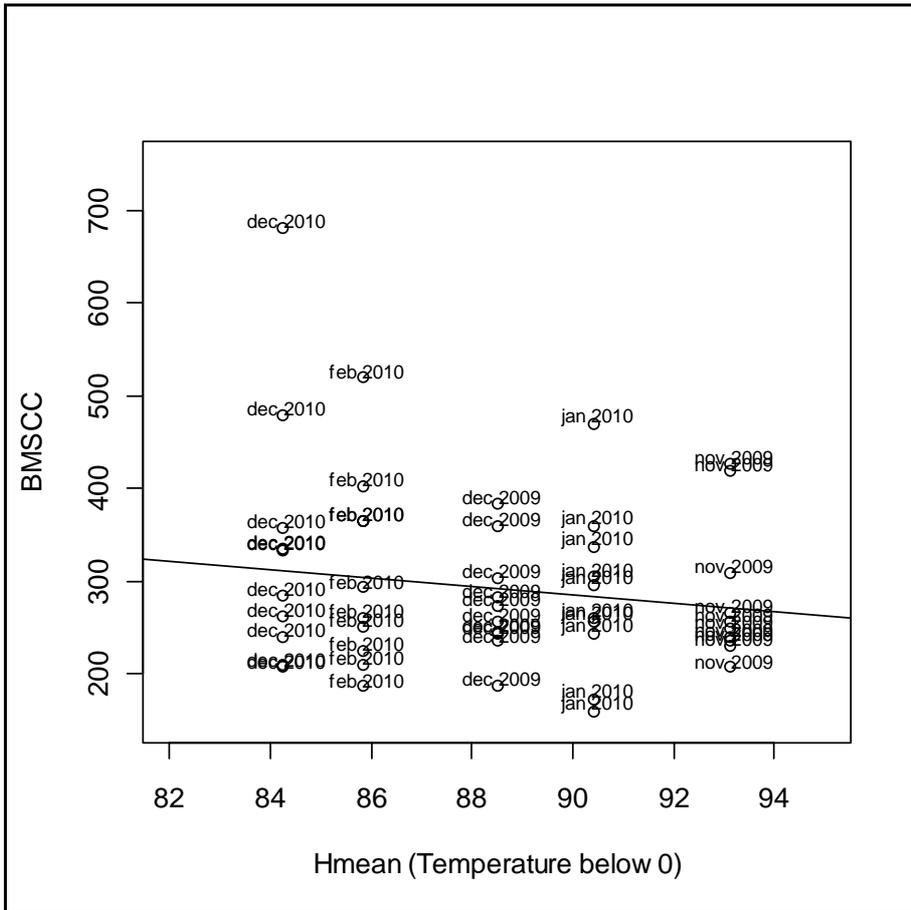


Figure 14 Mean BMSCC of dairy herds in periods with temperature below 0° C and humidity between 84% and 94%

A further control considering each herd separately, shows that the effect of decreasing BMSCC values for increasing humidity (at low temperature values) is less pronounced with respect to the one predicted by the overall model, with the exception of dairy herd H (Fig. 15).

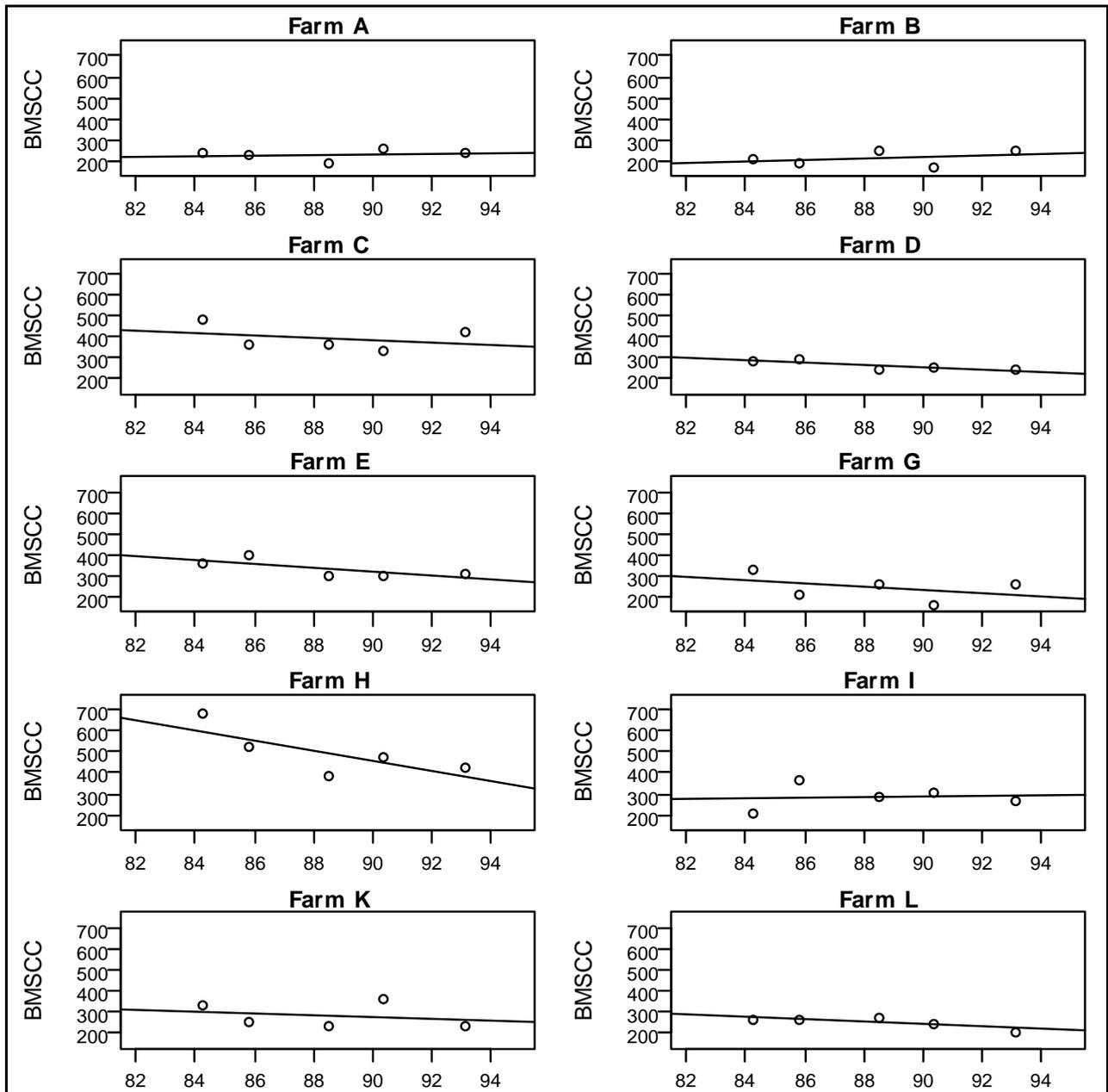


Figure 15 Same as Fig.14, divided by dairy farms and with tendency line

However, even removing the “herd H” observations from the data, we could not find a change of both, bi-dimensional and three-dimensional graphs from the overall model.

As observed in the results, the model fits well for all herds except for herd H. An explanation for this result, is that at the beginning of the study (Fig. 16), in herd H the milk of high SCC cows was not shipped, but was used for calves nutrition.

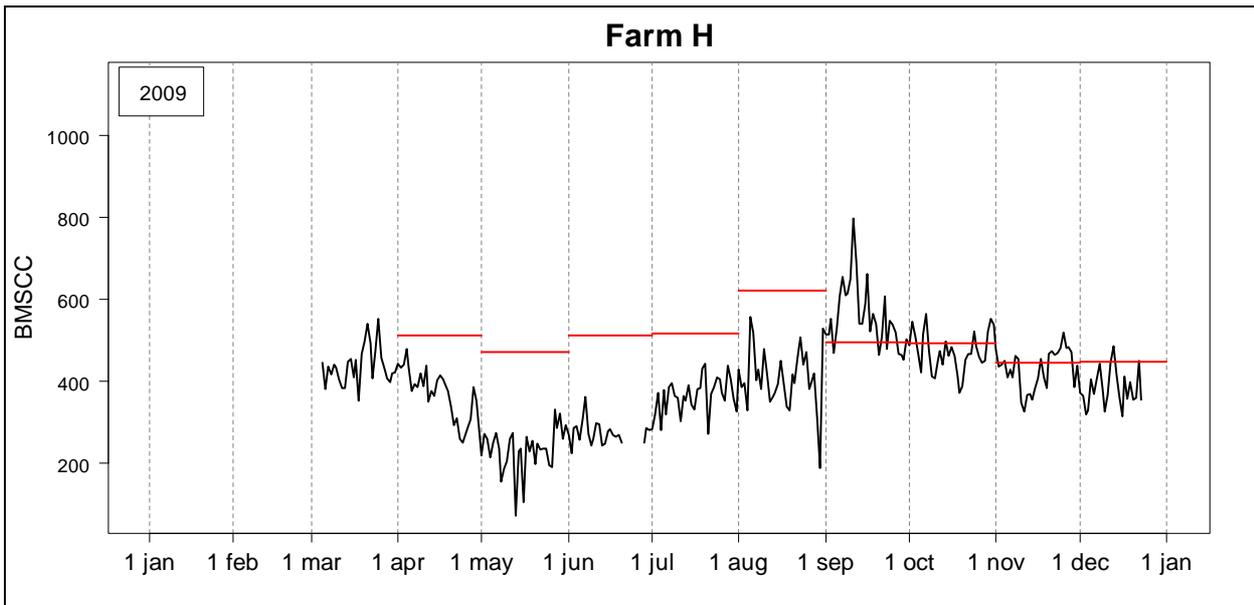


Figure 16 Estimated monthly (red line) and measured daily (black line) BMSCC for farm H in 2009

The analyses of individual SCC including all milking cows, performed by Associazione Provinciale Allevatori di bestiame (APA) di Bergamo (Fig. 17) shows that mean SCC and Linear Score (LS) of the herd from March to September 2009 are much higher than the BMSCC measured on milk shipped. Since September 2009 this practice was interrupted for economic reasons.

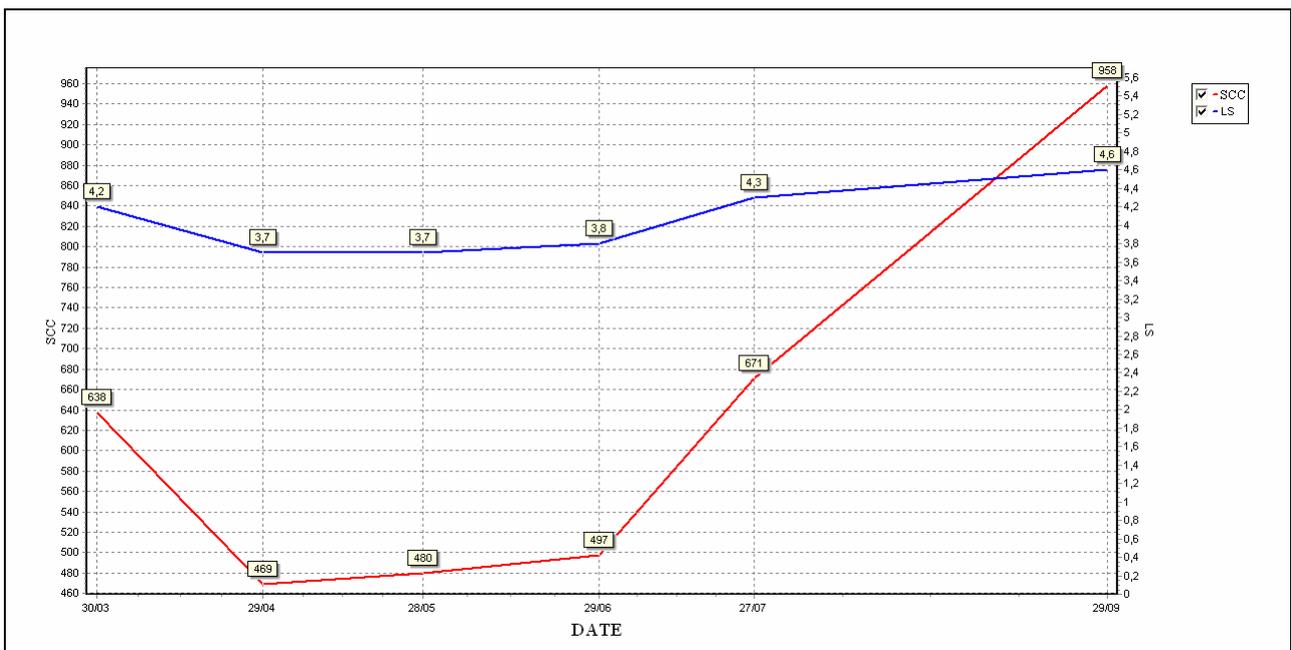


Figure 17 Somatic cell count and Linear score of farm H measured by APA

The explanation for the behavior of BMSCC in summer 2010 (Fig. 18) is probably related to an outbreak of IBR. Animals suffering from a clinical form of IBR can be reasonably more susceptible to intramammary infections, thus increasing both BMSCC and clinical mastitis.

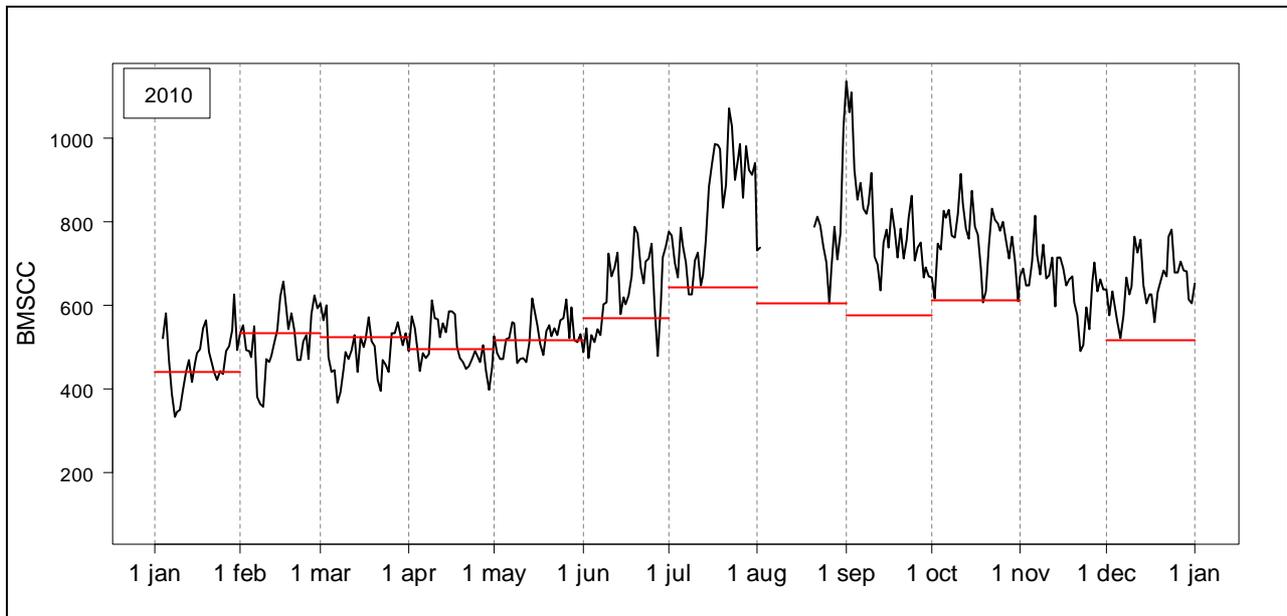


Figure 18 Estimated monthly (red line) and measured daily (black line) BMSCC for farm H in 2010

Fig. 19 from the database of management software of herd H, shows that actually in summer 2010 a greater number of clinical mastitis was recorded, compared to previous and following periods.

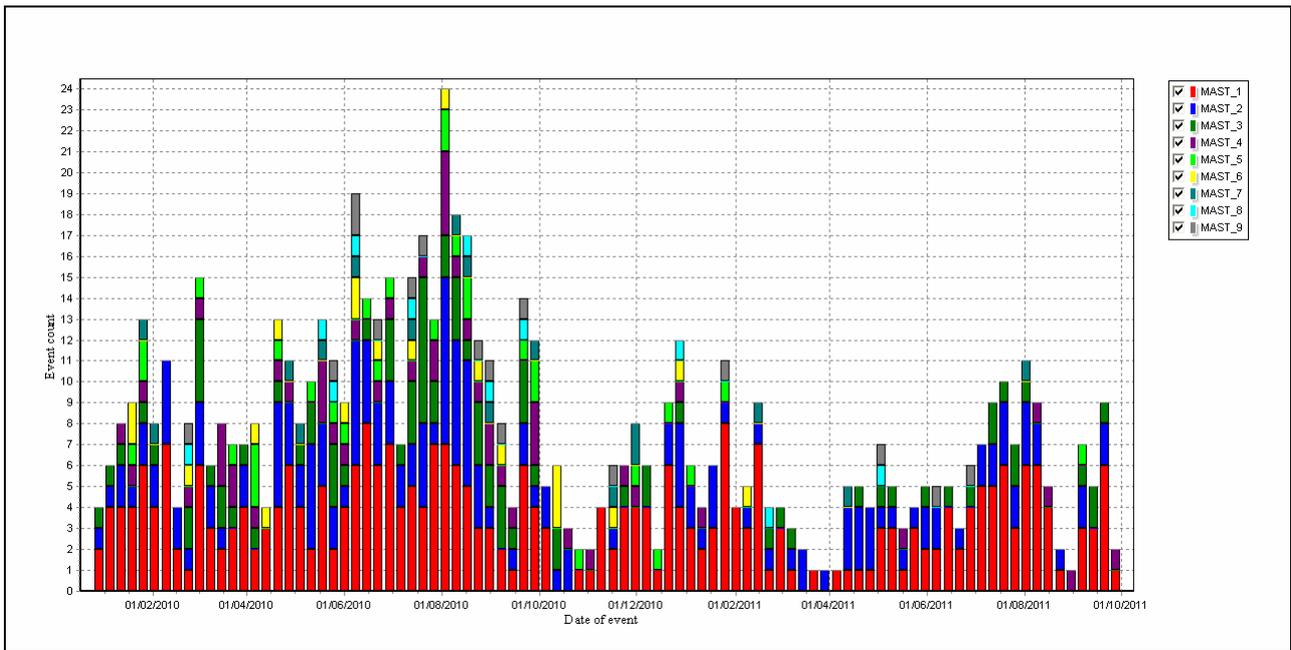


Figure 19 Clinical mastitis recorded on farm H from January 2010 to October 2011

The model may be used for quality control of herds highlighting herds with a marked different behaviour with respect to the average one predicted by the model, which is actually what happens for herd H. Moreover, such a regression model may be used as a statistical based tool alternative to THI for prediction of BMSCC.

CONCLUSIONS

The model described in this research allows us to study the impact of mean monthly temperature and humidity on mean BMSCC of commercial dairy herds in Lombardy. For the first time relative humidity and temperature are considered as predictors of BMSCC in a flexible regression model. The model constitutes a solid basis for further study of the relationship between daily temperature and humidity, and daily BMSCC. It was not possible to fully explain the behavior of the variable response in the presence of high humidity and low temperatures. Further studies and investigations are needed to validate the results obtained. Moreover the model will allow to set up a quality control on dairy farm that allows to predict the impact on BMSCC using atmospheric temperature and humidity data. Hence it will be possible to provide evidence-based advice to dairy farmers with the use of control charts created on the basis of our statistical model.

REFERENCES

1. Schalm, O. W.; Carroll, E. J.; Jain, N. C., *Le mastiti della bovina*. Edagricole: Bologna, 1975.
2. *Current concepts of bovine mastitis*. National Mastitis Council: Madison WI, 1996; p 64 pp.
3. Reneau, J. K., Effective use of dairy herd improvement somatic cell counts in mastitis control. *Journal of Dairy Science* 1986, 69, 1708-1720.
4. Dohoo, I. R.; Leslie, K. E., Evaluation of changes in somatic cell counts as indicators of new intramammary infections. *Preventive Veterinary Medicine* 1991, 10, 225-237.
5. Hogeveen, H.; Huijps, K.; Lam, T. J. G. M., Economic aspects of mastitis: new developments. *New Zealand Veterinary Journal* 2011, 59, 16-23.
6. Allore, H. G.; Erb, H. N., Partial budget of the discounted annual benefit of mastitis control strategies. *Journal of Dairy Science* 1998, 81, 2280-2292.
7. Hagnestam-Nielsen, C.; Emanuelson, U.; Berglund, B.; Strandberg, E., Relationship between somatic cell count and milk yield in different stages of lactation. *Journal of Dairy Science* 2009, 92, 3124-3133.
8. Halasa, T.; Nielen, M.; Roos, A. P. W. d.; Hoorne, R. v.; Jong, G. d.; Lam, T. J. G. M.; Werven, T. v.; Hogeveen, H., Production loss due to new subclinical mastitis in Dutch dairy cows estimated with a test-day model. *Journal of Dairy Science* 2009, 92, 599-606.
9. Halasa, T.; Huijps, K.; steras, O.; Hogeveen, H., Economic effects of bovine mastitis and mastitis management: a review. *Veterinary Quarterly* 2007, 29, 18-31.
10. Halasa, T.; Nielen, M.; Huirne, R. B. M.; Hogeveen, H., Stochastic bio-economic model of bovine intramammary infection. *Livestock Science* 2009, 124, 295-305.
11. Fetrow, J., Mastitis: An Economic Consideration. In *39th Annual Meeting*, National Mastitis Council: Madison, WI, 2000; pp 30-47.

12. Schällibaum, M., Impact of SCC on the Quality of Fluid Milk and Cheese. In *40th Annual Meeting*, National Mastitis Council: Reno, Nevada, 2001; pp 38-46.
13. Cha, E.; Bar, D.; Hertl, J. A.; Tauer, L. W.; Bennett, G.; Gonzalez, R. N.; Schukken, Y. H.; Welcome, F. L.; Grohn, Y. T., The cost and management of different types of clinical mastitis in dairy cows estimated by dynamic programming. *Journal of Dairy Science* 2011, 94, 4476-4487.
14. Hagnestam-Nielsen, C.; Stergaard, S., Economic impact of clinical mastitis in a dairy herd assessed by stochastic simulation using different methods to model yield losses. *Animal* 2009, 3, 315-328.
15. Klei, L.; Yun, J.; Sapru, A.; Lynch, J.; Barbano, D.; Sears, P.; Galton, D., Effects of milk somatic cell count on Cottage cheese yield and quality. *Journal of Dairy Science* 1998, 81, 1205-1213.
16. Ma, Y.; Ryan, C.; Barbano, D. M.; Galton, D. M.; Rudan, M. A.; Boor, K. J., Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk. *Journal of Dairy Science* 2000, 83, 264-274.
17. Grohn, Y. T.; Gonzalez, R. N.; Wilson, D. J.; Hertl, J. A.; Bennett, G.; Schulte, H.; Schukken, Y. H., Effect of pathogen-specific clinical mastitis on herd life in two New York State dairy herds. *Preventive Veterinary Medicine* 2005, 71, 105-125.
18. Taylor, K.; Bryan, M. A., Demonstrating to dairy farmers the impact of mastitis on reproduction. In *Mastitis control: from science to practice. Proceedings of International Conference, 30 September - 2 October 2008*, Lam, T. J. G. M., Ed. The Hague, Netherlands, 2008; pp 407-414.
19. Schrick, F. N.; Hockett, M. E.; Saxton, A. M.; Lewis, M. J.; Dowlen, H. H.; Oliver, S. P., Influence of subclinical mastitis during early lactation on reproductive parameters. *Journal of Dairy Science* 2001, 84, 1407-1412.

20. Loeffler, S. H.; Vries, M. J. d.; Schukken, Y. H., The effects of time of disease occurrence, milk yield, and body condition on fertility of dairy cows. *Journal of Dairy Science* 1999, 82, 2589-2604.
21. Hansen, P. J.; Soto, P.; Natzke, R. P., Mastitis and fertility in cattle - Possible involvement of inflammation or immune activation in embryonic mortality. *American Journal of Reproductive Immunology* 2004, 51, 294-301.
22. Malinowski, E., Mastitis as a cause of reproductive disorders in cows. *Medycyna Weterynaryjna* 2004, 60, 793-797.
23. Soto, P.; Natzke, R. P.; Hansen, P. J., Identification of possible mediators of embryonic mortality caused by mastitis: actions of lipopolysaccharide, prostaglandin F₂alpha, and the nitric oxide generator, sodium nitroprusside dihydrate, on oocyte maturation and embryonic development in cattle. *American Journal of Reproductive Immunology* 2003, 50, 263-272.
24. Allore, H. G.; Erb, H. N.; Schruben, L. W.; Oltenacu, P. A., A simulation of strategies to lower bulk tank somatic cell count below 500,000 per milliliter. *Journal of Dairy Science* 1998, 81, 694-702.
25. Allore, H. G.; Schruben, L. W.; Erb, H. N.; Oltenacu, P. A., Design and validation of a dynamic discrete event stochastic simulation model of mastitis control in dairy herds. *Journal of Dairy Science* 1998, 81, 703-717.
26. Eshraghi, H. R.; Zeitlin, I. J.; Fitzpatrick, J. L.; Ternent, H.; Logue, D., The release of bradykinin in bovine mastitis. *Life Sciences* 1999, 64, 1675-1687.
27. Milne, M. H.; Nolan, A. M.; Cripps, P. J.; Fitzpatrick, J. L., Assessment and alleviation of pain in dairy cows with clinical mastitis. *Cattle Practice* 2003, 11, 289-293.
28. Kemp, M. H.; Nolan, A. M.; Cripps, P. J.; Fitzpatrick, J. L., Animal-based measurements of the severity of mastitis in dairy cows. *Veterinary Record* 2008, 163, 175-179.

29. Huxley, J. N.; Whay, H. R., Current attitudes of cattle practitioners to pain and the use of analgesics in cattle. *Veterinary Record* 2006, 159, 662-668.
30. Laven, R. A.; Huxley, J. N.; Whay, H. R.; Stafford, K. J., Results of a survey of attitudes of dairy veterinarians in New Zealand regarding painful procedures and conditions in cattle. *New Zealand Veterinary Journal* 2009, 57, 215-220.
31. Wieneke, A. A.; Roberts, D.; Gilbert, R. J., Staphylococcal food poisoning in the United-Kingdom, 1969-90. *Epidemiology and Infection* 1993, 110, 519-531.
32. Rosec, J. P.; Guiraud, J. P.; Dalet, C.; Richard, N., Enterotoxin production by staphylococci isolated from foods in France. *International Journal of Food Microbiology* 1997, 35, 213-221.
33. Ote, I.; Taminiau, B.; Duprez, J. N.; Dizier, I.; Mainil, J. G., Genotypic characterization by polymerase chain reaction of *Staphylococcus aureus* isolates associated with bovine mastitis. *Veterinary Microbiology* 2011, 153, 285-292.
34. Cullor, J. S., Risks and prevention of contamination of dairy products. *Revue Scientifique Et Technique De L Office International Des Epizooties* 1997, 16, 472-481.
35. Roberts, D., Sources of infection - food. *Lancet* 1990, 336, 859-861.
36. Stephan, R.; Kuhn, K., Prevalence of verotoxin-producing *Escherichia coli* (VTEC) in bovine coli mastitis and their antibiotic resistance patterns. *Journal of Veterinary Medicine. Series B* 1999, 46, 423-427.
37. Dufour, S.; Frechette, A.; Barkema, H. W.; Mussell, A.; Scholl, D. T., Effect of udder health management practices on herd somatic cell count. *Journal of Dairy Science* 2011, 94, 563-579.
38. Barkema, H. W.; Ploeg, J. D. v. d.; Schukken, Y. H.; Lam, T. J. G. M.; Benedictus, G.; Brand, A., Management style and its association with bulk milk somatic cell count and incidence rate of clinical mastitis. *Journal of Dairy Science* 1999, 82, 1655-1663.

39. Koldeweij, E.; Emanuelson, U.; Janson, L., Relation of milk production loss to milk somatic cell count. *Acta Veterinaria Scandinavica* 1999, 40, 47-56.
40. Schukken, Y. H.; Barkema, H. W.; Lam, T. J. G. M.; Zadoks, R. N., Improving udder health on well managed farms: mitigating the 'perfect storm'. *Mastitis control: from science to practice. Proceedings of International Conference, The Hague, Netherlands, 30 September - 2 October 2008* 2008, 21-35.
41. Elbers, A. R. W.; Miltenburg, J. D.; Lange, D. d.; Crauwels, A. P. P.; Barkema, H. W.; Schukken, Y. H., Risk factors for clinical mastitis in a random sample of dairy herds from the southern part of the Netherlands. *Journal of Dairy Science* 1998, 81, 420-426.
42. Neijenhuis, F.; Barkema, H. W.; Hogeveen, H.; Noordhuizen, J., Relationship between teat-end callosity and occurrence of clinical mastitis. *Journal of Dairy Science* 2001, 84, 2664-2672.
43. Plozza, K.; Lievaart, J. J.; Potts, G.; Barkema, H. W., Subclinical mastitis and associated risk factors on dairy farms in New South Wales. *Australian Veterinary Journal* 2011, 89, 41-46.
44. Steeneveld, W.; Hogeveen, H.; Barkema, H. W.; Broek, J. v. d.; Huirne, R. B. M., The influence of cow factors on the incidence of clinical mastitis in dairy cows. *Journal of Dairy Science* 2008, 91, 1391-1402.
45. Elmoslemany, A. M.; Keefe, G. P.; Dohoo, I. R.; Wichtel, J. J.; Stryhn, H.; Dingwell, R. T., The association between bulk tank milk analysis for raw milk quality and on-farm management practices. *Preventive Veterinary Medicine* 2010, 95, 32-40.
46. Fadlelmoula, A.; Fahr, R. D.; Anacker, G.; Swalve, H. H., The management practices associated with prevalence and risk factors of mastitis in large scale dairy farms in Thuringia-Germany. 1: Environmental factors. *Australian Journal of Basic and Applied Sciences* 2007, 1, 619-624.

47. Pinedo, P.; Risco, C.; Melendez, P., A retrospective study on the association between different lengths of the dry period and subclinical mastitis, milk yield, reproductive performance, and culling in Chilean dairy cows. *Journal of Dairy Science* 2011, 94, 106-115.
48. Waage, S.; Sviland, S.; degaard, S. A., Identification of risk factors for clinical mastitis in dairy heifers. *Journal of Dairy Science* 1998, 81, 1275-1284.
49. Barkema, H. W.; Schukken, Y. H.; Lam, T. J. G. M.; Beiboer, M. L.; Benedictus, G.; Brand, A., Management practices associated with low, medium, and high somatic cell counts in bulk milk. *Journal of Dairy Science* 1998, 81, 1917-1927.
50. Barkema, H. W.; Schukken, Y. H.; Lam, T. J. G. M.; Beiboer, M. L.; Benedictus, G.; Brand, A., Management practices associated with the incidence rate of clinical mastitis. *Journal of Dairy Science* 1999, 82, 1643-1654.
51. Barnouin, J.; Bord, S.; Bazin, S.; Chassagne, M., Dairy management practices associated with incidence rate of clinical mastitis in low somatic cell score herds in France. *Journal of Dairy Science* 2005, 88, 3700-3709.
52. Hansson, H.; Szczensa-Rundberg, M.; Nielsen, C., Which preventive measures against mastitis can increase the technical efficiency of dairy farms? *Animal* 2011, 5, 632-640.
53. Dufour, S.; Dohoo, I.; Devries, T.; Scholl, D., Improving mastitis control programs through identification of risk factors related to the incidence of subclinical intramammary infections. *Mastitis control: from science to practice. Proceedings of International Conference, The Hague, Netherlands, 30 September - 2 October 2008* 2008, 211-218.
54. Dohmen, W.; Neijenhuis, F.; Hogeveen, H., Relationship between udder health and hygiene on farms with an automatic milking system. *Journal of Dairy Science* 2010, 93, 4019-4033.
55. Lehenbauer, T.; Jones, T.; Collar, L., The impact of free-stall housing on somatic cell counts in bulk tank milk. *Bovine Practitioner* 1998, 32, 13-18.

56. Sant'Anna, A. C.; Costa, M. J. R. P. d., The relationship between dairy cow hygiene and somatic cell count in milk. *Journal of Dairy Science* 2011, 94, 3835-3844.
57. Svensson, C.; Nyman, A. K.; Waller, K. P.; Emanuelson, U., Effects of housing, management, and health of dairy heifers on first-lactation udder health in Southwest Sweden. *Journal of Dairy Science* 2006, 89, 1990-1999.
58. Gautam, R.; Bani-Yaghoub, M.; Neill, W. H.; Dopfer, D.; Kaspar, C.; Ivanek, R., Modeling the effect of seasonal variation in ambient temperature on the transmission dynamics of a pathogen with a free-living stage: example of *Escherichia coli* O157:H7 in a dairy herd. *Preventive Veterinary Medicine* 2011, 102, 10-21.
59. Pocknee, B. R., The impact of heat stress on mastitis. In *British Mastitis Conference 2007, Warwickshire, UK, 10th October 2007*, 2007; pp 43-52.
60. Cincovic, M. R.; Belic, B.; Radovic, I., Heat stress of dairy cows - etiopathogenesis and prevention Toplotni stres mlecnih krava - etiopatogeneza i prevencija. *Agrozanje - Agro-knowledge Journal* 2010, 11, 99-105.
61. Pusta, D.; Pasca, I.; Sobolu, R., The influence of the environment on milks' quantity and quality. *International Journal of Agriculture: Research and Review* 2011, 1, 147-150.
62. Hillerton, J. E.; Pankey, J. W.; Pankey, P., Effect of over-milking on teat condition. *Journal of Dairy Research* 2002, 69, 81-84.
63. Reinemann, D. J.; Bade, R.; Zucali, M.; Spanu, C.; Ruegg, P. L., Understanding the influence of machine milking on teat defense mechanisms. *Mastitis control: from science to practice. Proceedings of International Conference, The Hague, Netherlands, 30 September - 2 October 2008* 2008, 323-331.
64. Rasmussen, M. D., Influence of switch-level of automatic cluster removers on milking performance and udder health. *Journal of Dairy Research* 1993, 60, 287-297.

65. Barkema, H. W.; Schukken, Y. H.; Lam, T. J. G. M.; Beiboer, M. L.; Wilmink, H.; Benedictus, G.; Brand, A., Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell counts. *Journal of Dairy Science* 1998, 81, 411-419.
66. Riekerink, R. G. M. O.; Barkema, H. W.; Kelton, D. F.; Scholl, D. T., Incidence rate of clinical mastitis on Canadian dairy farms. *Journal of Dairy Science* 2008, 91, 1366-1377.
67. Andre, G.; Engel, B.; Berentsen, P. B. M.; Vellinga, T. V.; Lansink, A. G. J. M. O., Quantifying the effect of heat stress on daily milk yield and monitoring dynamic changes using an adaptive dynamic model. *Journal of Dairy Science* 2011, 94, 4502-4513.
68. Gantner, V.; Mijic, P.; Kuterovac, K.; Solic, D.; Gantner, R., Temperature-humidity index values and their significance on the daily production of dairy cattle. *Mljekarstvo* 2011, 61, 56-63.
69. Bohmanova, J.; Misztal, I.; Cole, J. B., Temperature-humidity indices as indicators of milk production losses due to heat stress. *Journal of Dairy Science* 2007, 90, 1947-1956.
70. West, J. W., Effects of heat-stress on production in dairy cattle. *Journal of Dairy Science* 2003, 86, 2131-2144.
71. Collier, R. J.; Beede, D. K.; Thatcher, W. W.; Israel, L. A.; Wilcox, C. J., Influences of environment and its modification on dairy animal health and production. *Journal of Dairy Science* 1982, 65, 2213-2227.
72. Ferreira, R. M.; Ayres, H.; Chiaratti, M. R.; Ferraz, M. L.; Araujo, A. B.; Rodrigues, C. A.; Watanabe, Y. F.; Vireque, A. A.; Joaquim, D. C.; Smith, L. C.; Meirelles, F. V.; Baruselli, P. S., The low fertility of repeat-breeder cows during summer heat stress is related to a low oocyte competence to develop into blastocysts. *Journal of Dairy Science* 2011, 94, 2383-2392.
73. Crescio, M. I.; Forastiere, F.; Maurella, C.; Ingravalle, F.; Ru, G., Heat-related mortality in dairy cattle: a case crossover study. *Preventive Veterinary Medicine* 2010, 97, 191-197.

74. Morse, D.; DeLorenzo, M. A.; Wilcox, C. J.; Collier, R. J.; Natzke, R. P.; Bray, D. R., Climatic effects on occurrence of clinical mastitis. *Journal of Dairy Science* 1988, 71, 848-853.
75. Singh, K. B.; Nauriyal, D. C.; Oberoi, M. S.; Baxi, K. K., Studies on occurrence of clinical mastitis in relation to climatic factors. *Indian Journal of Dairy Science* 1996, 49, 534-536.
76. Shearer, J. K.; Bray, D. R., Maintaining udder health and milk quality during periods of hot weather. *Dairy, Food and Environmental Sanitation* 1995, 15, 368-370.
77. Riekerink, R. G. M. O.; Barkema, H. W.; Stryhn, H., The effect of season on somatic cell count and the incidence of clinical mastitis. *Journal of Dairy Science* 2007, 90, 1704-1715.
78. Green, M. J.; Bradley, A. J.; Newton, H.; Browne, W. J., Seasonal variation of bulk milk somatic cell counts in UK dairy herds: investigations of the summer rise. *Preventive Veterinary Medicine* 2006, 74, 293-308.
79. Shearer, J. K.; Beede, D. K., Thermoregulation and physiological responses of dairy cattle in hot weather. *Agri-Practice* 1990, 11, 5...17.
80. Lacetera, N.; Bernabucci, U.; Ronchi, B.; Nardone, A., Body condition score, metabolic status and milk production of early lactating dairy cows exposed to warm environment. *Rivista di Agricoltura Subtropicale e Tropicale* 1996, 90, 43-55.
81. Elvinger, F.; Natzke, R. P.; Hansen, P. J., Interactions of heat stress and bovine somatotropin affecting physiology and immunology of lactating cows. *Journal of Dairy Science* 1992, 75, 449-462.
82. Nardone, A.; Lacetera, N.; Bernabucci, U.; Ronchi, B., Composition of Colostrum from Dairy Heifers Exposed to High Air Temperatures during Late Pregnancy and the Early Postpartum Period. *Journal of Dairy Science* 1997, 80, 838-844.

83. Segnalini, M.; Nardone, A.; Bernabucci, U.; Vitali, A.; Ronchi, B.; Lacetera, N., Dynamics of the temperature-humidity index in the Mediterranean basin. *International Journal of Biometeorology* 2011, 55, 253-263.
84. Akyuz, A.; Boyaci, S.; Cayli, A., Determination of critical period for dairy cows using temperature humidity index. *Journal of Animal and Veterinary Advances* 2010, 9, 1824-1827.
85. Tian, P.; Cai, S.; Yao, W., Effects of temperature and humidity index on milk yield of Holstein cow. *China Dairy Cattle* 2002, 13-15.
86. Jansen, J., Mastitis and farmer mindset: towards effective communication strategies to improve udder health management on Dutch dairy farms. *Mastitis and farmer mindset: toward effective communication strategies to improve udder health management on Dutch dairy farms* 2010, 168 pp.
87. Steuten, C. D. M.; Jansen, J.; Renes, R. J.; Aarts, M. N. C.; Lam, T. J. G. M., Effective communication with 'hard-to-reach' farmers. *Mastitis control: from science to practice. Proceedings of International Conference, The Hague, Netherlands, 30 September - 2 October 2008* 2008, 389-395.
88. Kuiper, D.; Jansen, J.; Renes, R. J.; Leeuwis, C.; Zwaag, H. v. d., Social factors related to mastitis control practices: the role of dairy farmers' knowledge, attitude, values, behaviour and networks. *Mastitis in dairy production: current knowledge and future solutions* 2005, 576-582.
89. Lam, T. J. G. M.; Jansen, J.; Borne, B. H. P. v. d.; Renes, R. J.; Hogeveen, H., What veterinarians need to know about communication to optimise their role as advisors on udder health in dairy herds. *New Zealand Veterinary Journal* 2011, 59, 8-15.
90. Kristensen, E.; Jakobsen, E. B., Challenging the myth of the irrational dairy farmer; understanding decision-making related to herd health. *New Zealand Veterinary Journal* 2011, 59, 1-7.

91. *Udder health and communication Proceedings of the international conference 25-27 October 2011 Utrecht the Netherlands*. First ed.; Wageningen Academic Publishers: Wageningen, 2011; p 428.
92. Noordhuizen, J. P. T. M.; Cannas da Silva, J.; Boersema, J. S. C.; Vieira, A., *Applying HACCP-based Quality Risk Management on Dairy Farms*. Wageningen Academic Publishers: Wageningen, 2008; p 311.
93. Jansen, J.; Steuten, C. D. M.; Renes, R. J.; Aarts, N.; Lam, T. J. G. M., Debunking the myth of the hard-to-reach farmer: effective communication on udder health. *Journal of Dairy Science* 2010, 93, 1296-1306.
94. Reneau, J. K.; Lukas, J., Using statistical process control methods to improve herd performance. *Veterinary Clinics of North America, Food Animal Practice* 2006, 22, 171-193.
95. Vries, A. d.; Conlin, B. J., Economic value of timely determination of unexpected decreases in detection of estrus using control charts. *Journal of Dairy Science* 2003, 86, 3516-3526.
96. Vries, A. d.; Reneau, J. K., Application of statistical process control charts to monitor changes in animal production systems. *J Anim Sci*. 2010, 88, E11-24. Epub 2010 Jan 15.
97. Radostits, O. M., *Herd health : food animal production medicine*. 3rd ed.; W.B. Saunders: Philadelphia, 2001; p xi, 884.
98. Niza-Ribeiro, J.; Noordhuizen, J. P. T. M.; Menezes, J. C., Capability index - a statistical process control tool to aid in udder health control in dairy herds. *Journal of Dairy Science* 2004, 87, 2459-2467.
99. Lukas, J. M.; Hawkins, D. M.; Kinsel, M. L.; Reneau, J. K., Bulk tank somatic cell counts analyzed by statistical process control tools to identify and monitor subclinical mastitis incidence. *Journal of Dairy Science* 2005, 88, 3944-3952.

100. Lukas, J. M.; Reneau, J. K.; Kinsel, M. L., Predicting somatic cell count standard violations based on herd's bulk tank somatic cell count. Part I: Analyzing variation. *Journal of Dairy Science* 2008, 91, 427-432.
101. Lukas, J. M.; Reneau, J. K.; Munoz-Zanzi, C.; Kinsel, M. L., Predicting somatic cell count standard violations based on herd's bulk tank somatic cell count. Part II: Consistency index. *Journal of Dairy Science* 2008, 91, 433-441.
102. R_Development_Core_Team *R: A Language and Environment for Statistical Computing*, R Foundation for Statistical Computing: Vienna, Austria, 2011.
103. Testa, F.; Tranquillo, M.; Casula, A.; Moroni, P., Effect of time delay from sample collection and sample analysis on bulk milk somatic cell count in dairy herds. In *European Buiatrics Forum, Palais du Pharo, Marseille, 1-3 December 2009*, Maillard, R.; Navetat, H., Eds. 2009; p 210.
104. Zhang, D.; Lin, X., Hypothesis testing in semiparametric additive mixed models. *Biostatistics (Oxford, England)* 2003, 4, 57-74.
105. Wood, S. N., *Generalized Additive Models An Introduction with R*. Chapman & Hall/CRC Taylor & Francis Group: Boca Raton, FL, 2006.
106. Ali, A. K. A.; Shook, G. E., An optimum transformation for somatic cell concentration in milk. *Journal of Dairy Science* 1980, 63, 487-490.
107. Dakic, A.; Pintic, N.; Poljak, F.; Novosel, A.; Strucic, D.; Jelen, T.; Pintic, V., Influence of year season on somatic cell count in cow milk collected for the market. Utjecaj godisnjeg doba na broj somatskih stanica u kravljem mlijeku isporucenom za trziste. In *Stocarstvo*, 2006; Vol. 60, pp 35-39.

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